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An fMRI study of the prefrontal activity during the performance of a working memory task in premutation carriers of the fragile X mental retardation 1 gene with and without fragile X-associated tremor/ataxia syndrome (FXTAS)

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ABSTRACT

Premutation alleles of the fragile X mental retardation 1 gene (*FMR1*) are associated with the risk of developing fragile X-associated tremor/ataxia syndrome (FXTAS), a late-onset neurodegenerative disorder that involves neuropsychiatric problems and executive and memory deficits. Although abnormal elevation of *FMR1* mRNA has been proposed to underlie these deficits, it remains unknown which brain regions are affected by the disease process of FXTAS and genetic molecular mechanisms associated with the *FMR1* premutation. This study used functional magnetic resonance imaging (fMRI) to identify deficient neural substrates responsible for altered executive and memory functions in some *FMR1* premutation individuals. We measured fMRI BOLD signals during the performance of verbal working memory from 15 premutation carriers affected by FXTAS (PFX+), 15 premutation carriers unaffected by FXTAS (PFX−), and 12 matched healthy control individuals (HC). We also examined correlation between brain activation and *FMR1* molecular variables (CGG repeat size and mRNA levels) in premutation carriers. Compared with HC, PFX+ and PFX− showed reduced activation in the right ventral inferior frontal cortex and left premotor/dorsal inferior frontal cortex. Reduced activation specific to PFX + was found in the right premotor/dorsal inferior frontal cortex. Regression analysis combining the two premutation groups demonstrated significant negative correlation between the right ventral inferior frontal cortex activity and the levels of *FMR1* mRNA after excluding the effect of disease severity of FXTAS. These results indicate altered prefrontal cortex activity that may underline executive and memory deficits affecting some individuals with *FMR1* premutation including FXTAS patients.

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1. Introduction

Mutations of the fragile X mental retardation 1 (*FMR1*) gene are the genetic cause of fragile X syndrome (FXS) (Verkerk et al., 1991), the most common inherited form of mental retardation. Large expansions of the CGG trinucleotide repeat in the full mutation range (>200 CGG repeats) consequently result in transcriptional silencing of the *FMR1* gene and deficiency of the *FMR1* protein (FMRP) (Fu et al., 1991; Pieretti et al., 1991). The smaller expansions of about 55–200 repeats are referred to as the premutation. It has been known that carriers of *FMR1* premutation alleles have the risk of developing fragile X-associated tremor/ataxia syndrome (FXTAS), a late-onset neurodegenerative disorder. Although FXTAS has been principally characterized as a movement disorder with intention tremor and gait ataxia, cognitive decline and psychiatric problems are also parts of its core symptoms (Bacalman et al., 2006; Hagerman et al., 2001; Jacquemont et al., 2003). Since FXTAS has not been diagnosed in individuals with the full mutation, it has been suggested that the molecular mechanism underlying this disease is distinct from that observed in FXS. Although its pathogenetic mechanism is still unclear, an RNA toxic "gain-of-function" model has been proposed based on the observation of abnormally elevated *FMR1* mRNA in the premutation carriers in the presence of relatively normal levels of FMRP (Hagerman and Hagerman, 2004; Hagerman et al., 2001; Kenneson et al., 2001; Tassone et al., 2000).

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To date, a number of cognitive impairments have been documented in FXTAS patients. A recent study used an extensive neuropsychological test battery for male FXTAS patients and unaffected premutation carriers and found that both groups performed worse than control males on executive cognitive functioning and declarative learning and memory (Grigsby et al., 2008). Another study examined individual subcomponents of working memory (i.e. verbal, spatial, and central executive memory) and again observed impairments of executive memory in patients with FXTAS and in premutation individuals unaffected with FXTAS (Cornish et al., 2009). Although the cognitive and behavioral problems associated with asymptomatic premutation carriers still remain controversial (see Hunter et al., 2008a,b for negative findings), these studies indicate that, among other cognitive profiles, executive and memory functions are particularly impaired in patients with FXTAS. Because a number of previous functional imaging studies on healthy individuals have shown that areas in the prefrontal cortex play crucial roles in executive and memory functions (Budson and Price, 2005; Cabeza and Nyberg, 2000), these observations indicate deficient prefrontal cortex functioning in FXTAS patients. Although altered hippocampal functions were indicated both by histological studies of patients with FXTAS (Greco et al., 2002, 2006) and by our recent functional magnetic imaging (fMRI) study of the unaffected premutation males, there have been no functional imaging studies that have targeted deficient prefrontal functions of patients with FXTAS or unaffected premutation carriers.

The objective of the present study is to identify neural correlates of deficient executive and memory functions that are observed in some individuals with *FMR1* premutation carriers. For this purpose, we use fMRI and measure cortical activity from premutation carriers with and without FXTAS during the performance of a verbal working memory task, which is known to activate extensive areas in the prefrontal cortex. By performing group comparisons including matched healthy controls, we aim to identify areas in the prefrontal cortex that are impaired in FXTAS. We also investigate the possible association between cortical activity, CGG repeat size and *FMR1* mRNA levels to understand the genetic molecular variables that contribute to increased risk of prefrontal deficiency in some of the *FMR1* premutation carriers.

2. Materials and methods

2.1. Participants

We recruited a total of 44 participants (22 females) aged between 33 and 75 for the study. All participants had verbal-scale IQ (Wechsler Adult Intelligence Scale-Third edition) of higher than 80 and normal or corrected-to-normal vision. Of these, data from two participants (one female) were removed due to data loss. We included 12 healthy control (HC) participants, 15 participants with the premutation with FXTAS (PFX+), and 15 participants with the premutation without FXTAS participants (PFX−) for fMRI analyses (Table 1). *FMR1* allele status was confirmed in all participants by DNA testing. In this study, the premutation range was defined as those with a CGG repeat size of larger than 50, but less than 200. The CGG repeat size and *FMR1* mRNA levels were measured in each participant following the procedures described elsewhere (Koldewyn et al., 2008). Two premutation participants (one PFX+ and one PFX−) had missing mRNA data. No premutation individuals were mosaic for CGG repeat size. For participants with CGG repeat count within the premutation range, a trained physician (RJH) scored the severity of FXTAS on a scale ranging from 0 to 6 (as described by Adams et al., 2007; Jacquemont et al., 2003). Premutation carriers with FXTAS scores of 0 or 1 were placed in the PFX- group, while those with FXTAS scores of 2–5 were designated as PFX+. Patients with resting tremor were excluded from the recruitment. Participant demographic information, including FXTAS score and molecular data, are shown in Table 1. The three groups were matched for education years ($F = 0.08$, df = 2, 39, $p = 0.92$), full scale IQ ($F = 1.42$, $p = 0.25$), and verbal-scale IQ ($F = 0.75$, $p = 0.48$). Ages of HC and PFX+ were closely matched ($t = 0.04$, df = 25, $p = 0.96$). Although PFX− participants tended to be younger than the other two groups, main effect of group did not reach the significant level ($F = 2.89$, $p = 0.07$). Participants in the PFX− group were recruited through screening of family pedigrees of probands with fragile X syndrome. In the PFX+ group, because we were specifically seeking patients with FXTAS, this group is biased for patients with neurological problems. Controls were recruited either from the local community through the University of California Davis Medical Center or were unaffected individuals in families affected by fragile X. All participants were right-handed except for two PFX+ participants and one PFX−. Neurological examinations on all HC participants were normal, including absence of tremor and ataxia. Healthy control participants received a semi-structured clinical interview conducted by a trained research assistant, which was used to rule out medical disease that would affect the central nervous system including alcoholism, stroke or brain trauma. The study was carried out in accordance with the latest version of the Declaration of Helsinki. All participants gave written informed consent before participating in the study. The protocol was approved by the institutional review board at the University of California, Davis.

2.2. Working memory task

Participants performed a verbal working memory task during fMRI scanning. Each trial consisted of three different phases: (i) the presentation of a 2×3 matrix of 6 alphabetical letters at the center of a screen for 3 s during which the participants were asked to remember the letters ("encoding"); (ii) the display of a fixation cross for 5 s ("rehearsal"); and (iii) the presentation of one alphabetical letter for 1 s ("retrieval"). A fixation cross was presented for 1 s before the start of the next trial. Participants were instructed to press a button only when the single letter presented during the retrieval period matched one of the six letters shown during the encoding period. A button press was required on 23 out of 48 total trials. The trials of button press and withhold were randomly presented. Presentation of stimuli and collection of responses were performed using Presentation (Neurobehavioral Systems, Albany, CA) implemented on a PC. The behavioral logfiles were not generated for two PFX+ participants, one PFX− subject, and one HC subject because of technical errors. All participants practiced the task before entering the MRI scanner.

2.3. fMRI data acquisition

All MRI data was acquired on a 1.5 T GE Signa Horizon LX NV/I MRI system package (GE Medical Systems, Milwaukee, WI) using a phased array whole-head coil. A single-shot gradient echo-planar imaging (EPI) sequence (TE: 32 ms, TR: 2000 ms, flip angle: 90°, FOV: 22 cm, slice thickness: 4 mm with 1 mm slice gap, matrix: 64×64) was used to acquire the functional images. The EPI volume was composed of 27 axial slices covering the entire brain. 244 volumes were acquired during a single run (8 min 8 s), and the first two images were discarded to allow for T1 equilibration. Additionally, a high resolution T1-weighted spoiled grass gradient (SPGR) 3-D MRI sequence (128 slices, in-plane resolution: 0.86×0.86 mm, slice thickness: 1.3 mm) was administered. During the scan, a custom-built head holder was used to prevent movement during scanning.

Table 1
Statistics on demographic data including the FXTAS score and molecular data for healthy control, premutation carriers affected with FXTAS, and premutation carriers unaffected with FXTAS.

	Healthy Control (HC) ($N = 12$, 5 female)			Premutation with FXTAS (PFX+) ($N = 15$, 9 female)			Premutation without FXTAS (PFX−) ($N = 15$, 7 female)		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
Age	59.3	11.3	35–74	59.5	11.2	33–72	52.4	12.5	34–73
Full IQ	114.4	21.5	90–151	104.9	10.7	85–120	112.3	13.2	95–141
Verbal IQ	112.5	18.9	84–131	106.1	12.5	81–123	111.9	13.8	91–140
Years of education	15.7	4.2	10–21	15.4	2.8	8–21	15.9	2.50	12–18
FXTAS score		N.A.		2.86	0.99	2–5	0.26	0.45	0–1
CGG repeat	30.4	7.1	18–49	99.7	13.3	78–130	91.3	20.4	52–130
FMR1 mRNA	1.41	0.37	0.63–2.00	3.26	0.77	1.96–4.42	2.51	0.90	1.65–5.14

2.4. fMRI data preprocessing and analysis

Preprocessing and analysis of fMRI time-series data was performed using Statistical Parametric Mapping software (SPM5) (Wellcome Department of Cognitive Neurology, London, UK) running on MATLAB version 7.4.0 (The Mathworks, Inc., Natick, MA, USA). Imaging data was preprocessed in the following manner: 1) the functional images were realigned and unwarped, 2) functional images of each subject were coregistered to their SPGR anatomical image, 3) transformation matrix for the MNI template was calculated using their SPGR image, 4) functional data-series was normalized into the MNI template using the transformation matrix calculated during step 3, and 5) functional data was smoothed using an 8 mm FWHM Gaussian smoothing kernel. In a first-level general linear model analysis for individual participants, we used three separate regressors for encoding, rehearsal, and retrieval, each of which was set at 0 s, 4 s, and 8 s with reference to the onset of each trial, respectively. Task-related activation was modeled by convolving vectors representing the timing of these events with a canonical hemodynamic response function. For each participant, the contrast image of Encoding vs. (Rehearsal + Retrieval) was generated. In light of our purpose of obtaining measures of executive and memory functioning of the prefrontal cortex, we selected this contrast as optimal in our continuous working memory task, because the encoding period was the most demanding and it induced reliable activation in the prefrontal areas in each group (as reported in the Result section) whereas either Rehearsal vs. (Encoding + Retrieval) or Retrieval vs. (Encoding + Rehearsal) did not show any significant prefrontal activation. The contrast image was fed into a random-effects second-level analysis, in which the significant activation was assessed for each subject group of HC, PFX+, and PFX− separately. The statistical threshold was set at $p < 0.01$, corrected for multiple comparisons using the false discovery rate (Genovese et al., 2002).

After generating the activation map for each group, we performed Region of Interest (ROI) analyses to compare encoding-related activations among groups. We included the bilateral hippocampus, inferior frontal cortex, premotor cortex, anterior cingulate cortex, supplementary motor area as the ROIs, because a number of previous imaging studies replicated activation in these regions during working memory tasks using verbal materials (Cabeza and Nyberg, 2000; Smith and Jonides, 1999). In addition, we included the bilateral superior parietal cortex because our verbal working memory task involved executive processes as well as processing information about spatial configuration of letter strings. Lastly, we also included the calcarine sulcus in the ROIs as a control for low-level visual perception. We defined these ROIs functionally using the voxel of the local maximal *t*-value and its surrounding 26 voxels in a cross-group activation map generated by a random-effects second-level analysis of all the 42 participants ($p < 0.05$, corrected for multiple comparisons using the family-wise error rate; see Table 2 for the coordinate of each ROI). For each participant, we extracted the mean parameter estimate of activation of 27 voxels in the ROI using the contrast image of Encoding vs. (Rehearsal and Retrieval). Using the extracted value from each individual, we performed a three-factor analysis of variance (ANOVA) with group and gender as the between-subject factors and ROI as the within-subject factor. When either significant main effect of group or interaction of group x ROI was observed, we proceeded to perform follow-up ANOVA in each ROI examining the effect of group.

When the ROI analysis described above revealed deficient cortical activity in the premutation carriers, we conducted regression analyses to determine the effects of molecular variables associated with the *FMR1* gene (i.e. CGG repeat size and *FMR1* mRNA levels) on cortical activity in the deficient ROI(s). For this analysis, we extracted the parameter estimate value in the deficient ROI(s) from each member of the two premutation groups (PFX+ and PFX−) following the same step described in the previous section. We excluded the data of HC to ensure that any linear effect of the molecular variables is not attributable to a group effect. The extracted parameter estimate was adjusted for the effects of gender and the severity of FXTAS, the latter of which was estimated by using the FXTAS score of the individual premutation member (Table 1). After regressing out the effect of these two factors, the simple regression analysis using either CGG repeat size or mRNA levels was performed.

Table 2
Clusters of significant activations during the encoding period in cross-group analysis and the MNI coordinates of the regions of interest.

Area	Cluster size	MNI coordinates			*t*-value
		x	*y*	*z*	
Occipital, posterior parietal, and temporal cortex	19 389				
Calcarine sulcus		2	−84	−4	17.1
L. Superior parietal cortex		−22	−68	44	12.4
R. Superior parietal cortex		22	−60	56	11.2
L. Hippocampus		−20	−32	−4	10.9
R. Hippocampus		22	−32	−2	9.2
Thalamus	1838	4	−2	14	9.6
Right frontal cortex	1537				
R. ventral Inferior frontal cortex		52	10	0	7.0
R. dorsal inferior frontal/premotor cortex		52	4	28	6.0
L. ventral Inferior frontal cortex	175	−52	12	0	6.9
L. dorsal inferior frontal/premotor cortex	1074	−44	−4	34	7.4
Medial frontal cortex	602				
Anterior cingulate cortex		−2	12	38	8.1
Supplementary motor area		0	2	56	7.2

3. Results

3.1. Behavioral results

We included both correct rejections and hits for correct responses. All three groups performed nearly equally on the working memory task. The accuracy rate was 75.2 ± 7.4% (mean ± standard deviation), 72 ± 13.6%, and 78.2 ± 11.5 % for the HC, PFX+, and PFX−, respectively. There was no effect of group in a one-way ANOVA ($F = 1.09$, df = 2, 36, $p = 0.35$). The mean reaction time (calculated using only trials with hits) was 1688.5 ± 141.2 ms, 1730.5 ± 184.2 ms, 1760.5 ± 61.8 ms for the HC, PFX+, and PFX− groups, respectively. There was no significant effect of group on reaction time ($F = 0.92$, $p = 0.41$).

3.2. Whole-brain analyses within-group

We performed the whole-brain analysis for activation during encoding in each group (HC, PFX+, and PFX−) separately. In all three groups, we observed significant activation in the bilateral hippocampus, inferior frontal cortex, premotor cortex, anterior cingulate cortex, and supplementary motor area (Fig. 1 and Supplementary Table 1). These patterns of activation are consistent with previous studies of encoding-related activity of the verbal materials (Cabeza and Nyberg, 2000; Smith and Jonides, 1999). Although activation in those areas was significant in each of the three groups, the spatial extent of activation in the lateral prefrontal cortex was the most prominent in HC (Fig. 1). We also observed strong activations in areas extending from the primary visual cortex to the lateral occipital cortex and the parieto-occipital cortex in each group. The strong activation in the visual cortex and the ventral high-order visual areas may reflect increased demands of visual processing of strings of six letters during encoding. The activation in the parieto-occipital cortex may reflect mnemonic processing for visuospatial information involved in this task by presenting letter strings in a 2 × 3 matrix (Cabeza and Nyberg, 2000). Significant activation in these regions was confirmed in cross-group analysis combining the three groups (Fig. 1). We observed two major activation foci in the lateral prefrontal cortex: one focus extended between dorsal part of the inferior frontal cortex and premotor cortex (dIFC/PMC), and the other focus was found in the ventral part of the inferior frontal cortex (vIFC).

3.3. ROI analyses

ANOVA showed a significant interaction of group and ROI ($F = 1.62$, df = 20, 360, $p < 0.05$), as well as a main effect of ROI ($F = 28.0$, df = 10, 360, $p < 0.001$). There were no significant main effect of gender ($F = 0.08$, df = 1, 36, $p > 0.5$) or interaction of gender and ROI ($F = 0.88$, df = 10, 360, $p > 0.5$). To further explore the interaction of group and ROI, we performed a follow-up one-factor ANOVA within each ROI to examine group differences. Among areas in the prefrontal cortex and its adjacent structures, a significant main effect of group was found only in the right vIFC and the bilateral dIFC/PMC (Fig. 2). Post-hoc tests revealed that PFX+ showed significantly reduced activation in the right dIFC/PMC compared with HC (Fig. 2). Compared with HC, activation in the right vIFC and left dIFC/PMC was reduced in both PFX+ and PFX− (Fig. 2). In contrast, the left vIFC, anterior cingulate cortex and supplementary motor area showed comparable activation among the three groups (Fig. 2). We did not observe significant group difference in the bilateral hippocampus, superior parietal cortex, the calcarine sulcus (Fig. 2). There was a trend-level effect in the right superior parietal cortex ($p = 0.08$).

3.4. Correlation analyses using molecular measures of FMR1 and severity of FXTAS

Among the ROIs, the right vIFC and the left dIFC/PMC showed reduced activation in the two premutation groups. This observation raises the possibility that cortical activity of this region may be influenced by genetic molecular factors associated with *FMR1* premutation status. In order to test this possibility, we performed regression analysis using either CGG repeat size or *FMR1* mRNA level as an explanatory variable for activity in these regions. Using estimates of activity adjusted for the effect of the severity of FXTAS and gender (seeMaterials and Methods), we found significant negative effect of *FMR1* mRNA levels on the right vIFC activity ($r = -0.38$, $p < 0.05$; see Fig. 3). There was no significant effect of CGG repeat size in this region ($r = -0.04$, $p > 0.5$). We observed no

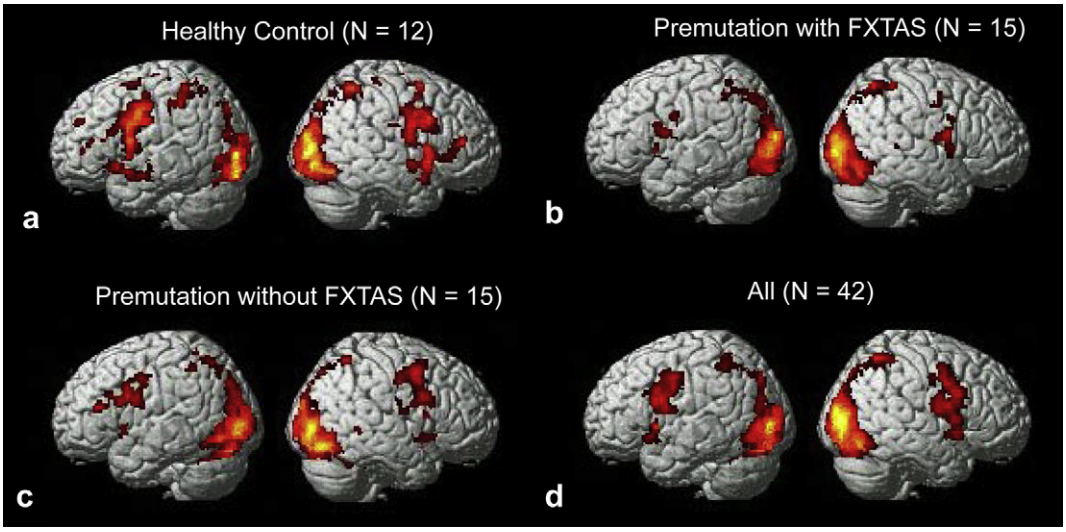

Fig. 1. Lateral surface rendering of activation during encoding in verbal working memory identified by group analysis. (a) Healthy control (HC) (b) Premutation carriers affected with FXTAS (PFX+) (c) Premutation carriers unaffected with FXTAS (PFX−). (d) Cross-group map combining all three groups. For the individual group maps, statistical threshold was set at $p < 0.01$, corrected for multiple comparisons using the false discovery rate. For the cross-group map, the statistical threshold was set at $p < 0.05$, corrected for multiple comparisons using the family-wise error rate.

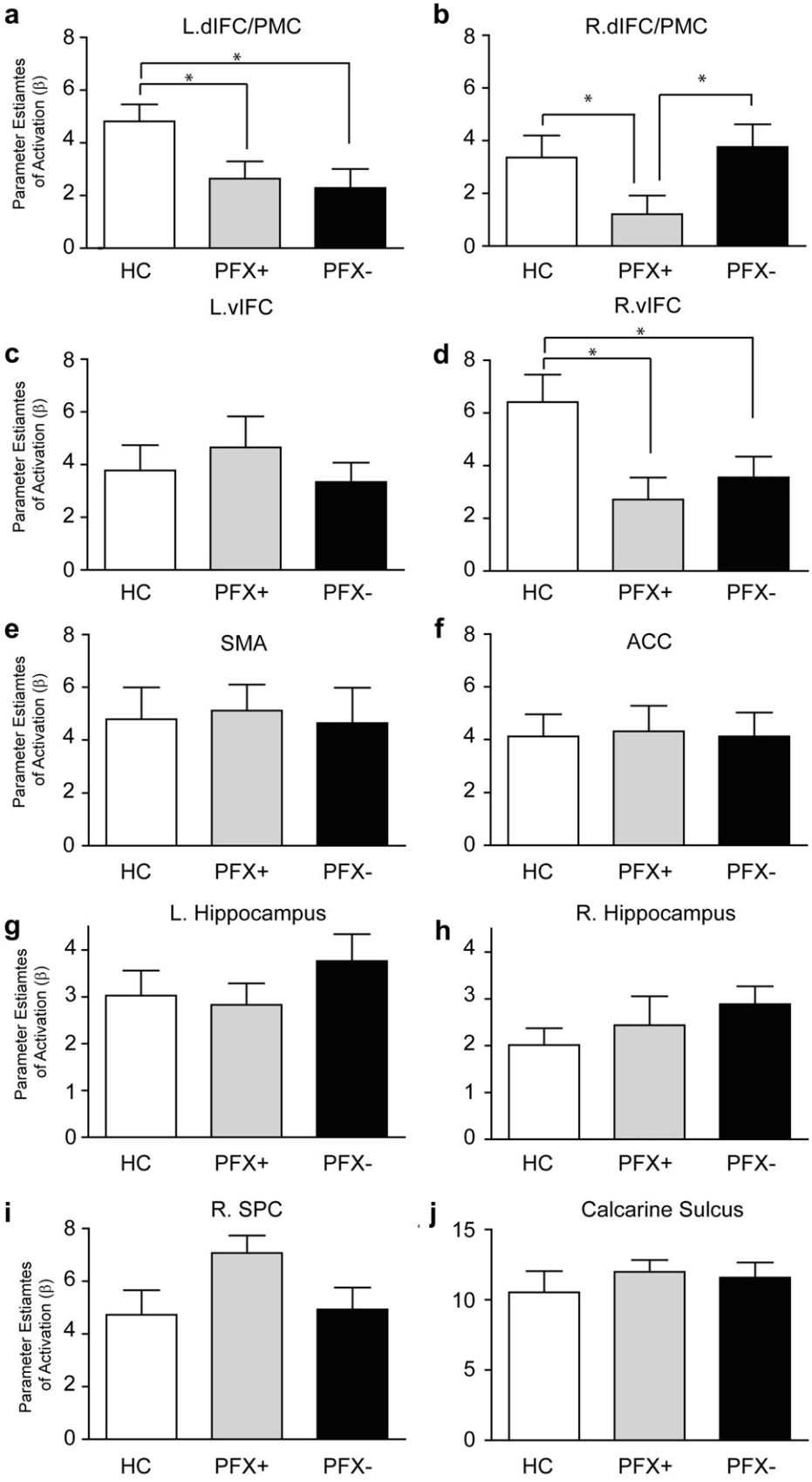

Fig. 2. Comparison of activation among groups in the regions of interest (ROIs) analysis. Activations in (a) left dorsal inferior frontal cortex/premotor cortex (dIFC/PMC), (b) right dIFC/PMC, (c) left ventral inferior frontal cortex (vIFC), (d) right vIFC, (e) anterior cingulate cortex (ACC), (f) supplementary motor area (SMA), (g) left hippocampus, (h) right hippocampus, (i) right superior parietal cortex, (j) calcarine sulcus. Error bars indicate the standard error of mean. The left superior parietal cortex was included in the ROIs, but the graph is not shown here (No significant main effect of group, $p > 0.1$). A significant difference between group is shown with an asterisk (*) ($p < 0.05$).

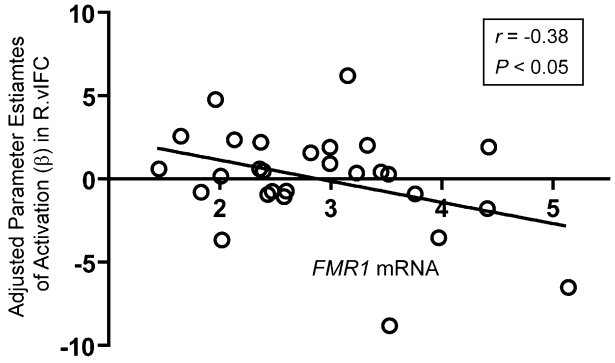

Fig. 3. Significant negative correlation of activity in the right ventral inferior frontal cortex with the levels of *FMR1* mRNA. Cortical activity was adjusted for the effects of the severity of FXTAS and gender.

significant effect of either CGG repeat size ($r = -0.22$, $P = 0.24$) or mRNA levels ($r = 0.05$, $p > 0.5$) on the left dIFC/PMC activity.

4. Discussion

The present study is the first to use fMRI to identify altered prefrontal activity in fragile X premutation carriers affected with FXTAS. Compared with the control group, the FXTAS patients showed reduced activation in the right vIFC, and the bilateral dIFC/PMC. Because these brain structures are critical for working memory and other executive functions (Cabeza and Nyberg, 2000; Smith and Jonides, 1999), deficient activity in these areas may be the neural correlate for the altered executive function and control of working memory that has been reported in patients with FXTAS. The right vIFC and left dIFC/PMC showed significantly reduced activation in premutation carriers with and without FXTAS. Regression analysis combining the two premutation groups revealed significant negative effects of *FMR1* mRNA levels on cortical activity in the right vIFC. These results identify compromised prefrontal regions responsible for deficient executive and memory processes in some *FMR1* premutation carriers, including those with FXTAS.

Because neuropsychiatric and neurological problems of FXTAS were recognized only recently, in vivo brain imaging studies of the *FMR1* premutation population have been scarce. A few MRI studies have examined structural abnormalities in male FXTAS patients (Brunberg et al., 2002) and premutation carriers of both genders (Moore et al., 2004; Murphy et al., 1999). Evidence from these studies indicates abnormalities in the cerebellum, thalamic nuclei, and hippocampal-amygdala complex. Our recent volumetric study compared male premutation carriers with and without FXTAS and observed advanced pathology of FXTAS in measures of the gross brain structures, including the whole brain, cerebrum, cerebellum, and ventricles (Cohen et al., 2006). The similar but a milder pattern of advanced pathology was also confirmed in female patients by our subsequent study (Adams et al., 2007). However, evidence from functional imaging studies was needed to identify specific neural correlates for cognitive decline in FXTAS.

Areas in the lateral and medial prefrontal cortex and its adjacent structures are critical for executive functions and control of memory. Our findings elucidate which of these areas are affected in some carriers of the fragile X premutation. The premutation carriers who were affected by FXTAS showed reduced activation in the bilateral dIFC/PMC and right vIFC. Reduced activation in the bilateral dIFC/PMC may explain the behavioral observation that FXTAS patients have general working memory deficits, including phonological loop impairments (Cornish et al., 2009). The right vIFC was shown to be negatively correlated with *FMR1* mRNA levels even after removing the effect of the severity of FXTAS. Previous functional imaging and brain lesion studies have shown critical roles of the right IFC in several executive functions involving response inhibition (Aron et al., 2004; Konishi et al., 1999). Therefore, reduced activation in this region might underlie such deficits in individuals with the premutation. Although this possibility is consistent with some of the past neuropsychological reports that both affected and unaffected premutation carriers are impaired in control of memory and executive functions including response inhibition (Cornish et al., 2009; Grigsby et al., 2008), it still remains unclear whether the non-FXTAS premutation individuals display significant deficits in these functions (Hunter et al., 2008b). It is possible that the executive cognitive problems are associated with a limited part of asymptomatic premutation population, which makes convergence among studies difficult. Our finding of significant negative impact of mRNA levels on the right vIFC activity suggests the need for future large-scale studies to investigate the association between executive functions and *FMR1* mRNA levels.

The advanced pathological processes in the hippocampus have been indicated by the previous histological studies of FXTAS patients (Greco et al., 2006; Greco et al., 2002). Evidence of hippocampal dysfunction has also been observed in our previous fMRI study of memory recall in men with the premutation who were unaffected by FXTAS (Koldewyn et al., 2008). Whereas the task in that study was crucially dependent on the hippocampus (recall of the visual objects encoded one day before), verbal working memory has been traditionally conceptualized as operations on information actively maintained in the phonological loop, which is primarily subserved by the anterior language system of the bilateral premotor and dorsal inferior frontal cortex rather than the hippocampus (Baddeley, 1992; Paulesu et al., 1993). Although the present verbal working memory study did not reveal deficient hippocampal activity in either premutation group, it is possible that such abnormalities become more apparent when the task is more dependent on the hippocampus. Further studies, including post-mortem pathological studies in unaffected premutation carriers, will be useful in addressing the possible functional abnormality of hippocampus.

In our continuous working memory task design, we compared activation during encoding with the other two memory phases to examine the executive and memory functions in the prefrontal cortex. Because areas in the prefrontal cortex are often involved in multiple memory phases (Cabeza and Nyberg, 2000), this contrast examines encoding-related activation modulated by increased task demands of memorizing multiple letters with reference to the elevated level of activity during the other two memory phases. Although the encoding phase in our design was made particularly demanding by simultaneous presentation of six different letters to remember, the contrast resulted in excluding activity for crucial working memory processes, such as short-term maintenance of information. Therefore, it is possible that there are important areas for working memory, such as the dorsolateral prefrontal cortex, that are absent in the activation pattern of this study. Further, the encoding-related activation was modeled in a fixed-interval of 10 s, which was shorter than the optimal value of 12–14 s for the fixed-interval event-related fMRI design (Bandettini and Cox, 2000). Lastly, because activation during encoding would reflect not only mnemonic processes but also combinations of multiple factors including executive processes, attention, and novelty-effects, we do not claim that the prefrontal activation identified by our analysis reflects specific processes in working memory. Further functional imaging studies will be necessary to examine possible deficiency in specific components in working memory processes of the *FMR1* premutation carriers. Despite these limitations, robust activation in

our prefrontal ROIs in the control group indicates that the present analysis served our goal of examining prefrontal functions of broad executive and memory processes.

Because this study was conducted in the context of seeing participants in a fragile X clinic, we cannot rule out the possibility of ascertainment bias in our sample, particularly in our PFX+ group, for which we were seeking participants who were showing signs of neurological decline. Therefore, the question of how representative our results are of carriers of the fragile X premutation in the general population is not currently known. Further, with the limited sample size in the present study, we chose to combine both male and female participants in each subject group for group comparison. Given the clinical observation that FXTAS primarily affects males (Amiri et al., 2008), as well as the fact that the gene of interest is on the X chromosome, future studies are needed to address possible gender-specific effects of the premutation on cortical activity in larger population. A recent behavioral study showed more deteriorating effects of aging on inhibitory control in the male premutation carriers than control males (Cornish et al., 2008). It is possible that effects of aging may also have an interaction with gender of the premutation carriers. Future studies with larger sample sizes would allow for systematic investigation of the effects and interactions of gender and aging on cortical activity of the premutation carriers.

To conclude, this study presented the first fMRI evidence for altered prefrontal functions in patients with FXTAS and for effects of *FMR1* mRNA levels on cortical functioning in the fragile X premutation carriers. Our findings provide bases for future studies addressing neural bases underlying behavioral deficiency and heterogeneity observed in the *FMR1* mutation spectrum.

Contributors

Author Hashimoto analyzed fMRI data and wrote the first draft of the manuscript. Author Backer analyzed behavioral data and co-wrote the first draft of the manuscript. Author Tassone analyzed and provided the molecular data from blood samples. Author Hagerman assessed the patients clinically and provided input on interpretation of study findings. Author Rivera conceptualized and designed the study and supervised the writing of the draft and the analysis of behavioral and fMRI data.

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Conflicts of interest

Author R. Hagerman receives funding from Seaside Therapeutics, Novartis, Roche, Forest, Johnson and Johnson, and Curemark for treatment trials in those with fragile X syndrome and/or autism. All other authors declare that they have no conflicts of interest.

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Appendix. Supplementary material

Supplementary material associated with this article can be found in the online version, at doi:10.1016/j.jpsychires.2010.04.030.

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