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Jessica Ezzell Hunter Date

Genetic epidemiology of phenotypes associated with *FMR1* premutation alleles

by

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An abstract of a dissertation submitted to the Faculty of the Graduate School of Emory University in partial fulfillment of the requirements for the degree of Doctor of Philosophy

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Abstract

Genetic epidemiology of phenotypes associated with *FMR1* premutation alleles

by Jessica Ezzell Hunter

The 5' untranslated region of the fragile X mental retardation gene, *FMR1*, contains a highly polymorphic CGG repeat. The most common alleles contain 40 repeats or less. Rare expansions of this repeat are associated with a spectrum of disorders. Repeats of 200 or more, termed full mutation alleles, are associated with hypermethylation and subsequent loss of expression of the *FMR1* gene. The loss of *FMR1* expression results in fragile X syndrome (FXS), the most common inherited mental retardation syndrome. Repeats of 55 to 199, termed premutation alleles, are associated with varying levels of transcript and protein product. Phenotypes known to be associated with premutation alleles include a tremor-ataxia syndrome (FXTAS), affecting roughly 30% of male carriers over the age of 50, and primary ovarian insufficiency (FXPOI), affecting roughly 20% of carrier females. Any global neuropsychological and neurobehavioral impact of carrying a premutation allele has been unclear in adult carriers under the age of 50. This dissertation presents research focused on determining phenotypes associated with premutation alleles among males and females in the largest study population to date in order to ask the question: in the absence of FXTAS or perhaps before the onset of FXTAS, what is the neuropsychological and/or neurobehavioral impact of carrying a premutation allele among younger adults? Results of these studies indicate subtle phenotypes associated with premutation alleles among

males and females, including increased symptoms associated with depression and self-concept as well as increased inattention. However, these results find no evidence for an increased risk of any clinical disorder. Lastly, in order to determine the extent to which background genetics might be involved in the variable penetrance of phenotypes associated with premutation alleles, the first study analyzing familial aggregation of FXPOI was performed. Results of this study showed significant familial aggregation of age at menopause, a proxy for ovarian function, after adjustment for *FMR1* genotype. In future studies, this methodology can also be applied to the other phenotype known to be associated with premutation alleles, FXTAS.

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Table of Contents

Tables and Figures

Chapter 2.

Chapter 3.

Chapter 4.

Chapter 5.

Chapter 1

Introduction to *FMR1* and *FMR1*-related phenotypes

The fragile X mental retardation 1 gene (*FMR1*; OMIM 30955) is located on the long arm of the X chromosome (Xq27.3). *FMR1* was identified in 1991 as the gene responsible for fragile X syndrome (FXS; OMIM 300624) (Verkerk et al., 1991). Individuals with FXS were shown to carry abnormal triplet expansions that led to the hypermethylation of the gene (Kremer et al., 1991; Oberle et al., 1991; Verkerk et al., 1991). *FMR1* codes for an RNA binding protein, fragile X mental retardation protein (FMRP), which functions in translational regulation at neuron synapses and is required for proper neurological development (Garber et al., 2008). Dynamic mutations in this gene are responsible for an array of molecular and phenotypic consequences.

Structure and Function of *FMR1*

FMR1 is 38 kilobases in length, consists of 17 exons, and codes for a 4.4 kilobase mRNA (Eichler et al., 1993). The 5' untranslated region (5' UTR) of exon 1 contains a highly polymorphic CGG repeat which is interspersed with single AGG interruptions at roughly every 10 repeats (Kunst & Warren, 1994). Located 250 basepairs upstream of CGG repeat is a CpG island that when methylated, silences the gene (Eichler et al., 1993). *FMR1* is expressed in many fetal and adult tissues including brain and testes (Abitbol et al., 1993).

FMR1 codes for an RNA-binding protein, FMRP, which plays a role in translation of various transcripts in dendrites of neurons (Ashley et al., 1993a). FMRP associates with polyribosomes to suppress translation of the mRNAs bound (Li et al., 2001). FMRP binds to transcripts via specific tertiary structures (Darnell et al., 2001; Darnell et al., 2005) and regulates translation of proteins associated with neuronal development, including FMRP itself (Brown et al., 2001). In 2004, Bear et al. proposed the 'mGluR theory' which proposed that FMRP acts in response to a metabotropic glutamate receptor (mGluR) (Bear et al., 2004).

FMR1 Allele Classification

The most common alleles of *FMR1* contain less than 40 repeats and are stably inherited across generations (Snow et al., 1993). In rare cases, these repeats can become unstable and expand (Fu et al., 1991). Based on the risk of these CGG repeat expansions, allelic groups have been characterized. Repeat length cutoffs for each group are not well-defined and tend to vary across research studies. However, the American College of Medical Genetics (ACMG) has published clinical guidelines defining allelic classes which will be discussed below (Maddalena et al., 2001).

Alleles with repeats of 200 or longer, termed full mutation alleles, are associated with FXS. These alleles have an estimated prevalence of about 1/2500 to 1/4000 among males and females in the general population (Turner et al., 1996; Crawford et al., 1999; Pesso et al., 2000; Toledano-Alhadef et al., 2001).

Alleles in the range of 55 to 199, termed premutation alleles, are meiotically unstable across generations (Fu et al., 1991; Oberle et al., 1991). The definition of this allelic range arose not from the presence of a particular clinical phenotype, but from the risk of expanding to a full mutation allele in a single generation with maternal transmission. Prevalence rates have been estimated at roughly 1/800 in males and roughly 1/250 in females (Rousseau et al., 1995; Dombrowski et al., 2002).

Alleles in the range of 45 to 54 repeats, termed intermediate or grey zone alleles, may or may not be unstable when inherited across generations (Nolin et al., 2003). If unstable, at least two generations would be required to expand to a full mutation allele.

FMR1 CGG Repeat Instability

Premutation alleles can expand to larger premutation alleles or to full mutation alleles. Only maternal alleles are at risk of expansion from a premutation to a full mutation allele (Yu et al., 1992; Snow et al., 1993). This lack of paternal inheritance is due to the inability of sperm to tolerate the full mutation expansion (Malter et al., 1997; Ashley-Koch et al., 1998). The risk of a premutation allele expanding to a full mutation in the next generation increases with increasing repeat length (Yu et al., 1992; Snow et al., 1993; Sherman et al., 1996). The shortest premutation allele known to expand to a full mutation in one generation is 59 (Nolin et al., 2003). Other factors, such as presence and location of AGG interruptions also affect instability of alleles (Crawford et al., 2000; Weisman-Shomer et al., 2000; Sullivan et al., 2002). For example, AGG interruptions in

most premutation alleles are only located in the 5' region with the 3' end consisting only of CGG repeats (Eichler et al., 1994; Crawford et al., 2000).

FMR1 Full Mutation Alleles

Molecular Correlates

Full mutation alleles are hypermethylated resulting in the loss of FMRP expression (Oberle et al., 1991; Pieretti et al., 1991). Specifically, hypermethylation of the CpG island in the promoter region upstream of the CGG repeat results in the loss of *FMR1* transcription early in embryogenesis (Sutcliffe et al., 1992). This loss of FMRP is responsible for phenotypes associated with FXS. There are exceptions where full mutation alleles demonstrate varying levels of abnormal methylation. Incomplete methylation allows some expression of FMRP, which results in milder presentation of phenotypes associated with methylated full mutation alleles (McConkie-Rosell et al., 1993; Tassone et al., 1999). In addition, methylated full mutation alleles can still produce residual levels of transcript (Tassone et al., 2001). However, studies have shown that these transcripts cannot be translated efficiently (Feng et al., 1995; Primerano et al., 2002), which is consistent with the absence of FMRP production.

Fragile X Syndrome

The loss of FMRP results in fragile X syndrome (FXS) (Ashley et al., 1993b), one of the most common forms of inherited mental retardation. FXS was the first genetic disease identified as being caused by a repeat instability. The vast majority of cases,

roughly 98%, are the result of carrying a full mutation allele. However, other mutations of the *FMR1* gene that results in the loss of FMRP expression are known to cause FXS, including missense and splice site mutations as well as deletions (e.g.(Gedeon et al., 1992; De Boulle et al., 1993; Coffee et al., 2008)). Average age of diagnosis is typically around 36 months of age. The most common clinical manifestation of FXS is mild to severe intellectual disability (Fisch et al., 2002). The cognitive profile includes deficits with short-term memory, particularly with complex information, visuospatial skills, and speech (Cornish et al., 2004). The clinical presentation is variable with the most common features being hyperactivity, attention deficits, and autistic behaviors (Cornish et al., 2004). Physical features are typically subtle and may include macroorchidism, long faces, large ears, and a prominent jaw. Females carrying a full mutation are typically more mildly affected due to the X-linked nature of the gene (Bennetto et al., 2001). In addition, mosaic males and carriers of unmethylated full mutations can also produce residual levels of FMRP, resulting in a milder phenotype (Kaufmann et al., 1999; Loesch et al., 2004).

FMR1 **Premutation Alleles**

Molecular Correlates

In contrast to full mutation alleles, premutation alleles remain unmethylated and are transcribed. Thus transcripts are available for translation to produce the protein product, FMRP. However, the processes of transcription and translation are altered in carriers of premutation alleles. First, transcript levels are elevated across the premutation

repeat length range (Tassone et al., 2000a; Tassone et al., 2000b; Kenneson et al., 2001; Primerano et al., 2002; Allen et al., 2004; Garcia-Alegria et al., 2007; Tassone et al., 2007b). Allen et al. (2004) reported significant linear relationships between premutation repeat length and mRNA levels in a large sample of males and females. Most recently, Garcia-Alegria et al. (2007) reported a non-linear association between repeat length and transcript levels, with the highest levels of transcript being associated with premutation alleles greater than 100 (Garcia-Alegria et al., 2007). The increase in transcript has been demonstrated to be due to increased transcription (Tassone et al., 2007b) rather than an increase in transcript stability (Tassone et al., 2000b). In addition, repeat expansions in the premutation range are associated with an upstream shift in transcription initiation (Beilina et al., 2004). However, despite increased levels of transcripts in the premutation range, protein levels are reduced (Tassone et al., 2000b; Tassone et al., 2000c; Kenneson et al., 2001; Primerano et al., 2002). This reduction in FMRP is due to reduced translation efficiency of the mRNA containing the expanded repeat (Feng et al., 1995; Primerano et al., 2002; Chen et al., 2003).

Phenotypes Associated with Premutation Alleles

After the identification of *FMR1* and the discovery that premutation alleles remain unmethylated, and thus are expressed, carriers were assumed to be unaffected clinically. However, premutation alleles are now associated with the risk of development of two distinct clinical disorders: fragile X-associated tremor/ataxia syndrome (FXTAS; OMIM 300623) and fragile X-associated primary ovarian insufficiency (FXPOI) (Sherman, 2000; Hagerman et al., 2001). These disorders are not present in carriers of

hypermethylated full mutation alleles, and are thus not likely due to reduced levels of FMRP. Currently, FXTAS has been suggested to be due, either directly or indirectly, to a toxic gain-of-function of the transcripts containing the expanded premutation length repeats (Hagerman & Hagerman, 2004). However, the penetrance of FXTAS and FXPOI is not complete in that not all carriers of premutation alleles are affected (Sherman, 2000; Jacquemont et al., 2004). This is likely due to a complex etiology and the potential involvement of background genetics warrants investigation.

FXTAS in Male Carriers of Premutation Allele

In 2001 a progressive intention tremor disorder associated with cognitive deficits and global brain atrophy was described in five older males who carried premutation alleles with repeats ranging from 78 to 98 (Hagerman et al., 2001). Further study has revealed a characteristic clinical presentation among males with FXTAS, primarily a late-onset progressive gait ataxia and/or intention tremor with other associated features including neuropsychological decline, psychiatric symptoms, and autonomic dysfunction (Jacquemont et al., 2003; Hessl et al., 2005; Bacalman et al., 2006; Bourgeois et al., 2007; Grigsby et al., 2007; Allen et al., 2008; Brega et al., 2008). Cognitive deficits are variable and likely are due to an initial impairment in executive functioning (Brega et al., 2008). Patients with FXTAS also have characteristic radiological findings, mainly increased bilateral signal intensities of the middle cerebellar peduncles on T2-weighted MRI (Brunberg et al., 2002; Jacquemont et al., 2003; Cohen et al., 2006). Greco et al. (2002) was the first to report the presence of intranuclear inclusions in neurons and astrocytes in post-mortem brain tissue of males affected by FXTAS (Greco et al., 2002).

These inclusions contain *FMR1* mRNA (Tassone et al., 2004) as well as a large number of other transcripts (Iwahashi et al., 2006). Inclusions have also been reported in other tissues such as pituitary and testicular tissue from men with FXTAS (Greco et al., 2007).

FXTAS is a late-onset disorder, typically affecting a subset of premutation males over the age of 50 (Jacquemont et al., 2004; Allen et al., 2008). In a retrospective analysis of 55 men affected by FXTAS, motor dysfunction presented first with a median age of onset of 60 (Leehey et al., 2007). One epidemiological study of 40 men carrying premutation alleles and 59 men with unexpanded alleles from families with a history of FXS indicated a 30% risk of developing FXTAS for male premutation carriers over the age of 50 with the risk increasing with increasing age (Jacquemont et al., 2004). Analyses of potential associations between premutation allele repeat length and onset of FXTAS has demonstrated that the vast majority (85%) of male carriers with FXTAS had premutation alleles with 70 repeats or more (Jacquemont et al., 2006) while longer repeat lengths likely predict earlier onset of motor symptoms (Tassone et al., 2007a). In a more recent study, Allen et al. (2008) demonstrated that males over the age of 50 with repeat lengths less than 70 had a lifetime prevalence of ataxia and tremor of 20% and 30%, respectively, while males with repeat lengths in the higher premutation range had a lifetime prevalence of ataxia and tremor of 50% and 45%, respectively (Allen et al., 2008).

The excess levels of expanded transcript have a toxic effect on neurons leading to FXTAS (Hagerman & Hagerman, 2004). Jin et al. (2003) demonstrated that the expanded CGG alone was sufficient to cause neurodegeneration in *Drosophila* models (Jin et al., 2003). More recently RNA-binding proteins have been shown to be

sequestered by their interaction with the expanded CGG repeat, thus preventing their normal function (Jin et al., 2007; Sofola et al., 2007). One likely model would involve sequestration of proteins that interact with the 5'UTR of the transcript. *FMR1* transcript has also been detected in nuclear inclusions seen in neurons of patients with FXTAS (Tassone et al., 2004) as have at least one CGG-interacting protein (Jin et al., 2007). Variability in this phenotypic expression among premutation carriers is likely due to background genetics or environmental factors.

FXTAS in Female Carriers of Premutation Alleles

FXTAS is more prevalent in males who carry premutation alleles, but has also been reported in females (Hagerman et al., 2004; Zuhlke et al., 2004; Berry-Kravis et al., 2005). The decreased prevalence in females is likely due to the presence of a second X chromosome carrying an unexpanded *FMR1* allele (Berry-Kravis et al., 2005; Jacquemont et al., 2005). As seen in males, intranuclear inclusions have been detected in port-mortem brain tissues of females affected by FXTAS (Hagerman et al., 2004) however, clinical manifestations and radiological findings are milder (Adams et al., 2007).

FXPOI

Among women, premutation alleles are associated with a spectrum of impaired ovarian function. Previous studies have reported that roughly 20% of women who carry a premutation allele develop premature ovarian failure (POF), or the cessation of menses before the age of 40 (Sherman, 2000). This is a relative risk of about 20-fold over that

seen in the general population. In addition, among carriers still cycling, elevated follicle stimulated hormone (FSH) levels have been detected, indicating reduced ovarian function (Hundscheid et al., 2001; Welt et al., 2004). More recently, Rohr et al. (2008) provided evidence that a reduced level of Anti-Mullerian hormone (AMH) is an earlier indicator of early ovarian decline (Rohr et al., 2008). Premutation women have been predicted to have on average a five year earlier age at menopause compared to women from the general population (Murray et al., 2000; Sullivan et al., 2005). However, the diagnosis of POF does not account for all variability in ovarian function among women who carry a premutation, thus recently this phenotype was changed to fragile X-associated primary ovarian insufficiency (FXPOI) (Abrams, 2007; Welt, 2008).

Though all carriers of premutation alleles are at risk of ovarian insufficiency, analysis of repeat length association between ovarian function and premutation alleles has demonstrated a non-linear association. Several studies have provided evidence that the most 'at risk' population is women with premutation alleles in the range of about 80 to 99 (Sullivan et al., 2005; Ennis et al., 2006; Allen et al., 2007).

Other Phenotypes

Neuropsychological and neurobehavioral phenotypes in addition to those co-occurring with movement problems in FXTAS have been analyzed in numerous studies with conflicting results. Thus any global cognitive or emotional impact of carrying a premutation allele in adults not affected by FXTAS is currently unclear. The results of these studies and potential reasons for the conflicting results will be discussed at length in Chapter 2.

Family and Maternal Stress Associated with Premutation Alleles

In addition to the molecular and biological associations with premutation alleles, it is important to keep in mind the environment that premutation carriers live in. Specifically, premutation females are at risk of having a child with FXS which could have psychosocial consequences. Several studies have reported significant associations with the child's behavior and maternal stress (Johnston et al., 2003; Abbeduto et al., 2004; Bailey et al., 2008). In addition, Hall et al. (2007) demonstrated that behavior issues from children in the household without FXS have an equal impact on maternal distress, suggesting an additive effect of behavior problems from children with and without FXS on maternal distress (Hall et al., 2007). In addition, family relationships have been reported to have an impact on maternal well-being (Johnston et al., 2003; Abbeduto et al., 2004; Bailey et al., 2008). However, mothers of children with FXS have reported increased family conflicts compared to mothers of children with other developmental disorders (Lewis et al., 2006).

Summary

Expansions of the CGG repeat contained in the 5' UTR of *FMR1* are associated with a spectrum of molecular aberrations and phenotypic consequences. Full mutation alleles, with repeats of 200 or longer, are associated with hypermethylation of *FMR1* and the subsequent loss of gene expression (Oberle et al., 1991; Pieretti et al., 1991; Sutcliffe et al., 1992). The loss of the protein product, FMRP, results in fragile X syndrome, the

most common inherited mental retardation disorder (Ashley et al., 1993b). Premutation alleles, with repeats in the range of about 55 to 199, remain unmethylated but are associated with increased levels of transcript and decreased levels of protein (Tassone et al., 2000a; Tassone et al., 2000b; Kenneson et al., 2001; Allen et al., 2004). These alleles are associated with increased risks of a late-onset movement disorder, FXTAS, and primary ovarian insufficiency, FXPOI (Sherman, 2000; Hagerman et al., 2001).

Focus of This Dissertation

The work presented here is aimed towards the study of phenotypes associated with premutation alleles in addition to FXTAS and FXPOI. Specifically, neuropsychological and neurobehavioral phenotypes among younger adult male and female premutation carriers are analyzed. Further, the background genetics that might contribute to the variable expression of one phenotype associated with premutation alleles, FXPOI, is quantified.

Chapter 2 is a literature review summarizing the history of studies analyzing premutation associations with neuropsychological and neurobehavioral phenotypes among adults under the age of 50. Pitfalls in study design and inconsistent results are discussed emphasizing the need for further research. My contributions to this work included the literature search, development of the inclusion criteria, compilation of the data from the studies included in the review, interpretation of the findings, as well as manuscript preparation and publication. This work has been accepted for publication (Hunter et al., 2008a).

Chapter 3 presents work analyzing premutation allele associations with neurobehavioral phenotypes among adult males and females under the age of 50. My contributions to this work include the selection of outcome variables for analysis, data clean-up, variable coding, identification and application of the correlated data analysis for repeat length associations, formation of the cluster structures, follow-up contact with all participants to identify those with a FXS child, formation of a strategy to adjustment for multiple testing, interpretation of findings, follow-up contact with refusals, as well as manuscript preparation and publication. This work has been published (Hunter et al., 2008b).

Chapter 4 presents work analyzing *FMR1* repeat length associations with neuropsychological phenotypes among young adult males and females. My contributions to this work include the selection of outcome variables for analysis, data clean-up, variable coding, imputation of missing data, application of principal component analysis and confirmatory factor analyses, identification and application of the correlated data analysis for repeat length associations, formation of the cluster structures, follow-up contact with all participants to identify those with a FXS child, formation of a strategy to adjustment for multiple testing, interpretation of findings, follow-up contact with refusals, as well as manuscript preparation and publication. This work has been accepted for publication (Hunter et al., 2008c).

Chapter 5 presents a study utilizing a statistical method to analyze familial aggregation of FXPOI, one of the phenotypes known to be associated with *FMR1* premutation alleles. This method quantifies the contribution of background genetics to variable age at menopause among female carriers of premutation alleles. My

contributions to this work included the selection of the statistical approach, data clean-up, pedigree coding and structure, variable coding, implementation of the familial aggregation models, interpretation of the findings, as well as manuscript preparation and publication. This work has been published (Hunter et al., 2008d).

References

Abbeduto L, Seltzer MM, Shattuck P, Krauss MW, Orsmond G, Murphy MM. 2004. Psychological well-being and coping in mothers of youths with autism, Down syndrome, or fragile X syndrome. *Am J Ment Retard 109*:237-254.

Abitbol M, Menini C, Delezoide AL, Rhyner T, Vekemans M, Mallet J. 1993. Nucleus basalis magnocellularis and hippocampus are the major sites of FMR-1 expression in the human fetal brain. *Nat Genet 4*:147-153.

Abrams L. 2007. From POF to POI: Evolution of a Term. *National Fragile X Foundation Quarterly 29*.

Adams JS, Adams PE, Nguyen D, Brunberg JA, Tassone F, Zhang W, Koldewyn K, Rivera SM, Grigsby J, Zhang L, DeCarli C, Hagerman PJ, Hagerman RJ. 2007. Volumetric brain changes in females with fragile X-associated tremor/ataxia syndrome (FXTAS). *Neurology 69*:851-859.

Allen EG, He W, Yadav-Shah M, Sherman SL. 2004. A study of the distributional characteristics of FMR1 transcript levels in 238 individuals. *Hum Genet 114*:439-447.

Allen EG, Juncos J, Letz R, Rusin M, Hamilton D, Novak G, Shubeck L, Tinker SW, Sherman SL. 2008. Detection of early FXTAS motor symptoms using the CATSYS computerised neuromotor test battery. *J Med Genet 45*:290-297.

Allen EG, Sullivan AK, Marcus M, Small C, Dominguez C, Epstein MP, Charen K, He W, Taylor KC, Sherman SL. 2007. Examination of reproductive aging milestones among women who carry the FMR1 premutation. *Hum Reprod 22*:2142-2152.

Ashley-Koch AE, Robinson H, Glicksman AE, Nolin SL, Schwartz CE, Brown WT, Turner G, Sherman SL. 1998. Examination of factors associated with instability of the FMR1 CGG repeat. *Am J Hum Genet 63*:776-785.

Ashley CT, Jr., Wilkinson KD, Reines D, Warren ST. 1993a. FMR1 protein: conserved RNP family domains and selective RNA binding. *Science 262*:563-566.

Ashley CT, Sutcliffe JS, Kunst CB, Leiner HA, Eichler EE, Nelson DL, Warren ST. 1993b. Human and murine FMR-1: alternative splicing and translational initiation downstream of the CGG-repeat. *Nat Genet 4*:244-251.

Bacalman S, Farzin F, Bourgeois JA, Cogswell J, Goodlin-Jones BL, Gane LW, Grigsby J, Leehey MA, Tassone F, Hagerman RJ. 2006. Psychiatric phenotype of the fragile X-associated tremor/ataxia syndrome (FXTAS) in males: newly described fronto-subcortical dementia. *J Clin Psychiatry 67*:87-94.

Bailey DB, Jr., Sideris J, Roberts J, Hatton D. 2008. Child and genetic variables associated with maternal adaptation to fragile X syndrome: a multidimensional analysis. *Am J Med Genet A 146A*:720-729.

Bear MF, Huber KM, Warren ST. 2004. The mGluR theory of fragile X mental retardation. *Trends Neurosci 27*:370-377.

Beilina A, Tassone F, Schwartz PH, Sahota P, Hagerman PJ. 2004. Redistribution of transcription start sites within the FMR1 promoter region with expansion of the downstream CGG-repeat element. *Hum Mol Genet 13*:543-549.

Bennetto L, Pennington BF, Porter D, Taylor AK, Hagerman RJ. 2001. Profile of cognitive functioning in women with the fragile X mutation. *Neuropsychology 15*:290-299.

Berry-Kravis E, Potanos K, Weinberg D, Zhou L, Goetz CG. 2005. Fragile X-associated tremor/ataxia syndrome in sisters related to X-inactivation. *Ann Neurol 57*:144-147.

Bourgeois JA, Cogswell JB, Hessl D, Zhang L, Ono MY, Tassone F, Farzin F, Brunberg JA, Grigsby J, Hagerman RJ. 2007. Cognitive, anxiety and mood disorders in the fragile X-associated tremor/ataxia syndrome. *Gen Hosp Psychiatry 29*:349-356.

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. 2008. The primary cognitive deficit among males with fragile X-associated tremor/ataxia syndrome (FXTAS) is a dysexecutive syndrome. *J Clin Exp Neuropsychol*:1-17.

Brown V, Jin P, Ceman S, Darnell JC, O'Donnell WT, Tenenbaum SA, Jin X, Feng Y, Wilkinson KD, Keene JD, Darnell RB, Warren ST. 2001. Microarray identification of FMRP-associated brain mRNAs and altered mRNA translational profiles in fragile X syndrome. *Cell 107*:477-487.

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

Chen LS, Tassone F, Sahota P, Hagerman PJ. 2003. The (CGG)n repeat element within the 5' untranslated region of the FMR1 message provides both positive and

negative cis effects on in vivo translation of a downstream reporter. *Hum Mol Genet 12*:3067-3074.

Coffee B, Ikeda M, Budimirovic DB, Hjelm LN, Kaufmann WE, Warren ST. 2008. Mosaic FMR1 deletion causes fragile X syndrome and can lead to molecular misdiagnosis: a case report and review of the literature. *Am J Med Genet A 146A*:1358-1367.

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. 2006. Molecular and imaging correlates of the fragile X-associated tremor/ataxia syndrome. *Neurology 67*:1426-1431.

Cornish K, Sudhalter V, Turk J. 2004. Attention and language in fragile X. *Ment Retard Dev Disabil Res Rev 10*:11-16.

Crawford DC, Meadows KL, Newman JL, Taft LF, Pettay DL, Gold LB, Hersey SJ, Hinkle EF, Stanfield ML, Holmgreen P, Yeargin-Allsopp M, Boyle C, Sherman SL. 1999. Prevalence and phenotype consequence of FRAXA and FRAXE alleles in a large, ethnically diverse, special education-needs population. *Am J Hum Genet 64*:495-507.

Crawford DC, Wilson B, Sherman SL. 2000. Factors involved in the initial mutation of the fragile X CGG repeat as determined by sperm small pool PCR. *Hum Mol Genet 9*:2909-2918.

Darnell JC, Fraser CE, Mostovetsky O, Stefani G, Jones TA, Eddy SR, Darnell RB. 2005. Kissing complex RNAs mediate interaction between the Fragile-X mental retardation protein KH2 domain and brain polyribosomes. *Genes Dev 19*:903-918.

Darnell JC, Jensen KB, Jin P, Brown V, Warren ST, Darnell RB. 2001. Fragile X mental retardation protein targets G quartet mRNAs important for neuronal function. *Cell 107*:489-499.

De Boulle K, Verkerk AJ, Reyniers E, Vits L, Hendrickx J, Van Roy B, Van den Bos F, de Graaff E, Oostra BA, Willems PJ. 1993. A point mutation in the FMR-1 gene associated with fragile X mental retardation. *Nat Genet 3*:31-35.

Dombrowski C, Levesque S, Morel ML, Rouillard P, Morgan K, Rousseau F. 2002. 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 11*:371-378.

Eichler EE, Holden JJ, Popovich BW, Reiss AL, Snow K, Thibodeau SN, Richards CS, Ward PA, Nelson DL. 1994. Length of uninterrupted CGG repeats determines instability in the FMR1 gene. *Nat Genet 8*:88-94.

Eichler EE, Richards S, Gibbs RA, Nelson DL. 1993. Fine structure of the human FMR1 gene. *Hum Mol Genet 2*:1147-1153.

Ennis S, Ward D, Murray A. 2006. Nonlinear association between CGG repeat number and age of menopause in FMR1 premutation carriers. *Eur J Hum Genet 14*:253-255.

Feng Y, Zhang F, Lokey LK, Chastain JL, Lakkis L, Eberhart D, Warren ST. 1995. Translational suppression by trinucleotide repeat expansion at FMR1. *Science 268*:731-734.

Fisch GS, Simensen RJ, Schroer RJ. 2002. Longitudinal changes in cognitive and adaptive behavior scores in children and adolescents with the fragile X mutation or autism. *J Autism Dev Disord 32*:107-114.

Fu YH, Kuhl DP, Pizzuti A, Pieretti M, Sutcliffe JS, Richards S, Verkerk AJ, Holden JJ, Fenwick RG, Jr., Warren ST, et al. 1991. Variation of the CGG repeat at the fragile X site results in genetic instability: resolution of the Sherman paradox. *Cell 67*:1047-1058.

Garber KB, Visootsak J, Warren ST. 2008. Fragile X syndrome. *Eur J Hum Genet 16*:666-672.

Garcia-Alegria E, Ibanez B, Minguez M, Poch M, Valiente A, Sanz-Parra A, Martinez-Bouzas C, Beristain E, Tejada MI. 2007. Analysis of FMR1 gene expression in female premutation carriers using robust segmented linear regression models. *Rna 13*:756-762.

Gedeon AK, Baker E, Robinson H, Partington MW, Gross B, Manca A, Korn B, Poustka A, Yu S, Sutherland GR, et al. 1992. Fragile X syndrome without CCG amplification has an FMR1 deletion. *Nat Genet 1*:341-344.

Greco CM, Hagerman RJ, Tassone F, Chudley AE, Del Bigio MR, Jacquemont S, Leehey M, Hagerman PJ. 2002. Neuronal intranuclear inclusions in a new cerebellar tremor/ataxia syndrome among fragile X carriers. *Brain 125*:1760-1771.

Greco CM, Soontrapornchai K, Wirojanan J, Gould JE, Hagerman PJ, Hagerman RJ. 2007. Testicular and pituitary inclusion formation in fragile X associated tremor/ataxia syndrome. *J Urol 177*:1434-1437.

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. 2007. Impairment of executive cognitive functioning in males with fragile X-associated tremor/ataxia syndrome. *Mov Disord 22*:645-650.

Hagerman PJ, Hagerman RJ. 2004. The fragile-X premutation: a maturing perspective. *Am J Hum Genet 74*:805-816.

Hagerman RJ, Leavitt BR, Farzin F, Jacquemont S, Greco CM, Brunberg JA, Tassone F, Hessl D, Harris SW, Zhang L, Jardini T, Gane LW, Ferranti J, Ruiz L, Leehey MA, Grigsby J, Hagerman PJ. 2004. Fragile-X-associated tremor/ataxia syndrome (FXTAS) in females with the FMR1 premutation. *Am J Hum Genet 74*:1051-1056.

Hagerman RJ, Leehey M, Heinrichs W, Tassone F, Wilson R, Hills J, Grigsby J, Gage B, Hagerman PJ. 2001. Intention tremor, parkinsonism, and generalized brain atrophy in male carriers of fragile X. *Neurology 57*:127-130.

Hall SS, Burns DD, Reiss AL. 2007. Modeling family dynamics in children with fragile x syndrome. *J Abnorm Child Psychol 35*:29-42.

Hessl D, Tassone F, Loesch DZ, Berry-Kravis E, Leehey MA, Gane LW, Barbato I, Rice C, Gould E, Hall DA, Grigsby J, Wegelin JA, Harris S, Lewin F, Weinberg D, Hagerman PJ, Hagerman RJ. 2005. Abnormal elevation of FMR1 mRNA is associated with psychological symptoms in individuals with the fragile X premutation. *Am J Med Genet B Neuropsychiatr Genet 139*:115-121.

Hundscheid RD, Braat DD, Kiemeney LA, Smits AP, Thomas CM. 2001. Increased serum FSH in female fragile X premutation carriers with either regular menstrual cycles or on oral contraceptives. *Hum Reprod 16*:457-462.

Hunter J, Abramowitz A, Rusin M, Sherman S. 2008a. Is there evidence for neuropsychological and neurobehavioral phenotypes among adults without FXTAS who carry the FMR1 premutation? A review of current literature. *Genet Med* [In press].

Hunter JE, Allen EG, Abramowitz A, Rusin M, Leslie M, Novak G, Hamilton D, Shubeck L, Charen K, Sherman SL. 2008b. Investigation of phenotypes associated with mood and anxiety among male and female fragile x premutation carriers. *Behav Genet 38*:493-502.

Hunter JE, Allen EG, Abramowitz A, Rusin M, Leslie M, Novak G, Hamilton D, Shubeck L, Charen K, Sherman SL. 2008c. No evidence for a difference in neuropsychological profile among carriers and non-carriers of the FMR1 premutation in adults under the age of 50. *Am J Hum Genet* [In press].

Hunter JE, Epstein MP, Tinker SW, Charen KH, Sherman SL. 2008d. Fragile X-associated primary ovarian insufficiency: evidence for additional genetic contributions to severity. *Genet Epidemiol 32*:553-559.

Iwahashi CK, Yasui DH, An HJ, Greco CM, Tassone F, Nannen K, Babineau B, Lebrilla CB, Hagerman RJ, Hagerman PJ. 2006. Protein composition of the intranuclear inclusions of FXTAS. *Brain 129*:256-271.

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. 2003. Fragile X premutation tremor/ataxia syndrome: molecular, clinical, and neuroimaging correlates. *Am J Hum Genet 72*:869-878.

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. 2004. Penetrance of the fragile X-associated tremor/ataxia syndrome in a premutation carrier population. *Jama 291*:460-469.

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

Jacquemont S, Orrico A, Galli L, Sahota PK, Brunberg JA, Anichini C, Leehey M, Schaeffer S, Hagerman RJ, Hagerman PJ, Tassone F. 2005. Spastic paraparesis, cerebellar ataxia, and intention tremor: a severe variant of FXTAS? *J Med Genet 42*:e14.

Jin P, Duan R, Qurashi A, Qin Y, Tian D, Rosser TC, Liu H, Feng Y, Warren ST. 2007. Pur alpha binds to rCGG repeats and modulates repeat-mediated neurodegeneration in a Drosophila model of fragile X tremor/ataxia syndrome. *Neuron 55*:556-564.

Jin P, Zarnescu DC, Zhang F, Pearson CE, Lucchesi JC, Moses K, Warren ST. 2003. RNA-mediated neurodegeneration caused by the fragile X premutation rCGG repeats in Drosophila. *Neuron 39*:739-747.

Jin P, Duan R, Qurashi A, Qin Y, Tian D, Rosser TC, Liu H, Feng Y, Warren ST. 2007. Pur alpha binds to rCGG repeats and modulates repeat-mediated neurodegeneration in a Drosophila model of fragile X tremor/ataxia syndrome. *Neuron 55*:556-564.

Johnston C, Hessl D, Blasey C, Eliez S, Erba H, Dyer-Friedman J, Glaser B, Reiss AL. 2003. Factors associated with parenting stress in mothers of children with fragile X syndrome. *J Dev Behav Pediatr 24*:267-275.

Kaufmann WE, Abrams MT, Chen W, Reiss AL. 1999. Genotype, molecular phenotype, and cognitive phenotype: correlations in fragile X syndrome. *Am J Med Genet 83*:286-295.

Kenneson A, Zhang F, Hagedorn CH, Warren ST. 2001. Reduced FMRP and increased FMR1 transcription is proportionally associated with CGG repeat number in intermediate-length and premutation carriers. *Hum Mol Genet 10*:1449-1454.

Kremer EJ, Pritchard M, Lynch M, Yu S, Holman K, Baker E, Warren ST, Schlessinger D, Sutherland GR, Richards RI. 1991. Mapping of DNA instability at the fragile X to a trinucleotide repeat sequence p(CCG)n. *Science 252*:1711-1714.

Kunst CB, Warren ST. 1994. Cryptic and polar variation of the fragile X repeat could result in predisposing normal alleles. *Cell 77*:853-861.

Leehey MA, Berry-Kravis E, Min SJ, Hall DA, Rice CD, Zhang L, Grigsby J, Greco CM, Reynolds A, Lara R, Cogswell J, Jacquemont S, Hessl DR, Tassone F, Hagerman R, Hagerman PJ. 2007. Progression of tremor and ataxia in male carriers of the FMR1 premutation. *Mov Disord 22*:203-206.

Lewis P, Abbeduto L, Murphy M, Richmond E, Giles N, Bruno L, Schroeder S, Anderson J, Orsmond G. 2006. Psychological well-being of mothers of youth with fragile X syndrome: syndrome specificity and within-syndrome variability. *J Intellect Disabil Res 50*:894-904.

Li Z, Zhang Y, Ku L, Wilkinson KD, Warren ST, Feng Y. 2001. The fragile X mental retardation protein inhibits translation via interacting with mRNA. *Nucleic Acids Res 29*:2276-2283.

Loesch DZ, Huggins RM, Hagerman RJ. 2004. Phenotypic variation and FMRP levels in fragile X. *Ment Retard Dev Disabil Res Rev 10*:31-41.

Maddalena A, Richards CS, McGinniss MJ, Brothman A, Desnick RJ, Grier RE, Hirsch B, Jacky P, McDowell GA, Popovich B, Watson M, Wolff DJ. 2001. Technical standards and guidelines for fragile X: the first of a series of disease-specific supplements to the Standards and Guidelines for Clinical Genetics Laboratories of the American College of Medical Genetics. Quality Assurance Subcommittee of the Laboratory Practice Committee. *Genet Med* 3:200-205.

Malter HE, Iber JC, Willemsen R, de Graaff E, Tarleton JC, Leisti J, Warren ST, Oostra BA. 1997. Characterization of the full fragile X syndrome mutation in fetal gametes. *Nat Genet 15*:165-169.

McConkie-Rosell A, Lachiewicz AM, Spiridigliozzi GA, Tarleton J, Schoenwald S, Phelan MC, Goonewardena P, Ding X, Brown WT. 1993. Evidence that methylation of the FMR-I locus is responsible for variable phenotypic expression of the fragile X syndrome. *Am J Hum Genet 53*:800-809.

Murray A, Ennis S, MacSwiney F, Webb J, Morton NE. 2000. Reproductive and menstrual history of females with fragile X expansions. *Eur J Hum Genet 8*:247-252.

Nolin SL, Brown WT, Glicksman A, Houck GE, Jr., Gargano AD, Sullivan A, Biancalana V, Brondum-Nielsen K, Hjalgrim H, Holinski-Feder E, Kooy F,

Longshore J, Macpherson J, Mandel JL, Matthijs G, Rousseau F, Steinbach P, Vaisanen ML, von Koskull H, Sherman SL. 2003. Expansion of the fragile X CGG repeat in females with premutation or intermediate alleles. *Am J Hum Genet 72*:454-464.

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

Pesso R, Berkenstadt M, Cuckle H, Gak E, Peleg L, Frydman M, Barkai G. 2000. Screening for fragile X syndrome in women of reproductive age. *Prenat Diagn 20*:611-614.

Pieretti M, Zhang FP, Fu YH, Warren ST, Oostra BA, Caskey CT, Nelson DL. 1991. Absence of expression of the FMR-1 gene in fragile X syndrome. *Cell 66*:817-822.

Primerano B, Tassone F, Hagerman RJ, Hagerman P, Amaldi F, Bagni C. 2002. Reduced FMR1 mRNA translation efficiency in fragile X patients with premutations. *Rna 8*:1482-1488.

Rohr J, Allen EG, Charen K, Giles J, He W, Dominguez C, Sherman SL. 2008. Anti-Mullerian hormone indicates early ovarian decline in fragile X mental retardation (FMR1) premutation carriers: a preliminary study. *Hum Reprod*.

Rousseau F, Rouillard P, Morel ML, Khandjian EW, Morgan K. 1995. Prevalence of carriers of premutation-size alleles of the FMRI gene--and implications for the population genetics of the fragile X syndrome. *Am J Hum Genet 57*:1006-1018.

Sherman SL. 2000. Premature ovarian failure in the fragile X syndrome. *Am J Med Genet 97*:189-194.

Sherman SL, Meadows KL, Ashley AE. 1996. Examination of factors that influence the expansion of the fragile X mutation in a sample of conceptuses from known carrier females. *Am J Med Genet 64*:256-260.

Snow K, Doud LK, Hagerman R, Pergolizzi RG, Erster SH, Thibodeau SN. 1993. Analysis of a CGG sequence at the FMR-1 locus in fragile X families and in the general population. *Am J Hum Genet 53*:1217-1228.

Sofola OA, Jin P, Qin Y, Duan R, Liu H, de Haro M, Nelson DL, Botas J. 2007. RNA-binding proteins hnRNP A2/B1 and CUGBP1 suppress fragile X CGG premutation repeat-induced neurodegeneration in a Drosophila model of FXTAS. *Neuron 55*:565-571.

Sullivan AK, Crawford DC, Scott EH, Leslie ML, Sherman SL. 2002. Paternally transmitted FMR1 alleles are less stable than maternally transmitted alleles in the common and intermediate size range. *Am J Hum Genet 70*:1532-1544.

Sullivan AK, Marcus M, Epstein MP, Allen EG, Anido AE, Paquin JJ, Yadav-Shah M, Sherman SL. 2005. Association of FMR1 repeat size with ovarian dysfunction. *Hum Reprod 20*:402-412.

Sutcliffe JS, Nelson DL, Zhang F, Pieretti M, Caskey CT, Saxe D, Warren ST. 1992. DNA methylation represses FMR-1 transcription in fragile X syndrome. *Hum Mol Genet 1*:397-400.

Tassone F, Adams J, Berry-Kravis EM, Cohen SS, Brusco A, Leehey MA, Li L, Hagerman RJ, Hagerman PJ. 2007a. CGG repeat length correlates with age of

onset of motor signs of the fragile X-associated tremor/ataxia syndrome (FXTAS). *Am J Med Genet B Neuropsychiatr Genet 144*:566-569.

Tassone F, Beilina A, Carosi C, Albertosi S, Bagni C, Li L, Glover K, Bentley D, Hagerman PJ. 2007b. Elevated FMR1 mRNA in premutation carriers is due to increased transcription. *Rna 13*:555-562.

Tassone F, Hagerman RJ, Chamberlain WD, Hagerman PJ. 2000a. Transcription of the FMR1 gene in individuals with fragile X syndrome. *Am J Med Genet 97*:195-203.

Tassone F, Hagerman RJ, Ikle DN, Dyer PN, Lampe M, Willemsen R, Oostra BA, Taylor AK. 1999. FMRP expression as a potential prognostic indicator in fragile X syndrome. *Am J Med Genet 84*:250-261.

Tassone F, Hagerman RJ, Taylor AK, Gane LW, Godfrey TE, Hagerman PJ. 2000b. Elevated levels of FMR1 mRNA in carrier males: a new mechanism of involvement in the fragile-X syndrome. *Am J Hum Genet 66*:6-15.

Tassone F, Hagerman RJ, Taylor AK, Hagerman PJ. 2001. A majority of fragile X males with methylated, full mutation alleles have significant levels of FMR1 messenger RNA. *J Med Genet 38*:453-456.

Tassone F, Hagerman RJ, Taylor AK, Mills JB, Harris SW, Gane LW, Hagerman PJ. 2000c. Clinical involvement and protein expression in individuals with the FMR1 premutation. *Am J Med Genet 91*:144-152.

Tassone F, Iwahashi C, Hagerman PJ. 2004. FMR1 RNA within the intranuclear inclusions of fragile X-associated tremor/ataxia syndrome (FXTAS). *RNA Biol 1*:103-105.

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

Turner G, Webb T, Wake S, Robinson H. 1996. Prevalence of fragile X syndrome. *Am J Med Genet 64*:196-197.

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

Weisman-Shomer P, Cohen E, Fry M. 2000. Interruption of the fragile X syndrome expanded sequence d(CGG)(n) by interspersed d(AGG) trinucleotides diminishes the formation and stability of d(CGG)(n) tetrahelical structures. *Nucleic Acids Res 28*:1535-1541.

Welt CK. 2008. Primary ovarian insufficiency: a more accurate term for premature ovarian failure. *Clin Endocrinol (Oxf) 68*:499-509.

Welt CK, Smith PC, Taylor AE. 2004. Evidence of early ovarian aging in fragile X premutation carriers. *J Clin Endocrinol Metab 89*:4569-4574.

Yu S, Mulley J, Loesch D, Turner G, Donnelly A, Gedeon A, Hillen D, Kremer E, Lynch M, Pritchard M, et al. 1992. Fragile-X syndrome: unique genetics of the heritable unstable element. *Am J Hum Genet 50*:968-980.

Zuhlke C, Budnik A, Gehlken U, Dalski A, Purmann S, Naumann M, Schmidt M, Burk K, Schwinger E. 2004. FMR1 premutation as a rare cause of late onset ataxia--evidence for FXTAS in female carriers. *J Neurol 251*:1418-1419.

Chapter 2

Is there evidence for neuropsychological and neurobehavioral phenotypes among adults without FXTAS who carry the *FMR1* premutation? A review of current literature.

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This chapter is a review of a subset of the literature devoted to the investigation of neuropsychological phenotypes associated with *FMR1* premutation alleles in order to determine if a specific phenotypic profile emerges among younger adult carriers (between ages 18-50). The question of whether or not younger premutation carriers are negatively affected cognitively and/or behaviorally is an important one in the field of *FMR1*-associated disorders for two reasons: 1) in spite of conflicting reports, the general view in the community is that a significant neuropsychological phenotype does exist and 2) given the later onset disorder of *FMR1*-premutation associated tremor/ataxia (FXTAS) which usually occurs after age 50, an earlier onset of symptoms may be represented by an altered neuropsychological profile. This review provides the first objective review of this

field of literature. My contributions to this work include the literature search for relevant studies, formation of appropriate inclusion criteria for studies in the review, compilation of the data from the 16 studies identified, interpretation of the findings, as well as manuscript preparation and publication.

This review is currently in press with *Genetics in Medicine*: Hunter et al. (2009) 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: [In press] © American College of Medical Genetics. This manuscript has been reprinted with permission of Lippincott Williams & Wilkins.

Further studies regarding this topic have been published since the acceptance of this manuscript. An addendum to this chapter addressing these studies can be found at the end of this chapter.

Abstract

Carriers of expanded, but unmethylated, premutation alleles of the fragile X mental retardation gene (*FMR1*) are at risk for a late-onset tremor/ataxia syndrome, mostly affecting men over age 50. However, the general neuropsychological and neurobehavioral impact of carrying a premutation allele in younger adults not affected by the tremor/ataxia syndrome remains unclear. Past studies have utilized varying study designs resulting in inconsistent conclusions. To better understand the current evidence of the influence of the premutation on such traits in adult carriers, we reviewed the literature and identified 16 studies that met conservative inclusion criteria, including molecular measures of the *FMR1* CGG triplet repeat length and standard measures of neurobehavioral and neurocognitive phenotypes. A review of these studies is presented to assess the evidence for possible premutation-associated neuropsychological deficits among adult men and women who do not meet diagnostic criteria of the tremor/ataxia syndrome. Results of these studies, as well as possible reasons for inconsistent conclusions, are discussed. The primary conclusion from this review is the need for further research using a standard protocol in a large multi-site project to ensure the necessary sample size.

Key Words: *FMR1*, CGG repeat, premutation, fragile X syndrome, FXTAS, neuropsychology, cognition, anxiety, depression

Introduction

The X-linked fragile X mental retardation gene (*FMR1*) contains a triplet CGG repeat in the 5' UTR region that is associated with the mental retardation syndrome, fragile X (FXS).[1-3] The most common alleles of *FMR1* contain less than 40 repeats and are stable when transmitted from generation to generation.[4, 5] Due to mechanisms that are presently unclear, the triplet repeat can become unstable and expand from one generation to the next. Expansion to greater than 200 repeats results in hypermethylation of the *FMR1* gene and subsequent loss of gene expression.[6-9] The loss of the protein product, FMRP, is responsible for FXS.[8, 10] Males with FXS typically have mild to severe mental retardation, developmental delay, hyperactivity, social anxiety and other anxiety disorders, and autistic-like features. In addition, males with FXS display a pattern of memory deficits, particularly for short-term, or working memory, and visual memory.[11-14]

Expanded, but unmethylated, repeats in the range of about 55-200 are unstable across generations[5] and are associated with increasing levels of transcript and decreasing levels of FMRP[15-18]. These *FMR1* alleles, termed premutation alleles, have recently been found to be associated with a late-onset fragile X-associated tremor ataxia syndrome (FXTAS), mostly affecting men after the age of 50.[19, 20] Men with FXTAS typically develop a progressive tremor and/or ataxia and experience cognitive decline, loss of executive function and short-term memory, as well as irritability and anxiety.[19, 20]

Thus cognitive, memory, and executive function impairments as well as neurobehavioral issues are shared phenotypes of FXS and FXTAS for males, and to a lesser degree for females due to the X-linked nature of *FMR1*. Further, FXS is the result of a lack of FMRP expression[8] while symptoms of FXTAS are caused by a toxic gain of

function of the expanded *FMR1* transcript present in premutation allele carriers.[21] Given these phenotypes associated with *FMR1* as well as the molecular phenotypes associated with premutation alleles, specifically increased transcript levels and reduced FMRP[15-18], global impairment in neuropsychological functioning associated with premutation alleles may be expected among adult carriers of the premutation. In addition, brain anomalies have been reported among carriers of premutation alleles that do not meet diagnostic criteria of FXTAS.[22-24] Aside from potential biological causes of neuropsychological and neurobehavioral phenotypes, the potential impact of environmental factors, including the stress of raising a child with FXS and the stigma of carrying a genetic mutation, should be considered as well.

Many studies have been conducted to characterize potential cognitive or behavioral deficits among premutation carriers. However many were done prior to the characterization of FXTAS. For those whose study populations included premutation carriers over the age of 50, any reported deficits are difficult to interpret as general impairments among premutation carriers or as impairments resulting from inclusion of carriers affected by FXTAS. Among all studies, results tend to be contradictory and many are compromised by poor study design. Also, most studies have utilized female study populations when males would be more informative due to the X-linked nature of *FMR1*.

Thus, the strength of evidence in support of a phenotype among premutation adults is unclear. In spite of the conflicting results from the published studies, anecdotal information suggesting significant deficits has become relevant in the clinical setting and in the fragile X community. This information is perpetuated in families and can result in

potentially needless stress and anxiety. The purpose of this review is to critically evaluate the current literature for evidence of neuropsychological phenotypes among adults who carry the premutation in the absence of FXTAS. Using strict inclusion criteria outlined in METHODS we identified 16 studies that examine these phenotypes associated with premutation alleles in adult carriers.

Methods

Articles for this review were identified by performing literature searches in PubMed and MEDLINE. The key words used in database searches in varying combinations were: *FMR1*, premutation, fragile X, neuropsychology, and phenotype. Articles published before 11/2006 were included in the search and were limited to those published in English. In order to select the articles to be included in the review, the abstracts were reviewed and the full text was retrieved for those that were relevant. Additional articles were identified through reviewing bibliographies of retrieved articles. Initial inclusion criteria for articles in this review were: (1) standard molecular measures of repeat length were used, including PCR and/or Southern blotting of the CGG repeat region of *FMR1*, (2) subjects were directly assessed using standardized, valid, and reliable measures of neuropsychological and neurobehavioral phenotypes (i.e. any analysis that used family report methods or unstructured self-report methods were not included), (3) subjects were limited to those 18 years of age or older, (4) a non-carrier comparison group was referenced in the study, which could include family controls, general population controls, and/or a normative sample, (5) statistical methods with reported P values were used, and (6) the article was published in a peer-reviewed journal.

Nineteen published studies met the inclusion criteria outlined above. However, several issues remained to be addressed.

First, a proportion of the 19 articles that met the criteria above included study participants over the age of 50 who carried the premutation. Unless subjects were assessed for the presence or absence of FXTAS symptoms, the inclusion of these older subjects, particularly men, with the premutation could compromise the study results. This is due to the fact that older carriers of the premutation may have cognitive deficits and behavioral changes associated with FXTAS [20, 25]. Thus it would be difficult to conclude whether any deficits detected among carriers were due to the late-onset neurodegeneration associated with FXTAS or due to a global impairment of carrying a premutation allele. In order to minimize this potential complication, all studies that included premutation men over the age of 50 were excluded. This reduced the number of studies in the review from 19 to 16. While women with a premutation are also at risk of developing FXTAS, studies that met all other inclusion criteria outlined above, but included women over the age of 50, were nonetheless included in this review. We made this exception as FXTAS appears to act as an X-linked recessive disorder: it is significantly less common among women compared with men who carry the premutation and symptoms of FXTAS for women are much milder than those among men.[26]

Second, the focus of some articles retrieved was to characterize phenotypes of full mutation carriers, while a premutation carrier group was included in the study for comparison purposes. As long as the premutation group was in some way compared to some control group, these papers were included because they likely represent an unbiased measurement of premutation phenotypes.

Third, neuropsychological measures were classified into specific cognitive domains in order to present the results in a coherent manner. However, the classification of several of the measures was difficult given the multiplicity of domains they assess. The authors have attempted to address this issue by providing the reader with the names of the specific tasks utilized by each study presented here.

Finally, several of the retrieved papers contained overlapping samples. In order to avoid reporting more than once on the same subjects, findings from the most recent publication were reported, unless different phenotype assessments were used.

In order to compare the magnitude of statistically significant results across studies, effect sizes were provided or calculated using reported data when appropriate. If a correlation was calculated in the study, the corresponding r value is provided. According to Cohen, r values of 0.1, 0.3, and 0.5 are considered small, medium, and large effects, respectively.[27] If two groups were compared in an analysis, the reported mean scores and standard deviations were used to calculate Cohen's d, where values of 0.2, 0.5, and 0.8 are considered small, medium, and large effects, respectively.[27] Where a multiple regression was performed, Cohen's f^2 has been calculated using the reported squared multiple correlation (R^2) for the independent variable tested.[27] Here, values of 0.02, 0.15, and 0.35 are considered small, medium, and large effects, respectively.[27] For chi square tests to compare frequencies of diagnoses between groups, Cramer's Φ was calculated. For 2x2 tables, as used in the analyses summarized here, this value will be the same as Cohen's ω, where values of 0.10, 0.30, 0.50 are considered small, medium, and large effects, respectively.[27]

Results

In total, 16 studies were included in this review. In an attempt to clearly summarize results, measures used in each study were categorized into five broad cognitive and emotional categories: general intelligence, memory, executive functioning, spatial abilities, and psychiatric phenotypes. Several measures were difficult to categorize since the functions they were designed to measure might overlap two or more cognitive processes, but every effort was made to pick the most appropriate category. Table I lists the abbreviations for the measures used and Tables II-VI summarize the sample groups and results for each category of measures. Results for analyses on males and females are presented separately.

Females

General intelligence (Table II)

The most commonly used measure of overall cognitive functioning in the studies reviewed here was the Weschler Adult Intelligence Scale (WAIS). This widely used test provides a Full Scale IQ (FSIQ), a Verbal IQ (VIQ), and a Performance IQ (PIQ). One of the most common approaches among these studies has been to ascertain women with the premutation and compare the mean score of this group to the mean score of one or more control groups. Of the studies that evaluated women, none detected a significant difference in FSIQ, PIQ, or VIQ scores between groups[13, 28-31] with the exception of Allen et al. (2005)[32]. They reported a significantly lower VIQ mean score for premutation carriers compared with non-carriers, although repeat length only explained 4% of the variance.[32]

The WAIS also provides individual subtest scores. Though the subscales are part of an intelligence battery, individually they are not measures of overall intelligence, but rather specific factors of intelligence. Scores on these subtests have been compared between groups of controls and premutation carriers. Several studies have found no significant differences in mean scores on individual subtests between premutation carriers and controls[28, 29], while Franke et al. (1991)[33] found that the premutation carriers scored significantly lower for Vocabulary, Arithmetic, Verbal Comprehension, and Object Assembly.

Using the Wide Range Achievement Test (WRAT), a measure of academic achievement, Lachiewicz et al. (2006)[34] reported that premutation carriers scored significantly lower than standardized norms on the Arithmetic scale, but not on the Reading or Spelling scales.

Another common approach to analyze the impact of carrying the premutation on cognitive functioning has been to examine linear relationships with repeat length and cognitive scores. Although most studies have noted no significant correlation between repeat length and IQ scores[28, 29, 35, 36] or cognitive subscale scores[28, 29, 33], two studies did detect significant correlations. Allen et al. (2005)[32] detected a significant linear association between VIQ and both repeat length and transcript level in an analysis that included both carriers and non-carriers. Lachiewicz et al. (2006)[34] noted a significant positive correlation between repeat length and WRAT Arithmetic scores among women. This suggests that Arithmetic subscores increased with repeat length, although, as a whole, the premutation group scored significantly below the standardized norm. This preliminary finding suggests that women with higher repeat premutation alleles may be

less affected than those with smaller repeat premutation alleles. However the authors emphasize the need to confirm these unexpected results in an independent sample due to the limited range of premutation alleles among participants.

Memory (Table III)

No significant differences in memory function between premutation carriers and non-carriers were detected in studies reviewed here. Thompson et al. (1994)[31] had a sample of 12 carriers and found that the mean score for the group for verbal memory subscales of the Wechsler Memory Scale - Revised (WMS-R) were within the average range. Using other measures of memory, Franke et al. (1999)[33] compared two groups of women with a premutation (65 carriers who were mothers of children with FXS and 12 carriers without a child with FXS) to two control groups (18 non-carrier siblings of the carrier mothers and 39 non-carrier mothers of children with autism). No significant differences in mean scores were detected between the premutation groups and control groups. In addition no significant correlations between repeat length and memory score were detected. Finally, Bennetto et al. (2001)[13] detected no significant differences in mean scores for verbal or visual memory between groups of 96 carriers and 37 non-carrier controls from families with a history of FXS.

Executive function (Table IV)

None of the studies reviewed here detected any deficits in executive functioning among premutation carriers. Comparing executive function scores between premutation carriers and non-carriers, four studies found no significant mean score differences between groups.[13, 30, 31, 33] In addition, Franke et al. (1999)[33] found no significant correlation between repeat length and test scores.

Spatial ability (Table V)

No deficits were detected in any of the studies using measures of visual/spatial skills, visual/motor skills, visual-spatial perception, and/or visual-spatial organization.[13, 30, 31] Comparing carriers with non-carriers, no significant group mean score differences were detected.[13, 30] In addition, Thompson et al. (1994)[31] determined that the mean score of a group of 12 premutation carriers was within the normal range. None of these studies analyzed the correlation of repeat length with spatial ability scores.

Neuropsychiatric symptoms (Table VI)

Using combinations of neuropsychiatric interviews and behavioral questionnaires, three studies reviewed here detected no significant increased risk for emotional morbidity among carriers of the premutation when compared to non-carrier controls nor any significant correlations with repeat length and neurobehavior variables.[30, 37, 38] Reiss et al. (1993)[30] did find an increased rate of stereotypy-habit disorder in the group of premutation carriers who were mothers of children with FXS, but concluded that the presence of this behavior in the absence of other psychiatric issues did not indicate a clinical mental health problem.

Thompson et al. (1994)[31] reported on a group of 12 premutation carriers and noted that although they did not have an increased rate of schizotypal features, the group had a higher rate of depression (75%) than would be expected in the general population. However, this result is compromised by the clinical ascertainment methods of the study as well as the lack of a comparison group. In addition, comparison to the general population rates of depression may not be appropriate, as mothers of special needs children are known to have increased rates of depression.[39] In support of this finding,

Franke et al. (1998)[35] found a significantly increased frequency of anxiety and depression disorders among a group of 61 premutation carriers who were mothers of children with FXS compared to 42 non-carriers who were mothers of children with autism and to 18 non-carrier family controls. However, no significant differences were detected between 17 premutation carriers who were not mothers of children with FXS and the control groups, indicating that the emotional morbidity could be due to raising a child with FXS. The authors attempted to address this by determining the mean age at onset of the mood disorders/psychiatric diseases. Onset tended to be earlier than the mean age of the mother when their child was diagnosed with FXS suggesting that the disorders were most likely unrelated to raising a child with special needs.

Johnston et al. (2001)[29] studied carriers separated into two groups based on repeat length (66 women with less than 100 repeats and 19 women with more than 100 repeats). Results indicated that the group with the larger repeat sizes had significantly higher mean scores for depression and interpersonal sensitivity, but not anxiety or overall symptomology. Results also showed a significant positive correlation with repeat length and depression scores. However there was not a control group and the tests were not adjusted for raising a child with FXS.

The most recent study to analyze emotional morbidity among premutation carriers is Hessl et al. (2005)[36]. Women with the premutation with and without symptoms of FXTAS were assessed for psychiatric symptomology using a symptom checklist (SCL-90-R). Those without symptoms of FXTAS displayed a significantly increased risk of emotional morbidity compared to normative controls. No significant correlations with

repeat length, *FMR1* mRNA levels, or protein levels were noted among premutation carriers.

Males

General intelligence (Table II)

Allen et al. (2005)[32] analyzed cognitive functioning among premutation carriers and found no significant differences in FSIQ, PIQ, or VIQ scores when compared to non-carrier controls, although sample sizes were small. Hessl et al. (2005)[36] noted a significant negative correlation between IQ score and repeat length among premutation carriers, but did not detect significant correlations between IQ scores and *FMR1* mRNA or FMRP levels. Unfortunately, the premutation group in the study of Hessl et al. (2005)[36] included both men with and without FXTAS symptoms. Therefore, no conclusions can be made about the neurocognitive functioning of premutation carriers outside the context of FXTAS.

Neuropsychiatric symptoms (Table VI)

The only study to analyze emotional morbidity among men with the premutation is Hessl et al. (2005)[36]. Carriers with and without symptoms of FXTAS were assessed for psychiatric symptomology using the SCL-90-R symptom checklist. Premutation carriers without symptoms of FXTAS displayed a significantly increased risk of emotional morbidity compared to normative controls. Further, the severity of symptoms was significantly correlated with *FMR1* mRNA levels, but not repeat length or protein level. For most scales, the strongest correlation was noted among men who carried the premutation, but did not have FXTAS.

Discussion

Since the discovery of the dynamic repeat sequence mutation in the *FMR1* gene, there has been interest in understanding the influence of this repeat expansion on neuropsychological and neurobehavioral outcomes. This interest was fueled by the significant discovery of FXTAS, a premutation-associated late-onset neurodegenerative disorder.[19, 20] For premutation carriers aged 18-50 years, many studies have been performed to understand the genotype/phenotype correlations. These results have been conflicting.

The primary objective of this report was to review the current literature and identify studies on the neuropsychological phenotype of adults who carry the *FMR1* premutation that fit strict criteria based on participant eligibility, molecular diagnosis of the premutation, and study design. Based on these studies, we asked: Does a pattern of neurocognitive and neurobehavioral deficits emerge in premutation carriers not affected by FXTAS? The primary finding is that no specific pattern of neurocognitive or neurobehavioral deficits emerges. For females, none of the studies reviewed here reported deficits in executive functioning, memory, or spatial ability among carriers of premutation alleles. Importantly, no studies that fit our strict criteria for inclusion were available to assess these domains among males. In addition, no deficits were noted in verbal functioning among females (Table II) with the exception of two studies: one identified deficits of medium effect size[33] while the other found only those of small effect size[32]. Similarly, other deficits detected among neuropsychological domains identified in single studies were of small to medium effect sizes (Table II).

In regard to neurobehavior phenotypes, some studies suggest an increased risk of emotional morbidity[29, 31, 35, 36], particularly for depression and anxiety disorders, compared to controls, while other studies indicate a lack of phenotype among premutation carriers[30, 31] (Table VI). The difficulty to determine if depression and anxiety results from the emotional toll of being a carrier and having a child with FXS or results from the effect of the premutation allele is noted by most of these studies. For example, Franke et al. (1998)[35] examined the onset of depression among women with respect to the diagnosis of their child with FXS. However, it may be necessary to conduct prospective studies among those at risk for carrying the premutation, as it is difficult to take into account when the environment of a woman who has a child with behavior problems and other issues associated with FXS begins to become stressful. Irrespective, among those studies that detected a phenotype, the largest effect sizes were found by Hessl et al. (2005)[36] for the obsessive-compulsive scale in men and women and for somatization among women. All other effect sizes were small to medium.[27]

Although further investigation is certainly warranted, particularly among males, the presence of global cognitive impairment or severe psychiatric morbidity is unlikely based on the effect sizes of the deficits summarized here. However, this conclusion needs to be considered within the context of the criteria we used for including published studies. We used strict inclusion criteria as outlined in the METHODS section and excluded studies on those less than 18 years of age and/or greater than 50 years of age. As symptoms of FXTAS usually occur after age 50, studies were excluded when they might have unknowingly included men who had symptoms of FXTAS. The exception to this was Hessl et al. (2005)[36] since the subjects were assessed for FXTAS status. Of

those studies that were excluded based on this criterion, several had results that are worth noting. Loesch and colleagues in a series of publications reported cognitive deficits and behavioral issues in men and women who carried the premutation whose ages ranged from roughly 5 to 80.[11, 40, 41] Moore et al. (2004)[42] reported deficits in executive functioning and memory in a sample of 20 men who carried the premutation with a mean age of roughly 53. Finally, Cornish et al. (2005)[43] reported group differences in social cognition between a sample of men who were carriers of the premutation and controls ranging from age 18 to 69. However, the possibility that these deficits were detected due to the inclusion of subjects affected by FXTAS rather than due to a general impairment associated with premutation alleles cannot be ruled out. The decision to include studies with women over the age of 50 was difficult, particularly because women who are carriers of the premutation are known to be at risk of FXTAS.[26] However, this risk is much lower than that for male carriers. In addition, women are likely to be less severely affected due to the X-linked nature of *FMR1*.

The exclusion of publications that included study participants under the age of 18 resulted in the exclusion of the two studies that analyzed the autism spectrum disorders.[44, 45] Autism spectrum disorders have not been assessed directly among younger adult premutation carriers. Although these excluded studies are not included in the body of the review, the possibility that premutation carriers are at an increased risk of autism spectrum disorders cannot be ruled out.

It is important to point out that even with strict criteria for inclusion, several of the studies in this review had methodological weaknesses. Most significantly, the majority of studies have modest sample sizes, limiting the power to detect phenotypes particularly

if the effect size is small. In addition, as pointed out by Franke et al. (1999)[33] in their own analysis, the possibility of a statistical difference being detected by chance is worth considering given the number of statistical tests conducted.

The results of several studies are also complicated by a lack of proper controls. For example, the comparison of mean scores of premutation carriers to normative samples is a practice that does not control for ascertainment biases and other complications that inadvertently occur in studies of this type. In the case of fragile X specifically, it does not control for the psychosocial impact of raising a child with FXS. This is an important point, particularly when considering the neurobehavioral domain. Overall, the interpretation and comparison of results across studies is complicated by varying study design, including different ascertainment strategies, phenotype measurement, and definition of a premutation allele. These differences likely contribute to the variable outcomes of the studies.

Lastly, many studies ascertained participants from pediatric and genetic clinics. These participants may not be representative of all carriers of premutation alleles. Socioeconomic status may limit access to these clinics. Further, participation may be influenced by attributes of the phenotypes themselves. For example, a person struggling with social interaction may be less likely to participate in any studies.

Conclusions and Future Directions

The one strong conclusion drawn from this review is that more research is needed, particularly for men. Most studies to date have focused in female carriers as they are more frequent in the population, 1/250 compared to 1/1000 in male carriers.[46] However,

the likelihood of detecting a phenotype among premutation carriers should be higher among men due to the X-linked nature of the gene.

It will be important to limit studies to adult subjects under the age of 50 in order to distinguish any deficits detected from those associated with the onset of FXTAS. The potential that any phenotypes detected in premutation carriers could constitute early signs of FXTAS is an intriguing one, particularly if these early signs are predictive of clinical outcomes of FXTAS. Identification of such early signs may enable preventative treatments in the future, thus avoiding the significant problems associated with FXTAS. This possibility could best be addressed with longitudinal studies in men who are carriers of the premutation. In addition, studies that analyze associations between FMRP levels and phenotypes could provide evidence on whether these phenotypes share molecular etiology to FXS due to decreased protein levels in higher premutation groups.

In addition, more widespread ascertainment strategies are needed to address issues related to the phenotypes being assessed. For example, cognitive impairments, depression, and issues with social interactions will likely impact personal relationships and/or the ability or choice to conceive and raise a child. Therefore, ascertainment through a child with FXS limits the premutation allele carriers included in the study to those who have been able to maintain a personal relationship and mate. In general, it may be difficult to assess such phenotypes, as they may influence participation in a study. This emphasizes the importance of ascertaining controls in the same manner as cases to minimize the potential bias.

The psychosocial burden of raising a child with FXS is an issue that requires attention in the study design. Several studies have included a control group consisting of

mothers of children with special needs. However, those that have developmentally disabled child might also carry other genetic factors that adversely affect cognitive functioning and psychiatric phenotype. This is likely more pertinent to mothers of children with an unknown etiology, such as autism, rather than children of non-inherited disorders such as Down Syndrome.

Finally, while most studies limit their analyses to phenotype associations with repeat length, the mechanism of CNS involvement might better be represented by the use of other molecular measures, including levels of *FMR1* mRNA as well as FMRP.

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References

1. Yu S, Pritchard M, Kremer E, et al. Fragile X genotype characterized by an unstable region of DNA. *Science.* May 24 1991;252(5010):1179-1181.
2. Verkerk AJ, Pieretti M, Sutcliffe JS, 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.* May 31 1991;65(5):905-914.
3. Oberle I, Rousseau F, Heitz D, et al. Instability of a 550-base pair DNA segment and abnormal methylation in fragile X syndrome. *Science.* May 24 1991;252(5010):1097-1102.
4. Snow K, Doud LK, Hagerman R, Pergolizzi RG, Erster SH, Thibodeau SN. Analysis of a CGG sequence at the FMR-1 locus in fragile X families and in the general population. *Am J Hum Genet.* Dec 1993;53(6):1217-1228.
5. Fu YH, Kuhl DP, Pizzuti A, et al. Variation of the CGG repeat at the fragile X site results in genetic instability: resolution of the Sherman paradox. *Cell.* Dec 20 1991;67(6):1047-1058.
6. Sutcliffe JS, Nelson DL, Zhang F, et al. DNA methylation represses FMR-1 transcription in fragile X syndrome. *Hum Mol Genet.* Sep 1992;1(6):397-400.
7. McConkie-Rosell A, Lachiewicz AM, Spiridigliozzi GA, et al. Evidence that methylation of the FMR-I locus is responsible for variable phenotypic expression of the fragile X syndrome. *Am J Hum Genet.* Oct 1993;53(4):800-809.
8. Pieretti M, Zhang FP, Fu YH, et al. Absence of expression of the FMR-1 gene in fragile X syndrome. *Cell.* Aug 23 1991;66(4):817-822.

9. Bell MV, Hirst MC, Nakahori Y, et al. Physical mapping across the fragile X: hypermethylation and clinical expression of the fragile X syndrome. *Cell.* Feb 22 1991;64(4):861-866.

10. Ashley CT, Jr., Wilkinson KD, Reines D, Warren ST. FMR1 protein: conserved RNP family domains and selective RNA binding. *Science.* Oct 22 1993;262(5133):563-566.

11. Loesch DZ, Hay DA, Mulley J. Transmitting males and carrier females in fragile X--revisited. *Am J Med Genet.* Jul 15 1994;51(4):392-399.

12. Cornish KM, Munir F, Cross G. Differential impact of the FMR-1 full mutation on memory and attention functioning : a neuropsychological perspective. *J Cogn Neurosci.* Jan 1 2001;13(1):144-150.

13. Bennetto L, Pennington BF, Porter D, Taylor AK, Hagerman RJ. Profile of cognitive functioning in women with the fragile X mutation. *Neuropsychology.* Apr 2001;15(2):290-299.

14. Jakala P, Hanninen T, Ryynanen M, et al. Fragile-X: neuropsychological test performance, CGG triplet repeat lengths, and hippocampal volumes. *J Clin Invest.* Jul 15 1997;100(2):331-338.

15. 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.* Jan 2000;66(1):6-15.

16. Tassone F, Hagerman RJ, Taylor AK, et al. Clinical involvement and protein expression in individuals with the FMR1 premutation. *Am J Med Genet.* Mar 13 2000;91(2):144-152.

17. 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.* Jul 1 2001;10(14):1449-1454.

18. Primerano B, Tassone F, Hagerman RJ, Hagerman P, Amaldi F, Bagni C. Reduced FMR1 mRNA translation efficiency in fragile X patients with premutations. *Rna.* Dec 2002;8(12):1482-1488.

19. Hagerman RJ, Leehey M, Heinrichs W, et al. Intention tremor, parkinsonism, and generalized brain atrophy in male carriers of fragile X. *Neurology.* Jul 10 2001;57(1):127-130.

20. Jacquemont S, Hagerman RJ, Leehey M, et al. Fragile X premutation tremor/ataxia syndrome: molecular, clinical, and neuroimaging correlates. *Am J Hum Genet.* Apr 2003;72(4):869-878.

21. Hagerman PJ, Hagerman RJ. The fragile-X premutation: a maturing perspective. *Am J Hum Genet.* May 2004;74(5):805-816.

22. Cohen S, Masyn K, Adams J, et al. Molecular and imaging correlates of the fragile X-associated tremor/ataxia syndrome. *Neurology.* Oct 24 2006;67(8):1426-1431.

23. Loesch DZ, Litewka L, Churchyard A, Gould E, Tassone F, Cook M. Tremor/ataxia syndrome and fragile X premutation: diagnostic caveats. *J Clin Neurosci.* Mar 2007;14(3):245-248.

24. Loesch DZ, Cook M, Litewka L, et al. A low symptomatic form of neurodegeneration in younger carriers of the FMR1 premutation, manifesting typical radiological changes. *J Med Genet.* Mar 2008;45(3):179-181.

25. Grigsby J, Brega AG, Jacquemont S, et al. Impairment in the cognitive functioning of men with fragile X-associated tremor/ataxia syndrome (FXTAS). *J Neurol Sci.* Oct 25 2006;248(1-2):227-233.

26. Hagerman RJ, Leavitt BR, Farzin F, et al. Fragile-X-associated tremor/ataxia syndrome (FXTAS) in females with the FMR1 premutation. *Am J Hum Genet.* May 2004;74(5):1051-1056.

27. Cohen J. A power primter. *Psychological Bulletin.* 1992;112(1):155-159.

28. Allingham-Hawkins DJ, Brown CA, Babul R, et al. Tissue-specific methylation differences and cognitive function in fragile X premutation females. *Am J Med Genet.* Aug 9 1996;64(2):329-333.

29. Johnston C, Eliez S, Dyer-Friedman J, et al. Neurobehavioral phenotype in carriers of the fragile X premutation. *Am J Med Genet.* Nov 1 2001;103(4):314-319.

30. Reiss AL, Freund L, Abrams MT, Boehm C, Kazazian H. Neurobehavioral effects of the fragile X premutation in adult women: a controlled study. *Am J Hum Genet.* May 1993;52(5):884-894.

31. Thompson NM, Gulley ML, Rogeness GA, et al. Neurobehavioral characteristics of CGG amplification status in fragile X females. *Am J Med Genet.* Dec 15 1994;54(4):378-383.

32. Allen EG, Sherman S, Abramowitz A, et al. Examination of the effect of the polymorphic CGG repeat in the FMR1 gene on cognitive performance. *Behav Genet.* Jul 2005;35(4):435-445.

33. Franke P, Leboyer M, Hardt J, et al. Neuropsychological profiles of FMR-1 premutation and full-mutation carrier females. *Psychiatry Res.* Oct 11 1999;87(2-3):223-231.

34. Lachiewicz AM, Dawson DV, Spiridigliozzi GA, McConkie-Rosell A. Arithmetic difficulties in females with the fragile X premutation. *Am J Med Genet A.* Apr 1 2006;140(7):665-672.

35. Franke P, Leboyer M, Gansicke M, et al. Genotype-phenotype relationship in female carriers of the premutation and full mutation of FMR-1. *Psychiatry Res.* Aug 17 1998;80(2):113-127.

36. Hessl D, Tassone F, Loesch DZ, et al. Abnormal elevation of FMR1 mRNA is associated with psychological symptoms in individuals with the fragile X premutation. *Am J Med Genet B Neuropsychiatr Genet.* Nov 5 2005;139(1):115-121.

37. Sobesky WE, Taylor AK, Pennington BF, et al. Molecular/clinical correlations in females with fragile X. *Am J Med Genet.* Aug 9 1996;64(2):340-345.

38. Sobesky WE, Pennington BF, Porter D, Hull CE, Hagerman RJ. Emotional and neurocognitive deficits in fragile X. *Am J Med Genet.* Jul 15 1994;51(4):378-385.

39. Lewis P, Abbeduto L, Murphy M, et al. Psychological well-being of mothers of youth with fragile X syndrome: syndrome specificity and within-syndrome variability. *J Intellect Disabil Res.* Dec 2006;50(Pt 12):894-904.

40. Loesch DZ, Bui QM, Grigsby J, et al. Effect of the fragile X status categories and the fragile X mental retardation protein levels on executive functioning in males and females with fragile X. *Neuropsychology.* Oct 2003;17(4):646-657.

41. Loesch DZ, Huggins RM, Bui QM, et al. Effect of fragile X status categories and FMRP deficits on cognitive profiles estimated by robust pedigree analysis. *Am J Med Genet A.* Sep 15 2003;122(1):13-23.

42. Moore CJ, Daly EM, Schmitz N, et al. A neuropsychological investigation of male premutation carriers of fragile X syndrome. *Neuropsychologia.* 2004;42(14):1934-1947.

43. Cornish K, Kogan C, Turk J, et al. The emerging fragile X premutation phenotype: evidence from the domain of social cognition. *Brain Cogn.* Feb 2005;57(1):53-60.

44. Aziz M, Stathopulu E, Callias M, et al. Clinical features of boys with fragile X premutations and intermediate alleles. *Am J Med Genet B Neuropsychiatr Genet.* Aug 15 2003;121(1):119-127.

45. Farzin F, Perry H, Hessl D, et al. Autism spectrum disorders and attention-deficit/hyperactivity disorder in boys with the fragile X premutation. *J Dev Behav Pediatr.* Apr 2006;27(2 Suppl):S137-144.

46. Crawford DC, Acuna JM, Sherman SL. FMR1 and the fragile X syndrome: human genome epidemiology review. *Genet Med.* Sep-Oct 2001;3(5):359-371.

47. Sobesky WE, Hull CE, Hagerman RJ. Symptoms of schizotypal personality disorder in fragile X women. *J Am Acad Child Adolesc Psychiatry.* Feb 1994;33(2):247-255.

48. Riddle JE, Cheema A, Sobesky WE, et al. Phenotypic involvement in females with the FMR1 gene mutation. *Am J Ment Retard.* May 1998;102(6):590-601.

49. Simon JA, Keenan JM, Pennington BF, Taylor AK, Hagerman RJ. Discourse processing in women with fragile X syndrome: evidence for a deficit establishing coherence. *Cognitive Neuropsychology.* 2001;18(1):1-18.

50. Franke P, Maier W, Hautzinger M, et al. Fragile-X carrier females: evidence for a distinct psychopathological phenotype? *Am J Med Genet.* Aug 9 1996;64(2):334-339.

Table I. Abbreviations for assessment tools used in reviewed studies.

Neuropsychological Assessments	
CBT	Corsi's Block-Tapping Test
CNT	Contingency Naming Test
HNTLA	Hiskey-Nebraska Test of Learning Aptitude
HRD	Hebb's Recurring Digits
JLO	Judgement of Line Orientation
ROF	Rey Osterrieth Figure
RRT	Reverse Reaction Time Test
SCWT	Stroop Color Word Test
TOH	Tower of Hanoi
TMT	Trail Making Test
VFT	Verbal Fluency Test
WAIS-III	Wechsler Adult Intelligence Scale - III
WAIS-R	Wechsler Adult Intelligence Scale-Revised
WCST	Wisconsin Card Sorting Task
WJ-R	Woodcock-Johnson Revised
WMS-R	Wechsler Memory Scale-Revised
WRAT-3	Wide Range Achievement Test - 3
Neurobehavior Assessments	
BPRS	Brief Psychiatric Rating Scale
CS	Chapman Scales
DIGS	Diagnostic Interview for Genetics Studies
HSCL-90	Hopkins Symptoms Checklist
SADS-L	Schedule for Affective Disorders and Schizophrenia - Lifetime Version
SIDP	Structured Interview for DSM-III Personality
SIS	Structured Interview for Schizotypy
MMPI-2	Minnesota Multiphasic Personality Inventory - 2
NEO-PI	NEO Personality Inventory
PDE	Personality Disorder Examination
PSRS	Psychotic Spectrum Rating Scale
SCL-90-R	Symptom Checklist-90 Revised

Table II. Summary of findings: General intelligence assessment in females and males.

Article Citation	Study Group(s)[a]	Comparison Group(s)[a]	Ascertainment	Measures[b]	Results
Females					
Reiss et al. (1993)[30]	34 PM, with FXS child (age: 39.7±7.3)	41 NC, with DD child (age: 39.0 ±6.6)	Cytogenetic records of FXS relative.	WAIS-R	• No significant differences in group means for FSIQ, VIQ, PIQ, verbal subtest scores, or performance subtest scores.
Thompson et al. (1994)[31,c]	5 FM 12 PM (age range: 20-49)	Normative sample to determine clinical range	FXS child in clinic.	WAIS-R	• The mean FSIQ, VIQ, and PIQ scores for PM carriers within average range.
Allingham-Hawkins et al. (1996)[28]	14 PM (age range: 30-65)	Normative sample to determine clinical range	FXS families.	WAIS-R	• Mean scores for FSIQ, VIQ, PIQ, and factor deviation IQs for verbal comprehension, perceptual organization, and freedom from distractibility were within a normal range. • No significant correlation between FSIQ score and repeat length detected.
Franke et al. (1998)[35,e]	13 FM, with FXS child (age: 35.9±10.0) 61 PM, with FXS child (age: 39.5±9.8) 17 PM, without FXS child (age: 40.1±15.0)	18 NC, siblings of FXS mothers (age: 31.7±12.1) 42 NC, with autistic child (age: 47.6±7.8)	Self-help groups and genetic counseling services.	WAIS-R	• No significant correlation between repeat length and FSIQ among PM carriers.
Franke et al. (1999)[33,e]	11 FM, with FXS child (age: 35.7±10.9) 65 PM, with FXS child (age: 39.8±9.4) 14 PM, without FXS child (age: 34.9±12.9)	18 NC, siblings of mothers (age: 32.0±11.9) 39 NC, with autistic child (age: 47.5±8.6)	Self-help groups and genetic counseling services.	WAIS-R	• Mean scores of PM mothers significantly lower than scores for NC groups for vocabulary (Cohens d=0.55, p<0.01), arithmetic (Cohen's d=0.73, p<0.01), verbal comprehension (Cohen's d=0.54, p,0.01), and object assembly (Cohens d=0.69, p<0.01) • No significant differences for

					information, digit span, similarities, digit symbol, picture completion, block design, or picture arrangement subtests. • Scores for PM mothers and PM non-mothers not significantly different. • Scores not significantly correlated with repeat length.
Johnston et al. (2001)[29]	85 PM (66 with <100 repeats; 19 with >100 repeats) (age range: 30-51)	Normative sample to determine clinical range	Child with FXS.	WAIS-III	• All PM mean scores for FSIQ, VIQ, PIQ, vocabulary, information, comprehension, arithmetic, digit span, picture arrangement, picture completion, block design, matrix reasoning, coding or symbol search within the normal range. • Two PM groups not different in scores. • No correlation between IQ and repeat length.
Bennetto et al. (2001)[13,d]	32 FM 96 PM (age range: 18-45)	37 NC, from FXS families (age range: 18-45)	Children's hospital as relatives of FXS individual.	WAIS-R	• PM scores not significantly different from controls for FSIQ, VIQ, or PIQ.
Allen et al. (2005)[32]	84 PM (age range: 18-50)	74 NC (age range: 18-50)	General population and FXS families.	WAIS-III	• PM scores not significantly different than NC for FSIQ and PIQ, but PM did score significantly lower than NC for VIQ (Cohen's d=0.44, p=0.05). • Significant negative linear association between VIQ and repeat length (Cohen's f2=0.04, p=0.01) and mRNA levels (Cohen's f^2=0.02, p=0.04). • Significant linear associations between repeat length and VIQ subtest

					scores for verbal comprehension index (Cohen's f^2=0.03, p=0.01), similarities (Cohen's f^2=0.03, p=0.01), information (Cohen's f^2=0.03, p=0.01), working memory index (Cohen's f^2=0.02, p=0.05), and letter-number sequencing (Cohen's f^2=0.02, p=0.04), but not vocabulary, arithmetic, or digit span.
Hessl et al. (2005)[36]	122 PM, without FXTAS (age: 49.9±12.8) 22 PM, with FXTAS (age: 63.1±12.8)	PM group not compared to a NC group or normative sample	FXS families.	WAIS-III	• Multiple regression analysis detected no significant effects of repeat length, protein, or transcript on FSIQ.
Lachiewicz et al. (2006)[34]	8 FM (age: 32.1±12.8) 39 PM (age: 36.7±8.7)	Normative sample to determine clinical range	FXS clinic.	WRAT-3	• PM scored significantly lower than standardized norms for arithmetic (Cohen's d=0.73, p<0.01), but not reading ability or spelling skills. • Among PM, significant correlation between repeat length and arithmetic scores detected (r=0.48, p<0.01).
Males					
Allen et al. (2005)[32]	19 PM (age range: 18-50)	24 NC (age range: 18-50)	General population and FXS families.	WAIS-III	• PM scores not significantly different from NC.
Hessl et al. (2005)[36]	26 PM, without FXTAS (age: 56.6±12.5) 42 PM, with FXTAS (age: 67.1±7.1)	PM group not compared to a NC group or normative sample	FXS families.	WAIS-III	• Significant negative correlation between FSIQ and repeat length (r=-0.32, p<0.05) among all PM males, but not *FMR1* mRNA or FMRP.

FM=full mutation; PM=premutation; NC=non-carrier; DD=developmentally disabled; FXS=fragile X syndrome; FXTAS=Fragile X-association tremor/ataxia syndrome

[a]Where available, either mean age (±SD) or age range of group presented.

[b]Measure names and abbreviations are presented in Table VI.

[c]Object of study was to analyze neuropsychological profile of FM carriers.

[d]Subject groups overlap with Sobesky, et al. (1994)[47], Sobesky et al. (1994)[38], Sobesky et al. (1996)[37], Riddle et al. (1998)[48], and Simon et al. (2001)[49]. All 6 papers looked at WAIS-R scores and, with the exception of Simon et al. (2001)[49], found no mean score differences for FSIQ, VIQ, or PIQ. In addition, the Sobesky et al. (1994)[38] and Sobesky et al. (1996)[37] did not detect a correlation with repeat length and IQ score. Only results of the most recent paper, Bennetto et al. (2001)[13], are shown here.

[e]Franke et al. (1996)[50], Franke et al. (1998)[35], and Franke et al. (1999)[33] contain overlapping subject populations. Franke et al. (1996)[50] and Franke et al. (1998)[35] analyzed repeat length effects on FSIQ and found no significant effects. Only the results of Franke et al. (1998)[35] are shown. Franke et al. (1999)[33] analyzed PIQ and VIQ subscales, so these results are shown also.

Table III. Summary of findings: Memory assessment in females.

Article Citation	Study Group(s)[a]	Comparison Group(s)[a]	Ascertainment	Measures[b]	Results
Thompson et al. (1994)[31,c]	5 FM 12 PM (age range: 20-49)	Normative sample to determine clinical range	FXS child in clinic.	WMS-R	• PM scored within average range for verbal memory.
Franke et al. (1999)[33]	11 FM, with FXS child (age: 35.7±10.9) 65 PM, with FXS child (age: 39.8±9.4) 14 PM, without FXS child (age: 34.9±12.9)	18 NC, siblings of FXS mothers (age: 32.0±11.9) 39 NC, with autistic child (age: 47.5±8.6)	Self-help groups and genetic counseling services.	HRD CBT	• No significant differences between mean scores for PM and NC groups for memory tests. • No significant correlations between scores and repeat length.
Bennetto et al. (2001)[13]	32 FM 96 PM (age range: 18-45)	37 NC, from FXS families	Children's hospital as relatives of FXS individual.	WMS-R	• No significant differences in scores between PM and NC, for both verbal and visual memory.

FM=full mutation; PM=premutation; NC=non-carrier; FXS=fragile X syndrome

[a]Where available, either mean age (±SD) or age range of group presented.

[b]Measure names and abbreviations are presented in Table IV.

[c]Object of study was to analyze neuropsychological profile of FM carriers.

Table IV. Summary of findings: Executive function assessment in females.

Article Citation	Study Group(s)[a]	Comparison Group(s)[a]	Ascertainment	Measures[b]	Results
Reiss et al. (1993)[30]	34 PM, with FXS child (age: 39.7±7.3)	41 NC, with DD child (age: 39.0±6.6)	Cytogenetic records of FXS relative.	TMT	• PM scores did not differ significantly from NC.
Thompson et al. (1994)[31,c]	5 FM 12 PM (age range: 20-49)	Normative sample to determine clinical range	FXS child in clinic.	WCST RRT	• PM mean scores within average range.
Franke et al. (1999)[33]	11 FM, with FXS child (age: 35.7±10.9) 65 PM, with FXS child (age: 39.8±9.4) 14 PM, without FXS child (age: 34.9±12.9)	18 NC, siblings of FXS mothers (age: 32.0±11.9) 39 NC, with autistic child (age: 47.5±8.6)	Self-help groups and genetic counseling services.	WCST TMT TOH SCWT VFT d2 test	• No significant differences between mean scores of PM and NC groups. • No significant correlations with scores and repeat length.
Bennetto et al. (2001)[13,d]	32 FM 96 PM (age range: 18-45)	37 NC, from FXS families (age range: 18-45)	Children's hospital as relatives of FXS individual.	WCST CNT	• No significant group differences in mean scores between PM and NC.

FM=full mutation; PM=premutation; NC=non-carrier; DD=developmentally disabled; FXS=fragile X syndrome

[a]Where available, either mean age (±SD) or age range of group presented.

[b]Measure names and abbreviations are presented in Table VI.

[c]Object of study was to analyze neuropsychological profile of FM carriers.

[d]Sobesky et al. (1994)[38], Sobesky et al. (1996)[37], and Bennetto et al. (2001)[13] all analyzed executive function scores on overlapping subject populations. Only the most recent study is shown here. All found no significant differences between PM and control groups. In addition, Sobesky et al. (1994)[38] and Sobesky et al. (1996)[37] did not detect a correlation with executive function score and repeat length.

Table V. Summary of findings: Spatial ability assessment in females.

Article Citation	Study Group(s)[a]	Comparison Group(s)[a]	Ascertainment	Measures[b]	Results
Reiss et al. (1993)[30]	34 PM, with FXS child (age: 39.7±7.3)	41 NC, with DD child (age: 39.0±6.6)	Cytogenetic records of FXS relative.	HNTLA	• No significant group differences in mean scores for block construction and spatial reasoning tasks between PM and NC.
Thompson et al. (1994)[31,c]	5 FM 12 PM (age range: 20-49)	Normative sample to determine clinical range	FXS child in clinic.	JLO ROF	• Mean PM scores within average range for visual-spatial perception and organization.
Bennetto et al. (2001)[13]	32 FM 96 PM (age range: 18-45)	37 NC, from FXS families (age range: 18-45)	Children's hospital as relatives of FXS individual.	WJ-R	• No significant differences in means scores between PM and NC for spatial relations subtest.

FM=full mutation; PM=premutation; NC=non-carrier; DD=developmentally disabled; FXS=fragile X syndrome

[a]Where available, either mean age (±SD) or age range of group presented.

[b]Measure names and abbreviations are presented in Table VI.

[c]Object of study was to analyze neuropsychological profile of FM carriers.

Table VI. Summary of findings: Neurobehavior assessment in females and males.

Article Citation	Study Group(s)[a]	Comparison Group(s)[a]	Ascertainment	Measures[b]	Results
Females					
Reiss et al. (1993)[30]	34 PM, with FXS child (age: 39.7±7.3)	41 NC, with DD child (age: 39.0±6.6)	Cytogenetic records of FXS relative.	Modified SADS-L interview Partial SIDP BPRS PSRS HSCL-90 NEO-PI	• No significant group differences in symptom severity or psychiatric diagnoses of major depression, dysthymia, bipolar disorder, psychotic disorder, social phobia, generalized anxiety disorder, schizotypal personality disorder, avoidant personality disorder, psychiatric disturbances, and personality traits. • Stereotypy-habit behavior more common in PM (Φ=0.30, $p<0.05$). • No significant association between repeat length and behavior.
Sobesky et al. (1994)[38,c,d]	21 FM 64 PM (age range: 18-45)	61 NC, with DD child 25 NC, from FXS families (age range: 18-45)	Records from FXS child at a children's hospital.	SADS-L interview	• Among PM, no significant increase in diagnostic rates of major depression syndrome, dysthymia, social phobia, or generalized anxiety disorder when compared to controls. • No significant correlation between repeat length and neurobehavior variables.
Thompson et al. (1994)[31,c]	5 FM 12 PM (age range: 20-49)	Normative sample to determine clinical range	FXS child in clinic.	SADS-L	• PM did not show increased rates of schizotypal features, but had higher rates of depression (75%) than would be expected in the general population.
Sobesky et al.	29 FM	35 NC, from FXS families	Records from	SIS interview	• No significant differences in

(1996) [37,c,d]	92 PM (age range: 18-45)	(age range: 18-45)	FXS child at a children's hospital.	MMPI-2	emotional traits between PM and NC. • No significant correlation with repeat length and scores.
Franke et al. (1998)[35,e]	13 FM, with FXS child (age: 35.9±10.0) 61 PM, with FXS child (age: 39.5±9.8) 17 PM, with FXS child (age: 40.1±15.0)	18 NC, siblings of FXS mothers (age: 31.7±12.1) 42 NC, with autistic child (age: 47.6±7.8)	Self-help groups and genetic counseling services.	DIGS PDE CS	• PM mothers had significantly increased frequency of anxiety disorders, including social phobia, compared to mothers of autistic children (Φ=0.18, p=0.05) and PM siblings without FXS children (Φ=0.25, p=0.02). • PM mothers diagnosed with major depressive episodes more often compared to non-mother PM females (p=0.03) and family controls (p<0.01). [f] • No significant differences between PM mothers and NC for psychoses, substance abuse, or personality disorders.
Johnston et al. (2001)[29]	85 PM (66 with <100 repeats; 19 with >100 repeats) (age range: 30-51)	Normative sample to determine clinical range	Child with FXS.	SCL-90-R	• Mean scores within normal range. • Significant positive correlation between repeat length and depression after adjusting for age (r=0.22, p=0.04). • Higher repeat group had significantly higher mean score for depression (Cohen's d=0.50, p=0.04) and interpersonal sensitivity (Cohen's d=0.56, p=0.02), but not anxiety or global severity of symptoms.
Hessl et al.	122 PM, without FXTAS	Normative sample to determine	FXS families.	SCL-90-R	• PM without symptoms of FXTAS

(2005)[36]	(age: 49.9±12.8) 22 PM, with FXTAS (age: 63.1± 12.8)	clinical range			scored significantly higher on obsessive-compulsive (Cohen's d=0.32, p<0.01), phobic anxiety (Cohen's d=0.25, p=0.01), and paranoid ideation (Cohen's d=0.25, p<0.01) scales. • PM with symptoms of FXTAS scored significantly higher on somatization (Cohen's d=0.86, p<0.01), obsessive-compulsive (Cohen's d=1.00, p<0.001), interpersonal sensitivity (Cohen's d=0.54, p0.05), depression (Cohen's d=0.74, p<0.01), psychoticism (Cohen's d=0.55, p<0.05), and global severity scales (Cohen's d=0.71, p0.01). • Among all PM, no significant correlations with repeat length, mRNA levels, or protein levels detected for any scales. • Significant association between mRNA level and anxiety scores for PM with X activation ratios less than 0.5 (r=0.57, p<0.001).
Males					
Hessl et al. (2005)[36]	26 PM, without FXTAS (age: 56.6±12.5) 42 PM, with FXTAS (age: 67.1±7.1)	Normative sample to determine clinical range	FXS families.	SCL-90-R	• PM without symptoms of FXTAS scored significantly higher on obsessive-compulsive (Cohen's d=0.89, p<0.0001) and psychoticism (Cohen's d=0.52, p<0.05) scales as well as overall symptom severity (Cohen's d=0.53, p<0.05) . • PM with symptoms of FXTAS scored significantly higher on somatization (Cohen's =0.51,

					p<0.01), obsessive-compulsive (Cohen's d=0.80, p<0.0001), interpersonal sensitivity (Cohen's d=0.44, p<0.01), depression (Cohen's d=0.68, p<0.001), anxiety (Cohen's d=0.55, p<0.01), phobic anxiety (Cohen's d=0.57, p<0.01), psychoticism (Cohen's d=0.47, p,0.01), and global severity scales (Cohen's d=0.65, p=0.01). • Among all PM, mRNA levels significantly positively correlated with somatization (r=0.38, p<.01), obsessive-compulsive (r=0.47, p<0.001), interpersonal sensitivity (r=0.38, p<0.01), depression (r=0.44, p<0.001), anxiety (r=0.41, p<0.01), hostility (r=0.42, p<0.01), paranoid ideation (r=0.45, p<0.001), psychoticism (r=0.50, p<0.001), global severity index (r=0.45, p<0.001) but not phobic anxiety, with correlation stronger in PM without FXTAS symptoms. • Paranoid ideation significantly positively correlated with CGG repeat (r=0.39, p<0.01). • No significant correlations with protein level.

FM=full mutation; PM=premutation; NC=non-carrier; DD=developmentally disabled; FXS=fragile X syndrome; FXTAS=Fragile X-association tremor/ataxia syndrome

[a]Where available, either mean age (±SD) or age range of group presented.

[b]Measure names and abbreviations are presented in Table VI.

[c]Object of study was to analyze neuropsychological profile of FM carriers.

[d]Sobesky et al. (1994)[47], Sobesky et al. (1994)[38], and Sobeksy et al. (1996)[37] used overlapping subject populations to analyze neurobehavioral phenotypes. Sobesky et al. (1994)[38] used the SADS-L interview and the MMPI-2, Sobesky et al. (1994)[47] used the SIS interview, and Sobesky et al. (1996)[37] used the MMPI-2 and the SIS interview. SADS-L results are shown from Sobesky et al. (1994)[38] and the most recent MMPI-2 and SIS results are shown from Sobesky et al. (1996)[37].

[e]Franke et al. (1996)[50] and Franke et al. (1998)[35] used overlapping subject populations to analyze neurobehavioral phenotypes using the DIGS interview. In addition, Franke et al. (1998)[35] used the PDE and Chapman Scale. The most recent study is shown.

[f]Could not calculate effect sizes based on data provided.

Addendum to Chapter 2

Since the publication of the manuscript above other studies have been published analyzing these phenotypes among premutation carriers not affected by FXTAS that also meet the criteria for inclusion. However, results continue to vary widely among research groups and with differing study design. Kogan et al. (2007)[1] reported neuropsychiatric scores for 40 males who carried a premutation but did not have FXTAS, 22 non-carrier family controls, and 43 non-carrier males. This study found no effect of the premutation on mood and anxiety, psychotic disorders, autistic symptoms, or schizotypal personality issues. However, an increased risk of working memory deficits as part of attention deficit hyperactivity disorder among the premutation group compared to controls was reported. Cornish et al. (2008)[2] analyzed a group of 40 premutation males compared to non-carrier males and reported no significant differences between the groups for full scale IQ, performance IQ, verbal IQ, sustained attention, visual working memory, and visual-spatial functioning but did report significant differences for selective attention. Curiously, they did report that premutation males under the age of 50 without motor symptoms of FXTAS significantly differed from controls for response inhibition while premutation males over the age of 50 without motor symptoms of FXTAS did not significantly differ from controls. This could indicate that inhibition symptoms precede motor symptoms of FXTAS. Grigsby et al. (2008)[3] reported executive functioning and verbal memory deficits among a sample of 28 male carriers of the premutation without FXTAS compared to controls. However, no difference for mental status, general intellectual functioning, working memory, remote recall of information, verbal learning,

language, information processing, visuospatial functioning, or temporal sequencing were detected. In a study of 43 women who carried a premutation allele, Minquez et al. (2008)[4] reported significantly lower scores for full scale IQ and performance IQ for premutation carriers compared to non-carrier controls, but not for verbal IQ. And lastly, two studies analyzed psychiatric symptoms among mothers of children with FXS who were also carriers of the premutation, but had different conclusions. Roberts et al. (2008)[5] reported that 93 premutation mothers had a significantly higher frequency of current agoraphobia without panic disorder, lifetime major depressive disorder, and lifetime panic disorder without agoraphobia but a significantly lower frequency of current and lifetime specific phobia, lifetime social phobia, and lifetime post-traumatic stress disorder compared to a control group. While Rodriguez-Revenga et al. (2008)[6] reported no difference in psychiatric symptoms between 34 mothers of children with FXS who were also carriers of the premutation allele and 39 non-carrier mothers of children with mental retardation.

Given the results of these additional studies, the conclusions of the review paper above remain the same. In addition, though significant differences between premutation and control groups are reported, the effect sizes are not always large and the mean scores of premutation groups are not necessarily in the clinical range. The effect sizes for the significant differences between premutation and controls groups for executive functioning, logical memory immediate recall, and logical memory delayed recall were medium (Cohen's $f^{2=}0.15$), small (Cohen's $f^{2=}0.11$), and medium (Cohen's $f^{2=}0.18$), respectively.[3] The effect sizes for difference in FSIQ (Cohen's d=0.52) and PIQ were medium and small (Cohen's d=0.48), respectively.[4] Elevated frequencies of lifetime

major depressive disorder, lifetime panic disorder, and current agoraphobia among premutation carriers had small effect sizes (Cohen's ω of 0.05, 0.08, and 0.05, respectively).[5] Only the difference in scores for working memory was large (Cohen's d 0.84).[1] Scores were not available for effect size calculations for Cornish et al. (2008)[2]. In studies of neuropsychological and neurobehavioral phenotypes, it is important to keep in mind that the use of statistics to detect differences in scores between groups is only a rough tool and significant differences do not necessarily indicate the presence of a clinical disorder or deficit. In addition, the lack of differences between groups should be equally emphasized.

References

1. Kogan CS, Turk J, Hagerman RJ, Cornish KM. Impact of the Fragile X mental retardation 1 (FMR1) gene premutation on neuropsychiatric functioning in adult males without fragile X-associated Tremor/Ataxia syndrome: A controlled study. *Am J Med Genet B Neuropsychiatr Genet.* Dec 28 2007.
2. Cornish KM, Li L, Kogan CS, et al. Age-dependent cognitive changes in carriers of the fragile X syndrome. *Cortex.* Jun 2008;44(6):628-636.
3. Grigsby J, Brega AG, Engle K, et al. Cognitive profile of fragile X premutation carriers with and without fragile X-associated tremor/ataxia syndrome. *Neuropsychology.* Jan 2008;22(1):48-60.
4. Minguez M, Ibanez B, Ribate MP, et al. Risk of cognitive impairment in female premutation carriers of fragile X premutation: Analysis by means of robust segmented linear regression models. *Am J Med Genet B Neuropsychiatr Genet.* Jun 18 2008.
5. Roberts JE, Bailey DB, Jr., Mankowski J, et al. Mood and anxiety disorders in females with the FMR1 premutation. *Am J Med Genet B Neuropsychiatr Genet.* Jun 13 2008.
6. Rodriguez-Revenga L, Madrigal I, Alegret M, Santos M, Mila M. Evidence of depressive symptoms in fragile-X syndrome premutated females. *Psychiatr Genet.* Aug 2008;18(4):153-155.

Chapter 3

Investigation of phenotypes associated with mood and anxiety among male and female fragile X premutation carriers.

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This chapter describes research analyzing *FMR1* repeat length associations with self-reported neurobehavioral scores among the largest study population of males and females to date. My contributions to this work include the selection of outcome variables for analysis, data clean-up, variable coding, identification and application of the correlated data analysis for repeat length associations, formation of the cluster structures, follow-up contact with all participants to identify those with a FXS child, formation of a strategy to adjustment for multiple testing, interpretation of findings, follow-up contact with refusals, as well as manuscript preparation and publication.

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Abstract

The fragile X disorder spectrum, due to a CGG expansion in *FMR1*, includes fragile X syndrome (>200 repeats) and the premutation-associated disorders of ovarian insufficiency and tremor/ataxia syndrome (~55-199 repeats). Altered neurobehavioral profiles including variation of phenotypes associated with mood and anxiety may be expected among younger premutation carriers given this spectrum of disorders. However, previous studies have produced conflicting findings, providing the motivation to examine these phenotypes further. We investigated measures of mood and anxiety in 119 males and 446 females age 18-50 ascertained from families with a history of fragile X syndrome and from the general population. Scores were analyzed using a linear model with repeat length as the main predictor, adjusting for potential confounders. Repeat length was not associated with anxiety, but was marginally associated with depression and negative affect in males and negative affect only in females. These results suggest that premutation carriers may be at risk for emotional morbidity; however, phenotypic differences were subtle and of small effect size.

Key words: FXTAS; FMR1; premutation; CGG repeat; neurobehavior; depression; anxiety

Introduction

Fragile X mental retardation 1 gene (*FMR1*) is located near the end of the long arm of the X chromosome and contains a highly polymorphic CGG repeat in the 5' UTR of exon 1. The most common alleles for *FMR1* contain fewer than 40 repeats (Snow et al., 1993). In rare cases, the repeat can become unstable and expand. If the repeat number exceeds 200, termed full mutation, the gene becomes hypermethylated and no gene product, FMRP, is made due to transcriptional silencing (Sutcliffe et al., 1992). This loss of FMRP is responsible for fragile X syndrome (FXS), the most common identified form of heritable mental retardation (Pieretti et al., 1991). FXS has a prevalence of roughly 1 in 4000 for males and1 in 8000 for females (Crawford et al., 2001). The clinical presentation of males with FXS is variable, but the most common phenotypes include mild to severe mental retardation, developmental delay, hyperactivity, social anxiety and other anxiety disorders, and autistic-like features. As a group, females are more mildly affected due to X-inactivation (Reiss & Dant, 2003).

Intermediate alleles, about 45-54 repeats, may or may not be stable during transmission from parent to child and do not expand to a full mutation in one generation. Premutation alleles are defined as unmethylated alleles with repeat numbers in the range of 55-199 that are unstable during transmission and can lead to a full mutation in one to three generations (Maddalena et al., 2001). The smallest repeat to expand to a full mutation in one generation is 59 repeats (Nolin et al., 2003). About 1 in 250 females and 1 in 800 males carry one of these high repeat alleles, termed premutation alleles (Crawford et al., 2001). Premutation alleles remain unmethylated, therefore *FMR1* is transcriptionally active and produces FMRP. *FMR1* mRNA levels linearly increase

across the premutation range due higher rates of transcription as a results of a mechanism that is presently not understood (Tassone et al., 2000a; Tassone et al., 2000b; Kenneson et al., 2001; Tassone & Hagerman, 2003; Allen et al., 2004; Garcia-Alegria et al., 2007). However, a negative association has been found between FMRP and repeat size in premutation carriers due to a decreased translation efficiency of the mRNA as the repeat size increases (Feng et al., 1995; Tassone et al., 2000b; Kenneson et al., 2001; Primerano et al., 2002; Tassone & Hagerman, 2003).

Two phenotypes are associated with these premutation alleles. Males with the premutation who are over the age of 50 are at risk for a neurodegenerative tremor/ataxia syndrome (FXTAS). This disorder is very rare in females who carry the premutation allele. However, female carriers of the premutation are at an increased risk of primary ovarian insufficiency (FXPOI) (Sherman, 2000; Abrams, 2007; Welt, 2007). FXTAS and FXPOI have not been found to be associated with the full mutation, thus they are not associated with a lack of the *FMR1* protein product. For FXTAS, converging evidence indicates that the phenotype is a result of the toxic effect of the expanded repeat length in the *FMR1* mRNA (Hagerman & Hagerman, 2004).

Numerous studies have investigated neuropsychological phenotypes among premutation allele carriers. Conflicting results have been reported and a definitive profile fails to emerge (for review, see Hunter et al., In press). Most of these studies were conducted prior to the identification of FXTAS, have primarily utilized small samples with varying ascertainment methods and phenotype measurement modalities, lack proper controls, and concentrate solely on female premutation carriers. The use of female study

populations makes interpretation of the results difficult due to the X-linked nature of *FMR1*.

Several studies have concluded that premutation allele among females lacks a detectable neuropsychological phenotype (e.g., (Reiss et al., 1993; Thompson et al., 1994; Bennetto et al., 2001)). Other studies, some with both males and females participants, have concluded that premutation allele carriers manifest milder forms of clinical features seen in FXS, including learning disabilities, cognitive deficits, developmental delay, and attention deficits, as well as physical features such as prominent ears and flexible finger joints (e.g., (Hull & Hagerman, 1993; Cornish et al., 2005)). One study suggested that premutation allele carriers may to be at a higher risk of autism spectrum disorders (Aziz et al., 2003), although this has not been confirmed.

An increased risk of anxiety and mood disorders among premutation allele carriers has not been established. Some studies have reported a lack of phenotype (e.g., (Reiss et al., 1993; Sobesky et al., 1996)), while others have reported repeat length associations with psychiatric symptoms (e.g., (Franke et al., 1998; Johnston et al., 2001)). More recently, Hessl et al. (2005) found that *FMR1* transcript level, but not repeat length or FMRP levels, was significantly associated with increased severity of psychiatric symptoms in males, independent of FXTAS status.

In 2005 we published a study examining cognition among 66 men and 217 women with varying *FMR1* repeat lengths (Allen et al., 2005). We reported that women who were carriers of premutation alleles had significantly lower verbal IQ scores compared to non-carriers. Here, we examine phenotypes associated with mood and anxiety among carriers of fragile X premutation alleles in the largest study population to

date consisting of 119 men and 446 women. All study participants were between the ages of 18 and 50 at the time of testing. Thus, any phenotypes detected here would most likely not be due to the presence of FXTAS, but would potentially indicate a more global impairment among premutation carriers in general.

Methods

Study population

A large sample of study participants were recruited from the general population and from families with a history of FXS. The study population was the result of a 78% participation rate and included males and females with repeat sizes ranging from 20 to 180. Participants from the general population were recruited from Atlanta area hospitals, churches, universities, technical schools, corporations, sports events, and health fairs. Recruitment from families with a known history of FXS was pursued to enrich the sample population with carriers of expanded alleles. FXS families were identified through clinics, internet postings, FXS parent groups, and word of mouth. Participation was limited to those aged 18 to 50 years whose primary language was English. The majority of participants were unrelated, while some were ascertained from the same pedigree. In the female sample, there were 47, 14, 8, 3, and 2 families with 2, 3, 4, 6, and 7 female participants, respectively. In the male sample, there are 11, 1, 1, and 1 families with 2, 3, 4, and 5 participants, respectively. The remaining were singletons. Thus, overall 446 women were ascertained from 320 families and 119 men from 99 families. The protocols and consent forms for ascertainment were approved by the Institutional

Review Board at Emory University. For more information on study population ascertainment, see Allen, et al. (2005).

In an effort to create roughly equal sized groups for analysis, particularly for males, we used the following allele group definitions: intermediate allele = 41-60 repeats and premutation allele = 61-199 repeats. Although these differ slightly from those proposed for a clinical application (i.e., those based on risk for instability) (Sherman et al., 2005), they are similar to previous studies used to examine *FMR1* mRNA levels (Allen et al., 2004; Garcia-Alegria et al., 2007). At this point in time, there is no biological underpinning for any of these definitions, particularly with respect to risk for neuropsychological or neurobehavioral phenotypes. Thus, we used those outlined above to better balance sample sizes.

Data collection

Each study participant was asked to complete a medical history questionnaire and a neuropsychological test battery that included the Wechsler Adult Intelligence Scale 3rd Edition (WAIS-III) to determine IQ scores as well as several widely-used self-report inventories of mood and anxiety described below. Test administrators were blind to the subject's *FMR1* genotype as well as family history of FXS. For molecular analysis to determine CGG repeat size of *FMR1*, participants were asked to provide a blood or buccal brush sample.

Measurement of IQ and phenotypes associated with mood and anxiety

Symptoms of depression were measured with The Center for Epidemiologic Studies Depression Scale (CES-D) (Radloff, 1977). The CES-D consists of 20 items rated on a four-point scale, indicating how frequently each symptom was experienced in the past week (0 = rarely or none of the time, 1 = some or a little of the time, 2 = occasionally or a moderate amount of time, and 3 = all of the time). Total scores can range from 0 to 60, with higher scores indicating higher levels of emotional distress associated with depression. Scores of 16 or more suggest clinically-significant depression. The CES-D has high internal consistency, with a value of about 0.85 for the general population and about 0.91 for a patient sample. The test-retest reliability is moderate with a value of about 0.58. CES-D scores were obtained for all participants.

The State-Trait Anxiety Inventory (STAI) is a two-part inventory used to measure levels of current anxiety (state anxiety) and general anxiety susceptibility (trait anxiety) (Spielberger, 1983). Each subscale consists of 20 items, each rated on a four-point scale. The state anxiety subscale measures the severity of current anxiety symptoms (1 = not at all, 2 = somewhat, 3 = moderately so, and 4 = very much so). The trait anxiety subscale measures the frequency of anxiety symptoms experienced in general (1 = almost never, 2 = sometimes, 3 = often, and 4 = almost always). STAI state and trait anxiety scores range from 20 to 80, with higher scores indicating higher levels of anxiety. The STAI has good internal consistency, ranging from 0.86 to 0.96. Test-retest reliability is highly dependent on the subject population and can range from 0.65 to 0.86 for the trait anxiety subscale and 0.16 to 0.62 for the state anxiety subscale. This inventory was added to the

test battery after the initiation of study participant recruitment, thus state anxiety and trait anxiety scores for 54 male and 174 female participants were not obtained.

The Social Phobia and Anxiety Inventory (SPAI) is a two-part inventory used to measure symptoms of social phobia in various social situations (Turner, 1996). The social phobia subscale consists of 32 items rated on a seven-point scale indicating how frequently symptoms of social phobia are experienced in various social situations (0 = never to 6 = always). The agoraphobia subscale consists of 13 items rated on the same seven-point scale. By subtracting the social phobia and agoraphobia subscores, this test is capable of distinguishing pure social phobia from social distress due to panic disorder with agoraphobia. Higher subscale scores and "difference" scores reflect higher levels of anxiety. An agoraphobia subscale score of 39 or above is indicative of possible panic disorder, while a "difference" score of 80 or above is indicative of probable social phobia. The SPAI has high internal consistencies with a value of 0.96 for the social phobia subscale and 0.85 for the agoraphobia subscale. Test-retest reliability ranges from 0.74 to 0.86, depending on the subscale. SPAI scores for two female participants were incomplete and thus unavailable for analysis.

General and specific emotional states were measured with The Positive and Negative Affect Schedule (PANAS), a 60-item scale (Watson, 1994). Two broad affective states, negative and positive, are each measured by 10 items, all on a five-point scale indicating the extent to which each emotion was felt in the past year (1 = very slightly or not at all, 2 = a little, 3 = moderately, 4 = quite a bit, and 5 = extremely). Using the same five-point scale, the remaining 40 items are used to measure 11 specific affective states: fear, sadness, guilt, hostility, shyness, fatigue, surprise, joviality, self-

assurance, attentiveness, and serenity. The PANAS has internal consistencies ranging from 0.72 to 0.94, depending on the subscale and study population. Test-retest reliabilities range from 0.51 to 0.68. The PANAS questionnaire was incomplete for one male subject, and thus his subscale scores were not available.

Lastly, each subject's full-scale IQ and verbal IQ was measured as part of the neuropsychological test battery using the Wechsler Adult Intelligence Scale 3rd Edition (WAIS-III) (Wechsler, 1997).

Laboratory methods

FMR1 CGG repeat number

Each study participant was asked to provide a blood or buccal brush sample for molecular analysis. For more information on molecular analysis, see Allen, et al. (2005). Briefly, DNA was extracted from samples with the Qiagen QiAmp DNA Blood Mini Kit. A fluorescent-sequencer method using an ABI Prism 377 DNA sequencer was used to determine *FMR1* CGG repeat length (Meadows et al., 1996). When no repeat length band for males or only one band for females was present, an alternative PCR-based, hybridization technique was used to identify larger premutation or full mutation alleles (Brown et al., 1993). For heterozygous females, CGG repeat length from the larger repeat allele was used in subsequent statistical analyses.

Statistical analysis

Descriptive statistics for the study population are shown in Table I, with male and female data shown separately. The demographic variables included age at the time of

testing (continuous variable), ethnicity (dichotomous variable: 0 = Caucasians and Asians, 1 = other ethnicities), education level reached at the time of testing (dichotomous variable: 0 = high school completed or less, 1 = some college completed or more), household income level at time of testing (dichotomous variable: 0 = less than $50,000, 1 = $50,000 or more), full-scale IQ (continuous variable), method of ascertainment (dichotomous variable: 0 = recruited from families with a known history of FXS, 1 = recruited from the general population), and anxiety or depression medication use at the time of testing (dichotomous variable: 0 = not taking anxiety/depression medications, 1 = taking anxiety/depression medications). Analysis of variance was used to test for repeat length group differences for continuous demographic variables while chi square tests were used for dichotomous demographic variables. Significant differences between repeat length groups were noted for race and ascertainment source among male participants and for these same two variables plus age at testing, level of household income, and the use of anxiety and/or depression medication at the time of testing for female participants (Table I). Thus, all models for emotional outcomes were adjusted for age, income, medication use, race, and ascertainment source.

For each test analyzed, males and females were modeled separately due to the X-linked nature of *FMR1*. The distributions of scores for each measure were tested for normality. Scores were transformed, if necessary, to produce a normal distribution for further analysis. A natural logarithm transformation was needed for the STAI state and trait anxiety and for the PANAS general negative affect scores. A square root transformation was required for the CES-D and the SPAI social phobia and agoraphobia scores.

Scores were analyzed using general linear regression equations modeled for correlated outcomes. This approach was used to adjust for correlated data that may have occurred among relatives from the same family due to shared environmental or genetic factors. In addition, this approach is robust to the varying family cluster sizes among our sample population. Length of the *FMR1* repeat was used as the main predictor of mood and anxiety scores and was classified in two ways. First, repeat length was used as a continuous variable. Second, subjects were divided into three groups based on their repeat length: non-carriers (40 repeats or less), intermediate allele carriers (41 to 60 repeats) and premutation allele carriers (61 to 199 repeats). In this analysis, repeat length classes were used as the predictor with the non-carrier group as the reference group. A Tukey's post hoc analysis was performed to identify differences in adjusted mean scores among repeat length groups. All interaction terms that consisted of a covariate and repeat length, either as a continuous or as a class variable, were tested for each model.

The psychosocial stress of raising a child with FXS could contribute to any emotional morbidity detected in our analyses. This possibility was addressed in two ways. First, the analyses were repeated including adjustment for having a FXS child. Second, premutation carriers were divided into two groups: those with a FXS child and those without a FXS child. Analysis of covariance (ANCOVA) was used to test for mean score differences between the two groups.

Although many statistical tests were performed, adjustment for multiple testing was not straightforward due to the correlation among the eight mood and anxiety outcome scores. Further, scores were tested in two consecutive models, one with repeat length as a continuous variable and one with repeat length as a categorical variable, so

these tests cannot be considered independent due to the correlation between these two repeat length variables. Thus, we present the results using a significance level of $p<0.05$, but provide all p-values, and discuss the results in this context. Further discussion of the influence of multiple testing on interpretation of results is provided in Discussion. In addition, we calculated the effect size for each significant mean score difference between repeat length groups using Cohen's d score (Cohen, 1992). According to Cohen, values of 0.2, 0.5, and 0.8 indicate small, medium, and large effect sizes, respectively (Cohen, 1992). All statistical analyses were performed using the PROC MIXED procedure on the SAS System for Windows, Release 8.2.

Results

For males, positive associations were detected between repeat length and depression scores (CES-D, p=0.03; Table II) and general negative affect scores of the PANAS (p=0.04, Table II). No associations were observed for repeat length and state anxiety, trait anxiety, positive affect, social phobia, or agoraphobia scores (Table II). Using repeat length group as a predictor, no mean score differences among repeat length groups were seen for any outcome measure (Table III). For all models, all interaction terms for the repeat length were tested to analyze any modifier effects of the confounders. No interaction terms were significant for any model for any confounder, including age.

For females, a positive association was seen between repeat length and general negative affect scores (p=0.04; Table II), similar to the results among males. This association was also indicated when repeat length group was used as a predictor variable: premutation carriers scored higher than non-carriers although the effect size was small

(Cohen's d=0.36, p=0.02; Table III). However, unlike males, there was no association observed with depression scores. No associations were observed for state anxiety, trait anxiety, positive affect, social phobia, or agoraphobia scores (Table II) nor were there any group mean differences (Table III).

The PANAS also provides subscores for specific emotions. In order to follow up on the association of repeat length and general negative affect among males and females, the subscores were analyzed for all of the specific negative emotions tested by the PANAS: fear, sadness, guilt, and hostility. Results of this analysis are shown in Tables IV and V. Among males, positive linear associations were detected with sadness (p=0.03) and guilt (p=0.01), but not fear or hostility. In addition, the premutation group had a higher mean score for guilt compared to the non-carrier group, with a medium effect size (Cohen's d=0.78, p=0.03). Among females, linear associations with repeat size were not detected for any of the four specific emotion scores. However, the premutation group did have higher mean scores for fear (Cohen's d=0.30, p=0.05) and hostility (Cohen's d=0.28, p=0.05) compared to the non-carrier group, though with small effect sizes.

To investigate the clinical implications of scores, diagnostic rates provided by the relevant measures were analyzed. The CES-D measure provides a cutoff value for the diagnosis of probable depression, while the SPAI provides cutoff values for probable social phobia and probable panic disorder. Although the means did not exceed the diagnostic cutoff for any of the repeat length groups, the distribution of the frequency of participants who scored above this cutoff score was examined (Table VI). For probable depression, the rates differed by group for males (Fisher's Exact test: p=0.0093), but not

females. For probable social phobia, the frequency of those exceeding the cutoff increased with increasing repeat length group for females (Fisher's Exact test: p=0.0004). Finally, for probable panic disorder, premutation males had higher rates compared to non-carriers (Fisher's Exact test: p=0.0095).

Any phenotypes detected in this study could potentially be due to the psychosocial impact of raising a child with FXS. ANCOVA and linear regression analysis were performed with adjustment for raising a child with FXS in addition to other significant covariates. This adjustment had no effect on the statistical outcomes. Carriers of the premutation were then divided into two groups (those with and those without a child with FXS) and mean scores between the two groups were compared using ANCOVA. No score differences were detected for any mood or anxiety test.

Discussion

The purpose of this study was to examine phenotypes associated with mood and anxiety that may be associated with CGG repeat size or allele class status of the *FMR1* gene among younger adults, those who are at low risk for the clinical expression of FXTAS. Two primary strengths of this study were the relatively large sample size compared with other published studies and the ascertainment strategy that did not involve the fragile X-associated spectrum disorders. Specifically, we identified premutation carriers through families with a known diagnosis of a child with FXS, not because of their own symptoms, and we excluded subjects over the age of 50 in order to avoid the inclusion of premutation carriers with FXTAS. However, we must acknowledge that an ascertainment bias probably exists for any study of mood and anxiety phenotypes, since

those who agree to participate in a research study may be less likely to have clinical mood and anxiety problems than those who do not. This would be true for both non-carriers and carriers of the premutation.

Our analyses did not detect any repeat length associations with social phobia, agoraphobia, or state or trait anxiety. However, we identified a subtle association between *FMR1* CGG repeat size and emotional phenotypes in males and females. Specifically, repeat length had a linear association with negative affect in males and females and with depression in males only (Tables II and III). Though negative affect and depression represent two different factors, they are related. Increased negative affect is highly associated with depression along with decreased positive affect. However, no repeat length associations with positive affect were detected. In addition, negative affect is highly associated with anxiety, but no repeat length associations were detected in males or females with regard to anxiety. Other factors, such as age, race, and medication use, also contributed to the variation in emotional phenotype in our study, although we adjusted for these variables when examining the repeat length effects.

In a follow-up analysis of negative emotions from the PANAS, premutation males reported increased feelings of guilt and sadness compared to non-carriers while premutation females were at an increased risk of feeling fear and hostility (Tables IV and V). These contradictory results makes interpretation difficult, as one might expect the profile of negative emotions to be the same between males and females if it were related to the premutation effect. Further, an increased score for guilt could be expected among premutation females due to their risk of passing on an expanded allele which results in

having a child with FXS. Therefore, the significant association between guilt scores and repeat length among males and not females is contrary to expectation.

These results support those of other recent studies that have reported emotional morbidity among premutation carriers (Dorn et al., 1994; Franke et al., 1998; Johnston et al., 2001; Hessl et al., 2005). Though the differences detected here are statistically significant at $p<0.05$, they are subtle and might not indicate a susceptibility to a clinical disorder. Indeed, all mean scores differences between female repeat length groups were of small effect size while the mean score difference noted among male repeat length groups was of medium effect size (Cohen, 1992). Further, it is important to note that the mean scores for the premutation group were not within the diagnostic range for probable depression, probable social phobia, and panic disorder. However, as a group, both males and females with the premutation did show higher rates of probable social phobia and panic disorder, and males with the premutation also showed higher rates of probable depression (Table VI).

In reporting results above, we used a significance level of 0.05 based on an unadjusted p-value, which is most likely too liberal due to multiple testing. However, the adjustment to the p-value to accommodate multiple testing is not straightforward: 1) the mood and anxiety outcome variables are correlated among samples and 2) the two sets of analyses defining *FMR1* repeat length predictor in two ways (binary and continuous) are correlated. In an attempt to examine the effect of these influences on the p-values, we used the Cheverud-Nyholt estimate (Cheverud, 2001; Nyholt, 2004) to obtain an estimate of the number of effective tests given the correlation among the eight outcome measures. We found that the effective number of tests for the male and female samples would be

6.1 and 6.2, respectively. Using these results and applying the Bonferroni correction, significance at the 0.05 level would be indicated if the test outcome had an associated $p<0.0082$ (0.05/6.1) and $p<0.0081$ (0.05/6.2) for male and female analyses, respectively. With this adjustment, none of the results presented here remain statistically significant. Further adjustment to account for modeling each outcome measure twice, using repeat length as a continuous variable and repeat length as a categorical variable, would only increase the number of effective tests and lower the required p-value for statistical significance. Thus, all findings reported are only marginally significant and must be confirmed in independent studies. This and the small effect sizes, together, emphasize the subtlety of the phenotypic differences observed in this study.

A strength of this study was the use of measurements that provide scores related to severity of symptoms associated with psychiatric disorders, not just to the presence or absence of a clinical disorder. However, our use of self-report questionnaires provides only a 'snapshot' of mental health at the time of testing, rather than a lifetime occurrence of a mental disorder. This is an important point, as most disorders, including depression and anxiety, tend to be episodic. In addition, self-report assumes the subjects retain insight into their mental health, irrespective of their situation on the day of testing.

In an effort to control for any effect of the psychosocial stress involved in raising a child with FXS, we performed additional analyses. We were not able to show that raising a child with FXS accounted for any of the mood and anxiety phenotype differences that we observed among women with and without the premutation. However, there are other factors potentially related to carrying the premutation (e.g., being a carrier

and not having children, guilt of carrying a mutation, etc) that could influence mood and anxiety for which we could not account in our analyses.

The effect of age on emotional morbidity cannot be ignored, especially in the context of premutation carriers who are at risk for late onset FXTAS. Most often, clinical motor symptoms of FXTAS have an onset around mid 50s to 60 years of age (Jacquemont et al., 2004). However, signs of cognitive impairment may precede motor symptoms (Grigsby et al., 2006). Our study population was limited to those ages 18 to 50 years. We suggest that the subtle emotional phenotypes reported here are most likely not due to the psychosocial stress of potentially having FXTAS. However, we cannot disregard the possibility that these phenotypes may be precursors to FXTAS. We tested this possibility by including age as a covariate in all analyses and did not detect any interaction between age and repeat length.

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References

Abrams L. 2007. From POF to POI: Evolution of a Term. *National Fragile X Foundation Quarterly 29*.

Allen EG, He W, Yadav-Shah M, Sherman SL. 2004. A study of the distributional characteristics of FMR1 transcript levels in 238 individuals. *Hum Genet 114*:439-447.

Allen EG, Sherman S, Abramowitz A, Leslie M, Novak G, Rusin M, Scott E, Letz R. 2005. Examination of the effect of the polymorphic CGG repeat in the FMR1 gene on cognitive performance. *Behav Genet 35*:435-445.

Aziz M, Stathopulu E, Callias M, Taylor C, Turk J, Oostra B, Willemsen R, Patton M. 2003. Clinical features of boys with fragile X premutations and intermediate alleles. *Am J Med Genet B Neuropsychiatr Genet 121*:119-127.

Bennetto L, Pennington BF, Porter D, Taylor AK, Hagerman RJ. 2001. Profile of cognitive functioning in women with the fragile X mutation. *Neuropsychology 15*:290-299.

Brown WT, Houck GE, Jr., Jeziorowska A, Levinson FN, Ding X, Dobkin C, Zhong N, Henderson J, Brooks SS, Jenkins EC. 1993. Rapid fragile X carrier screening and prenatal diagnosis using a nonradioactive PCR test. *Jama 270*:1569-1575.

Cheverud JM. 2001. A simple correction for multiple comparisons in interval mapping genome scans. *Heredity 87*:52-58.

Cohen J. 1992. A power primer. *Psychological Bulletin 112*:155-159.

Cornish K, Kogan C, Turk J, Manly T, James N, Mills A, Dalton A. 2005. The emerging fragile X premutation phenotype: evidence from the domain of social cognition. *Brain Cogn 57*:53-60.

Crawford DC, Acuna JM, Sherman SL. 2001. FMR1 and the fragile X syndrome: human genome epidemiology review. *Genet Med 3*:359-371.

Dorn MB, Mazzocco MM, Hagerman RJ. 1994. Behavioral and psychiatric disorders in adult male carriers of fragile X. *J Am Acad Child Adolesc Psychiatry 33*:256-264.

Feng Y, Zhang F, Lokey LK, Chastain JL, Lakkis L, Eberhart D, Warren ST. 1995. Translational suppression by trinucleotide repeat expansion at FMR1. *Science 268*:731-734.

Franke P, Leboyer M, Gansicke M, Weiffenbach O, Biancalana V, Cornillet-Lefebre P, Croquette MF, Froster U, Schwab SG, Poustka F, Hautzinger M, Maier W. 1998. Genotype-phenotype relationship in female carriers of the premutation and full mutation of FMR-1. *Psychiatry Res 80*:113-127.

Garcia-Alegria E, Ibanez B, Minguez M, Poch M, Valiente A, Sanz-Parra A, Martinez-Bouzas C, Beristain E, Tejada MI. 2007. Analysis of FMR1 gene expression in female premutation carriers using robust segmented linear regression models. *Rna 13*:756-762.

Grigsby J, Brega AG, Jacquemont S, Loesch DZ, Leehey MA, Goodrich GK, Hagerman RJ, Epstein J, Wilson R, Cogswell JB, Jardini T, Tassone F, Hagerman PJ. 2006. Impairment in the cognitive functioning of men with fragile X-associated tremor/ataxia syndrome (FXTAS). *J Neurol Sci*.

Hagerman PJ, Hagerman RJ. 2004. The fragile-X premutation: a maturing perspective. *Am J Hum Genet 74*:805-816.

Hessl D, Tassone F, Loesch DZ, Berry-Kravis E, Leehey MA, Gane LW, Barbato I, Rice C, Gould E, Hall DA, Grigsby J, Wegelin JA, Harris S, Lewin F, Weinberg D, Hagerman PJ, Hagerman RJ. 2005. Abnormal elevation of FMR1 mRNA is associated with psychological symptoms in individuals with the fragile X premutation. *Am J Med Genet B Neuropsychiatr Genet 139*:115-121.

Hull C, Hagerman RJ. 1993. A study of the physical, behavioral, and medical phenotype, including anthropometric measures, of females with fragile X syndrome. *Am J Dis Child 147*:1236-1241.

Hunter J, Abramowitz A, Rusin M, Sherman S. submission. Neuropsychological and neurobehavioral phenotypes among FMR1 premutation allele carriers: a review of current literature. *Behav Genet* [In press].

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. 2004. Penetrance of the fragile X-associated tremor/ataxia syndrome in a premutation carrier population. *Jama 291*:460-469.

Johnston C, Eliez S, Dyer-Friedman J, Hessl D, Glaser B, Blasey C, Taylor A, Reiss A. 2001. Neurobehavioral phenotype in carriers of the fragile X premutation. *Am J Med Genet 103*:314-319.

Kenneson A, Zhang F, Hagedorn CH, Warren ST. 2001. Reduced FMRP and increased FMR1 transcription is proportionally associated with CGG repeat number in intermediate-length and premutation carriers. *Hum Mol Genet 10*:1449-1454.

Maddalena A, Richards CS, McGinniss MJ, Brothman A, Desnick RJ, Grier RE, Hirsch B, Jacky P, McDowell GA, Popovich B, Watson M, Wolff DJ. 2001. Technical standards and guidelines for fragile X: the first of a series of disease-specific supplements to the Standards and Guidelines for Clinical Genetics Laboratories of the American College of Medical Genetics. Quality Assurance Subcommittee of the Laboratory Practice Committee. *Genet Med 3*:200-205.

Meadows KL, Pettay D, Newman J, Hersey J, Ashley AE, Sherman SL. 1996. Survey of the fragile X syndrome and the fragile X E syndrome in a special education needs population. *Am J Med Genet 64*:428-433.

Nolin SL, Brown WT, Glicksman A, Houck GE, Jr., Gargano AD, Sullivan A, Biancalana V, Brondum-Nielsen K, Hjalgrim H, Holinski-Feder E, Kooy F, Longshore J, Macpherson J, Mandel JL, Matthijs G, Rousseau F, Steinbach P, Vaisanen ML, von Koskull H, Sherman SL. 2003. Expansion of the fragile X CGG repeat in females with premutation or intermediate alleles. *Am J Hum Genet 72*:454-464.

Nyholt DR. 2004. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. *Am J Hum Genet 74*:765-769.

Pieretti M, Zhang FP, Fu YH, Warren ST, Oostra BA, Caskey CT, Nelson DL. 1991. Absence of expression of the FMR-1 gene in fragile X syndrome. *Cell 66*:817-822.

Primerano B, Tassone F, Hagerman RJ, Hagerman P, Amaldi F, Bagni C. 2002. Reduced FMR1 mRNA translation efficiency in fragile X patients with premutations. *Rna 8*:1482-1488.

Radloff LS. 1977. The CES-D scale: a self-report depression scale for research in the general population. *Applied Psychological Measurement 1*:385-401.

Reiss AL, Dant CC. 2003. The behavioral neurogenetics of fragile X syndrome: analyzing gene-brain-behavior relationships in child developmental psychopathologies. *Development and psychopathology 15*:927-968.

Reiss AL, Freund L, Abrams MT, Boehm C, Kazazian H. 1993. Neurobehavioral effects of the fragile X premutation in adult women: a controlled study. *Am J Hum Genet 52*:884-894.

Sherman S, Pletcher BA, Driscoll DA. 2005. Fragile X syndrome: diagnostic and carrier testing. *Genet Med 7*:584-587.

Sherman SL. 2000. Premature ovarian failure in the fragile X syndrome. *Am J Med Genet 97*:189-194.

Snow K, Doud LK, Hagerman R, Pergolizzi RG, Erster SH, Thibodeau SN. 1993. Analysis of a CGG sequence at the FMR-1 locus in fragile X families and in the general population. *American journal of human genetics 53*:1217-1228.

Sobesky WE, Taylor AK, Pennington BF, Bennetto L, Porter D, Riddle J, Hagerman RJ. 1996. Molecular/clinical correlations in females with fragile X. *Am J Med Genet 64*:340-345.

Spielberger C. 1983. *Manual for the state-trait anxiety inventory for adults (Form Y).* Redwood City, CA: Mind Garden.

Sutcliffe JS, Nelson DL, Zhang F, Pieretti M, Caskey CT, Saxe D, Warren ST. 1992. DNA methylation represses FMR-1 transcription in fragile X syndrome. *Hum Mol Genet 1*:397-400.

Tassone F, Hagerman PJ. 2003. Expression of the FMR1 gene. *Cytogenet Genome Res 100*:124-128.

Tassone F, Hagerman RJ, Taylor AK, Gane LW, Godfrey TE, Hagerman PJ. 2000a. Elevated levels of FMR1 mRNA in carrier males: a new mechanism of involvement in the fragile-X syndrome. *Am J Hum Genet 66*:6-15.

Tassone F, Hagerman RJ, Taylor AK, Mills JB, Harris SW, Gane LW, Hagerman PJ. 2000b. Clinical involvement and protein expression in individuals with the FMR1 premutation. *Am J Med Genet 91*:144-152.

Thompson NM, Gulley ML, Rogeness GA, Clayton RJ, Johnson C, Hazelton B, Cho CG, Zellmer VT. 1994. Neurobehavioral characteristics of CGG amplification status in fragile X females. *Am J Med Genet 54*:378-383.

Turner SM, Beidel, D. C., Dancu, C. V. 1996. *Social phobia and anxiety inventory: manual*. Toronto, Ont.: Multi-Health Systems, Inc.

Watson D, Clark, L. A. 1994. *Manual for the positive and negative affect schedule (Expanded form).* Iowa City, IA: University of Iowa.

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

Welt CK. 2007. Primary ovarian insufficiency: a more accurate term for premature ovarian failure. *Clin Endocrinol (Oxf).*

Table I: Demographic data of study male and female study participants stratified by *FMR1* repeat length group.

Gender	Repeat Length Group	N	Mean Age (SD)	Ethnicity % Caucasian /Asian	Education % college or higher	Income % $50,000 or higher	Mean FSIQ (SD)	Ascertainment % GP	Anxiety/depression medication use % on meds
males	**All**	119	35.8 (9.4)	78.2 [a,c]	77.3	67.3	110.4 (14.6)	48.7 [b]	5.0
	NC	61	36.2 (8.9)	82.0	83.3	69.0	110.2 (12.7)	44.3	8.2
	IM	32	33.6 (10.3)	59.4	78.1	64.5	110.9 (18.2)	96.9	3.1
	PM	26	37.8 (9.3)	92.3	61.5	66.7	110.2 (14.5)	0.0	0.0
females	**All**	446	35.1 (9.5) [b]	76.4 [b,d]	86.1	61.4 [b]	107.9 (13.2)	38.6 [b]	14.3 [b]
	NC	97	32.7 (10.0)	58.8	92.8	56.3	109.0 (13.8)	82.5	7.2
	IM	94	32.0 (11.1)	59.6	87.2	47.8	106.6 (15.2)	89.4	8.6
	PM	255	37.1 (8.2)	89.4	83.1	68.4	108.0 (12.1)	3.1	18.8

NC = non-carriers; IM = intermediate allele carriers; PM = premutation allele carriers; SD = standard deviation; FSIQ = full scale intelligence quotient; GP = general population.

[a] $p<0.05$ for comparison among repeat groups

[b] $p< 0.005$ for comparison among repeat groups

[c] Male participants consisted of 78.2% Caucasian, 0% Asian, 19.3% African American, and 2.5% Hispanic subjects.

[d] Female participants consisted of 75.5% Caucasian, 0.9% Asian, 18.4% African American, 3.4% Hispanic , and 1.8% 'other' ethinicity subjects.

Table II: Results from the general linear model using *FMR1* repeat length as the main predictor of neurobehavior phenotypes.

Gender	Measure	Subscale	β estimates	p value
males	**CES-D**	**depression**	0.2601	0.03
	STAI	**state anxiety**	0.0800	0.40
		trait anxiety	0.1292	0.30
	PANAS	**negative affect**	0.1914	0.04
		positive affect	-0.2360	0.08
	SPAI	**social phobia**	0.0837	0.49
		agoraphobia	0.1462	0.22
		"pure" social phobia	0.0162	0.88
females	**CES-D**	**depression**	0.0323	0.45
	STAI	**state anxiety**	-0.0333	0.72
		trait anxiety	0.1047	0.15
	PANAS	**negative affect**	0.0883	0.04
		positive affect	-0.0353	0.64
	SPAI	**social phobia**	-0.0319	0.64
		agoraphobia	0.0951	0.08
		"pure" social phobia	-0.0034	0.96

CES-D = Centers for Epidemiologic Studies Depression Scale; STAI = State-Trait Anxiety Inventory; PANAS = Positive and Negative Affect Schedule; SPAI = Social Phobia and Anxiety Inventory.

Table III: Results from the general linear model results using indicator variables to compare *FMR1* repeat length groups as the main predictors of neurobehavior phenotypes.

Gender	Measure	Subscale	β estimates	p value	Adjusted group means
males	**CES-D**	**depression**	NC: ref IM: 0.20 PM: 0.25	NC: ref IM: 0.11 PM: 0.08	NC: 2.55 IM: 3.17 PM: 3.36
	STAI	**state anxiety**	NC: ref IM: 0.07 PM: 0.03	NC: ref IM: 0.72 PM: 0.76	NC: 3.4 IM: 3.5 PM: 3.4
		trait anxiety	NC: ref IM: -0.02 PM: 0.13	NC: ref IM: 0.93 PM: 0.39	NC: 3.45 IM: 3.44 PM: 3.53
	PANAS	**negative affect**	NC: ref IM: 0.07 PM: 0.17	NC: ref IM: 0.55 PM: 0.19	NC: 2.87 IM: 2.92 PM: 3.01
		positive affect	NC: ref IM: -0.07 PM: -0.30	NC: ref IM: 0.48 PM: 0.07	NC: 37.35 IM: 36.44 PM: 32.96
	SPAI	**social phobia**	NC: ref IM: 0.10 PM: 0.09	NC: ref IM: 0.31 PM: 0.45	NC: 6.99 IM: 7.57 PM: 7.53
		agoraphobia	NC: ref IM: 0.08 PM: 0.17	NC: ref IM: 0.43 PM: 0.15	NC: 3.06 IM: 3.35 PM: 3.76
		"pure" social phobia	NC: ref IM: 0.13 PM: 0.02	NC: ref IM: 0.86 PM: 0.24	NC: 6.09 IM: 6.79 PM: 6.21
females	**CES-D**	**depression**	NC: ref IM: 0.02 PM: 0.02	NC: ref IM: 0.82 PM: 0.71	NC: 3.01 IM: 3.09 PM: 3.07
	STAI	**state anxiety**	NC: ref IM: -0.03 PM: -0.08	NC: ref IM: 0.79 PM: 0.48	NC: 3.5 IM: 3.5 PM: 3.4
		trait anxiety	NC: ref IM: -0.03 PM: 0.11	NC: ref IM: 0.71 PM: 0.27	NC: 3.53 IM: 3.51 PM: 3.59
	PANAS	**negative affect**	NC: ref IM: 0.01 PM: 0.22	NC: ref IM: 0.89 PM: 0.02	NC: 2.88 * IM: 2.89 PM: 3.04 *
		positive affect	NC: ref IM: -0.04 PM: -0.01	NC: ref IM: 0.53 PM: 0.95	NC: 35.74 IM: 35.11 PM: 35.65
	SPAI	**social phobia**	NC: ref IM: 0.07 PM: 0.01	NC: ref IM: 0.19 PM: 0.94	NC: 7.65 IM: 8.10 PM: 7.68
		agoraphobia	NC: ref IM: 0.10 PM: -0.06	NC: ref IM: 0.09 PM: 0.40	NC: 3.91 IM: 4.31 PM: 3.71
		"pure" social phobia	NC: ref IM: 0.05 PM: 0.02	NC: ref IM: 0.34 PM: 0.75	NC: 6.35 IM: 6.71 PM: 6.48

ref = reference group; CES-D = Centers for Epidemiologic Studies Depression Scale; STAI = State-Trait Anxiety Inventory; PANAS = Positive and Negative Affect Schedule; SPAI = Social Phobia and Anxiety Inventory; NC = non-carriers; IM = intermediate allele carriers; PM = premutation allele carriers.

Table IV: Post hoc analysis to further explore negative emotion subscale scores from the Positive and Negative Affect Schedule. Results are obtained from the general linear model results using *FMR1* repeat length as the main predictor.

Gender	Subscale	β estimates	p value
males	**fear**	0.1468	0.23
	sadness	0.1981	0.03
	guilt	0.2901	0.01
	hostility	0.1286	0.28
females	**fear**	0.0112	0.87
	sadness	0.0013	0.98
	guilt	0.0429	0.55
	hostility	0.0566	0.16

Table V: General linear model results using indicator variables to compare *FMR1* repeat length groups as the main predictors. Follow-up on specific negative emotion subscale scores from the Positive and Negative Affect Schedule.

Gender	Subscale	β estimates	p value	Adjusted group means
males	**fear**	NC: ref IM: -0.04 PM: 0.18	NC: ref IM: 0.18 PM: 0.66	NC: 10.62 IM: 10.24 PM: 12.35
	sadness	NC: ref IM: 0.12 PM: 0.22	NC: ref IM: 0.33 PM: 0.08	NC: 8.96 IM: 10.16 PM: 11.29
	guilt	NC: ref IM: 0.23 PM: 0.30	NC: ref IM: 0.04 PM: 0.03	NC: 9.01 [a,b] IM: 11.33 [a] PM: 12.20 [b]
	hostility	NC: ref IM: -0.01 PM: 0.16	NC: ref IM: 0.99 PM: 0.23	NC: 11.68 IM: 11.67 PM: 13.52
females	**fear**	NC: ref IM: -0.01 PM: 0.18	NC: ref IM: 0.93 PM: 0.05	NC: 10.62 [b] IM: 10.57 [a] PM: 12.21 [a,b]
	sadness	NC: ref IM: 0.01 PM: 0.07	NC: ref IM: 0.41 PM: 0.83	NC: 9.99 IM: 10.12 PM: 10.63
	guilt	NC: ref IM: -0.01 PM: 0.13	NC: ref IM: 0.98 PM: 0.11	NC: 10.19 IM: 10.17 PM: 11.45
	hostility	NC: ref IM: 0.02 PM: 0.16	NC: ref IM: 0.70 PM: 0.05	NC: 11.28 [b] IM: 11.53 PM: 12.85 [b]

NC = non-carriers; IM = intermediate allele carriers; PM = premutation allele carriers; ref = reference group

[a,b] group mean scores are different at the p=0.05 level.

Table VI: Clinical diagnoses determined from the Center for Epidemiologic Studies Depression Scale (CES-D) and social phobia and panic disorder scales of the Social Phobia and Anxiety Inventory (SPAI) by gender and repeat length group.

Gender	Repeat Length Group	CES-D probable depression	SPAI probable social phobia	SPAI probable panic disorder
males	**All**, n=119	22 (18.5%) [b]	14 (11.8%)	3 (2.5%) [b]
	NC, n=61	5 (8.2%)	6 (9.8%)	0 (0.0%)
	IM, n=32	10 (31.3%)	3 (9.4%)	0 (0.0%)
	PM, n=26	7 (26.9%)	5 (19.2%)	3 (11.5%)
females	**All**, n=446 [a]	115 (25.8%)	69 (15.5%) [c]	27 (6.1%)
	NC, n=97	23 (23.7%)	5 (5.2%)	3 (3.1%)
	IM, n=94	25 (26.6%)	11 (11.7%)	6 (6.4%)
	PM, n=255	67 (26.3%)	53 (20.9%)	18 (7.1%)

NC = non-carriers; IM = intermediate allele carriers; PM = premutation allele carriers.

[a] SPAI scores were unavailable for 2 female participants, 1 PM and 1 NC.

[b] Fisher's Exact Test, $p<0.05$

[c] Fisher's Exact Test, $p<0.005$

Chapter 4

No evidence for a difference in neuropsychological profile among carriers and non-carriers of the *FMR1* premutation in adults under the age of 50.

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This chapter describes research analyzing *FMR1* repeat length associations with neuropsychological scores among the largest study population of males and females to date. My contributions to this work include the selection of outcome variables for analysis, data clean-up, variable coding, imputation of missing data, application of principal component analysis and confirmatory factor analyses, identification and application of the correlated data analysis for repeat length associations, formation of the cluster structures, follow-up contact with all participants to identify those with a FXS child, formation of a strategy to adjustment for multiple testing, interpretation of findings, follow-up contact with refusals, as well as manuscript preparation and publication.

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Abstract

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 using 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 non-carriers. 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.

Key words: FXTAS; FMR1; premutation; CGG repeat; fragile X

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 due to 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. (2008)[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 using strategies to reduce potential participation biases, these results suggest that 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 using 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 non-carriers 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 I. This test battery was designed to assess a broad range of abilities. Twenty-one outcome scores from these tests were used in this analysis (Table I). 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 sixty-six items and provides an inconsistency index and nine 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. Twelve (8.7%) men and seventeen (3.4%) women had missing scores for the CAARS. In addition, eight (5.8%) men and twenty-nine (5.7%) women had inconsistency index scores of 8 or greater which is indicative of potential inconsistency of the responses. Thus, 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 alarms, indicate the number of times the subject presses the space bar in the absence of the appropriate stimulus. Twelve (8.7%) men and forty-one (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 second 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 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 non-word 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 seconds to complete the task. The number of items correctly completed from the three tasks is used to compute an "interference" score which 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 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 only told 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. Six (4.3%) men and twenty-four (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 while 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 using 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 using 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 using 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 using 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 6 (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 using 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. (2005).[45]

Statistical analysis

Male and female participants were separated into three groups based on their repeat length allele: non-carriers (≤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 II lists demographic data stratified by gender and repeat group. Repeat group differences for the demographic variables shown were tested using 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 dataset, the three repeat groups differed on ethnicity (% Caucasian/Asian) (X^2=12.42, df=2, p<0.01). For the female dataset, the three repeat groups differed on age (F=15.52, p<0.01), ethnicity (% Caucasian/Asian) (X^2=48.56, df=2, p<0.01), and household income (% ≥$50,000) ($X^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 due to possible learning disability. However, no differences in reading abilities were detected for the male or female dataset. 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 ≥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 III. 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 using the EM algorithm.

In order to further reduce the number of variables analyzed, a principal component analysis (PCA) followed by varimax rotation was used. Since 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 due to 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 twenty-one variables (Table IV). 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 IV).

Since the new factor structure was obtained using data from all participants, confirmatory factor analysis was performed on the male and female datasets 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 datasets, respectively.

Factor scores for all participants were computed for each participant using the scoring coefficients calculated by the PCA. The six factor scores were analyzed as outcome variables using 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 due to 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: non-carriers, 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 II). 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 dataset 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 since 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 using 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 using *FMR1* repeat length as a continuous variable as the main predictor are shown in Table V. For both the male and female datasets, repeat length as a continuous variable was not a statistically significant predictor for any of the six factor scores using the Bonferonni correction for multiple testing (i.e., $p<0.01$). For the female dataset, 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 V). Both models indicated positive linear associations between repeat length and these two factor scores, indicative of reduced processing speed 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 VI. For the male dataset, repeat length was not a statistically significant predictor of any of the factors scores. In addition, using Tukey's post hoc analysis to compare the adjusted group means, factor scores did not differ significantly among repeat

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

As shown in Table IV, 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 VII. The premutation group scored marginally significantly higher than non-carriers for inattention and memory, impulsivity and emotional lability, and problems with self-concept, but not 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 non-carrier 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 non-linear 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 non-carriers (≤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 non-carrier group (repeats ≤40) for any of the six factors among the male dataset (p values of 0.18, 0.75, 0.43, 0.77, 0.24, and 0.67, respectively) or among the female dataset (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 dataset with imputation of missing values was a good fit for the dataset 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 dataset reached significance, while among the female dataset, 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 twenty-one primary outcome scores derived from the eight neuropsychological tests were used in a principal component analysis to construct a six-factor model (Table IV). Factor loadings of the original twenty-one 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 IV).

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

Our data suggested that females with the premutation reported significantly more severe symptoms associated with ADHD than did non-carriers. 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 the non-carrier 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 non-carriers 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 since 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 dataset. Another explanation could be that since the CAARS is a self-report questionnaire, women report the symptoms associated with ADHD differently than 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 ≥ 100 repeats may be more likely to manifest symptoms related to the mutation since 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. (2008)[30] and Grigsby et al (2008)[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 non-carriers. Cornish et al. (2008)[30] found no differences among carriers and non-carriers under the age of 50 for general intelligence, sustained attention, visual spatial function, or visual memory function. Similarly, Grigsby et al (2008)[31] found no differences among premutation carriers without FXTAS and non-carriers 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. (2008)[30] found a significant deficit in response inhibition, a component of executive function, among men under age 50. Grigsby et al. (2008)[31] found that carriers without FXTAS performed worse than non-carriers 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 as Cornish et al. (2008) found that the difference between carriers and non-carriers for response inhibition deficits increased with increasing age. Secondly, 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 ≥ 100 repeat alleles, increasing the power of detecting small effect sizes. Thirdly, 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. Fourthly, 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. (2000) reported decreased feelings of self-concept among carriers compared to non-carriers 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 non-carrier 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 non-carriers 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 premutation 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

Online Mendelian Inheritance in Man (OMIM),

http://www.ncbi.nlm.nih.gov.proxy.library.emory.edu/Omim/

References

1. Ashley CT, Sutcliffe JS, Kunst CB, Leiner HA, Eichler EE, Nelson DL, Warren ST (1993) Human and murine FMR-1: alternative splicing and translational initiation downstream of the CGG-repeat. Nat Genet 4:244-251

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

3. Feng Y, Zhang F, Lokey LK, Chastain JL, Lakkis L, Eberhart D, Warren ST (1995) Translational suppression by trinucleotide repeat expansion at FMR1. Science 268:731-734

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

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

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

7. Primerano B, Tassone F, Hagerman RJ, Hagerman P, Amaldi F, Bagni C (2002) Reduced FMR1 mRNA translation efficiency in fragile X patients with premutations. Rna 8:1482-1488

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

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

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

11. Tassone F, Hagerman PJ (2003) Expression of the FMR1 gene. Cytogenet Genome Res 100:124-128

12. Tassone F, Beilina A, Carosi C, Albertosi S, Bagni C, Li L, Glover K, Bentley D, Hagerman PJ (2007) Elevated FMR1 mRNA in premutation carriers is due to increased transcription. Rna 13:555-562

13. Sherman SL (2000) Premature ovarian failure in the fragile X syndrome. Am J Med Genet 97:189-194

14. Welt CK (2008) Primary ovarian insufficiency: a more accurate term for premature ovarian failure. Clin Endocrinol (Oxf) 68:499-509

15. Wittenberger MD, Hagerman RJ, Sherman SL, McConkie-Rosell A, Welt CK, Rebar RW, Corrigan EC, Simpson JL, Nelson LM (2007) The FMR1 premutation and reproduction. Fertil Steril 87:456-465

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

17. 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 (2004) Penetrance of the fragile X-associated tremor/ataxia syndrome in a premutation carrier population. Jama 291:460-469

18. 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 (2003) Fragile X premutation tremor/ataxia syndrome: molecular, clinical, and neuroimaging correlates. Am J Hum Genet 72:869-878

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

20. Greco CM, Hagerman RJ, Tassone F, Chudley AE, Del Bigio MR, Jacquemont S, Leehey M, Hagerman PJ (2002) Neuronal intranuclear inclusions in a new cerebellar tremor/ataxia syndrome among fragile X carriers. Brain 125:1760-1771

21. Berry-Kravis E, Lewin F, Wuu J, Leehey M, Hagerman R, Hagerman P, Goetz CG (2003) Tremor and ataxia in fragile X premutation carriers: blinded videotape study. Ann Neurol 53:616-623

22. Bacalman S, Farzin F, Bourgeois JA, Cogswell J, Goodlin-Jones BL, Gane LW, Grigsby J, Leehey MA, Tassone F, Hagerman RJ (2006) Psychiatric phenotype of the fragile X-associated tremor/ataxia syndrome (FXTAS) in males: newly described fronto-subcortical dementia. J Clin Psychiatry 67:87-94

23. Hagerman RJ, Leavitt BR, Farzin F, Jacquemont S, Greco CM, Brunberg JA, Tassone F, Hessl D, Harris SW, Zhang L, Jardini T, Gane LW, Ferranti J, Ruiz L, Leehey MA, Grigsby J, Hagerman PJ (2004) Fragile-X-associated tremor/ataxia syndrome (FXTAS) in females with the FMR1 premutation. Am J Hum Genet 74:1051-1056

24. Zuhlke C, Budnik A, Gehlken U, Dalski A, Purmann S, Naumann M, Schmidt M, Burk K, Schwinger E (2004) FMR1 premutation as a rare cause of late onset ataxia--evidence for FXTAS in female carriers. J Neurol 251:1418-1419

25. Berry-Kravis E, Potanos K, Weinberg D, Zhou L, Goetz CG (2005) Fragile X-associated tremor/ataxia syndrome in sisters related to X-inactivation. Ann Neurol 57:144-147

26. Biancalana V, Toft M, Le Ber I, Tison F, Scherrer E, Thibodeau S, Mandel JL, Brice A, Farrer MJ, Durr A (2005) FMR1 premutations associated with fragile X-associated tremor/ataxia syndrome in multiple system atrophy. Arch Neurol 62:962-966

27. Jacquemont S, Orrico A, Galli L, Sahota PK, Brunberg JA, Anichini C, Leehey M, Schaeffer S, Hagerman RJ, Hagerman PJ, Tassone F (2005) Spastic paraparesis, cerebellar ataxia, and intention tremor: a severe variant of FXTAS? J Med Genet 42:e14

28. Hunter J, Abramowitz A, Rusin M, Sherman S (In press) Is there evidence for neuropsychological and neurobehavioral phenotypes among adults without FXTAS who carry the FMR1 premutation? A review of current literature. Genet Med [In press].

29. Kogan CS, Turk J, Hagerman RJ, Cornish KM (2008) Impact of the Fragile X mental retardation 1 (FMR1) gene premutation on neuropsychiatric functioning in adult males without fragile X-associated Tremor/Ataxia syndrome: a controlled study. Am J Med Genet B Neuropsychiatr Genet 147B:859-872

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

31. 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 (2008) Cognitive profile of fragile X premutation carriers with and without fragile X-associated tremor/ataxia syndrome. Neuropsychology 22:48-60

32. Minguez M, Ibanez B, Ribate MP, Ramos F, Garcia-Alegria E, Fernandez-Rivas A, Ruiz-Parra E, Poch M, Alonso A, Martinez-Bouzas C, Beristain E, Tejada MI (2008) Risk of cognitive impairment in female premutation carriers of fragile X

premutation: Analysis by means of robust segmented linear regression models. Am J Med Genet B Neuropsychiatr Genet

33. Roberts JE, Bailey DB, Jr., Mankowski J, Ford A, Sideris J, Weisenfeld LA, Heath TM, Golden RN (2008) Mood and anxiety disorders in females with the FMR1 premutation. Am J Med Genet B Neuropsychiatr Genet
34. Rodriguez-Revenga L, Madrigal I, Alegret M, Santos M, Mila M (2008) Evidence of depressive symptoms in fragile-X syndrome premutated females. Psychiatr Genet 18:153-155
35. Conners CK, Erhardt D, Sparrow EP (1999) Conners Adult ADHD Rating Scales (CAARS) Technical Manual. Multi-Health Systems Inc., North Tonawanda, N.Y.
36. Halperin JM (1991) The clinical assessment of attention. Int J Neurosci 58:171-182
37. Benton AL, Hamsher KD (1976) Multilingual Aphasia Examination. University of Iowa Press, Iowa City
38. Golden C (1978) Stroop Color and Word Test: A Manual for Clinical and Experimental Uses. Wood Dale, IL
39. Reitan R (1986) Trail Making Test: Manual for Administration and Scoring. Reitan Neuropsychology Laboratory, Tucson, AZ
40. Heaton R, G. Cheline, J. Talley, G. Kay, G. Curtiss (1993) Wisconsin Card Sorting Test Manual: Revised and expanded. Psychological Assessment Resources, Odessa, FL
41. Wechsler D (1997) Wechsler memory scale, 3rd edition manual. The Psychological Corporation, San Antonio

42. Wechsler D (1997) Wechsler adult intelligence scale, 3rd edition manual. The Psychological Corporation, San Antonio

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

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

45. Allen EG, Sherman S, Abramowitz A, Leslie M, Novak G, Rusin M, Scott E, Letz R (2005) Examination of the effect of the polymorphic CGG repeat in the FMR1 gene on cognitive performance. Behav Genet 35:435-445

46. Sherman S, Pletcher BA, Driscoll DA (2005) Fragile X syndrome: diagnostic and carrier testing. Genet Med 7:584-587

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

48. Hunter JE, Allen EG, Abramowitz A, Rusin M, Leslie M, Novak G, Hamilton D, Shubeck L, Charen K, Sherman SL (2008) Investigation of Phenotypes Associated with Mood and Anxiety Among Male and Female Fragile X Premutation Carriers. Behav Genet 38:493-502

49. Kaiser HF (1974) An index of factorial simplicity. Psychometrika 39:31-36

50. Garcia-Alegria E, Ibanez B, Minguez M, Poch M, Valiente A, Sanz-Parra A, Martinez-Bouzas C, Beristain E, Tejada MI (2007) Analysis of FMR1 gene

expression in female premutation carriers using robust segmented linear regression models. Rna 13:756-762

51. McConkie-Rosell A, Spiridigliozzi GA, Sullivan JA, Dawson DV, Lachiewicz AM (2000) Carrier testing in fragile X syndrome: effect on self-concept. Am J Med Genet 92:336-342

Table I. 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)

Table II. 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	18-50	18-50	18-50	18-50	18-50	18-50
		ANOVA: F=1.58, p=0.21				ANOVA: F=15.52, p<0.01		
Ethnicity								
% Caucasian	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	3.0	0.0	1.8	1.7	4.2	1.1
		X^2=12.42, df=2, p<0.01				X^2=48.56, df=2, p<0.01		
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	15.2	16.7	15.2	18.8	6.3	16.7
		X^2=4.64, df=2, p=0.10				X^2=4.72, df=2, p=0.10		
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	12.9	28.6	20.0	18.4	15.2	22.3
		X^2=0.80, df=2, p=0.67				X^2=12.61, df=2, p<0.01		
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	63-121	75-119	51-122	70-121	65-122	51-121
		ANOVA: F=0.26, p=0.77				ANOVA: F=2.61, p=0.07		

NC = non-carriers; 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: one missing race, eight missing income, and three missing WRAT scores.

[b] Among female participants: sixteen missing race, seventeen missing income, and six missing WRAT scores.

Table III. 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

Table IV. Structure of six factors derived from principal component analysis with varimax rotation and associated factor loadings which represent correlations between the new factors and the original neuropsychological measures from both male and female participants. Factors loadings >0.40 (shown in bold) were used to interpret the new factors.

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**

Table V: Results from the general linear model using *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 VI: Results from the general linear model results using indicator variables to compare *FMR1* repeat length groups as the main predictors.

Gender	**Factor**	**Adjusted Group Means**			**P Value**
		NC	**IM**	**PM**	
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 ($p<0.01$)

Table VII: Analysis of individual CAARS scores to follow up on mean factor 4 score differences between female non-carrier and premutation carrier groups.

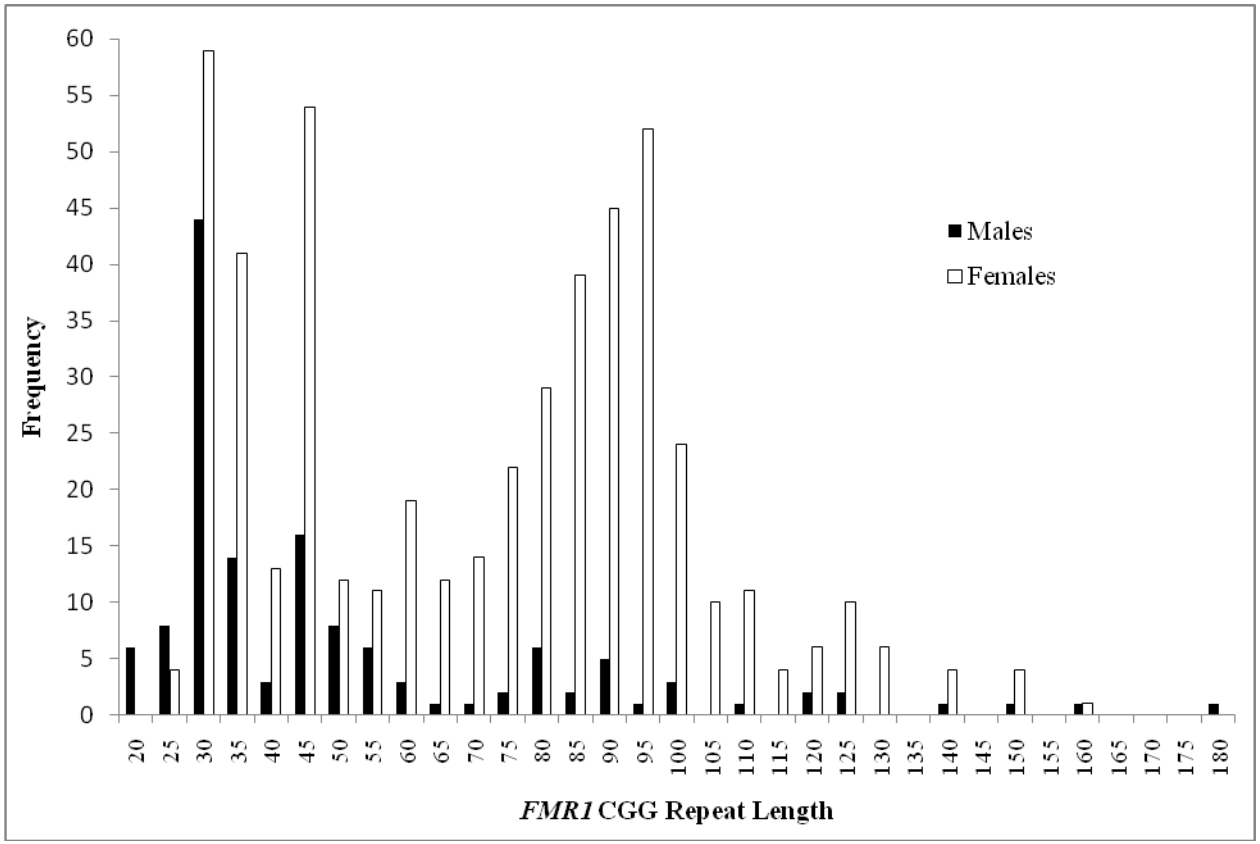
CAARS Subscale	Symptoms Assessed	Adjusted Group Means			P Value
		NC	IM	PM	
A	Inattention and memory	47.90[a]	49.36	51.18[a]	0.02
B	Hyperactivity and restlessness	49.03	49.43	49.84	0.72
C	Impulsivity and emotional lability	44.23[b]	45.77	47.95[b]	0.02
D	Problems with self-concept	44.73[c]	45.75	47.58[c]	0.05

[a] mean factor scores marginally significantly different (p=0.01)

[b] mean factor scores marginally significantly different (p=0.01)

[c] mean factor scores marginally significantly different (p=0.02)

Figure I. Distribution of *FMR1* CGG repeat lengths for all male and female participants.



Chapter 5

Fragile X-associated primary ovarian insufficiency (FXPOI): evidence for additional genetic contributions to severity.

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This chapter describes the use of a method to analyze familial aggregation of one of the phenotypes known to be associated with *FMR1* premutation alleles in order to determine the contribution of background genetics to variable expression of these phenotypes. My contributions to this work included the selection of the statistical approach, data clean-up, pedigree coding and structure, variable coding, implementation of the familial aggregation models, interpretation of the findings, as well as manuscript preparation and publication.

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Abstract

The fragile X mental retardation gene (*FMR1*) contains a CGG repeat sequence in its 5' untranslated region that can become unstable and expand in length from generation to generation. Alleles with expanded repeats in the range of ~55-199, termed premutation alleles, are associated with an increased risk for fragile-X-associated primary ovarian insufficiency (FXPOI). However, not all women who carry the premutation develop FXPOI. To determine if additional genes could explain variability in onset and severity, we used a random-effects Cox proportional hazards model to analyze age at menopause on 680 women from 225 families who have a history of fragile X syndrome and 321 women from 219 families from the general population. We tested for the presence of a residual additive genetic effect after adjustment for *FMR1* repeat length, race, smoking, body mass index, and method of ascertainment. Results showed significant familial aggregation of age at menopause with an estimated additive genetic variance of 0.55 to 0.96 depending on the parameterization of *FMR1* repeat size and definition of age at menopause (*P*-values ranging 0.0002 and 0.0027). This is the first study to analyze familial aggregation of FXPOI. This result is important for proper counseling of women who carry *FMR1* premutation alleles and for guidance of future studies to identify additional genes that influence ovarian insufficiency.

Key words: CGG repeat; triplet repeat; fragile X syndrome; premature ovarian failure

Introduction

The X-linked fragile X mental retardation gene (*FMR1*) contains a highly polymorphic CGG repeat in the 5' untranslated region (5'UTR) of exon 1 [Ashley, et al. 1993; Verkerk, et al. 1991]. The most common alleles contain roughly 30 repeats [Fu, et al. 1991]. When mutated, this triplet repeat becomes unstable and expands with transmission from one generation to the next. To date, expansion of this repeat has been linked to several clinical disorders.

First, expansions of 200 repeats or more, termed full mutations, are associated with aberrant hypermethylation which results in a loss of *FMR1* expression [Bell, et al. 1991; McConkie-Rosell, et al. 1993; Sutcliffe, et al. 1992]. The subsequent loss of the protein product, FMRP, results in a mental retardation syndrome, fragile X (FXS) [Pieretti, et al. 1991]. Males with full mutations tend to be more severely affected compared to females with full mutations due to the X-linked nature of the gene.

Second, repeats in the range of about 55-199 as defined by the American College of Medical Genetics [Sherman, et al. 2005] and termed premutation alleles, remain unmethylated and are associated with increasing levels of the *FMR1* transcripts and decreasing levels of FMRP [Kenneson, et al. 2001; Primerano, et al. 2002; Tassone and Hagerman 2003; Tassone, et al. 2000a; Tassone, et al. 2000b]. Older males (>50 years) who carry the premutation are at an increased risk for fragile X-associated tremor/ataxia syndrome(FXTAS) while premutation females are at an increased risk of fragile X-associated primary ovarian insufficiency (FXPOI) [Hagerman, et al. 2003; Sherman 2000]. Neither of these disorders is seen in conjunction with FXS, and is therefore

thought to be caused by a toxic gain-of-function effect of increased levels of the expanded *FMR1* transcripts.

Roughly 20% of women who carry premutation alleles experience premature ovarian failure (POF), clinically defined as the cessation of menstrual periods before the age of 40 [Sherman 2000]. This risk is about 20 times higher than that seen in the general population, with the highest risk being for premutation alleles in the range of about 80-100 repeats [Allen, et al. 2007; Sullivan, et al. 2005]. In the study of Allen et al., the mean age at menopause for carriers with 59-79 repeats was 48.5 ± 0.7, for those with 80-100 repeats was 44.9 ± 0.6, and those with >100 repeats was 47.5 ± 1.2. These reduced menopause ages compared with 52.3 ± 0.5 found among non-carriers. In addition, premutation females who are still cycling have increased levels of FSH, an indicator of reduced ovarian function, compared to non-carriers [Hundscheid, et al. 2001; Murray, et al. 1999; Sullivan, et al. 2005; Welt, et al. 2004] and altered cycle characteristics [Allen, et al. 2007; Welt, et al. 2004]. Thus it is recommended that the term "ovarian insufficiency" be applied to this condition to capture the observation that women who carry the premutation have traits associated with reduced ovarian function [Abrams 2007; Welt 2007].

The effect of the *FMR1* premutation on reducing age at menopause is the most common single major gene effect known to date. However, not all women who carry a premutation allele experience FXPOI. Presently, neither the etiology of FXPOI nor the cause of the variation in phenotype is understood. We know that repeat size variation within the premutation allele range explains a significant proportion of the variation in FXPOI [Allen, et al. 2007; Ennis, et al. 2006; Sullivan, et al. 2005]. Another likely source

of phenotype variation is background genes that, in conjunction with the effects of the *FMR1* alleles, could either decrease or increase the age of onset and severity of FXPOI. Large heritability estimates for the age at menopause support this hypothesis. For example, estimates of heritability of age at natural menopause range from 31% to 85% depending on the type of sample (twins, mother-daughter, sister pairs) and other sample attributes (e.g., age structure, geographical region) [de Bruin, et al. 2001; Murabito, et al. 2005; Snieder, et al. 1998; Treloar, et al. 1998; van Asselt, et al. 2004; Vink and Boomsma 2005].

This is the first study to examine heritability of age at menopause, a reflection of the level of severity of ovarian insufficiency, in the presence of a single gene effect, the *FMR1* premutation, known to influence ovarian function. Here, we ask if there are residual additive genetic effects that influence onset and severity of ovarian insufficiency after adjusting for the effects of *FMR1* repeat length. Our study was based on 230 families with a history of FXS as well as 219 families from the general population. For analysis, we used a random-effect version of the Cox Proportional Hazards model that allows for shared additive genetic effects within families [Pankratz, et al. 2005]. This model takes variable age at onset into account and includes censored measurements, thus maximizing the information derived from our study population.

Materials and Methods

Study Participants

Study participants were ascertained from families with a history of FXS to enrich the sample with varying repeat lengths as well as from the general population (see

Sullivan et al. (2005) for a review of ascertainment protocols.) Once a proband was identified, female relatives were also invited to participate in the study. Participants were aged 18-92 and had English as their primary language. All participants were asked to complete a reproductive history questionnaire and provide a biological sample, either buccal or blood, for repeat length determination. For our analysis, we had reproductive history information from 680 women from 225 FXS families and 321 women from 219 general population families. The protocols and consent forms for ascertainment were approved by the Institutional Review Board at Emory University.

Reproductive History Questionnaire

All study participants were asked to complete a reproductive history questionnaire. Depending on the participant's availability, the questionnaire was administered in person, over the telephone, or through the mail. The questionnaire included items regarding demographic information, such as date of birth and ethnicity, as well as information on potential confounders such as body mass index and smoking history. The bulk of the questionnaire pertained to menstrual cycle history and hormone medication use.

Determination of Age at Menopause

Menopause is defined as the cessation of menses for at least one year. However, pinpointing the exact age at which menopause occurs can be problematic due to the duration of the transition as well as the common use of hormone medication at the start of menopause symptoms which might allow the woman to continue cycling. Thus, age at

menopause for each study participant was determined in three ways using the same strategy outlined in Sullivan et al. (2005) (Table I). The first definition of age at menopause uses the self-reported age at menopause without taking into account hormone use. This definition scheme allowed us to define data points for 197 participants and censor data points for 804 participants. The second definition of age at menopause defines age at menopause as either the self-reported age at menopause or the age at which the participant started using hormone replacement therapy (HRT). This definition scheme was the most liberal and allowed us to define data points for 262 participants and censor data points for 739 participants. The third definition of age at menopause is the most conservative, with participants being censored at the start of HRT or oral contraceptive (OC) use. This definition scheme allowed us to define data points for 161 participants and censor data points for 840 participants.

FMR1 Repeat Length Measurement

All participants were asked to provide a biological sample, either buccal or blood. DNA was extracted using the Qiagen QiAmp DNA Blood Mini Kit. A fluorescent-sequencer method is used to determine *FMR1* CGG repeat length [Meadows, et al. 1996]. Briefly, flourescent-labeled primers are used to PCR amplify across the repeat region and the resulting product is run on an automated sequencer. Repeat lengths up to 90 can be determined with this method. In the event that a single band was detected in females, indicating either homozygous status or the presence of a larger repeat band from the second allele, an alternative PCR-based, hybridization technique was used [Brown, et al.

1993]. For heterozygous females, the CGG repeat length from the larger repeat allele was used in subsequent statistical analyses.

Statistical Analysis

We conducted statistical analyzes using a random-effect version of the Cox proportional hazards model that allows for shared effects due to polygenes [Pankratz, et al. 2005]. For the i^{th} subject, we defined the hazard function for menopause at time t as:

$$\lambda_i(t) = \lambda_0(t) \cdot \exp(X_i\beta + b_i)$$

where λ_o represents an unspecified baseline hazard function, X_i represents a design vector for the fixed effects of *FMR1* repeat length and additional confounders with related parameter vector β, and b_i represents the subject's random effect due to shared polygenes within the family. We assume that the additive genetic random effects among all subjects follow a multivariate normal distribution with mean zero and the variance-covariance matrix Σ, which we model using:

$$\Sigma = 2\Phi\sigma^2_p$$

where σ^2_p is the variance due to additive genetic effects and Φ is the kinship matrix (which depends on the familial relationships among subjects). Therefore, the introduction of b_i allows for correlation in age of menopause among related subjects, with such correlation assumed to be due solely to polygene effects.

Using this model scheme, we tested the hypothesis that there are no additional polygene effects beyond *FMR1* repeat size major gene effect that contribute to age at menopause (H_O: $\sigma_p^2=0$ vs. H_A: $\sigma_p^2>0$) by maximizing the likelihoods under both the null and alternative hypotheses. We then construct a likelihood-ratio statistic, which

asymptotically follows a $0.5\chi_1^2 : 0.5\chi_0^2$ distribution under H_O [Self and Liang 1987]. We rejected H_O assuming a significance threshold of 0.05.

We analyzed the data using age at menopause as the outcome variable, based on the three definitions outlined in Table I. For *FMR1* repeat length, we modeled the predictor as either a continuous variable or a four-level categorical variable (non-carriers = <59 repeats, low premutation group = 59-79 repeats, middle premutation group = 80-100 repeats, and high premutation group = 101-199 repeats). This second approach accounts for the non-linear relationship between age at menopause and repeat length [Allen, et al. 2007; Sullivan, et al. 2005]. These definitions, although somewhat arbitrary, were based on the risk to expand to the full mutation in one generation [Nolin, et al. 2003]. We also used these to be comparable to our previous studies. To determine which parameterization of *FMR1* repeat length best fit the data, we calculated the Akaike Information Criterion (AIC) and compared the models for each definition of age at menopause.

We initially examined potential confounders to assess significant differences among repeat classes using analysis of variances (ANOVA) for continuous variables and chi square analysis for dichotomous variables (Table II). Twenty-four participants did not self-report race, 28 were missing information on history of smoking, and 17 were missing body mass index (BMI) data. The mean age at time of interview differed significantly among repeat classes, although it was not a confounder. This finding is expected as younger participants are likely to have higher repeat sizes due to the expansion bias from parent to offspring. Race and ascertainment source also differed significantly among repeat groups, with the higher repeat groups being composed mostly

of Caucasians from families with a history of FXS. Thus we included race and ascertainment as potential confounders in the model. Smoking and BMI were not significantly different between classes. However, as these factors are known to affect age at menopause, we kept them in the model. Thus, within the random-effects Cox Proportional Hazards model, we adjusted for confounders consisting of race (dichotomous variable: 0 = non-white, 1 = white), history of smoking in "packyears" (continuous variable calculated using period of time the subject reported smoking in years multiplied by the number of cigarette packs smoked a day), BMI (continuous variable), and method of ascertainment (0 = ascertained from families with a known history of FXS, 1 = ascertained from families in the general population).

All analyses were run using the 'kinship' statistics package [Therneau and Atkinson 2007] in R 2.4.1.

Results

We initiated our studies by confirming the association between *FMR1* repeat size and age at menopause after adjusting for confounders, including race and ascertainment site, and for covariates known to affect age at menopause, namely smoking and BMI. Using the random-effect Cox proportional hazards model, we found statistically significant evidence for *FMR1* repeat size measured as a continuous variable (Table III). With each increase in a single repeat, there was an increased risk of earlier age at menopause by approximately 1%. This finding was consistent across the various definitions of age at menopause. We also examined models that adjusted for repeat length as a four-level categorical variable with the expectation that this parameterization

may fit the data better due to the non-linear association between repeat length and age at menopause. As expected, we found that the mid-size premutation group had the highest risk of earlier age at menopause (about four times that of the non-carrier group) and low- and high-repeat size groups had an increased risk of about two times the non-carrier group (Table IV).

Next, we examined the variation in age of menopause due to residual additive genetic effects, after adjusting for the major gene effect due to *FMR1* repeat length and confounders. Independent of our definition of age at menopause, we found significant evidence of a additive genetic component for age at menopause using either the continuous *FMR1* predictor (Table III) or the categorical *FMR1* predictor (Table IV). We found that the estimated variance due to additive genetic effects changed based on the definition of age at menopause and on the coding of the repeat length covariate (continuous vs. indicator variables). Using self-reported age at menopause (which does not incorporate hormone use), we estimated the additive genetic variance component to be 0.55 (P=0.0221) using the continuous *FMR1* repeat-length variable (Table III) and 0.64 (P=0.0040) using the categorical *FMR1* variable (Table IV). Interestingly, use of the definitions of age at menopause that incorporate information about use of HRT increased the estimate of the additive genetic variance component. When the definition of age at menopause included age at the start of HRT, the additive genetic variance component was estimated to be 0.82 (P=0.0005) using the continuous *FMR1* repeat-length variable (Table III) and 0.75 (P=0.0002) using the categorical *FMR1* variable (Table IV). Likewise, when we censored age at menopause at the initiation of HRT, the estimated

additive genetic variance component increased to 0.96 (P=0.0027) using the continuous *FMR1* variable and 0.87 (P=0.0023) using the categorical *FMR1* variable.

We compared the AIC values of the models that included *FMR1* repeat length as a continuous variable and as a repeat length group categorical variable to determine which fitted the data best. For each definition of age at menopause, the categorical parameterization was better. The difference in AIC values for the model including the continuous versus categorical variables was 18.52, 21.99, and 19.04 for the three definitions, respectively.

The Cox regression model does not allow for an estimate of heritability. Therefore, familial aggregation of age at menopause was quantified using measures described by Pankratz, et al. (2005). In particular, we can determine the estimated range of the hazard ratio for age of menopause among subjects by exponentiating the square root of the additive genetic variance estimate. In the case of *FMR1* repeat length as categorical variables for the most "conservative" definition of age at menopause (i.e., censoring at start of HRT use), the estimate of the additive genetic variance component is 0.87, giving a subject-specific hazard ratio based on genetic relationships that is, on average, 2.54 times larger or smaller than the overall hazard ratio for age at menopause. This indicates a substantial familial aggregation of age at menopause due to shared polygenes, even after adjusting for the major effect of the *FMR1* repeat length.

Discussion

In this study, we found significant evidence for an additive genetic component for age at menopause after adjusting for the influential effects of the *FMR1* premutation

allele. We took a comprehensive approach to maximize the information from a sample of 1001 women from 444 families using a random-effect version of the Cox proportional hazards model allowing for shared polygenes. We were also able to include information on other sources of variation including race, history of smoking, BMI, and ascertainment source.

In our previous studies [Allen, et al. 2007; Ennis, et al. 2006; Sullivan, et al. 2005], a significant nonlinear association between repeat size and ovarian insufficiency was identified. The repeat size alleles that carry the highest risk are those in the mid-range of ~80-99, not the highest premutation repeat sizes (i.e., 100-200 repeats). Those carriers with 80-99 repeats compared with non-carriers have: increased rates of infertility, a seven-year reduction in mean age at menopause, and a consequently increased prevalence of POF (32% vs. 1% in the general population) that initiates at younger ages. Carriers of both smaller and larger premutation repeat sizes also suffer from ovarian insufficiency, but not to as great an extent. To incorporate this nonlinear effect, we parameterized repeat length as a four-level categorical using repeat size groups and found that it fit the data better than did the continuous variable. We suggest that the estimates of the additive genetic component are more accurate when the *FMR1* major gene effect is adjusted using the indicator variables.

One of the important limitations of this study concerns the reliability of self-reported age-at-menopause. Pinpointing an event that has a long transition period and is somewhat ambiguous depending on symptoms is difficult. This is particularly true for a cross-sectional survey. Moreover, many women are prescribed hormone medication as soon as symptoms of menopause occur and may continue to cycle until medication is

stopped. Some women on hormone replacement therapy (HRT) may have had the ability to continue cycling naturally and some may not; HRT use masks this distinction. This, of course, complicates the ability to define a specific menopausal age. However, if it is measured similarly among those with and without the premutation, analyses will identify important patterns. To address possible effects of the use of HRT in defining age at menopause, we conducted the analyses using the three definitions of menopausal age that incorporate hormone medication use. In all models, estimates of the additive genetic variance component were significant and ranged from 0.64 to 0.87, assuming a categorical modeling of *FMR1* repeat length. The highest estimates of the familial genetic component were obtained using the most conservative definition of age at menopause that censored data at the initiation of HRT. It is possible that other non-genetic factors account for the increased estimate of familial aggregation when using this definition. For example, use of HRT for menopausal symptoms may be a shared phenomenon within families.

This is the first study to analyze familial aggregation of ovarian insufficiency among families with a history of FXS. Overall, the models outlined in this study provide significant evidence that the onset of *FMR1*-associated ovarian insufficiency, as marked by age at menopause, is controlled in part by additive genetic effects. This finding is important for two reasons. First, the average age at menopause within a family should be taken into consideration in addition to repeat size when counseling a woman who carries the premutation. These two attributes should help to determine the woman's risk for ovarian insufficiency that may interfere with fertility. Second, this evidence for additional

genes that influence age of menopause motivates the next stage to research: identification of additional genes that are involved in ovarian function.

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References

Abrams L. 2007. From POF to POI: Evolution of a Term. National Fragile X Foundation Quarterly 29(22).

Allen EG, Sullivan AK, Marcus M, Small C, Dominguez C, Epstein MP, Charen K, He W, Taylor KC, Sherman SL. 2007. Examination of reproductive aging milestones among women who carry the FMR1 premutation. Hum Reprod 22(8):2142-52.

Ashley CT, Sutcliffe JS, Kunst CB, Leiner HA, Eichler EE, Nelson DL, Warren ST. 1993. Human and murine FMR-1: alternative splicing and translational initiation downstream of the CGG-repeat. Nat Genet 4(3):244-51.

Bell MV, Hirst MC, Nakahori Y, MacKinnon RN, Roche A, Flint TJ, Jacobs PA, Tommerup N, Tranebjaerg L, Froster-Iskenius U and others. 1991. Physical mapping across the fragile X: hypermethylation and clinical expression of the fragile X syndrome. Cell 64(4):861-6.

Brown WT, Houck GE, Jr., Jeziorowska A, Levinson FN, Ding X, Dobkin C, Zhong N, Henderson J, Brooks SS, Jenkins EC. 1993. Rapid fragile X carrier screening and prenatal diagnosis using a nonradioactive PCR test. Jama 270(13):1569-75.

de Bruin JP, Bovenhuis H, van Noord PA, Pearson PL, van Arendonk JA, te Velde ER, Kuurman WW, Dorland M. 2001. The role of genetic factors in age at natural menopause. Hum Reprod 16(9):2014-8.

Ennis S, Ward D, Murray A. 2006. Nonlinear association between CGG repeat number and age of menopause in FMR1 premutation carriers. Eur J Hum Genet 14(2):253-5.

Fu YH, Kuhl DP, Pizzuti A, Pieretti M, Sutcliffe JS, Richards S, Verkerk AJ, Holden JJ, Fenwick RG, Jr., Warren ST and others. 1991. Variation of the CGG repeat at the fragile X site results in genetic instability: resolution of the Sherman paradox. Cell 67(6):1047-58.

Hagerman PJ, Greco CM, Hagerman RJ. 2003. A cerebellar tremor/ataxia syndrome among fragile X premutation carriers. Cytogenet Genome Res 100(1-4):206-12.

Hundscheid RD, Braat DD, Kiemeney LA, Smits AP, Thomas CM. 2001. Increased serum FSH in female fragile X premutation carriers with either regular menstrual cycles or on oral contraceptives. Hum Reprod 16(3):457-62.

Kenneson A, Zhang F, Hagedorn CH, Warren ST. 2001. Reduced FMRP and increased FMR1 transcription is proportionally associated with CGG repeat number in intermediate-length and premutation carriers. Hum Mol Genet 10(14):1449-54.

McConkie-Rosell A, Lachiewicz AM, Spiridigliozzi GA, Tarleton J, Schoenwald S, Phelan MC, Goonewardena P, Ding X, Brown WT. 1993. Evidence that methylation of the FMR-I locus is responsible for variable phenotypic expression of the fragile X syndrome. Am J Hum Genet 53(4):800-9.

Meadows KL, Pettay D, Newman J, Hersey J, Ashley AE, Sherman SL. 1996. Survey of the fragile X syndrome and the fragile X E syndrome in a special education needs population. Am J Med Genet 64(2):428-33.

Murabito JM, Yang Q, Fox C, Wilson PW, Cupples LA. 2005. Heritability of age at natural menopause in the Framingham Heart Study. J Clin Endocrinol Metab 90(6):3427-30.

Murray A, Webb J, MacSwiney F, Shipley EL, Morton NE, Conway GS. 1999. Serum concentrations of follicle stimulating hormone may predict premature ovarian failure in FRAXA premutation women. Hum Reprod 14(5):1217-8.

Nolin SL, Brown WT, Glicksman A, Houck GE, Jr., Gargano AD, Sullivan A, Biancalana V, Brondum-Nielsen K, Hjalgrim H, Holinski-Feder E and others. 2003. Expansion of the fragile X CGG repeat in females with premutation or intermediate alleles. Am J Hum Genet 72(2):454-64.

Pankratz VS, de Andrade M, Therneau TM. 2005. Random-effects Cox proportional hazards model: general variance components methods for time-to-event data. Genet Epidemiol 28(2):97-109.

Pieretti M, Zhang FP, Fu YH, Warren ST, Oostra BA, Caskey CT, Nelson DL. 1991. Absence of expression of the FMR-1 gene in fragile X syndrome. Cell 66(4):817-22.

Primerano B, Tassone F, Hagerman RJ, Hagerman P, Amaldi F, Bagni C. 2002. Reduced FMR1 mRNA translation efficiency in fragile X patients with premutations. Rna 8(12):1482-8.

Self SG, Liang K-Y. 1987. Asymptotic Properties of Maximum Likelihood Estimators and Likelihood Ratio Tests Under Nonstandard Conditions. Journal of the American Statistical Association 82(398):605-610.

Sherman S, Pletcher BA, Driscoll DA. 2005. Fragile X syndrome: diagnostic and carrier testing. Genet Med 7(8):584-7.

Sherman SL. 2000. Premature ovarian failure in the fragile X syndrome. Am J Med Genet 97(3):189-94.

Snieder H, MacGregor AJ, Spector TD. 1998. Genes control the cessation of a woman's reproductive life: a twin study of hysterectomy and age at menopause. J Clin Endocrinol Metab 83(6):1875-80.

Sullivan AK, Marcus M, Epstein MP, Allen EG, Anido AE, Paquin JJ, Yadav-Shah M, Sherman SL. 2005. Association of FMR1 repeat size with ovarian dysfunction. Hum Reprod 20(2):402-12.

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

Tassone F, Hagerman PJ. 2003. Expression of the FMR1 gene. Cytogenet Genome Res 100(1-4):124-8.

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

Tassone F, Hagerman RJ, Taylor AK, Mills JB, Harris SW, Gane LW, Hagerman PJ. 2000b. Clinical involvement and protein expression in individuals with the FMR1 premutation. Am J Med Genet 91(2):144-52.

Therneau TM, Atkinson B. 2007. Kinship: mixed-effects Cox models, sparse matrices, and modeling data from large pedigrees. R package version 1.1.0-15 (available at http://www.r-project.org/).

Treloar SA, Do KA, Martin NG. 1998. Genetic influences on the age at menopause. Lancet 352(9134):1084-5.

van Asselt KM, Kok HS, Pearson PL, Dubas JS, Peeters PH, Te Velde ER, van Noord PA. 2004. Heritability of menopausal age in mothers and daughters. Fertil Steril 82(5):1348-51.

Verkerk AJ, Pieretti M, Sutcliffe JS, Fu YH, Kuhl DP, Pizzuti A, Reiner O, Richards S, Victoria MF, Zhang FP and others. 1991. Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. Cell 65(5):905-14.

Vink J, Boomsma D. 2005. Modeling age at menopause. Fertil Steril 83(4):1068.

Welt CK. 2007. Primary ovarian insufficiency: a more accurate term for premature ovarian failure. Clin Endocrinol (Oxf).

Welt CK, Smith PC, Taylor AE. 2004. Evidence of early ovarian aging in fragile X premutation carriers. J Clin Endocrinol Metab 89(9):4569-74.

Table I. Three different definitions of age at menopause based on self-reported menstrual cycle history and hormone use.

Self-reported age at menopause
stopped cycling due to:
natural menopause = self-reported menopause age
other reasons (i.e. hysterectomy) = censored at age at last menses
still cycling = censored at age at interview
Age at menopause or start of HRT
stopped cycling due to:
natural menopause with no HRT use before menopause = self-reported menopause age
natural menopause with HRT use before menopause = age started HRT
other reason with no HRT use = censored at age at last menses
other reason with HRT use = age started HRT
still cycling:
using HRT = age started HRT
not using HRT = censored at age of interview
Age at menopause censoring at start of HRT
stopped not cycling due to:
natural menopause with no HRT use before menopause = self-reported menopause age
natural menopause with HRT use before menopause = censored at age started HRT
other reason with no HRT use = censored at age at last menses
other reason with HRT use = censored at age started HRT
still cycling:
using HRT or OC = censored at age started HRT or OC
not using HRT or OC = censored at age of interview

HRT = hormone replacement therapy; OC = oral contraceptives

Table II. Study population demographic information. Participants were divided into four groups based in *FMR1* repeat length. Repeat groups differed significantly with mean age at interview, self-reported race, and ascertainment source (* $p<0.0001$).

Repeat Group	**N**	**Mean Age (SD)**	**Race (% white)**	**Smoking History (% ever smoked)**	**Hormone Use (% using hormones)**	**Mean BMI (SD)**	**Ascertainment (% from GP)**
all participants	1001	43.3 (15.0) *	82.4 *	35.9	38.4	27.0 (6.7)	32.1 *
NC (<59 repeats)	541	42.4 (15.9)	74.9	33.3	38.9	27.0 (7.0)	57.1
low PM carriers (59-79 repeats)	134	49.1 (15.3)	90.1	34.3	36.4	27.6 (5.8)	9.0
mid PM carriers (80-100 repeats)	248	43.3 (12.6)	91.3	41.5	39.6	26.9 (6.7)	0.0
high PM carriers (101-199 repeats)	78	38.8 (11.7)	93.3	38.5	34.2	25.8 (6.2)	0.0

NC = non-carriers; PM = premutation; SD = standard deviation; BMI = body mass index; GP = general population

Table III. Results of Cox proportional hazard mixed models based on self-reported age at menopause, adjusting for *FMR1* repeat length as a continuous variable. Models are also adjusted for race, history of smoking in packyears, hormone use at time of interview, BMI (body mass index), and ascertainment source (FXS families vs. general population). A significance level of p=0.05 was used.

Definition of Menopause	Covariates in Model	Risk Ratio [a]	p value	σ_p^2 Estimate	Likelihood Statistic
Self-reported age at menopause	repeat length	1.01	0.0012	0.55	4.05, p=0.0221
	race	0.60	0.1100		
	packyears	1.02	0.0006		
	BMI	0.97	0.0900		
	ascertainment source	0.43	0.0004		
Age at menopause or start of HRT	repeat length	1.01	0.0032	0.82	11.03, p=0.0005
	race	0.81	0.4800		
	packyears	1.01	0.0075		
	BMI	0.97	0.0280		
	ascertainment source	0.54	0.0040		
Age at menopause censoring at start of HRT	repeat length	1.01	0.0012	0.96	7.78, p=0.0027
	race	0.55	0.0910		
	packyears	1.02	0.0015		
	BMI	0.97	0.0880		
	ascertainment source	0.38	0.0007		

[a] Risk ratios are based on the exponentiation of the β estimate for each covariate

Table IV – Results of Cox proportional hazard models based on self-reported age at menopause, adjusting for *FMR1* repeat length with indicator variables for repeat length groups. Models are also adjusted for race, history of smoking in packyears, hormone use at time of interview, BMI (body mass index), and ascertainment source (FXS families vs. general population). A significance level of p=0.05 was used.

Definition of Menopause	Covariates in Model	Risk Ratio [a]	p value	σ_p^2 Estimate	Likelihood Statistic
Self-reported age at menopause	NC (reference)	----	----	0.64	7.04, p=0.0040
	low PM carriers	2.29	0.0018		
	mid PM carriers	4.10	<0.0001		
	high PM carriers	2.17	0.0530		
	race	0.54	0.0530		
	packyears	1.02	0.0047		
	BMI	0.98	0.1100		
	ascertainment source	0.63	0.0780		
Age at menopause or start of HRT	NC (reference)	----	----	0.75	12.73, p=0.0002
	low PM carriers	2.37	0.0004		
	mid PM carriers	3.53	<0.0001		
	high PM carriers	1.43	0.3500		
	race	0.77	0.3600		
	packyears	1.01	0.0062		
	BMI	0.97	0.0260		
	ascertainment source	0.78	0.3000		
Age at menopause censoring at start of HRT	NC (reference)	----	----	0.87	8.03, p=0.0023
	low PM carriers	3.53	<0.0001		
	mid PM carriers	4.26	<0.0001		
	high PM carriers	2.68	0.0250		
	race	0.480	0.0390		

	packyears	1.02	0.0082		
	BMI	0.97	0.0680		
	ascertainment source	0.61	0.1100		

NC = non-carriers; PM = premutation

[a] Risk ratios are based on the exponentiation of the β estimate for each covariate.

Chapter 6

Conclusions and Future Directions

The main goal of the work presented in this dissertation was to study phenotypes associated with *FMR1* premutation alleles. Specifically, analyzing a large study population of males and females with varying repeat lengths, we concluded that there is minimal, if any, clinical neuropsychological or neurobehavior impact of carrying a premutation allele among adults under the age of 50. Further, the work presented here utilizes a statistical method to quantify the contribution of background genetics to variable expression of one of the phenotypes known to be associated with premutation alleles, fragile X-associated primary ovarian insufficiency (FXPOI).

Neuropsychological and neurobehavioral phenotypes among premutation carriers not affected by FXTAS.

We analyzed phenotypes associated with cognition as well as mood and anxiety among male and female carriers of *FMR1* premutation alleles under the age of 50. With a larger study population than previously reported, we tested associations of neuropsychological and neurobehavioral scores with CGG repeat length, both for linear associations across the repeat length range as well as mean score differences between the non-carrier group and premutation group.

Analysis of repeat length associations with cognitive domains revealed that the premutation carriers in the male or females datasets were not significantly impaired compared to non-carriers. However, among the female dataset, there were significantly increased scores for self-report inattention among the premutation group. Increased inattention can be associated with executive functioning deficits. However, the mean score for the female premutation group was not in the clinically significant range. Further, in a follow-up analysis comparing carriers with premutation alleles of >100 repeats to non-carriers, no significant differences were detected. This provides evidence that increased transcript levels and decreased amounts of protein, molecular phenotypes associated with these high repeat premutation alleles, are not associated with cognitive impairment. All together, these results led us to conclude that younger adult premutation carriers are unlikely to be significantly impaired neuropsychologically.

Analysis of neurobehavioral scores revealed repeat length associations with negative affect scores among the male and female participants and with depression scores among the male participants only. However, these differences were subtle and of small effect size. In addition, once the analyses were adjusted for multiple testing, none of the results were significant. Due to the large study population analyzed and the recruitment strategy that minimized ascertainment biases, we concluded that an increased risk of mood or anxiety disorders among premutation carriers compared to a person from the general population was not likely.

In future studies, recruitment of male and female participants will continue in order to enlarge the study population available for analysis. However, given the strong evidence presented here for a lack of neuropsychological or mood phenotype among

younger premutation carriers, future analyses will be focused on longitudinal studies of phenotypes across age in order to address the potential that cognitive or mood phenotypes emerge as a precursor to the onset of motor symptoms in FXTAS.

Familial aggregation of phenotypes associated with premutation alleles.

The aim of this study was to utilize a random-effects Cox proportional hazards model to determine if additional genes could explain variability in onset and severity of FXPOI. This study was the first to determine familial aggregation of a phenotype known to be associated with premutation alleles. We analyzed ages at menopause for 680 women from families with a history of FXS and 321 women the general population. Results indicated a significant familial aggregation of age at menopause with an estimated additive genetic variance of 0.55 to 0.96 depending on the parameterization of *FMR1* repeat size and definition of age at menopause.

Further studies will be focused on using this statistical model to determine familial aggregation of another phenotype known to be associated with premutation alleles, FXTAS. The effect of additional genes could explain the variability in the onset and severity of this disorder as well. In addition, given the strong evidence that additional genes play a role in the onset of FXPOI, future studies will be directed at the identification of these genes. Depending on effect sizes, genome wide association studies or linkage studies could be proposed.

Final Remarks

Results of past studies have indicated that male and female premutation carriers display neurobehavioral and neuropsychological impairments [see Hunter et al. (2008) for review]. However, analysis of our large study population did not support these past reports. More than direct *FMR1* molecular factors, such as repeat length and transcript levels, the family environment of a carrier of a premutation allele deserves further study. In other words, instead of the direct impact of carrying a premutation allele, the impairments detected in past studies could be due to the psychosocial impact of raising a child with FXS, taking care of a parent affected with FXTAS, or the emotional impact of dealing with issues of infertility. One potential would be to quantify these stressors as well as coping mechanisms and include these variables in future models of neuropsychological and neurobehavioral phenotypes. Also, the study of phenotypes among children who carry a premutation would be informative due to the absence of these stressors. However, without more evidence of a presence of a phenotypic profile among adults, a study of children would be unwarranted. In addition, future studies will be directed at studying the phenotypic course of FXTAS and FXPOI in order to identify further risk factors and early signs of onset thus providing targets for early intervention or prevention.

References

Hunter J, Abramowitz A, Rusin M, Sherman S. 2008. Is there evidence for neuropsychological and neurobehavioral phenotypes among adults without FXTAS who carry the FMR1 premutation? A review of current literature. *Genet Med* [In press].