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No Evidence for a Difference in Neuropsychological Profile among Carriers and Noncarriers of the *FMR1* Premutation in Adults under the Age of 50

Jessica Ezzell Hunter,[1] Emily Graves Allen,[1] Ann Abramowitz,[2] Michele Rusin,[3] Mary Leslie,[1] Gloria Novak,[1] Debra Hamilton,[1] Lisa Shubeck,[1] Krista Charen,[1] and Stephanie L. Sherman[1,*]

The 5′ untranslated region of the fragile X mental retardation gene, *FMR1*, contains a polymorphic CGG repeat. Expansions of this repeat are associated with a spectrum of disorders. Full mutation alleles, repeats ≥ 200, are associated with fragile X syndrome. Premutation alleles, repeats of ~55–199, are associated with a tremor-ataxia syndrome most commonly in older males and primary ovarian insufficiency in females. However, the neuropsychological impact of carrying a premutation allele is presently unclear in younger adults. In this study, we analyzed neuropsychological scores for 138 males and 506 females ascertained from the general population and from families with a history of fragile X syndrome. Subjects were age 18–50 years and had varying repeat lengths. Neuropsychological scores were obtained from measures of general intelligence, memory, and executive functioning, including attention. Principal component analysis followed by varimax rotation was used to create independent factors for analysis. These factors were modeled for males and females separately via a general linear model that accounted for correlation among related subjects. All models were adjusted for potential confounders, including age at testing, ethnicity, and household income. Among males, no repeat length associations were detected for any factor. Among females, only a significant association with repeat length and self-report attention ($p < 0.01$) was detected, with premutation carriers self-reporting significantly more attention-related problems compared to noncarriers. No significant interactions between repeat length and age were detected. Overall, these results indicate the lack of a global neuropsychological impact of carrying a premutation allele among adults under the age of 50.

Introduction

The X-linked fragile X mental retardation gene, *FMR1* (MIM 309550), contains a CGG repeat in the 5′ untranslated region.[1] The most common alleles contain less than 40 repeats. In rare cases, this repeat can become unstable and expand from one generation to the next.[2] Expanded alleles of *FMR1* are associated with a spectrum of disorders.

Expansions of 200 repeats or more, termed full mutation alleles, typically result in hypermethylation and subsequent silencing of *FMR1*.[3–5] These alleles are associated with fragile X mental retardation syndrome (FXS [MIM 300624]).[6] Individuals with FXS present with a wide range of phenotypic severity, including mild to severe intellectual disabilities, with females typically more mildly affected because of the X-linked nature of *FMR1*.

Alleles with repeats in the range of about 55–199, termed premutation alleles, remain unmethylated and are thus expressed. However, these alleles are associated with increased levels of mRNA as well as decreased protein levels as measured in blood.[7–12] Roughly 20% of females who carry premutation alleles have fragile X-associated primary ovarian insufficiency (FXPOI).[13–15] In addition, roughly 30% of males over the age of 50 who carry premutation alleles will develop a tremor/ataxia disorder (FXTAS [MIM 300623]).[16–18] FXTAS is characterized by a progressive intention tremor and/or ataxia, cognitive deficits, psychiatric symptoms, and brain atrophy.[16,18–22] Females who carry premutation alleles have also been reported with symptoms of FXTAS, but have reduced penetrance and possibly a different presentation compared to males.[23–27]

The presence of additional phenotypes associated with premutation alleles distinct from FXPOI or FXTAS is unclear. Hunter et al.[28] reviews past studies that report neuropsychological phenotypes among carriers of premutation alleles not affected by FXTAS. Many of these studies were conducted prior to the characterization of FXTAS. Thus, any phenotypes reported could be due to inclusion of older carriers of premutation alleles with FXTAS. In addition, many studies are compromised by small samples sizes, ascertainment biases associated with participant recruitment, and the use of inappropriate control groups. More recent published studies have overcome many of these obstacles, but results still do not converge on a particular profile.[29–34]

The goal of this study was to characterize neuropsychological phenotypes among male and female younger adults who carry an *FMR1* premutation allele in order to ask the question: before the possible onset of FXTAS, what is the neuropsychological impact of carrying a premutation allele? The results of the study indicate a lack of a definitive neuropsychological impact of the premutation allele among both males and females. Given the notable strengths of this study, including the recruitment of the largest study population to date via strategies to reduce potential participation biases, these results suggest that

[1]Department of Human Genetics, [2]Department of Psychology, Emory University School of Medicine, Atlanta, GA 30322, USA; [3]Independent practice 1776 Briarcliff Road, NE, Atlanta, GA 30306, USA
*Correspondence: ssherma@emory.edu
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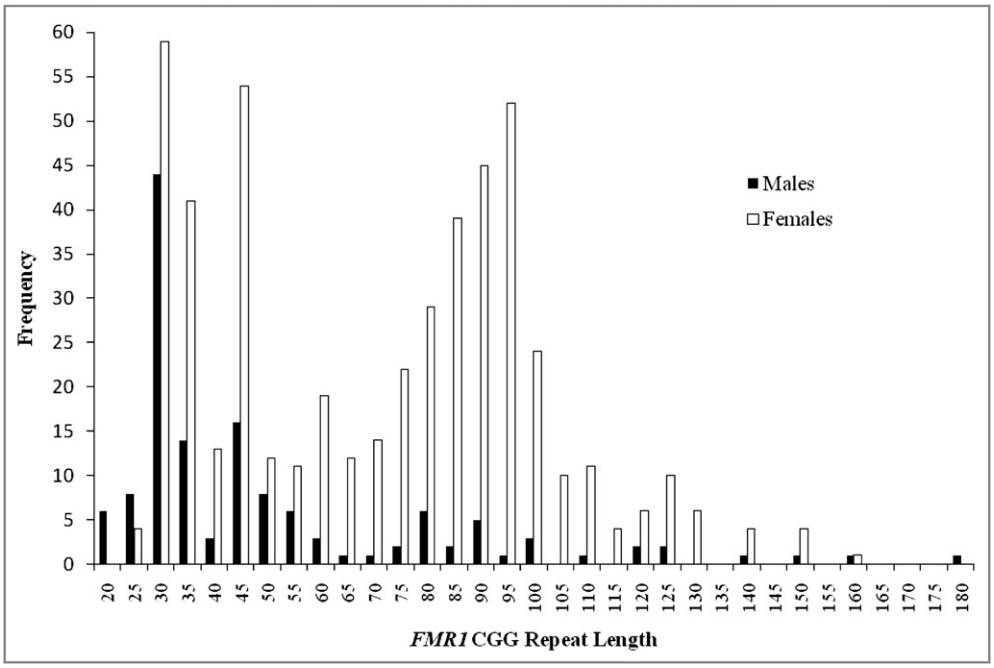

Figure 1. Distribution of *FMR1* CGG Repeat Lengths for All Male and Female Participants

the *FMR1* premutation allele probably acts as a quantitative trait locus (QTL) in the sense that it may contribute a weak effect on neuropsychological measures among young adults, but by itself does not have a major gene effect.

Subjects and Methods

Study Population

Study participants were identified with two recruitment strategies. First, subjects from the general population were recruited from a variety of Atlanta area public sites such as churches, universities, sports events, and health fairs. Second, in order to enrich the study sample with carriers of expanded *FMR1* alleles, participants were recruited from families with a known history of FXS through clinics, internet postings, FXS parent groups, and word of mouth. Once a family was identified with FXS, all family members were screened for the premutation and identified carriers and noncarriers were invited to participate. Participants were aged 18 to 50 years, had *FMR1* alleles of less than 200 repeats (Figure 1), and spoke English as their primary language. 506 women were ascertained from 348 pedigrees and 138 men from 112 pedigrees. The protocols and consent forms for ascertainment were approved by the Institutional Review Board at Emory University.

Measurement of Neuropsychological Phenotypes

Study participants were asked to complete the eight neuropsychological tests listed in Table 1. This test battery was designed to assess a broad range of abilities. 21 outcome scores from these tests were used in this analysis (Table 1). Test administrators were blind to each subject's *FMR1* genotype as well as family history of FXS.

The Conners' Adult ADHD Rating Scales self-report long form (CAARS-S:L) was used to assess symptoms associated with ADHD.[35] The CAARS consists of 66 items and provides an inconsistency index and 9 subscale scores: four factor-derived subscale scores, three DSM-IV ADHD symptom subscales, and an ADHD index. The four factor-derived subscale scores are based on ADHD-related symptoms and behaviors and were included in this analysis. The "A" subscale assesses inattention and memory problems, the "B" subscale assesses hyperactivity and restlessness, the "C" subscale assesses impulsivity and emotional lability, and the "D" subscale assesses problems with self-concept. Gender- and age-adjusted t scores were used for analysis. 12 (8.7%) men and 17 (3.4%) women had missing scores for the CAARS. In addition, 8 (5.8%) men and 29 (5.7%) women had inconsistency index scores of 8 or greater, which is indicative of potential inconsistency of the responses. Therefore, these scores were removed from the analysis.

The Continuous Performance Test (CPT) was used to assess sustained attention and vigilance.[36] This computer-based task requires the subject to detect infrequent targets and respond to them by pressing the space bar whenever the same four-digit number appears in the screen twice in a row. Two types of test errors were used in this analysis: omissions and commissions. Omissions, or inattention errors, indicate the number of times the subject does not press the space bar after the appropriate stimulus and reflects failures of sustained attention. Commissions (errors of impulsivity or false alarm) indicate the number of times the subject presses the space bar in the absence of the appropriate stimulus. 12 (8.7%) men and 41 (8.1%) women were missing scores for the CPT.

The Controlled Oral Word Association Test (COWA-T) is a measure of verbal fluency.[37] The COWA-T is comprised of three parts where subjects are asked to generate as many words as possible that begin with the letters F, A, and S in three subsequent 60 s sessions. The number of words provided in the three parts were added and converted to age- and education-adjusted t scores. One (0.7%) male was missing scores for the COWA-T.

The Stroop Color and Word Test (SCWT) is a sensorimotor speed-efficiency task that measures the ability to suppress common responses, an aspect of executive functioning.[38] The SCWT consists of three subtests: the word test, the color test, and the

Table 1. List of Neuropsychological Measures and 21 Outcome Variables Used in Analysis

Domain	Test Name	Outcome Variables
Attention	Connors' Adult ADHD Rating Scales (CAARS)	4 factor-derived subscales: A t score: inattention/memory B t score: hyperactivity/restlessness C t score: impulsivity/emotional lability D t score: problems with self-concept
Executive functioning	Continuous Performance Test (CPT)	number of omissions (OM); number of commissions (COM)
	Controlled Oral Word Association Test (COWA-T)	overall FAS t score (FAS)
	Stroop Color and Word Test (SCWT)	interference t score (INT)
	Trail Making Test (TMT)	part A: seconds to complete task part B: seconds to complete task
	Wisconsin Card Sorting Test (WCST)	number of perseverative errors (PE)
Verbal memory	Wechsler Memory Scale 3rd Edition (WMS-III)	logical memory age-adjusted scaled scores: immediate recall (LM1) delayed recall (LM2) delayed recognition (LM3)
Visual memory	Wechsler Memory Scale 3rd Edition (WMS-III)	Visual reproduction age-adjusted scaled scores: immediate recall (VR1) delayed recall (VR2) Visual reproduction raw score: delayed recognition (VR3)
General intelligence	Wechsler Adult Intelligence Scale, 3rd Edition (WAIS-III)	Factor index age-adjusted standard scores: verbal comprehension (VCI) perceptual organization (POI) working memory (WMI) processing speed (PSI)

color-word test. The word test requires the subject to read color names printed in black ink. The color test requires the subject to name the color of the ink used to print the nonword string "XXXX." The color-word test requires the subject to name the color of the ink the words are printed in and not read the words. Subjects are given 45 s to complete the task. The number of items correctly completed from the three tasks is used to compute an "interference" score that reflects the ability to suppress the interfering stimuli. Interference scores are converted to age-adjusted t scores. Three (2.2%) men and two (0.4%) women had missing scores for the SCWT.

The Trail Making Test (TMT) assesses visual scanning, attention, and mental flexibility.[39] The task consists of two trials with different complexities: part A involves visuomotor tracking of numbers 1 through 23 and part B involves the shifting of cognitive sets while visuomotor tracking between numbers and letters. The scores for parts A and B are the time in seconds used to complete each task. One (0.7%) man was missing the TMT part A score, two (1.4%) men were missing the TMT part B score, and three (0.6%) women were missing scores for both TMT parts A and B.

The Wisconsin Card Sorting Test (WCST) assesses mental flexibility and the ability to adapt strategies to changing conditions.[40] The WCST involves matching response cards to a set of stimulus cards based on either the number of shapes on the card, the color of the shapes, or the shapes themselves. However, the participant is not told the sorting principal and is told only whether each match was correct or incorrect. After a number of consecutive correct matches, the sorting principal changes and the participant must shift to a new sorting strategy. In this analysis, the number of perseverative errors was used as the outcome score. 6 (4.3%) men and 24 (4.7%) women were missing scores for the WCST.

The Wechsler Memory Scale 3rd Edition (WMS-III) assesses short- and long-term recall and recognition of verbal and visual information.[41] The logical memory subtest involves recollection of brief stories and the visual reproduction subtest involves recollection of visual patterns. Logical memory and visual reproduction subtest scores for immediate recall, delayed recall, and delayed recognition were used in this analysis. Raw scores are converted to age-adjusted scaled scores for all scores except the logical memory delayed recognition score. Two (1.4%) men were missing all scores for visual reproduction as well as logical memory immediate and delayed recall. Four (2.9%) men were missing scores for logical memory delayed recall. Six (1.2%) women were missing scores for visual reproduction immediate and delayed recall and logical memory immediate recall. Seven (1.4%) women were missing scores for logical memory delayed recall and delayed recognition. Eight (1.6%) women were missing scores for visual reproduction delayed recognition.

The Wechsler Adult Intelligence Scale 3rd Edition (WAIS-III) is an intelligence battery that measures verbal and nonverbal cognitive functioning.[42] The battery provides four factor index scores that capture the main themes or dimensions of the underlying performance. The verbal comprehension index (VCI) is calculated with three subtests (vocabulary, similarities, and information) and measures general verbal skills, such as verbal fluency, ability to understand and use verbal reasoning, and verbal knowledge. The perceptual organization index (POI) is calculated with three subtests (picture completion, block design, and matrix reasoning) and assesses the ability to examine a problem, draw upon visual-motor and visual-spatial skills, organize thoughts, create solutions, and then test them. The working memory index (WMI) is calculated with three subtests (arithmetic, digit span, and letter-number sequencing) and assesses ability to memorize new information, hold it in short-term memory, concentrate, and manipulate that information to complete a task. The processing speed index (PSI) is calculated with two subtests (digit symbol-coding and symbol search) and assesses skills of focusing attention and quickly scanning, discriminating between, and sequentially ordering visual

information. All index scores were converted to age-adjusted standard scores. One (0.7%) man was missing scores for VCI and POI. Two (1.4%) men were missing scores for WMI and PSI. Six (1.2%) women were missing scores for VCI, POI, WMI, and PSI.

The Wide Range Achievement Test 3 (WRAT-3) reading subscale requires the participant to correctly pronounce a set of words while reading them aloud. Raw scores from the reading subtest were converted to age-standard scores and grade equivalents. Three (2.2%) men and six (1.2%) women were missing WRAT-3 scores.

Laboratory Method

FMR1 CGG Repeat Number

All study participants were asked to provide a blood or buccal brush sample for molecular analysis of *FMR1* repeat length. DNA was extracted from samples with the QIAGEN QiAmp DNA Blood Mini Kit and analyzed with an ABI Prism 377 DNA fluorescent-sequencer.[43] For males or females with a larger premutation allele and for homozygous females, an alternative PCR-based, hybridization technique was used.[44] For heterozygous females, the CGG repeat length from the larger repeat allele was used as the main predictor in subsequent statistical analyses. For more information on molecular analysis, see Allen et al.[45]

Statistical Analysis

Male and female participants were separated into three groups based on their repeat length allele: noncarriers ($\leq$40 repeats), intermediate allele carriers (41–60 repeats), and premutation allele carriers (>60 repeats). To date, repeat length definitions with respect to clinical application have been based on instability, not on neuropsychological or neurobehavioral phenotype associations.[46] Thus, we used the definitions outlined above to better balance sample sizes and to be consistent with previous studies.[47,48]

Table 2 lists demographic data stratified by gender and repeat group. Repeat group differences for the demographic variables shown were tested with analysis of variances for continuous variables and chi-square tests for dichotomous variables. Any variables that differed across repeat groups would be included in models as potential confounders. Categories for ethnicity, income, and education were collapsed to create dichotomous variables. For the male data set, the three repeat groups differed on ethnicity (% white/Asian) ($\chi^2 = 12.42$, df = 2, $p < 0.01$). For the female data set, the three repeat groups differed on age ($F = 15.52$, $p < 0.01$), ethnicity (% white/Asian) ($\chi^2 = 48.56$, df = 2, $p < 0.01$), and household income (% $\geq$ \$50,000) ($\chi^2 = 12.61$, df = 2, $p < 0.01$).

WRAT-3 reading scores across repeat groups were analyzed to account for potential confounding on test performance resulting from possible learning disability. However, no differences in reading abilities were detected for the male or female data set. In addition, discrepancies between IQ and achievement scores, an additional indicator of learning disability, were analyzed among repeat groups. The mean differences between these scores as well as the frequency of participants who had a difference between scores greater than one standard deviation (discrepancy score $\geq$ 25) did not differ significantly between repeat groups.

Unadjusted mean scores for the 21 outcome scores stratified by gender and repeat length group are shown in Table 3. Distributions of all scores were tested for normality. Scores were transformed, if necessary, to produce a normal distribution for further analysis. A natural logarithm transformation was performed on CPT omission and commission scores, TMT parts A and B scores, and WCST perseverative error scores. Missing data points were estimated with the EM algorithm.

In order to further reduce the number of variables analyzed, a principal component analysis (PCA) followed by varimax rotation was used. Because the factor structure was not expected to vary between males and females, the data from all participants were used to create the new factors. The Kaiser-Meyer Olkin measure of sampling adequacy was 0.87. This was well above the cutoff of 0.50 to indicate PCA is appropriate for these data because of the significant correlation among the 21 variables.[49] Examination of eigenvalues and scree plots suggested a model of six independent factors based on the original 21 variables (Table 4). A cutoff value of 0.40 for factor loadings was used for inclusion of a variable in interpretation of each factor. This six-factor model accounted for 65.2% of the total variance of the original 21 variables (Table 4).

Because the new factor structure was obtained with data from all participants, confirmatory factor analysis was performed on the male and female data sets separately to ensure the six-factor model was a good fit. Several measures were used to determine the fit of the six-factor structure, including the goodness of fit index (GFI) where a value of greater than 0.90 is indicative of a good fit of the model. The GFI values were 0.90 and 0.95 for the male and female data sets, respectively.

Factor scores for all participants were computed for each participant with the scoring coefficients calculated by the PCA. The six factor scores were analyzed as outcome variables via general linear regression equations modeled for correlated outcomes. This approach was used to adjust for correlated outcome values that may have occurred among relatives from the same family because of shared environmental or genetic factors. In addition, this approach is robust to the varying family cluster sizes among our sample population. The main predictor of these models was *FMR1* repeat length and was classified in two ways. First, repeat length was used as a continuous variable to analyze linear associations between factor scores and repeat length. Second, repeat length was used as a categorical variable to compare mean scores across the three repeat groups: noncarriers, intermediate allele carriers, and premutation allele carriers. A Tukey's post hoc analysis was performed to test for adjusted factor mean score differences among repeat length groups. In order to account for any potential confounding, all models were adjusted for age, race, and income (Table 2). All interaction terms between repeat variables and covariates were tested for each model.

Lastly, to ensure that the imputation of missing data points did not affect the factor structure or the results of the analyses, a confirmatory factor analysis was performed to test the fit of the six-factor model on the data set containing the missing data points before imputation. In addition, the models analyzing repeat length as a predictor of factor scores were repeated where individual factor scores had been removed for participants that were missing data for the specific measures used to interpret that factor.

A simple Bonferonni correction was used to adjust for multiple testing because the six new factors were uncorrelated. Thus a cutoff value of $p = 0.01$ was used to indicate significance in these analyses. All statistical analyses were performed with the PROC MI, PROC PRINCOMP, PROC CALIS, and PROC MIXED procedures on the SAS System for Windows, Release 9.1.

Results

Results from the models with *FMR1* repeat length as a continuous variable as the main predictor are shown in

Table 2. Demographic Data of Study Male and Female Study Participants Stratified by *FMR1* Repeat Length Group

	Males[a]				Females[b]			
Group	**All**	**NC**	**IM**	**PM**	**All**	**NC**	**IM**	**PM**
N	138	75	33	30	506	117	96	293
Age (in years)								
Mean	35.9	36.6	33.4	36.8	35.3	33.3	31.9	37.2
SD	9.3	8.8	10.2	9.2	9.4	9.8	11.1	8.1
Range	18–50	20–50 ANOVA: F=1.58, p=0.21	18–50	18–50	18–50	18–50 ANOVA: F=15.52, p<0.01	18–50	18–50
Ethnicity								
% white	78.8	82.4	57.6	93.3	76.3	61.2	59.4	88.5
% Asian	0.0	0.0	0.0	0.0	0.8	2.6	1.0	0.0
% African American	17.5	13.5	39.4	3.3	17.6	30.2	34.4	6.5
% Hispanic	2.2	2.7	0.0	3.3	3.5	4.3	1.0	4.0
% other	1.5	1.4 χ^2=12.42, df=2, p<0.01	3.0	0.0	1.8	1.7 χ^2=48.56, df=2, p<0.01	4.2	1.1
Education								
% HS/GED not completed	0.7	0.0	3.0	0.0	0.2	0.0	0.0	0.3
% HS/GED completed	15.2	12.0	12.1	26.7	10.9	7.7	8.3	13.0
% trade/vocational school	4.4	2.7	6.1	6.7	3.6	1.7	4.2	4.1
% college not completed	38.4	42.7	42.4	23.3	36.8	38.5	51.0	31.4
% college completed	27.5	30.7	21.2	26.7	33.4	33.3	30.2	34.5
% graduate/professional school	13.8	12.0 χ^2=4.64, df=2, p=0.10	15.2	16.7	15.2	18.8 χ^2=4.72, df=2, p=0.10	6.3	16.7
Household Income								
% <$10,000	1.5	1.4	3.2	0.0	3.7	2.6	8.7	2.5
% $10–25,000	7.7	8.5	9.7	3.6	8.6	14.0	9.8	6.0
% $25–50,000	20.0	16.9	22.6	25.0	24.7	23.7	32.6	22.6
% $50–75,000	25.4	22.5	29.0	28.6	22.7	27.2	19.6	21.9
% $75–100,000	16.9	15.5	22.6	14.3	20.3	14.0	14.1	24.7
% >$100,000	28.5	35.2 χ^2=0.80, df=2, p=0.67	12.9	28.6	20.0	18.4 χ^2=12.61, df=2, p<0.01	15.2	22.3
WRAT-3								
Mean	102.3	102.8	102.1	101.0	102.2	104.2	101.9	101.6
SD	11.6	9.8	15.4	11.6	10.7	9.8	12.6	10.2
Range	63–121	77–120 ANOVA: F=0.26, p=0.77	63–121	75–119	51–122	70–121 ANOVA: F=2.61, p=0.07	65–122	51–121

Abbreviations: NC, noncarriers; IM, intermediate allele carriers; PM, premutation allele carriers; SD, standard deviation; HS, high school; GED, General Education Development; WRAT-3, Wide Range Achievement Test 3.

[a] Among male participants: 1 missing race, 8 missing income, and 3 missing WRAT scores.

[b] Among female participants: 16 missing race, 17 missing income, and 6 missing WRAT scores.

Table 5. For both the male and female data sets, repeat length as a continuous variable was not a statistically significant predictor for any of the six factor scores with the Bonferonni correction for multiple testing (i.e., $p < 0.01$). For the female data set, repeat length was marginally statistically significant as a predictor for processing speed (factor 3, $p = 0.05$) and self-reported inattention and impulsivity (factor 4, $p = 0.02$) (Table 5). Both models indicated positive linear associations between repeat length and these two factor scores, indicative of reduced processing speed

Table 3. Unadjusted Mean Scores on Neuropsychological Measures by Gender and Repeat Group

Neuropsychological Outcome Measures		Males					Females				
		N	All	NC	IM	PM	N	All	NC	IM	PM
CAARS	A	118	47.9	47.0	50.0	48.0	460	49.9	47.7	48.8	51.1
	B	118	50.4	49.5	50.5	52.8	460	49.5	49.1	49.1	49.8
	C	118	45.1	45.0	44.7	45.8	460	46.6	43.8	45.2	48.1
	D	118	45.8	45.2	47.3	45.7	460	46.4	44.0	44.7	47.8
CPT	OM	126	5.7	4.9	6.0	7.5	465	5.3	5.8	5.9	4.9
	COM	126	9.0	10.7	6.5	7.0	465	8.0	7.1	10.6	7.5
COWA-T	FAS	137	46.5	47.0	46.6	45.3	506	47.1	47.7	47.1	46.9
SCWT	INT	135	51.5	52.1	50.5	51.2	504	51.2	50.5	50.8	51.5
TMT	A	137	23.2	23.1	24.2	22.5	503	22.0	21.4	21.9	22.2
	B	136	55.3	54.4	55.0	57.8	503	52.4	54.3	53.4	51.3
WCST	PE	132	9.3	9.5	8.1	10.4	482	9.3	8.0	10.2	9.5
WMS-III	LM1	136	10.5	10.6	10.3	10.6	500	11.3	11.2	10.7	11.6
	LM2	136	10.9	10.9	10.8	11.1	499	11.9	11.8	11.4	12.1
	LM3	134	26.3	26.2	26.3	26.4	499	27.0	27.0	26.9	27.0
	VR1	136	9.0	8.7	9.4	9.3	500	8.8	8.8	8.6	8.8
	VR2	136	10.7	10.6	11.3	10.4	500	10.4	10.7	10.4	10.4
	VR3	136	11.5	11.4	11.5	11.4	498	11.0	11.1	10.8	10.9
WAIS-III	VCI	137	109.8	109.8	110.8	108.8	500	106.3	109.4	106.6	105.1
	POI	137	113.5	113.5	112.0	115.3	500	109.1	108.3	107.1	110.2
	WMI	136	105.2	105.7	103.1	106.4	500	102.5	103.1	102.0	102.4
	PSI	136	101.4	101.5	102.2	100.1	500	108.3	108.3	106.5	109.0

and higher levels of symptoms associated with self-reported ADHD.

For the models where repeat length as a categorical variable was used as the main predictor, adjusted mean scores for the three repeat classes and associated p values are shown in Table 6. For the male data set, repeat length was not a statistically significant predictor of any of the factors scores. In addition, with Tukey's post hoc analysis to compare the adjusted group means, factor scores did not differ significantly among repeat groups. For the

Table 4. Structure of Six Factors Derived from Principal Component Analysis with Varimax Rotation and Associated Factor Loadings that Represent Correlations between the New Factors and the Original Neuropsychological Measures from Both Male and Female Participants

Factor		1	2	3	4	5	6
Factor Interpretation		Visual Processing and Memory	Verbal Comprehension and Memory	Processing Speed	Self-Report Inattention and Impulsivity	Sustained Attention	Response Fluency
CAARS	A	0.04	0.00	0.16	**0.82**	0.02	−0.01
	B	−0.07	−0.14	−0.08	**0.71**	−0.03	0.06
	C	0.00	−0.03	−0.05	**0.82**	0.12	−0.01
	D	−0.05	0.09	0.14	**0.73**	−0.03	−0.09
CPT	OM	−0.06	−0.10	0.19	0.00	**0.79**	−0.08
	COM	−0.20	−0.16	0.08	0.10	**0.75**	−0.19
COWA-T	FAS	0.03	0.30	−**0.42**	0.03	−0.10	**0.45**
SCWT	INT	0.16	−0.05	−0.08	−0.05	−0.08	**0.79**
TMT	A	−0.18	−0.04	**0.79**	0.15	0.00	0.01
	B	−0.21	−0.17	**0.76**	0.00	0.22	−0.13
WCST	PE	−0.31	−0.09	0.30	−0.03	**0.44**	0.18
WMS-III	LM1	0.16	**0.87**	−0.16	−0.02	−0.17	0.06
	LM2	0.17	**0.89**	−0.08	−0.01	−0.15	0.06
	LM3	0.13	**0.80**	−0.17	−0.06	−0.02	−0.05
	VR1	**0.82**	0.20	−0.09	−0.05	−0.04	0.06
	VR2	**0.80**	0.14	−0.11	−0.04	−0.08	0.12
	VR3	**0.76**	0.08	−0.19	−0.04	−0.16	0.03
WAIS-III	VCI	0.39	**0.45**	−0.23	0.01	−0.09	0.37
	POI	**0.61**	0.12	−0.37	0.06	−0.22	0.21
	WMI	0.32	0.29	−**0.47**	0.01	−0.23	0.34
	PSI	0.15	0.20	−**0.71**	−0.07	−0.22	0.12
% of variance explained		**29.7**	**11.5**	**8.1**	**6.6**	**5.0**	**4.3**

Factors loadings >0.40 (shown in bold) were used to interpret the new factors.

Table 5. Results from the General Linear Model with *FMR1* Repeat Length as the Main Predictor

Gender	Factor	Standardized β Estimates	p Value
Males	1: Visual processing and memory	−0.05	0.52
	2: Verbal comprehension and memory	0.03	0.75
	3: Processing speed	−0.03	0.78
	4: Self-report inattention and impulsivity	0.06	0.42
	5: Sustained attention	0.10	0.15
	6: Response fluency	−0.01	0.84
Females	1: Visual processing and memory	−0.09	0.08
	2: Verbal comprehension and memory	<0.01	0.96
	3: Processing speed	0.10	0.05
	4: Self-report inattention and impulsivity	0.11	0.02
	5: Sustained attention	−0.02	0.71
	6: Response fluency	0.02	0.60

Table 6. Results from the General Linear Model Results via Indicator Variables to Compare *FMR1* Repeat Length Groups as the Main Predictors

		Adjusted Group Means			
Gender	Factor	NC	IM	PM	p Value
Males	1: Visual processing and memory	0.16	0.50	0.20	0.36
	2: Verbal comprehension and memory	−0.32	−0.30	−0.29	0.99
	3: Processing speed	0.19	0.25	0.31	0.83
	4: Self-report inattention and impulsivity	−0.10	0.05	0.02	0.69
	5: Sustained attention	−0.10	0.03	0.13	0.51
	6: Response fluency	0.24	0.28	0.11	0.74
Females	1: Visual processing and memory	0.04	−0.00	−0.13	0.33
	2: Verbal comprehension and memory	0.10	−0.02	0.12	0.57
	3: Processing speed	−0.10	−0.12	−0.03	0.74
	4: Self-report inattention and impulsivity	−0.20[a]	−0.03	0.17[a]	0.01
	5: Sustained attention	0.01	0.11	−0.07	0.42
	6: Response fluency	−0.05	−0.04	−0.10	0.88

[a] Mean factor scores significantly different ($p < 0.01$).

female data set, repeat length was a marginally statistically significant predictor for self-reported inattention and impulsivity (factor 4, $p = 0.01$) (Table 6). With the Tukey's post hoc analysis, the adjusted mean scores for this factor were significantly higher for the premutation group compared to the noncarrier group ($p < 0.01$). These results indicate increased severity of self-reported symptoms associated with inattention and impulsivity.

As shown in Table 4, the inattention and impulsivity factor (factor 4) is heavily loaded by the four CAARS subscale scores that assess symptoms associated with ADHD. In order to follow up the above results of more severe symptoms among females with the premutation, adjusted mean scores for the four CAARS subscales were compared among females for the three repeat groups. Results are shown in Table 7. The premutation group scored marginally significantly higher than did noncarriers for inattention and memory, impulsivity and emotional lability, and problems with self-concept, but not for hyperactivity and restlessness.

In order to assess what these results might indicate clinically, the frequency of female participants who had a CAARS subscale t score of 65 or greater was analyzed across repeat groups, where a t score of 65 or greater is indicative of elevated symptoms.[35] We used generalized estimating equation (GEE) models to analyze this frequency across repeat groups while adjusting for covariates. The premutation group did not differ significantly from the noncarrier group for the frequency of scoring above this clinical significant cut-off value for the CAARS subscale A (OR = 3.31; 95% CI 0.86 to 12.71; $p = 0.08$), B (OR = 1.19; 95% CI 0.42 to 3.37; $p = 0.74$), C (OR = 4.59; 95% CI 0.85 to 24.65; $p = 0.08$), or D (OR = 2.76; 95% CI 0.74 to 10.33; $p = 0.13$). However, the point estimates of the ORs were >1 for all subscales with the highest point estimates for inattention and memory, impulsivity and emotional lability, and problems with self-concept, similar to the results above.

Any phenotypes detected among females who carry a premutation allele could potentially be due to the psychosocial stress of raising a child with FXS. Therefore, in a follow-up analysis, a new covariate was added to the ADHD models for females to indicate whether or not the participant was a mother of a child with FXS. Among the female participants who carried a premutation allele, 162 were mothers of a child with FXS and 103 were known to not have a child with FXS. However, for the linear models with repeat length as a continuous variable, this covariate was not a significant predictor of factor 4 scores ($p = 0.48$) or the CAARS ADHD subscores A ($p = 0.16$), B ($p = 0.73$), C ($p = 0.21$), or D ($p = 0.95$). This covariate was also not a significant predictor in the models with repeat length as a categorical model for factor 4 scores ($p = 0.31$) or the CAARS subscores A ($p = 0.56$), B ($p = 0.36$), C ($p = 0.13$), or D ($p = 0.78$). In addition, among female carriers of premutations, mean scores did not differ between those with and without children with fragile X for factor 4 ($p = 0.34$) or the CAARS subscores A ($p = 0.61$), B ($p = 0.39$), C ($p = 0.16$), or D ($p = 0.79$).

In addition, a nonlinear association or "threshold" effect between repeat length and factors scores is possible. Carriers with ≥100 repeats could be more likely to manifest neuropsychological symptoms given the significantly increased levels of *FMR1* transcript and, importantly, the decreased levels of FMRP in this repeat range.[8,10,50] Therefore, in a second follow-up analysis, premutation carriers with repeats ≥100 were compared to noncarriers (≤40 repeats) in models of all six factor scores. In the male sample, 10 of the 30 premutation carriers had repeats ≥100 and, in the female sample, 70 of the 293 premutation carriers had repeats ≥100. The premutation group with ≥100 repeats did not score significantly different compared to the noncarrier group (repeats ≤ 40) for any of the six factors among the male data set (p values of 0.18, 0.75, 0.43,

Table 7. Analysis of Individual CAARS Scores to Follow Up on Mean Factor 4 Score Differences between Female Noncarrier and Premutation Carrier Groups

CAARS Subscale	Symptoms Assessed	Adjusted Group Means			p Value
		NC	IM	PM	
A	inattention and memory	47.90[a]	49.36	51.18[a]	0.02
B	hyperactivity and restlessness	49.03	49.43	49.84	0.72
C	impulsivity and emotional lability	44.23[a]	45.77	47.95[a]	0.02
D	problems with self-concept	44.73[b]	45.75	47.58[b]	0.05

[a] Mean factor scores marginally significantly different ($p = 0.01$).
[b] Mean factor scores marginally significantly different ($p = 0.02$).

0.77, 0.24, and 0.67, respectively) or among the female data set (p values of 0.05, 0.37, 0.03, 0.04, 0.95, and 0.08, respectively).

Tests of all interaction terms between the covariates and *FMR1* repeat length variables, both continuous and categorical, were not significant. This indicates that none of the covariates, including age, modify the effect of repeat length on neuropsychological scores.

Finally, a confirmatory factor analysis indicated that the six-factor model obtained from the data set with imputation of missing values was a good fit for the data set with missing data points (GFI = 0.95). In addition, models run with missing factor scores where the original outcome score which loaded onto a particular factor provided similar patterns of significant associations between repeat length and factor scores. None of the factor models for the male data set reached significance, whereas among the female data set, three models reached marginal significance: models with repeat length as a continuous variable as a predictor of factor 3 ($p = 0.03$) and factor 4 ($p = 0.03$) and the model with repeat length as a categorical variable as a predictor of factor 4 ($p = 0.02$). Thus, there is no evidence that the imputation of missing data altered the analyses.

Discussion

The purpose of this study was to investigate potential effects of *FMR1* premutation alleles on neuropsychological performance among younger adult males and females. The presence of a neuropsychological phenotype in the absence of FXTAS or perhaps before the onset of FXTAS is presently unclear. Recruitment strategies utilized in this study have successfully limited potential ascertainment biases while attaining the largest study population to date.

All participants were administered a neuropsychological test battery that included assessments of attention, executive functioning, visual and verbal memory, and general intelligence. The 21 primary outcome scores derived from the eight neuropsychological tests were used in a principal component analysis to construct a six-factor model (Table 4). Factor loadings of the original 21 variables were used to interpret the new factors: visual processing and memory, verbal comprehension and memory, processing speed, self-report inattention and impulsivity, sustained attention, and response fluency (Table 4).

Overall, there was no statistically significant association of the six neuropsychological factor scores with *FMR1* repeat length in the male data set, defined either as a continuous variable or by repeat size class, after adjustment for multiple testing. This was true also for the female data set, with the exception of one marginally significant finding that was further explored.

Our data suggested that females with the premutation reported significantly more severe symptoms associated with ADHD than did noncarriers. This was reflected by the positive association of repeat length with factor 4, which was interpreted as self-reported inattention and impulsivity. In addition, the premutation group had a significantly higher mean factor 4 score than did the noncarrier group. Factor 4 was heavily loaded by the four subscale scores of the CAARS. Post-hoc analyses suggested that females with the premutation scored higher than noncarriers on the CAARS subscales that assessed inattention and memory, impulsivity and emotional lability, and problems with self-concept, but not hyperactivity and restlessness. However, it is important to note that because a t score of 65 or higher is indicative of elevated symptoms,[35] the mean scores of all repeat groups are in the normal range, including the premutation group. In addition, the frequency of participants who scored above this cutoff value did not statistically differ across repeat groups for any of the CAARS subscores. Therefore, these results suggest that females with the premutation may be at risk for increased severity of some symptoms associated with ADHD, but not necessarily the presence of clinical ADHD. The elevated mean score for problems with self-concept among female carriers of premutation alleles is consistent with our findings in a recent study on this population, where scores for general negative affect were elevated in premutation carriers.[48]

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 ADHD. One explanation could be that these phenotypes are not due directly to *FMR1* repeat length, but instead to the psychosocial stress of raising a child with FXS. However, tests of a covariate representing raising a child with FXS was not a significant

predictor of ADHD scores among the female data set. Another explanation could be that because the CAARS is a self-report questionnaire, women report the symptoms associated with ADHD differently than do men. A third explanation could be that the increased sample size among females compared with males allowed for greater power to detect smaller differences.

Previous studies have suggested that individuals with $\geq$100 repeats may be more like to manifest symptoms related to the mutation because increased levels of *FMR1* transcript as well as decreased levels of FMRP are evident.[8,10,50] In our exploratory analyses on a subset of individuals with these large repeats, we found no evidence for neuropsychological impairment.

Comparison of our results to the most recently published studies is encouraging.[29–31] Cornish et al.[30] and Grigsby et al. [31] are the largest of these recently published studies examining neuropsychological functioning among premutation males without FXTAS. Overall, the major findings of these two studies are similar to ours: most neuropsychological measures that were administered were not significantly different among adult carriers without FXTAS and noncarriers. Cornish et al. [30] found no differences among carriers and noncarriers under the age of 50 for general intelligence, sustained attention, visual spatial function, or visual memory function. Similarly, Grigsby et al. [31] found no differences among premutation carriers without FXTAS and noncarriers in general intelligence, working memory, remote recall of information, verbal learning, language, information processing, visual-spatial functioning, or temporal sequencing. Both studies did find executive function deficits among premutation carriers, a phenotype that we did not observe. For example, Cornish et al. [30] found a significant deficit in response inhibition, a component of executive function, among men under age 50. Grigsby et al. [31] found that carriers without FXTAS performed worse than noncarriers on executive cognitive functioning and some aspects of verbal learning and memory.

There are several possible explanations for these differing results. First, the age distribution of participants varied across studies. This is important because Cornish et al. [30] found that the difference between carriers and noncarriers for response inhibition deficits increased with increasing age. Second, the repeat length distribution in each sample may differ. Although we found no association with repeat length, even among those with the highest repeats, other studies may have a larger proportion of carriers with $\geq$100 repeat alleles, increasing the power of detecting small effect sizes. Third, the neuropsychological measures and the use of composite scores differed across studies; one measure may have a higher probability of tapping into a specific domain than another. Fourth, the variability in results could be due to different sizes of study populations and recruitment strategies. Lastly, all studies conducted many statistical tests and significant differences could be due to chance, particularly if the study does not adjust for multiple testing.

There are some potential limitations to our study. First, though the neuropsychological testers were blind to the *FMR1* repeat length status of the participants and participants are asked to not disclose this status to testers, the participants typically knew their status prior to testing, particularly those recruited from families with a history of FXS. This could impact how these participants respond to the self-report questionnaires, particularly those assessing self-concept. McConkie-Rosell et al. reported decreased feelings of self-concept among carriers compared to noncarriers after learning about carrier status.[51] This could explain the results in this study regarding the increased CAARS subscores for problems with self-concept, but not the increased CAARS subscores for inattention and memory and impulsivity and emotional lability. Further, it is possible that carriers familiar with recent literature citing neuropsychological and neurobehavioral deficits among carriers without FXTAS might be biased in their responses to the self-report questionnaires. Second, though every effort was made to limit ascertainment biases, there is the potential that those that agree to participate and complete the neuropsychological test battery might be less likely to have cognitive deficits or inattention issues. However, this would be true for both carrier and noncarrier recruits.

Despite these potential limitations, the results of this study are encouraging. Given the large study population, particularly for females, and the limited ascertainment biases associated with recruitment, the lack of performance differences on neuropsychological assessments between carriers and noncarriers is monumental in the study of fragile X-associated phenotypes. These results indicate that in the absence of FXTAS, there is no global neuropsychological impact of carrying a premutation allele, at least among those $<$50 years of age. Importantly, these results are consistent with the larger, recent studies that have tried to overcome study design problems. These findings are clinically important to families with fragile-X spectrum disorders. On average, young adults, and by inference, children who carry the permutation, should be assured that the premutation form of the *FMR1* gene is only one of many genes that contribute to their neuropsychological strengths and hurdles.

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Web Resources

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