

# Impact of the Fragile X Mental Retardation 1 (*FMR1*) Gene Premutation on Neuropsychiatric Functioning in Adult Males Without Fragile X-Associated Tremor/Ataxia Syndrome: A Controlled Study

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**Fragile X Syndrome is the most common heritable form of mental retardation caused by silencing of the *FMR1* gene, which arises from intergenerational trinucleotide repeat expansion leading to full mutation. An intermediary carrier condition, known as the *premutation*, is characterized by expansion up to 200 repeats without concomitant gene silencing. This prevalent allelic variant was initially thought to be free of phenotypic effects. However, recent reports have identified a degenerative disease, Fragile X-associated Tremor/Ataxia Syndrome (FXTAS) in older men as well as premature ovarian failure in women. Previously reports are inconsistent regarding the neuropsychiatric phenotype associated with *premutation* due to small sample sizes, ascertainment bias, lack of adequate control groups, administration of measures with poor psychometric properties, and the confounding effects of FXTAS. We addressed these problems by conducting a controlled study of male carriers (n = 40) of the *premutation* without manifest symptoms of FXTAS, comparing their responses on specific, reliable, and valid measures of neuropsychiatric functioning to those of individuals with shared family environment (n = 22) and non-carrier comparison males (n = 43). Multivariate analyses revealed that the *premutation* confers significant risk for working memory difficulties, an associated feature of Attention-Deficit Disorder. Furthermore, both the family controls and men with**

**premutation exhibited higher rates of Alcohol Abuse as compared to non-carrier control men. These findings highlight the importance of recognizing the distinct phenotypic outcomes that characterize the Fragile X *premutation* and the subtle risk factors that can act as precursors to more significant psychiatric impairment.**

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**KEY WORDS:** Fragile X syndrome; FMRP; FXTAS; working memory; alcohol abuse

Please cite this article as follows: Kogan CS, Turk J, Hagerman RJ, Cornish KM. 2008. Impact of the Fragile X Mental Retardation 1 (*FMR1*) Gene Premutation on Neuropsychiatric Functioning in Adult Males Without Fragile X-Associated Tremor/Ataxia Syndrome: A Controlled Study. *Am J Med Genet Part B* 147B:859–872.

## INTRODUCTION

Fragile X syndrome (FXS) has been identified as the most common heritable form of mental retardation affecting nearly 1 in 4,000 males and 1 in 9,000 females [Turner et al., 1996; Crawford et al., 2001]. Beyond the relatively high prevalence of this condition, FXS has garnered additional interest because of its unique single gene etiology, which has allowed for fruitful exploration of genotype-phenotype relationships. FXS is caused by expansion of a trinucleotide (CGG)<sub>n</sub> repeat region within the 5' untranslated region of the Fragile X Mental Retardation 1 (*FMR1*) gene (Online Mendelian Inheritance in Man<sup>®</sup> [OMIM] 309550; Johns Hopkins University, Baltimore, MD) located on the X chromosome [Oberle et al., 1991; Pieretti et al., 1991; Verkerk et al., 1991]. Expansion occurs in successive steps across generations, which is reflected in the nomenclature used to describe the various genotypes. Representative samples have demonstrated that *normal* CGG repeat sizes correspond to between 7 and 54, with 30 repeats found on the most common allele [O'Donnell and Warren, 2002]. A *premutation* is considered present when the trinucleotide repeat region expands to between 55 and 200. Finally, the *full mutation* condition is considered present when individuals harbor greater than 200 CGG repeats. Typically such large expansions result in methylation of the *FMR1* promoter region, thereby preventing mRNA transcription and the expression of its associated protein, FMRP. The lack of FMRP has been attributed to the wide range of deficits associated with

Grant sponsor: Center for Disease Control; Grant number: U10/CCU92513; Grant sponsor: NICHD; Grant number: #HD02274.

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Received 14 June 2007; Accepted 31 October 2007

DOI 10.1002/ajmg.b.30685

FXS including mental retardation [Hagerman and Hagerman, 2002].

Initially, the *premutation* was believed to be medically and psychologically benign. However, several case reports prompted researchers to examine the possibility of the existence of phenotypic features specific to the *premutation* condition [Hagerman et al., 1996, 2001]. The hypothesis was put forth that individuals with the *premutation* might manifest similar impairments as are observed in the *full mutation* condition, albeit less severe. An alternative hypothesis is derived from more recent molecular findings suggesting that whereas similar brain regions might be susceptible to *premutation* expansion as in the *full mutation*, the mechanisms of impairment may differ, thereby leading to, at times, divergent phenotypic effects. Specifically, it appears that although individuals with the *premutation* have expression levels of FMRP that are sometimes lower than observed in individuals with the *normal* allele, there is also evidence of an increase in *FMR1* mRNA levels, as much as by an order of magnitude, a molecular event that is largely restricted to the *premutation* condition, except in those cases of the *full mutation* where the allele remains unmethylated [Tassone et al., 2000a,b]. The increase in mRNA levels has been hypothesized to lead to a toxic "gain-of-function" in neurons and astrocytes, which results in the characteristic nuclear inclusions, brain atrophy, and white matter disease observed in *post-mortem* brain tissue [Hagerman et al., 2004; Greco et al., 2006]. Therefore, it is equally plausible that individuals with the *full mutation* and those with the *premutation* will evidence overlapping phenotypic features as much as it is likely that they will display genotype-specific features.

Evidence for a unique *premutation* phenotype, at the physiological level, comes from two findings. Firstly, female carriers appear to have significant increased risk for developing premature ovarian failure [Allingham-Hawkins et al., 1999]. Secondly, male and, occasionally, female *premutation* carriers also have a risk of developing a degenerative motor condition known as fragile X-associated tremor/ataxia syndrome (FXTAS), which manifests in later life [Allingham-Hawkins et al., 1999; Hagerman et al., 2001; Leehey et al., 2003]. Neither of these conditions has been observed in the *full mutation* condition.

Although limited, there is emerging evidence suggesting that individuals with the *premutation* do indeed demonstrate an associated profile of cognitive strengths and weaknesses that in part mirrors the *full mutation*. In particular, findings from these studies suggest that *premutation* expansion leads to impairments in working memory [Cornish et al., submitted], inhibitory control [Cornish et al., in press], social cognition [Cornish et al., 2005], along with deficits in planning of goal-directed behavior and in executive functions [Loesch et al., 2003a,b].

Individuals affected by the *full mutation* also have a characteristic neuropsychiatric phenotype. In particular, individuals with FXS have been reported to exhibit higher prevalence of symptoms of social anxiety, communication disorders, autism spectrum disorders, and Attention-Deficit Hyperactivity Disorder [Bregman et al., 1988; Cohen et al., 1988, 1989, 1991; Sudhalter et al., 1990; Reiss and Freund, 1992; Turk, 1992; Kerby and Dawson, 1994; Mazzocco et al., 1998; Hatton et al., 2003; Kau et al., 2004; Kaufmann et al., 2004]. Therefore, much like the recently identified *premutation*-associated cognitive phenotype, it is possible that there exists a specific profile of psychiatric or psychological problems associated with *premutation* expansion. It is also possible that *premutation* carriers manifest a profile of psychiatric and psychological difficulties distinct from *full mutation* individuals. Elucidating this putative profile is critically important because unlike the *full mutation*, prevalence of the *premuta-*

*tion* is relatively high, estimated at affecting 1 in 813 men [Dombrowski et al., 2002] and 1 in 259 women [Rousseau et al., 1995]. Unfortunately, studies conducted to date examining the psychiatric and psychological symptoms associated with the *premutation* condition are fraught with methodological limitations, particularly for male *premutation* carriers. Briefly, studies have been limited by small sample sizes [Thompson et al., 1994; Myers et al., 2001; Aziz et al., 2003; Goodlin-Jones et al., 2004; Farzin et al., 2006], ascertainment bias [e.g., Aziz et al., 2003] failure to differentiate between FXTAS affected and non-carrier individuals [e.g., Dorn et al., 1994], and lack of appropriate control groups [Rousseau et al., 1994; Thompson et al., 1994; Johnston et al., 2001; Myers et al., 2001; Aziz et al., 2003; Goodlin-Jones et al., 2004; Moore et al., 2004; Hessel et al., 2005]. Therefore, our knowledge of the psychiatric and psychological phenotype of individuals with the *premutation*, in particular of men, is limited.

The aim of the present study is to elucidate the psychiatric profile of men diagnosed as *premutation* carriers of Fragile X who do not manifest physical symptoms of FXTAS. We set out to address limitations of previous research by including a large sample of *premutation* men (forty), appropriate family control participants who have shared a similar environment to those with the *premutation*, and psychometrically sound measures of specific psychiatric/psychological domains of function. Furthermore, we have focused on non-symptomatic carriers (i.e., without FXTAS) to guard against the potential confound of a medical illness, which is known to deleteriously impact brain function [Bacalman et al., 2006] and thereby, result in the manifestation of psychiatric/psychological problems secondary to FXTAS. An ancillary goal of the present study is to examine the relationship between CGG repeat size, a measure of *premutation* severity, and the various measures of psychiatric and psychological functioning.

## MATERIALS AND METHODS

### Participants

Group 1 comprised adult men identified as *premutation* carrier status according to standard protocols (see DNA analysis). These individuals were recruited through the UK Clinical Genetic Service Centres and the UK Fragile X Society on the sole basis that they had a relative who had been diagnosed with the *full mutation*. As such, they were not seeking medical services for symptoms related to FXTAS or any other condition. All participants were self-identified as Caucasian (indigenous white British). Group 2 comprised non-carrier adult men who are members of families within which there was at least one identified proband. These individuals were matched to the *premutation* men, within a 5-year window and served as a critical comparison group intended to control for environmental effects associated with altered family interactions due to having to care for one or more children or grandchildren with a diagnosis of Fragile X Syndrome. Group 3 comprised non-carrier adult males with no family history of Fragile X. These participants were recruited from the local general population and matched individually according to age to the *premutation* group. Furthermore, these individuals were assessed for carrier status and all were found to be negative for *premutation* expansion.

Consistent with the objective of the present study, which was to investigate the psychiatric profile of *premutation* carrier men who do not manifest symptoms of the Fragile X-Associated Tremor/Ataxias Syndrome, all participants were screened for such symptoms using the self-report neurology questionnaire (see Neurology Questionnaire Section). A highly conservative cut score was chosen (i.e., scores > 0) in order to ensure that none of the participants manifested symptoms of tremor or

TABLE I. Age and IQ of the Participant Groups

	Mean $\pm$ SD		
	Premutation (n = 40)	Family control (n = 20)	Non-family control (n = 43)
Age	45.03 $\pm$ 14.27	40.80 $\pm$ 12.41	43.84 $\pm$ 13.87
WASI			
Verbal scale IQ	101.5 $\pm$ 17.2	107.3 $\pm$ 20.6	108.0 $\pm$ 12.8
Performance scale IQ	105.5 $\pm$ 12.5	114.2 $\pm$ 13.2	109.8 $\pm$ 11.0
Full scale IQ	104.1 $\pm$ 14.9	113.7 $\pm$ 12.8	110.2 $\pm$ 13.6

ataxia. Therefore, with this criterion, eight participants from Group 1, two from Group 2, and five from Group 3 were excluded from further analyses. Therefore, 40 men with the premutation, 20 non-carrier family control men, and 43 non-carrier non-family control men were included in subsequent analyses. Age and IQ data for each of the groups is presented in Table I.

### DNA Analysis

Genomic DNA was isolated from 5 ml of peripheral blood by standard protocols. Direct PCR was carried out using the following primers: forward, 5'-CACGACGTTGTAACGACACGGAGGCGCCGCTGCCAGG-3' and reverse 5'-GAGAGGTGGGCTGCGGGCGCT-3', modified from Wang et al. [1995] at 0.5 pmol final concentration. Conditions were as follows: final concentration 1 mM MgCl<sub>2</sub>, dATP, dCTP, and dTTP at 0.2 mM, 7-deaza-GTP (Amersham Pharmacia Biotech, Piscataway, NJ) at 0.4 mM supplemented with 5% DMSO in a total volume of 20  $\mu$ l [Wang et al., 1995]. Cycling conditions were 32 cycles at 67°C annealing. Products were separated on PAGE gels and visualized by silver staining according to standard protocols. Where the premutation was visible following PCR the repeat size was calculated according to size markers and by electrophoresing the products in size order and aligning the stutter bands. Southern blotting was carried out according to standard protocols on genomic DNA using a double restriction enzyme digest of *Eco*R1 (NEB) and the methylation sensitive enzyme *Eag*1 (NEB) and probed with Ox1.9 [Knight et al., 1993].

Relative sizing for each participant was accomplished using DNA from female control with known repeat size. Where possible, repeat sizes derived from single bands (SB) were compared to those obtained from direct PCR. Repeat sizes of those individuals who gave a result on direct PCR and on SB were congruent. Blots were over-exposed to detect any evidence of mosaicism against a known mosaic control. A premutation is defined here as an allele between 55 CGG repeats up to approximately 200 repeats without any evidence of abnormal methylation. Mosaicism was considered present when there was evidence of a methylated cell line as well as an unmethylated premutation cell line.

### Neurology Questionnaire

Participants self-reported neurological symptoms on a questionnaire derived from Jacquemont et al. [2004]. The neurology questionnaire assesses two domains of functioning. These are, (1) Tremor: questions were asked regarding the presence, characteristics, and time-of-onset of tremors and (2) Gait and lower extremities: questions were asked related to the onset of balance problems, recent falls, and walking distance. The questionnaire was completed over the phone or in person. For the purpose of the survey, symptoms were scored

as present if noticed by the respondent, with clarification of the questions or characterization of the symptoms being provided by the interviewing physician as necessary. The participant gave the final answers. The convergent validity of this questionnaire was previously evaluated by comparison with blind videotape scoring of matched clinical neurological evaluations and found to be highly congruent [Jacquemont et al., 2004].

### Marital Status, Education Level, Employment Status, and Medical History

In order to examine possible group differences in marital status, education level, employment status, and medical history the following demographic information was gathered from each participant (Table II). For marital status, each participant was asked whether they were: (i) married, (ii) single and never married, (iii) living with a partner, or (iv) divorced. For education level, each participant was asked whether they had completed either (i) a graduate degree, (ii) an undergraduate degree, (iii) a higher diploma, (iv) their A levels, (v) their O levels, (vi) left secondary school, or (v) had fewer than 11 years of schooling. In order to assess employment status, the Standard Occupational Classification—Volume 3 was administered. Therefore, participants were asked whether they were employed in: (i) a professional occupation, (ii) a managerial or technical occupation, (iii) a skilled occupation—manual and non-manual labor, (iv) an occupation requiring partly skilled labor, or (v) an occupation not requiring skilled labor. Medical history for significant illnesses was assessed by asking each participant whether they had suffered from any of the following medical conditions: (i) infectious or parasitic infection (ii) neoplasms, (iii) endocrine, metabolic, or immune system disease, (iv) blood or diseases of the blood-forming organs, (v) mental disorders, (vi) nervous system or sense organ disease, (vii) circulatory system disease, (viii) respiratory system disease, (ix) digestive system disease, (x) skin or subcutaneous tissue disease, (xi) musculoskeletal system or connective tissue disease, (xii) congenital anomalies, (xiii) conditions originating in the perinatal period, (xiv) symptoms, signs and ill-defined conditions, (xv) injury and poisoning, (xvi) other factors influencing health status. Furthermore, information was gathered regarding whether participants had ever experienced any significant back or head injuries. Finally, participants were asked about whether they were currently taking any medications.

### Cognitive Assessment

General cognitive ability was assessed with the Wechsler [1999] Abbreviated Scale of Intelligence [WASI], which comprises four subtests of the WAIS-III and provides acceptable estimates of Verbal, Performance, and Full Scale IQ scores. Each participant was administered these subtests by a

TABLE II. Demographic Information and Medical History for the Participant Groups

	Frequency (%)		
	<i>Premutation</i> (n = 40)	<i>Family control</i> (n = 20)	<i>Non-family control</i> (n = 43)
Marital status			
Married	67.50	40.00	51.20
Single and never married	17.50	35.00	34.90
Living with a partner	2.50	10.00	2.30
Divorced	7.50	5.00	9.30
Separated	5.00	10.00	2.30
Education			
University level	7.50	40.00	37.25
Secondary only	20.00	15.00	23.25
Less than secondary	72.50	45.00	39.50
Employment status			
Employed	67.50	80.00	55.80
Unemployed	5.00	5.00	14.00
Retired at normal age	2.50	—	—
Retired early	5.00	—	9.30
Retired—ill health	5.00	5.00	7.00
Training	2.50	10.00	11.60
Self employed	12.50	—	2.30
Social class			
Professional	15.00	20.00	4.70
Managerial or technical	10.00	25.00	14.00
Skilled labor	27.50	40.00	44.20
Partly skilled labor	30.00	—	27.90
Non-skilled labor	17.50	15.00	9.30
Medical history			
Infections	5.00	—	7.00
Neoplasms	0.00	—	4.70
Endocrine, metabolic, or immune	5.00	5.00	2.30
Blood	2.50	—	—
Mental	7.50	—	—
Nervous system	5.00	5.00	—
Circulatory	2.50	—	—
Respiratory	5.00	10.00	2.30
Digestive	—	—	2.30
Skin or subcutaneous	2.50	—	—
Musculoskeletal	2.50	—	—
Congenital	—	—	—
Perinatal	—	—	—
Ill-defined conditions	—	—	—
Injury or poisoning	—	—	—
Other	—	—	—
Injuries			
Back	—	—	—
Head	5.0	15.0	11.6
Neck	5.0	5.0	7.0
Medications			
Yes	30.0	15.0	14.0

trained psychometrician. The means and standard deviations for group scores on the IQ measures are provided in Table I.

### Psychological and Psychiatric Assessment

A comprehensive battery of well-validated and reliable self-report measures was administered at study visits. We examined psychiatric and psychological functioning within specific domains that have previously been shown to be impaired in individuals affected by Fragile X Syndrome. In particular, we evaluated participants for evidence of symptoms in the following areas: mood and anxiety, attention-deficit hyperactivity, negative symptoms of psychotic disorders, autistic spectrum disorders, drug and/or alcohol abuse and dependence, and schizotypal personality. Descriptions of the specific measures administered and their psychometric properties are provided below.

### Mood and Anxiety

**Malaise inventory.** The Malaise Inventory (MI) is a commonly used 24-item self-report screening tool developed by Rutter et al. [1970] to evaluate the construct of psychological distress, also described as negative affect. This measure asks participants to indicate “yes” or “no” to items presented allowing a total score to be calculated by adding on point for each item endorsed as “yes” [Rutter et al., 1970]. Items included in the inventory assess a broad range of psychological symptoms (e.g., depression anxiety, and anger) as well as a number of somatic complaints (e.g., backache, stomachache, and rheumatism). Exploratory factor analysis for this measure supports a two-factor solution, with a first factor reflecting psychological symptoms and a second, reflecting physical symptoms [Rodgers et al., 1999]. Indeed, a 15-item subscale has been extracted from this inventory to form a strictly

psychological subscale [Grant et al., 1990]. However, other findings suggest that the psychological subscale has somewhat weaker internal consistency and is no better at discriminating between groups with and without psychiatric problems when compared with the full scale [Rodgers et al., 1999]. The MI full scale has been shown to have acceptable internal consistency with coefficient alpha values ranging from 0.77 to 0.81. Furthermore, the MI has good external validity, adequately discriminating among groups of individuals with affective disorders, major depressive disorder, those requiring hospital contact for mental health reasons both in the past year and past 5 years, those taking psychotropic medications, and those who have contacted their family physician for mental health reasons. In the present study, group differences for the full scale, the 15-item psychological subscale, and 8-item physical subscale were included in analyses. A separate analysis of the psychological subscale was conducted to address concerns that those caring for disabled adults, such as premutation carriers with disabled children or grandchildren, are more likely to demonstrate elevations in the MI total score, which has been attributed to the higher prevalence of physical concerns among this population rather than psychological distress per se [Rodgers et al., 1999].

**Hospital anxiety and depression scale.** The Hospital Anxiety and Depression Scale (HADS) is a 14-item self-report screening tool comprising seven anxiety and seven depression items [Zigmond and Snaith, 1983]. The latter permit separate scores to be obtained for anxiety and depression subscales in addition to the total score. The HADS assesses for the presence of psychopathology, specifically within the population of individuals already diagnosed with a medical condition (e.g., premutation status). Participants are presented with items that they can rate on 4-point Likert-type scales. Despite the two logically differentiated subscales articulated by Zigmond and Snaith [1983], more recent studies suggest that the HADS is tri-dimensional, composed of factors assessing depressive symptoms, "psychic anxiety," and "psychomotor agitation" [Friedman et al., 2001]. Despite the better fit of items to a three-factor solution, the original anxiety and depression subscales still retain high internal consistency with coefficient alphas at 0.81 and 0.90, respectively [Martin et al., 2006]. As such, the original 7-item subscales were included in the analyses conducted in the present study.

**Leibowitz social anxiety scale—self-report.** The Leibowitz Social Anxiety Scale (LSAS) is a self-report measure that evaluates the degree to which a person experiences both fear and avoidance of 24 specific social situations often problematic for socially anxious individuals [Leibowitz, 1987]. Participants are asked to use a 4-point Likert-type scale to indicate their level of fear as well as their level of avoidance for each of situation. For scoring and interpretation purposes, the individual item values can be partitioned in several ways. Therefore, the fear and avoidance total scale scores can be further divided in to participants' scores for situations that involve social interaction and those that involve performance. The internal consistency for the various subscales of the LSAS, as measured by coefficient alpha, in both clinical and non-clinical samples ranges from 0.73 for the Avoidance of Performance subscale to 0.91 for the Total Fear score [Fresco et al., 2001]. This suggests overall adequate internal consistency of this measure. Furthermore, both convergent and discriminant validity for the LSAS are good [Fresco et al., 2001].

**Rosenberg self-esteem scale.** The Rosenberg Self-Esteem Scale [RSES; Rosenberg, 1965] is a commonly used 10-item self-report measure designed to evaluate a unidimensional construct of self-esteem also referred to as self-worth. Participants' endorse items upon a 4-point Likert-style scale. The scale has been used for a broad range of applications in

both clinical and non-clinical samples and has been found to be related to depression when data from the National Comorbidity Study were examined [Schmitz et al., 2003]. Factor analyses suggest a two-factor structure of the RSES, reflecting the design of the instrument into five positively valenced items, that is, measuring self-enhancement, and five negatively-valenced items, that is, measuring self-derogation [e.g., Shahani et al., 1990]. However, the unidimensional total score is still considered to have adequate psychometric properties of internal consistency and construct validity. As such, the full scale score was used in subsequent analyses.

### Attention-Deficit Hyperactivity

The Brown Attention-Deficit Disorder Scale for Adults [Brown ADD; Brown, 1996] is a 40-item self-report measure that assesses for the presence of diagnostic features associated with and often co-morbid with Attention-Deficit/Hyperactivity Disorder (ADHD). Thus, items probe for endorsement of criteria outlined in the DSM-IV-TR definition of ADHD as well as symptoms of disorders often co-morbid with ADHD such as learning disorders and mood disturbance [Brown, 1996]. For scoring and interpretation purposes, the items comprising the Brown ADD have been grouped in to five separate clusters reflecting domains of functioning that are often problematic for individuals with ADHD. Thus, this instrument measures, (i) organizing and activating to work, (ii) sustaining attention and concentration, (iii) sustaining energy and effort, (iv) managing affective interference, and (v) utilizing working memory and accessing recall. The coefficient alphas for the various clusters ranges from 0.79 for the utilizing working memory and accessing recall cluster to 0.92 for the sustaining attention and concentration cluster, suggesting adequate internal consistency. The Brown ADD total score and cluster scores also have excellent discriminant validity, with positive cases showing elevations in total and cluster scores. The range of effect size differences between clinical and non-clinical groups for the various scores is 2.17 for the utilizing working memory and accessing recall and 2.97 for the total score [using Cohen's *d*; Brown, 1996].

### Negative Symptoms Associated With Psychotic Disorders

The Scale for the Assessment of Negative Symptoms [SANS; Andreasen and Olsen, 1982] is a 20-item clinician-rated measure that assesses the presence and severity of negative symptoms associated with schizophrenia. These symptoms are grouped according to five separate domains of functioning, which are: (i) affective flattening and blunting, (ii) alogia, (iii) avolition-apathy, (iv) anhedonia-asociality, and (v) attentional impairment. The clinician scores the severity of each item on a 6-point Likert-type scale drawing on information obtained by interviewing the participant, chart review, and collateral information. Additionally, the clinician makes a global severity rating for each of the five SANS domains of functioning. The internal consistency for the five subscales is acceptable, with the exception of the inattention subscale ( $\alpha = 0.51$ ). The remaining scales have coefficient alphas ranging from 0.64 for the avolition-apathy subscale to 0.93 for the affective flattening or blunting subscale [Mueser et al., 1994]. However, support for separate scoring according to the five subscales is equivocal [Moscarelli et al., 1987]. A factor analytic study using data obtained from multiple hospital sites suggest that the SANS may actually tap three factors, which correspond to the affective flattening or blunting subscale, the avolition-apathy and anhedonia-asociality subscales, and the alogia and inattention subscales [Mueser et al., 1994]. However, these

factors were highly correlated. The SANS based on a three-factor structure has also been shown to have good construct validity [Sayers et al., 1996]. The original five subscale scores as well as the total score were used in subsequent analyses.

### Autistic Symptoms

The Autism Spectrum Quotient (AQ) is a self-report screening tool designed to obtain an estimate of the degree to which an individual of average intelligence manifests autistic traits [Baron-Cohen et al., 2001]. In the present study, we were interested in evaluating whether participants with pre-mutation status were more likely to demonstrate symptoms of Asperger Syndrome. Participants were presented with 50 statements about different domains of social functioning that are divided into the following logical subscales: social skills, attention switching, attention to detail, communication, and imagination. Each subscale comprises ten statements, which participants can endorse according to their perception of their own behavior along a 4-point Likert-type scale ranging from agree, slightly agree, slightly disagree, to definitely disagree. In addition to individual subscale scores, an overall score can be derived. The AQ total score has been demonstrated to adequately differentiate between patients receiving a diagnosis of Asperger Syndrome and those who do not [Woodbury-Smith et al., 2005]. Using a cut-off score of 26 results in a sensitivity measure of 0.95 and a specificity measure of 0.52.

### Schizotypal Personality

The Schizotypal Personality Questionnaire (SPQ) is a 74-item self-report measure that was designed to measure symptoms associated with Schizotypal Personality Disorder according to DSM-IV-TR criteria [Raine, 1991]. Participants are asked to indicate the presence of a symptom by endorsing "yes" or the absence of a symptom by endorsing "no." Each "yes" response results in one point being added to the total score. The SPQ was divided into subscales that reflect the DSM-IV-TR criteria. Thus, the subscales are: (i) ideas of reference, (ii) excessive social anxiety, (iii) odd beliefs or magical thinking, (iv) unusual perceptual experiences, (v) odd or eccentric behavior, (vi) no close friends, (vii) odd speech, (viii) constricted affect, and (ix) suspiciousness. Confirmatory factor analysis reveals that the SPQ has three underlying factors, which are, cognitive-perceptual deficits, interpersonal deficits, and disorganized behavior [Raine et al., 1994]. Therefore, scores for the three factors can be derived from the subscale scores. Coefficient alpha values for the total score are between 0.90 and 0.91 and ranges from 0.71 to 0.78 for the nine subscales. Furthermore, the SPQ appears to have adequate discriminant validity particularly for differentiating between individuals presenting with a frank psychotic disorder and those with Schizotypal Personality Disorder [Raine, 1991].

### Drug and Alcohol Abuse and Dependence

Participants were first administered the two screening questions from the Structured Clinical Interview for DSM-IV-TR (SCID-I). Those participants satisfying positive screening criteria were then administered the Substance Abuse Disorder module of the SCID-I. The SCID-I is semi-structured interview that assesses whether participants meet DSM-IV-TR diagnostic criteria for alcohol and/or drug abuse or dependence. The SCID-I is, arguably, the instrument with the highest discriminant validity available for identifying positive cases of psychopathology [Kranzler et al., 1995, 1996]. Furthermore, both test-retest and interrater reliability for this instrument are extremely good [Zanarini et al., 2000; Zanarini and Frankenburg, 2001].

## STATISTICAL ANALYSES

The continuous multiple dependent variables related to cognitive ability, psychiatric symptoms and personality dimensions were analyzed by way of separate one-way multivariate analyses of variance (MANOVAs). The independent variable for each of these analyses was Group, which has three levels (i.e., Group 1: Premutation men, Group 2: Family control men, and Group 3: Non-family control men). To minimize Type I error, dependent variables were clustered logically for analyses within separate MANOVAs as follows: *Cluster 1*: Malaise Inventory—2 subscales, Hospital Anxiety and Depression Scale—2 subscales, Liebowitz Social Anxiety Scale, and Rosenberg Self-Esteem Scale, *Cluster 2*: The Brown ADD subscales, *Cluster 3*: the Asperger's Quotient subscales, *Cluster 4*: the Schizotypal Personality Questionnaire, and *Cluster 5*: the Scale for the Assessment of Negative Symptoms. For all MANOVAs a significance level of 0.05 was adopted. The logical clustering of measures was verified using the residual Sum-of-Squares and Cross Products correlation matrices provided as output from the SPSS general linear model option. The criterion was adopted such that pair-wise correlation values greater than 0.3 between each of the measures included in each MANOVA were considered sufficiently high to justify inclusion of each test or subtest within a cluster [Tabachnick and Fidell, 2007].

All categorical or frequency data were analyzed with  $\chi^2$  tests and a significance level of 0.05 was adopted.  $\chi^2$  tests were conducted to evaluate potential differences among the groups across the various demographic variables (i.e., marital status, education level, employment status, and medical history). Groups were also compared in terms of the frequency of individuals who met diagnostic criteria for alcohol and/or drug abuse or dependence. For post-hoc analyses, we computed the adjusted residuals from the  $\chi^2$ -test and used  $\pm 1.96$  SD as a threshold to determine where significant differences existed between observed and expected values. Furthermore, groups were compared on specific measures of interest that have published cut off scores for clinical significance or so-called "caseness."

### Procedure

Participants received informed consent according to procedures approved by regional and local ethics committees. The participants were administered all psychological and psychiatric measures in a quiet and comfortable setting ensuring confidentiality. Generally, the measures were administered in participants' own homes. Feedback was not given on individual performance.

## RESULTS

The potentially confounding effects of significant differences in Age, IQ, the various demographic variables, and medical history were examined prior to the analyses of the dependent measures of interest. A one-way independent groups ANOVA revealed a non-significant difference for Age [ $F_{(2,100)} = 0.631$ ,  $P = 0.534$ , n.s.] among the three participant groups. Furthermore, Pearson correlations between age and the various psychiatric and psychological variables were all non-significant ( $r$  values ranging from  $-0.166$  to  $0.148$ , all  $P > 0.05$ ). A one-way MANOVA with Group as independent measure and Verbal and Performance IQ scores as a dependent measures was also conducted and revealed no significant differences among the groups [Wilks'  $F = 2.205$ ,  $P = 0.07$ ].

Frequency data for the various demographic and medical history variables were first verified for adequate cell size numbers before  $\chi^2$  analyses were performed. Whereas the full data is presented in Table II, for the  $\chi^2$  analyses, for

statistical analyses categories were collapsed for the following variables to ensure a minimum of five participants per cell: Marital Status, Employment Status, Social Class, Level of Education, Significant Illnesses, and Significant Accidents. As such, marital status was categorized according to whether participants were either (i) married or living with a partner or (ii) other, which included single, divorced, or widowed. Employment status was categorized according to whether participants were either (i) employed, self-employed, or training or (ii) unemployed or retired. Social Class was categorized according to whether participants were employed as either (i) professionals, managers, or having technical training, (ii) skilled laborers, or (iii) partially skilled or unskilled laborers. Level of education was categorized according to whether participants had either completed (i) graduate, undergraduate, or a higher diploma, (ii) secondary only, (iii) less than secondary. Significant illnesses were categorized according to whether participants had either (i) experienced a significant illness in their lifetime or (ii) had not experienced a significant illness in their lifetime. Significant accidents were categorized according to whether participants either (i) had experienced a significant accident in their lifetime or (ii) had not experienced a significant accident in their lifetime. The three groups did differ significantly on the newly categorized Level of Education variable (Pearson  $\chi^2 = 11.334$ ,  $P = 0.023$ ). Specifically, the premutation group as compared to both control groups had significantly fewer than expected individuals who reported having completed higher education and significantly more than expected individuals who reported having not completed high school or having less than 11 years of education. To control for the potentially confounding effect of Level of Education of the various dependent measures of interest, this variable was included as a covariate for all subsequent analyses.  $\chi^2$  analyses conducted on the remaining newly categorized variables revealed no significant differences for any of the demographic or medical history variables among the groups. Therefore these were not considered in subsequent analyses.

### Psychiatric and Psychological Measures

Mean adjusted scores and standard errors for each group for each of the clusters analyzed are presented in Table III.

**Cluster 1: mood and anxiety.** For this cluster, data was only available from 30 premutation, 19 family control, and 39 non-family control participants because the questionnaires comprising the cluster were only introduced after a pilot study was completed on the remaining questionnaires in the study. The one-way MANOVA, with Level of Education as a covariate, conducted on the two factors of the MI, two factors of the HADS, the single factor of the RSE, and two factors of the LSAS (i.e., Total Fear and Total Avoidance) revealed that whereas the covariate (Level of Education) was significantly different among groups [Wilks'  $F = 2.165$ ,  $P = 0.046$ ], the premutation group did not differ from the two control groups [Wilks'  $F = 0.742$ ,  $P = 0.738$ ] on any of the mood and anxiety related dependent measures of interest. Results from the Residual Sum-of-Squares and Cross Products matrix for the MANOVA revealed that the HADS—Depression subscale and the Physical subscale of the MI had correlations less than the expected 0.3 with the other measure subscales included. As such, these subscales were analyzed in separate one-way ANOVAs with Level of Education as a covariate. Neither of these additional analyses revealed significant differences among the groups (HADS—Depression:  $F = 1.058$ ,  $P = 0.351$ ; Physical scale—MI:  $F = 0.890$ ,  $P = 0.414$ ).

**Cluster 2: attention deficit hyperactivity.** A one-way MANOVA, with Level of Education as a covariate, included the five cluster T scores and revealed no significant difference for

the covariate [Wilks'  $F = 1.719$ ,  $P = 0.138$ ], but a significant difference among the groups for the dependent measures [Wilks'  $F = 2.484$ ,  $P = 0.008$ ]. Examination of the individual clusters revealed that the T score for the *Utilizing working memory and accessing recall* cluster was significantly different among the groups [ $F_{(2,99)} = 4.120$ ,  $P = 0.019$ ]. Post-hoc Scheffé tests with T scores for *Utilizing working memory and accessing recall* as dependent measure revealed significant differences between the premutation and family control participants [ $P = 0.004$ ] as well as the premutation and non-family control participants [ $P = 0.05$ ].

Although group differences were revealed between the premutation group and the two control groups on the T scores for *Utilizing working memory and accessing recall* cluster, we sought to examine whether the premutation group included a greater number of individuals with T scores in the clinical range as defined by published norms (i.e., above 65), which reflects severe impairment on the functions assessed by this cluster. As such, a  $\chi^2$  analysis was conducted to examine whether the number of "cases" differed among the groups. The analysis revealed that the percentage of individuals with premutation expansion who reported clinically significant elevations was significantly greater than expected as compared to both the family and non-family control participants [Pearson  $\chi^2 = 14.745$ ,  $P = 0.001$ ; Premutation group: 3.7 SD greater than expected number fell in the clinically significant ranges]. Thirty-five percent of the individuals with premutation as compared to 0% of the family controls and 9% of the non-family controls reported clinically significant elevations on the *Utilizing working memory and accessing recall* cluster subscale.

Although Level of Education was included as a covariate, an exploratory analysis was conducted to examine the potential influence of this variable on the significant difficulties observed in the T Score for the *Utilizing working memory and accessing recall* cluster, specifically, for the premutation group. That is, we sought to test the idea that greater impairments in working memory were associated with poorer academic achievement. Indeed, this correlation was significant [ $r(40) = 0.354$ ,  $P = 0.025$ ].

**Cluster 3: negative symptoms associated with psychotic disorders.** The one-way MANOVA, with Level of Education as a covariate, conducted on the five factors of the SANS revealed that whereas the covariate (Level of Education) was significantly different among groups [Wilks'  $F = 3.524$ ,  $P = 0.006$ ], the premutation group did not differ from the two control groups [Wilks'  $F = 1.031$ ,  $P = 0.419$ ] on any of the subscales related to the dependent measures of interest.

**Cluster 4: autistic symptoms.** The one-way MANOVA, with Level of Education as a covariate, conducted on the five factors of the AQ revealed that whereas the covariate (Level of Education) was significantly different among groups [Wilks'  $F = 3.910$ ,  $P = 0.003$ ], the premutation group did not differ from the two control groups [Wilks'  $F = 0.559$ ,  $P = 0.846$ ] on any of the subscales related to the dependent measures of interest.

**Cluster 5: schizotypal personality.** The one-way MANOVA, with Level of Education as a covariate, conducted on the nine factors of the SPQ revealed that whereas the covariate (Level of Education) was significantly different among groups [Wilks'  $F = 2.775$ ,  $P = 0.007$ ], the premutation group did not differ from the two control groups [Wilks'  $F = 1.137$ ,  $P = 0.322$ ] on any of the subscales related to the dependent measures of interest. Mean scores and standard deviations for each group are presented in Table III. Data were also analyzed according to the three-factor solution described by Raine et al. [1994]. Therefore, a one-way MANOVA, with Level of Education as a covariate, was conducted on the three factors of the SPQ (i.e., cognitive-perceptual deficits, interpersonal deficits, and

TABLE III. Psychiatric and Psychological Measures With Level of Education as Covariate

Measure	Adjusted means $\pm$ SE		
	Premutation (n = 40)	Family control (n = 20)	Non-family control (n = 43)
Cluster 1 <sup>a</sup>			
Malaise inventory			
Psychological subscale	2.82 $\pm$ 0.43	1.67 $\pm$ 0.53	1.76 $\pm$ 0.37
Physical subscale	1.12 $\pm$ 0.24	0.85 $\pm$ 0.30	0.63 $\pm$ 0.20
Hospital anxiety and depression scale			
Anxiety subscale	5.96 $\pm$ 0.62	5.23 $\pm$ 0.77	5.51 $\pm$ 0.53
Depression subscale	3.85 $\pm$ 0.51	2.83 $\pm$ 0.63	3.69 $\pm$ 0.43
Leibowitz social anxiety scale			
Fear/anxiety total	18.24 $\pm$ 2.61	15.34 $\pm$ 3.21	13.14 $\pm$ 2.21
Avoidance total	14.20 $\pm$ 2.48	14.15 $\pm$ 3.06	11.29 $\pm$ 2.11
Rosenberg self-esteem	19.37 $\pm$ 1.44	22.32 $\pm$ 1.77	22.17 $\pm$ 1.22
Cluster 2*			
Brown ADD scales (T score)			
Activation subscale	57.47 $\pm$ 1.66	56.94 $\pm$ 2.31	55.19 $\pm$ 1.56
Attention subscale	57.86 $\pm$ 1.71	54.12 $\pm$ 2.39	59.10 $\pm$ 1.61
Effort subscale	57.91 $\pm$ 1.47	54.44 $\pm$ 2.06	54.41 $\pm$ 1.39
Affect subscale	57.14 $\pm$ 1.66	53.51 $\pm$ 2.32	57.20 $\pm$ 1.57
Memory subscale <sup>a</sup>	61.45 $\pm$ 1.63	53.51 $\pm$ 2.28	57.05 $\pm$ 1.54
Cluster 3			
Asperger's quotient			
Social skills	3.27 $\pm$ 0.34	2.85 $\pm$ 0.48	3.10 $\pm$ 0.32
Attention switching	5.20 $\pm$ 0.38	4.92 $\pm$ 0.53	4.36 $\pm$ 0.36
Attention to detail	4.51 $\pm$ 0.37	4.38 $\pm$ 0.52	4.24 $\pm$ 0.35
Communication	2.94 $\pm$ 0.31	2.62 $\pm$ 0.43	2.30 $\pm$ 0.29
Imagination	3.70 $\pm$ 0.30	3.59 $\pm$ 0.41	3.13 $\pm$ 0.28
Cluster 4			
Schizotypal personality questionnaire			
Ideas of reference	1.40 $\pm$ 0.30	0.83 $\pm$ 0.38	1.27 $\pm$ 0.26
Excessive social anxiety	2.75 $\pm$ 0.45	2.75 $\pm$ 0.56	1.75 $\pm$ 0.39
Odd beliefs magical thoughts	0.95 $\pm$ 0.21	0.56 $\pm$ 0.26	0.69 $\pm$ 0.18
Unusual perceptual experience	0.93 $\pm$ 0.22	1.08 $\pm$ 0.27	0.79 $\pm$ 0.19
Odd/eccentric behavior	1.27 $\pm$ 0.29	0.83 $\pm$ 0.36	0.89 $\pm$ 0.25
No close friends	3.04 $\pm$ 0.45	2.01 $\pm$ 0.56	2.19 $\pm$ 0.39
Odd speech	2.77 $\pm$ 0.41	3.10 $\pm$ 0.51	1.61 $\pm$ 0.36
Constricted affect	2.14 $\pm$ 0.35	1.72 $\pm$ 0.44	1.75 $\pm$ 0.30
Suspiciousness	1.87 $\pm$ 0.35	1.38 $\pm$ 0.43	1.52 $\pm$ 0.30
Cluster 5			
Scale for the assessment of negative symptoms (SANS)			
Affective	0.27 $\pm$ 0.09	0.25 $\pm$ 0.12	0.09 $\pm$ 0.08
Alogia	0.11 $\pm$ 0.05	0.02 $\pm$ 0.07	0.05 $\pm$ 0.04
Avolition	0.09 $\pm$ 0.08	0.06 $\pm$ 0.11	0.10 $\pm$ 0.07
Anhedonia	0.14 $\pm$ 0.09	0.39 $\pm$ 0.13	0.16 $\pm$ 0.09
Attention	0.04 $\pm$ 0.07	0.26 $\pm$ 0.09	0.17 $\pm$ 0.06

<sup>a</sup>Sample sizes for this cluster were 30 premutation men, 19 family controls, and 39 non-family controls.

\*Significant at  $P < 0.05$ .

disorganized behavior). The analysis revealed no significant differences among the groups [Wilks'  $F = 1.402$ ,  $P = 0.216$ ].

**Drug and alcohol abuse/dependence diagnoses.** Participant's self-report of alcohol use frequency was obtained and initially categorized as: Never, Occasionally, Weekly, and Daily. The Never and Occasionally categories were combined in order to meet the required 5 participants per cell for the  $\chi^2$  analysis. A  $\chi^2$ -test revealed a significant difference among the groups [Pearson  $\chi^2 = 11.198$ ,  $P = 0.024$ ]. Results of a post-hoc analysis revealed a significantly greater than expected proportion of individuals in the family control group reported consuming alcohol on a weekly basis than never or occasionally as compared to the premutation and non-carrier normal control groups (Table IV). None of the other pair wise comparisons were significantly different.

We also obtained independent confirmation of participant's alcohol use patterns from a clinician interview (SCID-I) that allowed us to make a DSM-IV-TR diagnosis of Alcohol Abuse and Dependence. In contrast to the alcohol use self-report

measure, which does not assess for diagnostic criteria of Substance Use Disorders, results of the clinician interview indicate that both the premutation and the family control groups exhibit significantly greater than expected numbers of individuals meeting criteria for Alcohol Abuse [ $\chi^2 = 6.929$ ,  $P = 0.031$ ; *Non-family control group*: 2.6 SD lower than expected number did not meet diagnostic criteria; Table IV]. When examining the more severe diagnosis of Alcohol Dependence, no significant differences among the groups were revealed [ $\chi^2 = 1.526$ ,  $P = 0.466$ ]. Similarly, no significant differences among the groups were observed for either Drug Abuse or Dependence [Abuse:  $\chi^2 = 0.287$ ,  $P = 0.866$ ; Dependence:  $\chi^2 = 0.363$ ,  $P = 0.834$ ].

#### Association Between CGG Repeat Size and Psychiatric/Psychological Measures

In order to evaluate the possibility of a relationship between CGG repeat size and the severity of psychiatric and

TABLE IV. Substance Use History for the Participant Groups

	Frequency (%)		
	Premutation (n = 40)	Family control (n = 20)	Non-family control (n = 43)
Self-report			
Never/occasionally	42.5	15.0	53.5
Weekly	37.5	70.0 <sup>a</sup>	27.9
Daily	20.0	15.0	18.6
SCID-I interview (DSM-IV-TR diagnosis)			
Alcohol abuse	32.5 <sup>a</sup>	35.0 <sup>a</sup>	11.6
Alcohol dependence	2.5	10.0	4.7
Drug abuse	2.5	5.0	4.7
Drug dependence	2.5	5.0	2.3

<sup>a</sup>Significant,  $SD > 1.96$ .

psychological variables, the potentially confounding variables of Age, IQ, and the various demographic and medical history variables were first examined by splitting the data file by group and then calculating bivariate correlations between CGG repeat size and each variable for the premutation group alone. No significant correlations were found between the CGG repeat size and any of the potentially confounding variables ( $r$  values ranged from  $-0.239$  to  $0.267$ , all  $P$  values  $> 0.10$ ).

After adjusting for multiple statistical tests, analyses of the Pearson correlations between CGG repeat size and each of the psychiatric/psychological measures described above revealed no significant correlations for the premutation group. One measure, the T Score for *Sustaining attention and concentration* was significantly correlated with CGG repeat size in the expected direction [ $r(33) = -0.346$ ,  $P = 0.048$ ]. However, after the correction for multiple tests, it failed to attain significance.

## DISCUSSION

This is the first controlled study to evaluate the hypothesis that there is a unique psychiatric/psychological phenotype in FXTAS-asymptomatic male carriers of the Fragile X *premutation*. The results presented here suggest that when psychometrically sound measures are administered to assess specific domains of psychosocial functioning, male *premutation* carriers exhibit similar levels of psychopathology as both age-matched non-carrier family members with whom they have shared an environment as well as age-matched non-carrier non-family members. One exception noted was a significant elevation on one subscale of the Brown Attention-Deficit Disorder Scale for Adults [Brown, 1996], the *Utilizing working memory and accessing recall* cluster, where *premutation* men reported, on average, more problems in this domain. Furthermore, a greater proportion of *premutation* men as compared to men in both comparison groups scored in the clinical range on this subscale. Finally, although *premutation* men were found to have higher rates of DSM-IV-TR diagnoses of Alcohol Abuse as compared to non-family controls, similar rates were observed in the family control group.

The molecular effects of the Fragile X *premutation* in men differ from those associated with the *full mutation*. Unlike individuals with the *full mutation*, *premutation* carriers continue to express FMRP, albeit sometimes in diminished levels [Kenneson et al., 2001; Tassone et al., 2004a,b]. Furthermore, *premutation* carriers appear to have the added molecular phenotype of increased expression levels of *FMR1* mRNA [Tassone et al., 2000a, 2004a]. Hagerman et al. [2004] have conjectured that the surplus of *FMR1* mRNA may be responsible for the specific brain pathology observed in *premutation* carriers with FXTAS through a toxic “gain-of-

function” mechanism [Greco et al., 2006]. The downstream effects of these molecular mechanisms on psychiatric/psychological functioning have not yet been fully elucidated. However, it is possible that the unique molecular events affecting *premutation* carriers results in a psychiatric and psychological profile that differs from what has been described in the *full mutation* condition. However, it is equally plausible that because the same populations of neurons rely on *FMR1* gene activity in both individuals with the *premutation* and the *full mutation*, these individuals may share a common psychiatric/psychological phenotype. In the present study, we sought to evaluate these two possible hypotheses.

Notwithstanding methodological flaws, findings from previous studies would seem to support the idea of an overlapping, yet milder psychiatric/psychological phenotype in males with the *premutation* (Table V). Of the studies that do report significant elevations in the prevalence of psychopathology in *premutation* males, difficulties shared in both the *full mutation* and *premutation* conditions include inattention, impulsivity and hyperactivity [Dorn et al., 1994; Aziz et al., 2003], anxiety [Dorn et al., 1994; Aziz et al., 2003; Hessler et al., 2005], symptoms of psychoticism [Hessler et al., 2005], and behaviors associated with the autistic spectrum [Aziz et al., 2003; Goodlin-Jones et al., 2004; Cornish et al., 2005; Farzin et al., 2006]. Similarly, our finding of significant impairments in working memory among male participants with *premutation* expansion are consistent with working memory impairments described in males with the *full mutation* [Munir et al., 2000].

Other studies, all of which were limited to women with the *premutation* but included adequate comparison groups have produced mixed results. In one study, women who had cared for a developmentally disabled child (not Fragile X) were included [Reiss et al., 1993], in another study *premutation* women who had never had to care for a child with the Fragile X *full mutation* were included [Franke et al., 1998], and in a third study both types of comparison individuals were included [Sobesky et al., 1994]. Whereas, two of the studies [Cohen et al., 1988; Franke et al., 1998; Johnston et al., 2001] reported a mild psychiatric phenotype (stereotypy-habit disorder in former and social phobia and avoidant personality in the latter) similar to the *full mutation* condition, the third found no differences in emotional functioning [Sobesky et al., 1994].

In sum, most studies that report a psychiatric/psychological phenotype find overlapping, yet much less severe symptomatology with the *premutation* condition. Of these studies, many are limited by methodological problems and the remainder have focused exclusively on female carriers of the *premutation*. The present study has addressed many of the methodological concerns of earlier research and has provided results supporting a mild psychiatric/psychological phenotype that

TABLE V. Previous Studies Examining the Psychiatric/Psychological Phenotype in People With the Fragile X Premutation

Reference	N	Instruments used	Control group(s)	N	Phenotype
<b>Females</b>					
Reiss et al. [1993]	34	SADS-L, NEO-PI HSCL-90	Women without <i>premutation</i> caring for a developmentally disabled child	41	No group differences
Thompson et al. [1994]	12	SADS-L	None	—	9/12 met criteria for Depression
Sobesky et al. [1994]	64	SADS-L, MMPI-2	(1) Family control women; (2) women caring for a developmentally disabled child	(1) 25 (2) 61	No group differences
Franke et al. [1998]	61	DIGS, PDS	(1) Women with the <i>premutation</i> but no affected children; (2) Women without <i>premutation</i> caring for a developmentally disabled child (autism)	(1) 17 (2) 42	Higher rates of social phobia and avoidant personality disorder
Johnston et al. [2001]	85	SCL-90-R	None	—	No group differences; CGG repeat size correlated with SCL-90-R depression subscale score
<b>Males</b>					
Dorn et al. [1994]	24	Family informant interview; retrospective reporting by <i>premutation</i> daughters	Women with the <i>premutation</i> whose fathers were not carriers	—	Higher rates of ADHD; poorer parental bonding; higher rates of alcohol abuse; higher rates of OCD; higher rates of abuse
Aziz et al. [2003]	10	DBC-P, conners, MRC HBS, parent account of childhood including: autistic symptoms hyperactivity	None	—	8/10 with psychiatric symptoms spectrum; anxiety; antisocial behavior
Moore et al. [2004]	20	BDI, BAI, Y-BOCS general health questionnaire	Unaffected	20	No group differences
Bacalman et al. [2006]	14	NPI	Compared men with the <i>premutation</i> and FXTAS to those without FXTAS	14	Men with FXTAS had higher rates of agitation/aggression, depression, apathy, disinhibition, and irritability
Farzin et al. [2006]	27	Conners', SCQ, ADOS, ADI-R	Compared proband boys, with family non-proband controls with <i>premutation</i> and family controls without <i>premutation</i>	16	Higher rates of ADHD symptoms and ASD in both <i>premutation</i> groups
Hessl et al. [2007]	12	fMRI amygdala activation to fearful stimuli	Unaffected	13	Reduced amygdala activation in men with <i>premutation</i> . Activation correlated with SCL-90-R GSI scores
<b>Males and females</b>					
Myers et al. [2001]	M:7 F:7	CBCL/4-18	Unaffected	14	No group differences
Goodlin-Jones et al. [2004]	M:4 F:2	ADOS-G, CARS, SCQ, ADI-R	Compared those with ASD and those without	—	Greater impairment in co-morbid group
Hessl et al. [2005]	M: 68 F: 144	SCL-90-R	None	—	SCL-90-R GSI, obsessive-compulsive, and psychoticism scores higher in <i>premutation</i> groups and correlated with <i>FMR1</i> levels

SADS-L, schedule for affective disorders and schizophrenia—lifetime version; BDI, beck depression inventory; BAI, beck anxiety inventory; Y-BOCS, Yale–Brown obsessive-compulsive scale; DBC-P, developmental behavior checklist—parent version; Conners', Conner's parent rating scale; MRC HBS, MRC schedule of handicaps, behavior, and skills; GHQ, general health questionnaire; CBCL, child behavior check list; HSCL-90 and SCL-90-R, (hospital) symptom checklist-90 (R), DIGS; PDS; MMPI-2; NEO-PI NEO-personality inventory; ADOS-G, autism diagnostic observation scale—general; CARS, childhood autism rating scale; SCQ, social communication questionnaire; ADI-R, autism diagnostic interview—revised; NPI, neuropsychiatric inventory.

overlaps with the *full mutation* phenotype only in the domain of working memory for men with the *premutation*.

One study reported that *premutation* carrier men appear to have higher rates of DSM-III diagnosed Alcohol Abuse as reported by their *premutation* daughters using the family

informant retrospective methodology [Dorn et al., 1994]. This result is unique to *premutation* carriers and has not been observed in individuals with the *full mutation*. Indeed, the finding is consistent with our result of higher rates of Alcohol Abuse among men with the *premutation*. However, we

included a group of family control men, who were presumably exposed to a similar family milieu that included psychosocial stressors associated with caring for a developmentally disabled child or grandchild. Family control individuals manifest similar rates of Alcohol Abuse suggesting that family environment may better explain the results of Dorn et al. [1994] and argues against a unique psychiatric/psychological phenotype associated with the *premutation*.

With respect to the finding reported here of significant elevations on the *Utilizing working memory and accessing recall* cluster (working memory) of the Brown ADD Scale, several points are noteworthy. First, of all the subscales on the Brown ADD, which includes items assessing aspects of organization and planning, sustained attention and concentration, persistent effort and energy, and affective difficulties in social interaction, only the subscale assessing working memory was significantly elevated for the *premutation* group. Unlike males with the *full mutation*, who exhibit many signs of inattention, impulsivity, and hyperactivity [Bregman et al., 1988] as well as working memory impairments [Munir et al., 2000], men with the *premutation* appear to have a circumscribed deficit in working memory. The working memory cluster includes items that evaluate the degree of a respondent's forgetfulness (e.g., forgetting appointments, misplacing needed items, forgetting what one was going to say or write) as well as his ability to encode and access learned information [Brown, 1996]. Such a selective weakness in working memory might explain the overall lower level of education attained by the *premutation* group as a whole. Indeed, working memory deficits are associated with learning disabilities, which if not identified early affect academic performance [Hoerig et al., 2002]. Although a causal link cannot be established with the present data, interestingly, a correlational analysis supports the idea that individuals with more severe self-reported working memory problems achieve lower levels of education.

Second, one might speculate that the reported working memory deficits are secondary to the effects of chronic alcohol use and abuse. However, this interpretation seems unlikely given that the family control group had similarly high rates of Alcohol Abuse as in the *premutation* group, which occurred alongside normal working memory abilities. Further support against this notion is provided by a one-way ANOVA with Group as independent variable, working memory T Score as dependent variable, and Level of Education and Alcohol Abuse diagnosis as covariates. This analysis revealed a significant main effect ( $F = 3.412$ ;  $P = 0.037$ ) but no effect of the Alcohol Abuse diagnosis covariate ( $F = 0.509$ ;  $P = 0.477$ , n.s.). A Post-hoc Scheffé test revealed that the *premutation* group report significantly higher scores on the working memory cluster than the two comparison groups. This finding suggests that the working memory deficits reported by the men with the *premutation* are likely directly related to the neuropathological effects consequent to CGG repeat expansion.

Third, findings from our neuropsychological investigations of *premutation* carrier men provide convergent experimental evidence to complement findings of the present study. Consistent with the self-reported working memory deficits reported here, results from measures of working memory (i.e., spatial-span forward and backward, and letter-number sequencing) indicate that *premutation* status carries with it an increased risk for working memory deficits that worsen with age [Cornish et al., in press].

Examination of the summary statistics for the various measures administered (Table III) reveals a consistent pattern whereby the *premutation* men obtain mean scores in the expected direction if a pervasive psychiatric/psychological phenotype were indeed present. However, the *premutation* group also exhibited greater variability in their scores as compared to the two control groups. We therefore wondered

whether the observed variability might be obscuring actual group differences. As such, where possible, we analyzed our data by applying published clinical cut-off scores (i.e., separating normal functioning from clinically significant impairment) for each of the measures. This categorical approach to our data set produced no additional significant differences among the three groups analyzed beyond what had already been revealed from the MANOVAs (i.e., a working memory deficit).

One way to interpret the greater variance in scores among the *premutation* men is to speculate that a portion of that variability can be explained by one of several molecular factors. For example, it is possible that individuals with more severe *premutation* molecular phenotypes are more likely to exhibit psychiatric/psychological symptoms. To test this idea, we obtained CGG repeat expansion sizes from the region at the 5' end of the *FMR1* gene. Correlational analyses were conducted to evaluate the potential relationship between CGG repeat size and levels on the psychiatric/psychological measures administered. Our results are consistent with Thompson et al. [1994] who found no correlation between CGG repeat size and psychopathology as assessed by a semi-structured clinical interview of female *premutation* carriers as well as with results from Hessel et al. [2005] who found no correlation between repeat size and scores on the Symptom Check List [SCL-90-R; Derogatis, 1994] in both male and female *premutation* carriers. Our results do however differ from Johnston et al. [2001] who reported a significant relationship between repeat size and scores on the Depression subscale of the SCL-90-R in women with the *premutation*. Despite the significant correlation, Johnston et al. [2001] found the scores on this subscale to be in the normal range indicating that the *premutation* status does not confer any additional risk in manifesting a mood disorder. Furthermore, the authors failed to include a proper control group making these findings difficult to interpret. Therefore, CGG repeat length does not appear to contribute to psychiatric/psychological symptoms severity in *premutation* males.

Hessel et al. [2005] have argued that despite the relatively strong link between CGG repeat size and *FMR1* mRNA levels, the latter may be a more appropriate molecular correlate of *premutation* severity. They reported that male *premutation* carriers exhibit significant correlations between *FMR1* mRNA levels (but not CGG repeat size) and scores on the Global Severity Index of the SCL-90-R, a measure of psychological distress, as well as with the Obsessive-Compulsive and Psychoticism subscales of that same measure. Hessel et al. [2005] also report even higher correlations among a non-FXTAS group of men, which they propose may reflect prodromal symptoms of FXTAS among those men with high levels of *FMR1* mRNA. This association may also contribute to the variability we observed in our non-FXTAS group. Therefore, some of those *premutation* men with higher scores on the psychiatric/psychological measures may be at greater risk for developing FXTAS. Notwithstanding the statistically significant correlations Hessel et al. [2005] report, the authors failed to include a family control group, making it difficult to know whether family environmental factors might better explain the high proportion of *premutation* carriers who met criteria for "caseness" on the Global Severity Index, the Psychoticism subscale, and/or the Obsessive-Compulsive scale.

The greater variability in measures of psychological/psychiatric functioning among *premutation* carriers may also be attributable to individual variability in the downstream targets of FMRP. This is a reasonable assumption because FMRP acts as an mRNA chaperone for a variety of genes [Brown et al., 2001] as well as a negative regulator of translation for the transcripts to which it binds [Laggerbauer et al., 2001]. Variability in these genes and the functional impact of their different alleles may well alter an individual's

susceptibility to changes in *FMR1* mRNA and FMRP levels resulting from the *premutation* expansion. Identifying downstream targets of FMRP that are also known to be associated with psychological/psychiatric conditions may help account for the observed variability in our data.

The present study has addressed many of the shortcomings of previous studies that have sought to examine psychiatric/psychological functioning in male carriers of the *premutation*. First, unlike almost all the studies conducted to date on male carriers we included a proper comparison group of non-carrier family members to control for family environmental effects. Second, many studies were conducted prior to the discovery of FXTAS and therefore may have reported positive finding that are better explained as secondary to a neurodegenerative disorder. One difficulty in interpreting these results is the psychosocial stressor of having a diagnosis of a degenerative disease, which likely affects psychological functioning independent of any underlying brain pathology. We therefore limited our sample to individuals without any positive signs of FXTAS using a well-validated instrument to establish conservative exclusion criteria. Third, unlike several of the previous studies that used measures requiring retrospective family informant reports by interview or questionnaire [Dorn et al., 1994; Rousseau et al., 1994; Aziz et al., 2003] we used self-report instruments. Fourth, unlike many studies that have resorted to general screening measures of psychopathology (e.g., SCL-90-R), we also employed specific measures of functioning within a psychiatric/psychological domain. Fifth, and related to instrument selection, we employed measures with good psychometric properties.

With regard to instruments, it is important to highlight that conclusions drawn in several earlier reports examining the psychiatric/psychological phenotype of individuals with the *premutation* have used the SCL-90-R as the only measure of psychopathology. This is problematic because the validities associated with the subscales of this test are poor and a large proportion of score variability can actually be accounted for by one factor, which is best characterized as psychological distress [Brophy et al., 1988]. For example, Hessler et al. [2005] concluded from the finding of significant elevations on the SCL-90-R Obsessive-Compulsive scale, that men with the *premutation* might share a phenotype with males with the *full mutation* and perhaps individuals with autistic spectrum disorders that include, "prominent obsessive-compulsive features and social withdrawal." However, the Obsessive-Compulsive subscale is an extremely poor predictor of obsessive-compulsive symptoms with unacceptable divergent validity [Woody et al., 1995]. A similar problem with instrument psychometrics arises in the Johnston et al. [2001] study, which examined subscale scores on the Depression subscale of the SCL-90-R in women with the *premutation*. Also in a follow-up study, Hessler et al. [2007] documented reduced amygdala activation by fMRI to fearful faces in men with the *premutation* which was correlated with their SCL-90-R scores, a measure of psychological distress [Hessler et al., 2007]. We encourage future research to focus on inclusion of instruments that possess acceptable psychometric properties for the construct they purport to evaluate.

In conclusion, men with the Fragile X *premutation* report clinically significant problems in working memory alongside relatively normal rates of psychopathology when family environment is taken in to consideration. These findings are extremely important given the relatively high prevalence of this condition (i.e., 1 in 813) and the potential to overlook the impact of a subtle psychiatric/psychological phenotype on functioning. This study highlights the importance of including a group of family control men in order to draw correct conclusions about the relative risks of psychopathology within this population. Furthermore, the point is raised that although most authors have assumed that the *premutation* psychiatric/

psychological phenotype should be similar, albeit milder, than what has been described in the *full mutation*, it is also possible that the *premutation* results in unique features that relate to the molecular mechanisms underlying this condition. Our results suggest a minimal psychiatric/psychological *premutation* phenotype that is consistent with the *full mutation* phenotype only in the domain of working memory. Future studies should examine the psychiatric/psychological phenotype using other methodologies besides self-report, which may be biased by factors such as group differences in the interpretation of the questions posed, use of a single method, and differences in social desirability among the groups. Alternative methods (e.g., informant or clinical interview) would serve to provide convergent data on the findings presented here. Furthermore, subsequent studies should include measures of CGG repeat size, *FMR1* mRNA levels, and other relevant molecular variables to try to identify subgroups of men with the *premutation* who are at higher risk for developing psychopathology. Finally, as Hessler et al. [2005] have alluded to, it would be useful to compare FXTAS affected individuals with those diagnosed with a similarly debilitating degenerative condition to examine the hypothesis of a syndrome specific pattern of psychological disturbance. Therefore, future studies need to elucidate the contribution of the direct effects of toxic levels of *FMR1* mRNA on psychiatric and psychological functioning observed in individuals with FXTAS as well of those symptoms that reflect the impact of having to contend with a degenerative and life-threatening illness.

#### ACKNOWLEDGMENTS

This research was supported by grants to KC and JT from the Wellcome Trust, UK, from the Center for Disease Control U10/CCU92513 and the NICHD #HD02274 to RH, and from the University of Ottawa to CK. We express our sincerest thank you to the all the regional genetics centers that took part in the study and to the fragile X Society for their support in recruitment as well as to the participants who kindly gave of their time. Finally, we thank Dr. Dwayne Schindler for his assistance with the statistical analyses.

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