Title

Altered neural activity of magnitude estimation processing in adults with the fragile X premutation

Journal

Journal of Psychiatric Research, 47(12)

ISSN

0022-3956

Authors

Kim, So-Yeon
Hashimoto, Ryu-ichiro
Tassone, Flora
et al.

Publication Date

2013-12-01

DOI

10.1016/j.jpsychires.2013.08.014

Peer reviewed

Published in final edited form as:
J Psychiatr Res. 2013 December ; 47(12): . doi:10.1016/j.jpsychires.2013.08.014.

Altered neural activity of magnitude estimation processing in adults with the fragile X premutation

So-Yeon Kim[1,5], **Ryu-ichiro Hashimoto**[2], **Flora Tassone**[3], **Tony J. Simon**[3,5], and **Susan M. Rivera**[1,3,4,*]

[1]Center for Mind and Brain, University of California, Davis

[2]Dept. of Language Sciences, Tokyo Metropolitan University, Tokyo

[3]MIND Institute, University of California, Davis

[4]Dept. of Psychology, University of California, Davis

[5]Dept. of Psychiatry and Behavioral Sciences, University of California, Davis

Abstract

Mutations of the fragile X mental retardation 1 (*FMR1*) gene are the genetic cause of fragile X syndrome (FXS). Expanded CGG trinucleotide repeat (> 200 repeats) result in transcriptional silencing of the *FMR1* gene and deficiency/absence of the *FMR1* protein (FMRP). Carriers with a premutation allele (55–200 CGG repeats) are often associated with mildly reduced levels of FMRP and/or elevated levels of *FMR1* mRNA, and are associated with the risk of developing a neurodegenerative disorder known as fragile X-associated tremor/ataxia syndrome (FXTAS). While impairments in numerical processing have been well documented in FXS, recent behavioral research suggests that premutation carriers also present with subtle but significant impairments in numerical processing. Using fMRI, the current study examined whether asymptomatic adults with the premutation would show aberrant neural correlates of magnitude estimation processing in the fronto-parietal area. Using a magnitude estimation task, we demonstrated that activity in the intraparietal sulcus and inferior frontal gyrus, associated with magnitude estimation processing, was significantly attenuated in premutation carriers compared to their neurotypical counterparts despite their comparable behavioral performance. Further, multiple regression analysis using CGG repeat size and *FMR1* mRNA indicated that increased CGG repeat size is a primary factor for the decreased fronto-parietal activity, suggesting that reduced FMRP, rather than a toxic gain-of-function effect from elevated mRNA, contributes to altered neural activity of magnitude estimation processing in premutation carriers. In conclusion, we provide the first evidence on the aberrant neural correlates of magnitude estimation processing in premutation carriers accounted for by their *FMR1* gene expression.

*Corresponding author: Susan M. Rivera, Ph.D, at Center for Mind and Brain, University of California, Davis, 267 Cousteau Place, Davis, CA 95618, USA., Tel.: +1 530 747 3802; Fax: +1 530 297 4603., srivera@ucdavis.edu.

Contributors

Author Kim analyzed behavioral and fMRI data and wrote the first draft of the manuscript. Author Hashimoto designed and scripted the behavioral task and analyzed fMRI data. Author Tassone analyzed and provided the molecular data from blood samples. Author Simon conceptualized the study and supervised the writing of the draft. Author Rivera conceptualized and designed the study and supervised the writing of the draft and the analysis of behavioral and fMRI data.

1. Introduction

Fragile X syndrome (FXS) is the most common inherited form of intellectual disability, resulting from a trinucleotide repeat expansion in the 5′ untranslated region of the fragile X mental retardation 1 (*FMR1*) gene located at Xq27.3, affecting approximately 1 in 2500 individuals (Fernandez-Carvajal et al., 2009). When the CGG expansion exceeds 200 repeats (i.e., full mutation), it results in hypermethylation of the *FMR1* gene and subsequent loss of the gene protein product, *fragile X mental retardation protein (FMRP)*. Individuals with expanded, but unmethylated, repeats (55–200 repeat) are categorized as fragile X premutation carriers, and are associated with elevated levels of *FMR1* mRNA and mildly reduced levels of FMRP (Garcia-Arocena and Hagerman, 2010; Hagerman and Hagerman, 2004; Kenneson et al., 2001; Tassone et al., 2000b). The prevalence of the premutation is relatively common (1 of 130–250 females and 1 of 250–800 males (Hagerman et al., 2009)), and about 40% of male and 8–16% of female adults with premutation allele (>50 years old) may develop a late-onset neurodegenerative disorder known as fragile X-associated tremor/ataxia syndrome (FXTAS) (Jacquemont et al., 2004), which is associated with tremors, gait ataxia, and executive function impairments (Bourgeois et al., 2009).

Until recently, it has been widely regarded that asymptomatic (i.e., non-FXTAS), young adults with the premutation are unaffected in their cognitive processing (Snow et al., 1993). However, recent studies have reported evidence indicating that, although the effect may be very subtle, presence of the premutation allele might modify neural development and/or cognitive function similar to that which is seen in individuals with FXS or FXTAS. For example, Keri and Benedek (2009; 2012) have found that, like in the case of FXS, premutation carriers present with mild dysfunction in motion perception, detection of spatial location, and visuomotor coordination. Further, these researchers recently demonstrated that subtle visual dysfunction in individuals with the premutation was significantly explained by FMRP level, suggesting possible neurodevelopmental changes in the low-level visual processing in the premutation with genetic contributions— i.e., expanded CGG length (Keri & Benedek, 2012). Furthermore, consistent with evidence on deficits in dorsal-stream visual processing in people with FXS (e.g., Kogan et al., 2004a, b), Hocking and colleagues (2012) demonstrated that asymptomatic male premutation carriers with high CGG repeat size (100 < CGG < 200) performed significantly worse than normal controls on a dot test of visuospatial working memory (WM) after accounting for effects of age and IQ on the performance. Such findings indicate that individuals with premutation alleles may have subtle cognitive dysfunctions which are a much milder form of those seen in FXS, and which have little, if any, effect on their everyday cognitive functioning.

Like deficits in dorsal-stream visual processing, quantitative and magnitude estimation processing have also been identified as one of the core cognitive functions affected in people with the full mutation (FXS). Specifically, females with FXS often reveal arithmetic difficulties in IQ tests (Bennetto et al., 2001; Grigsby et al., 1990; Kemper et al., 1986; Miezejeski et al., 1986), and a previous study showed impairments on complex arithmetic tests (e.g., 3-operamd arithmetic equations) in females with FXS both at behavioral and neural levels (Rivera et al., 2002). Interestingly, recent studies have also reported subtle impairments in arithmetic performance in females with the premutation. For example, using the Wide Range Achievement test, Lachiewicz and colleagues (2006) found that females with the premutation showed significant difficulty in arithmetic tests compared to tests for reading and spelling. Although the results from Lachiewicz et al. (2006) must be considered with a caution because the study was a retrospecitve review of the clinical experience rather than a prospective controlled study, it is still worth noting that the reported deficit in mathematics were positively correlated with CGG repeat length in premutation carriers, indicating a significant dose-response of *FMR1* gene on arithmetic processing in the

premutation. Likewise, recent studies from our group also found a dosage effect of the *FMR1* gene (indexed by CGG repeat size) on basic magnitude processing in females with the premutation, although no significant performance differences were found in either enumeration or magnitude estimation task between premutation and control groups (Goodrich-Hunsaker et al., 2011a, b).

Despite such recent behavioral evidence on effect of *FMR1* gene expression on behavioral performance of quantitative processing in asymptomatic premutation carriers, to date there has been no published study on the neural substrates of numerical/magnitude processing in premutation carriers. In fact, aberrant neural correlates responding to other cognitive functions have been found in asymptomatic adults with the premutation. Structurally, it has been found that asymptomatic adult carriers reveal significantly reduced grey matter density in the amygdala-hippocampal complex (Moore et al., 2004), reduced hippocampal volumes (Jakala et al., 1997), and a negative correlation between CGG repeat length and brain volume (Cohen et al., 2006). In functional brain imaging studies, researchers have demonstrated reduced activity in brain regions, such as hippocampus, in premutation carriers relative to age and IQ matched controls while performing a recall task, despite comparable behavioral performance on the task between the two groups (Koldewyn et al., 2008). Motivated by aforementioned studies reporting atypical brain activity for some of cognitive functions and also inspired by recent behavioral findings on the effect of *FMR1* gene expression on magnitude processing in premutation carriers, the present study investigated whether asymptomatic adult carriers show atypical neural correlates of magnitude estimation processing modulated by *FMR1* gene expressions.

To examine neural correlates of basic magnitude estimation processing, we used a well-established magnitude comparison task developed by Ansari and colleagues (Ansari and Dhital, 2006; Holloway and Ansari, 2010; Holloway et al., 2010; Price et al., 2007). The task requires magnitude comparison processing for non-symbolic numerical quantities. The "numerical distance effect" in this task is measured by comparing performance in a smaller numerical difference condition to that of a larger difference condition. Previous neuroimaging studies using this task have found strong fronto-parietal activation in response to the magnitude comparison processing, with great emphasis on activation in intraparietal sulcus (IPS) and inferior frontal gyrus (IFG) (Ansari and Dhital, 2006; Holloway and Ansari, 2009; Holloway et al., 2010). Based on these previous findings, we expected to find a distance effect in the fronto-parietal areas including bilateral IPS and IFG, and hypothesized that asymptomatic adults with the premutation would show attenuated brain activity in such areas associated with the distance effect compared to neurotypical counterparts. Further, we expected to find a dosage effect of *FMR1* gene expression on reduced fronto-parietal activation associated with distance effect in premutation carriers.

2. Methods

2.1. Participants

Twenty-eight adults with the premutation (Premutation group; mean age: 32.3 years, Female: 16) and 29 neurotypical age- and gender-matched controls (NT group; mean age: 30.6 years, Female: 14) with normal or corrected-to-normal vision participated in the current study. Full-Scale IQ (FSIQ) was obtained using the Wechsler Adult Intelligence Scale (WAIS-III; Wechsler, 1997) or the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999).

Four females in the premutation group and two females and three males in the NT group were excluded from further analyses due to excessive movement (> 3mm) in the scanner or due to failure to complete the fMRI scan. The two groups did not differ in age, $t(46)=.74$,

$p>.05$, FSIQ, $t(43)=.77$, $p>.05$, nor gender (i.e., each group had 12 females). None of the participants in the premutation group were mosaic for either repeat size or methylation, and allele status was confirmed by *FMR1* DNA testing. Group demographic statistics, FSIQ, and *FMR1* data are shown in Table 1. Participants were recruited through the NeuroTherapeutics Research Institute (NTRI) at the Medical Investigation of Neurodevelopmental Disorders (MIND) Institute of the University of California Davis Medical Center. The Institutional Review Board at the UC Davis approved the experimental protocol. Prior to the experiment, each participant provided informed written consent and all were compensated $25 for participation in the MRI scan. Screening for MR safety was also completed on the day of scanning to ensure eligibility for MR. None of the participants were taking psychotropic medications or medications known to affect MR signal.

2.2. Molecular Genetics Measures

Genomic DNA was isolated from peripheral blood lymphocytes using standard methods (Puregene Kit; Gentra Inc). CGG sizing and methylation status were assessed by Southern Blot and polymerase chain reaction (PCR) analyses as detailed in Tassone et al. (2008). Analysis and calculation of the repeat size for both analyses were carried out using an Alpha Innotech FluorChem 8800 Image Detection System (Tassone et al., 2008). As previously described (Tassone et al., 2000), all quantifications of *FMR1* mRNA were performed using a 7900 Sequence detector (Applied Biosystems).

2.3. Imaging methods and analyses

2.3.1. Brain image acquisition—All imaging data were acquired on a Siemens 3 Tesla Trio scanner with Echospeed gradients and a Siemens 8-channel whole head coil located at the UC Davis Imaging Research Center. Visual stimuli were projected onto a screen and viewed on an MR compatible mirror mounted above the participant's head. For each participant, an anatomical scan was acquired using a high resolution T1-weighted MPRAGE sequence (TR = 2500 ms, TE = 4.33 ms, flip angle = 7°, FOV = 243× 243 mm, 208 slices, 0.9 mm slice thickness) to aid in localization, co-registration, and normalization of functional data. For the functional runs, imaging was performed using a T2*-weighted gradient echo planar pulse sequence (TR = 2500 ms, TE = 30 ms, flip angle = 90°). Each brain volume was composed of 34 axial slices (FOV = 218 × 218, matrix = 64 × 64, 3.4 × 3.4 × 3.4 mm resolution) aligned to the AC–PC line, collected interleaved, inferior to superior. For all functional runs, data from the first two volumes were discarded to allow for stabilization of magnetic fields.

2.3.2. Image processing and statistical analyses—Imaging data were preprocessed using SPM 5 (Wellcome Department of Cognitive Neurology, London) run within Matlab (Matlab Mathwork, Inc., Natick, MA). For preprocessing, data were slice-time corrected for acquisition order, realigned and unwarped to correct for motion across runs. Next, the images were spatially normalized (with trilinear interpolation and preserving the intensities of the original images) to the SPM EPI template corresponding to the MNI (Montreal Neurological Institute) defined standardized brain space, and then spatially smoothed with a Gaussian kernel of 5 mm FWHM. All participants who were included in the analyses moved less than 3 mm in x, y, or z planes. The time series were high pass filtered at 128 s.

Statistical analyses were performed using the general linear model for event-related designs in SPM 5. For each participant, a whole-brain voxel-wise analysis was conducted in which individual events were modeled as a canonical hemodynamic response. Each event type was first modeled for each participant using a fixed effects analysis. Then, the resulting least squares parameter estimates of the height of the modeled hemodynamic response for each condition were entered into random effects analyses. For all random effects analyses,

significant clusters of activation were determined using the joint expected probability of height $p < .01$ ($z > 2.33$ at the voxel-level) and an extent threshold of $p < .05$ (at least 220 consecutive voxels), yielding a clusterwise significance level of $p < .05$, corrected for multiple comparisons (Poline, et al., 1997).

2.4. fMRI magnitude estimation task and statistical analyses

We modeled our magnitude estimation task after that used in Ansari & Dhital (2006) (Figure 1). In the scanner, participants saw side-by-side displays of sets of rectangles of which each side of screen contained 1–9 white squares. The total area occupied by squares was equated between numerosities so that squares in both sides were equated for area. Furthermore, groups of squares in each trial were equidistantly presented from a central fixation cross. Participants were asked to indicate a set containing more rectangles by pressing a left or right button of an MR compatible button box. The numerical distance between groups of squares was 1, 2, 3, 5, 6, or 7. As in the previous study, the distances were categorized into small distances (1–3) and large distances (5–7) for the analysis, and the distance effect was calculated by comparing the accuracy and reaction time data between the small and large distance conditions. To achieve jitter in the time series, trials were presented with variable fixation intervals of 3900, 6400, or 8900 ms. Each participant completed two functional runs of this task and each run consisted of 54 trials and lasted for 6 minutes and 53 seconds. Half of the trials in each run were the small distance condition and the other half of them were the large distance condition, resulting in 54 small and 54 large distance trials for blood oxygenation level dependent (BOLD) response estimation.

All of the statistical tests including the between-group analyses and correlation analyses on demographic, FSIQ, molecular, and behavioral performance data were conducted using IBM SPSS statistics 20 (http://www.ibm.com/software/analytics/spss/products/statistics).

3. Results

3.1. Correlation between molecular variables and FSIQ

As previously found (Kenneson et al., 2001; Tassone et al., 2000a), CGG repeat length was positively correlated with *FMR1* mRNA level in the premutation group only (r=.47, p<.05). There was no significant relationship between FSIQ and CGG, nor between FSIQ and *FMR1* mRNA, in either NT or premutation groups (all $p > .05$).

3.2. Behavioral performance

To test behavioral performance in the magnitude estimation task, a 2 (distance, within-subjects factor) X 2 (group, between-subjects factor) mixed analysis of variance (ANOVA) was conducted on the accuracy data. The results showed a significant main effect of distance (F(1,46)=41.75, p<.05), indicating that both groups made fewer errors in the large distance condition than the small distance condition (i.e., distance effect; Table 2). Neither a main effect of group nor an interaction between distance and group was significant (F(1,46)=1.12, p>.05, F(1,46)=.70, p>.05, respectively), indicating that the two groups revealed comparable performance and patterns of distance effect. Correlation analyses using molecular variables showed no significant relationship between *FMR1* gene expression (i.e., CGG repeat size and *FMR1* mRNA) and the distance effect in either diagnostic group.

The same 2 × 2 mixed ANOVA on the reaction times (RTs) data showed a significant main effect of distance (F(1,46)=271.70, p<.05), indicating slower RT in the small than the large distance condition in both groups (Table 2). There was no significant main effect of group nor interaction between the two factors (F(1,46)=.78, p>.05, F(1,46)=.67, p>.05, respectively), confirming no speed-accuracy trade-off on comparable distance effect in two

groups. Finally, no significant correlation was found between *FMR1* gene expression and the distance effect in the RT data in either group.

3.3. Functional imaging results

3.3.1. Group analyses—Table 3 lists brain regions showing significant activation in each group for the distance effect (i.e., Small distance > Large distance). Consistent with findings in previous studies using magnitude estimation tasks, the NT group showed significantly activated clusters in bilateral inferior parietal lobule (IPL) encompassing bilateral IPS. Furthermore, activation in bilateral insula, bilateral dorsolateral prefrontal cortex (DLPFC), and bilateral inferior frontal gyrus (IFG) was also significant in the Small > Large distance comparison. The premutation group showed a similar pattern of brain activity for the distance effect, but the intensity of the activity was relatively weaker than in the NT group. In the premutation group, clusters revealing significant distance effect were found in the bilateral IPL including IPS, right IFG including right DLPFC and insula, right cingulate gyrus, and left insula.

A two-sample t-test was conducted to evaluate whether the premutation group showed attenuated fronto-parietal activity for the distance effect compared to the NT group. The analysis revealed significantly greater activation in bilateral IPL/IPS and left IFG in the NT group than the premutation group for the distance effect (Table 4). It is worth highlighting that the two groups showed comparable behavioral performance in the magnitude estimation task. That is, premutation carriers showed attenuated neural activity for the magnitude comparison processing compared to controls although this atypicality was not apparent at the behavioral level. The inversed contrast (Premutation > NT for Small > Large) did not reveal any suprathreshold clusters.

3.3.2. Multiple regression analyses using FMR1 gene expression—Our results indicated that the fronto-parietal activation related to the distance effect was significantly attenuated in the premutation group, suggesting a possible role of *FMR1* gene expression playing in the neural correlates of the magnitude estimation processing. To determine the relative and independent effects of the *FMR1* molecular measures on brain activity, we ran multiple regression analysis in SPM using CGG repeat size and *FMR1* mRNA level as two factors and brain activation for the distance effect (i.e., activated regions in the Small > Large contrast) in the premutation group as a dependent variable. Because the two factors were positively correlated with each other in the premutation group, we residualized the two gene expression variables for regression analyses in order to obtain the component of one independent variable that cannot be explained or predicted by the other independent variable (Allen, 1997). The residualized predictor was then used in the regression analyses. The test of multicollinearity in the model confirmed that the two molecular predictors were independent (i.e., Tolerance = 1; Variance Inflation Factor (VIF) = 1).

Results from the regression analysis showed that when ruling out the effect of CGG repeat size, no cluster in the brain associated with the distance effect was significantly explained by *FMR1* mRNA level. In contrast, the results indicated that CGG repeat size was a primary factor to explain the reduced fronto-parietal activation in the premutation group associated with the distance effect, after accounting for the effect of *FMR1* mRNA (Table 5; Figure 2). Specifically, while controlling for the effect of *FMR1* mRNA, we found a significantly negative relationship between CGG repeat size in premutation carriers and their brain activation associated with the distance effect in bilateral IPS (R^2_{adj}=.53, r=−.75, β=−.07, p<.05 and R^2_{adj}=.36, r=−.65, β=−.06, p<.05 for right and left IPS, respectively), right IFG (R^2_{adj}=.47, r=−.72, β=−.05, p<.05), bilateral MFG (R^2_{adj}=.44, r=−.69, β=−.04, p<.05 and R^2_{adj}=.55, r=−.77, β=−.02, p<.05 for right and left MFG, respectively), and right SFG

(R^2_{adj}=.37, r=−.65, β=−.03, p<.05). Of note, the Shapiro-Wilk normality test revealed that the distributions of the molecular variables did not significantly violate the assumption of normality, $p > .87$. Thus, the results do not appear to be driven by a subset of participants with high (or low) CGG repeat sizes.

4. Discussion

The current study provides novel evidence of significant modulation of *FMR1* gene expression on neural correlates of the numerical distance effect in asymptomatic adults with the premutation. Specifically, we replicated well-established findings of fronto-parietal activation for the numerical distance effect in our neurotypical group, and this effect was significantly attenuated in asymptomatic adults with the premutation. Importantly, behavioral performance in the two groups was not significantly different for either accuracy or RT, indicating that both groups showed strong and comparable distance effects in the magnitude estimation task. Nonetheless, we found that the premutation group showed significantly reduced neural activity in the brain regions associated with the distance effect, namely IPS and IFG, suggesting reduced specialization of the fronto-parietal regions for magnitude estimation processing in premutation carriers. Further, we found that CGG repeat size in the carriers can account for significant variances in neural activation in the fronto-parietal areas, including bilateral IPS, related to the distance effect.

Unlike a previous behavioral study from our group (Goodrich-Hunsaker et al., 2011a), we did not find significant modulation effect of *FMR1* gene expression on our behavioral measures for the distance effect. Specifically, the previous study showed a subtle, but significant effect of CGG repeat size on the distance effect in the premutation. The different findings may be due to different task designs for testing the numerical distance effect. Specifically, following the well-established previous fMRI studies on magnitude estimation processing (Ansari and Dhital, 2006), the current study categorized each difference trial into two conditions (i.e., Small vs. Large) and compared the two conditions to test the distance effect. In contrast, the previous behavioral study used two vertical bars and the distances were defined by height differences between the two bars (i.e., 1, 2, 3, 5, 6, or 7 cm). Importantly, the previous study focused their analysis on the smallest differences (1- to 2-cm), and found the significant effect of CGG repeat size only for those small distance differences. Because the current fMRI study was not designed to separately test the smallest differences, we are unable to replicate the previous behavioral findings of CGG repeat size modulation on behavioral measures of distance effect. However, our results from multiple regression analyses on brain activity indeed cumulate evidence on doseresponse of *FMR1* gene expression (CGG repeat size) on the distance effect in premutation carriers.

Previously, authors in our group demonstrated that the neural network for mental arithmetic processing was affected in females with the full mutation (Rivera et al., 2002). Specifically, in response to increasing arithmetic complexity (i.e., going from 2-operand to 3-operand problems), participants with FXS did not recruit, to the same extent, the fronto-parietalnetwork known to be involved in arithmetic processing in unaffected individuals. Furthermore, that study reported a significantly positive modulation effect of FMRP on the neural correlates of arithmetic processing in the more complex condition (i.e., 3-operand condition) in the FXS group, providing evidence of a direct relationship between decreased FMRP and impairments in mental arithmetic performance in persons with FXS.

The current study expands the previous findings in the full mutation to the premutation, and provides novel evidence of a modulation effect of *FMR1* gene expression on neural correlates of magnitude estimation processing in non-FXTAS premutation carriers. Here, we used CGG repeat size and *FMR1* mRNA level, to investigate effects of two related but

distinct genetic factors on brain activity for numerical distance effect in adults with the premutation. Critically, the multiple regression analysis using both measures together indicated that the reduced activity in bilateral IPS and inferior and middle frontal gyrus, associated with the distance effect, was more strongly related to increased CGG repeat expansion than to abnormal elevation of *FMR1* mRNA. There are thought to be two molecular mechanisms associated with the premutation. First, increased CGG repeat may lead to reduction in FMRP, mimicking aspects of the full mutation phenotype. Alternatively, elevated levels of mRNA is thought to lead to an RNA toxic gain-of-function, which is likely to associated with the development of FXTAS (Bourgeois et al., 2009; Tassone et al., 2004). Our results suggest that altered neural correlates of magnitude estimation processing in the fronto-parietal area is possibly due to the reduced level of FMRP in the premutation carriers and is independent of FXTAS progression. However, followup studies with FMRP measurements, and with individuals with FXTAS, are necessary to elucidate the relation between altered brain functions for magnitude processing and distinct premutation phenotypes.

Despite the novel findings on altered neural correlates of magnitude estimation function in adults with the premutation, some limitations to the current study merit further discussion. First, one could question whether the current findings of reduced IPS activity in premutation carriers are due to differences in spatial attention or response selection, rather than to atypical magnitude estimation processing in the carriers. Although IPS has been recognized as a key area for numerical distance effect in numerous studies (Ansari and Dhital, 2006; Cohen Kadosh et al., 2005; Dehaene et al., 2003; Fias et al., 2003; Holloway and Ansari, 2009; Holloway et al., 2010; Pinel et al., 2001), the region has also been reported as a part of attentional network (Corbetta et al., 2000; Kim and Hopfinger, 2010; Yantis et al., 2002). Without a comparison task specifically targeting spatial attention, it is hard to disentangle the process of spatial attention from the numerical distance effect in the current task design. However, findings from our within- and between-group analyses support our claim that the reduced IPS activity found in the premutation group reflects attenuated IPS specialization for numerical processing in premutation carriers, rather than dysfunctions related to other cognitive processing such as spatial attention. Specifically, along with IPS, both groups showed significant neural activity in areas associated with attentional orientation such as DLPFC, anterior cingulate cortex (ACC), and anterior insula (Menon and Uddin, 2010), suggesting that brain regions for both magnitude comparison processing and spatial attention were recruited to perform our cognitive task in both groups. However, when comparing the two groups, only IPS and IFG were found to be significant in the contrast of Neurotypical > Premutation for Small > Large distance, which are the areas repeatedly found to be involved in numerical distance effect (Ansari and Dhital, 2006; Dehaene et al., 2003) and the development of numerical representation (Cantlon et al., 2009; Diester and Nieder, 2007). Furthermore, the differences in brain activity between the two groups cannot likely be explained by differences in response selection or task demands since the behavioral performance between the neurotypical and premutation groups were equivalent. Thus, it is reasonable to conclude that the present finding of attenuated IPS and IFG activation in the premutation is associated with atypical specialization of those areas for the magnitude comparison, although a future study with comparison tasks which target attentional processing would help to further validate our findings.

Another limitation in the current study has to do with the molecular measurements that were available to us. As in many of previous studies with FXS, the current *FMR1* molecular measures were ascertained from blood samples, and therefore may not necessarily reflect what would have been found in brain tissue. Lastly, although it is reasonable to assume that increased CGG repeat expansion is closely related to FMRP level in the premutation group, we cannot definitively conclude that the aberrant brain activation in the premutation group is

exclusively due to reduced FMRP level without direct measurements of FMRP in our sample. Future research with direct FMRP measurement will allow us to test whether reduced FMRP is indeed a primary factor for the atypical involvement of the fronto-parietal regions for the distance effect in individuals with the premutation.

Acknowledgments

We express our gratitude to all individuals who participated in this research. We would like to thank Yingratana Bella McLennan and Ling Wong for help with data collection.

Funding

Funding for this study was provided by NIH grants RL1NS062412, TL1DA024854, and UL1DE019583; the NIH had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

References

Allen, MP. Understanding regression analysis. New York: Plenum Press; 1997.

Ansari D, Dhital B. Age-related changes in the activation of the intraparietal sulcus during nonsymbolic magnitude processing: an event-related functional magnetic resonance imaging study. Journal of Cognitive Neuroscience. 2006; 18:1820–1828. [PubMed: 17069473]

Bourgeois JA, Coffey SM, Rivera SM, Hessl D, Gane LW, Tassone F, et al. A review of fragile X premutation disorders: expanding the psychiatric perspective. Journal of Clinical Psychiatry. 2009; 70:852–862. [PubMed: 19422761]

Cantlon JF, Libertus ME, Pinel P, Dehaene S, Brannon EM, Pelphrey KA. The neural development of an abstract concept of number. Journal of Cognitive Neuroscience. 2009; 21:2217–2229. [PubMed: 19016605]

Cohen, Kadosh R.; Henik, A.; Rubinsten, O.; Mohr, H.; Dori, H.; van de Ven, V., et al. Are numbers special? The comparison systems of the human brain investigated by fMRI. Neuropsychologia. 2005; 43:1238–1248. [PubMed: 15949508]

Cohen S, Masyn K, Adams J, Hessl D, Rivera S, Tassone F, et al. Molecular and imaging correlates of the fragile X-associated tremor/ataxia syndrome. Neurology. 2006; 67:1426–1431. [PubMed: 17060569]

Corbetta M, Kincade JM, Ollinger JM, McAvoy MP, Shulman GL. Voluntary orienting is dissociated from target detection in human posterior parietal cortex. Nature Neuroscience. 2000; 3:292–297.

Dehaene S, Piazza M, Pinel P, Cohen L. Three parietal circuits for number processing. Cognitive Neuropsychology. 2003; 20:487–506. [PubMed: 20957581]

Diester I, Nieder A. Semantic associations between signs and numerical categories in the prefrontal cortex. PLoS Biology. 2007; 5:e294. [PubMed: 17973578]

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

Fias W, Lammertyn J, Reynvoet B, Dupont P, Orban GA. Parietal representation of symbolic and nonsymbolic magnitude. Journal of Cognitive Neuroscience. 2003; 15:47–56. [PubMed: 12590842]

Garcia-Arocena D, Hagerman PJ. Advances in understanding the molecular basis of FXTAS. Human Molecular Genetics. 2010; 19:R83–R89. [PubMed: 20430935]

Goodrich-Hunsaker NJ, Wong LM, McLennan Y, Srivastava S, Tassone F, Harvey D, et al. Young adult female fragile X premutation carriers show age- and genetically-modulated cognitive impairments. Brain and Cognition. 2011; 75:255–260. [PubMed: 21295394]

Goodrich-Hunsaker NJ, Wong LM, McLennan Y, Tassone F, Harvey D, Rivera SM, et al. Adult Female Fragile X Premutation Carriers Exhibit Age- and CGG Repeat Length-Related Impairments on an Attentionally Based Enumeration Task. Frontiers in Human Neuroscience. 2011; 5:63. [PubMed: 21808616]

Grigsby JP, Kemper MB, Hagerman RJ, Myers CS. Neuropsychological dysfunction among affected heterozygous fragile X females. American Journal of Medical Genetics. 1990; 35:28–35. [PubMed: 1689106]

Hagerman PJ, Hagerman RJ. The fragile-X premutation: a maturing perspective. American Journal of Human Genetics. 2004; 74:805–816. [PubMed: 15052536]

Hagerman RJ, Berry-Kravis E, Kaufmann WE, Ono MY, Tartaglia N, Lachiewicz A, et al. Advances in the treatment of fragile X syndrome. Pediatrics. 2009; 123:378–390. [PubMed: 19117905]

Hocking DR, Kogan CS, Cornish KM. Selective spatial processing deficits in an at-risk subgroup of the fragile X premutation. Brain and Cognition. 2012; 79:39–44. [PubMed: 22417865]

Holloway ID, Ansari D. Mapping numerical magnitudes onto symbols: the numerical distance effect and individual differences in children's mathematics achievement. Journal of Experimental Child Psychology. 2009; 103:17–29. [PubMed: 18513738]

Holloway ID, Ansari D. Developmental specialization in the right intraparietal sulcus for the abstract representation of numerical magnitude. Journal of Cognitive Neuroscience. 2010; 22:2627–2637. [PubMed: 19929327]

Holloway ID, Price GR, Ansari D. Common and segregated neural pathways for the processing of symbolic and nonsymbolic numerical magnitude: an fMRI study. Neuroimage. 2010; 49:1006–1017. [PubMed: 19666127]

Jacquemont S, Hagerman RJ, Leehey MA, Hall DA, Levine RA, Brunberg JA, et al. Penetrance of the fragile X-associated tremor/ataxia syndrome in a premutation carrier population. JAMA: The Journal of the American Medical Association. 2004; 291:460–469. [PubMed: 14747503]

Jakala P, Hanninen T, Ryynanen M, Laakso M, Partanen K, Mannermaa A, et al. Fragile-X: neuropsychological test performance, CGG triplet repeat lengths, and hippocampal volumes. Journal of Clinical Investigation. 1997; 100:331–338. [PubMed: 9218509]

Kaufmann L, Koppelstaetter F, Delazer M, Siedentopf C, Rhomberg P, Golaszewski S, et al. Neural correlates of distance and congruity effects in a numerical Stroop task: an event-related fMRI study. Neuroimage. 2005; 25:888–898. [PubMed: 15808989]

Kemper MB, Hagerman RJ, Ahmad RS, Mariner R. Cognitive profiles and the spectrum of clinical manifestations in heterozygous fra (X) females. American Journal of Medical Genetics. 1986; 23:139–156. [PubMed: 3953643]

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. Human Molecular Genetics. 2001; 10:1449–1454. [PubMed: 11448936]

Keri S, Benedek G. Visual pathway deficit in female fragile X premutation carriers: a potential endophenotype. Brain and Cognition. 2009; 69:291–295. [PubMed: 18789568]

Keri S, Benedek G. Why is vision impaired in fragile X premutation carriers? The role of fragile X mental retardation protein and potential *FMR1* mRNA toxicity. Neuroscience. 2012; 206:183–189. [PubMed: 22266345]

Kim SY, Hopfinger JB. Neural basis of visual distraction. Journal of Cognitive Neuroscience. 2010; 22:1794–1807. [PubMed: 19702467]

Kogan CS, Boutet I, Cornish K, Zangenehpour S, Mullen KT, Holden JJ, et al. Differential impact of the FMR1 gene on visual processing in fragile X syndrome. Brain. 2004a; 127:591–601. [PubMed: 14736752]

Kogan CS, Bertone A, Cornish K, Boutet I, Der Kaloustian VM, Andermann E, et al. Integrative cortical dysfunction and pervasive motion perception deficit in fragile X syndrome. Neurology. 2004b; 63:1634–1639. [PubMed: 15534248]

Koldewyn K, Hessl D, Adams J, Tassone F, Hagerman PJ, Hagerman RJ, et al. Reduced Hippocampal Activation During Recall is Associated with Elevated FMR1 mRNA and Psychiatric Symptoms in Men with the Fragile X Premutation. Brain Imaging and Behavior. 2008; 2:105–116. [PubMed: 19430586]

Lachiewicz AM, Dawson DV, Spiridigliozzi GA, McConkie-Rosell A. Arithmetic difficulties in females with the fragile X premutation. American Journal of Medical Genetics. Part A. 2006; 140:665–672.

Menon V, Uddin LQ. Saliency, switching, attention and control: a network model of insula function. Brain Structure & Function. 2010; 214:655–667. [PubMed: 20512370]

Miezejeski CM, Jenkins EC, Hill AL, Wisniewski K, French JH, Brown WT. A profile of cognitive deficit in females from fragile X families. Neuropsychologia. 1986; 24:405–409. [PubMed: 3736823]

Moore CJ, Daly EM, Tassone F, Tysoe C, Schmitz N, Ng V, et al. The effect of pre-mutation of X chromosome CGG trinucleotide repeats on brain anatomy. Brain. 2004; 127:2672–2681. [PubMed: 15483045]

Pinel P, Dehaene S, Riviere D, LeBihan D. Modulation of parietal activation by semantic distance in a number comparison task. Neuroimage. 2001; 14:1013–1026. [PubMed: 11697933]

Poline JB, Worsley KJ, Evans AC, Friston KJ. Combining spatial extent and peak intensity to test for activations in functional imaging. Neuroimage. 1997; 5:83–96. [PubMed: 9345540]

Price GR, Holloway I, Rasanen P, Vesterinen M, Ansari D. Impaired parietal magnitude processing in developmental dyscalculia. Current Biology. 2007; 17:R1042–R1043. [PubMed: 18088583]

Rivera SM, Menon V, White CD, Glaser B, Reiss AL. Functional brain activation during arithmetic processing in females with fragile X Syndrome is related to FMR1 protein expression. Human Brain Mapping. 2002; 16:206–218. [PubMed: 12112763]

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. American Journal of Human Genetics. 1993; 53:1217–1228. [PubMed: 7902673]

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. American Journal of Human Genetics. 2000a; 66:6–15. [PubMed: 10631132]

Tassone F, Hagerman RJ, Taylor AK, Mills JB, Harris SW, Gane LW, et al. Clinical involvement and protein expression in individuals with the FMR1 premutation. American Journal of Medical Genetics. 2000b; 91:144–152. [PubMed: 10748416]

Tassone F, Iwahashi C, Hagerman PJ. FMR1 RNA within the intranuclear inclusions of fragile X-associated tremor/ataxia syndrome (FXTAS). RNA Biology. 2004; 1:103–105. [PubMed: 17179750]

Tassone F, Pan R, Amiri K, Taylor AK, Hagerman PJ. A rapid polymerase chain reaction-based screening method for identification of all expanded alleles of the fragile X (FMR1) gene in newborn and high-risk populations. The Journal of Molecular Diagnostics. 2008; 10:43–49. [PubMed: 18165273]

Wechsler, D. WAIS-III administration and scoring manual. San Antonio, TX: The Psychological Corporation; 1997.

Wechsler, D. Wechsler abbreviated scale of intelligence. San Antonio: Harcourt Assessment; 1999.

Yantis S, Schwarzbach J, Serences JT, Carlson RL, Steinmetz MA, Pekar JJ, et al. Transient neural activity in human parietal cortex during spatial attention shifts. Nature Neuroscience. 2002; 5:995–1002.

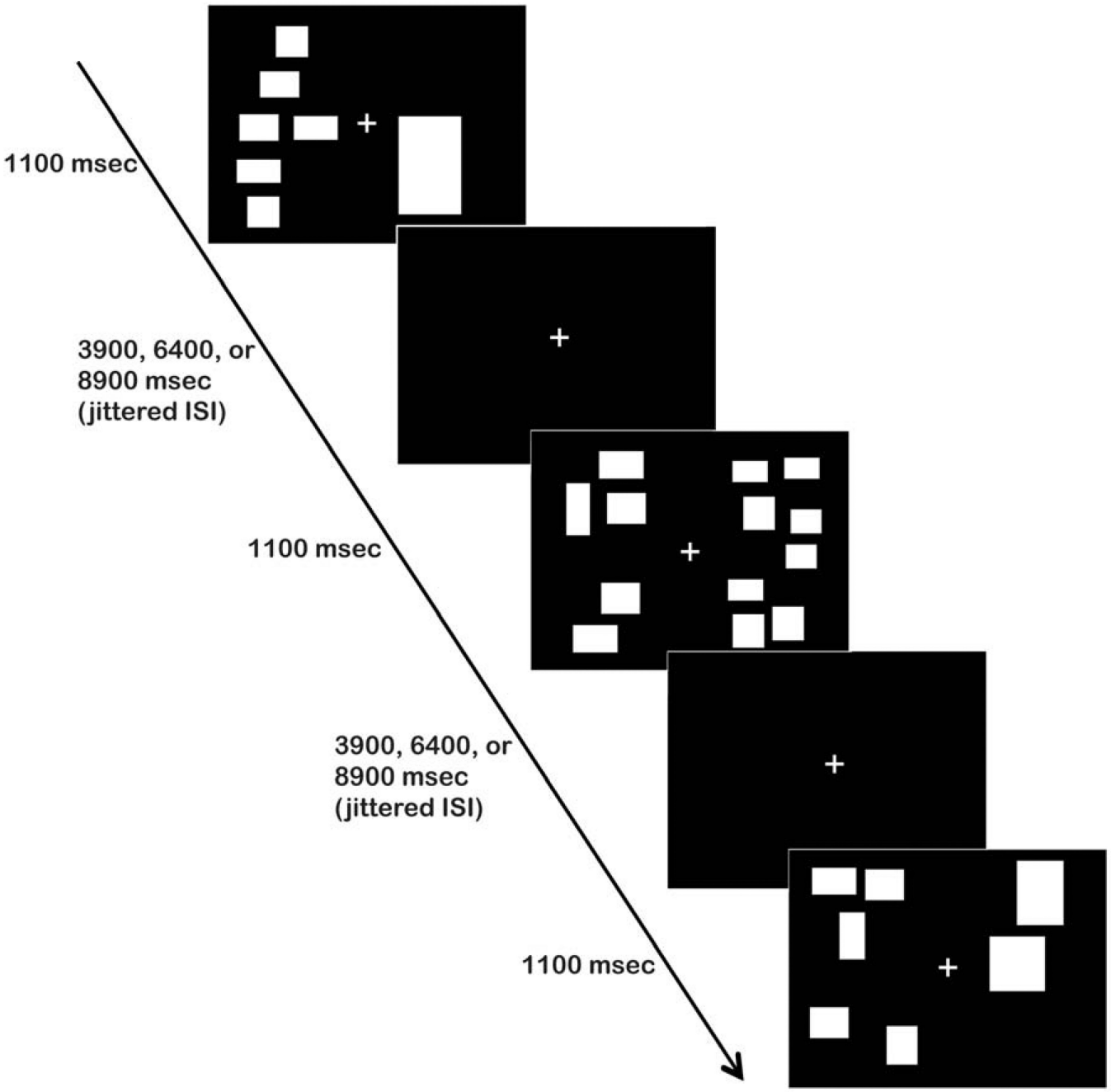

Figure 1.
Trial sequence in the magnitude estimation task. In an fMRI scanner, participants saw side-by-side displays of sets of rectangles of which each side of screen contained 1–9 white squares. Participants were asked to indicate a set containing more rectangles by pressing a left or right button of an MR compatible button box. The numerical distance between groups of squares was either 1, 2, or 3 (i.e., small distance condition), or 5, 6, or 7 (i.e., large distance condition). To achieve jitter in the time series, trials were presented with variable fixation intervals of 3900, 6400, or 8900 ms.

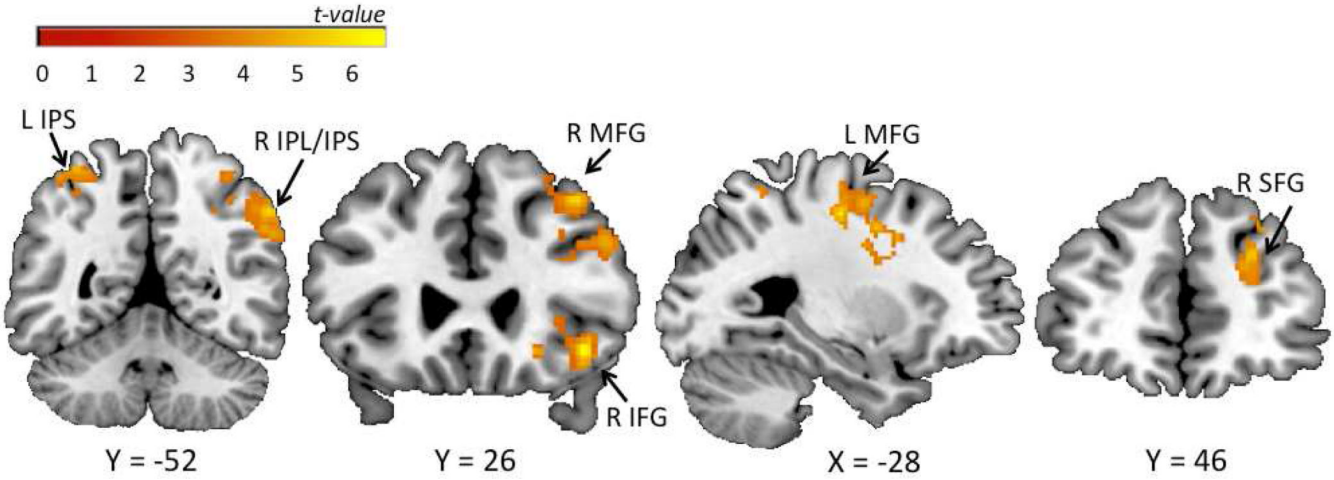

Figure 2.
Fronto-parietal activation showing a negative correlation with CGG repeat size in the premutation group in the Small > Large contrast, after accounting for the effect of *FMR1* mRNA.

Table 1

Group demographic and *FMR1* data

	NT			Premutation		
	N	**Mean**	**Range**	**N**	**Mean**	**Range**
Age	24	30.75 (6.19)	21–39	24	32.0 (5.46)	20–40
CGG repeat size	23	29.7* (5.6)	10–43	24	93.6* (22.6)	52–141
***FMR1* mRNA**	20	1.54* (0.23)	1.12–1.98	24	2.52* (0.73)	1.55–4.9
FSIQ	22	119.0 (16.1)	89–155	24	115.5 (12.5)	77–134

Numbers in parenthesis indicate standard deviation.

* indicates significant mean differences between the two groups ($p<.05$).

Table 2

Mean accuracy and response times for magnitude estimation task

	NT		Premutation	
	Accuracy (% correct)	**Reaction Time (msec)**	**Accuracy (% correct)**	**Reaction Time (msec)**
Large Distance	99.6 (0.8)	617 (107)	98.7 (3.6)	613 (81)
Small Distance	95.8 (3.4)	812 (157)	95.9 (5.8)	789 (98)
Distance Effect	−3.9 (3.4)	195 (88)	−2.8 (3.7)	176 (66)

Numbers in parenthesis indicate standard deviation.

Table 3

Brain areas that showed significant distance effect in each group

Group	Brain regions	No. of voxels in cluster	Peak coordinates	t-value	Corrected p-value of cluster
NT	R insula (BA13)	8863	32 24 0	9.62	<.001
	R cingulate gyrus		4 22 40	9.28	
	R IFG (BA47)		48 18 2	8.35	
	R DLPFC (BA9)		38 20 36	6.08	
	R ACC (BA32)		10 28 22	5.73	
	L insula (BA13)	1941	−38 16 2	8.36	<.001
	L IFG (BA9)		−44 2 32	6.59	
	L IFG (BA44)		−52 16 16	3.03	
	R IPL (BA40)	4451	46 −48 46	7.63	<.001
	R IPL/IPS (BA40)		42 −54 54	6.89	
	R IPS/SPL (BA7)		34 −58 52	6.34	
	R IPS/SPL (BA7)		28 −58 46	6.21	
	R precuneus (BA7)		28 −60 52	6.16	
	R IPS		30 −62 38	5.99	
	R SMG		58 −44 32	5.35	
	R angular gyrus		28 −66 46	5.18	
	L IPL (BA40)	2606	−46 −42 48	6.22	<.001
	L IPS/SPL (BA7)		−32 −60 50	6.11	
	L IPS		−30 −52 48	5.27	
	L SPL (BA7)		−30 −58 58	4.69	
	L precuneus		−24 −66 42	4.62	
	L SMG (BA40)		−60 −48 38	3.28	
	R Thalamus	912	10 −12 6	5.21	<.001
	R pulvinar		10 −26 6	3.21	
	R lateral globus		20 −6 2	2.94	
	L Thalamus	520	−10 6 −2	4.87	<.001
	L pulvinar		−18 −28 8	2.88	

Group	Brain regions	No. of voxels in cluster	Peak coordinates	t-value	Corrected p-value of cluster
	L middle frontal gyrus	423	−34 −2 52	4.77	0.002
	L DLPFC/MFG	271	−42 28 28	4.57	0.035
Premutation	R IPL (BA40)	1768	44 −46 48	6.79	<.001
	R IPS		36 −44 40	5.83	
	R precuneus		30 −70 40	5.73	
	R SPL (BA7)		24 −62 46	4.26	
	R angular gyrus		36 −56 48	3.95	
	R IFG	1364	46 4 24	6.56	<.001
	R IFG (BA45)		54 12 22	4.55	
	R insula (BA13)		40 14 8	4.47	
	R MFG/DLPFC (BA9)		56 14 36	4.09	
	R IFG (BA47)		36 18 −6	3.67	
	R cingulate gyrus	1539	4 26 38	5.72	<.001
	L cingulate gyrus		−2 22 44	5.17	
	R medial frontal gyrus		4 18 50	4.8	
	L medial frontal gyrus		−2 12 48	4.64	
	R ACC		10 30 24	4.1	
	R MFG		26 4 50	3.95	
	R SFG (BA6)		20 8 54	3.6	
	R ACC (BA24)		12 22 28	3.45	
	L insula	465	−40 14 2	5.51	<.001
	L IPL	305	−42 −40 40	4.81	0.008
	L IPL (BA40)		−42 −46 42	4.1	
	L IPS		−34 −46 50	4.04	
	L cuneus	305	−20 −70 8	4.47	0.008
	L cuneus (BA18)		−8 −80 14	3.37	
	R posterior cingulate	238	18 −62 6	5.01	0.035
	R cuneus		14 −68 4	4.65	

All clusters significant at $p<.05$ corrected.

Table 4

Brain areas that showed greater distance effect in the NT than the premutation group

Contrast	Brain regions	No. of voxels in cluster	Peak coordinates	t-value	Corrected p-value of cluster
NT>Premutation	L precentral gyrus (BA6)	301	−52 0 36	4.83	0.033
for Small>Large	L IFG (BA9)		−50 6 34	4.04	
	L MFG (BA9)		−48 8 40	3.52	
	L precentral gyrus		−54 −8 42	2.83	
	R IPS	426	52 −38 46	3.69	0.004
	R IPL/SMG (BA40)		54 −34 46	3.65	
	R IPL/IPS (BA40)		38 −50 46	3.55	
	R IPL/IPS		42 −56 50	3.18	
	R SMG		58 −40 30	2.97	
	R angular gyrus		34 −62 42	2.95	
	L IPL (BA40)	330	−44 −42 46	3.63	0.02
	L IPL/IPS		−44 −56 54	3.43	
	L SPL/IPS (BA7)		−28 −62 46	3.24	
	L IPL/IPS (BA40)		−40 −50 46	3.12	
	L SPL/IPS		−36 −62 50	3.04	
	L SPL/IPS (BA7)		−28 −54 44	3.04	
	L SMG		−36 −50 36	2.76	
	L precuneus		−22 −58 40	2.7	

All clusters significant at $p<.05$ corrected.

Table 5

Brain areas that showed significant effect of CGG repeat size (controlling out the effect of *FMR1* mRNA) on attenuated activation associated with the distant effect in the premutation group

Contrast	Brain regions	No. of Voxels in cluster	Peak coordinates	t-value	Corrected p-value of cluster
Effect of CGG	L precentral gyrus	565	−32 0 44	6.4	<.001
	L MFG		−28 −16 48	6.16	
	L MFG		−30 −16 54	4.69	
	R IFG	776	44 24 −6	6.28	<.001
	R IFG		46 18 −12	5.99	
	R IFG		32 10 −14	4.98	
	R IPL	4215	56 −54 42	6.06	<.001
	R cingulate gyrus		8 −18 34	5.71	
	R IPL		52 −44 28	5.57	
	R supramarginal gyrus		52 −40 34	4.78	
	R IPS/SPL		32 −62 58	4.63	
	R angular gyrus		58 −50 32	4.59	
	R SFG	261	26 46 26	4.85	0.013
			38 40 36	4.54	
	R MFG	543	40 26 46	4.75	<.001
	R MFG		48 26 30	4.34	
	L IPS/SPL	248	−36 −52 62	4.66	0.018
	L IPL		−50 −42 40	4.27	
	L supramarginal gyrus		−46 −44 32	3.95	

All clusters significant at $p<.05$ corrected.