American Journal on Intellectual and Developmental Disabilities

FMR1 CGG Repeats and Stress Influence Self-Reported Cognitive Functioning in Mothers

--Manuscript Draft--

Manuscript Number:	AJIDD-D-21-00003R2
Article Type:	Research Report
Keywords:	stress; executive function; memory; FMR1; CGG repeats
Corresponding Author:	Nell Maltman University of Wisconsin-Madison Madison, WI UNITED STATES
First Author:	Nell Maltman
Order of Authors:	Nell Maltman
	Leann Smith DaWalt, PhD
	Jinkuk Hong, PhD
	Mei Wang Baker, MD
	Elizabeth M. Berry-Kravis, MD, PhD
	Murray H Brilliant, PhD
	Marsha Mailick, PhD
Manuscript Region of Origin:	UNITED STATES
Abstract:	Variation in the FMR1 gene may affect aspects of cognition, such as executive function and memory. Environmental factors, such as stress, may also negatively impact cognitive function; however, is not yet known whether these two factors together have additive or interaction effects. Participants included 1035 mothers of children with and without intellectual and developmental disabilities. Participants completed self-report measures of executive function, memory, stress (i.e., parenting status, life events), and provided DNA to determine CGG repeat length (ranging from 18 to 123 CGGs). Stress significantly predicted greater self-reported difficulties in executive function and the likelihood of memory problems. CGG repeat number independently predicted executive functioning and memory difficulties, suggesting additive effects of genetic variation and environmental stress exposure.

Abstract

Variation in the *FMR1* gene may affect aspects of cognition, such as executive function and memory. Environmental factors, such as stress, may also negatively impact cognitive functioning. Participants included 1053 mothers of children with and without developmental disabilities. Participants completed self-report measures of executive function, memory, and stress (i.e., life events, parenting status), and provided DNA to determine CGG repeat length (ranging from 7 to 192 CGGs). Stress exposure significantly predicted greater self-reported difficulties in executive function and the likelihood of memory problems. Cubic CGG effects independently predicted executive function and memory difficulties, suggesting effects of both genetic variation and environmental stress exposure on cognitive functioning.

Keywords: stress, cognitive functioning, executive function, memory, *FMR1*, CGG repeats

FMR1 CGG Repeats and Stress Influence Self-Reported Cognitive Functioning in Mothers

The fragile X mental retardation 1 (*FMR1*) gene plays a significant role in cognitive development and functioning across the lifespan. A full mutation expansion of more than 200 cytosine-guanine-guanine (CGG) trinucleotide repeats in the 5' untranslated region of the *FMR1* mRNA causes the *FMR1* promoter to be fully methylated, interferes with protein production, and results in fragile X syndrome (FXS). FXS is the most common inherited, single gene cause of intellectual disability (Nolin et al., 1996). Relatedly, a premutation (PM) expansion (between 55-200 CGG repeats) of *FMR1* can cause a range of clinical and subclinical phenotypes, including fragile X-associated primary ovarian insufficiency (FXPOI), fragile X-associated tremor/ataxia syndrome (FXTAS), difficulties with executive function, and elevated rates of mood and anxiety symptoms, and mothers with a PM can go on to have a child with FXS (Hagerman & Hagerman, 2021; Movaghar et al., 2019; Sullivan et al., 2005; Wheeler et al., 2017). Optimal functioning of the *FMR1* gene and expression of its protein product, FMRP, are essential for normal neurodevelopment and synaptic function (Darnell et al., 2011). There is evidence to suggest variability in cognitive functioning across the CGG range of *FMR1* below the full mutation (e.g., 6 to 200 repeats) (Hong et al., 2021; Klusek et al., 2018; Mailick et al., 2017), though most prior work has focused on variation among PM carriers (Birch et al., 2016; Hippolyte et al., 2014; Shelton, Cornish, & Fielding, 2017; Shelton et al., 2015; Shelton et al., 2016). Additionally, cognitive functioning may be impacted by stress, such as experiencing stressful life events or parenting a child with a developmental disability. The purpose of the present study is to further investigate how quantitative variation in *FMR1* CGG repeats and exposure to environmental stressors (i.e., life events, parenting a child with a developmental disability) contribute to cognitive functioning.

FMR1-related Variability and Cognitive Functioning

Cognitive functioning encompasses a variety of domains that involve an individual's ability to learn and solve problems, as well as attend and respond to the environment (Harada et al., 2013). Two cognitive domains, executive function and memory, entail higher-order mental processes required for goal-directed behavior (e.g., planning, inhibition, performance monitoring) and retrieval (Diamond, 2013). Much of the prior work on *FMR1*-related variability and cognition has centered around individuals with CGG expansions (Grigsby et al., 2014; Shelton et al., 2015; Shelton et al., 2016), with evidence suggesting that individuals with the *FMR1* PM may have difficulties with executive function (Brega et al., 2008; Cornish et al., 2011; Grigsby et al., 2008; Kraan et al., 2014; Shelton et al., 2014) and memory (Grigsby et al., 2008; Hippolyte et al., 2014; Moore et al., 2004; Shelton, Cornish, & Fielding, 2017). These difficulties have been shown to be associated with *FMR1* CGG repeat length, with both linear and curvilinear effects noted (Klusek et al., 2020; Klusek et al., 2018). Importantly, however, relationships between CGG repeat length and cognitive functioning have also been noted *across* the CGG range below the full mutation (< 26 CGGs up to 200 repeats) (Hong et al., 2021; Klusek et al., 2018; Mailick, Hong, Rathouz, et al., 2014), indicating that genetic factors associated with cognition are not restricted to CGG expansions.

There remains some controversy regarding the extent to which variation in cognitive functioning may be directly the result of the repeat expansions, or alternatively may be explained by ascertainment bias, namely the inclusion in research of individuals with CGG expansions who also have children or family members with FXS, or participants who have other FX-related disorders, such as FXPOI or FXTAS (Gossett et al., 2016; Hunter et al., 2008; Wheeler et al., 2014). To better tease apart these effects, one potentially fruitful approach is to examine the

FMR1 CGG range (normal through PM expansions) in relationship to variation in executive function and memory. Among the few studies to have done so, Hunter et al. (2008) examined whether *FMR1* CGG repeat length on the long allele predicted aspects of executive function and memory. For women, greater CGG repeat length was predictive of higher levels of self-reported inattention and impulsivity, as well as direct-assessment processing speed, but did not predict other factors (e.g., memory, response fluency). Though these findings were interpreted as marginal after correcting for multiple comparisons, this and several clinical reports (Debrey et al., 2016; Hall et al., 2011; Hall et al., 2020) set the stage for continued exploration of variability in cognitive functioning along the CGG repeat continuum.

Stress and Cognitive Functioning

Environmental sources of stress (e.g., life events, parenting a child with a disability) have been associated with similar aberrations in executive function in diverse populations across the lifespan regardless of genetic status (Diamond, 2013; Heyman & Hauser-Cram, 2015; Liston et al., 2009; Lupien et al., 2009; Op den Kelder et al., 2017; Shields et al., 2017), and have been found to be associated with cognitive dysfunction (including poorer episodic memory) (Song et al., 2016). In a meta-analysis, Luhmann et al. (2012) found that life events, such as divorce or the birth of a child, had negative effects on cognitive functioning.

Parenting stress, or adverse psychological responses to parenting obligations (Deater-Deckard, 1998), has been observed at increased rates in parents of children with disabilities due to unique and chronic caregiving demands. Meta-analyses (Barroso et al., 2018; Hayes & Watson, 2013) suggest that parents of children with developmental disabilities (DDs) experience higher rates of parenting stress than parents of typically developing children. Parents of adult children with DDs have been exposed to this unique stressor for many years (Seltzer et al.,

2011), which has been reported to adversely affect parents' cognition. For example, prior work suggests that mothers of children with autism spectrum disorder (ASD), FXS, and other DDs are more likely to report memory problems than mothers of typically developing individuals (Lovell et al., 2014; Song et al., 2016). Importantly, most studies of parenting stress have not selected participants based on genetics, nor included genetic information as part of their study procedures (Barker et al., 2011; Cousino & Hazen, 2013; Fischer, 1990; Lovell et al., 2014). Nevertheless, these findings suggest that objective measures of stress may be implicated in cognitive functioning, including executive function and memory, among mothers of children with DDs, independent of their genetic status.

In other research, environmental stressors have been found to interact with maternal genetic factors when predicting cognitive functioning. For instance, Mailick et al. (2017) found that parents of adult children with disabilities who had *FMR1* CGG repeats either above or below the population mean (i.e., 30 repeats $\pm$ 2SDs on the target allele) reported greater difficulties with daily cognitive functioning, and in particular memory and problem solving, within the past month than parents who did not have adult children with disabilities. Prior work also suggests that individuals at the lower end of the CGG distribution (i.e., the low zone; <26 CGGs on the short allele) may also be susceptible to environmental effects on cognition. A recent study evaluated gene by environment interactions among low zone and normal-range (26-40 CGGs, homozygous alleles) mothers of children with and without disabilities (Mailick et al., 2020). Significant gene by environment interactions indicated that low zone mothers who had children with disabilities had greater limitations in executive function than low zone mothers whose children did not have disabilities. In contrast, mothers with normal-range CGG repeats did not differ in executive function based on stress exposure. The present study builds on this prior work

by exploring both independent and synergistic effects of genetic and environmental influences on cognition in a sample of mothers in the low through PM range of CGGs.

Past research has suggested that mothers may be more negatively affected by parenting stress than fathers (Pelchat et al., 2007; Pelchat et al., 2003; Skreden et al., 2012). Therefore, the present study focused on mothers and examined the effects of environmental stressors (i.e., life events, parenting a child with a DD) on executive function and memory in mothers with low zone through the PM range of CGGs. In addition to stress, it is possible that other factors may contribute to variability in executive function and memory, namely age and education (Fjell et al., 2017; Harada et al., 2013; Klusek et al., 2020; Taylor et al., 2018). These individual factors were therefore incorporated into the study as covariates to account for sociodemographic features that may influence variation in cognitive functioning. We hypothesized that environmental stressors, as well as *FMR1* CGG repeat number, would predict executive function and memory difficulties, net of age and education.

We used three approaches to evaluate the nature of the association between CGG repeat number, stress, and cognition. First, we evaluated linear effects of both stress and CGG repeat length; if significant, such effects would suggest an additive, independent influence of CGG repeat length on cognition above and beyond stress. Second, drawing from prior literature that has examined CGG effects on cognition from low through expanded repeats (Klusek et al., 2020; Klusek et al., 2018; Mailick et al., 2020; Mailick et al., 2017; Mailick, Hong, Rathouz, et al., 2014), we assessed curvilinear CGG effects (i.e., quadratic and cubic effects), which would suggest that individuals within a particular CGG range may be more susceptible to the effects of stress on cognition. Our third approach was to examine interactions between stress and CGG

repeat length. Significant interaction effects would signify that stress either enhances or diminishes effects of CGG repeat length.

Methods

Participants and Procedures

Recruitment and Sample Selection.

The sampling plan for this research was designed to include a sufficient number of participants from the low end of the CGG repeat range through PM expansions to evaluate genotype-phenotype associations. The number of CGG repeats in *FMR1* in the human population is not evenly distributed across the CGG range and is highly polymorphic (Eichler et al., 1995; Fu et al., 1991). The peak value for CGG repeats is 30, with >90% of individuals having fewer than 40 repeats and the lowest number of repeats ever reported being 6 (Brown et al., 1993; Chen et al., 2003; Fu et al., 1991; Snow et al., 1993). In the current research, we investigate the phenotypic associations of variation in CGG repeats by treating repeat number as a continuous variable.

Participants included 1053 women with CGG repeats ranging from 7 to 192. The majority of these participants were drawn from the Marshfield Clinic Personalized Medicine Research Project (PMRP) (McCarty et al., 2005), a 20,000-person population-based biobank. Individuals had enrolled in this biobank in the early 2000s and provided written informed consent to allow researchers access to their DNA and electronic health records, and to be contacted for additional data collection. Per IRB, research results were not returned to participants, nor were the results entered into their medical record or provided to health care personnel. Over half of the PMRP members were female (n = 11,556) and DNA was available for 99.7% of them.

For a previous investigation (Maenner et al., 2013), the DNA samples of all PMRP members were screened for *FMR1* CGG repeats. This screening made it possible to recruit participants across the CGG repeat range, with adequate numbers of individuals at the lower and higher ends of the range. We invited all those who had at least one allele in the low zone (defined here as below 26 CGG repeats; Mailick et al., 2020; Weghofer et al., 2012) (Mailick et al., 2020; Weghofer et al., 2012)to participate. Additionally, based on a power analysis, a random sample of females with normal-range CGGs (homozygous alleles, 26-40 repeats) was selected for inclusion in the present research. Thus, by design, the recruited sample included all females in the population biobank who had expanded or low numbers of CGGs on either allele, and a random sample of females in the normal range. The response rate of the recruited females from the full PMRP sample was 77.7%.

From this pool, we restricted participants in the current study to mothers of biological or adopted children because of our interest in how stress affects parents, and further to mothers who either had a child diagnosed with a DD or whose children did not have disabilities (n = 912). Their *FMR1* CGGs ranged from 7 – 192 repeats. Only a small number (n = 34) had CGG repeats in the PM range (55-192 CGG repeats), one of whom had PM/full mutation mosaicism.

To enrich the range of *FMR1* CGG repeats, the current sample was augmented by the inclusion of clinically-ascertained mothers of children diagnosed with FXS (n = 140). Participants from clinically-ascertained samples were recruited from fragile X clinics, via local media, newsletters, brochures, and disability registries (Mailick, Hong, Greenberg, et al., 2014; Mailick et al., 2018). The range of CGG repeats for these mothers was 67-186, and 17 of these mothers had PM/full mutation mosaicism. Thus, the range of CGG repeats among all 1053 mothers in the present study was 7 to 192. The inclusion of both population-based and clinically-

ascertained participants is consistent with prior work that has evaluated the influence of the *FMR1* CGG repeat range on health-related variables by combining samples from diverse sources or different studies (Albizua et al., 2017; Allen et al., 2020; Allen et al., 2004; Sullivan et al., 2005).

The data from the majority of participants in the present study (69.9% of the present sample, $n = 736$) have never been published before, namely measures of executive function and memory drawn from participants in the Marshfield Personalized Medicine Research Project. Other investigations from our group have focused on clinically-ascertained PM carriers, many of whom are included in the present study (Klusek et al., 2020; Mailick et al., 2018; Mailick et al., 2020). However, the goal of the present study – to evaluate the impact on cognition of stress exposure and CGG repeats (measured continuously from 7 to 192 repeats) – did not overlap with our prior research.

The Institutional Review Boards at the University of Wisconsin-Madison and Marshfield Clinic approved all procedures and all participants signed informed consents.

Measures

Participants completed a questionnaire that provided information on whether they had a child with a DD, as well as all other non-genetic measures for the current study.

Stress.

Life Events. Participants reported life events (positive and negative) that they personally experienced during the past year (adapted from Abidin's Parenting Stress Index; Abidin, 2012) (Abidin, 2012). Participants selected events from a list of 22 items, such as divorce, going into debt, and the birth of a child. Higher scores indicate a greater number of personal life events.

Parenting Status. Participants reported whether their child had a DD (0 = no, 1= yes). Children in the present study averaged 28 years of age, and thus when we refer to children we are indicating that they are the mothers' sons and daughters, not indicating a particular stage of life. Children were considered to have a DD based on the Diagnostic and Statistical Manual – 5th edition, including FXS, attention deficit hyperactivity disorder, learning disabilities (e.g., dyslexia), autism spectrum disorder, Down syndrome, cerebral palsy, and intellectual disability (American Psychiatric Association, 2013). See **Table 1** for further details.

[INSERT TABLE 1 HERE]

Cognitive Functioning.

Executive Function. Participants completed the Behavior Rating Inventory of Executive Function-Adult Version (BRIEF-A) (Roth et al., 2005; Roth et al., 2013), a well-validated self-report measure of executive function in daily life for adults. The BRIEF-A consists of 75-items that yields an overall raw score of executive function (Global Executive Composite; GEC), made up of two indices: Behavior Regulation Index (BRI) and Metacognitive Index (MI). Participants indicated the extent to which they experienced problems across nine domains: Inhibit, Shift, Emotional Control, Self-Monitor, Initiate, Working Memory, Plan/Organize, Task Monitor, and Organization of Materials, which together comprise the GEC. Each item was rated from 1 (never) to 3 (often). Raw scores for each domain were converted into t-scores, with higher scores suggestive of greater executive difficulties in daily life. T-scores that exceeded 65 on any domain indicated clinically-significant executive dysfunction in that area. The present study used the GEC t-score as the indicator of self-reported executive function.

The BRIEF-A was previously standardized on a representative population sample of 1136 adults with Cronbach α coefficients ranging from .93-.96 and test-retest reliability ranging

from .93-.94 across domains, with utility demonstrated in both clinical and non-clinical samples (Christ et al., 2010; Rabin et al., 2006; Roth et al., 2005; Roth et al., 2013). The BRIEF-A has been shown to correlate significantly with direct-assessment measures of executive function (e.g., go/no go and trail making tests) in healthy adults (Erkkila et al., 2018) and in individuals with disorders associated with executive dysfunction (Grane et al., 2014; Rouel et al., 2016; Solsnes et al., 2014), indicating that the BRIEF-A is an ecologically valid measure of executive function.

The BRIEF-A was standardized on participants ages 18-90. Since there were six participants in the present sample who were over the age of 90, we checked all findings excluding these participants, which did not change results. Therefore, the findings reported below include all participants.

Self-reported Memory Problems. Participants answered the question: *Do you have problems with memory?* Participants responded as 0 (no problems with memory), 1 (problems with memory, but not diagnosed by a health professional), or 2 (diagnosed memory problems).

FMR1-related Variation.

DNA samples were obtained from cheek swabs and blood samples from all participants, and were analyzed for CGG repeats in *FMR1*. Assays were completed at the Wisconsin State Laboratory of Hygiene under the supervision of Mei Wang Baker, MD and the Rush University Medical Center Molecular Diagnostics Laboratory under the supervision of Elizabeth Berry-Kravis, MD, PhD, using procedures described previously (Klusek et al., 2020; Maenner et al., 2013; Mailick et al., 2018; Seltzer et al., 2012).

Target Allele Selection. For all participants, the assays yielded CGG repeat data on the *FMR1* gene on both X chromosomes. Because we did not have activation ratio data, one X

chromosome was selected for analysis in the present study as follows. We followed the approach of Hunter et al. (2012), which was also taken in our prior work (Hong et al., 2021; Mailick et al., 2017). We selected the *longer* allele in women who had one expanded (i.e., > 40 CGGs) and one normal allele (i.e., between 26 and 40 CGG repeats; n = 397) and in the five cases who had two expanded alleles. We selected the *shorter* allele in women who had one low allele (i.e., < 26 CGGs) and one normal allele (n = 191) and in women who had two low alleles (n = 79). We *randomly selected* one allele for analysis in the present study in women who had two normal alleles (n = 260), and also for those with one low allele and one expanded allele (n = 121). As noted previously, prior preliminary work suggests possible effects of low zone alleles (Hong et al., 2021; Mailick et al., 2020) and PM alleles (Klusek et al., 2020) on cognition with the measures used here. As we did not have activation ratio data to determine which allele was active, random allele selection for these individuals was implemented to reduce bias. A description of allelic distributions is presented in **Table 2**. We refer to the selected allele as the 'target' allele.

[INSERT TABLE 2 HERE]

Statistical Analyses

Statistical analyses were performed using IBM SPSS Statistics, version 27 (IBM Corp., 2019). Descriptive statistics and Pearson correlations among all study variables are presented in **Table 3**. Maternal age and education were controlled in all subsequent analyses. Education was ordinally coded on the following scale: 1 (less than high school), 2 (high school degree), 3 (college degree or equivalent), 4 (master's degree or above).

As noted previously, we evaluated stress and CGG effects in three ways based on prior research that reported mixed results regarding the form of the associations between these

variables and cognition – linear effects of stress and CGGs, curvilinear (quadratic and cubic) effects of CGGs, and interactions between stress and CGG terms (Cornish et al., 2011; Hong et al., 2021; Hunter et al., 2008; Klusek et al., 2020; Klusek et al., 2018; Mailick et al., 2020; Mailick et al., 2017; Mailick, Hong, Rathouz, et al., 2014). For executive function and memory problems, the primary analyses involved two hierarchical regressions (one for each type of stressor) that assessed the prediction that stress and *FMR1* CGG repeat length would each contribute to self-reported executive function difficulties or memory problems.

For both executive function and memory problem models, maternal age, education, and each stress measure (i.e., life events, parenting a child with a DD) were entered into the first block. To evaluate the linear effect of CGG repeats, the number of CGGs on the target allele was entered into the second block. To evaluate curvilinear effects, in the third block of each regression analysis, a quadratic term (CGG squared) was included; a fourth block included a cubic CGG term. To evaluate interaction effects, in the final block of each regression analysis, an interaction term between each measure of stress and CGG repeat length (linear, quadratic, and cubic) was included. Tables including the interaction effects appear in Supplementary Materials, as none of the interaction effects were significant. Effect sizes (f^2) for the multiple regression analyses are interpreted as small (.02), medium (.15), and large (.39) (Cohen, 1988). Regression diagnostics were completed using Cook's *D* based on the criteria $D>4=(1-k-n)$ and no outliers were observed.

Results

Descriptive Findings

Participants' ages ranged from 28-96 years (M = 57.03, SD = 15.20). Almost all mothers self-identified as White (99.0%). The majority of the mothers (63%) had graduated from college. Their children ranged in age from <1 year to 71 years (M = 28.44, SD = 16.53). The number of

children in each family ranged from 1 to 8 (M = 1.02, SD = 1.22). Of the mothers included in this study, 31.1% had a child with a DD. Most participants (62.1%) had experienced at least one life event in the past year (M = 1.23, SD = 1.41, Range = 0 - 9). GEC t-scores on the BRIEF-A ranged from 35 to 93 (M = 50.01, SD = 10.48), similar to the normative population (Roth et al., 2005; Roth et al., 2013). Only 9.6% of participants exceeded clinical cutoff on the GEC (i.e., t-score > 65). Approximately 25% of participants (n=266) self-reported a memory problem (see **Table 3**).

Correlations among study variables are depicted in **Table 3.** Notably, the objective stressors were significantly inter-correlated (r = .170, p < .001). A follow-up t-test revealed that mothers of children with a DD had experienced significantly more life events (M = 1.59, Range = 0-8) from the past year than mothers of children who did not have a DD (M = 1.07, Range = 0-9; t(532.94) = -5.09, p < .001, d = .35). Both executive function difficulty and memory problems were significantly associated with each stressor and CGG repeat length (p-values < .001). Executive function difficulty and memory problems were significantly correlated with each other, with moderate effects (r = .359; p < .001).

[INSERT TABLE 3 HERE]

Multiple Regressions

Executive Function.

As shown in **Table 4** total number of life events significantly predicted higher executive function problems (b = 1.560, p < .001) with small effects (f^2 = .07), with additional significant cubic CGG effects (b = -.0002, p =.004, f^2 = .010). No significant effects were observed in the interaction models (bs < -.003, p-values >.134). (See Supplementary Materials for tables including interaction effects.)

Greater executive function difficulties were also significantly predicted by parenting a child with a DD (b = 3.435, p < .001) with small effects (f^2 = .048), with additional significant cubic CGG effects (b = -.00002, p =.036, f^2 = .066). No significant interaction effects were found (bs < .003, p-values >.062).

[INSERT TABLE 4 HERE]

For descriptive purposes, **Figure 1** illustrates the cubic association of CGG repeats and executive function. Mothers with CGG repeats at the low end of the CGG distribution (7-25 CGGs) and those in the mid-range (~80-110 CGGs) had higher levels of executive function limitations. Conversely, mothers with the highest CGGs (>110) had lower levels of executive function limitations.

[INSERT FIGURE 1 HERE]

Figure 1. Cubic association between CGG repeat length and executive function difficulty.

Memory Problems. As shown in **Table 5,** total number of life events significantly predicted self-reported memory problems (b = .036, p = .001, f^2 = .037), with significant cubic CGG effects (b = - .0000009, p < .001, f^2 = .025). Self-reported memory problems were also predicted by parenting status (b = .175, p <.001, f^2 = .055), with significant cubic CGG effects (b = -.0000007, p = .003, f^2 = .065). No significant findings were observed in the interaction models for life events or parenting status (bs < .0002, p-values > .052).

[INSERT TABLE 5 HERE]

For descriptive purposes, **Figure 2** illustrates the cubic association of CGG repeat length and memory problems. Mothers with CGG repeats at the low end of the CGG distribution (7-25 CGGs) and mothers with CGG repeats between 100 and 130 had a greater likelihood of memory problems than mothers with CGGs in the normative range and those with CGGs >130.

[INSERT FIGURE 2 HERE]

Figure 2. Cubic association between CGG repeat length and self-reported memory problems. Self-reported memory problems reflect the following scale: 0 (no memory problems), 1 (problems with memory, but not diagnosed by a health professional), 2 (diagnosis of memory problems).

Covariates. Both age and education were significant predictors of executive function difficulty and self-reported memory problems across all models that included CGG repeat length (p-values $\leq .040$).

Discussion

The present study evaluated the influence of *FMR1* CGG repeat length (between 7-192 CGGs) and environmental stressors on self-reported cognitive functioning (i.e., executive function and memory) in mothers. Importantly, CGG repeat length and environmental stressors (life events, parenting a child with a DD) independently predicted variability in cognition across all models after age and level of education were controlled. To date, this study represents the largest sample in which the association between cognitive functioning, stress, and *FMR1* CGG repeat length has been studied. By taking a continuous approach to evaluating *FMR1*-related effects on cognitive functioning, and by assessing mothers of non-disabled children as well as children with a diverse range of DDs, this study advances understanding of how both environmental and genetic factors influence self-reported cognitive functioning at the population level.

The *FMR1* gene plays an essential role in brain development and functioning across the lifespan (Allen et al., 2005; Cornish et al., 2008; Darnell et al., 2011; Hocking et al., 2019; Huber et al., 2002; Shelton, Cornish, Clough, et al., 2017). Historically, examination of behavioral phenotypes associated with *FMR1*-related variability (e.g., CGG repeat length) have largely focused on individuals with FXS, the gray zone, or the PM, with some exceptions (Hunter et al.,

2008; Kim et al., 2019; Klusek et al., 2018; Mailick et al., 2017). Many prior assessments of cognition associated with CGG expansions in the *FMR1* gene involved group comparisons, typically between PM carriers and individuals with the normal range of CGGs (i.e., less than 41 repeats) (Hippolyte et al., 2014; Hunter et al., 2008; Shelton, Cornish, & Fielding, 2017; Shelton et al., 2016). With a continuous analysis of CGG repeats across a wider range, as in the present study, the interpretation of the relationship between *FMR1*-related variation and phenotypic expression can be advanced. Our findings revealed cubic effects of CGG repeats on executive function and self-reported memory problems, but not interactions with stress across all models.

Though prior work has noted that mothers of children with FXS, who are themselves carriers of the *FMR1* PM, may exhibit differences in some aspects of cognitive functioning (Shelton, Cornish, & Fielding, 2017; Shelton et al., 2014; Shelton et al., 2016), our findings indicate cognitive heterogeneity within this group. Specifically, cubic CGG effects were observed in predicting executive function and self-reported memory problems, whereby mothers with CGGs in the PM mid-range (~80-110 CGGs) had more difficulties with executive function and memory problems, but those at the higher end of the range (>110 repeats) had fewer difficulties.

The CGG effects on executive function are somewhat similar to those observed in past research within the PM range. Klusek et al. (2020) found quadratic CGG effects on BRIEF-A Inhibit subscale scores among PM carriers (who overlap in part with this study sample) such that females with > 110 repeats evidenced fewer difficulties with executive function compared to those with mid-range CGG repeats (~80-110). Moreover, prior work has shown divergent patterns of performance on items from the BRIEF-A among PM carriers who have PM/full mutation mosaicism (who overlap with this study sample; Mailick et al., 2018) (Mailick et al.,

2018). That is, PM carriers who had full mutation mosaicism had fewer problems with executive function than PM carriers without full mutation mosaicism (Mailick et al., 2018). Thus, perhaps in part due to PM/full mutation mosaicism, individuals at the higher range of the CGG distribution may be somewhat protected against difficulties in cognitive functioning. The CGG effects observed in the present study reflect small, but significant associations with cognition, and although these patterns do not indicate clinically meaningful differences, they instead provide insight into how CGG repeat length may influence cognition incrementally in the general population, consistent with recent work (Hong et al., 2021).

The lack of interaction effects indicates that in the present analysis, stress did not have an enhancing or mitigating effect when evaluated across the wide range of the CGG distribution. Rather, stress and CGGs appear to have independent influences on cognition. One reason why the present results may diverge from past research on PM expansions is that only 13.4% of the sample members had CGG repeats in the PM range, and thus the incremental, additive effects of CGG repeats across a much wider range (7-192 repeats) became evident. The use of a continuous measure of CGG repeats from the low through PM range was a fundamental aspect of the present research design, and is an approach that has been recently used to evaluate genotype-phenotype associations in the general population (Hong et al., 2021). We pursued a continuous (vs. categorical) approach because comparisons between specific categories of the CGG distribution might have obscured the quantitative association of CGGs and outcomes, especially as not all categories have universally recognized cut-off points (e.g., gray zone, low zone). It is also possible that our allele selection method, particularly for those with both low and expanded alleles, may have influenced results. However, we did not have activation ratio data that would

indicate which allele was active. Thus, randomly selecting one allele was an attempt to reduce bias and support our goal of examining CGG effects across the low through expanded range.

Limitations in executive function and memory problems represent distinct aspects of cognition. Higher scores on the BRIEF-A reflect difficulty sitting still and waiting, the propensity to make untactful remarks, and the tendency to complete tasks in a hurried manner. In contrast, the endorsement of memory problems reflects the self-perception that one has difficulties with everyday memory, and not necessarily a clinical diagnosis of memory problems. Importantly, the present results suggest that CGG repeat number from low through the PM range is predictive of cognitive variation above and beyond both demographic factors (age and education) and environmental stressors. Replication of the current findings is necessary, and research examining the basic biological functions of the *FMR1* CGG repeat is needed to fully understand these effects.

The results confirm past findings that environmental stressors affect cognitive functioning (Diamond, 2013; Heyman & Hauser-Cram, 2015; Song et al., 2016). First, life events were related to cognitive functioning limitations. The life events endorsed by participants encompassed a wide range, such as the birth of a child, increased income, and moving to a new home. A prior meta-analysis of the relationship between life events and subjective well-being (including cognitive well-being) found that cognitive well-being varied in response to the presence of life events, both positive and negative, which may simply be an indication that *life change* is stressful and can affect cognition (Luhmann et al., 2012). Second, parenting a child with a DD was adversely associated with both executive function and memory problems. Our findings also revealed that mothers of children with DDs experienced more life events during the past year than mothers of typically developing children, indicating more environmental stressors

for these individuals. These findings are likely not attributable to parenting a child with FXS specifically, as the majority of mothers of children with DDs reported other diagnoses (e.g., autism, ADHD, etc.). Nevertheless, it is possible that child behaviors, rather than the child's condition itself, may have influenced stress in different ways among the mothers included here. Given the range of diagnoses within the children with DDs, there may have been varying levels of problem behaviors. Unfortunately, measures of child behavior problems were not available for the population-based sample in the present research, so this possibility could not be tested here.

In addition to stress and CGG repeat effects, age and education each significantly contributed to variance in cognitive functioning, consistent with prior research (Fjell et al., 2017; Harada et al., 2013; Klusek et al., 2020). Prior work shows that age-related cognitive problems are most pronounced for individuals with lower levels of education (Taylor et al., 2018). The present research suggests that studies of the relationships between variation in the *FMR1* CGG repeat number and behavioral phenotypes should consider additional individual and environmental factors to accurately evaluate the magnitude of *FMR1*-related influences.

Study Strengths, Limitations, and Future Directions

This study had several notable strengths. First, the availability of DNA and *FMR1* CGG repeat assays across the range of CGG repeats (up to 192) enabled robust examination of the effects of *FMR1* repeat-related variability on self-reported cognitive functioning. Second, we had a large sample size, which drew primarily from a population-based biobank. Third, this study was strengthened by consideration of objective, environmental stressors including life events and parenting a child with a DD, providing a thorough test of the research aims evaluated here. Finally, the DD diagnoses in these children were diverse, further contributing to the generalizability of study findings.

This study also had some limitations. Although the sample was diverse with regards to age and the range of *FMR1* repeat-related variation, the participants in the sample were racially and ethnically homogenous. Additionally, many prior reports of associations between cognitive functioning and *FMR1* CGG expansions have included direct-assessment measures, whereas the present study relied on self-report. The study's large sample size precluded direct testing of >1000 individuals. Although PM carriers constituted only a small portion of the present sample, it has been suggested that such individuals may over-report symptoms not evidenced on neurological exam (Birch et al., 2016; Hall et al., 2016). However, there is also extant literature confirming significant associations between cognitive functioning and CGG repeat length using both direct-assessment and self-report measures across the CGG range (Hunter et al., 2008; Kraan et al., 2014), suggesting the validity of self-reported results. Indeed, we observed heterogeneity of cognitive functioning within the PM sample showing that individuals at the higher end of the CGG distribution (who also had PM/full mutation mosaicism) performed similarly to, and in some cases better than, individuals below the PM range of the CGG distribution. Interestingly, Hunter et al. (2008) discussed the possibility that individuals who participate in research may be *less* likely to have cognitive difficulties.

Another limitation of the present study is that the only *FMR1*-related biomarker available for the study participants was CGG repeat number. Inclusion of *FMR1* activation ratio, mRNA, and FMRP levels would greatly enhance understanding of the processes investigated here, such as the influence of PM/full mutation mosaicism on cognitive functioning. Additionally, interpretation of these findings can only be extrapolated to mothers. Studies of males and/or fathers, or women who do not have children, across the CGG range would clarify generalizability of these findings. Finally, the participants in the present study were recruited

using multiple methods, including drawing from a 20,000-person population-based biobank and via a national sample of PM carriers who were identified clinically after a child was diagnosed with FXS. Some prior research similarly used cohorts recruited via multiple sources (Albizua et al., 2017; Allen et al., 2020; Allen et al., 2004; Sullivan et al., 2005). Although this approach made it possible to include participants with repeats ranging from 7 to 192 CGGs, in future research, it would be advantageous to use a single method of recruitment across diverse samples, but that would require access to much larger population biobanks.

Conclusions

Findings from the present study highlight the importance of separately considering the role of stress and *FMR1*-related variability in studies of cognitive functioning. Both stress and CGG repeat length independently predicted variation in self-reported executive function and the likelihood of memory problems. Mothers of children with a range of DDs, as well as mothers experiencing other sources of stress, face challenges with cognitive functioning. It is critical that clinicians who work with families of individuals with DDs consider these factors, particularly as they relate to the demands placed on mothers to support their child. Future work should incorporate multiple dimensions of *FMR1*-related biomarkers and objective cognitive testing to advance understanding of genotype-phenotype associations at the population level.

References

Abidin, R. R. (2012). *Parenting stress index* (4th Edition ed.). PAR.

Albizua, I., Rambo-Martin, B. L., Allen, E. G., He, W., Amin, A. S., & Sherman, S. L. (2017). Women who carry a fragile X premutation are biologically older than noncarriers as measured by telomere length. *Am J Med Genet A*, *173*(11), 2985-2994. https://doi.org/10.1002/ajmg.a.38476

Allen, E. G., Charen, K., Hipp, H. S., Shubeck, L., Amin, A., He, W., . . . Sherman, S. L. (2020). Clustering of comorbid conditions among women who carry an FMR1 premutation. *Genet Med*. https://doi.org/10.1038/s41436-019-0733-5

Allen, E. G., He, W., Yadav-Shah, M., & Sherman, S. L. (2004). A study of the distributional characteristics of FMR1 transcript levels in 238 individuals. *Hum Genet*, *114*(5), 439-447. https://doi.org/10.1007/s00439-004-1086-x

Allen, E. G., Sherman, S., Abramowitz, A., Leslie, M., Novak, G., Rusin, M., . . . Letz, R. (2005). Examination of the effect of the polymorphic CGG repeat in the FMR1 gene on cognitive performance. *Behav Genet*, *35*(4), 435-445. https://doi.org/10.1007/s10519-005-2792-4

American Psychiatric Association (Ed.). (2013). *Diagnostic and statistical manual of mental disorders* (5th Edition ed.).

Barker, E. T., Hartley, S. L., Seltzer, M. M., Floyd, F. J., Greenberg, J. S., & Orsmond, G. I. (2011). Trajectories of emotional well-being in mothers of adolescents and adults with autism. *Dev Psychol*, *47*(2), 551-561. https://doi.org/10.1037/a0021268

Barroso, N. E., Mendez, L., Graziano, P. A., & Bagner, D. M. (2018). Parenting Stress through the Lens of Different Clinical Groups: a Systematic Review & Meta-Analysis. *J Abnorm Child Psychol*, *46*(3), 449-461. https://doi.org/10.1007/s10802-017-0313-6

Birch, R. C., Hocking, D. R., & Trollor, J. N. (2016). Prevalence and predictors of subjective memory complaints in adult male carriers of the FMR1 premutation. *Clin Neuropsychol*, *30*(6), 834-848. https://doi.org/10.1080/13854046.2016.1145905

Brega, A. G., Goodrich, G., Bennett, R. E., Hessl, D., Engle, K., Leehey, M. A., . . . 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*, *30*(8), 853-869. https://doi.org/10.1080/13803390701819044

Brown, W. T., Houck, G. E., Jr., Jeziorowska, A., Levinson, F. N., Ding, X., Dobkin, C., . . . Jenkins, E. C. (1993). Rapid fragile X carrier screening and prenatal diagnosis using a nonradioactive PCR test. *JAMA*, *270*(13), 1569-1575.

Chen, L. S., Tassone, F., Sahota, P., & Hagerman, P. J. (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*(23), 3067-3074. https://doi.org/10.1093/hmg/ddg331

Christ, S. E., Kanne, S. M., & Reiersen, A. M. (2010). Executive function in individuals with subthreshold autism traits. *Neuropsychology*, *24*(5), 590-598. https://doi.org/10.1037/a0019176

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences*. Routledge Academic.

Cornish, K. M., Hocking, D. R., Moss, S. A., & Kogan, C. S. (2011). Selective executive markers of at-risk profiles associated with the fragile X premutation. *Neurology*, *77*(7), 618-622. https://doi.org/10.1212/WNL.0b013e3182299e59

Cornish, K. M., Li, L., Kogan, C. S., Jacquemont, S., Turk, J., Dalton, A., . . . Hagerman, P. J. (2008). Age-dependent cognitive changes in carriers of the fragile X syndrome. *Cortex, 44*(6), 628-636. https://doi.org/10.1016/j.cortex.2006.11.002

Cousino, M. K., & Hazen, R. A. (2013). Parenting stress among caregivers of children with chronic illness: a systematic review. *J Pediatr Psychol, 38*(8), 809-828. https://doi.org/10.1093/jpepsy/jst049

Darnell, J. C., Van Driesche, S. J., Zhang, C., Hung, K. Y., Mele, A., Fraser, C. E., . . . Darnell, R. B. (2011). FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism. *Cell, 146*(2), 247-261. https://doi.org/10.1016/j.cell.2011.06.013

Deater- Deckard, K. (1998). Parenting Stress and Child Adjustment: Some Old Hypotheses and New Questions. *Clinical Psychology: Science and Practice, 5*(3), 314-332. https://doi.org/10.1111/j.1468-2850.1998.tb00152.x

Debrey, S. M., Leehey, M. A., Klepitskaya, O., Filley, C. M., Shah, R. C., Kluger, B., . . . Hall, D. A. (2016). Clinical Phenotype of Adult Fragile X Gray Zone Allele Carriers: a Case Series. *Cerebellum, 15*(5), 623-631. https://doi.org/10.1007/s12311-016-0809-6

Diamond, A. (2013). Executive functions. *Annu Rev Psychol, 64*, 135-168. https://doi.org/10.1146/annurev-psych-113011-143750

Eichler, E. E., Kunst, C. B., Lugenbeel, K. A., Ryder, O. A., Davison, D., Warren, S. T., & Nelson, D. L. (1995). Evolution of the cryptic FMR1 CGG repeat. *Nat Genet, 11*(3), 301-308. https://doi.org/10.1038/ng1195-301

Erkkila, M., Perakyla, J., & Hartikainen, K. M. (2018). Executive Functions and Emotion-Attention Interaction in Assessment of Brain Health: Reliability of Repeated Testing With Executive RT Test and Correlation With BRIEF-A Questionnaire. *Front Psychol, 9*, 2556. https://doi.org/10.3389/fpsyg.2018.02556

Fischer, M. (1990). Parenting stress and the child with attention deficict hyperactivity disorder. *Journal of Clinical Child Psychology, 19*, 337-346. https://doi.org/10.1207/s15374424jccp1904_5

Fjell, A. M., Sneve, M. H., Grydeland, H., Storsve, A. B., & Walhovd, K. B. (2017). The Disconnected Brain and Executive Function Decline in Aging. *Cereb Cortex, 27*(3), 2303-2317. https://doi.org/10.1093/cercor/bhw082

Fu, Y. H., Kuhl, D. P., Pizzuti, A., Pieretti, M., Sutcliffe, J. S., Richards, S., . . . et al. (1991). Variation of the CGG repeat at the fragile X site results in genetic instability: resolution of the Sherman paradox. *Cell, 67*(6), 1047-1058. https://doi.org/10.1016/0092-8674(91)90283-5

Gossett, A., Sansone, S., Schneider, A., Johnston, C., Hagerman, R., Tassone, F., . . . Hessl, D. (2016). Psychiatric disorders among women with the fragile X premutation without children affected by fragile X syndrome. *Am J Med Genet B Neuropsychiatr Genet, 171*(8), 1139-1147. https://doi.org/10.1002/ajmg.b.32496

Grane, V. A., Endestad, T., Pinto, A. F., & Solbakk, A. K. (2014). Attentional control and subjective executive function in treatment-naive adults with Attention Deficit Hyperactivity Disorder. *PLoS One, 9*(12), e115227. https://doi.org/10.1371/journal.pone.0115227

Grigsby, J., Brega, A. G., Engle, K., Leehey, M. A., Hagerman, R. J., Tassone, F., . . . Reynolds, A. (2008). Cognitive profile of fragile X premutation carriers with and without fragile X-associated tremor/ataxia syndrome. *Neuropsychology, 22*(1), 48-60. https://doi.org/10.1037/0894-4105.22.1.48

Grigsby, J., Cornish, K., Hocking, D., Kraan, C., Olichney, J. M., Rivera, S. M., . . . Yang, J. C. (2014). The cognitive neuropsychological phenotype of carriers of the FMR1 premutation. *J Neurodev Disord*, *6*(1), 28. https://doi.org/10.1186/1866-1955-6-28

Hagerman, R., & Hagerman, P. (2021). Fragile X-associated tremor/ataxia syndrome: pathophysiology and management. *Curr Opin Neurol*. https://doi.org/10.1097/WCO.0000000000000954

Hall, D., Berry-Kravis, E., Zhang, W., Tassone, F., Spector, E., Zerbe, G., . . . Leehey, M. A. (2011). FMR1 gray-zone alleles: association with Parkinson's disease in women? *Mov Disord*, *26*(10), 1900-1906. https://doi.org/10.1002/mds.23755

Hall, D., Nag, S., Ouyang, B., Bennett, D. A., Liu, Y., Ali, A., . . . Berry-Kravis, E. (2020). Fragile X Gray Zone Alleles Are Associated With Signs of Parkinsonism and Earlier Death. *Mov Disord*. https://doi.org/10.1002/mds.28086

Hall, D., Todorova-Koteva, K., Pandya, S., Bernard, B., Ouyang, B., Walsh, M., . . . Berry-Kravis, E. (2016). Neurological and endocrine phenotypes of fragile X carrier women. *Clin Genet*, *89*(1), 60-67. https://doi.org/10.1111/cge.12646

Harada, C. N., Natelson Love, M. C., & Triebel, K. L. (2013). Normal cognitive aging. *Clin Geriatr Med*, *29*(4), 737-752. https://doi.org/10.1016/j.cger.2013.07.002

Hayes, S. A., & Watson, S. L. (2013). The impact of parenting stress: a meta-analysis of studies comparing the experience of parenting stress in parents of children with and without autism spectrum disorder. *J Autism Dev Disord*, *43*(3), 629-642. https://doi.org/10.1007/s10803-012-1604-y

Heyman, M., & Hauser-Cram, P. (2015). Negative life events predict performance on an executive function task in young adults with developmental disabilities. *J Intellect Disabil Res*, *59*(8), 746-754. https://doi.org/10.1111/jir.12181

Hippolyte, L., Battistella, G., Perrin, A. G., Fornari, E., Cornish, K. M., Beckmann, J. S., . . . Jacquemont, S. (2014). Investigation of memory, executive functions, and anatomic correlates in asymptomatic FMR1 premutation carriers. *Neurobiol Aging*, *35*(8), 1939-1946. https://doi.org/10.1016/j.neurobiolaging.2014.01.150

Hocking, D. R., Loesch, D. Z., Trost, N., Bui, M. Q., Hammersley, E., Francis, D., . . . Storey, E. (2019). Total and Regional White Matter Lesions Are Correlated With Motor and Cognitive Impairments in Carriers of the FMR1 Premutation. *Front Neurol*, *10*, 832. https://doi.org/10.3389/fneur.2019.00832

Hong, J., DaWalt, L., Baker, M. W., Berry-Kravis, E. M., & Mailick, M. R. (2021). Is FMR1 CGG repeat number polymorphism associated with phenotypic variation in the general population? Report from a cohort of 5499 adults. *Frontiers in Psychiatry*. https://doi.org/10.3389/fpsyt.2021.727085

Huber, K. M., Gallagher, S. M., Warren, S. T., & Bear, M. F. (2002). Altered synaptic plasticity in a mouse model of fragile X mental retardation. *Proc Natl Acad Sci U S A*, *99*(11), 7746-7750. https://doi.org/10.1073/pnas.122205699

Hunter, J. E., Allen, E. G., Abramowitz, A., Rusin, M., Leslie, M., Novak, G., . . . Sherman, S. L. (2008). No evidence for a difference in neuropsychological profile among carriers and noncarriers of the FMR1 premutation in adults under the age of 50. *Am J Hum Genet*, *83*(6), 692-702. https://doi.org/10.1016/j.ajhg.2008.10.021

Hunter, J. E., Leslie, M., Novak, G., Hamilton, D., Shubeck, L., Charen, K., . . . Sherman, S. L. (2012). Depression and anxiety symptoms among women who carry the FMR1 premutation: impact of raising a child with fragile X syndrome is moderated by CRHR1

polymorphisms. *Am J Med Genet B Neuropsychiatr Genet*, *159B*(5), 549-559. https://doi.org/10.1002/ajmg.b.32061

IBM Corp. (2019). *IBM SPSS Statistics, Version 26.0*. In IBM Corp.

Kim, K., Hessl, D., Randol, J. L., Espinal, G. M., Schneider, A., Protic, D., . . . Hagerman, P. J. (2019). Association between IQ and FMR1 protein (FMRP) across the spectrum of CGG repeat expansions. *PLoS One*, *14*(12), e0226811. https://doi.org/10.1371/journal.pone.0226811

Klusek, J., Hong, J., Sterling, A., Berry-Kravis, E., & Mailick, M. R. (2020). Inhibition deficits are modulated by age and CGG repeat length in carriers of the FMR1 premutation allele who are mothers of children with fragile X syndrome. *Brain Cogn*, *139*, 105511. https://doi.org/10.1016/j.bandc.2019.105511

Klusek, J., Porter, A., Abbeduto, L., Adayev, T., Tassone, F., Mailick, M. R., . . . Roberts, J. E. (2018). Curvilinear Association Between Language Disfluency and FMR1 CGG Repeat Size Across the Normal, Intermediate, and Premutation Range. *Front Genet*, *9*, 344. https://doi.org/10.3389/fgene.2018.00344

Kraan, C. M., Hocking, D. R., Georgiou-Karistianis, N., Metcalfe, S. A., Archibald, A. D., Fielding, J., . . . Cornish, K. M. (2014). Impaired response inhibition is associated with self-reported symptoms of depression, anxiety, and ADHD in female FMR1 premutation carriers. *Am J Med Genet B Neuropsychiatr Genet*, *165B*(1), 41-51. https://doi.org/10.1002/ajmg.b.32203

Liston, C., McEwen, B. S., & Casey, B. J. (2009). Psychosocial stress reversibly disrupts prefrontal processing and attentional control. *Proc Natl Acad Sci U S A*, *106*(3), 912-917. https://doi.org/10.1073/pnas.0807041106

Lovell, B., Elliot, H., Liu, C. C., & Wetherell, M. A. (2014). Memory failures for everyday tasks in caregivers of children with autism. *Res Dev Disabil*, *35*(11), 3057-3061. https://doi.org/10.1016/j.ridd.2014.07.019

Luhmann, M., Hofmann, W., Eid, M., & Lucas, R. E. (2012). Subjective well-being and adaptation to life events: a meta-analysis. *J Pers Soc Psychol*, *102*(3), 592-615. https://doi.org/10.1037/a0025948

Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nat Rev Neurosci*, *10*(6), 434-445. https://doi.org/10.1038/nrn2639

Maenner, M. J., Baker, M. W., Broman, K. W., Tian, J., Barnes, J. K., Atkins, A., . . . Mailick, M. R. (2013). FMR1 CGG expansions: prevalence and sex ratios. *Am J Med Genet B Neuropsychiatr Genet*, *162B*(5), 466-473. https://doi.org/10.1002/ajmg.b.32176

Mailick, M., Hong, J., DaWalt, L. S., Greenberg, J. S., Movaghar, A., Baker, M. W., . . . Brilliant, M. H. (2020). FMR1 Low Zone CGG Repeats: Phenotypic Associations in the Context of Parenting Stress. *Front Pediatr*, *8*, 223. https://doi.org/10.3389/fped.2020.00223

Mailick, M., Hong, J., Greenberg, J., Dawalt, L. S., Baker, M. W., & Rathouz, P. J. (2017). FMR1 genotype interacts with parenting stress to shape health and functional abilities in older age. *Am J Med Genet B Neuropsychiatr Genet*, *174*(4), 399-412. https://doi.org/10.1002/ajmg.b.32529

Mailick, M., Hong, J., Greenberg, J., Smith, L., & Sherman, S. (2014). Curvilinear association of CGG repeats and age at menopause in women with FMR1 premutation expansions. *Am J Med Genet B Neuropsychiatr Genet*, *165B*(8), 705-711. https://doi.org/10.1002/ajmg.b.32277

Mailick, M., Hong, J., Rathouz, P., Baker, M. W., Greenberg, J. S., Smith, L., & Maenner, M. (2014). Low-normal FMR1 CGG repeat length: phenotypic associations. *Front Genet*, *5*, 309. https://doi.org/10.3389/fgene.2014.00309

Mailick, M., Movaghar, A., Hong, J., Greenberg, J. S., DaWalt, L. S., Zhou, L., . . . Berry-Kravis, E. (2018). Health profiles of mosaic versus non-mosaic FMR1 premutation carrier mothers of children with fragile X syndrome. *Front Genet*, *9*, 173. https://doi.org/10.3389/fgene.2018.00173

McCarty, C. A., Wilke, R. A., Giampietro, P. F., Wesbrook, S. D., & Caldwell, M. D. (2005). Marshfield Clinic Personalized Medicine Research Project (PMRP): design, methods and recruitment for a large population-based biobank. *Per Med*, *2*(1), 49-79. https://doi.org/10.1517/17410541.2.1.49

Moore, C. J., Daly, E. M., Schmitz, N., Tassone, F., Tysoe, C., Hagerman, R. J., . . . Murphy, D. G. (2004). A neuropsychological investigation of male premutation carriers of fragile X syndrome. *Neuropsychologia*, *42*(14), 1934-1947. https://doi.org/10.1016/j.neuropsychologia.2004.05.002

Movaghar, A., Page, D., Brilliant, M., Baker, M. W., Greenberg, J., Hong, J., . . . Mailick, M. R. (2019). Data-driven phenotype discovery of FMR1 premutation carriers in a population-based sample. *Sci Adv*, *5*(8), eaaw7195. https://doi.org/10.1126/sciadv.aaw7195

Nolin, S. L., Lewis, F. A., Ye, L. L., Houck, G. E., Glicksman, A. E., Limprasert, P., . . . Brown, W. T. (1996). Familial transmission of the FMR1 CGG repeat. *Am J Hum Genet*, *59*(6), 1252-1261.

Op den Kelder, R., Ensink, J. B. M., Overbeek, G., Maric, M., & Lindauer, R. J. L. (2017). Executive function as a mediator in the link between single or complex trauma and posttraumatic stress in children and adolescents. *Qual Life Res*, *26*(7), 1687-1696. https://doi.org/10.1007/s11136-017-1535-3

Pelchat, D., Lefebvre, H., & Levert, M. J. (2007). Gender differences and similarities in the experience of parenting a child with a health problem: current state of knowledge. *J Child Health Care*, *11*(2), 112-131. https://doi.org/10.1177/1367493507076064

Pelchat, D., Lefebvre, H., & Perreault, M. (2003). Differences and similarities between mothers' and fathers' experiences of parenting a child with a disability. *J Child Health Care*, *7*(4), 231-247. https://doi.org/10.1177/13674935030074001

Rabin, L. A., Roth, R. M., Isquith, P. K., Wishart, H. A., Nutter-Upham, K. E., Pare, N., . . . Saykin, A. J. (2006). Self- and informant reports of executive function on the BRIEF-A in MCI and older adults with cognitive complaints. *Arch Clin Neuropsychol*, *21*(7), 721-732. https://doi.org/10.1016/j.acn.2006.08.004

Roth, R. M., Isquith, P. K., & Gioia, G. A. (2005). *Behavior rating inventory of executive function-adult version*. Psychological Assessment Resources.

Roth, R. M., Lance, C. E., Isquith, P. K., Fischer, A. S., & Giancola, P. R. (2013). Confirmatory factor analysis of the Behavior Rating Inventory of Executive Function-Adult version in healthy adults and application to attention-deficit/hyperactivity disorder. *Arch Clin Neuropsychol*, *28*(5), 425-434. https://doi.org/10.1093/arclin/act031

Rouel, M., Raman, J., Hay, P., & Smith, E. (2016). Validation of the Behaviour Rating Inventory of Executive Function - Adult Version (BRIEF-A) in the obese with and without binge eating disorder. *Eat Behav*, *23*, 58-65. https://doi.org/10.1016/j.eatbeh.2016.07.010

Seltzer, M. M., Baker, M. W., Hong, J., Maenner, M., Greenberg, J., & Mandel, D. (2012). Prevalence of CGG expansions of the FMR1 gene in a US population-based sample. *Am J*

Med Genet B Neuropsychiatr Genet, *159B*(5), 589-597. https://doi.org/10.1002/ajmg.b.32065

Seltzer, M. M., Floyd, F., Song, J., Greenberg, J., & Hong, J. (2011). Midlife and aging parents of adults with intellectual and developmental disabilities: impacts of lifelong parenting. *Am J Intellect Dev Disabil*, *116*(6), 479-499. https://doi.org/10.1352/1944-7558-116.6.479

Shelton, A. L., Cornish, K., Clough, M., Gajamange, S., Kolbe, S., & Fielding, J. (2017). Disassociation between brain activation and executive function in fragile X premutation females. *Hum Brain Mapp*, *38*(2), 1056-1067. https://doi.org/10.1002/hbm.23438

Shelton, A. L., Cornish, K., & Fielding, J. (2017). Long term verbal memory recall deficits in fragile X premutation females. *Neurobiol Learn Mem*, *144*, 131-135. https://doi.org/10.1016/j.nlm.2017.07.002

Shelton, A. L., Cornish, K., Kraan, C., Georgiou-Karistianis, N., Metcalfe, S. A., Bradshaw, J. L., . . . Fielding, J. (2014). Exploring inhibitory deficits in female premutation carriers of fragile X syndrome: through eye movements. *Brain Cogn*, *85*, 201-208. https://doi.org/10.1016/j.bandc.2013.12.006

Shelton, A. L., Cornish, K. M., Godler, D. E., Clough, M., Kraan, C., Bui, M., & Fielding, J. (2015). Delineation of the working memory profile in female FMR1 premutation carriers: the effect of cognitive load on ocular motor responses. *Behav Brain Res*, *282*, 194-200. https://doi.org/10.1016/j.bbr.2015.01.011

Shelton, A. L., Cornish, K. M., Kraan, C. M., Lozano, R., Bui, M., & Fielding, J. (2016). Executive Dysfunction in Female FMR1 Premutation Carriers. *Cerebellum*, *15*(5), 565-569. https://doi.org/10.1007/s12311-016-0782-0

Shields, G. S., Moons, W. G., & Slavich, G. M. (2017). Better executive function under stress mitigates the effects of recent life stress exposure on health in young adults. *Stress*, *20*(1), 75-85. https://doi.org/10.1080/10253890.2017.1286322

Skreden, M., Skari, H., Malt, U. F., Pripp, A. H., Bjork, M. D., Faugli, A., & Emblem, R. (2012). Parenting stress and emotional wellbeing in mothers and fathers of preschool children. *Scand J Public Health*, *40*(7), 596-604. https://doi.org/10.1177/1403494812460347

Snow, K., Doud, L. K., Hagerman, R., Pergolizzi, R. G., Erster, S. H., & Thibodeau, S. N. (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*(6), 1217-1228.

Solsnes, A. E., Skranes, J., Brubakk, A. M., & Lohaugen, G. C. (2014). Executive functions in very-low-birth-weight young adults: a comparison between self-report and neuropsychological test results. *J Int Neuropsychol Soc*, *20*(5), 506-515. https://doi.org/10.1017/S1355617714000332

Song, J., Mailick, M. R., Greenberg, J. S., Ryff, C. D., & Lachman, M. E. (2016). Cognitive Aging in Parents of Children with Disabilities. *J Gerontol B Psychol Sci Soc Sci*, *71*(5), 821-830. https://doi.org/10.1093/geronb/gbv015

Sullivan, A. K., Marcus, M., Epstein, M. P., Allen, E. G., Anido, A. E., Paquin, J. J., . . . Sherman, S. L. (2005). Association of FMR1 repeat size with ovarian dysfunction. *Hum Reprod*, *20*(2), 402-412. https://doi.org/10.1093/humrep/deh635

Taylor, A., Bouldin, E. D., & McGuire, L. C. (2018). Subjective cognitive decline among adults aged ≥45 years—United States, 2015-2016. *Morbidity and Mortality Weekly Report*, *67*, 753-757. https://doi.org/http://dx.doi.org/10.15585/mmwr.mm6727a1

Weghofer, A., Tea, M. K., Barad, D. H., Kim, A., Singer, C. F., Wagner, K., & Gleicher, N. (2012). BRCA1/2 mutations appear embryo-lethal unless rescued by low (CGG n<26) FMR1 sub-

genotypes: explanation for the "BRCA paradox"? *PLoS One*, *7*(9), e44753. https://doi.org/10.1371/journal.pone.0044753

Wheeler, A., Bailey, D. B., Berry-Kravis, E., Greenberg, J., Losh, M., Mailick, M., . . . Hagerman, R. (2014). Associated features in females with an FMR1 premutation. *J Neurodev Disord*, *6*(1), 30. https://doi.org/10.1186/1866-1955-6-30

Wheeler, A., Raspa, M., Hagerman, R., Mailick, M., & Riley, C. (2017). Implications of the FMR1 Premutation for Children, Adolescents, Adults, and Their Families. *Pediatrics*, *139*(Suppl 3), S172-S182. https://doi.org/10.1542/peds.2016-1159D

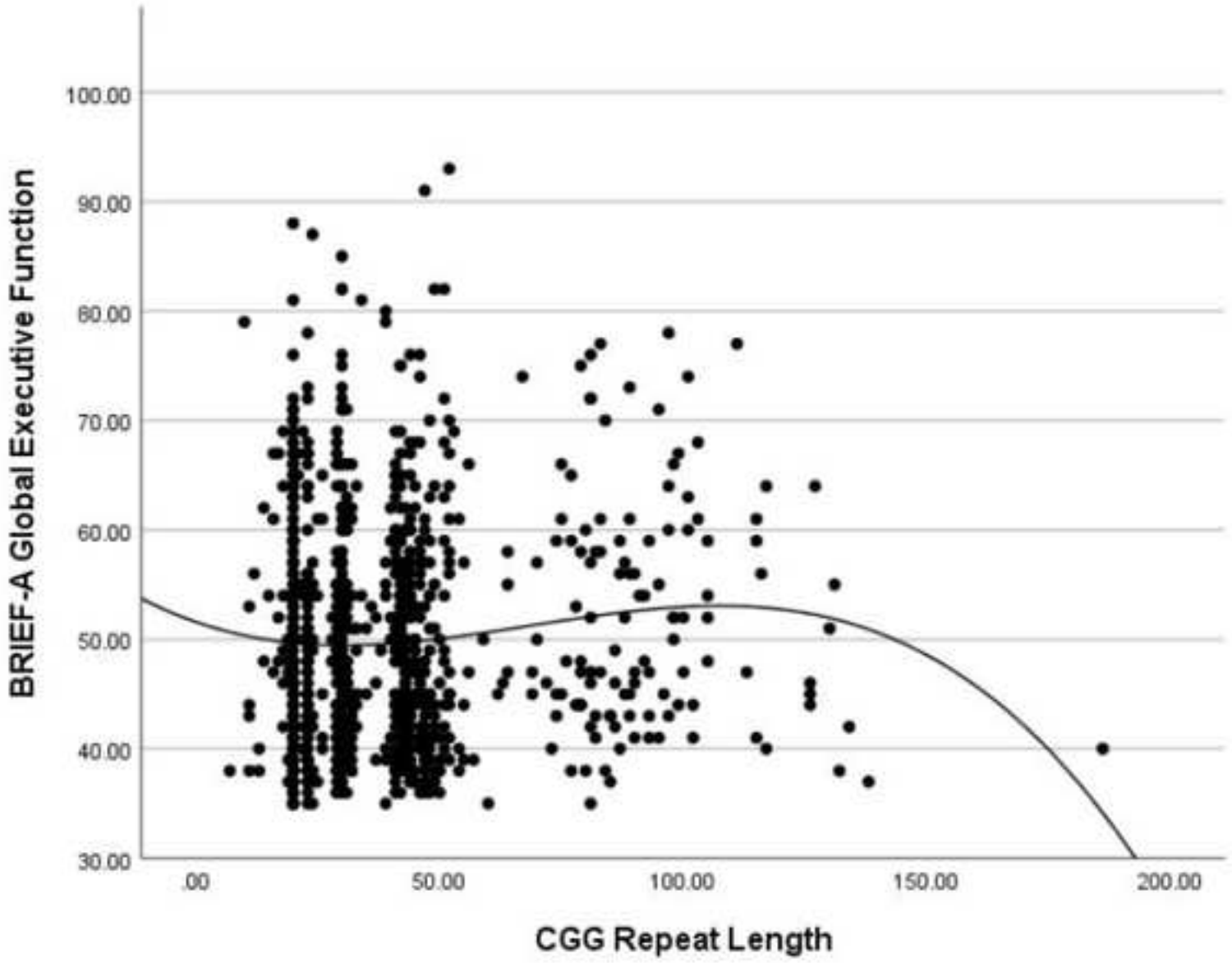

Figure 1. Cubic association between CGG repeat length and executive function difficulty.

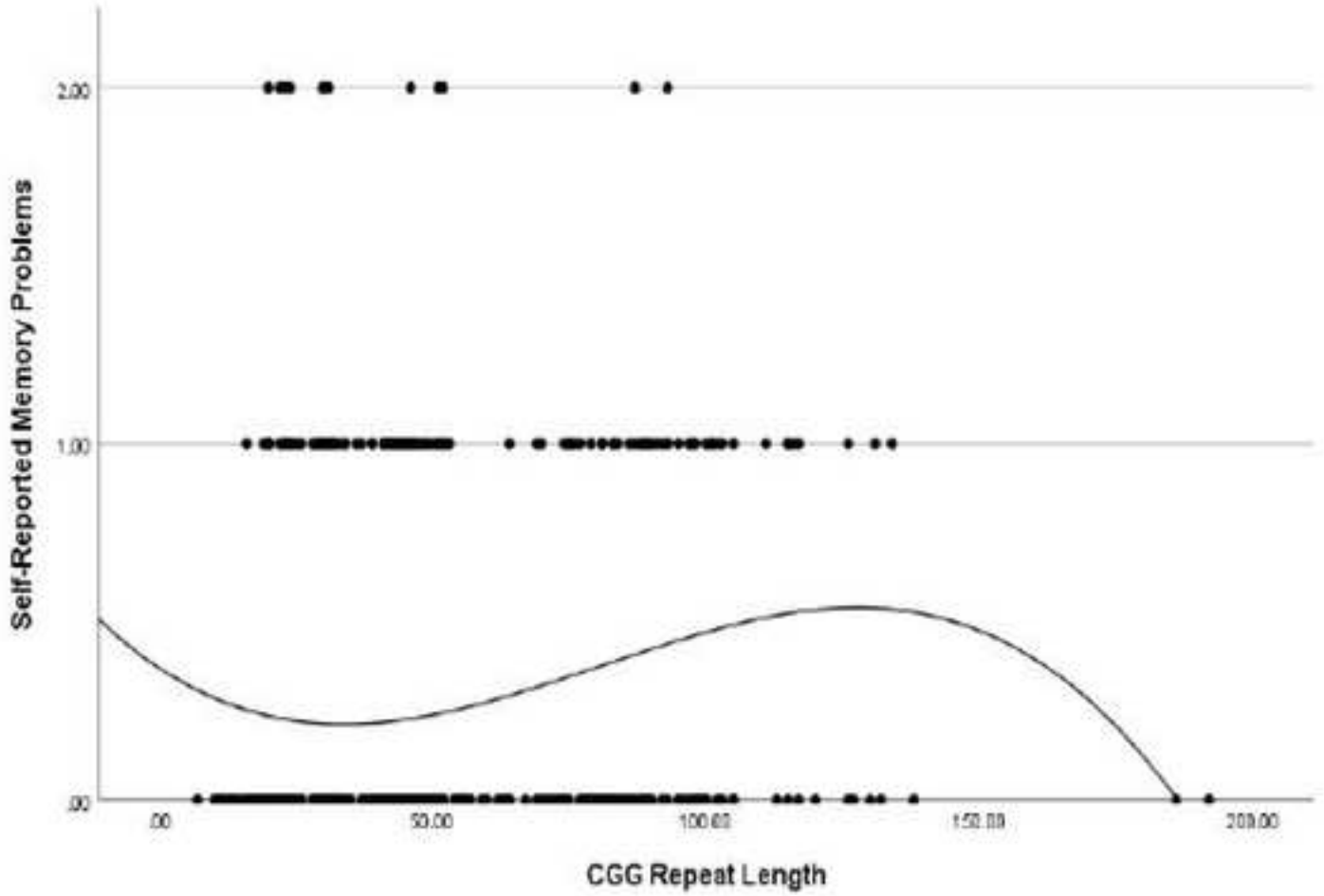

Figure 2. Cubic association between CGG repeat length and self-reported memory problems. Self-reported memory problems reflect the following scale: 0 (no memory problems), 1 (problems with memory, not diagnosed by a health professional), 2 (diagnosis of memory problems).

Table 1. Conditions of children of participating mothers

Condition	Frequency	Percentage (%)
None	721	68.5%
Fragile X Syndrome[1]	141	13.4%
ADHD	116	11.0%
Learning disabilities	28	2.7%
Other DDs[2]	25	2.3%
Autism spectrum disorders	22	2.1%
Total	1053	100.0%

[1] 140/141 cases of FXS were derived from the clinically-ascertained sample.
[2] including cerebral palsy, Down syndrome, intellectual disabilities.
Note: Some mothers in the present study had more than one child with a DD (developmental disability). The conditions of the children within each family were independently reviewed by three experienced raters (authors LSD, MM, and JH) and the condition determined to be most severe was reported above in Table 1.

Table 2. Target allele selection method and CGG repeat lengths across the CGG allele types.

CGG Allele Types (Long Allele-Short Allele)	**Target Allele Selection Method**	**Target (Selected) Allele** *M*(*SD*), Range	**Non-Target (Non-Selected) Allele** *M*(*SD*), Range
Low-Low (n = 79)	CGG Shorter Allele	19.56 (2.52) 7-24	21.29 (1.46) 18-25
Normal-Low (n = 191)	CGG Shorter Allele	20.92 (2.52) 10-25	31.27 (3.13) 26-40
Normal-Normal (n = 260)	Randomly Selected	30.85 (2.69) 26-40	30.52 (2.28) 26-40
Gray-Low (n = 75)	Randomly Selected	33.33 (11.75) 20-52	33.47 (12.69) 9-53
Gray-Normal (n = 276)	CGG Longer Allele	44.97 (3.36) 41-54	31.30 (2.37) 26-40
Premutation-Low (n = 46)	Randomly Selected	46.56 (32.63) 16-115	61.47 (34.65) 17-123
Premutation-Normal (n = 121)	CGG Longer Allele	90.24 (22.34) 55-192	30.26 (1.59) 27-40
Premutation-Gray (n = 5)	CGG Longer Allele	89.40 (22.55) 56-115	44.40 (3.44) 41-50

Note: Alleles are characterized based on the following CGG repeat ranges: Low (<26), Normal (26-40), Gray (41-54), Premutation (55-200).

Table 3. Correlations between cognitive functioning, age, education, stress, and CGG repeat length

Variable	Age	Education	Parenting Status	Life Events	CGG Target Allele	Self-Reported Memory Problems	BRIEF-A GEC
Age	--						
Education	-.282***	--					
Parenting Status	-.120***	.166***	--				
Life Events	-.164**	-.020	.170***	--			
CGG Target Allele	.011	.142***	.406***	<.001	--		
Self-Reported Memory Problems	.107***	-.093**	.156***	.131***	.119***	--	
BRIEF-A GEC	.113***	-.122***	.119***	.199***	.066***	.359***	--
M (SD)	57.01 (15.17)	(1) 3.0% (2) 34.0% (3) 47.2% (4) 15.7%	31.5%[#]	1.23 (1.41)	40.20 (23.40)	.25 (.43)	49.99 (10.48)

Note: Education was coded as 1 (less than high school), 2 (high school degree), 3 (college degree or equivalent), 4 (master's degree or above). Parenting status is dichotomized (0 = no child with a DD, 1 = child with a DD). Participant *N*s ranged from 983 to 1053. GEC: Global executive composite.
***$p < .001$; **$p < .010$
[#]Represents percentage of mothers of a child with a DD

Table 4. Results of Ordinary Least Square (OLS) Regression Models: Stress and CGG Repeat Length Predict Global Executive Function.

A. Life Events (*n*=945)	Model 1			Model 2			Model 3		
	b	S.E.	*p*	*b*	S.E.	*p*	*b*	S.E.	*p*
(Constant)	46.648	2.180	<.001	45.940	2.191	<.001	50.299	2.951	<.001
Age	.083	.023	<.001	.079	.023	.001	.080	.023	.001
Maternal Ed.	-1.176	.464	.011	-1.378	.469	.003	-1.559	.472	.001
Life Events (Self)	1.560	.239	<.**001**	1.563	.238	<.001	1.542	.237	<.001
CGG (Linear)	--	--	--	.036	.014	**.011**	-.213	.107	.047
CGG (Quadratic)	--	--	--	--	--	--	.004	.002	.007
CGG (Cubic)	--	--	--	--	--	--	-.0002	.000	.**004**
R^2	.063			.069			.078		
B. Parenting Status (*n*=979)	Model 1			Model 2			Model 3		
	b	S.E.	*p*	*b*	S.E.	*p*	*b*	S.E.	*p*
(Constant)	49.627	2.083	<.001	49.412	2.097	<.001	51.965	2.902	<.001
Age	.069	.023	.003	.067	.023	.004	.066	.023	.004
Maternal Ed.	-1.699	.463	<.001	-1.752	.467	<.001	-1.867	.469	<.001
Parenting Status	3.435	.712	<.**001**	3.159	.774	<.001	2.904	.799	<.001
CGG (Linear)	--	--	--	.014	.016	.364	-.139	.109	.203
CGG (Quadratic)	--	--	--	--	--	--	.003	.002	.075
CGG (Cubic)	--	--	--	--	--	--	-.00002	.000	.**036**
R^2	.045			.049			.051		

Note: Significant effects of key predictors noted in **bold**.

Table 5. Results of Ordinary Least Square (OLS) Regression Models: Stress and CGG Repeat Length Predict Memory Problems.

A. Life Events	Model 1			Model 2			Model 3		
(n=993)	*b*	S.E.	*p*	*b*	S.E.	*p*	*b*	S.E.	*p*
(Constant)	.065	.094	.492	.013	.094	.889	.295	.125	.889
Age	.004	.001	<.001	.004	.001	<.001	.004	.001	<.001
Maternal Ed.	-.028	.020	.162	-.042	.020	.039	-.053	.020	.040
Life Events (Self)	.036	.010	**.001**	.036	.010	.001	.034	.010	.001
CGG (Linear)	--	--	--	.003	.001	**<.001**	-.012	.004	.190
CGG (Quadratic)	--	--	--	--	--	--	.0002	.00006	.978
CGG (Cubic)	--	--	--	--	--	--	-.0000009	.0000002	<**.001**
R^2	.029			.046			.060		
B. Parenting Status	Model 1			Model 2			Model 3		
(n=1034)	*b*	S.E.	*p*	*b*	S.E.	*p*	*b*	S.E.	*p*
(Constant)	.126	.087	.150	.099	.088	.257	.304	.120	.011
Age	.004	.001	<.001	.003	.001	.001	.003	.001	.001
Maternal Ed.	-.050	.019	.011	-.054	.020	.005	-.062	.020	.002
Parenting Status	.175	.030	<**.001**	.145	.033	<.001	.123	.034	<.001
CGG (Linear)	--	--	--	.002	.001	**.019**	-.009	.004	.029
CGG (Quadratic)	--	--	--	--	--	--	.0002	.00006	.005
CGG (Cubic)	--	--	--	--	--	--	-.0000007	.0000002	**.003**
R^2	.049			.054			.062		

Note: Significant effects of key predictors noted in **bold**.

Supplementary Materials

Table S1. Results of Ordinary Least Square (OLS) Regression Models: Stress and CGG Repeat Length Do Not Predict Global Executive Function.

A. Life Events (n=945)	Model 1		
	b	S.E.	*p*
(Constant)	51.184	3.411	<.001
Age	.080	.023	.001
Maternal Ed.	-1.610	.471	.001
Life Events (Self)	2.522	1.604	.116
CGG Linear	-.238	.138	.086
CGG Quadratic	.004	.002	.033
CGG Cubic	-.00002	.000008	.025
CGG x LE (Linear)	-.090	.092	.329
CGG x LE (Quadratic)	.002	.001	.145
CGG x LE (Cubic)	-.00001	.000007	.063
R^2	.086		

B. Parenting Status (n=979)	Model 1		
	b	S.E.	*p*
(Constant)	51.015	3.308	<.001
Age	.065	.023	.005
Maternal Ed.	-1.870	.469	<.001
Parenting Status	5.172	3.957	.192
CGG Linear	-.086	.150	.566
CGG Quadratic	.002	.002	.268
CGG Cubic	-.00004	.000009	.051
CGG x LE (Linear)	-.120	.194	.536
CGG x LE (Quadratic)	.001	.002	.536
CGG x LE (Cubic)	.00002	.00003	.600
R^2	.051		

Table S2. Results of Ordinary Least Square (OLS) Regression Models: Stress and CGG Repeat Length Do Not Predict Self-Reported Memory Problems

A. Life Events (n=993)	Model 1		
	b	S.E.	*p*
(Constant)	.283	.147	.054
Age	.003	.001	.001
Maternal Ed.	-.053	.020	.009
Life Events (Self)	.052	.066	.439
CGG Linear	-.009	.006	.117
CGG Quadratic	.0002	. 00009	.526
CGG Cubic	-.0000007	.0000003	.055
CGG x LE (Linear)	-.002	.004	.053
CGG x LE (Quadratic)	.00005	.00006	.364
CGG x LE (Cubic)	-.0000003	.0000003	.359
R^2	.065		
B. Parenting Status (n=1034)	Model 1		
	b	S.E.	*p*
(Constant)	.187	.139	.177
Age	.003	.001	.001
Maternal Ed.	-.062	.020	.001
Parenting Status	.311	.165	.059
CGG Linear	-.002	.006	.782
CGG Quadratic	.00008	.00009	.378
CGG Cubic	-.0000009	.0000003	.005
CGG x PS (Linear)	-.011	.008	.158
CGG x PS (Quadratic)	.0001	.00009	.106
CGG x PS (Cubic)	.000002	.000002	.455
R^2	.070		