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Psychological Symptoms Correlate With Reduced Hippocampal Volume in Fragile X Premutation Carriers

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Fragile X-associated tremor/ataxia syndrome (FXTAS) is a neurodegenerative disorder occurring in male and occasional female carriers of a premutation expansion (55–200 CGG repeats) of the fragile X mental retardation 1 gene (*FMR1*). This study assessed the relationship between hippocampal volume and psychological symptoms in carriers, both with and without FXTAS, and controls. Volumetric MRI measures, clinical staging, cognitive testing, molecular analysis, and measures of psychological symptoms were performed for female premutation carriers both with FXTAS ($n = 16$, age: 57.50 ± 12.46) and without FXTAS ($n = 17$, age: 44.94 ± 11.23), in genetically normal female controls ($n = 8$, age: 50.63 ± 11.43), male carriers with FXTAS ($n = 34$, age: 66.44 ± 6.77) and without FXTAS ($n = 21$, age: 52.38 ± 12.11), and genetically normal male controls ($n = 30$, age: 57.20 ± 14.12). We examined the relationship between psychological symptom severity and hippocampal volume, as well as correlations with molecular data. We found a significant negative correlation between total hippocampal volume and anxiety in female carriers, with and without FXTAS. This finding was mainly driven by the significant negative correlation between right hippocampal volume and anxiety. Other anxiety-related subscales also correlated with the right hippocampus in females. In male carriers with and without FXTAS, only paranoid ideation negatively correlated with hippocampal volume. Female premutation carriers demonstrated a negative association between hippocampal volume and the severity of anxiety-related psychological symptoms. Though the presentation of FXTAS symptoms is less common in females, anxiety-related problems are common both prior to and after the onset of FXTAS, and may be related to hippocampal changes.

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INTRODUCTION

The fragile X premutation, which involves a CGG repeat expansion (55–200 repeats) on the fragile X mental retardation 1 (*FMR1*) gene, is associated with emotional problems [Franke et al., 1998; Cornish et al., 2005; Hessl et al., 2005], neurocognitive deficits [Loesch et al., 2003; Moore et al., 2004; Grigsby et al., 2006a,b], primary ovarian insufficiency (POI) [Allingham-Hawkins et al., 1999; Murray et al., 2000; Sullivan et al., 2005], and the fragile X-associated tremor/ataxia syndrome (FXTAS) [Hagerman et al., 2001; Jacquemont et al., 2003, 2007; Loesch et al., 2005a; Berry-Kravis et al., 2007; Leehey et al., 2007].

The characteristic features of FXTAS are progressive gait ataxia and intention tremor, with onset typically occurring after the age of 50 [Hagerman et al., 2001; Jacquemont et al., 2003; Leehey et al., 2007]. Other symptoms of FXTAS include memory and executive function deficits, which may progress to cognitive impairment and dementia [Bacalman et al., 2006; Bourgeois et al., 2006; Grigsby et al., 2006a], and peripheral neuropathy [Jacquemont et al., 2003; Hagerman et al., 2007]. Recent studies involving the volumetric analysis of structural brain MRI showed that individuals with FXTAS may incur brain atrophy, often with subcortical and periventricular white matter disease, with a high prevalence (58%) of white matter disease in the middle cerebellar peduncles (MCP) [Brunberg et al., 2002; Loesch et al., 2005b; Cohen et al., 2006; Adams et al., 2007]. Neuropathological examinations of patients with FXTAS have revealed intranuclear inclusions in neurons and astroglia of the cerebrum, brain stem, and spinal cord, with the highest density of inclusions in the hippocampi [Greco et al., 2002, 2006].

In rats, hippocampal function can be divided into two distinct regions: dorsal (corresponding to the posterior hippocampus in primates) and ventral (anterior hippocampus in primates). Whereas the dorsal hippocampus is involved in certain types of learning and memory [Moser et al., 1993], the ventral region may have more of a role in anxiety-related behaviors [Moser et al., 1993; Bannerman et al., 2003]. Moreover, ventral hippocampus emotional processing is distinct from that of the amygdala, which is related specifically to fear [Bannerman et al., 2004]. Studies of the action of anxiolytic drugs, and of hippocampal and amygdala lesions, have led to hypotheses that the septo-hippocampal system is an important piece of the behavioral inhibition system, and thus in anxiety and anxiety-related disorders [Gray, 1982; Gray and McNaughton, 2000; McNaughton and Gray, 2000; Bannerman et al., 2004]. Given that males with the premutation have reduced hippocampal activation during memory recall tasks, presumably because of dysfunction in the posterior hippocampus, which also correlated with psychological symptoms [Koldewyn et al., 2008], it is possible that the anterior hippocampus in premutation carriers is also affected (and thus, level of anxiety). Additionally, reduced hippocampal volume has been shown in several mood disorders, including major depression disorder [Bremner et al., 2000; Beyer and Krishnan, 2002] and post-traumatic stress disorder [Smith, 2005]. It was recently reported that the risk of major depression in female premutation carriers is increased compared to controls, and is associated with smaller CGG repeat size [Roberts et al., 2009]. However, the size of the hippocampus in those carriers with psychiatric problems has not been studied.

FXTAS affects approximately 40% of male premutation carriers over the age of 50 in families ascertained through probands with fragile X syndrome (FXS), but is less frequent in females (8–16%) [Hagerman et al., 2004; Zuhlke et al., 2004; Berry-Kravis et al., 2005; Jacquemont et al., 2005; O'Dwyer et al., 2005; Coffey et al., 2008; Rodriguez-Revenga et al., 2009]. This disparity may be due to the presence of a second, non-expanded X chromosome which, if active in a significant percentage of cells in the brain, may provide a protective effect with respect to FXTAS symptoms [Berry-Kravis et al., 2005; Jacquemont et al., 2005]. It has been demonstrated that females with FXTAS present with relatively larger brain region volumes (less atrophy) than males with FXTAS, though a trend of reduced hippocampal volume with increased FXTAS symptoms has been observed in females [Adams et al., 2007]. In addition, it has been demonstrated that individuals (both male and female) with FXTAS have an increased incidence of psychological symptoms, including anxiety [Hessl et al., 2005]. These data, in conjunction with the high prevalence of the premutation allele in the general population, approximately 1 per 130 in females [Pesso et al., 2000; Hagerman, 2008] and 1 per 250–810 in the male population [Hagerman, 2008], underscore the need for further study of premutation carriers and the extent and effects of reduced hippocampal size.

METHODS

Subjects

Patients were selected for the study through their familial relationship with children with FXS. Female relatives of proband children with FXS were recruited for testing to identify those who exhibit symptoms of FXTAS. Presence of the premutation (*FMR1* allele ranging between 55 and 200 CGG repeats) was determined using PCR and Southern blot DNA analysis. Genetically normal relatives of proband children and patients with FXTAS were recruited as control subjects. Two additional male controls were recruited to match the age and education levels of the premutation carriers. All study participants signed informed consent for this study, which was approved by the Institutional Review Boards at the University of California at Davis Medical Center and the University of Colorado at Denver and Health Sciences Center.

Our analysis included 126 subjects. Of these subjects, 17 were females with the premutation but without FXTAS symptoms (non-FXTAS), 16 were females with the premutation affected with FXTAS, according to previously defined criteria [Jacquemont et al., 2003], and 8 were genetically normal female controls. The remainder of the subjects ($n = 85$) were male, which included 21 male non-FXTAS premutation carriers, 34 males with the premutation and with FXTAS, and 30 genetically normal controls.

The mean age between males and females was significantly different among both the FXTAS and non-FXTAS carrier groups

TABLE I. Description of Characteristics of 126 Subjects

	Female			Male			
	N	Mean	SD	N	Mean	SD	*P*-value (*t*-value)[a]
Age							
FXTAS	16	57.50	12.46	34	66.44	6.77	0.011 (2.60)
Non-FXTAS	17	44.94	11.23	21	52.38	12.11	0.047 (2.01)
Control	8	50.63	11.43	30	57.20	14.12	0.148 (1.45)
Years since symptom onset							
FXTAS	16	7.48	10.19	33	7.83	5.71	0.901 (0.13)
Symptom[b] onset age							
FXTAS	16	50.00	15.13	33	58.67	7.15	0.041 (2.22)
Activation ratio							
FXTAS	16	0.54	0.18				0.952[c] (−0.06)
Non-FXTAS	15	0.54	0.12				
CGG repeat							
FXTAS	16	95.19	14.18	33	92.76	18.86	0.652 (−0.45)
Non-FXTAS	15	96.40	20.17	21	88.52	32.33	0.233 (−1.20)
Control	8	32.00	6.89	24	28.42	4.82	0.652 (−0.41)
FMR1 mRNA							
FXTAS	14	3.04	1.23	30	3.41	0.82	0.259 (1.14)
Non-FXTAS	13	2.49	0.70	21	3.13	1.63	0.071 (1.82)
Control	7	1.43	0.19	19	1.34	0.36	0.850 (−0.19)

	Medication (SSRI and/or SNRI)				
	Yes	No	Yes	No	*P*-value
FXTAS	12	4	14	20	0.035
Non-FXTAS	6	11	6	15	0.734
Control	2	6	3	27	0.279

[a]*P*- and *t*-values pertain to comparison between females and males within each group.
[b]Symptoms include ataxia and/or intention tremor.
[c]Comparison of activation ratio of FXTAS and non-FXTAS female groups.

($P = 0.011$ and $P = 0.047$, respectively). The subgroups (FXTAS, non-FXTAS, controls) within each gender differed in age as well (see Table I). The age of onset of FXTAS symptoms for males and females (58.67 ± 7.15, 50.00 ± 15.13, respectively) was also significantly different ($P = 0.041$).

Although there was no significant difference in psychotropic medication taken (SSRI, SNRI, or SSNRI) between females and males among the control and non-FXTAS groups, a significantly higher proportion of females with FXTAS were on psychotropic medication relative to males with FXTAS (75.0% vs. 41.2%, $P = 0.035$).

There were no differences in IQ within each gender between the control and non-FXTAS groups, although there were differences in the males between the FXTAS group and both the non-FXTAS and control groups (mean/SD: 104.0/15.0, 116.7/15.8, 116.0/17.4, respectively).

Years since symptom onset, CGG repeat size, and *FMR1* mRNA levels were not significantly different between males and females. Also, the mean activation ratios (AR) for females with and without FXTAS were not significantly different. Table I summarizes the characteristics of the 126 study subjects in more detail.

Neuroimaging

Structural MR images were acquired using a 1.5 T GE Signa Horizon LX Echospeed system. Acquisition parameters are as follows:

Coronal 3D spoiled gradient recalled echo (IR-prepped SPGR) acquisition, T1 weighted: Coronal plane, 3D acquisition, gradient recalled echo, RF spoiled, TR 9.1 msec, spatial resolution: $0.9375 \times 0.9375 \times 1.5\,mm^3$ thickness.

High resolution FLAIR (same orientation as axial spin echo): Oblique axial plane, 2D acquisition, inversion recovery spin echo, TE 144 msec, TR 11,000 msec, TI 2,250 msec, 14 slices/acquisition, 2 interleaved acquisitions, resolution $0.9375 \times 0.9375 \times 3\,mm^3$ thickness, 0 mm interslice on reconstructed image.

Volumetric analysis was performed on the MR images using a custom computer program operating on a UNIX, Solaris platform (Quanta 6.1). Evaluation involved operator-guided tracing of the dura mater, and subsequent removal of non-brain elements. The resulting cranial vault was deemed total cranial volume (TCV) and used to normalize other brain volumes (by dividing the hippocampal volume by TCV). TCV analysis was performed on axial FLAIR images.

Hippocampal volumes were obtained using the coronal 3D SPGR images. Through operator-guided tracing, including the CA1–CA4 fields, the dentate gyrus, and the subicular complex, left and right hippocampal volumes were determined. The volumes from the hippocampi were summed together to obtain a total hippocampal volume.

Intrarater reliability was determined using intraclass correlation coefficients (ICCs) with a minimum score of 0.97 needed for reliability. ICCs were 0.99 for TCV, 0.98 for cerebral volume, 0.98 for right hippocampal volume, 0.97 for left hippocampal volume, and 0.99 for volume of white matter hyperintensity. A single rater (J.A.) performed all of the analysis, and was blinded to subjects' experimental condition, molecular status, and demographic information.

Clinical Evaluation

The diagnosis of FXTAS was made after a thorough medical, neurological, and radiological examination using criteria for definite or probable FXTAS, as previously published [Jacquemont et al., 2003]. After examination, a FXTAS clinical staging score was given to each patient with the premutation. This 7-point rating scale, as previously reported [Bacalman et al., 2006; Grigsby et al., 2006a], measures functional impairment, as follows: 0—normal functioning; 1—subtle or questionable tremor and/or balance problems; 2—minor, but clear tremor and/or balance problems producing no significant interference with activities of daily living (ADLs); 3—moderate tremor and/or balance problems with at least occasional falls and significant interference in ADLs; 4—severe tremor and/or balance problems requiring the use of a cane or walker; 5—use of a wheelchair on a daily basis; 6—bedridden.

Subjects were administered the Wechsler Adult Intelligence Scale—Third Edition [Wechsler, 1997], providing a measure of verbal IQ (VIQ) and performance IQ (PIQ).

Molecular Analysis

DNA analysis: genomic DNA was isolated from peripheral blood leucocytes (5 ml of whole blood) using standard methods (Puregene Kit; Gentra Systems, Inc., Minneapolis, MN). Southern blot and PCR analyses were performed as previously described [Tassone et al., 2008]. Analysis and calculation of the repeat size (for both Southern blot and PCR analysis), methylation status, and activation ratio were carried out using an Alpha Innotech FluorChem 8800 Image Detection System, as previously described [Tassone et al., 2008]. The activation ratio in females expresses the percent of cells that carry the normal allele on the active X chromosome and is calculated by the ratio of the intensity of the normal unmethylated band over the sum of the intensities of the unmethylated and methylated normal bands [Tassone et al., 1999].

FMR1 mRNA levels: All quantifications of *FMR1* mRNA were performed using 7900 Sequence Detectors (Applied Biosystems Inc., Foster City, CA), as previously described [Tassone et al., 2007b].

Psychological Symptoms

Psychological symptoms were determined through the use of the Symptom Checklist-90-Revised (SCL-90-R) [Derogatis, 1994], which is a self-report of current psychological symptoms. There are 90 questions, each evaluated on a 5-point scale of level of distress felt by the patient (5 being the most distressed). The questions are clustered into the following symptom categories: Somatization, obsessive–compulsive tendencies, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation, and psychoticism. These categories are combined in the Global Severity Index (GSI) to obtain an overall level of psychological symptoms. The SCL-90-R has been used previously to evaluate individuals with the premutation [Johnston et al., 2001; Hessl et al., 2005; Hessl et al., 2007]. The psychometrics of the SCL-90-R has been previously discussed by our research group [Hessl et al., 2005].

Statistical Analysis

Comparisons of patient characteristics between gender and diagnostic groups (FXTAS, non-FXTAS, control) were performed using the analysis of variance for continuous variables and Fisher's exact test for categorical variables. The primary analyses and comparisons of psychological symptoms on the SCL-90-R among gender and diagnostic groups (FXTAS, non-FXTAS, control) were performed using linear regression models (ANCOVA), adjusting for both age and whether patients were on psychotropic medication (yes or no). The individual ANCOVA models for the diagnostic groups were compared at (the modeled) ages of 50 and 60 years. These two comparisons help illustrate the age-specific differences in the models with regard to psychological symptoms, as well as the differing symptom trajectories of the various diagnostic groups. A subset of the SCL-90-R data was previously reported [Hessl et al., 2005], where analysis focused on comparison to published norms. This subset included 32 of the males with the premutation (23 with FXTAS and 9 without FXTAS) and 8 of the females with the premutation (4 with and 4 without FXTAS).

Partial Pearson correlations were used to assess associations between psychological measures and hippocampal volume in carriers with FXTAS, adjusted for age and stratified by sex. It should be noted that the stratification by sex controls for the use of psychotropic medication, as a significantly higher proportion of females with FXTAS were taking psychotropic medications as compared to males with FXTAS (see Subjects Section). Reported *P*-values are for two-sided tests at level 0.05. The Benjamini–Hochberg method for controlling false discovery rate (FDR) was used to adjust the *P*-values for multiple tests within the regression analysis, as well as the partial correlation analysis. A *P*-value that is significant after FDR adjustment is marked by an asterisk throughout the text, although we present and discuss results from both adjusted and unadjusted *P*-values. For analysis of right, left, and total (right and left combined) hippocampal size, the structures were normalized by TCV prior to statistical analyses. It should be noted that all statistical analyses were also performed using logarithm-transformed data, to better satisfy statistical assumptions of the methods used. The results based on log-transformation were the same as for the raw data. Therefore, we report results based on the untransformed (raw) data.

Due to the differences among groups, regression analyses accounted for both the effects of age and the use of psychotropic medication. We also explored models that adjusted for IQ. The conclusion from these models was that IQ was not a significant confounder ($P > 0.05$), except in the case of social phobia. In either case (with or without IQ accounted for), however, the results of the regression models were the same. Therefore, we chose to present the results for the simpler models, without IQ.

Statistical analyses were performed with the use of SAS software, version 9.1 (SAS Institute, Cary, NC).

RESULTS

Psychological Involvement

Age- and gender-specific comparisons show differences in psychological symptoms after controlling for medication taken, as summarized below.

Psychological symptoms in females. Prior to FDR adjustments, females with FXTAS compared to controls, showed significantly elevated levels of obsessive–compulsive behaviors, interpersonal sensitivity, depression, anxiety (Fig. 1), phobic anxiety, paranoid ideation, and GSI at both ages of comparison (50 and 60 years of age). We also found a significant increase in psychological affectedness between females with FXTAS and non-FXTAS female carriers in somatization, obsessive–compulsive traits, interpersonal sensitivity, GSI (all at age 50), and anxiety (ages 50 and 60). There was no difference between non-FXTAS carriers and controls on any of the SCL-90-R subscales.

After FDR adjustment, there were significant differences between females with FXTAS and controls in obsessive–compulsive characteristics and anxiety (ages 50 and 60). The females with FXTAS, compared to non-FXTAS females, demonstrated significantly higher levels of anxiety and obsessive–compulsive behaviors (age 50). For details, see Table II.

Psychological symptoms in males. Male premutation carriers with FXTAS exhibited elevated levels of somatization (ages 50 and 60) relative to both non-FXTAS and control subjects. The FXTAS group also showed elevated levels of anxiety (compared to non-FXTAS males and controls; Fig. 1), and obsessive–compulsive behaviors, depression, and GSI (compared to non-FXTAS males).

Results based on post-FDR adjustments suggest that males with FXTAS exhibit significantly more obsessive–compulsive behaviors when compared to non-FXTAS carriers (ages 50 and 60), and anxiety when compared to controls (age 60). For further details, see Table III.

It should be noted that the psychological symptoms of non-FXTAS carriers and controls are quite similar for both males and females, though the sample size of the female controls is small.

Association of Psychological Measures and Hippocampal Size

All observed associations between psychological measures and hippocampal volumes were in a negative direction: an increased level of the symptom was associated with decreased hippocampal size.

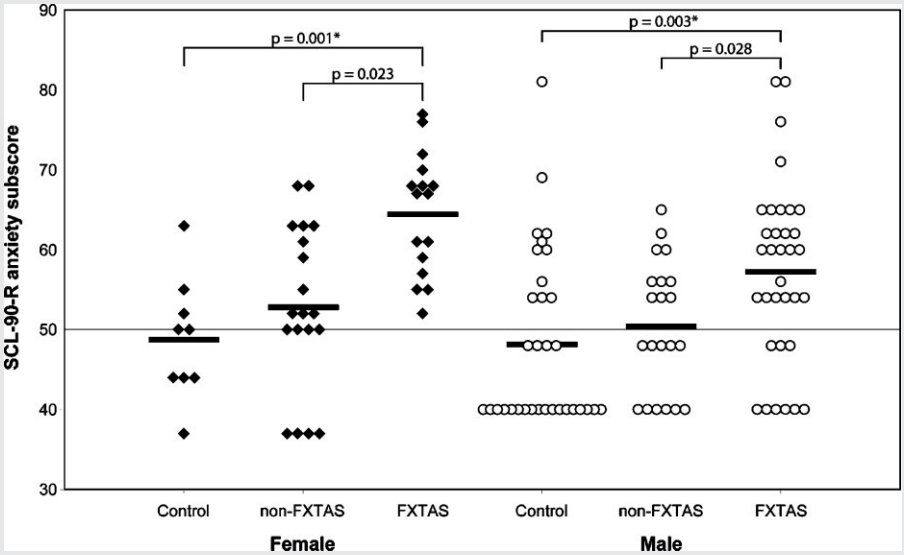

FIG. 1. Comparison of anxiety score on SCL-90-R for males and females, with and without FXTAS, and controls. Closed diamonds indicate female data points, while open circles depict male data points. *P*-values are for comparisons of predicted means at the age of 60 (fitted ANCOVA model). We found female premutation carriers with FXTAS had significantly higher predicted scores at age 60 in the ANCOVA model as compared to female carriers without FXTAS ($P = 0.023$), and controls ($P = 0.001$*). In the same analysis, male premutation carriers with FXTAS also had higher levels of anxiety compared to male carriers without FXTAS ($P = 0.028$) and controls ($P = 0.003$*).
***P*-value retains significance following correction for multiple comparisons.**

TABLE II. Comparison of Psychological Scores of 41 Female Subjects

Comparison groups[a]	Difference	SE	*P*-value[b]
Somatization			
F–N age 50	10.143	3.965	0.012
F–C age 50	9.601	4.722	0.044
Obsessive–compulsive			
F–N age 50	9.770	3.649	0.009*
F–C age 50	14.234	4.347	0.001*
F–C age 60	13.970	4.379	0.002*
Interpersonal sensitivity			
F–N age 50	9.417	4.266	0.029
F–C age 50	11.939	5.081	0.021
F–C age 60	12.166	5.118	0.019
Depression			
F–C age 50	9.881	4.533	0.031
F–C age 60	10.397	4.567	0.025
Anxiety			
F–N age 50	10.014	3.513	0.005*
F–N age 60	8.907	3.877	0.023
F–C age 50	11.804	4.184	0.006*
F–C age 60	14.557	4.215	0.001*
Phobic anxiety			
F–C age 50	8.734	3.953	0.029
F–C age 60	9.154	3.983	0.023
Paranoid ideation			
F–C age 50	12.546	4.841	0.011
F–C age 60	13.803	4.877	0.006
Psychoticsim			
F–C age 50	9.753	4.706	0.041
Global severity index			
F–N age 50	7.687	3.818	0.046
F–C age 50	10.762	4.547	0.020
F–C age 60	11.251	4.581	0.016

*P-value significant after correction for multiple comparisons.
[a]Groups include FXTAS (F), non-FXTAS (N), and controls (C).
[b]*T*-value = difference/SE.

TABLE III. Comparison of Psychological Scores of 85 Male Subjects

Comparison groups[a]	Difference	SE	*P*-value[b]
Somatization			
F–N age 50	11.418	3.950	0.005
F–N age 60	7.052	3.260	0.033
F–C age 50	9.764	3.882	0.013
F–C age 60	6.263	2.885	0.032
Obsessive–compulsive			
F–N age 50	10.675	3.635	0.004*
F–N age 60	7.943	3.001	0.009*
Interpersonal sensitivity			
F–N age 50	8.112	4.249	0.059
F–N age 60	6.878	3.507	0.052
Depression			
F–N age 50	8.280	3.791	0.031
F–N age 60	6.744	3.129	0.033
Anxiety			
F–N age 50	7.535	3.499	0.033
F–N age 60	6.428	2.888	0.028
F–C age 60	7.644	2.556	0.003*
Psychoticsim			
F–N age 50	9.115	3.936	0.022
Global severity index			
F–N age 50	9.366	3.803	0.015
F–N age 60	7.902	3.139	0.013

*P-value significant after correction for multiple comparisons.
[a]Groups include FXTAS (F), non-FXTAS (N), and controls (C).
[b]*T*-value = difference/SE.

Males with FXTAS showed possible correlations between total hippocampal volume and GSI ($r = -0.356$, $P = 0.042$), interpersonal sensitivity ($r = -0.406$, $P = 0.019$), and paranoid ideation ($r = -0.313$, $P = 0.076$), none of which were significant following FDR adjustment. However, graphic analysis suggests that a single influential data point masked the associations between psychological measures and hippocampal volumes. Analysis excluding this subject indicated stronger associations between total hippocampal volume and GSI ($r = -0.499$, $P = 0.004^*$), interpersonal sensitivity ($r = -0.475$, $P = 0.006^*$), and paranoid ideation ($r = -0.587$, $P < 0.001^*$), as well as newly significant correlations with phobic anxiety, depression and obsessive–compulsive tendencies. Results from secondary (exploratory) analyses of psychological measures and left and right hippocampal size are similar to the analyses based on total hippocampal size. See Table IV.

The analysis for females with FXTAS suggests there are no associations between hippocampal size and any of the subscales on the SCL-90-R, though the sample size is small ($n = 16$). However, when all females with the premutation (both with and without FXTAS) were analyzed together, we observed significant associations between hippocampal size and anxiety, phobic anxiety and obsessive–compulsive traits (Table IVB). Most of these correlations seemed to be driven by the right hippocampus, especially with anxiety ($P < 0.001^*$; Fig. 2) and phobic anxiety ($P = 0.001^*$).

In the equivalent analysis of all male premutation carriers (Table IVC), the only association with total hippocampal volume that was significant following FDR adjustment was paranoid ideation ($r = -0.404$, $P = 0.003^*$).

TABLE IV. Correlations Between Psychological Symptoms and Hippocampal Volume

	Hippocampal volume, correlation R (*P*-value)		
Psychological symptom	**Total**	**Right**	**Left**
(A) Male carriers w/FXTAS[a]			
Global severity index	−0.499 (0.004*)	−0.439 (0.012*)	−0.503 (0.003*)
Interpersonal sensitivity	−0.475 (0.006*)	−0.488 (0.005*)	−0.401 (0.023*)
Phobic anxiety	−0.390 (0.029*)	−0.362 (0.042)	−0.365 (0.040)
Obsessive–compulsive	−0.430 (0.014*)	−0.363 (0.041)	−0.450 (0.010*)
Depression	−0.405 (0.021*)	−0.341 (0.056)	−0.425 (0.015*)
Paranoid ideation	−0.587 (<0.001*)	−0.570 (<0.001*)	−0.533 (0.002*)
(B) Female premutation carriers[b]			
Global severity index	−0.332 (0.063)	−0.458 (0.008*)	−0.144 (ns)
Anxiety	−0.519 (0.002*)	−0.634 (<0.001*)	−0.314 (ns)
Phobic anxiety	−0.383 (0.030)	−0.551 (0.001*)	−0.140 (ns)
Obsessive–compulsive	−0.349 (0.050)	−0.472 (0.006*)	−0.161 (ns)
Depression	−0.270 (ns)	−0.367 (0.039)	−0.123 (ns)
Paranoid ideation	−0.219 (ns)	−0.364 (0.0407)	−0.025 (ns)
(C) Male premutation carriers[a]			
Global severity index	−0.301 (0.027)	−0.306 (0.025)	−0.269 (0.049)
Interpersonal sensitivity	−0.264 (0.053)	−0.293 (0.032)	−0.210 (ns)
Obsessive–compulsive	−0.281 (0.040)	−0.287 (0.035)	−0.250 (0.069)
Paranoid ideation	−0.404 (0.003*)	−0.417 (0.002*)	−0.354 (0.009)

ns = non-significant *P*-value >0.05.
**P*-value significant after correction for multiple comparisons.
[a]Analysis without the single influential data point (male with FXTAS). See text.
[b]Results for subset analysis for females with FXTAS (n = 16) were not significant. See text.

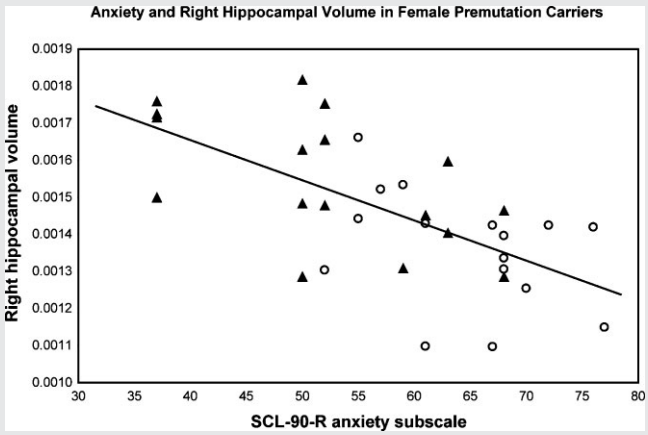

FIG. 2. Comparison of anxiety score on the SCL-90-R with right hippocampal volume (normalized by TCV) in female premutation carriers (both with and without FXTAS). Closed triangles indicate female premutation carriers without FXTAS, whereas open circles represent female carriers with FXTAS. We found that anxiety score correlated significantly with the size of the right hippocampus (R = −0.634, *P* < 0.001*). The same was true for several other subscales on the SCL-90-R. **P*-value retains significance following correction for multiple comparisons.

Neither male nor female controls showed associations between SCL-90-R scores and hippocampal volume ($P > 0.1$ for all control comparisons). The female control sample size was small (n = 8), so these data should be interpreted with caution.

Association of Hippocampal Size and Molecular Measures

Correlations of hippocampal size to CGG repeat size and *FMR1* mRNA levels were examined, stratified by sex and affectedness (FXTAS or non-FXTAS). Significant negative associations with CGG repeat size were observed for total ($r = -0.375$, $P = 0.034$) and left ($r = -0.430$, $P = 0.014$) hippocampal volumes in males with FXTAS. There were no statistically significant associations between mRNA and hippocampal volume for male carriers, either with or without FXTAS. Female carriers with FXTAS showed a negative association between right hippocampal size and CGG repeat size ($r = -0.535$, $P = 0.040$). For female carriers without FXTAS, there was a negative association between mRNA and left hippocampal volume ($r = -0.637$, $P = 0.026$). None of the associations with molecular measures were significant following FDR adjustment.

DISCUSSION

The results from this study demonstrate an increase in psychological symptoms in premutation carriers with FXTAS, as compared to

controls and carriers without FXTAS, concurring with previous studies [Hessl et al., 2005; Bacalman et al., 2006; Bourgeois et al., 2009]. We show that females with the premutation have significantly increased levels of obsessive–compulsive behaviors and anxiety, as well as, to a lesser extent, other psychological symptoms, compared to controls. Some of these symptoms have been seen in previous studies of female carriers without FXTAS [Sobesky et al., 1996; Franke et al., 1998; Johnston et al., 2001]. We also report that males with FXTAS have significant levels of psychological symptoms, including obsessive–compulsive traits, somatization, and anxiety. Though it has been documented that females are, to some degree, protected from the effects of FXTAS by the presence of a second, healthy X chromosome [Adams et al., 2007; Berry-Kravis et al., 2007], it seems that those females with FXTAS exhibit a profile of psychological symptoms similar to males. In fact, our results indicate that the psychological symptoms of female premutation carriers with FXTAS may be more pronounced than those of their male counterparts.

In female premutation carriers, we found several significant negative correlations between hippocampal volume and psychological symptoms (Table IVC), particularly those involved with anxiety. Interestingly, the hippocampal associations seemed to be driven by the right hippocampus, where the correlations were particularly strong, even after FDR adjustment. There is some evidence of lateral differences in function within the limbic region, with the left amygdala involved in cognitive processing and the right more involved in autonomic responses [Skuse, 2006], as well as the left hippocampus being smaller than the right in major depression [Bremner et al., 2000; Mervaala et al., 2000]. Our hippocampal associations also seemed to be driven by the females with the premutation but without FXTAS, indicating that emotional and hippocampal dysfunction may occur prior to the emergence of FXTAS in females.

In the corresponding analysis with male premutation carriers, we found that males with FXTAS showed an association between size of the hippocampi and several psychological problems (Table IVB). When we grouped all the male premutation carriers together (as we did in the females), we found a few weak correlations, only one of which (paranoid ideation) was still significant after FDR adjustment. Unlike in females, the correlations did not seem to be driven by a particular side of the brain, nor by carriers without FXTAS. These data suggest that even though females show a similar psychological symptom trajectory to males after incurring FXTAS, they seem to be affected psychologically prior to the development of FXTAS as well. It is probable that psychological dysfunction stems from both gross structural abnormalities (i.e., reduced overall hippocampal volume) and cellular abnormalities [Greco et al., 2002, 2006; Arocena et al., 2005], with gender-specific susceptibilities.

We also examined associations between hippocampal volume and *FMR1* molecular data. We found a negative correlation between CGG repeat size and total and left hippocampal volume in males with FXTAS. We found a similar correlation with CGG repeat length and right hippocampal volume in females with FXTAS. Though mRNA levels correlated with left hippocampal volume in female carriers without FXTAS, this result should be interpreted with caution, given the small sample size.

It has been previously reported that carriers are at increased risk of major depression with smaller CGG repeat size [Roberts et al., 2009]. It has also been shown that age of onset of FXTAS symptoms correlates positively with CGG repeat length [Tassone et al., 2007a], as does symptom severity [Leehey et al., 2008]. These findings, in addition to our data, seem disparate. Previous studies of FXTAS have shown greater neuroanatomical involvement, including inclusion formation, CNS atrophy and white matter disease, with larger CGG repeat number [Cohen et al., 2006; Greco et al., 2006; Adams et al., 2007]. There is an increase in the prevalence of POI with a larger CGG repeat number until approximately 100–120 repeats, at which point there is a decline in prevalence [Sullivan et al., 2005]. In the higher repeat end of the premutation, there is a mild decrease of FMRP [Tassone et al., 2000] which may alleviate some of the RNA toxicity of the premutation. This may also be true of emotional difficulties and FXTAS symptoms, since both FXTAS and POI are considered to be related to RNA toxicity. Our study had five males and four females with CGG repeat lengths >120. This number may have been enough to skew the data slightly (if it is indeed true that RNA toxicity is alleviated somewhat past 120 CGG repeats), making correlations less significant, but it did not allow us to discern a CGG drop-off point in prevalence.

In summary, we found an increase in psychological problems in both male and female premutation carriers with FXTAS. We also found evidence that non-FXTAS female premutation carriers are affected psychologically, and that affectedness correlates with the size of the hippocampi. It is known that chronic stress and associated alterations of the hypothalamic–pituitary–adrenal axis can lead to hippocampal atrophy [Sapolsky, 2000]. Perhaps the lives of female carriers are more stressful than males as a result of the challenges of raising children with FXS [Johnston et al., 2003]. Recent studies in the premutation mouse have demonstrated a great rise in cortisol levels with increased stress compared to control mice [Brouwer et al., 2008]. Enhanced levels of cortisol have yet to be documented in human carriers, but stress is often seen clinically, which is likely related to the history of psychological symptoms [Hessl et al., 2005]. The limbic system may be particularly susceptible to RNA toxicity from the premutation, as there is a higher rate of transcription for the *FMR1* mutation in this area as compared to other areas of the brain [Hagerman and Hagerman, 2004; Tassone et al., 2004]. It has also been shown that a high density of intranuclear inclusions occurs within the hippocampi of premutation carriers with FXTAS, compared to other brain areas [Greco et al., 2002, 2006].

Further evidence of hippocampal involvement with the premutation has been shown in males with the premutation but without FXTAS, who demonstrated decreased activation of the hippocampi during memory recall, which was also associated with psychological symptom severity [Koldewyn et al., 2008]. It is likely that RNA toxicity has an effect on the hippocampus even before the onset of FXTAS symptoms, as emotional problems in carriers without FXTAS have been shown to correlate with elevations in *FMR1* mRNA [Hessl et al., 2005].

Our data suggest that females with the premutation (both with and without FXTAS) have significant psychological problems and (relatively) smaller hippocampi than male carriers. This may be related to direct RNA toxicity and perhaps the effect of stress on

neuronal survival. However, for females who develop FXTAS, it is likely that the developmental course of their symptoms will be less severe because of the protective effect of the extra X chromosome [Hagerman et al., 2004; Adams et al., 2007; Berry-Kravis et al., 2007].

Our findings also have treatment implications, including the need to reduce stress and alleviate or treat psychological problems in premutation carriers [Hagerman et al., 2008]. The use of therapy, exercise, and pharmacological treatment of anxiety, depression and OCD symptoms is warranted, when clinically indicated, with the added benefit of the latter two interventions stimulating stem cells to repair the brain [Jacobs et al., 2000; Santarelli et al., 2003; Hagerman et al., 2008]. Further research is necessary regarding early intervention for premutation involvement prior to and with the onset of FXTAS.

Although these data paint an intriguing picture of the fragile X premutation, several factors limit us from making some definitive conclusions. One such limitation is the relatively small sample size of this study in the female cohort, especially in the female controls. Another limitation is, as with most observational studies, the unmeasured (unobserved) factors, such as the stress of raising a child with FXS, could be confounding. Also, as the SCL-90-R is a self -report measure, differential disclosure of psychological symptoms between genders cannot be discounted. Furthermore, while psychotropic medication is unlikely to reduce hippocampal volume, it was associated with psychological symptoms in this study (logically, if you are taking a psychological medication, you most likely have a psychological symptom). Though our analysis accounted for age, being the main confounder of brain volumetric and psychological symptom change, we were unable to stratify further by psychotropic medication (within diagnostic groups). Therefore, some of the psychological symptoms reported could have been dampened or improved by medication. However, we believe that these limiting factors cannot account for all of our results, as the evidence for hippocampal involvement in the fragile X premutation is continuing to mount. Larger studies will shed further light upon the results presented here.

REFERENCES

Adams JS, Adams PE, Nguyen D, Brunberg JA, Tassone F, Zhang W, Koldewyn K, Rivera SM, Grigsby J, Zhang L, et al. 2007. Volumetric brain changes in females with fragile X-associated tremor/ataxia syndrome (FXTAS). Neurology 69(9):851–859.

Allingham-Hawkins DJ, Babul-Hirji R, Chitayat D, Holden JJA, Yang KT, Lee C, Hudson R, Gorwill RH, Nolin SL, Glicksman A, et al. 1999. Fragile X premutation is a significant risk factor for premature ovarian failure: The international collaborative POF in fragile X study— Preliminary data. Am J Med Genet 83:322–325.

Arocena DG, Iwahashi CK, Won N, Beilina A, Ludwig AL, Tassone F, Schwartz PH, Hagerman PJ. 2005. Induction of inclusion formation and disruption of lamin A/C structure by premutation CGG-repeat RNA in human cultured neural cells. Hum Mol Genet 14(23):3661–3671.

Bacalman S, Farzin F, Bourgeois JA, Cogswell J, Goodlin-Jones BL, Gane LW, Grigsby J, Leehey MA, Tassone F, Hagerman RJ. 2006. Psychiatric phenotype of the fragile X-associated tremor/ataxia syndrome (FXTAS) in males: Newly described fronto-subcortical dementia. J Clin Psychiatry 67(1):87–94.

Bannerman DM, Grubb M, Deacon RM, Yee BK, Feldon J, Rawlins JN. 2003. Ventral hippocampal lesions affect anxiety but not spatial learning. Behav Brain Res 139(1–2):197–213.

Bannerman DM, Rawlins JN, McHugh SB, Deacon RM, Yee BK, Bast T, Zhang WN, Pothuizen HH, Feldon J. 2004. Regional dissociations within the hippocampus–memory and anxiety. Neurosci Biobehav Rev 28(3): 273–283.

Berry-Kravis E, Potanos K, Weinberg D, Zhou L, Goetz CG. 2005. Fragile X-associated tremor/ataxia syndrome in sisters related to X-inactivation. Ann Neurol 57(1):144–147.

Berry-Kravis E, Abrams L, Coffey SM, Hall DA, Greco C, Gane LW, Grigsby J, Bourgeois JA, Finucane B, Jacquemont S, et al. 2007. Fragile X-associated tremor/ataxia syndrome: Clinical features, genetics, and testing guidelines. Mov Disord 22(14):2018–2030.

Beyer JL, Krishnan KR. 2002. Volumetric brain imaging findings in mood disorders. Bipolar Disord 4(2):89–104.

Bourgeois JA, Farzin F, Brunberg JA, Tassone F, Hagerman P, Zhang L, Hessl D, Hagerman R. 2006. Dementia with mood symptoms in a fragile X premutation carrier with the fragile X-associated tremor/ataxia syndrome: Clinical intervention with donepezil and venlafaxine. J Neuropsychiatry Clin Neurosci 18(2):171–177.

Bourgeois JA, Coffey SM, Rivera SM, Hessl D, Gane LW, Tassone F, Greco C, Finucane B, Nelson L, Berry-Kravis E, et al. 2009. A review of fragile X premutation disorders: Expanding the psychiatric perspective. J Clin Psychiatry 70(6):852–862.

Bremner JD, Narayan M, Anderson ER, Staib LH, Miller HL, Charney DS. 2000. Hippocampal volume reduction in major depression. Am J Psychiatry 157(1):115–118.

Brouwer JR, Severijnen E, de Jong FH, Hessl D, Hagerman RJ, Oostra BA, Willemsen R. 2008. Altered hypothalamus-pituitary-adrenal gland axis regulation in the expanded CGG-repeat mouse model for fragile X-associated tremor/ataxia syndrome. Psychoneuroendocrinology 33(6):863–873.

Brunberg JA, Jacquemont S, Hagerman RJ, Berry-Kravis E, Grigsby J, Leehey M, Tassone F, Brown WT, Greco C, Hagerman PJ. 2002. Fragile X premutation carriers: Characteristic MR imaging findings in adult males with progressive cerebellar and cognitive dysfunction. Am J Neuroradiol 23(10):1757–1766.

Coffey SM, Cook K, Tartaglia N, Tassone F, Nguyen DV, Pan R, Bronsky HE, Yuhas J, Borodyanskaya M, Grigsby J, et al. 2008. Expanded clinical phenotype of women with the FMR1 premutation. Am J Med Genet Part A 146A(8):1009–1016.

Cohen S, Masyn K, Adams J, Hessl D, Rivera S, Tassone F, Brunberg J, DeCarli C, Zhang L, Cogswell J, et al. 2006. Molecular and imaging correlates of the fragile X-associated tremor/ataxia syndrome. Neurology 67(8):1426–1431.

Cornish KM, Kogan C, Turk J, Manly T, James N, Mills A, Dalton A. 2005. The emerging fragile X premutation phenotype: Evidence from the domain of social cognition. Brain Cogn 57(1):53–60.

Derogatis LR. 1994. Symptom Checklist-90-R (SCL-90-R): Administration, Scoring, and Procedures Manual. Minneapolis: National Computer Systems.

Franke P, Leboyer M, Gansicke M, Weiffenbach O, Biancalana V, Cornillet-Lefebre P, Croquette MF, Froster U, Schwab SG, Poustka F, et al. 1998. Genotype-phenotype relationship in female carriers of the premutation and full mutation of FMR-1. Psychiatry Res 80(2):113–127.

Gray JA. 1982. The neuropsychology of anxiety. Oxford: Oxford University Press.

Gray JA, McNaughton N. 2000. The neuropsychology of anxiety. Oxford: Oxford University Press.

Greco CM, Hagerman RJ, Tassone F, Chudley A, Del Bigio MR, Jacquemont S, Leehey M, Hagerman PJ. 2002. Neuronal intranuclear inclusions in a new cerebellar tremor/ataxia syndrome among fragile X carriers. Brain 125(8):1760–1771.

Greco CM, Berman RF, Martin RM, Tassone F, Schwartz PH, Chang A, Trapp BD, Iwahashi C, Brunberg J, Grigsby J, et al. 2006. Neuropathology of fragile X-associated tremor/ataxia syndrome (FXTAS). Brain 129(Pt1):243–255.

Grigsby J, Brega AG, Jacquemont S, Loesch DZ, Leehey MA, Goodrich GK, Hagerman RJ, Epstein J, Wilson R, Cogswell JB, et al. 2006a. Impairment in the cognitive functioning of men with fragile X-associated tremor/ataxia syndrome (FXTAS). J Neurol Sci 248(1–2):227–233.

Grigsby J, Leehey MA, Jacquemont S, Brunberg JA, Hagerman RJ, Wilson R, Epstein JH, Greco CM, Tassone F, Hagerman PJ. 2006b. Cognitive impairment in a 65-year-old male with the fragile X-associated tremor-ataxia syndrome (FXTAS). Cogn Behav Neurol 19(3):165–171.

Hagerman PJ. 2008. The fragile X prevalence paradox. J Med Genet 45(8): 498–499.

Hagerman PJ, Hagerman RJ. 2004. The fragile-X premutation: A maturing perspective. Am J Hum Genet 74(5):805–816.

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

Hagerman RJ, Leavitt BR, Farzin F, Jacquemont S, Greco CM, Brunberg JA, Tassone F, Hessl D, Harris SW, Zhang L, et al. 2004. Fragile-X-associated tremor/ataxia syndrome (FXTAS) in females with the *FMR1* premutation. Am J Hum Genet 74(5):1051–1056.

Hagerman RJ, Coffey SM, Maselli R, Soontarapornchai K, Brunberg JA, Leehey MA, Zhang L, Gane LW, Fenton-Farrell G, Tassone F, et al. 2007. Neuropathy as a presenting feature in fragile X-associated tremor/ataxia syndrome. Am J Med Genet Part A 143A(19):2256–2260.

Hagerman RJ, Hall DA, Coffey S, Leehey M, Bourgeois J, Gould J, Zhang L, Seritan A, Berry-Kravis E, Olichney J, et al. 2008. Treatment of fragile X-associated tremor ataxia syndrome (FXTAS) and related neurological problems. Clin Interv Aging 3(2):251–262.

Hessl D, Tassone F, Loesch DZ, Berry-Kravis E, Leehey MA, Gane LW, Barbato I, Rice C, Gould E, Hall DA, et al. 2005. Abnormal elevation of FMR1 mRNA is associated with psychological symptoms in individuals with the fragile X premutation. Am J Med Genet Part B 139B(1):115–121.

Hessl D, Rivera S, Koldewyn K, Cordeiro L, Adams J, Tassone F, Hagerman PJ, Hagerman RJ. 2007. Amygdala dysfunction in men with the fragile X premutation. Brain 130(Pt2):404–416.

Jacobs BL, Praag H, Gage FH. 2000. Adult brain neurogenesis and psychiatry: A novel theory of depression. Mol Psychiatry 5(3):262–269.

Jacquemont S, Hagerman RJ, Leehey M, Grigsby J, Zhang L, Brunberg JA, Greco C, Des Portes V, Jardini T, Levine R, et al. 2003. Fragile X premutation tremor/ataxia syndrome: Molecular, clinical, and neuroimaging correlates. Am J Hum Genet 72(4):869–878.

Jacquemont S, Orrico A, Galli L, Sahota PK, Brunberg JA, Anichini C, Leehey M, Schaeffer S, Hagerman RJ, Hagerman PJ, et al. 2005. Spastic paraparesis, cerebellar ataxia, and intention tremor: A severe variant of FXTAS? J Med Genet 42(2):e14.

Jacquemont S, Hagerman RJ, Hagerman PJ, Leehey MA. 2007. Fragile-X syndrome and fragile X-associated tremor/ataxia syndrome: Two faces of FMR1. Lancet Neurol 6(1):45–55.

Johnston C, Eliez S, Dyer-Friedman J, Hessl D, Glaser B, Blasey C, Taylor A, Reiss A. 2001. Neurobehavioral phenotype in carriers of the fragile X premutation. Am J Med Genet 103(4):314–319.

Johnston C, Hessl D, Blasey C, Eliez S, Erba H, Dyer-Friedman J, Glaser B, Reiss AL. 2003. Factors associated with parenting stress in mothers of children with fragile X syndrome. J Dev Behav Pediatr 24(4):267–275.

Koldewyn K, Hessl D, Adams J, Tassone F, Hagerman PJ, Hagerman RJ, Rivera SM. 2008. Reduced hippocampal activation during recall is associated with elevated FMR1 mRNA and psychiatric symptoms in men with the fragile X premutation. Brain Imaging Behav 2(2):105–116.

Leehey MA, Berry-Kravis E, Min SJ, Hall DA, Rice CD, Zhang L, Grigsby J, Greco CM, Reynolds A, Lara R, et al. 2007. Progression of tremor and ataxia in male carriers of the FMR1 premutation. Mov Disord 22(2): 203–206.

Leehey MA, Berry-Kravis E, Goetz CG, Zhang L, Hall DA, Li L, Rice CD, Lara R, Cogswell J, Reynolds A, et al. 2008. FMR1 CGG repeat length predicts motor dysfunction in premutation carriers. Neurology 70(16Pt 2):1397–1402.

Loesch DZ, Bui MQ, Grigsby J, Butler E, Epstein J, Huggins RM, Taylor A, Hagerman RJ. 2003. Effect of the fragile X status categories and the FMRP levels on executive functioning in fragile X males and females. Neuropsychology 17(4):646–657.

Loesch DZ, Churchyard A, Brotchie P, Marot M, Tassone F. 2005a. Evidence for, and a spectrum of, neurological involvement in carriers of the fragile X pre-mutation: FXTAS and beyond. Clin Genet 67(5): 412–417.

Loesch DZ, Litewka L, Brotchie P, Huggins RM, Tassone F, Cook M. 2005b. Magnetic resonance imaging study in older fragile X premutation male carriers. Ann Neurol 58(2):326–330.

McNaughton N, Gray JA. 2000. Anxiolytic action on the behavioural inhibition system implies multiple types of arousal contribute to anxiety. J Affect Disord 61(3):161–176.

Mervaala E, Fohr J, Kononen M, Valkonen-Korhonen M, Vainio P, Partanen K, Partanen J, Tiihonen J, Viinamaki H, Karjalainen AK, et al. 2000. Quantitative MRI of the hippocampus and amygdala in severe depression. Psychol Med 30(1):117–125.

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

Moser E, Moser MB, Andersen P. 1993. Spatial learning impairment parallels the magnitude of dorsal hippocampal lesions, but is hardly present following ventral lesions. J Neurosci 13(9):3916–3925.

Murray A, Ennis S, MacSwiney F, Webb J, Morton NE. 2000. Reproductive and menstrual history of females with fragile X expansions. Eur J Hum Genet 8(4):247–252.

O'Dwyer JP, Clabby C, Crown J, Barton DE, Hutchinson M. 2005. Fragile X-associated tremor/ataxia syndrome presenting in a woman after chemotherapy. Neurology 65(2):331–332.

Pesso R, Berkenstadt M, Cuckle H, Gak E, Peleg L, Frydman M, Barkai G. 2000. Screening for fragile X syndrome in women of reproductive age. Prenat Diagn 20(8):611–614.

Roberts JE, Bailey DB Jr, Mankowski J, Ford A, Sideris J, Weisenfeld LA, Heath TM, Golden RN. 2009. Mood and anxiety disorders in females with the FMR1 premutation. Am J Med Genet Part B 150B(1):130–139.

Rodriguez-Revenga L, Madrigal I, Pagonabarraga J, Xuncla M, Badenas C, Kulisevsky J, Gomez B, Mila M. 2009. Penetrance of FMR1 premutation associated pathologies in fragile X syndrome families. Eur J Hum Genet 17(10):1359–1362.

Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub N, Lee J, Duman R, Arancio O, et al. 2003. Requirement of hippocampal

neurogenesis for the behavioral effects of antidepressants. Science 301(5634):805–809.

Sapolsky RM. 2000. Glucocorticords and hippocampal atrophy in neuropsychiatric disorders. Arch Gen Psychiatry 57:925–935.

Skuse D. 2006. Genetic influences on the neural basis of social cognition. Philos Trans R Soc Lond B Biol Sci 361(1476):2129–2141.

Smith ME. 2005. Bilateral hippocampal volume reduction in adults with post-traumatic stress disorder: A meta-analysis of structural MRI studies. Hippocampus 15(6):798–807.

Sobesky WE, Taylor AK, Pennington BF, Bennetto L, Porter D, Riddle J, Hagerman RJ. 1996. Molecular-clinical correlations in females with fragile X. Am J Med Genet 64(2):340–345.

Sullivan AK, Marcus M, Epstein MP, Allen EG, Anido AE, Paquin JJ, Yadav-Shah M, Sherman SL. 2005. Association of FMR1 repeat size with ovarian dysfunction. Hum Reprod 20(2):402–412.

Tassone F, Hagerman RJ, Iklé DN, Dyer PN, Lampe M, Willemsen R, Oostra BA, Taylor AK. 1999. FMRP expression as a potential prognostic indicator in fragile X syndrome. Am J Med Genet 84(3):250–261.

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

Tassone F, Hagerman RJ, Garcia-Arocena D, Khandjian EW, Greco CM, Hagerman PJ. 2004. Intranuclear inclusions in neural cells with premutation alleles in fragile X associated tremor/ataxia syndrome. J Med Genet 41(4):e43.

Tassone F, Adams J, Berry-Kravis EM, Cohen SS, Brusco A, Leehey MA, Li L, Hagerman RJ, Hagerman PJ. 2007a. CGG repeat length correlates with age of onset of motor signs of the fragile X-associated tremor/ataxia syndrome (FXTAS). Am J Med Genet Part B 144B(4):566–569.

Tassone F, Beilina A, Carosi C, Albertosi S, Bagni C, Li L, Glover K, Bentley D, Hagerman PJ. 2007b. Elevated FMR1 mRNA in premutation carriers is due to increased transcription. RNA 13(4):555–562.

Tassone F, Pan R, Amiri K, Taylor AK, Hagerman PJ. 2008. 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. J Mol Diagn 10(1):43–49.

Wechsler D. 1997. Wechsler Adult Intelligence Scale—Third Edition: Administration and Scoring Manual. San Antonio: Harcourt Assessment, Inc.

Zuhlke C, Budnik A, Gehlken U, Dalski A, Purmann S, Naumann M, Schmidt M, Burk K, Schwinger E. 2004. FMR1 premutation as a rare cause of late onset ataxia—Evidence for FXTAS in female carriers. J Neurol 251(11):1418–1419.