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BROAD AUTISM SPECTRUM AND OBSESSIVE COMPULSIVE SYMPTOMS IN ADULTS WITH THE FRAGILE X PREMUTATION

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Abstract

Objective—Clinical observations and a limited number of research studies provide evidence that the fragile X premutation may confer risk for autism, executive dysfunction, and psychopathology. The link to autism spectrum symptoms and social cognition deficits with the premutation remains uncertain, and thus was the focus of the present investigation.

Method—Our sample included 131 individuals, 42 men/22 women with the *FMR1* premutation (mean age = 31.83 ± 8.59 years) with a normal neurological exam, and 48 men/19 women healthy age matched controls (mean age = 29.48 ± 7.29 years). Individuals completed a comprehensive neuropsychological battery with additional assessments for social cognition, broad autism spectrum, and obsessive-compulsive (OC) symptoms.

Results—Premutation carriers self-reported higher rates of autism-related symptoms (Autism Quotient; p=.001). Among males only, premutation carriers showed more atypical social interaction (p<.001) and stereotyped behavior (p=.014) during standardized clinical examination on the Autism Diagnostic Observation Schedule (ADOS) relative to controls. Female premutation carriers reported significantly higher rates of OC symptoms compared to control females (p=.012). Molecular measures defining the expanded premutation (*FMR1* CGG repeat length and/or mRNA) were significantly associated with a measure of theory of mind (Reading the Mind in the Eyes Task).

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Conclusions—The results of this study indicate a higher rate of broad autism spectrum symptoms in some males with the premutation and provide evidence for an obsessive-compulsive subtype in female premutation carriers.

Keywords

social cognition; fragile X premutation; broad autism spectrum phenotype; obsessive compulsive disorder

Introduction

Throughout the last decade of research on carriers of the *FMR1* premutation, the focus was mainly on clinical observations and reports of autism spectrum disorders (Aziz et al., 2003; Borghgraef, Steyaert, Deroo, Maes, & Fryns, 2004; Chonchaiya et al., 2012; Chonchaiya et al., 2010; Farzin et al., 2006b; Goodlin-Jones, Tassone, Gane, & Hagerman, 2004), working memory or executive function deficits (Cornish et al., 2009; Grigsby et al., 2007; Kraan, Hocking, Bradshaw, Georgiou-Karistianis, & Cornish, 2012; Loesch et al., 2015), obsessive compulsive symptoms (Hessl et al., 2005), and other psychiatric features (Bourgeois et al., 2009; Roberts et al., 2009). However, many of these studies have been limited by recruitment bias issues, have been case series, or have various methodology confounds, such as relying on the reports of parents of affected children or the lack of appropriate controls.

The prevalence of the premutation (55–200 CGG repeats in the promoter region in *FMR1* at Xq27.3) is relatively high in the general population, with approximately 1:130–250 females and 1:400–800 males (Fernandez-Carvajal et al., 2009; Hagerman, 2008; Seltzer, Baker, et al., 2012; Tassone et al., 2012). Given the relatively high prevalence, and increasing evidence of neuropsychiatric involvement, the clinical neuropsychologist is likely to encounter one or more patients with the fragile X premutation during the course of practice. An awareness of the unfolding understanding of the neuropsychiatric phenotype of carriers as empirical evidence becomes available will help the practitioner interpret assessment results providing added clarity to the patient on the cause of symptoms (understanding that the etiology may be multifactorial including genetic and environmental/developmental aspects) and highlight the potential need for referral to other specialists (e.g., neurology, psychiatry, autism specialists). With an added basic understanding the genetics and intergenerational transmission of the mutation and its phenotypes in families may also help the practitioner be more aware of when referral to a clinical geneticist is appropriate for *FMR1* testing.

The increase in CGG repeats is strongly correlated with significant up-regulation of *FMR1* mRNA levels, which leads to RNA toxicity. In addition, a lowering of *FMR1* protein (FMRP) expression levels can be observed in some carriers particularly in the upper premutation range (Hagerman, 2013). About 20% of female premutation carriers are affected by fragile X-associated primary ovarian insufficiency (FXPOI) (Sullivan et al., 2005; Welt, 2008; Wittenberger et al., 2007), with a non-linear relationship of FXPOI onset and CGG repeat size length (Peprah et al., 2010; Sullivan et al., 2005). Older premutation carriers of both genders (though the predominance is in men) can be affected by fragile X-

associated tremor/ataxia syndrome (FXTAS), a neurodegenerative disease characterized by intention tremor, ataxia, autonomic dysfunction, and cognitive decline (Hagerman et al., 2001; Hall et al., 2014). For individuals affected by FXTAS, CGG repeat length positively correlates with age of onset and severity of motor symptoms (Sevin et al., 2009; Tassone et al., 2007; Tassone et al., 2012). For individuals unaffected by FXTAS, the relationship of CGG repeat size and severity of neurocognitive symptoms remains controversial (Cornish et al., 2009; Hunter, Abramowitz, Rusin, & Sherman, 2009; Hunter, Sherman, Grigsby, Kogan, & Cornish, 2012).

One challenge of specifying the premutation phenotype is that problems in younger carriers are typically more subtle and very common in the general population, and not as obvious as in the full mutation fragile X syndrome (FXS), (Cornish et al., 2005; Farzin et al., 2006b; Hessl et al., 2005; Johnston et al., 2001; Roberts et al., 2009). Symptoms of hyperactivity, social deficits, and autism spectrum disorder (ASD), seizures, as well as anxiety and mood disorders can be present in premutation carriers of both genders (Chonchaiya et al., 2012; Aziz et al., 2003; Goodlin-Jones et al., 2004). Females with the premutation have been found to have higher rates of mood disorders and social phobia than the general population (Bourgeois et al., 2011; Kenna et al., 2013; Roberts et al., 2009) and those with FXTAS have higher rates of lifetime major depressive disorder, panic disorder, post-traumatic stress disorder, and specific phobia compared to the general population (Bourgeois et al., 2011).

The cognitive profile in premutation carriers predominantly shows executive function deficits (Fischer, Sananbenesi, Wang, Dobbin, & Tsai, 2007; Grigsby et al., 2014; Kogan & Cornish, 2010; Wang et al., 2013). Males with the premutation, in comparison with matched family and non-family controls, display a behavioral and cognitive profile similar to, but less severe than that observed in males with the fragile X full mutation (FXS), including impairment on a social cognition task (i.e., worse performance in reading emotion from the eyes), obsessive-compulsive traits, and executive function problems with inhibitory control (Cornish et al., 2005). While Cornish and colleagues attribute these deficits to cerebellar functioning, others have reported evidence that dysfunction or structural differences in the amygdala-hippocampus complex and associated structures of the limbic system are also involved. Recent evidence from our center showed that increased levels of *FMR1* mRNA and reduced *FMR1* protein (FMRP) are associated with functional alterations in limbic brain regions that mediate social cognition, memory, and emotion (Hessl et al., 2011).

In addition to potential impairment on facial affect recognition, some male carriers of the fragile X premutation show broad autism phenotype features including rigid adherence to routine and perseveration (Cornish et al., 2005), and a significant subgroup meet full diagnostic criteria for ASD (Aziz et al., 2003; Borghgraef et al., 2004; Chonchaiya et al., 2012; Farzin et al., 2006b; Goodlin-Jones et al., 2004). In recent years, several reviews and meta-analyses focused on the broad autism phenotype (BAP) in the general population. BAP is described as a milder manifestation of autism spectrum symptoms without meeting full diagnostic criteria (D'Cruz et al., 2013; Ruzich et al., 2015; Sasson, Nowlin, & Pinkham, 2013). The BAP has also been reported in females with the premutation with a focus on deficits in pragmatic language and personality features including rigidity and over-conscientiousness (Losh et al., 2012).

The current study focuses on social cognition, the BAP and molecular correlates in a non-clinic referred sample of individuals with the premutation and matched controls, with hypotheses that a) premutation carriers have greater BAP features and lower scores on measures of social cognition and b) that this aspect of the premutation phenotype is associated with elevated *FMR1* CGG and mRNA levels as measured in blood lymphocytes.

Method

Participants

The study sample included 131 individuals, 42 men and 22 women with the *FMR1* premutation (mean age = 31.8 ± 8.6 years) with normal neurological exam, and 48 men and 19 women healthy controls (mean age = 29.5 ± 7.3 years). Premutation carriers were not clinic referred and were primarily selected at random from existing pedigrees of probands with the fragile X full mutation or responded to flyers posted through the National Fragile X Foundation (www.nfxf.org). Healthy controls were recruited from the local general population and through postings at the University. Two of the control participants had clinically elevated symptoms on the autism measures (ADOS, AQ), and were excluded from further analyses to ensure an unaffected control group. Group status for each participant was confirmed through DNA testing as having 55–200 CGG repeats (carriers of the *FMR1* premutation), or having 5–44 CGG repeats (normal range, comparison group). The two study groups did not differ on age, IQ, gender, marital status, or ethnicity. To eliminate potential confounds of increased symptomology related to raising developmentally disabled children (Johnston et al. 2003, Abbeduto et al. 2004), the recruitment and exclusion strategy was such that none of the premutation carriers had children with the full mutation (fragile X syndrome) or other neurodevelopmental disorders, and no control participant was raising a child with neurodevelopmental disorders. Table 1 gives an overview of the study demographics. A subset of males from this study participated in a prior fMRI study (Hessl et al., 2011) that focused on the amygdala volume and function in relation to *FMR1* mRNA and FMRP.

Prior to study participation, each participant gave written informed consent. The study's protocol was approved by the Institutional Review Board at the University of California, Davis. All assessments were administered by psychologists, trained psychometrists, or high level graduate students. Assessors were usually blinded to the participant's molecular status; however in several cases the participants themselves revealed their diagnosis and rater blindness could not be guaranteed.

Molecular measures

Genomic DNA was isolated from peripheral blood lymphocytes (5 ml of whole blood using standard methods; Qiagen, Valencia, CA.). Premutation allele sizes were confirmed using Southern Blot and PCR analyses as previously described (Tassone et al., 2000; Tassone, Pan, Amiri, Taylor, & Hagerman, 2008). All non-carrier controls were documented to be negative for the mutations in the *FMR1* gene (< 45 CGG repeats). Individuals who had a gray zone allele (45–54 CGG repeats) or a full mutation mosaicism (in which some cells carry alleles both in the premutation and the full mutation range) were not included in this study. *FMR1*

mRNA involved expression levels were quantified by real-time qRT–PCR as described previously (Tassone et al., 2000).

Test materials

For testing the different areas of social cognition and psychopathology, a comprehensive test battery was administered to the participants. Social cognition and BAP was assessed with the Movie for Assessment of Social Cognition (Dziobek, 2006), the Reading the Mind in the Eyes Task (Baron-Cohen, Wheelwright, Hill, Raste, & Plumb, 2001), Autism Diagnostic Observation Schedule (Lord, Rutter, DiLavore, & Risi, 1999), the Autism Quotient (Baron-Cohen, Wheelwright, Skinner, Martin, & Clubley, 2001), and the Social Responsiveness Scale, Adult version (Constantino & Todd, 2005). For the area of psychopathology, the Symptom Checklist-90 Revised (Derogatis, 1994), the Beck Anxiety Inventory (Beck & Steer, 1993), and the Dimensional Yale–Brown Obsessive–Compulsive Scale (Rosario-Campos et al., 2006) were administered. The participants' intellectual ability was assessed with the Wechsler Adult Intelligence Scale, 3rd Edition (Wechsler, 1997).

Social Cognition and Broad Autism Spectrum Measures

Movie for Assessment of Social Cognition (MASC; Dziobek, 2006): The MASC is a video-based assessment of social cognition, especially Theory of Mind (ToM) (Dziobek, 2006). It requires the study participants to watch a 15 min series of video clips about four adults getting together for a dinner party. The video is paused 46 times and questions concerning the characters' feelings, thoughts, and intentions are probed. In addition, there are 5 control questions that assess how well the participant followed the storyline. We chose the MASC total correct score (maximum 46 points) as an indicator for a representation of ToM functioning. According to the authors, the average correct response for a healthy control group is 34.8 (SD 2.7), and for high functioning individuals on the Autism Spectrum 24.4 (SD 5.9). The internal consistency was reported with a Cronbach's Alpha of .82 to .84, and a test-retest correlation of .97.

Reading the Mind in the Eyes Task (Baron-Cohen, Wheelwright, Hill, et al., 2001 Raste, & Plumb, 2001): The Eyes Task-Revised is a more extensive version of the original test developed by Baron-Cohen and colleagues (Baron-Cohen, Wheelwright, Hill, et al., 2001). The stimuli for this test consist of 36 black and white photographs (2x5 in) of only the eye region of human faces showing various expressions taken from larger photographs in popular magazines. Participants were asked to look at each photograph one at a time and then choose a word from a choice of 4 that best describes what that individual may be thinking or feeling. For example, a black and white image of the eye region of a female character is presented, and the participant is asked to select one of the following words that may describe what the person may be feeling or thinking: "a) doubtful, b) affectionate, c) playful, d) aghast". During the test, participants were allowed to read through the glossary for word meanings of which they were unsure. The maximum score was 36. The average correct response mean for the general population, according to Baron-Cohen et al. (2001) was 26.2 (SD 3.6), with a minimal advantage for the female control group (M=26.4, SD 3.2), compared to the male control group (M=26.0, SD 4.2). A group of high functioning

individuals on the Autism Spectrum showed an average of 21.9 correct answers (SD 6.6). Internal consistency, validity, and test-retest reliability has been shown repeatedly.

Autism Spectrum Quotient (Baron-Cohen, Wheelwright, Skinner, et al., 2001): The AQ is a self-assessment screening instrument developed by Baron-Cohen and colleagues (Baron-Cohen, Wheelwright, Skinner, et al., 2001) to measure the degree to which an individual of normal intelligence shows autistic traits. Participants are presented with 50 statements in the domains of social skills, attention switching, attention to detail, communication, and imagination. Each subscale comprised 10 statements, with participants being asked on a Likert-type scale to agree, slightly agree, slightly disagree, or definitely disagree with each. In addition to individual sub- scores, an overall score can be derived. The total score ranges from 0–50, with scores > 32 considered to be on the ASD spectrum. An unaffected, IQ-matched control group showed an average score of 18.9 (SD 2.9).

Social Responsiveness Scale (Constantino & Todd, 2005): The original SRS is a quantitative measure of autistic traits in children and adolescents. In our study, we used a revised form to address social responsiveness in adulthood (SRS-A; Constantino and Todd 2005). Similar to the child version, the SRS-A has 65 Likert-scaled (0–3) items, and generates a singular scale with a maximum score of 195 for behavior shown in the last 6 months, with scores ranging from 0 (highly socially competent) to 195 (severely socially impaired), with scores above 80 suggesting a significant impairment in everyday social functioning. The questionnaire was completed by a spouse, friend, or relative who knows the participant well.

Psychopathology

Symptom Checklist-90-Revised (SCL-90-R; Derogatis, 1994): The SCL-90-R is a standardized self-report measure of psychological symptoms. 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 is an indicator of overall level of psychiatric disturbance. The scale results are given in T-scale (Mean 50, SD 10). T-scores above 70 are considered clinically significant.

Beck Anxiety Inventory (Beck & Steer., 1993): The BAI is a 21-question multiple-choice self-report inventory that is used for measuring the severity of an individual's anxiety. Each question has the same set of four possible answer choices, ranging from Not at all (0 points), to Severely (3 points). Summed scores from 0–21 indicate low anxiety, 22–35 moderate anxiety, and scores higher than 36 may be an indicator for clinically significant anxiety. For the purpose of our study, the BAI summed scores were converted into the T-scale (Mean=50, SD 10).

Dimensional Yale–Brown Obsessive–Compulsive Scale Self-report (DY-BOCS; Rosario-Campos et al., 2006): The DY-BOCS is a 88-item self-report measure of obsessive-compulsive symptoms: obsessions about harm due to aggression/injury/violence/ natural disasters and related compulsions; obsessions concerning sexual/moral/religious

obsessions and related compulsions; obsessions about symmetry/'just-right' perceptions, and compulsions to count or order/arrange; contamination obsessions and cleaning compulsions; obsessions and compulsions related to hoarding and miscellaneous obsessions and compulsions that relate to somatic concerns and superstitions, among other symptoms. In addition to the symptom checklist, the DY-BOCS self-report also includes items that ask the patient to assess the overall symptom severity in each of the dimensions for the previous week, on scales ranging from 0 (no symptoms) to 10 (symptoms are extremely troublesome). Participants are also asked about avoidance behaviors within each dimension. Main measure for the DY-BOCS is the Global OCD score. The validation study reports an average global severity score for adults with OCD symptoms of 19.6 (SD 4.8) (Rosario-Campos et al. 2006).

Statistical Analysis

To examine initial group differences in demographic, *FMR1* molecular measures and IQ, we performed two-way analyses of variance (ANOVA) with group (premutation vs. control) and gender as the independent variables. To examine categorical variables (e.g., marital status and race) by group, we used Chi-Square tests. To examine group differences in the primary measures of interest, two-way analyses of variance (ANOVA) were used, with group (premutation vs. control) and gender as the independent variables, and each of the social cognition tests and measures of the broad autism phenotype as the dependent variables. For measures that did not meet the ANOVA distributional assumptions (ADOS and D-YBOCS had skewed, non-normal distributions), we used Mann-Whitney U tests to first examine differences in scores between premutation and controls overall, and then followed-up with Mann-Whitney tests separately for males and females. The partial Eta squared (η^2) was used to represent effect size for parametric tests. To calculate associations between the molecular measures (CGG repeat size and *FMR1* mRNA) and the variables of social cognition and broad autism phenotype, Spearman's rank correlations were used, separately for premutation carriers and controls.

Results

Group differences in demographic, molecular genetic and intelligence measures

The two-way ANOVAs (group and gender as the independent variables) showed no significant main effects or interactions on age, verbal or performance IQ, or education level. Chi Square tests (separately done with males and females) showed no significant differences in race or marital status by group. As to be expected, premutation carriers had significantly larger CGG repeat sizes and *FMR1* mRNA levels than controls, with large effect sizes (see Table 1).

Broad Autism Phenotype

Group (premutation vs. control) by gender ANOVAs with MASC, AQ, Eyes Task, and SRS-A as dependent variables showed a significant main effect of group for AQ total score (premutation carriers showed significantly greater autism-related features than controls, F=10.80, p=.001, η^2 .080), and a main effect of gender for Eyes Task (females>males,

F=6.06, p = .015, η^2 .046), with females responding to more items correctly. There were no significant group differences for the MASC, or the SRS-A. Follow-up ANOVAs of the subscales of the AQ showed that the premutation group effects were specific to the Attention Switching (F=15.32, p<.001, η^2.110) and Communication (F=9.27, p=.003, η^2 .070) domains (see Table 2).

For the ADOS, the analysis showed significant elevations for social interaction and stereotyped and restricted behavior in male premutation carriers vs. male controls (U=565, p<.001 and Z=744, p=.014, respectively; Table 3). The communication score approached significance (p=.084). No significant premutation vs. control group differences were observed for females (p>.160).

Psychopathology

Two-way ANOVAs with group (premutation vs. control) and gender as the independent variables and each subscale of the SCL-90-R as the dependent variables showed significantly elevated scores for premutation carriers vs. controls on all scales (see Table S1), although the group averages mostly fell within the normal range (T 40–59), except for obsessive compulsive and interpersonal sensitivity averages being more than a standard deviation above normal for the female premutation carrier group (see supplemental Table S1). There was a main effect of gender for phobia (F=7.81, p=.006) and paranoia (F=6.78, p = .010) with males reporting higher scores than females. There were no significant interactions between gender and group on any of the SCL-90-R scores.

An ANOVA for the Beck Anxiety Inventory showed a main effect of group with elevated T-scores for premutation carriers (F=19.69, p<.001) but no main effect of gender or interaction between gender and group, (see supplemental Table S1).

The DY-BOCS data were not normally distributed, and the nonparametric Mann-Whitney tests demonstrated significantly elevated Global OCD scores for female premutation carriers vs. female controls (U=81, p=.012), but no group differences were observed for males (U=456, p=.62). See Table 3.

Correlation with molecular measures

The Spearman Rho rank correlations did not reveal significant correlations between CGG repeat size and the measures of broad autism phenotype, social cognition, or psychopathology in the control group. In the premutation group, CGG repeat size and *FMR1* mRNA were significantly correlated with the Eyes Task total score (r -.435, p < .001 and r = − .398, p = .003). Higher CGG repeat and mRNA were associated with a lower performance on reading emotion/thoughts from the eye region. To control for the multiple comparisons in our analyses, we adjusted the probability level using a family wise error rate and a Bonferroni procedure. For both methods, an alpha level = .002 for any given test would yield a general alpha level = .05 for the entire family of tests. According to this criterion, the correlations with the Eyes Task remained significant (adjusted p value .025). One should note, however, that both tests are highly conservative, thus, making our finding for Eyes Task total score particularly reliable.

Discussion

In this study, we sought to more thoroughly investigate the hypothesis that broad autism spectrum features, psychopathology, and social cognition deficits are an aspect of the fragile X premutation phenotype. The study focused on a non-clinic referred sample of younger men and women with the premutation unaffected by FXTAS, and without children with FXS and compared to an age-, gender-, and IQ-matched control group. The recruitment strategy for this study minimized ascertainment bias associated with enrolling a clinical population motivated to be seen for treatment or assessment, which has been a limitation of some prior studies in the field.

Interestingly, the measures of social cognition (Eyes task, MASC) did not show group differences between premutation carriers and controls. There was a gender effect in the Eyes task, with both female premutation carriers and female controls showing a slightly higher ability to identify the expressions correctly.

Both male and female premutation carriers showed elevated scores for the Autism Quotient, a self-assessment of ASD-related symptoms. Four premutation carriers scored above the cut-off score for autism spectrum disorders (AQ total score > 32). This was validated for the males with elevated Social Interaction and Restrictive/Repetitive Behavior scores on the ADOS, a gold-standard assessment for ASD symptoms. When applying the new ADOS, Module 4 scoring algorithm (Lord et al., 2012) , 4 premutation male carriers met ASD criteria. This appears consistent with the finding of a significant increase of behaviors associated with ASD in male premutation carriers who were primarily clinic referred (Aziz et al., 2003; Chonchaiya et al., 2012; Farzin et al., 2006a). Previous results from our group (Hessl et al., 2005) showed a self-reported preference for social isolation and, in an fMRI study, a blunted amygdala response while viewing or evaluating the emotions in human faces in male premutation carriers unaffected by FXTAS (Hessl et al., 2007; Hessl et al., 2011). Furthermore, blunted amygdala response was associated with psychological symptoms, particularly obsessive–compulsive traits and symptoms of ASD (Hessl et al., 2007).

The female premutation carriers had significantly higher rates of OC symptoms compared to control females. From a clinical perspective, we have often noted that mothers of children affected by FXS report OC symptoms. This was originally thought to be related to the anxiety of raising affected children. In our current study, none of the female premutation carriers have affected children but show elevated OC symptoms, which leads to a tentative hypothesis that in females, the premutation itself, and its influence on brain function may confer some risk for development of formal OCD, or OC personality traits. Based on the fact that the *FMR1* gene is located on the X chromosome, a more severe phenotype among male carriers would be expected. However, this pattern was not evident for the symptoms related to OCD. This may be an indicator for a specific female subtype of impairment. A study by Loesch (2015) showed a relationship of severity of psychiatric symptoms in female premutation carriers dependent on a non-linear effect of CGG repeat size within the premutation range, similar to the severity of FXPOI symptoms (Allen et al., 2008; Hunsaker et al., 2011; Sullivan et al., 2005) and previous reports of psychopathology in female carriers

(Roberts et al., 2009; Seltzer, Barker, et al., 2012). The mid-range CGG repeat size is associated with an increased severity of symptoms in these studies. Loesch and colleagues (2015) hypothesized that those differences may result from structural transcript errors in the mid-premutation range causing ineffective protein binding. In our study, however, we did not find an effect of CGG repeat size on the severity of symptoms of psychopathology, although given our small sample size, detecting non-linear effects would not be possible.

The study results are limited by the sample sizes that prevented a more complex statistical modelling. Furthermore, the limited number of participants from ethnic minority populations limits the generalizability of the results of the study. Furthermore, we were not able to obtain FMRP results, which could harbor more striking correlations between neuropsychological measures, as shown previously. Future work with larger sample sizes and comprehensive *FMR1*-related correlates, including sensitive FMRP assays will help to confirm these results and more clearly determine their molecular basis.

Finally, there remains the possibility of ascertainment bias for the enrolled study participants, because only a subset of carriers may have been capable of traveling to our center, and these individuals may be more likely to seek clinical impressions from our research team. However, it is also possible that premutation carriers who are more affected by social difficulties and anxiety would avoid travel and research participation, as has been reported to us by relatives during recruitment efforts. Future studies should include a population based sampling such as reported by Seltzer and colleagues (Seltzer, Baker, et al., 2012). Another strategy to increase the participation could be the use of assessments via telemedicine.

In summary, this study contributes further evidence for a higher rate of broad autism phenotype traits in males with the premutation and provides evidence for an OC subtype in female premutation carriers, perhaps similar to the personality features of rigidity and over-consciousness previously described by Losh and colleagues (Losh et al., 2012). These features may be subtle and below threshold for diagnosis of a formal DSM 5 diagnosis. Thus it is important for the clinician to differentiate personality traits without impairment in daily life from clinically meaningful symptoms of ASD or OCD that require treatment in this population. Although this study focused on a particular set of symptoms and phenotypic features, carriers of the premutation are at higher risk for executive dysfunction, mood and other anxiety disorders, and FXTAS as has been previously described; therefore the adult clinical neuropsychologist should be vigilant and screen for these psychiatric and neurological conditions as well. At this time, until more targeted treatments become available, the treatment of psychiatric problems in premutation carriers is no different than that of individuals without the premutation; however the potential stressors of multiple affected family members (e.g., children and siblings with FXS, parents/grandparents with FXTAS), the meaning of and response to passing on the mutation to offspring, and the individual's thoughts and emotions concerning risk for future neurodegenerative disease should be evaluated and addressed. It is also important to emphasize that individuals with the premutation may be high functioning, experience no clinically significant symptoms, and require no intervention. Our ongoing and other longitudinal studies will help to clarify whether psychiatric symptoms or particular traits that do occur in a subset of adults with the

premutation are associated with later neurological problems, as in FXTAS, or whether they are a developmental and independent manifestation of the phenotype. This information will contribute to the meaning of neuropsychological assessment results related to aging in premutation carriers and how this information is interpreted to patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Demographic, *FMR1* genetic, and intelligence measures by group.

	Control (C, n=65)		Premutation (P, n=64)		C vs. P (Males) p	C vs. P (Females) p
Gender	Males (n=46)	Females (n=19)	Males (n=42)	Females (n=22)	-	-
CGG	29.19 (4.57)	30.79 (2.41)	99.55 (33.61)	89.04 (22.36)	**<.001**	**<.001**
*FMR1*mRNA	1.36 (0.28)	1.31 (.22)	3.28 (1.39)	2.29 (.67)	**<.001**	**<.001**
Age	29.71 (7.52)	29.53 (7.96)	31.83 (9.14)	31.61 (6.75)	ns	ns
Caucasian	75%	84.21%	92.8%	91.3%	ns	ns
Married	37.5%	42.1%	45.2%	43.47%	ns	ns
VIQ	122.88 (16.34)	114.00 (11.97)	114.40 (16.01)	116.45 (14.18)	ns	ns
PIQ	118.83 (16.87)	118.42 (12.00)	114.31 (16.51)	121.73 (11.53)	ns	ns

Abbreviations: C=Control, P=Premutation, n= Sample size, p = Significance Level, CGG = CGG Repeat Size, FMR1mRNA = Fragile X Mental Retardation messenger RNA level, VIQ = Verbal IQ, PIQ = Performance IQ

Table 2

Group comparisons for social cognition and broad autism phenotype measures.

	Control (C) Mean (SD) (n=65)		Premutation (P) Mean (SD) (n=64)		Group	Gender	Group x Gender
	Male (N=46)	**Female (N=19)**	**Male (N=42)**	**Female (N=22)**	**F p η^2***	**F p η^2***	**F p η^2***
Eyes Task (Total)	27.20 (4.11)	28.40 (3.06)	26.67 (4.00)	29.00 (2.19)	.213 .645 *.000*	**6.06** **.015** ***.046***	.914 .341 *.005*
MASC (ToM total)	35.98 (4.22)	36.93 (2.96)	35.56 (4.55)	37.94 2.23)	.051 .822 *.001*	3.91 .051 *.037*	.743 .391 *.007*
SRS-A	29.07 (22.86)	29.37 (21.06)	37.44 (31.77)	32.83 21.21)	1.72 .192 *.014*	.175 .677 *.005*	.017 .897 *.000*
Autism Quotient (AQ Total)	15.13 (6.60)	11.95 (4.83)	19.24 7.85)	16.74 8.40)	**10.80** **.001** ***.080***	**4.41** **.038** ***.034***	.063 .802 *.001*
AQ Attention Switching	3.64 (2.20)	2.74 (1.85)	4.95 (2.13)	4.70 2.58)	**15.32** **<.001** ***.110***	1.94 .166 *.015*	.611 .436 *.005*
AQ Communication	1.89 (1.66)	1.00 (1.24)	2.80 (2.49)	2.35 1.92)	**9.27** **.003** ***.070***	3.27 .073 *.026*	.337 .562 *.003*

Note: *$\eta_2^2 \geq 0.10$ = small effect, $\eta_2^2 \geq 0.30$ = medium effect, $\eta_2^2 \geq 0.50$ = large effect Abbreviations: C=Control, P=Premutation, MASC=Movie for Assessment of Social Cognition, SRS-A=Social Responsiveness Scale Adulthood, AQ=Autism Quotient

Table 3

Autism Diagnostic Observation Schedule (ADOS Module 4) and Dimensional Yale–Brown Obsessive–Compulsive Scale (D-YBOCS) group differences.

	Controls Mean Rank		Premutation Mean Rank		Group (P vs. C)	Males Only	Females Only
	Male (N=46)	Female (N=19)	Male (N=42)	Female (N=22)	U p	U p	U p
ADOS - Communication	62.22	52.58	72.38	55.95	1716 .107	763 .084	181 .563
ADOS - Social Interaction	55.87	49.37	80.63	57.10	**1363** **<.001**	**565** **<.001**	169 .347
ADOS - Restricted /Repetitive Behavior	49.37	59.45	71.38	61.55	**1644** **.007**	**744** **.014**	171 .162
DY-BOCS - Global OCD	48.27	44.67	45.04	66.91	1034 .201	456 .618	**81** **.012**

Abbreviations: C=Control, P=Premutation, ADOS=Autism Diagnostic Observation Schedule, DY-BOCS-Dimensional Yale Brown Obsessive Compulsive Scale Self Report, OCD=Obsessive Compulsive