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Neuropsychological findings from older premutation carrier males and their non-carrier siblings from families with fragile X syndrome

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Abstract

Objective—Carriers of the FMR1 premutation allele are at a significantly increased risk for a late-onset neurodegenerative disorder, fragile X-associated tremor/ataxia syndrome (FXTAS). The primary features of FXTAS are late-onset intention tremor and gait ataxia. Previous reports have shown global deficits in neuropsychological measures among males with FXTAS, particularly those related to executive functioning. The purpose of this study was to investigate the neuropsychological profile among older males with the premutation who are at risk for FXTAS.

Method—Premutation carriers, 66 with motor symptoms and 23 without, and 18 non-carrier siblings were recruited from pedigrees diagnosed with fragile X syndrome, all over age 50. Subjects were examined with a neurological test battery to identify symptoms of FXTAS and a neuropsychological test battery to investigate cognitive and behavioral profiles. Linear regression

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and ANCOVA were used to determine the effect of the premutation on outcome measures adjusting for age and education.

**Results**—We identified a significant decrease in scores of general intelligence and a marginally significant decrease in scores of logical memory among premutation carrier males with motor symptoms compared to the non-carrier male siblings. We did not identify deficits in executive functioning in our sample of premutation carrier males with motor symptoms.

**Discussion**—Similar to other reports, we found that the *FMR1* premutation is associated with deficits in general intelligence and memory among older males with symptoms of FXTAS. However, our results differed in that we found no evidence of premutation-associated executive dysfunction. We provide possible explanations for this difference.

**Keywords**
Fragile X syndrome; *FMR1*; Tremor; Ataxia; Premutation

**Introduction**
Fragile X syndrome (FXS), the most commonly inherited form of intellectual and developmental disabilities, is caused by the loss of function of the *FMR1* protein (FMRP). In most cases, this loss is the result of an expansion of CGG repeats to over 200 repeats in the 5′ untranslated region of the FMR1 gene and subsequent hypermethylation of the region (Ashley, Wilkinson, Reines, & Warren, 1993). It is now evident that although premutation carriers (defined as individuals with 55-199 repeats by the American College of Medical Genetics (Sherman, Pletcher, & Driscoll, 2005)) do not manifest the symptoms of FXS, they do experience several distinct phenotypes. First, premutation females, particularly those with 80-100 CGG repeats, are at an increased risk for primary ovarian insufficiency (FXPOI) (Allen et al., 2007; Allingham-Hawkins et al., 1999; Cronister et al., 1991; Ennis, Ward, & Murray, 2006; Schwartz et al., 1994; Sullivan et al., 2005). Second, male carriers, and to a lesser extent female carriers, are at an increased risk for the fragile X-associated tremor/ataxia syndrome (FXTAS), especially those with >70 repeats (R. J. Hagerman et al., 2001; Jacquemont et al., 2003).

The defining characteristics of FXTAS are tremor, ataxia, and characteristic neuroimaging (MRI) signs (Jacquemont et al., 2003). Other associated signs include parkinsonism, neuropsychological dysfunction, autonomic dysfunction, and peripheral neuropathy (Jacquemont et al., 2003). Neuroimaging reveals changes in the periventricular white matter, subcortical white matter, and middle cerebellar peduncles (MCP) on T2-weighted MRI (Brunberg et al., 2002). The distinctive increased signal intensities in the MCPs have been incorporated into the definition of FXTAS, as they occur in about 60% of males with FXTAS (P. J. Hagerman & Hagerman, 2007).

*Post mortem* studies have revealed the presence of eosinophilic, ubiquitin-positive intranuclear inclusions in neurons and astrocytes throughout the central nervous system (Greco et al., 2002). There is a significant association between the number of CGG repeats and the number of inclusions in the neurons and astrocytes (Greco et al., 2006). Inclusion bodies have also been reported outside of the central nervous system, in both the pituitary gland and the testicles of men with FXTAS (Greco et al., 2007).

Recent studies have investigated cognition among FXTAS patients. Global deficits on tests of intelligence (Bacalman et al., 2006; Bourgeois et al., 2007; Grigsby et al., 2008; Grigsby et al., 2007) in addition to deficits in executive cognitive functioning have been reported (Grigsby et al., 2008; Grigsby, Brega et al., 2006; Grigsby et al., 2007; Grigsby, Leehey et
al., 2006). Sevin et al. (Sevin et al., 2009) identified an association with repeat size and cognitive impairment, particularly among premutation carriers with greater than 70 repeats.

We surveyed sibships in families with a diagnosis of FXS to recruit males at risk of carrying the premutation without regard to symptoms of FXTAS. We recruited 107 males (89 premutation carrier males and 18 non-carrier males) over age 50 years, and assessed motor symptoms of FXTAS including tremor and ataxia using a quantitative computerized neurological test battery. Participants also completed a neuropsychological test battery if eligible. We divided participants into three groups: premutation carriers with motor symptoms (tremor and/or ataxia) (N=66), premutation carriers without motor symptoms (N=23), and non-carriers (N=18) and tested for differences of general intelligence, memory, and executive function to corroborate findings from previous reports. We also compared groups for symptoms of depression or anxiety as measured by self-report questionnaires.

Methods

Study population

Our goal was to ascertain a sample of premutation carriers and their non-carrier siblings without respect to known symptoms of FXTAS. Thus, we invited all relatives in families who had a family member diagnosed with FXS to be tested for premutation carrier status. If a premutation male was identified in a sibship, he and all of his premutation and non-carrier siblings were recruited blind to symptoms of FXTAS. The only eligibility criteria were > 50 years of age and English as a primary language (required for our neuropsychological test battery). We identified and contacted 150 eligible males and enrolled 107 (71.3%). The non-enrolled subjects were comprised of 27 refusals (10 non-carriers, 7 premutation carriers, and 10 of unknown repeat size), 12 exclusions (1 non-carrier, 7 premutation carriers, and 4 of unknown repeat size), and 4 subjects we were unable to locate. Reasons for exclusion included very poor health or non-English speaking. The protocols and consent forms were approved by the Institutional Review Board at Emory University.

Data Collection

An initial medical history interview was used to screen participants for eligibility and to ascertain self-reported evidence of the major symptoms of FXTAS, namely tremor and ataxia. This initial screening was done by the project coordinator who was not blind to FXS family history. All eligible participants were then asked to provide a biological sample for testing or confirmation of carrier status, to complete the quantitative neurological test battery and, if eligible, to complete a neuropsychological test battery. For the neuropsychological testing, stricter criteria for eligibility were implemented. Participants were excluded if they endorsed heavy alcohol or drug use (3 premutation carriers), history of strokes (2 premutation carriers), brain trauma or loss of consciousness (4 premutation carriers and 1 non-carrier) or other conditions (7 premutation carriers: reasons include post-polio exposure, Guillain Barre syndrome, history of electroshock therapy, dementia (n=2), significant hearing impairment, and recent administration of neuropsychological tests) that could affect performance on these measures. Participants who did not speak English as their primary language were also excluded from neuropsychological testing (5 premutation carriers and 1 non-carrier). In addition, a subset of participants was only able to complete a shortened battery, primarily due to fatigue (5 premutation carriers and 2 non-carriers). All testing was done by trained psychometrists who were blind to carrier status. A total of 73 males were given the full neuropsychological battery. Testing was completed at the Emory site or at the home of the participant.
Neurological Test Battery

The neurological test battery is described in detail in Allen et al. (Allen et al., 2008). Briefly, the CATSYS 2000 system (www.catsys.dk), a portable testing system, was used to measure coordination ability, reaction time, tremor, and postural stability. Output variables used for analysis were selected based on their correlation with a neurologist’s clinical rating (Gerr, Letz, & Green, 2000). Participants were scored as positive for measures based on comparisons to normative populations.

Ataxia was assessed using a force plate attachment to the CATSYS system. Participants were asked to simply stand on the platform with their eyes open (two trials) or their eyes closed (two trials). The variable used to assess ataxia was sway velocity (mm/s). Participants who scored greater than one standard deviation above the normative population mean, or were unable to stand on the platform due to postural instability (n=6), were scored as positive for ataxia.

For tremor, three types of measurements were obtained using the CATSYS system. Each measurement was obtained from two trials for each hand using a stylus equipped with an accelerometer that measured movement in two dimensions. The variable analyzed was the tremor intensity, defined as the root mean square of acceleration, measured in m/s². The first task measured postural tremor (Postural Tremor I). Participants were asked to hold the stylus so that it was parallel to the floor with their little finger in front of their navel. They were instructed to relax their arm as much as they could without letting it touch their body. For the second task used to measure postural tremor (Postural Tremor II), participants were asked to stand with their arms extended perpendicular to their body. A stylus was affixed to their middle finger to detect any movement. Finally, intention tremor was measured by having participants hold a stylus as they would a pencil. They were asked to track the movement of a bar across the monitor of a computer without actually touching the screen. Positive tremor was defined as a score greater than one standard deviation above the mean in a normative population.

Fine motor dexterity was measured using the Lafayette Grooved Pegboard Test. Participants were asked to place directional (or grooved) pegs into a pegboard as quickly as possible. The time and the number of pegs dropped were recorded. A total mean time was calculated for the dominant and non-dominant hands.

Premutation carriers with motor symptoms were defined as subjects who scored more than one standard deviation above the mean of the normative population for ataxia or any of the three tremor measurements. We used a one standard deviation above normative means as we found this threshold to be most consistent with clinical neurological exam results (data not shown).

Neuropsychological Test Battery

General cognitive functioning was assessed using the North American Adult Reading Test (NAART)(Blair & Spreen, 1989; Nelson, 1982) and subtests of the Wechsler Adult Intelligence Scale - III (WAIS-III) (Wechsler, 1997a). The NAART provides a measure of pre-morbid IQ. WAIS-III subtests administered included Picture Completion, Digit Symbol-Coding, Similarities, Arithmetic, Matrix Reasoning, Digit Span, Information, Symbol Search, and Letter-Number Sequencing. Full scale IQ (FSIQ), Verbal IQ (VIQ), and Performance IQ (PIQ) were calculated from the subtests as recommended (Wechsler, 1997a). Cognitive decline was measured by subtracting the current IQ (WAIS-III VIQ or PIQ) from the respective pre-morbid IQ score (NAART VIQ or PIQ).
Memory was measured using the Wechsler Memory Scales – III (WMS-III) (Wechsler, 1997b). The Logical Memory, Visual Reproduction, and Word List subtests were administered, each with immediate recall, delayed recall, and delayed recognition (9 scores total).

Executive functioning was assessed using three instruments: 1) Delis – Kaplan Executive Function System (D-KEFS) (Delis, Kaplan, & Kramer, 2001), a version of the trail making test that reduces the effects motor dysfunction (the Number-Letter Switching scaled score was used for analyses); 2) Wisconsin Card Sorting Test 64 (WCST 64) (Heaton, Cheline, Talley, Kay, & Curtiss, 1993) (four t-scores were included in the analyses: Perseverative Errors, Perseverative Responses, Non-Perseverative Errors, and Conceptual Level Responses) and 3) Controlled Oral Word Association Test (COWAT) (Benton & Hamsher, 1976) (the overall t-score was used in analyses).

Symptoms of depression and anxiety were assessed using three self-report questionnaires. First, the Center for Epidemiologic Studies Depression Scale (CES-D) (Radloff, 1977) is a 20-item scale used to evaluate symptoms of depression. The Social Phobia and Anxiety Inventory (SPAI) (Turner, 1996) is a two-part inventory used to measure symptoms of social phobia in various situations. The two subscales measure social phobia and agoraphobia. Lastly, the State-Trait Anxiety Inventory (STAI) (Spielberger, 1983) is a two-part inventory used to measure levels of current anxiety (state anxiety) and general anxiety susceptibility (trait anxiety). For all three scales, higher scores indicate higher levels of the associated symptoms.

**Laboratory Methods**

DNA was extracted from buccal samples or blood using Qiagen QiAmp DNA Blood Mini Kit. \( FMR1 \) CGG repeat sizes were determined by a fluorescent-sequencer method, as described elsewhere (Meadows et al., 1996), using the ABI Prism 377 DNA Sequencer or ABI 3100 Genetic Analyzer. For male samples that did not amplify, a second PCR-based, hybridization technique was used (Brown et al., 1993) to identify a possible high repeat size band. The PCR reaction consisted of 1X PCR Buffer, 10% DMSO, 370 μM deazaG, 500 μM d(ACT), 0.3 μM each primer, 15 ng T4 gene 32, and 1.05U Roche Expand Long Taq. Primers for the \( FMR1 \) repeat region were C: 5'Cy5GCTCAGCTCCGTTTCGGTTTCACTTCCGGT3' and F: 5'AGCCCAGCTCCACGAGCTCCTCCA3' (Fu et al.). If a ladder of bands was seen in the PCR result, indicating mosaicism, a Southern blot was also done to assure there were no full mutation sized bands (Rousseau et al., 1991). Any participant with full mutation alleles was not included in the analysis.

**Statistical Methods**

Premutation carriers were defined as having ≥55 CGG repeats. Comparisons between premutation carriers with and without motor symptoms and non-carriers for demographic information (Table 1) were analyzed using Chi-square analysis, Fisher’s Exact test, or Analysis of Variance (ANOVA) where appropriate.

There was a maximum of 23 neurocognitive and neurobehavioral outcomes measures that we obtained from each participant. To reduce the number of variables being analyzed, a principal component analysis (PCA) followed by varimax rotation was used to develop factors using measures from each of the following areas: memory, executive functioning, depression and anxiety. IQ measures and cognitive decline variables were analyzed separately.
In the PCA model for memory, the Kaiser-Meyer Olkin measure of sampling adequacy was 0.84 indicating the usefulness of reducing the nine measures into a smaller number of factors. Three independent factors were chosen based on the proportion of variance accounted for and the interpretability of those factors. These three factors accounted for 78.2% of the total variance. In interpreting the rotated factor pattern, a score was said to load on a given factor if the factor loading was 0.40 or greater for that component. The three scores of Visual Reproduction and the delayed Word Lists Recall score loaded onto the first memory factor (Visual Reproduction Factor), the three scores of Logical Memory loaded onto the second memory factor (Logical Memory Factor), and the three scores of Word Lists loaded onto the third memory factor (Word Lists Memory Factor).

In the PCA model for EF, the Kaiser-Meyer Olkin measure of sampling adequacy was 0.69. There were two independent factors chosen from the 6 scores for EF; these scores explained 77.8% of the variance. The four scores from the WCST loaded onto the first EF factor (Mental Flexibility Factor). The second score included the D-KEFS score, COWAT score, the WCST Perseverative Errors and the Perseverative Responses (Attention and Verbal Fluency Factor).

The PCA model for depression and anxiety obtained from the self-reported questionnaire data had a Kaiser-Meyer Olkin measure of sampling adequacy of 0.73. Two independent factors explained 78.4% of the variance. The one score from the CES-D and the two scores from the STAI loaded onto the first factor score (Depression and Anxiety Factor) and the two scores from the SPAI loaded onto the second factor (Social Phobia Factor).

Linear regression analysis was used to test for an association with each individual measure or PCA factor and repeat size as a continuous variable (Table 2). All models were adjusted for age and education (binary variable for college completed or not). Interaction terms of age and repeat size as well as education and repeat size were tested for each model, but were not found to be significant covariates. Race/ethnicity was not adjusted for in statistical analyses due to the small number of non-Caucasian subjects; however, each statistical test was also tested using only Caucasian subjects to assure that conclusions did not change. Because the model estimates were essentially the same using both samples, we report results from models that include all subjects. To adjust for motor dysfunction, the score for the Grooved Pegboard on the dominant hand was included for models with measures that may include a motor component: PIQ, the WAIS-III subtests of Digit-Symbol Coding and Symbol Search, the PIQ decline, the WMS-III Visual Reproduction measures, and D-KEFS score. The results presented in Tables 2 and 3 include the $R^2$ from the full model including repeat size and all covariates, as well as the p-value, $R^2$, and standardized beta coefficient associated with the variable of repeat size in that full model.

To test these same variables (the individual measures and the PCA factors) for associations with motor symptoms, analysis of covariance (ANCOVA) was used to compare neuropsychological measures for premutation carriers with motor symptoms, premutation carriers without motor symptoms, and non-carriers. All models were adjusted for age and education. A Tukey’s Studentized range test was used as a post hoc test to determine which groups were significantly different from each other.

Generalized estimating equation (GEE) methods (Zeger & Liang, 1986) were tested to account for the dependent nature of measurements among family members. Results from the GEE models were essentially the same; thus, only results from the linear regression models are presented.

We used a Bonferonni correction to adjust for multiple testing. There were nine uncorrelated variables that were tested (VIQ measures, PIQ measures, three Memory factors, two
Executive Functioning factors, and two behavioral factors from the questionnaires). Thus, a cutoff value of 0.006 was used to indicate significance. We also conducted exploratory analyses on individual test scores and report p-values of those results <0.05, recognizing that these cannot be interpreted as statistically significant.

Effect sizes for repeat size from the multiple regression models were calculated using the partial correlation to determine $f^2$. All effect sizes were determined to be small for each of the tests performed ($f^2<0.15$) (J. Cohen, 1992).

Statistical analysis was done using SAS V9.

Results

We tested 89 premutation carrier males and 18 of their non-carrier brothers. Of these participants, 21 premutation males and two non-carrier males were not eligible for the neuropsychological test battery (see Methods). We divided participants into three groups: non-carriers (N=18), premutation carriers without motor symptoms (N=23), and premutation carriers with motor symptoms (N=66). There was no statistically significant difference between groups with respect to mean age, race/ethnicity, or education levels (Table 1). However, in our models, we included education because of the lower proportion of college graduates in the premutation groups. We also examined age as potential modifier variable; in these models, we included age and age by repeat size as an interaction term. The interaction term of age and repeat size was not significant in any of the models, and, thus, was not included in the final models.

Associations with FMR1 repeat size as a continuous variable

We began the analysis by examining the associations between each test score outlined in Table 2 and FMR1 repeat size as a continuous variable using linear regression. We found that the WAIS-III summary scores for VIQ and PIQ were associated with repeat size (Table 2; VIQ p=0.002; PIQ p=0.004) as well as measures for verbal (p=0.015) and performance cognitive decline (p=0.012). We conducted an exploratory analysis to determine whether a specific subdomain of the VIQ or PIQ played an important role in this finding, (Table 3). No specific pattern was identified; all subtests were marginally associated with repeat size (p<0.05) with the exception of Digit-Symbol Coding (p=0.193; Table 3).

None of the factors scores for memory, executive functioning, or behavior were statistically significantly associated with repeat size (Table 2).

Associations by repeat size group and FXTAS status

Based on the report of Grigsby et al. (Grigsby et al., 2008), premutation carriers with FXTAS showed a significantly different profile on cognitive testing compared to non-carriers. However, for most measures, carriers without symptoms of FXTAS were not different from non-carriers. To test this finding in our study sample, we divided our premutation carriers into groups based on the presence or absence of motor symptoms (ataxia and/or tremor) as measured by CATSYS 2000 quantitative system (see Methods).

We tested for differences in the three groups (non-carriers, premutation carriers without motor symptoms, and premutation carriers with motor symptoms) for each of our primary eleven outcome measures (Table 4). There was a marginally significant association between the group variable and VIQ (p=0.018; Table 4). Tukey’s analysis of between group differences revealed that the premutation carrier group with motor symptoms was significantly different from non-carriers (p=0.005). The difference between groups for PIQ did not reach significance (p=0.056; Table 2). However in a post-hoc analysis, we did find a
marginally significant difference between premutation carriers with motor symptoms and non-carriers (p=0.017). There were no significant differences detected for verbal cognitive decline (p=0.296) or performance cognitive decline (p=0.163) among the three groups (Table 4).

Using the memory PCA factors, a marginally significant difference was identified among the three groups for the Logical Memory Factor (p=0.031; Table 3). Using Tukey’s post-hoc test, we found that premutation carriers with motor symptoms were significantly different from non-carriers (p=0.009). The other two memory factors, Visual Reproduction Memory Factor and Word Lists Memory Factor, did not show any between-group differences.

No significant differences were seen for the comparison of groups for the EF PCA factors or for the self-report behavioral (depression, anxiety, and social phobia) questionnaire factor scores.

**Discussion**

The purpose of this study was to investigate the neuropsychological profile among older males with the premutation who are at risk for FXTAS. We recruited 107 males over age 50 without regard to symptoms of FXTAS: 18 non-carrier men, 23 premutation carrier men without detected motor symptoms, and 66 premutation carrier men with tremor and/or ataxia.

Overall, we found that cognitive performance as captured in VIQ and PIQ of the WAIS-III differed significantly by repeat size for men >50 years old: there was a statistically significant negative correlation between repeat size and cognitive performance (Table 2) and a marginally significant reduction in scores between premutation carriers with symptoms of FXTAS and non-carriers (Table 4). These results were consistent with the marginally significant association between verbal cognitive decline and performance cognitive decline and repeat size. This overall pattern is similar to that found in other studies which compared males with FXTAS and non-carriers (S. Cohen et al., 2006; Grigsby et al., 2008; Grigsby et al., 2007). In our previous report that focused on younger men between the ages of 18-50 years, we did not find an association of repeat size and cognitive function among males (Hunter et al., 2008). This suggests that the effect of the *FMR1* repeat size is more evident among an older population with or without overt symptoms of FXTAS. Two points should be made that may limit these conclusions. First, our non-carrier and asymptomatic premutation carrier groups are limited in size. Second, the non-carrier group had a mean VIQ and PIQ above the normal mean of 100, although it was not outside of one standard deviation. This may indicate a participation bias, a bias that should apply to all males irrespective of repeat size. This makes it an unlikely factor to affect the comparisons among groups.

Other studies have shown significant differences in other domains of Memory and Executive Function (EF) between carriers and non-carriers. Accordingly, we did further exploratory analyses, even though we did not find statistical significance in our primary comparisons. First, we tried to replicate the finding of Grigsby et al. (Grigsby et al., 2008). They found that Memory scores differed significantly between all three groups of men: carriers with motor symptoms were more affected than carriers without symptoms, and non-carriers performed the best. We noted a marginally significant difference for the Logical Memory factor score (Table 4) for premutation carriers with motor symptoms compared to non-carriers. The performance of premutation carriers without motor symptoms fell between these groups, although the differences between means did not reach significance. Again, this could be due to the small sample sizes in these two groups.
Surprisingly, we were not able to confirm a significant EF deficit among premutation carrier males, which has been observed in two different study samples (Brega et al., 2008; Cornish et al., 2008; Grigsby et al., 2008; Grigsby, Brega et al., 2006; Grigsby et al., 2007). Brega et al. (Brega et al., 2008) suggested that the impairment of other nonexecutive cognitive skills may be secondary to executive dysfunction. We used various statistical approaches to uncover differences in EF functioning among premutation carriers with and without motor symptoms. We examined a combination of tests to potentially tap into specific domains (Tables 2 and 4), as well as individual tests (data not shown), but did not find any differences among premutation carriers and non-carriers. One possible explanation for this discrepancy is that the tests used in each study measure different domains of EF. Cornish et al. (Cornish et al., 2008) used the Hayling Sentence Completion Task, the Stroop Color-Word Test, the Test of Everyday Attention – Map Search Task, and the Sustained Attention to Response Test. Grigsby et al. (Brega et al., 2008; Cornish et al., 2008; Grigsby et al., 2008; Grigsby, Brega et al., 2006; Grigsby et al., 2007) used the Behavioral Dyscontrol Scale, the COWAT, the Animal Naming Test and Stroop. In the current study, we use the WCST, the D-KEFS, and the COWAT. Thus, there is little overlap among specific tests, although most tap into the same domains. If it is true that only certain tests of EF identify differences by repeat size with or without motor symptoms, we suggest that the effect of repeat length on EF must be very specific. Also, the effect size for repeat length on each measure in our sample was small ($f^2$ < 0.15), thus, a sample size of 547 would be needed to detect a difference at $\alpha=0.05$ with power of 0.80 (J. Cohen, 1992). None of the studies, including our own, had sample sizes >50 in each group.

Another possible explanation for differences in our results could be related to differences in ascertainment of study samples or criteria for inclusion and exclusion. In that respect, one limitation of our study may be that we excluded individuals who reported any health problems that would influence their neuropsychological test results, for example brain trauma, stroke, and excessive use of recreational drugs or alcohol. We had more exclusions among premutation carriers: only 11% of non-carriers were excluded from neuropsychological testing compared to 24% of premutation carriers. If these exclusion criteria are somehow associated with men who have FXTAS (i.e., present as a “trigger” for onset or severity), our results will be conservative. For example, heavy alcohol use was endorsed by 3/89 premutation carriers and 0/18 non-carriers. As alcoholism is known to be negatively associated with executive function (Glass et al., 2009), exclusion of these subjects may influence results on executive function measures.

One difference in our study from others is that we used the CATSYS alone to define the group of premutation carriers with motor symptoms. Although tremor and ataxia are the primary symptoms of FXTAS and the CATSYS provides a sensitive measure of these, it differs from a full neurological exam to determine the diagnosis of FXTAS. It may define a group of premutation carriers with milder symptoms or earlier stages of FXTAS. Thus, the association of executive function with the premutation may be due to the duration of the disease. As mentioned above, if our exclusion criteria are biased toward symptoms associated with duration of the disease, then our ability to identify any executive function deficits will be reduced.

In summary, we have found that premutation carriers with motor symptoms have lower scores compared to their non-carriers siblings for measures of VIQ, PIQ, and Logical Memory. We did not identify any statistically significant differences between these groups for executive functioning or for the self-report questionnaires for depression and anxiety. Interestingly, most of the results do indicate that there is a decline with repeat size (Table 1 – negative standardized beta coefficients), but the effect size is small. Our future objectives are to increase the sample size, as well as obtain longitudinal testing results on existing
subjects in order to determine whether decline in neuropsychological functioning is a function of duration of FXTAS and thus a late-stage indicator of the disorder.

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References


Table 1

Demographic information on enrolled male subjects.

<table>
<thead>
<tr>
<th></th>
<th>Non-carriers</th>
<th>Premutation Carriers without motor symptoms</th>
<th>Premutation Carriers with motor symptoms</th>
</tr>
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<tbody>
<tr>
<td>N</td>
<td>18</td>
<td>23</td>
<td>66</td>
</tr>
<tr>
<td>Age</td>
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<td>63.5 ± 7.3</td>
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<tr>
<td>Race/Ethnicity</td>
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<td>0%</td>
<td>1.69%</td>
</tr>
<tr>
<td>Education (percentage college completed)</td>
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<td>20.0%</td>
<td>43.5%</td>
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<tr>
<td>Repeat Size Median (Range)</td>
<td>31 (20-54)</td>
<td>80 (55-150)</td>
<td>88 (55-180)</td>
</tr>
<tr>
<td>WAIS-III FSIQ Estimate*</td>
<td>114.4 ± 11.9 (N=14)</td>
<td>99.1 ± 13.9 (N=16)</td>
<td>100.2 ± 14.5 (N=44)</td>
</tr>
<tr>
<td>WAIS-III VIQ Estimate*</td>
<td>113.7 ± 14.5 (N=14)</td>
<td>98.2 ± 13.6 (N=16)</td>
<td>100.9 ± 13.8 (N=47)</td>
</tr>
<tr>
<td>WAIS-III PIQ Estimate*</td>
<td>110.0 ± 11.3 (N=14)</td>
<td>97.1 ± 15.3 (N=16)</td>
<td>97.6 ± 16.1 (N=43)</td>
</tr>
</tbody>
</table>

* WAIS-III IQ estimates were only available on the subset of subjects eligible for the full neuropsychological battery (see methods).
Table 2

Linear regression results using repeat size as a continuous variable. All models were adjusted for age and education.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>N</th>
<th>Overall Model R²</th>
<th>p-value for repeat size</th>
<th>Partial R² for repeat size</th>
<th>Standardized beta coefficient for repeat size</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIQ (WAIS-III)</td>
<td>73</td>
<td>0.32</td>
<td>0.002</td>
<td>0.13</td>
<td>−0.32</td>
</tr>
<tr>
<td>PIQ (WAIS-III) *</td>
<td>63</td>
<td>0.38</td>
<td>0.004</td>
<td>0.13</td>
<td>−0.31</td>
</tr>
<tr>
<td>V cognitive decline</td>
<td>70</td>
<td>0.07</td>
<td>0.015</td>
<td>0.09</td>
<td>0.29</td>
</tr>
<tr>
<td>P cognitive decline *</td>
<td>62</td>
<td>0.22</td>
<td>0.012</td>
<td>0.11</td>
<td>0.30</td>
</tr>
<tr>
<td>Memory Factor 1: Visual Reproduction Memory Factor *</td>
<td>65</td>
<td>0.07</td>
<td>0.201</td>
<td>0.03</td>
<td>−0.16</td>
</tr>
<tr>
<td>Memory Factor 2: Logical Memory Factor</td>
<td>70</td>
<td>0.10</td>
<td>0.130</td>
<td>0.03</td>
<td>−0.18</td>
</tr>
<tr>
<td>Memory Factor 3: Word Lists Memory Factor</td>
<td>70</td>
<td>&lt;0.01</td>
<td>0.758</td>
<td>&lt;0.01</td>
<td>−0.04</td>
</tr>
<tr>
<td>EF Factor 1: Mental Flexibility Factor</td>
<td>63</td>
<td>&lt;0.01</td>
<td>0.224</td>
<td>0.02</td>
<td>−0.16</td>
</tr>
<tr>
<td>EF Factor 2: Attention and Verbal Fluency Factor *</td>
<td>59</td>
<td>0.13</td>
<td>0.406</td>
<td>0.01</td>
<td>−0.10</td>
</tr>
<tr>
<td>Behavior Factor 1: Depression and Anxiety Factor</td>
<td>88</td>
<td>0.01</td>
<td>0.154</td>
<td>0.02</td>
<td>0.15</td>
</tr>
<tr>
<td>Behavior Factor 2: Social Phobia Factor Score</td>
<td>88</td>
<td>0.04</td>
<td>0.611</td>
<td>&lt;0.01</td>
<td>−0.05</td>
</tr>
</tbody>
</table>

* grooved pegboard dominant hand time included as a covariate
Table 3

WAIS-III subscales. Linear regression results using repeat size as a continuous variable. All models were adjusted for age and education.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>N</th>
<th>Overall Model R²</th>
<th>p-value for repeat size</th>
<th>Partial R² for repeat size</th>
<th>Standardized beta coefficient for repeat size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verbal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Similarities</td>
<td>74</td>
<td>0.33</td>
<td>0.010</td>
<td>0.09</td>
<td>−0.25</td>
</tr>
<tr>
<td>Arithmetic</td>
<td>73</td>
<td>0.15</td>
<td>0.024</td>
<td>0.07</td>
<td>−0.25</td>
</tr>
<tr>
<td>Digit Span</td>
<td>73</td>
<td>0.14</td>
<td>0.027</td>
<td>0.07</td>
<td>−0.25</td>
</tr>
<tr>
<td>Information</td>
<td>74</td>
<td>0.31</td>
<td>0.016</td>
<td>0.08</td>
<td>−0.24</td>
</tr>
<tr>
<td>Letter Number Sequencing</td>
<td>73</td>
<td>0.07</td>
<td>0.026</td>
<td>0.07</td>
<td>−0.26</td>
</tr>
<tr>
<td>Performance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Picture Completion</td>
<td>74</td>
<td>0.21</td>
<td>0.022</td>
<td>0.07</td>
<td>−0.25</td>
</tr>
<tr>
<td>Digit Symbol-Coding a</td>
<td>65</td>
<td>0.36</td>
<td>0.193</td>
<td>0.03</td>
<td>−0.13</td>
</tr>
<tr>
<td>Matrix Reasoning</td>
<td>72</td>
<td>0.14</td>
<td>0.081</td>
<td>0.07</td>
<td>−0.24</td>
</tr>
<tr>
<td>Symbol Search a</td>
<td>65</td>
<td>0.32</td>
<td>0.005</td>
<td>0.12</td>
<td>−0.30</td>
</tr>
</tbody>
</table>

*a* grooved pegboard dominant hand time included as a covariate
Table 4

Least-square means for ANCOVA models adjusted for age and education.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Overall model p-value</th>
<th>p-value for repeat size group</th>
<th>Non-carriers (N)</th>
<th>Premutation Carriers without motor symptoms (N)</th>
<th>Premutation Carriers with motor symptoms (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIQ (WAIS-III)</td>
<td>&lt;0.001</td>
<td>0.018</td>
<td>112.0 (14)</td>
<td>103.1 (15)</td>
<td>101.1 (44)**</td>
</tr>
<tr>
<td>PIQ (WAIS-III)</td>
<td>0.003</td>
<td>0.056</td>
<td>108.5 (14)</td>
<td>101.5 (15)</td>
<td>97.9 (40)**</td>
</tr>
<tr>
<td>V cognitive decline</td>
<td>0.399</td>
<td>0.296</td>
<td>−4.1 (14)</td>
<td>−0.6 (15)</td>
<td>0.8 (41)</td>
</tr>
<tr>
<td>P cognitive decline</td>
<td>0.269</td>
<td>0.163</td>
<td>0.9 (14)</td>
<td>5.4 (15)</td>
<td>8.7 (39)</td>
</tr>
<tr>
<td>Memory Factor 1: Visual Reproduction Memory Factor</td>
<td>0.998</td>
<td>0.978</td>
<td>0.03 (14)</td>
<td>−0.05 (15)</td>
<td>−0.02 (41)</td>
</tr>
<tr>
<td>Memory Factor 2: Logical Memory Factor</td>
<td>0.005</td>
<td>0.031</td>
<td>0.58 (14)</td>
<td>0.08 (15)</td>
<td>−0.20 (41)**</td>
</tr>
<tr>
<td>Memory Factor 3: Word Lists Memory Factor</td>
<td>0.471</td>
<td>0.577</td>
<td>−0.12 (14)</td>
<td>0.27 (15)</td>
<td>0.03 (41)</td>
</tr>
<tr>
<td>EF Factor 1: Mental Flexibility Factor</td>
<td>0.963</td>
<td>0.792</td>
<td>0.06 (14)</td>
<td>0.12 (14)</td>
<td>−0.09 (35)</td>
</tr>
<tr>
<td>EF Factor 2: Attention and Verbal Fluency Factor</td>
<td>0.014</td>
<td>0.375</td>
<td>0.31 (14)</td>
<td>−0.19 (14)</td>
<td>0.02 (35)</td>
</tr>
<tr>
<td>Behavior Factor 1: Depression and Anxiety Factor</td>
<td>0.298</td>
<td>0.242</td>
<td>−0.37 (17)</td>
<td>0.09 (19)</td>
<td>0.10 (52)</td>
</tr>
<tr>
<td>Behavior Factor 2: Social Phobia Factor Score</td>
<td>0.155</td>
<td>0.797</td>
<td>0.16 (17)</td>
<td>−0.06 (19)</td>
<td>0.01 (52)</td>
</tr>
</tbody>
</table>

** indicates significant between group differences compared to non-carriers using Tukey’s post hoc analysis.