Published in final edited form as:
Neuropsychology. 2012 March ; 26(2): 156–164. doi:10.1037/a0026799.

Capturing the fragile X premutation phenotypes: a collaborative effort across multiple cohorts

Jessica Ezzell Hunter[1], **Stephanie Sherman**[1], **Jim Grigsby**[2], **Cary Kogan**[3], and **Kim Cornish**[4]

[1]Department of Human Genetics, Emory University School of Medicine, Atlanta, Georgia

[2]Departments of Psychology and Medicine, University of Colorado Denver, Denver, Colorado

[3]School of Psychology, University of Ottawa, Ottawa, Ontario, Canada

[4]School of Psychology and Psychiatry, Monash University, Melbourne, Australia

Abstract

Objective—To capture the neuropsychological profile among male carriers of the *FMR1* premutation allele (55-200 CGG repeats) who do not meet diagnostic criteria for the late-onset fragile X-associated tremor/ataxia syndrome, FXTAS.

Method—We have initiated a multi-center collaboration that includes three independent cohorts totaling 100 carriers of the premutation and 216 non-carriers. The initial focus of this collaboration has been on executive function. Four executive function scores are shared among the three cohorts (Controlled Oral Word Association Test, Stroop Color-Word Test, and Wechsler backward digit span and letter-number sequencing) while additional executive function scores are available for specific cohorts (Behavior Dyscontrol Scale, Hayling Sentence Completion Test Part B, and Wisconsin Card Sorting Test). Raw scores were analyzed using statistical models that adjust for cohort-specific effects as well as age and education.

Results—Carriers scored significantly lower compared to non-carriers on the Stroop Color-Word Test ($p=0.01$), Hayling Sentence Completion Test Part B ($p<0.01$), and Behavioral Dyscontrol Scale ($p=0.03$), with the Hayling displaying a significant age-related decline ($p=0.01$), as assessed by an age and repeat length group interaction. Follow-up analysis of the collective data did not identify any specific age groups or repeat length ranges (i.e. low premutation=55-70 repeats, mid premutation=71-100 repeats, high premutation=101-199 repeats) that were associated with an increased risk of executive function deficits.

Conclusions—Preliminary analyses do not indicate global executive function impairment among male carriers without FXTAS compared to non-carriers. However, impairment in inhibitory capacity may be present among a sub-set of carriers, though the risk factors for this group do not appear to be related to age or repeat length.

Keywords

FMR1; premutation; CGG repeat; executive function; FXTAS

Corresponding Author: Kim Cornish, Ph.D., School of Psychology & Psychiatry, Monash University, Building 17, Wellington Road, Melbourne 3800, Australia, Tel +61 39902 0488, Fax +61 39905 3848, kim.cornish@monash.edu.

INTRODUCTION

The X-linked fragile X mental retardation 1 gene (*FMR1)* is associated with a group of disorders referred to as fragile X-associated disorders. *FMR1* contains a polymorphic CGG trinucleotide repeat sequence in the 5′ untranslated region of the gene, which can become unstable and expand from one generation to the next. Normal CGG repeat sizes are between 6 and 54 repeats, with 29 or 30 repeats as the most common (Y. H. Fu et al., 1991). An expansion greater than 200 repeats, termed a full mutation**,** generally results in hypermethylation and transcriptional silencing of the *FMR1* locus (Feng et al., 1995; Sutcliffe et al., 1992; Verkerk et al., 1991). The subsequent absence of the fragile X mental retardation protein **(**FMRP**)** is associated with fragile X syndrome (Pieretti et al., 1991). FMRP is specifically involved in synapse formation, maturation and function, and its absence results in the characteristic intellectual impairment and behavioral profile associated with fragile X syndrome (Till, 2010). Autistic features are present among a proportion of individuals with fragile X syndrome (Hatton et al., 2006); thus, the *FMR1* full mutation represents one of the few known single-gene causes of autism spectrum disorder (K. Cornish et al., 2008).

An expansion between 55 and 199 repeats, termed a premutation, is capable of expanding to a full mutation in a single generation when passed from mother to offspring, and thus is considered “carrier” status for fragile X syndrome (S. D. Fu et al., 1994; Oberle et al., 1991). The premutation is relatively common in the general population, found in about 1/100 to 1/250 females (Cronister et al., 2008; Toledano-Alhadef et al., 2001) and in about 1/250 to 1/800 males (Dombrowski et al., 2002; Fernandez-Carvajal et al., 2009) in a predominantly Caucasian sample. At the *molecular* level, individuals harboring premutation alleles, unlike those with the full mutation, produce 2-fold to 10-fold increased levels of *FMR1* mRNA, but reduced levels of FMRP (Allen et al., 2004; Kenneson et al., 2001; Peprah et al., 2010; Primerano et al., 2002; Tassone, Hagerman, Taylor, Gane, et al., 2000; Tassone, Hagerman, Taylor, Mills, et al., 2000).

In the last decade, the phenotype of carriers of the premutation has received considerable attention, spurred by the identification of a fragile X premutation-associated neurodegenerative disorder, fragile X-associated tremor/ataxia syndrome (FXTAS). This disorder, which occurs in approximately 40% of premutation males (> 50 years) and less than 10% of females (Coffey et al., 2008; Jacquemont et al., 2004), is characterized by a constellation of symptoms that include progressive gait ataxia and action tremor (Hagerman et al., 2001; Leehey et al., 2003), parkinsonism (Jacquemont et al., 2003), and executive dysfunction, notably affecting inhibitory control and working memory (Grigsby et al., 2008; Grigsby et al., 2007). Neuroradiologic abnormalities in FXTAS patients include generalized atrophy and white matter disease as well as a distinctive hyperintensity of the middle cerebellar peduncles on T-2 magnetic resonance imaging that is considered a diagnostic hallmark of FXTAS (Brunberg et al., 2002; S. Cohen et al., 2006). Neuropathologic changes include atrophy, spongiform changes in the middle cerebellar peduncles, Purkinje cell loss in the cerebellum, and characteristic neuronal intranuclear inclusions (Greco et al., 2002). Penetrance of FXTAS among premutation carriers is a function of age and repeat length, with roughly 30% of carriers over age 50 developing FXTAS and most carriers with FXTAS having greater than 70 repeats (Jacquemont, et al., 2004; Jacquemont et al., 2006).

To date, the cognitive profile of premutation carriers without FXTAS has remained unclear (Hunter et al., 2009). However, recent fMRI data indicate changes in prefrontal activity in premutation carriers, irrespective of FXTAS diagnosis, during performance of a working memory task (Hashimoto et al., 2010). These preliminary findings provide the first evidence

to suggest vulnerability of specific brain regions associated with neural networks mediating executive cognitive functioning among persons in the premutation range, irrespective of a diagnosis of FXTAS. In addition, accumulating findings indicate that among men with FXTAS, larger CGG repeat is associated with greater ataxia and action tremor (Leehey et al., 2008) and poorer executive function (Brega et al., 2008; K. M. Cornish et al., 2008). Therefore, it is of considerable clinical interest to determine whether there exists an age-related subpopulation with executive function impairments that can be identified among premutation carriers, perhaps prior to the onset of FXTAS. To date, individual studies have produced mixed results and all have been limited by relatively small samples sizes with variability in age range and CGG repeat distribution across studies (e.g., Grigsby *et al*., 2008, Cornish *et al*., 2008, Hunter *et al*., 2008).

In the present study, we analyzed data from a multi-center collaboration that included three independent cohorts totaling 100 carriers of the premutation and 216 non-carriers. The primary goal of this collaboration was to determine whether a profile of executive function deficits emerged among premutation carriers asymptomatic for FXTAS when analyzed in the largest sample to date, and, if present, whether these deficits emerged as a function of age or repeat length.

METHODS

Subjects

Subjects included in this collaboration were recruited as part of three independent studies initiated to assess neuropsychological phenotypes associated with *FMR1* premutation alleles. Though analyses from these independent cohorts have been previously published (Brega, et al., 2008; Cornish, et al., 2008; Grigsby, et al., 2008; Hunter et al., 2008), we have combined these cohorts for a large-scale analysis of executive function. Cohort information, including recruitment, is reported in Table 1.

FMR1 Genotyping

Biological samples were obtained from subjects within each cohort for molecular analysis of the *FMR1* CGG repeat length using methods previously published (cohort 1: Cornish et al., 2008, cohort 2: Hunter et al., 2008, cohort 3: Brega et al., 2008 and Grigsby et al., 2008). Briefly, cohort 1 was genotyped using a direct PCR method modified from Wang et al. (1995) and Southern Blot (Knight et al., 1993), cohort 2 was genotyped using a fluorescent-sequence method (Meadows et al., 1996) and a second PCR-based hybridization method when necessary to detect high-repeat alleles (Brown et al., 1993), and cohort 3 was genotyped with a PCR-based assay and Southern Blot.

Measurement of Executive Function

Since each cohort included in this collaboration came from an independently initiated study to assess neuropsychological profiles among adult male premutation carriers, test batteries were variable across cohorts. However, we were able to identify four measures of executive function shared across all three cohorts: Controlled Oral Word Association Test, Stroop Color and Word Test, and the Wechsler measures of backward digit span and letter-number sequencing. In order to expand the assessment of the executive function profile beyond the four measures above, we also identified one measure unique to each cohort to be included in the analyses: the Hayling Sentence Completion Test from cohort 1, the Wisconsin Card Sorting Test from cohort 2, and the Behavioral Dyscontrol Scale from cohort 3.

The Controlled Oral Word Association Test (COWAT) is a measure of verbal fluency that requires subjects to generate words that begin with the letters F, A, and S in three

subsequent 60 second sessions (Benton & Hamsher, 1976). The total number of words was used as the outcome score. One male from cohort 1 was missing a score for the COWAT.

The Stroop Color and Word Test is a measure of response inhibition (Golden, 1978). The color-word section is comprised of names of colors printed in a contrasting ink color (e.g. the word BLUE printed in red ink). The task requires subjects to name the color of the ink rather than read the word. Subjects are given 45 seconds to complete the task. This is the version of the Stroop utilized in the test batteries from cohorts 2 and 3. However, cohort 1 used a slightly different version, the Stroop Neuropsychological Screening Test, which is identical to the version above with the exception that subjects were allowed 120 seconds to complete the task (Trenerry et al., 1989). The number of items completed in the time allowed was used as the outcome variable. Three men from each of the three cohorts were missing scores for the Stroop.

Backward digit span and letter-number sequencing are both measures of working memory and were administered as subtests of the Wechsler Adult Intelligence Scale 3rd Edition (WAIS-III; Wechsler, 1997a) for cohorts 2 and 3 and the Wechsler Memory Scale 3rd Edition (WMS-III; Wechsler, 1997b) for cohort 1. For backward digit span, subjects were verbally provided increasingly longer sequences of numbers and were required to recall the digits in reverse order. For the letter-number sequencing, subjects were verbally provided a sequence of alternating letters and numbers (e.g. Q1B3J2) and were required to recall the numbers in numerical order followed by the letters in alphabetical order. The outcome score for each of these tasks was the raw score. Three men from cohort 2 and one man from cohort 3 were missing scores for backward digit span; three men from cohort 2 and 10 men from cohort 3 were missing scores for letter-number sequencing.

The Hayling Sentence Completion Test Part B is a measure of response inhibition (Burgess & Shallice, 1997). This task requires the subject to complete a sentence with a word that is incongruous with the meaning of the sentence (e.g. “The captain wanted to stay with the sinking…peanut.”). The outcome score for this task was the number of errors.

The Wisconsin Card Sorting Test (WSCT) assesses mental flexibility and involves matching response cards to a set of stimulus cards based on one of three features that changes across the course of administration: the number of shapes on the card, the color of the shapes, or the shapes themselves (Heaton, 1993). The WCST requires the participant to match cards correctly without knowing the sorting principle, being told after each response whether that match was correct or incorrect. After a fixed number (n=10) of consecutive correct matches, the sorting principle changes and the participant must shift to a new sorting strategy. The number of perseverative errors was used as the outcome score. Six men from cohort 2 have missing scores for the WCST.

The Behavioral Dyscontrol Scale (BDS) assesses behavioral self-regulation (Grigsby et al., 1992). The BDS requires subjects to complete seven items, which assess motor performance (two items for simple motor control, 2 items for inhibition, two items for motor learning, and one item for cognition and error detection) and one non-motor item, which assesses the capacity to shift attention. A final item is a rating by the examiner of the patient’s insight into the accuracy of his/her performance. The total BDS score (0-27 points) was used as the outcome score.

Statistical analysis

Subjects were assigned to one of two groups based on *FMR1* repeat length: non-carriers (<55 repeats) and premutation allele carriers (55-199 repeats). Table 2 lists age, education, and IQ data stratified by repeat group for individual cohorts and the combined sample.

Education was defined as a 6-level ordinal variable in all statistical models (1=some high school completed/no qualifications, 2=high school completed/O Levels, 3=technical or vocational school completed/A Levels, 4=some college completed/higher diploma, 5=college completed/first degree, and 6=Professional or graduate school completed/ postgraduate studies). For descriptive purposes, this variable was collapsed as a dichotomous variable [1=some higher education or less (classes 1, 2, 3, and 4 above), 2=college completed or more (classes 5 and 6 above)] (Table 2). IQ scores were obtained in cohort 1 using the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999) and in cohorts 2 and 3 using the WAIS-III (Wechsler, 1997a). Repeat group differences were tested using analysis of variances (ANOVA) for age and IQ and chi-square tests for education. Demographic variables that differed for repeat groups were included in models as potential confounders in addition to indicator variables for cohort.

All executive function scores were analyzed as outcome variables in models with *FMR1* repeat length as the main predictor, which was used both as a categorical and continuous variable in different analyses. First, repeat length was used as a dichotomous variable in analysis of covariance (ANCOVA) models to compare mean scores between the non-carrier and premutation groups. A Tukey's *post hoc* analysis was performed to test for adjusted mean score differences between groups. Second, repeat length was used as a continuous variable in linear regression models to analyze associations between scores and repeat length. For models where repeat length was a significant predictor, interaction terms between repeat variables and covariates were tested.

Two strategies were used to account for differences in Stroop administration between cohorts. In the models outlined above, indicator variables for cohort were included as covariates along with the demographic covariates in order to account for cohort-specific effects. Our second approach was to normalize scores across cohorts rather than include a cohort covariate; cohort-specific Z-scores were calculated and used as the outcome measure.

Whereas the models above query *FMR1* repeat length as a predictor of executive function score as a continuous variable, we also performed additional analyses to determine whether the premutation allele was associated with low executive function scores, defined as scoring greater than one standard deviation away from the mean on any measure, which might indicate executive function impairment. Means and standard deviations were calculated using scores from the entire sample, including carriers, non-carriers, and all cohorts. Scores from each measure were dichotomized (0=scored higher than one standard deviation below the mean or "unimpaired", 1=scored lower than one standard deviation from the mean or "impaired") and logistic regression was performed for each measure to determine whether the premutation group was more susceptible to a low score on executive function measures.

Phenotypes currently known to be associated with *FMR1* premutation alleles are not fully penetrant. Roughly 20% of female carriers of the premutation develop premature ovarian failure (Sherman, 2000) while roughly 30% of male carriers of the premutation develop FXTAS (Jacquemont, et al., 2004). Further, the penetrance of FXTAS appears to be a function of both age and repeat length, with males over the age of 50 with 70 repeats or more at the highest risk (Jacquemont, et al., 2006). Thus follow-up analyses were performed to test the hypothesis that the presence of executive dysfunction is determined by age and repeat length among premutation males. First, to identify a subset of premutation carriers with low executive function scores as a function of age and CGG repeat length, the subjects were further divided into four repeat groups (non-carrier: <55 repeats; low premutation: 55-70 repeats; mid premutation: 71-100 repeats; and high premutation: >100 repeats) and three age groups (<30 years of age, 30-49 years of age, and ≥50 years of age). The four measures shared across cohorts were tested for a significant interaction between these repeat

and age groups. Second, we compared premutation carriers with and without low executive function scores to determine if the two groups differed for age or repeat length.

All statistical analyses were performed using the PROC REG and PROC GLM procedures on the SAS System for Windows, Release 9.1.

RESULTS

Description of Study Cohorts

We found significant differences for demographic variables within and across cohorts (Table 2). Within cohort 1, the non-carrier and premutation groups differed in education level (χ^2=5.89, p=0.02) and IQ (t=−2.53, p=0.01). Within cohort 3, the non-carrier and premutation groups differed in age (t=−2.19, p=0.03). Across the three cohorts, the non-carrier and premutation groups differed in age (ANOVA; non-carrier: F=91.12, df=2, p<0.01; premutation: F=40.12, df=2, p<0.01), education (non-carrier: χ^2=69.84, df=10, p<0.01; premutation: χ^2=48.08, df=10, p<0.01), and IQ (ANOVA; non-carrier: F=7.71, df=2, p<0.01; premutation: F=3.98, df=2, p=0.02). In addition, the cohort 1 premutation group had significantly longer repeat lengths than did the cohort 3 premutation group (Wilcoxon Signed-Rank Test statistic=1166, p<0.01).

FMR1 repeat length as a predictor of executive function scores

Table 3 summarizes the results of models using *FMR1* repeat length as the main predictor of executive function scores, with categorical models using a repeat length group variable (non-carrier vs. premutation carriers) and linear models using repeat length as a continuous variable. Models were adjusted for age, education, and cohort effects. IQ was not included as a covariate as it was highly correlated with education (r=0.49, p<0.01). However, it should be noted that models run with IQ as a covariate rather than education did not change the conclusions of the analyses (data not shown).

In categorical models, the premutation group scored statistically significantly worse compared to non-carriers on the Stroop (partial R^2=2%, p=0.01), the Hayling (partial R^2=12%, p<0.01), and the BDS (partial R^2=7%, p=0.03). Significant linear associations between *FMR1* repeat length as a continuous variable in were detected for the Stroop (partial R^2=2%, p=0.02) and Hayling (partial R2=12%, p<0.01). The partial R^2 values were used to calculate Cohen's f^2, a measure of effect size (J. Cohen, 1992). We obtained f^2 values of 0.02, 0.14, and 0.08 for the Stroop, Hayling, and BDS scores, respectively, indicating small effect sizes of *FMR1* on these scores (J. Cohen, 1992).

To determine whether the significant associations between repeat length and executive function scores detected in the models above were modified by age or education, we tested interaction terms between these variables and repeat length for the Stroop, Hayling, and BDS. Age modified the effect of *FMR1* on Hayling scores in both categorical (p=0.01) and linear models (p<0.01), with premutation carriers scoring worse with age compared to non-carriers. Age also modified the effect of *FMR1* on Stroop scores for the linear models only (p=0.01), with participants scoring significantly worse with age as a function of increasing repeat length. Age did not modify the effect of *FMR1* on BDS scores (data not shown). Level of education did not modify the effect of *FMR1* on any of the scores (data not shown).

Because of the difference in test administration of the Stroop across cohorts (see METHODS), we conducted several analyses to examine potential cohort-specific effects. All Stroop models discussed above used raw Stroop outcome scores and were adjusted for cohort using an indicator variable covariate. In addition to *FMR1* acting as a significant predictor of Stroop scores (Table 3), this cohort variable was a significant predictor for both

categorical ($p<0.01$) and linear models ($p<0.01$). Analysis of interaction terms also indicated that cohort was a modifier of the significant association between Stroop scores and repeat length in linear models ($p=0.03$), thus we analyzed each cohort separately. In categorical models, premutation carriers did not score differently than non-carriers within cohorts 2 ($p=0.57$) or 3 ($p=0.34$), but did score differently within cohort 1 ($p=0.01$). In linear models, repeat length was not a significant predictor of scores within cohorts 2 ($p=0.71$) or 3 ($p=0.64$), but was a significant predictor of scores within cohort 1 ($p<0.01$). In the event that cohorts 2 and 3 were limited by power to detect a significant difference between the non-carrier and premutation groups, we combined data from these two cohorts and did not detect a group difference ($p=0.31$). In separate follow-up models, we used a different approach to adjust for this cohort effect by creating cohort-specific z-scores to normalize scores across all cohorts, rather than using raw scores as the outcome variable and adjusting for cohort as in the models above. In new models of the combined sample of all cohorts using these z-scores as the outcome variable, we found that the premutation group scored significantly lower than non-carriers ($p=0.03$).

FMR1 premutation allele as a predictor of "impaired" executive function

Executive function scores were dichotomized to indicate "impaired", defined as scoring greater than one standard deviation from the mean, and "unimpaired". Using logistic regression models, premutation group was not associated with a higher risk of "impaired" scores on any executive function measure adjusting for age, education and cohort (Table 4).

Identification of subset of premutation carriers at highest risk for "impaired" executive function

In secondary analyses to determine whether there was a subset of premutation carriers at a higher risk for "impaired" executive function scores based on repeat length or age, variables coding for four repeat groups and three age groups were created, and interaction terms between these group variables were tested (Table 5). Age by repeat length interaction terms were not significant for COWAT, backward digit span, and letter-number sequencing. However, this interaction between repeat and age groups was significant for the Stroop ($p=0.01$), with the largest repeat group (>100 repeats) scoring significantly worse than the non-carrier group (<55 repeats) in the oldest age group (≥50 years) only ($p=0.0072$).

In analyses of premutation carriers only, subjects who scored in the "impaired" range had significantly lower repeat lengths than those that scored in the "unimpaired" range for the Stroop test only ($p=0.01$). No differences in age were detected for premutation carriers who scored in the "impaired" range compared to the "unimpaired" range for any test (data not shown).

DISCUSSION

The present study represents the largest study sample to date of males who carry an *FMR1* premutation allele and are asymptomatic for FXTAS. Our study compiled data from three independent cohorts totaling 100 carriers and 216 non carriers. The main objective was to assess scores from four executive function measures shared across the three cohorts. Based on this collective analysis across shared measures, evidence for a generalized vulnerability for executive function deficits among premutation carriers was not detected, which is consistent with previously reported studies among female carriers (Bennetto et al., 2001; Franke et al., 1999). Although we did not detect any significant associations between the premutation allele on the COWA, backward digit span, and letter-number sequencing adjusting for education, age and cohort, we did detect a significant association with the Stroop, a measure of response inhibition for learned information. Further examination of this

association with the Stroop revealed a cohort-specific effect. Specifically, the association was limited to cohort 1, in which the protocol allowed subjects an additional 75 seconds to complete as many items as possible in contrast to that for cohorts 2 and 3 where subjects were given 45 seconds. One potential explanation would be that the additional time allowed for this version of the Stroop captured increased attentional fatigue with poor inhibitory control which manifested as poorer performance among premutation carriers compared to non-carriers in this study sample.

A secondary objective of this study was to assess additional measures unique to each cohort: the Hayling Sentence Completion Test for cohort 1, the Wisconsin Card Sorting Test for cohort 2, and the Behavioral Dyscontrol Scale (BDS) for cohort 3. We detected associations between the premutation and the Hayling, and the BDS, as previously published (Brega, et al., 2008; Cornish, et al., 2008; Grigsby, et al., 2008). In addition, an age by group interaction was reported for the Hayling task, such that premutation males, asymptomatic for FXTAS, performed disproportionately worse with increasing age compared to performance by control males.

These findings indicate a potential impairment in inhibitory capacity among a sub-set of premutation carriers, consistent with previously published reports that report executive function deficits among premutation carriers (Moore et al., 2004; Sevin et al., 2009). Furthermore, Sevin et al (2009) reported cognitive impairments that precede any evidence of FXTAS symptoms among a small proportion of their sample of premutation males. Emerging neuroimaging data also lends some support to the notion that the premutation may confer some *risk* for cognitive dysfunction. For example, in their recent fMRI study, Hashimoto et al (2010) found significant altered activation of the prefrontal cortex of premutation carriers when performing a simple working memory paradigm particularly in areas known to subserve executive functions including working memory (right inferior frontal, dorsolateral prefrontal, and premotor cortices), despite premutation carriers not scoring significantly different from controls. Developmental imaging data are needed to ascertain whether atypical changes in activation worsen with age or remain stable but slightly decreased across the lifespan in the premutation compared to non-carrier controls.

Overall, we found no general executive function deficit among male premutation carriers, though Stroop and Hayling analyses suggest a potential deficit in inhibitory control among a sub-set of male carriers. Certainly additional research is needed to further investigate inhibition among premutation carriers and identify the potential key factors involved in determining which individuals with the premutation are at greatest risk of exhibiting these inhibitory deficits, and if, indeed these are the same individuals most likely to develop FXTAS. Our exploratory analysis did not provide evidence for repeat size or age group to help define this high risk group. In contrast, other domains of executive function (e.g., working memory and cognitive flexibility) may not be as adversely affected in most premutation carriers and may only become discernable later in adulthood among premutation carriers who go on to develop FXTAS.

There are several limitations to this study presented here. First, each cohort was the product of independently initiated studies that varied in ascertainment methods and exclusion criteria. We accounted for potential confounders by adjusting models for variables that differed between cohorts (e.g. age) as well as an indicator variable for cohort, but there is the potential for additional effects not accounted for here. Second, we focused on measures of executive function that overlapped between the individual test batteries and, though the measures were identical across batteries with the exception of the Stroop task, there is the potential that the measures were administered differently within each cohort. Such differences could confound the results of this study. Lastly, we only used *FMR1* repeat

length as a predictor of executive function in this study, while additional molecular measures (e.g., *FMR1* mRNA level and FMRP production) might be more appropriate modifiers of the phenotypes.

Altogether, these findings highlight the need for future studies exploring specific executive functions among large samples of male premutation carriers. In addition, we hope that our collaboration will encourage more multi-site analyses in future studies, including the development of shared neuropsychological test batteries and ascertainment methods.

Acknowledgments

We would like to thank Drs. Michael Epstein and Karen Conneely for their assistance in the statistical analysis. We would also like to thank Drs. Darren Hocking and Emily Graves Allen for their comments on the final draft. Finally we would like to thank the study subjects who made this work possible. This work was supported by the Wellcome Trust and National Institutes of Child Health and Human Development grant R01 HD29909 and P30 HD24064, and the National Institute of Neurological Disorders and Stroke grant NS044299.

References

Allen EG, He W, Yadav-Shah M, Sherman SL. A study of the distributional characteristics of FMR1 transcript levels in 238 individuals. Hum Genet. 2004; 114(5):439–447. [PubMed: 14758538]

Bennetto L, Pennington BF, Porter D, Taylor AK, Hagerman RJ. Profile of cognitive functioning in women with the fragile X mutation. Neuropsychology. 2001; 15(2):290–299. [PubMed: 11324870]

Benton, AL.; Hamsher, KD. Multilingual Aphasia Examination. University of Iowa Press; Iowa City: 1976.

Brega AG, Goodrich G, Bennett RE, Hessl D, Engle K, Leehey MA, Bounds LS, Paulich MJ, Hagerman RJ, Hagerman PJ, Cogswell JB, Tassone F, Reynolds A, Kooken R, Kenny M, Grigsby J. The primary cognitive deficit among males with fragile X-associated tremor/ataxia syndrome (FXTAS) is a dysexecutive syndrome. J Clin Exp Neuropsychol. 2008:1–17.

Brown WT, Houck GE Jr. Jeziorowska A, Levinson FN, Ding X, Dobkin C, Zhong N, Henderson J, Brooks SS, Jenkins EC. Rapid fragile X carrier screening and prenatal diagnosis using a nonradioactive PCR test. Jama. 1993; 270(13):1569–1575. [PubMed: 8371467]

Brunberg JA, Jacquemont S, Hagerman RJ, Berry-Kravis EM, Grigsby J, Leehey MA, Tassone F, Brown WT, Greco CM, Hagerman PJ. Fragile X premutation carriers: characteristic MR imaging findings of adult male patients with progressive cerebellar and cognitive dysfunction. AJNR Am J Neuroradiol. 2002; 23(10):1757–1766. [PubMed: 12427636]

Burgess, P.; Shallice, T. The Hayling and Brixton Tests. Thames Valley Test Company; England: 1997.

Coffey SM, Cook K, Tartaglia N, Tassone F, Nguyen DV, Pan R, Bronsky HE, Yuhas J, Borodyanskaya M, Grigsby J, Doerflinger M, Hagerman PJ, Hagerman RJ. Expanded clinical phenotype of women with the FMR1 premutation. Am J Med Genet A. 2008; 146A(8):1009–1016. [PubMed: 18348275]

Cohen J. A power primer. Psychological Bulletin. 1992; 112(1):155–159. [PubMed: 19565683]

Cohen S, Masyn K, Adams J, Hessl D, Rivera S, Tassone F, Brunberg J, DeCarli C, Zhang L, Cogswell J, Loesch D, Leehey M, Grigsby J, Hagerman PJ, Hagerman R. Molecular and imaging correlates of the fragile X-associated tremor/ataxia syndrome. Neurology. 2006; 67(8):1426–1431. [PubMed: 17060569]

Cornish K, Turk J, Hagerman R. The fragile X continuum: new advances and perspectives. J Intellect Disabil Res. 2008; 52(Pt 6):469–482. [PubMed: 18444988]

Cornish KM, Li L, Kogan CS, Jacquemont S, Turk J, Dalton A, Hagerman RJ, Hagerman PJ. Age-dependent cognitive changes in carriers of the fragile X syndrome. Cortex. 2008; 44(6):628–636. [PubMed: 18472033]

Cronister A, Teicher J, Rohlfs EM, Donnenfeld A, Hallam S. Prevalence and instability of fragile X alleles: implications for offering fragile X prenatal diagnosis. Obstet Gynecol. 2008; 111(3):596–601. [PubMed: 18310361]

Dombrowski C, Levesque S, Morel ML, Rouillard P, Morgan K, Rousseau F. Premutation and intermediate-size FMR1 alleles in 10572 males from the general population: loss of an AGG interruption is a late event in the generation of fragile X syndrome alleles. Hum Mol Genet. 2002; 11(4):371–378. [PubMed: 11854169]

Feng Y, Zhang F, Lokey LK, Chastain JL, Lakkis L, Eberhart D, Warren ST. Translational suppression by trinucleotide repeat expansion at FMR1. Science. 1995; 268(5211):731–734. [PubMed: 7732383]

Fernandez-Carvajal I, Walichiewicz P, Xiaosen X, Pan R, Hagerman PJ, Tassone F. Screening for expanded alleles of the FMR1 gene in blood spots from newborn males in a Spanish population. J Mol Diagn. 2009; 11(4):324–329. [PubMed: 19460941]

Franke P, Leboyer M, Hardt J, Sohne E, Weiffenbach O, Biancalana VV, Cornillet-Lefebre P, Delobel B, Froster U, Schwab SG, Poustka F, Hautzinger M, Maier W. Neuropsychological profiles of FMR-1 premutation and full-mutation carrier females. Psychiatry Res. 1999; 87(2-3):223–231. [PubMed: 10579555]

Fu SD, Shen Y, Fan Y. [Unstable DNA sequence and methylation in fragile X syndrome]. Zhonghua Yi Xue Za Zhi. 1994; 74(10):611–614. 646–617. [PubMed: 7842338]

Fu YH, Kuhl DP, Pizzuti A, Pieretti M, Sutcliffe JS, Richards S, Verkerk AJ, Holden JJ, Fenwick RG, Warren ST, et al. Variation of the CGG repeat at the fragile X site results in genetic instability: resolution of the Sherman paradox. Cell. 1991; 67(6):1047–1058. [PubMed: 1760838]

Golden, C. Stroop Color and Word Test: A Manual for Clinical and Experimental Uses. Wood Dale, IL: 1978.

Grigsby J, Brega AG, Engle K, Leehey MA, Hagerman RJ, Tassone F, Hessl D, Hagerman PJ, Cogswell JB, Bennett RE, Cook K, Hall DA, Bounds LS, Paulich MJ, Reynolds A. Cognitive profile of fragile X premutation carriers with and without fragile X-associated tremor/ataxia syndrome. Neuropsychology. 2008; 22(1):48–60. [PubMed: 18211155]

Grigsby J, Brega AG, Leehey MA, Goodrich GK, Jacquemont S, Loesch DZ, Cogswell JB, Epstein J, Wilson R, Jardini T, Gould E, Bennett RE, Hessl D, Cohen S, Cook K, Tassone F, Hagerman PJ, Hagerman RJ. Impairment of executive cognitive functioning in males with fragile X-associated tremor/ataxia syndrome. Mov Disord. 2007; 22(5):645–650. [PubMed: 17266074]

Grigsby J, Kaye K, Robbins LJ. Reliabilities, norms and factor structure of the Behavioral Dyscontrol Scale. Percept Mot Skills. 1992; 74(3 Pt 1):883–892. [PubMed: 1608726]

Hagerman RJ, Leehey M, Heinrichs W, Tassone F, Wilson R, Hills J, Grigsby J, Gage B, Hagerman PJ. Intention tremor, parkinsonism, and generalized brain atrophy in male carriers of fragile X. Neurology. 2001; 57(1):127–130. [PubMed: 11445641]

Hashimoto RI, Backer KC, Tassone F, Hagerman RJ, Rivera SM. An fMRI study of the prefrontal activity during the performance of a working memory task in premutation carriers of the fragile X mental retardation 1 gene with and without fragile X-associated tremor/ataxia syndrome (FXTAS). J Psychiatr Res. 2010

Hatton DD, Sideris J, Skinner M, Mankowski J, Bailey DB Jr. Roberts J, Mirrett P. Autistic behavior in children with fragile X syndrome: Prevalence, stability, and the impact of FMRP. Am J Med Genet A. 2006

Heaton, R.; Cheline, G.; Talley, J.; Kay, G.; Curtiss, G. Wisconsin Card Sorting Test Manual: Revised and expanded. Psychological Assessment Resources; Odessa, FL: 1993.

Hunter JE, Abramowitz A, Rusin M, Sherman SL. Is there evidence for neuropsychological and neurobehavioral phenotypes among adults without FXTAS who carry the FMR1 premutation? A review of current literature. Genet Med. 2009; 11(2):79–89. [PubMed: 19265746]

Hunter JE, Allen EG, Abramowitz A, Rusin M, Leslie M, Novak G, Hamilton D, Shubeck L, Charen K, Sherman SL. No evidence for a difference in neuropsychological profile among carriers and noncarriers of the FMR1 premutation in adults under the age of 50. Am J Hum Genet. 2008; 83(6): 692–702. [PubMed: 19026394]

Jacquemont S, Hagerman RJ, Leehey M, Grigsby J, Zhang L, Brunberg JA, Greco C, Des Portes V, Jardini T, Levine R, Berry-Kravis E, Brown WT, Schaeffer S, Kissel J, Tassone F, Hagerman PJ. Fragile X premutation tremor/ataxia syndrome: molecular, clinical, and neuroimaging correlates. Am J Hum Genet. 2003; 72(4):869–878. [PubMed: 12638084]

Jacquemont S, Hagerman RJ, Leehey MA, Hall DA, Levine RA, Brunberg JA, Zhang L, Jardini T, Gane LW, Harris SW, Herman K, Grigsby J, Greco CM, Berry-Kravis E, Tassone F, Hagerman PJ. Penetrance of the fragile X-associated tremor/ataxia syndrome in a premutation carrier population. Jama. 2004; 291(4):460–469. [PubMed: 14747503]

Jacquemont S, Leehey MA, Hagerman RJ, Beckett LA, Hagerman PJ. Size bias of fragile X premutation alleles in late-onset movement disorders. J Med Genet. 2006

Kenneson A, Zhang F, Hagedorn CH, Warren ST. Reduced FMRP and increased FMR1 transcription is proportionally associated with CGG repeat number in intermediate-length and premutation carriers. Hum Mol Genet. 2001; 10(14):1449–1454. [PubMed: 11448936]

Knight SJ, Flannery AV, Hirst MC, Campbell L, Christodoulou Z, Phelps SR, Pointon J, Middleton-Price HR, Barnicoat A, Pembrey ME, et al. Trinucleotide repeat amplification and hypermethylation of a CpG island in FRAXE mental retardation. Cell. 1993; 74(1):127–134. [PubMed: 8334699]

Leehey MA, Berry-Kravis E, Goetz CG, Zhang L, Hall DA, Li L, Rice CD, Lara R, Cogswell J, Reynolds A, Gane L, Jacquemont S, Tassone F, Grigsby J, Hagerman RJ, Hagerman PJ. FMR1 CGG repeat length predicts motor dysfunction in premutation carriers. Neurology. 2008; 70(16 Pt 2):1397–1402. [PubMed: 18057320]

Leehey MA, Munhoz RP, Lang AE, Brunberg JA, Grigsby J, Greco C, Jacquemont S, Tassone F, Lozano AM, Hagerman PJ, Hagerman RJ. The fragile X premutation presenting as essential tremor. Arch Neurol. 2003; 60(1):117–121. [PubMed: 12533098]

Meadows KL, Pettay D, Newman J, Hersey J, Ashley AE, Sherman SL. Survey of the fragile X syndrome and the fragile X E syndrome in a special education needs population. Am J Med Genet. 1996; 64(2):428–433. [PubMed: 8844098]

Moore CJ, Daly EM, Schmitz N, Tassone F, Tysoe C, Hagerman RJ, Hagerman PJ, Morris RG, Murphy KC, Murphy DG. A neuropsychological investigation of male premutation carriers of fragile X syndrome. Neuropsychologia. 2004; 42(14):1934–1947. [PubMed: 15381024]

Oberle I, Rousseau F, Heitz D, Kretz C, Devys D, Hanauer A, Boue J, Bertheas MF, Mandel JL. Instability of a 550-base pair DNA segment and abnormal methylation in fragile X syndrome. Science. 1991; 252(5010):1097–1102.

Peprah E, He W, Allen E, Oliver T, Boyne A, Sherman SL. Examination of FMR1 transcript and protein levels among 74 premutation carriers. J Hum Genet. 2010; 55(1):66–68. [PubMed: 19927162]

Pieretti M, Zhang FP, Fu YH, Warren ST, Oostra BA, Caskey CT, Nelson DL. Absence of expression of the FMR-1 gene in fragile X syndrome. Cell. 1991; 66(4):817–822. [PubMed: 1878973]

Primerano B, Tassone F, Hagerman RJ, Hagerman P, Amaldi F, Bagni C. Reduced FMR1 mRNA translation efficiency in fragile X patients with premutations. Rna. 2002; 8(12):1482–1488. [PubMed: 12515381]

Sevin M, Kutalik Z, Bergman S, Vercelletto M, Renou P, Lamy E, Vingerhoets FJ, Di Virgilio G, Boisseau P, Bezieau S, Pasquier L, Rival JM, Beckmann JS, Damier P, Jacquemont S. Penetrance of marked cognitive impairment in older male carriers of the FMR1 gene premutation. J Med Genet. 2009; 46(12):818–824. [PubMed: 19542082]

Sherman SL. Premature ovarian failure in the fragile X syndrome. Am J Med Genet. 2000; 97(3):189–194. [PubMed: 11449487]

Sutcliffe JS, Nelson DL, Zhang F, Pieretti M, Caskey CT, Saxe D, Warren ST. DNA methylation represses FMR-1 transcription in fragile X syndrome. Hum Mol Genet. 1992; 1(6):397–400. [PubMed: 1301913]

Tassone F, Hagerman RJ, Taylor AK, Gane LW, Godfrey TE, Hagerman PJ. Elevated levels of FMR1 mRNA in carrier males: a new mechanism of involvement in the fragile-X syndrome. Am J Hum Genet. 2000; 66(1):6–15. [PubMed: 10631132]

Tassone F, Hagerman RJ, Taylor AK, Mills JB, Harris SW, Gane LW, Hagerman PJ. Clinical involvement and protein expression in individuals with the FMR1 premutation. Am J Med Genet. 2000; 91(2):144–152. [PubMed: 10748416]

Till SM. The developmental roles of FMRP. Biochem Soc Trans. 2010; 38(2):507–510. [PubMed: 20298211]

Toledano-Alhadef H, Basel-Vanagaite L, Magal N, Davidov B, Ehrlich S, Drasinover V, Taub E, Halpern GJ, Ginott N, Shohat M. Fragile-X carrier screening and the prevalence of premutation and full-mutation carriers in Israel. Am J Hum Genet. 2001; 69(2):351–360. [PubMed: 11443541]

Trenerry, M.; Crosson, B.; Deboe, J.; Leber, W. Stroop Neuropsychological Screening Test. Psychological Assessment Resources; Odessa, FL: 1989.

Verkerk AJ, Pieretti M, Sutcliffe JS, Fu YH, Kuhl DP, Pizzuti A, Reiner O, Richards S, Victoria MF, Zhang FP, et al. Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. Cell. 1991; 65(5):905–914. [PubMed: 1710175]

Wang Q, Green E, Bobrow M, Mathew CG. A rapid, non-radioactive screening test for fragile X mutations at the FRAXA and FRAXE loci. J Med Genet. 1995; 32(3):170–173. [PubMed: 7783163]

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

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

Wechsler, D. Wechsler abbreviated scale of intelligence. The Psychological Corporation; San Antonio, TX: 1999.

Table 1

Details on three cohorts included in the collaboration.

Cohort	Citation	Recruitment	Inclusion	Exclusion
1	Cornish et al. 2008	United Kingdom Clinical Genetics Services and Fragile X Society	Age 18-50 English-speaking	IQ less than 85, stroke, dementia
2	Hunter et al. 2008	FXS conferences and support groups, word of mouth, sports events, health fairs, churches	Age 18-50 English-speaking	Active substance abuse/addiction, head trauma with loss of consciousness, meningitis, oxygen deprivation, Multiple Sclerosis, Amyotrophic Lateral Sclerosis, Parkinson's disease, severe chronic fatigue syndrome, complicated migraines (temporary stroke-like effects) with progressive memory impairment, stroke or brain hemorrhage, brain surgery, seizure disorder, schizophrenia, active use of sedating medications
3	Grigsby et al. 2008, Brega et al. 2008	FXS study subjects at University of Colorado Denver and University of California Davis, FXS support groups, employees at CU Denver and Health Sciences Center	Over age 40 English-speaking	Significant speech/language deficits that make participation impossible, head trauma with loss of consciousness, epilepsy, demyelinating disease, family history of non-FXS genetic neurologic disorder, other neurologic disorders (e.g., stroke), complex medical comorbidities that may affect cognition, contraindications for MRI scanning (e.g., pacemaker, metal in body), schizophrenia, manic episodes, psychotic depression, alcoholism or significant drug use, medications affecting cognition

FXS=Fragile X Syndrome

Table 2

Demographic information for individual cohorts and the combined sample.

Cohort	NC (<55 repeats)					PM (≥55)				
	N	Median CGG (Q1,Q3)	Mean age (SD)	Education (% higher education)	Mean IQ	N	Median CGG (Q1,Q3)	Mean age (SD)	Education (% higher education)	Mean IQ
1	70	30 (29,31)	44.5 (14.4)^{ab}	45.7^{abd}	111.7 (11.8)^{bd}	30	106 (79,124)^{b}	43.7 (14.7)^{ab}	20.0^{abd}	104.9 (13.7)^{bd}
2	104	30 (30,41)	36.0 (9.1)^{ac}	82.7^{a}	110.4 (13.4)^{c}	34	88 (77,110)	35.4 (10.0)^{ac}	70.6^{a}	112.3 (15.6)
3	42	30 (29,33)	64.48 (3.0)^{bcd}	87.8^{b}	119.7 (14.5)^{bc}	36	77 (71,94)^{b}	59.3 (9.2)^{bcd}	88.6^{b}	114.8 (14.0)^{b}
All	216	30 (29,33)	44.3 (15.6)	71.6	112.6 (13.6)	100	88 (73,112)	46.5 (15.2)	61.6	110.9 (14.9)

NC=non-carriers; PM=premutation carriers; Q1: lower quartile; Q3: upper quartile; SD=standard deviation

^{a}Among cohorts, cohorts 1 and 2 significantly differ ($p<0.05$)

^{b}Among cohorts, cohorts 1 and 3 significantly differ ($p<0.05$)

^{c}Among cohorts, cohorts 2 and 3 significantly differ ($p<0.05$)

^{d}Within cohorts, NC and PM groups significantly differ ($p<0.05$)

Table 3

FMR1 repeat length as a predictor of executive function measures: categorical (non-carrier vs. premutation carrier) and linear models (repeat length as a continuous variable). Models adjusted for age, education, and cohort.

	Categorical Models				Linear Models		
	Least Squares Means		Repeat group partial R^2	p-value	Repeat length standardized β estimates	Repeat length partial R^2	p-value
Measure	NC	PM					
COWAT	41.56	39.45	0.01	0.16	−0.05	<0.01	0.41
Stroop	63.52	59.66	0.02	0.01	−0.06	0.02	0.02
Backward digit span	7.32	7.06	<0.01	0.41	−0.10	0.01	0.08
Letter-number sequencing	11.68	11.34	<0.01	0.31	−0.06	<0.01	0.27
Hayling	1.33	2.43	0.12	<0.01	0.36	0.12	<0.01
WCST	1.94	2.01	<0.01	0.54	0.07	0.01	0.38
BDS	20.57	18.84	0.07	0.03	−0.16	0.03	0.18

NC=non-carrier; PM=premutation carrier; COWAT=Controlled Oral Word Association Test; WCST=Wisconsin Card Sorting Test; BDS=Behavioral Dyscontrol Scale

Table 4

Percentage of *FMR1* premutation allele carriers with low scores on executive function measures, defined as scoring greater than one standard deviation away from the mean. Logistic regression models adjusted for age, education and cohort.

Measure	Repeat Group		Logistic Regression	
	NC	PM	Odds Ratio (95% CI)	p-value
COWAT	13.5%	22.0%	0.68 (0.36,1.31)	0.25
Stroop	8.7%	9.3%	1.36 (0.55,3.36)	0.51
Backward Digit Span	12.1%	13.3%	1.20 (0.56,2.56)	0.64
Letter-Number Sequencing	9.0%	14.7%	0.81 (0.37,1.79)	0.61
Hayling	22.9%	10.0%	2.25 (0.57,8.95)	0.25
WCST	11.0%	15.6%	0.69 (0.22,2.22)	0.53
BDS	9.5%	27.8%	0.32 (0.08,1.22)	0.10

NC=non-carrier; PM=premutation carrier; CI=confidence interval; COWAT=Controlled Oral Word Association Test; WCST=Wisconsin Card Sorting Test; BDS=Behavioral Dyscontrol Scale

Neuropsychology. Author manuscript; available in PMC 2013 March 1.

Table 5

Executive function measures stratified by *FMR1* CGG repeat length and age group: sample sizes and mean scores.

Repeat Group (# CGGs)	**NC: <55**			**Low PM: 55-70**			**Mid PM: 71-100**			**High PM: >100**		
Age Group (years)	**<30**	**30-49**	**≥50**	**<30**	**30-49**	**≥50**	**<30**	**30-49**	**≥50**	**<30**	**30-49**	**≥50**
N	41	99	73	6	5	7	6	18	25	6	16	10
COWAT	40.60	43.91	40.12	47.78	47.01	33.16	36.25	41.42	35.37	38.91	44.02	32.27
Stroop	76.58	68.47	61.87	80.93	59.47	58.47	70.86	70.02	58.99	76.14	65.00	44.50
Backward digit span	7.85	7.49	7.03	10.24	5.87	5.86	8.51	7.61	6.50	5.39	7.59	5.10
Letter-number sequencing	12.11	11.98	11.16	13.73	10.26	9.64	11.85	13.08	10.48	10.81	12.00	9.01

NC=non-carrier; PM=premutation carrier; COWAT=Controlled Oral Word Association Test