Volumetric brain changes in females with fragile X-associated tremor/ataxia syndrome (FXTAS)

J.S. Adams, BA
P.E. Adams, BS
D. Nguyen, PhD
J.A. Brunberg, MD
F. Tassone, PhD
W. Zhang, MD
K. Koldewyn, BA
S.M. Rivera, PhD
J. Grigsby, PhD
L. Zhang, MD, PhD
C. DeCarli, MD
P.J. Hagerman, MD, PhD
R.J. Hagerman, MD

Address correspondence and reprint requests to Dr. Randi Hagerman, M.I.N.D. Institute, University of California–Davis Medical Center, 2825 50th Street, Sacramento, CA 95817
randi.hagerman@ucdmc ucdavis.edu

ABSTRACT

Background: Fragile X-associated tremor/ataxia syndrome (FXTAS) is a late-onset neurodegenerative disorder occurring in male and rare female carriers of a premutation expansion (55 to 200 CGG repeats) of the fragile X mental retardation 1 (*FMR1*) gene.

Methods: Volumetric MRI studies, clinical staging, cognitive testing, and molecular analysis were conducted in 15 female premutation carriers affected by FXTAS (age 59.5 ± 10.3 years), 20 unaffected female carriers (43.3 ± 11.2 years), 11 genetically normal female controls (51.0 ± 10.3 years), 36 affected male carriers (65.0 ± 5.6 years), 25 unaffected male carriers (53.5 ± 12.5 years), and 39 male controls (58.0 ± 15.0 years). Female and male carriers with FXTAS were matched on duration of disease.

Results: We found less pronounced reductions of cerebellar volume and a lower incidence of involvement (symmetric high T2 signal) of the middle cerebellar peduncles (MCP sign) in females affected by FXTAS (13%) compared with affected males (58%). We found reduced brain volumes and increased white matter disease associated with the presence of FXTAS in females compared with female controls. We also observed significant associations between reduced cerebellar volume and both increased severity of FXTAS symptoms and increased length of the CGG repeat expansion in male premutation carriers, but not in females.

Conclusions: Females affected by fragile X-associated tremor/ataxia syndrome (FXTAS) demonstrated milder brain changes than affected males, although they showed a similar pattern of radiologic findings consistent with brain atrophy and white matter disease. FXTAS should be considered (by ordering fragile X DNA testing) in females who present with late-onset ataxia, action tremor, or neuropathy, particularly in those with a family history of mental retardation, autism, or premature ovarian failure. ***Neurology*® 2007;69:851–859**

Trinucleotide (CGG) repeat expansions in the 5′ untranslated region of the fragile X mental retardation 1 (*FMR1*) gene have recently been associated with clinical presentations other than fragile X syndrome, a common cause of mental retardation and autism. Individuals with CGG expansions in the premutation range (55 to 200 repeats), previously thought to be clinically unaffected, are now known to sometimes display forms of clinical involvement, including neurocognitive deficits,[1-4] emotional problems,[5-7] premature ovarian failure (POF),[8,9] and the fragile X-associated tremor/ataxia syndrome (FXTAS), a late-onset neurodegenerative disorder.[10-13]

Core features of FXTAS include intention tremor and gait ataxia, typically with onset after age 50 years.[10,11] Other features include memory and executive function deficits that may progress to cognitive impairment and dementia[3,4,14]; peripheral neuropathy[11]; and MRI findings of brain atrophy, often with subcortical and periventricular white matter

From the M.I.N.D. Institute (J.S.A., P.E.A., K.K., S.M.R., P.J.H., R.J.H.) and the Department of Pediatrics (J.S.A., R.J.H.), Public Health Sciences, Division of Biostatistics (D.N.), Radiology (J.A.B.), Biochemistry and Molecular Medicine (F.T., W.Z., P.J.H.), Neuroscience (K.K.), Psychology (S.M.R.), and Neurology (C.D., L.Z.), University of California, Davis, CA; and the Department of Medicine, University of Colorado at Denver and Health Sciences Center, Denver, CO (J.G.).

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disease, including white matter disease in the middle cerebellar peduncles (the MCP sign, present in approximately 59% of affected males).[15-18] Neuropathologic studies of FXTAS have documented intranuclear inclusions in neurons and astroglial cells throughout the cerebrum and brainstem.[19,20] Although FXTAS affects approximately 40% of male carriers older than 50 years in known fragile X families, it is thought to be rare in females, with only a few case studies reported.[21-25] The second X chromosome in females may be protective for involvement with FXTAS.[23,24]

Neither POF nor FXTAS is seen in individuals with fragile X syndrome, who have CGG expansions in the full mutation range (>200 repeats). In contrast to fragile X syndrome, which involves silencing of the *FMR1* gene, premutation carriers have normal or slightly reduced levels of the *FMR1* protein product (FMRP), but dramatically (twofold to eightfold) elevated levels of *FMR1* messenger RNA (mRNA).[26] It is hypothesized that the presence of elevated levels of expanded-repeat *FMR1* mRNA has a toxic "gain-of-function" effect,[27] akin to the pathogenic mechanism associated with myotonic dystrophy.[28] The presence of a pathogenic mechanism involving the premutation allele is of great importance given the high prevalence of the allele in the general population (1 in 813 males and 1 in 259 females).[29,30] Further studies of the effects of the premutation allele in both male and female carriers are therefore warranted.

METHODS

Subjects. Patients and controls were recruited through families with known fragile X syndrome or FXTAS in family members via a comprehensive cascade testing mechanism. Two additional male controls and one additional female control were recruited to match the age and education level of our affected premutation samples. 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 35 female premutation carriers (15 affected by FXTAS and 20 unaffected), 61 male premutation carriers (36 affected by FXTAS and 25 unaffected), and 11 female and 39 male controls (normal-range CGG repeats). Of the male premutation carriers, 59% (n = 36) were previously reported.[18] The main comparisons of interest were between diagnostic groups of females (affected vs unaffected carriers, affected vs normal CGG repeat controls), and between females and males affected by FXTAS. There was no difference in age between the affected female and affected male groups ($p = 0.12$), nor was there a difference in the time between the onset of symptoms related to FXTAS and the date of neuroimaging ($p = 0.56$). Unaffected female carriers (43.2 ± 11.2 years) were younger than affected females (59.5 ± 10.3 years; $p < 0.01$; table 1). This age difference is likely due to an increased incidence of FXTAS in aging premutation carriers, particularly those older than 50 years, which would diminish the pool of unaffected premutation carriers in this age range. Thus, in subsequent analyses we adjusted for the effect of age in regression analysis and analysis of covariance (ANCOVA). Among female premutation carriers, there was no difference in activation ratio between those with FXTAS and unaffected groups ($p = 0.54$). Among all premutation carriers, there was no difference in CGG repeat length between sexes or diagnostic groups (FXTAS or clinically uninvolved). There was also no significant difference in education level between any of our groups (table 1).

Neuroimaging. Structural magnetic resonance images were acquired using a 1.5-T GE Signa Horizon LX Echospeed system. The acquisition parameters were as follows:

Coronal three-dimensional spoiled gradient recalled echo (inversion recovery–prepped spoiled gradient recalled echo) acquisition, T1 weighted: coronal plane, three-dimensional acquisition, gradient recalled echo, radiofrequency spoiled, repetition time 9.1 msec, spatial resolution 0.9375 × 0.9375 × 1.5 mm thickness.

High-resolution fluid-attenuated inversion recovery (same orientation as axial spin echo): oblique axial plane, two-dimensional acquisition, inversion recovery spin echo, echo time 144 msec, repetition time 11,000 msec, inversion time 2,250 msec, 14 slices/acquisition, two interleaved acquisitions, resolution 0.9375 × 0.9375 × 3 mm thickness, 0-mm interslice on reconstructed image.

MRI quantification was performed using a custom-written computer program operating on a UNIX, Solaris platform (Quanta 6.1), as previously described.[18] All volumes were normalized to total cranial volume (TCV).

The TCV was segmented into CSF, brain matter, and white matter increased T2 signal intensity volume according to previously published methods,[31-34] and then further subdivided into regions of interest, including cerebrum, cerebellum, lateral ventricles, and third ventricle. Lateral and third ventricular volumes were summed for a total ventricular region of interest. Hippocampal volumes were quantified by operator-guided tracing as described previously.[33]

MRIs were reviewed with a neuroradiologist (J.A.B.), and the MCP sign was scored as present or absent.

Clinical evaluation. The diagnosis of FXTAS was made after a thorough medical, neurologic, and radiologic examination.[35] Patients met criteria for probable or definite FXTAS, as previously described.[15] After examination, a FXTAS clinical staging score was given to each patient with the premutation. This 7-point staging scale, as previously reported,[3,14] measures functional impairment, as follows: 0 = normal functioning; 1 = subtle or questionable tremor or balance problems with no interference in activities of daily living (ADLs); 2 = minor but clear tremor or balance problems producing minor interference with ADLs; 3 = moder-

Table 1 Characteristics of 146 study subjects

		Male (total n = 100)		Female (total n = 46)		
Characteristic		n	Mean ± SD	n	Mean ± SD	*p* Value
Age, y	Control	39	58.0 ± 15.0 (31–88)*	11	51.0 ± 10.3 (35–65)	0.08
	Unaffected	25	53.5 ± 12.5 (34–78)	20	43.2 ± 11.2 (30–71)	<0.01
	Affected	36	65.0 ± 5.6 (53–73)	15	59.5 ± 10.3 (42–74)	0.12
Education, y	Control	37	16.2 ± 3.2	3	16.0 ± 2.6	0.93
	Unaffected	24	16.3 ± 2.5	17	15.1 ± 3.8	0.20
	Affected	36	16.0 ± 3.0	14	15.1 ± 3.0	0.36
Age at symptom† onset, y	Affected	35	58.0 ± 6.6	15	51.0 ± 14.8	0.09
Years since symptom onset	Affected	35	6.8 ± 4.5	15	8.9 ± 11.0	0.56
CGG repeat	Control	32	29.4 ± 5.1	10	31.6 ± 6.1	0.74
	Unaffected	24	86.2 ± 30.4	18	93.3 ± 17.6	0.06
	Affected	35	93.7 ± 18.7	15	91.9 ± 12.4	0.80
FMR1 mRNA	Control	24	1.4 ± 0.4	9	1.4 ± 0.2	0.88
	Unaffected	22	3.3 ± 1.8	16	2.4 ± 0.7	0.01
	Affected	30	3.4 ± 0.9	14	2.8 ± 1.2	0.08
Activation ratio	Unaffected			18	0.56 ± 0.20	0.54‡
	Affected			15	0.58 ± 0.07	
IQ–verbal	Control	35	116.1 ± 17.0	7	111.6 ± 12.6	0.47
	Unaffected	25	115.8 ± 16.6	20	106.5 ± 12.7	0.04
	Affected	35	106.0 ± 15.2	15	104.3 ± 11.1	0.72
IQ–performance	Control	36	116.4 ± 14.5	7	115.6 ± 16.5	0.89
	Unaffected	24	115.5 ± 14.7	20	111.4 ± 11.5	0.34
	Affected	34	100.0 ± 15.3	15	103.1 ± 10.9	0.45

* Age range (min–max).
† Symptoms include intention tremor and ataxia.
‡ Comparison of the activation ratios of affected and unaffected female premutation carriers.
mRNA = messenger RNA.

ate tremor or balance problems with at least occasional falls and significant interference in ADLs; 4 = severe tremor or balance problems requiring the use of a cane or walker; 5 = use of a wheelchair on a daily basis; 6 = bedridden.

The Wechsler Adult Intelligence Scale III,[36] providing a measure of verbal IQ and performance IQ, was administered to subjects.

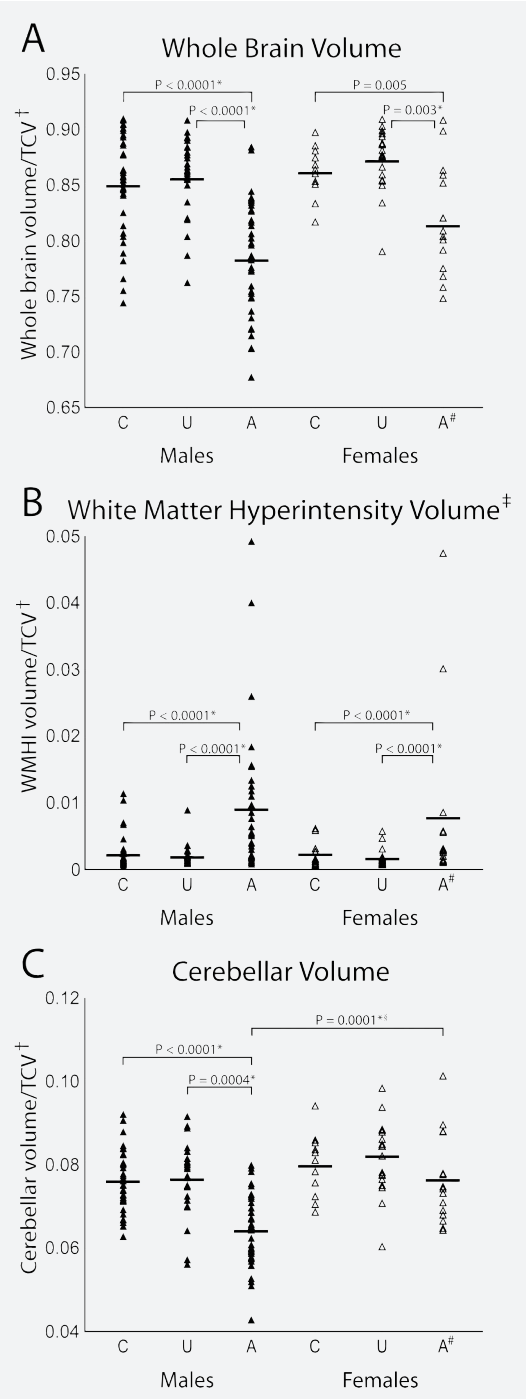
Molecular analysis. ***DNA analysis.*** Genomic DNA was isolated from peripheral blood leukocytes (5 mL of whole blood using standard methods (Puregene Kit; Gentra Inc.). Southern blot and PCR analyses were performed as previously described.[37,38] Analysis and calculation of the repeat size (for both Southern blot and PCR analysis), methylation status, and activation ratio were conducted by using an Alpha Innotech FluorChem 8800 Image Detection System. 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.[39]

FMR1 mRNA levels. All quantifications of *FMR1* mRNA were performed using 7700 and 7900 Sequence Detectors (PE Biosystems), as previously described.[26]

Statistical analysis. Univariate comparisons of patient characteristics between sex and diagnostic groups (control, unaffected, and affected with FXTAS) were performed with one-way analysis of variance for continuous variables and the Fisher exact test for the categorical variable MCP sign. The primary comparisons of MRI volumetric structures among sex and diagnostic groups were performed using ANCOVA, adjusting for age. Among those affected with FXTAS, we used ANCOVA adjusting for the year of symptom onset as well as age. Regression analyses with the independent variables, CGG repeat size, mRNA level, and age, were used to examine relationships with MRI volumetric structures and IQ scores in premutation carriers. Association/correlation between FXTAS score and MRI volumetric structures and IQ measures among patients with FXTAS were based on the (partial) Pearson correlation, adjusted for age.

Reported *p* values are two-sided and unadjusted. Significant *p* values (at level 0.05) after adjusting for multiple comparisons are marked by an asterisk (*) throughout the text. *P* value adjustments were made only for primary comparisons between male and female premutation carriers and among females. Comparisons among males in all analyses were considered secondary, because previous studies with male sub-

Figure 1 Comparison of whole brain (A), whole brain white matter increased T2 signal intensity (B), and cerebellar volumes between male and female controls, unaffected premutation carriers, and premutation carriers affected by FXTAS



Closed triangles represent males, and open triangles represent females. Unless otherwise noted, all *p* values are for comparisons of predicted means at age 70 years (fitted analysis of covariance model). We found significant differences within both sex groups between patients with fragile X-associated tremor/ataxia syndrome (FXTAS) and both controls and unaffected premutation carriers in whole brain volume and whole brain white matter increased T2 signal intensity volume, as well as within the male sex group in cerebellar volume. However, we did not find a significant reduction of cerebellar volume in females affected by FXTAS, as compared with controls or unaffected premutation carriers. Males and females with FXTAS differed in cerebellar volume ($p = 0.0001$*). † Total cranial volume. # Graphs depict brain region volumes of males and females, each divided into three groups: control subjects (C), unaffected premutation carriers (U), and premutation carriers affected by FXTAS (A). ‡ White matter hyperintensity (WMHI) volume indicates whole brain white matter increased T2 signal intensity volume. * *p* value retains significance after correction for multiple comparisons. § *p* value is for the comparison of mean volumes.

jects have been reported[18] and are not of direct (primary) interest in this study. *P* value adjustment was performed using the Sidak step-down method.

Because age is significantly associated with volumetric structures among diagnostic and sex groups, it was necessary to compare volumetric structures among these groups at specific ages in the ANCOVA model. Thus, we chose to examine volumetric differences among groups at ages 50, 60, and 70 years, covering a broad range of ages for the FXTAS patients.

All volumetric structures were normalized by total cranial volume before statistical analyses. All tests were performed at a significance level of 0.05. Statistical analyses were performed with the use of SAS software, version 9.1 (SAS Institute).

RESULTS **Sex differences in radiologic findings.** The incidence of a positive MCP sign was lower in females affected by FXTAS than in affected males, 13.3% (2/15) among affected females compared with 58.3% (21/36) among affected males ($p = 0.005$). In a direct comparison of brain volumes (normalized to TCV) between affected females and affected males, adjusting for age, we found greater cerebellar volume ($p = 0.0001$*) in the affected females (figure 1). We confirmed this finding with a subset model of these affected males and females (n = 50), with an additional adjustment for the time since symptom onset, finding an effect of sex on cerebellar volume ($p = 0.001$*).

Pairwise comparisons between individuals affected by FXTAS, unaffected (premutation) carriers, and controls were conducted for both males and females using the fitted ANCOVA model at ages 50, 60, and 70 years (n = 146). We found significantly smaller whole brain volumes and increased volume of white matter increased T2 signal intensity in affected females compared with both unaffected female carriers and controls (table 2 and figure 1). These differences were significant at age 70 years. In males, we found significantly smaller whole brain and cerebellar volumes, larger ventricular volume, and a greater volume of whole brain white matter increased T2 signal intensity in affected carriers when compared with both unaffected carriers and controls

Table 2 Volumes of several brain regions in 46 female study subjects

Brain structure[†]	Comparison group	Age, y	Difference[‡]	*p* Value[§]
Whole brain	Affected–control	50	−0.587 ± 1.398	0.6755
		60	−2.524 ± 1.297	0.0537
		70	−4.462 ± 1.552	0.0047
	Affected–unaffected	50	−0.202 ± 1.271	0.874
		60	−2.640 ± 1.283	0.0415
		70	−5.079 ± 1.671	0.0028*
White matter hyperintensity[‖]	Affected–control	50	0.155 ± 0.273	0.5708
		60	0.535 ± 0.253	0.0365
		70	1.226 ± 0.303	<0.0001*
	Affected–unaffected	50	0.111 ± 0.248	0.6557
		60	0.604 ± 0.251	0.0172
		70	1.319 ± 0.326	<0.0001*

Plus–minus values are estimated difference ± SE.
[†] All brain volumes were expressed as a percentage of total cranial volume in our analysis to account for differences in head size.
[‡] Differences were estimated based on a fitted analysis of covariance model at three different ages (50, 60, and 70 years).
[§] All reported *p* values are raw values taken before correction for multiple comparisons. *P* values that retained significance after the correction are marked with an asterisk (*).
[‖] Indicates whole brain white matter increased T2 signal intensity.

(table 3 and figure 1). These differences were generally significant at older age levels (e.g., at ages 60 and 70 years). The differences in whole brain, cerebellar, and ventricular volume between affected males and male controls were also evident at the youngest age level (age 50 years). In both males and females, no differences in hippocampal volume were found between the affected group and either the unaffected group or the control group. When comparing unaffected premutation carriers with controls, no difference was found in any measured volumetric parameter in males or females.

Correlation of clinical staging and quantitative findings. Among affected males (n = 36), we found an association between more advanced clinical stage (using the FXTAS clinical staging scale) and reduced cerebellar volume ($p = 0.005$*; figure 2), reduced hippocampal volume ($p = 0.009$*), increased ventricular volume ($p = 0.007$*), decreased performance IQ ($p = 0.002$*), and decreased verbal IQ ($p = 0.004$*). No significant association was found between clinical stage and either regional brain volumes or IQ scores in affected female premutation carriers (n = 15).

Correlation of molecular measures of *FMR1* and radiologic findings. Regression analyses, with CGG repeat number, *FMR1* mRNA level, and age as independent variables, were used to examine the relationship of molecular measures to brain volumes in male premutation carriers (n = 51). Similar analyses were used for female premutation carriers (n = 29), with the addition of activation ratio as a covariate.

CGG repeat length was associated with reduced cerebellar volume ($p = 0.021$; figure 2) and increased ventricular volume ($p = 0.047$) in male carriers, but not in female carriers ($p = 0.82$ for cerebellar volume; $p = 0.69$ for ventricular volume). Elevated levels of *FMR1* mRNA were associated with reduced hippocampal volume in both male ($p = 0.0035$) and female carriers ($p = 0.041$). However, these associations dropped out of significance after adjusting *p* values for multiple testing.

DISCUSSION These results demonstrate milder radiologic findings in females affected by FXTAS as compared with males. We document a significantly lower incidence of the MCP sign in females affected by FXTAS (13.3%) compared with affected males (58.3%). In a direct comparison of radiologic findings in females and males affected by FXTAS, we report less pronounced cerebellar volume loss in females after correcting for both age and time since symptom onset. It is likely that females are buffered from neurodegeneration associated with the premutation allele by a diluting effect of the second, normal X chromosome. However, we do not see significant sex differ-

Table 3 Volumes of several brain regions in 100 male study subjects

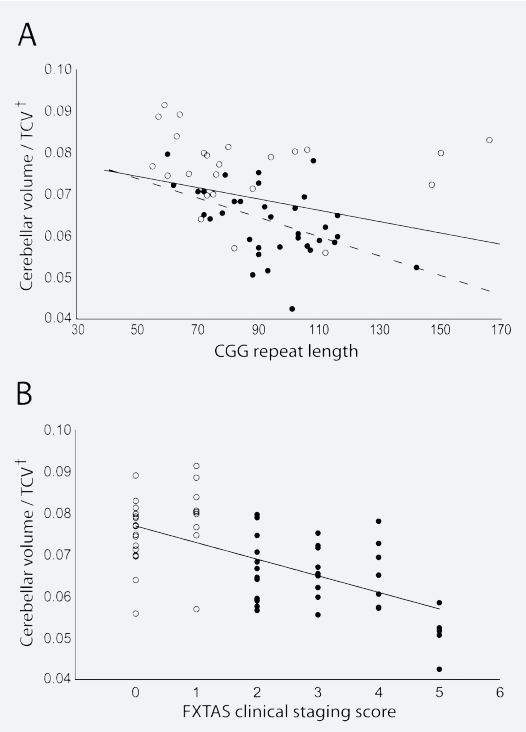
Brain structure[†]	Comparison group	Age, y	Difference[‡]	*p* Value[§]
Whole brain	Affected–control	50	−2.237 ± 1.221	0.069
		60	−4.175 ± 0.802	<0.0001*
		70	−6.113 ± 0.890	<0.0001*
	Affected–unaffected	50	−1.744 ± 1.260	0.1686
		60	−4.183 ± 0.924	<0.0001*
		70	−6.622 ± 1.111	<0.0001*
Cerebellum	Affected–control	50	−0.846 ± 0.313	0.0077
		60	−0.955 ± 0.205	<0.0001*
		70	−1.065 ± 0.228	<0.0001*
	Affected–unaffected	50	−0.845 ± 0.323	0.0099
		60	−0.941 ± 0.237	0.0001*
		70	−1.038 ± 0.285	0.0004*
Ventricular CSF	Affected–control	50	0.541 ± 0.415	0.1951
		60	1.238 ± 0.273	<0.0001*
		70	1.936 ± 0.303	<0.0001*
	Affected–unaffected	50	0.390 ± 0.429	0.3642
		60	1.158 ± 0.314	0.0003*
		70	1.925 ± 0.378	<0.0001*
White matter hyperintensity[‖]	Affected–control	50	−0.387 ± 0.238	0.1071
		60	0.304 ± 0.157	0.0544
		70	0.994 ± 0.174	<0.0001*
	Affected–unaffected	50	−0.385 ± 0.246	0.1202
		60	0.330 ± 0.180	0.0693
		70	1.045 ± 0.217	<0.0001*

Plus–minus values are estimated difference ± SE.
[†] All brain volumes were expressed as a percentage of total cranial volume in our analysis to account for differences in head size.
[‡] Differences were estimated based on a fitted analysis of covariance model at three different ages (50, 60, and 70 years).
[§] All reported *p* values are raw values taken before correction for multiple comparisons. *P* values that retained significance after the correction are marked with an asterisk (*).
[‖] Indicates whole brain white matter increased T2 signal intensity.

ences in clinical staging due to functional or adaptive differences, indicating that this protective effect may not spare females from clinical progression once they have the disease.

Similar to the pattern of radiologic findings previously reported in males with FXTAS, we report significantly reduced brain volumes and increased white matter disease in females affected by FXTAS compared with unaffected female carriers and female controls without the premutation. However, unlike males, females with FXTAS did not demonstrate significant differences in cerebellar or ventricular volume when compared with unaffected carriers or age-matched controls, again suggesting milder or different structural alteration of the brain in females compared with males. It should be noted that given the younger age of our unaffected female carrier sample and the age-dependent penetrance of FXTAS in males,[35] it is likely that this group contains some individuals who will develop FXTAS in the future. The fact that we see significant differences between the unaffected and affected groups after correcting for the effect of age and a lack of difference between unaffected and controls suggests that the radiologic changes observed are due to the presence of FXTAS rather than a developmental effect of the premutation. It should also be noted that the increased white matter disease observed among affected females was largely driven by two individuals that had particularly pronounced white matter changes. These individuals did not have particularly high scores on the FXTAS staging scale (both had a score of 3), nor did they have the MCP sign. However, at age 74 years, they were the oldest subjects in the

Figure 2 Comparisons between cerebellar volume and CGG repeat length (A), and FXTAS score (B), in male premutation carriers



Open circles represent unaffected premutation carriers (fragile X-associated tremor/ataxia syndrome [FXTAS] clinical staging score of 0 or 1), and closed circles represent premutation carriers with an FXTAS diagnosis (FXTAS clinical staging score of 2 to 6). The solid regression line is for all premutation carriers, and the dashed line is for males with FXTAS. Statistical analysis showed a correlation between reduced cerebellar volume and both increased FXTAS clinical staging score ($p = 0.005$*) and higher CGG repeat lengths ($p = 0.021$). † Total cranial volume.

female group, suggesting that the increase in white matter disease could be a result of exposure to RNA toxicity for several additional years.

We also observed significant associations between the severity of clinical symptoms of FXTAS and brain volumes in males. This finding validates the use of the FXTAS clinical staging scale as a clinical tool by providing a quantitative structural correlate to the functional impairment measured by this scale. We do not see a significant association between clinical staging of FXTAS and brain volumes in females. This is likely related to milder radiologic involvement in females.

Previous reports have found correlations between higher CGG numbers and reduced cerebellar volume, increased ventricular volume, and increased whole brain white matter disease in males affected with FXTAS.[18] Although we saw two of these correlations in males (cerebellar and ventricular volume), they did not hold up after the correction for multiple comparisons, and we did not observe any association between CGG number and brain volumes in females. The effect of CGG repeat number may be modified in females by the activation ratio, which is also variable between blood and brain. Therefore, a correction for activation ratio obtained from blood may not be reflective of the status of the different brain regions. Additionally, the protective effect in the brain may be enhanced by having a neuron or astrocyte with the normal X active in close proximity to a cell with an active mutated X. We did not see a difference in the activation ratio between females with FXTAS and unaffected carrier females. There are likely additional factors beyond activation ratio that influence FXTAS involvement in females. With respect to *FMR1* mRNA, there are differences in mRNA levels between blood and various brain regions, with the hippocampus having the highest levels.[40] It is interesting that a correlation existed between hippocampal volume and *FMR1* mRNA levels for both males and females before correction, warranting further study with a larger number of subjects.

The lack of a clear relationship between regional brain volumes and molecular measures of the *FMR1* gene or clinical staging in females may be related to the relatively small number of women with FXTAS in our study and the variability in the molecular measures, including the activation ratio among tissues. It is also possible that female premutation carriers exhibit structural brain changes that do not significantly reduce brain volume. Further studies are necessary to elucidate the relationship between brain structure, the *FMR1* gene, and the clinical presentation of FXTAS in female premutation carriers.

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