Published in final edited form as:

Mov Disord. 2011 June ; 26(7): 1329–1336. doi:10.1002/mds.23646.

Diffusion Tensor Imaging in Male Premutation Carriers of the Fragile X Mental Retardation Gene

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Abstract

Older male premutation carriers of the *FMRI* gene are associated with the risk of developing a late-onset neurodegenerative disorder, fragile X-associated tremor/ataxia syndrome (FXTAS). Although previous postmortem and *in vivo* MRI studies have indicated white matter pathology, the regional selectivity of abnormalities, as well as their relationship with molecular variables of the *FMRI* gene, has not been investigated. In this study, we used diffusion tensor imaging (DTI) to study male premutation carriers with and without FXTAS and healthy gender-matched controls. We performed a tract of interest analysis for fractional anisotropy (FA), axial and radial diffusivities of major white matter tracts in the cerebellar-brainstem and limbic systems. Compared with healthy controls, patients with FXTAS showed significant reductions of FA in multiple white matter tracts, including the middle cerebellar peduncle (MCP), superior cerebellar peduncle, cerebral peduncle, and the fornix and stria terminalis. Significant reduction of FA in these tracts were confirmed by a voxel-wise analysis using Tract-Based Spatial Statistics. Analysis of axial and radial diffusivities showed significant elevation of these measures in MCP even among premutation carriers without FXTAS. Furthermore, regression analyses demonstrated clear inverted U-shaped relationship between CGG repeat size and axial and radial diffusivities in MCP. These results provide new evidence from DTI for white matter abnormalities in the cerebellar-brainstem and limbic systems among individuals with the fragile X premutation, and suggest the involvement of molecular mechanisms related to the *FMRI* gene in their white matter pathology.

Keywords

DTI; cerebellum; *FMRI*; FXTAS

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Financial Disclosure/Conflict of Interest: Funded by NIH grants UL1DE019583, DA024854, HD036071, NINDS grant RLINS062412, NIA grants RL1AG032119, RL1AG032115, and NCRR UL1 RR024146. Dr Hagerman has received funding from Roche, Novartis, Seaside Therapeutics, Forest, Johnson and Johnson, and Neuropharm for treatment trials in fragile X or autism. There are no other potential conflicts of interest.

Fragile X-associated tremor/ataxia syndrome (FXTAS) is a late-onset neurodegenerative disorder that is caused by premutation expansions (55 – 200 CGG repeats) in the 5' untranslated region of the fragile X mental retardation gene (*FMRI*)¹. Males have higher risk of developing FXTAS than females, affecting more than one third of male premutation carriers older than 50 years of age². Although intention tremor and ataxia constitute the core clinical features of FXTAS, the main symptoms also include cognitive decline, autonomic dysfunction, neuropathy and psychiatric features including anxiety, depression, and apathy³.

The pathogenetic mechanism of FXTAS is not yet fully understood. However, an RNA “toxic” gain-of-function model has been supported by several lines of evidence including the observation of abnormally elevated *FMRI* mRNA levels for premutation alleles⁴. Previous postmortem histological studies examined the pathological processes in the FXTAS brain and revealed eosinophilic intranuclear inclusions in neurons and astrocytes throughout the cerebrum and brainstem with particularly pronounced concentration in the hippocampus⁵. Prominent neuropathological features were also found in the cerebellum, including spongiform changes in the deep cerebellar white matter^{5,6}.

There have been only a few *in vivo* MRI morphometric studies that have examined brain pathology in patients with FXTAS. The T2 hyperintensity signal in the middle cerebellar peduncle and periventricular zones has been reported as a neuroradiological hallmark of FXTAS⁷. Our previous MRI study has revealed volume reductions in the cerebrum, cerebellum, and brainstem in FXTAS^{8,9}. Similarly, a previous voxel-based morphometry study on male premutation carriers revealed reduced voxel density in several brain regions, including the cerebellum and amygdalo-hippocampal complex¹⁰. Together with the postmortem findings, the available evidence is suggestive of pronounced pathological changes in, but not limited to, the cerebellar-brainstem and limbic systems in the premutation carriers.

In the present study, we use diffusion tensor imaging (DTI) technology to examine the white matter abnormalities in male premutation carriers with and without FXTAS. DTI is a relatively new MRI tool for studying the white matter microarchitecture^{11,12}. Fractional anisotropy (FA) provides a useful measure for the degree of restriction of water diffusion in tract fibers. Recent studies indicated that axial diffusivity (magnitude of principal longitudinal diffusivity) and radial diffusivity (mean of diffusivity along the other two orthogonal directions) can be also informative because increases in the two measures may be selectively associated with different aspects of underlying white matter pathology, that is, axonal damage and demyelination¹³⁻¹⁵. Here, we employed an automated tract of interest (TOI) analysis for major white matter tracts in the cerebellar-brain and limbic systems by using the White Matter Parcellation Map (WMPM)^{16,17}. To compare results across multiple methodologies, we also performed Tract-Based Spatial Statistics (TBSS), a recently developed voxel-based analysis of DTI data in which issues of misregistration can be greatly circumvented¹⁸. We further performed regression analyses using molecular measures of the *FMRI* gene, namely, CGG repeat size and levels of mRNA elevation, in order to examine possible effects of these factors on white matter pathology.

METHODS

Participants

We examined the brains of a total of 71 male participants for this study (age: 40 – 79): 20 healthy control (HC) participants, 35 participants with the premutation with FXTAS (PFX+), and 16 participants with the premutation without FXTAS (PFX-). The demographic information is summarized in Table 1. In this study, the premutation range was defined as

those with a CGG repeat size of between 55 and 200. The CGG repeat size and *FMRI* mRNA were measured in each participant following the procedures described elsewhere¹⁹. There were four missing CGG repeat size values (all in the HC group) and seven missing mRNA values (six HC and one PFX+). An F-test and a subsequent post-hoc test showed that PFX+ group was significantly older than PFX- group ($F = 4.65$, $P = 0.012$). For participants with CGG repeat sizes within the premutation range, a trained physician (RJH) scored the severity of FXTAS on a scale ranging from 0 to 6²⁰. In this study, premutation carriers with FXTAS scores of 0 or 1 (borderline or questionable symptoms of tremor and/or ataxia) were automatically placed in the PFX- group, while those with FXTAS scores of 2 (clear tremor and/or ataxia) to 5 (severe tremor and/or ataxia and consistently using a wheelchair) were designated as PFX+. Subjects with the premutation were recruited through screening of fragile X pedigrees of probands with fragile X syndrome (48 families). Controls were recruited from the local community through the University of California, Davis Medical Center. All subjects gave their signed, written informed consent before participating in the study. The protocol was approved by the institutional review board at the University of California, Davis.

MRI Data Acquisition

All MRI data was acquired on a 1.5 T GE MR scanner (General Electric Medical Systems). Diffusion-weighted data were acquired by using an echo planar imaging sequence with the following parameters: TR = 8000 ms; TE = 78 ms; field of view = 220×220 mm²; in-plane resolution = 1.718×1.718 mm²; 19 axial slices with 4 mm thickness and 1 mm gap. The lowest slice was positioned at the bottom of the cerebellum in order to cover major white matter tracts in the cerebellum, brainstem, and the limbic system. This resulted in including a portion of the corpus callosum within a scanning range in most cases. The diffusion weighting was applied along 6 directions using a b-value of 1000s/mm². In addition to four diffusion-weighted images per direction (4×6), two no diffusion-weighted (b0) images were acquired at the beginning of the sequence, which resulted in 26 volumes per subject.

DTI Data Analysis

We used the WMPM (<http://cmrm.med.jhmi.edu/>) for our automated TOI analysis. The analysis consisted of three parts: (i) calculate the FA, axial, and radial diffusivity maps in the subject native space (ii) calculate the deformation field to transform the native space into the ICBM-152 space and apply the inverse deformation field to the WMPM and (iii) calculate the mean FA, axial, and radial diffusivity values of each ROI using the WMPM transformed into the native space.

We used FDT (FMRIB's Diffusion Tool) in the FSL toolbox to generate the FA, axial, and radial diffusivity images in the subject native space. First, the image series was corrected for eddy currents and head motion by using affine registration to the first b0 volume. After correcting for the rotation parameters of head motion for each image, diffusion tensors were fitted independently to each voxel and served to calculate the FA, axial and radial diffusivity maps in the individual subject.

We used SPM5 to warp the WMPM into the subject native space. First, the mean b0 volume of the individual subject and its binary mask image were coregistered into the ICBM-152 space. We then normalized the coregistered b0 image into the ICBM-152 space and obtained the deformation field. We used the coregistered binary mask image when performing the normalization in order to prevent normalization outside the scanning range. We inverted the normalization process using Deformation Utility included in the SPM5 package. The inverted field was applied to the WMPM to transform the ICBM standard space to the native subject space.

Of the 48 parcellated white matter tracts in the WMPM, ten tracts of the cerebellar-brainstem system and the limbic system were included in our TOIs: (1) middle cerebellar peduncle (MCP) (2) pontine crossing tract (3) inferior cerebellar peduncle (4) superior cerebellar peduncle (5) corticospinal tract (6) medial lemniscus (7) cerebral peduncle (8) fornix (column and body of fornix) (9) fornix/stria terminalis (10) cingulum in the levels of the hippocampus. The TOIs (1 – 7) and (8 – 10) were classified to the cerebellar-brainstem system and the limbic system, respectively. For each TOI, we calculated mean FA, axial, and radial diffusivity values from each individual using the transformed WMPM in the subject's native space. For this analysis, we used voxels with an FA value of larger than 0.2 to exclude cortical gray matter and cerebral spinal fluid^{18,21,22}. For a comparison with the TOI analysis, we also performed TBSS analyses¹⁸. Statistically significant voxels were identified by threshold-free cluster enhancement (TFCE)²³ with the threshold of family-wise error corrected $P < 0.05$ (see Supporting Information).

RESULTS

TOI Analysis

Using the extracted FA, axial, and radial diffusivity values in the TOIs, we performed separate two-way analysis of covariance (ANCOVA) with group (HC, PFX+, PFX-) as an inter-subject factor, DTI measure of TOIs (either FA, axial, or radial diffusivity) as an intra-subject factor, and age as a covariate. There were highly significant main effects of group in all the three measures (FA: $F = 9.75$, $P < 0.001$; axial diffusivity: $F = 14.57$, $P < 0.001$; radial diffusivity: $F = 16.67$, $P < 0.001$). For each DTI measure, we then performed follow-up one-way ANCOVAs with age as a covariate to examine group differences in each individual TOI. The Benjamini-Hochberg method was implemented to adjust for the multiple statistical tests, with the false discovery rate set at 5 %²⁴. Results are summarized in Table 2. For TOIs where a significant group effect was found, we performed post-hoc analyses (Holm-Sidak test) to compare between two groups. Compared with HC, PFX+ showed significant alternations in all the three measures in MCP, left superior cerebellar peduncle, bilateral cerebral peduncle, fornix, and bilateral fornix/stria terminalis (Table 2). Although FA did not show significant difference between HC and PFX- in any TOI, significant increases in axial and radial diffusivities for PFX- were identified in MCP and left cerebral peduncle (see Fig. 1 for individual plots of the three DTI measures in two representative TOIs: MCP and right cingulum in the levels of hippocampus).

TBSS Analysis

In the TBSS analysis, the contrast of HC vs. PFX+ revealed significant FA reductions for patients in multiple tracts in the cerebellar-brainstem and limbic systems as well as other white matter tracts (Fig. 2A). Table 3 summarizes significant voxels in the cerebellar-brainstem and limbic systems. All the TOIs that showed significant FA reduction in the TOI analysis were replicated in this analysis. There was no voxel that showed larger FA in PFX+ than HC. In the contrasts of PFX- vs. PFX+, voxels with significant FA reduction were found in fornix and fornix/stria terminalis in the limbic system, which replicated the TOI analysis, and in other white matter tracts such as the splenium of corpus callosum and posterior thalamic radiation (Fig. 2B and Table 3). No significant voxel showed PFX+ > PFX-. In the contrasts of HC vs. PFX-, no significant voxels were identified in either direction, consistent with TOI analysis. There was no significant voxel identified by simple regression analysis using either CGG repeat size, or *FMR1* mRNA level. In regression analysis using the FXTAS score, clusters with significant negative effect were found in multiple tracts including fornix and MCP (Fig. 2C and Table 3). Progressive pathological alternations in these tracts were also identified by significant positive correlation between

the FXTAS score and either axial or radial diffusivity (See Supporting Information Table S1).

Correlation Analysis Using Molecular Variables for DTI Measures in MCP

Among DTI measures in the TOIs, significant reductions of axial and radial diffusivities in MCP of premutation carriers are particularly noticeable (Table 2), indicating significant pathological processes in this tract. In order to investigate possible underlying molecular mechanisms, we performed regression analyses using CGG repeat size and level of *FMRI* mRNA for axial and radial diffusivities in the MCP combining PFX+ and PFX-. We found clear quadratic relationships of CGG repeat size with both axial and radial diffusivities (Fig. 3). A trend-level positive correlation was found between *FMRI* mRNA levels and axial diffusivity ($r = 0.26$; $P = 0.068$) whereas there was no clear correlation with radial diffusivity ($r = 0.176$; $P = 0.223$).

DISCUSSION

We found clear evidence of abnormalities in white matter integrity in the male premutation carriers with FXTAS. These individuals demonstrated marked reductions of FA in multiple white matter tracts: the MCP, superior cerebellar peduncle, and cerebral peduncle in the cerebellar-brainstem system, and the fornix and fornix/stria terminalis in the limbic system. FA reductions in these tracts were replicated in TBSS analyses. Axial and radial diffusivity values in the MCP and cerebral peduncle in both hemispheres were found to be increased in the unaffected premutation carriers, indicating incipient white matter pathology before the onset of the major symptoms. Regression analyses using molecular measures of the *FMRI* gene demonstrated clear inverted U-shaped relationships between CGG repeat size and axial and radial diffusivities in MCP. To our knowledge, the present study represents the first evidence, using DTI for assessing abnormalities in specific white matter tracts of *FMRI* premutation carriers with and without FXTAS, together with the effects of the *FMRI* molecular variables on alternations of white matter microstructure in these individuals.

Our analysis of FA values of the cerebellar peduncles indicated significant pathological processes in the MCP and superior cerebellar peduncles, which correspond to major afferent and efferent fibers of the cerebellum, respectively. It is possible that reduced FA values in the superior cerebellar peduncle reflect deficient output from the cerebellum as evidenced by neuropathological features of Purkinje cells in patients with FXTAS⁶. It is also noteworthy that, in the unaffected premutation carriers, significant alternations of MCP were identified only by axial and radial diffusivities but not by FA (Table 2). A similar observation was described by a recent DTI study that reported better utility of axial and radial diffusivity than FA as markers of neurodegeneration in amyotrophic lateral sclerosis²⁵. However, it remains unknown whether axial and radial diffusivities have better sensitivity for degenerative processes of *FMRI* premutation carriers in general or only for those occurring in specific white matter tracts.

In the brainstem, the cerebral peduncle showed significant FA reduction in the patients with FXTAS, although the bilateral corticospinal tract showed altered axial and radial diffusivities. In the WMPM, the boundary between the cerebral peduncle and corticospinal tract was set around the boundary between the mesencephalon and the pons. Our observation is partly consistent with the previous clinical MRI studies reporting the volume loss of the mesencephalon and pons in patients with FXTAS as well as reduced volume reduction in the total brainstem in both affected and unaffected premutation carriers^{7,8}. FA values of MCP and cerebral peduncle showed significant negative correlation with the severity of FXTAS (Table 3), indicating progressive pathological changes in the cerebellar and brainstem tracts.

In the limbic system, we observed significant alternations of FA, axial and radial diffusivities in the fornix and the bilateral fornix/stria terminalis in patients with FXTAS (Table 2). It is possible that abnormalities in these tracts may underlie psychiatric and psychological problems, such as depression and anxiety, and autonomic dysfunction in patients with FXTAS²⁶⁻²⁹. In TBSS analyses, the contrast of PFX- vs. PFX+ and regression analysis using the FXTAS score showed significant clusters in these limbic TOIs (Fig. 2B, 2C and Table 3). These alternations may underlie progressive deterioration of cognitive and psychological functions over the progress of FXTAS.

Our analysis using *FMRI* molecular variables demonstrated a quadratic relationship between CGG repeat size and axial and radial diffusivities in the MCP. Our findings imply that a different pathogenic mechanism in white matter may be at play for premutation carriers in the high repeat range. It is possible that a deficit in *FMRI* protein expression starts to play a “protective role” in high repeat carriers, for whom translational efficiency has been shown to decrease in the upper premutation range^{30,31}. For individuals in this range, then, the neurotoxic effect of elevated mRNA may be somewhat ameliorated. Less severe pathological process in the high repeat range was also indicated by analysis of FA (see Supporting Information Fig. S1). Previous studies indicated that axial and radial diffusivities are selectively associated with axonal damage and demyelination, respectively¹³⁻¹⁵, and past postmortem studies identified various forms of axonal and myelination abnormalities in the cerebellum of FXTAS. It would be interesting to examine in future postmortem and animal model studies the possible nonlinear relationship between CGG repeat size and pathological features of axon and myelin by including FMRP measures.

Recent studies have made a remarkable progress in the development of protocols for tractography of major fibers in the cerebellar and limbic systems³²⁻³⁶. Such tractography approaches can be particularly powerful for the FXTAS brain, as evidenced by successful identification of individual cerebellar peduncles in cerebellar neurodegenerative diseases³⁷. However, tractographic approaches were not optimal for our DTI parameter settings regarding the number of diffusion encoding directions as well as spatial resolution. As a complementary approach, we adopted TBSS analysis which replicated significant FA reductions in FXTAS obtained by our automated TOI analysis. On the other hand, TBSS detected significant alternations in tracts where TOI analysis did not find significant difference, including inferior cerebellar peduncle and cingulum in the levels of the hippocampus (Tables 2 and 3). We therefore suggest that our findings regarding those white matter tracts need to be reassessed by future studies adopting tractographic and manual drawing methods. We also note that, according to our previous study, volumetric measures of the cerebral and cerebellar volumes and white matter volumes were comparable between the control and unaffected premutation males⁸. Therefore, it is unlikely that misregistration caused by general brain atrophy explains our findings, particularly about the MCP where significant alternations of axial and radial diffusivities were found even in the unaffected premutation individuals.

To conclude, this study presented evidence from DTI for reduced white matter integrity in the cerebellar-brainstem and limbic systems of the male carriers of *FMRI* premutation alleles. Our findings that white matter abnormalities in the MCP are not only observable in patients with FXTAS, but already present even in unaffected premutation carriers is very significant and holds promise for the possibility of identification of patients for early targeted treatments for FXTAS. Future studies targeting the other critical white matter tracts will further advance our understanding of the neural bases of the various behavioral abnormalities of this genotype.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank Patrick Adams, who assisted with the MRI data collection, and most especially the individuals who participated in our brain imaging studies.

References

1. Hagerman RJ, Leehey M, Heinrichs W, et al. Intention tremor, parkinsonism, and generalized brain atrophy in male carriers of fragile X. *Neurology*. 2001; 57(1):127–130. [PubMed: 11445641]
2. Jacquemont S, Hagerman RJ, Leehey MA, et al. Penetrance of the fragile X-associated tremor/ataxia syndrome in a premutation carrier population. *Jama*. 2004; 291(4):460–469. [PubMed: 14747503]
3. Amiri K, Hagerman RJ, Hagerman PJ. Fragile X-associated tremor/ataxia syndrome: an aging face of the fragile X gene. *Arch Neurol*. 2008; 65(1):19–25. [PubMed: 18195136]
4. Hagerman PJ, Hagerman RJ. The fragile-X premutation: a maturing perspective. *Am J Hum Genet*. 2004; 74(5):805–816. [PubMed: 15052536]
5. Greco CM, Hagerman RJ, Tassone F, et al. Neuronal intranuclear inclusions in a new cerebellar tremor/ataxia syndrome among fragile X carriers. *Brain*. 2002; 125(Pt 8):1760–1771. [PubMed: 12135967]
6. Greco CM, Berman RF, Martin RM, et al. Neuropathology of fragile X-associated tremor/ataxia syndrome (FXTAS). *Brain*. 2006; 129(Pt 1):243–255. [PubMed: 16332642]
7. Brunberg JA, Jacquemont S, Hagerman RJ, et al. Fragile X premutation carriers: characteristic MR imaging findings of adult male patients with progressive cerebellar and cognitive dysfunction. *AJNR Am J Neuroradiol*. 2002; 23(10):1757–1766. [PubMed: 12427636]
8. Cohen S, Masyn K, Adams J, et al. Molecular and imaging correlates of the fragile X-associated tremor/ataxia syndrome. *Neurology*. 2006; 67(8):1426–1431. [PubMed: 17060569]
9. Adams JS, Adams PE, Nguyen D, et al. Volumetric brain changes in females with fragile X-associated tremor/ataxia syndrome (FXTAS). *Neurology*. 2007; 69(9):851–859. [PubMed: 17724287]
10. Moore CJ, Daly EM, Tassone F, et al. The effect of pre-mutation of X chromosome CGG trinucleotide repeats on brain anatomy. *Brain*. 2004; 127(Pt 12):2672–2681. [PubMed: 15483045]
11. Le Bihan D. Looking into the functional architecture of the brain with diffusion MRI. *Nat Rev Neurosci*. 2003; 4(6):469–480. [PubMed: 12778119]
12. Mori S, Zhang J. Principles of diffusion tensor imaging and its applications to basic neuroscience research. *Neuron*. 2006; 51(5):527–539. [PubMed: 16950152]
13. Song SK, Sun SW, Ju WK, Lin SJ, Cross AH, Neufeld AH. Diffusion tensor imaging detects and differentiates axon and myelin degeneration in mouse optic nerve after retinal ischemia. *Neuroimage*. 2003; 20(3):1714–1722. [PubMed: 14642481]
14. Song SK, Yoshino J, Le TQ, et al. Demyelination increases radial diffusivity in corpus callosum of mouse brain. *Neuroimage*. 2005; 26(1):132–140. [PubMed: 15862213]
15. Budde MD, Xie M, Cross AH, Song SK. Axial diffusivity is the primary correlate of axonal injury in the experimental autoimmune encephalomyelitis spinal cord: a quantitative pixelwise analysis. *J Neurosci*. 2009; 29(9):2805–2813. [PubMed: 19261876]
16. Hua K, Zhang J, Wakana S, et al. Tract probability maps in stereotaxic spaces: analyses of white matter anatomy and tract-specific quantification. *Neuroimage*. 2008; 39(1):336–347. [PubMed: 17931890]
17. Wakana S, Caprihan A, Panzenboeck MM, et al. Reproducibility of quantitative tractography methods applied to cerebral white matter. *Neuroimage*. 2007; 36(3):630–644. [PubMed: 17481925]

18. Smith SM, Jenkinson M, Johansen-Berg H, et al. Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. *Neuroimage*. 2006; 31(4):1487–1505. [PubMed: 16624579]
19. Tassone F, Hagerman RJ, Garcia-Arocena D, Khandjian EW, Greco CM, Hagerman PJ. Intranuclear inclusions in neural cells with premutation alleles in fragile X associated tremor/ataxia syndrome. *Journal of medical genetics*. 2004; 41(4):e43. [PubMed: 15060119]
20. Jacquemont S, Hagerman RJ, Leehey M, et al. Fragile X premutation tremor/ataxia syndrome: molecular, clinical, and neuroimaging correlates. *Am J Hum Genet*. 2003; 72(4):869–878. [PubMed: 12638084]
21. Snook L, Plewes C, Beaulieu C. Voxel based versus region of interest analysis in diffusion tensor imaging of neurodevelopment. *Neuroimage*. 2007; 34(1):243–252. [PubMed: 17070704]
22. Bonekamp D, Nagae LM, Degaonkar M, et al. Diffusion tensor imaging in children and adolescents: reproducibility, hemispheric, and age-related differences. *Neuroimage*. 2007; 34(2):733–742. [PubMed: 17092743]
23. Smith SM, Nichols TE. Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. *Neuroimage*. 2009; 44(1):83–98. [PubMed: 18501637]
24. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B*. 1995; 57:289–300.
25. Metwalli NS, Benatar M, Nair G, et al. Utility of axial and radial diffusivity from diffusion tensor MRI as markers of neurodegeneration in amyotrophic lateral sclerosis. *Brain Res*. 2010; 1348:156–164. [PubMed: 20513367]
26. Hessel D, Tassone F, Loesch DZ, et al. Abnormal elevation of FMR1 mRNA is associated with psychological symptoms in individuals with the fragile X premutation. *Am J Med Genet B Neuropsychiatr Genet*. 2005; 139B(1):115–121. [PubMed: 16184602]
27. Bourgeois JA, Coffey SM, Rivera SM, et al. A review of fragile X premutation disorders: expanding the psychiatric perspective. *The Journal of clinical psychiatry*. 2009; 70(6):852–862. [PubMed: 19422761]
28. Berry-Kravis E, Goetz CG, Leehey MA, et al. Neuropathic features in fragile X premutation carriers. *American journal of medical genetics*. 2007; 143(1):19–26. [PubMed: 17152065]
29. Roberts JE, Bailey DB Jr, Mankowski J, et al. Mood and anxiety disorders in females with the FMR1 premutation. *Am J Med Genet B Neuropsychiatr Genet*. 2009; 150B(1):130–139. [PubMed: 18553360]
30. Tassone F, Hagerman RJ, Taylor AK, Gane LW, Godfrey TE, Hagerman PJ. Elevated levels of FMR1 mRNA in carrier males: a new mechanism of involvement in the fragile-X syndrome. *Am J Hum Genet*. 2000; 66(1):6–15. [PubMed: 10631132]
31. Kenneson A, Zhang F, Hagedorn CH, Warren ST. Reduced FMRP and increased FMR1 transcription is proportionally associated with CGG repeat number in intermediate-length and premutation carriers. *Human molecular genetics*. 2001; 10(14):1449–1454. [PubMed: 11448936]
32. Concha L, Gross DW, Beaulieu C. Diffusion tensor tractography of the limbic system. *AJNR Am J Neuroradiol*. 2005; 26(9):2267–2274. [PubMed: 16219832]
33. Catani M, Jones DK, Daly E, et al. Altered cerebellar feedback projections in Asperger syndrome. *Neuroimage*. 2008; 41(4):1184–1191. [PubMed: 18495494]
34. Pugliese L, Catani M, Ameis S, et al. The anatomy of extended limbic pathways in Asperger syndrome: a preliminary diffusion tensor imaging tractography study. *Neuroimage*. 2009; 47(2):427–434. [PubMed: 19446642]
35. Aravamuthan BR, Muthusamy KA, Stein JF, Aziz TZ, Johansen-Berg H. Topography of cortical and subcortical connections of the human pedunculo-pontine and subthalamic nuclei. *Neuroimage*. 2007; 37(3):694–705. [PubMed: 17644361]
36. Jissendi P, Baudry S, Baleriaux D. Diffusion tensor imaging (DTI) and tractography of the cerebellar projections to prefrontal and posterior parietal cortices: A study at 3T. *Journal of Neuroradiology*. 2008; 35:9.
37. Taoka T, Kin T, Nakagawa H, et al. Diffusivity and diffusion anisotropy of cerebellar peduncles in cases of spinocerebellar degenerative disease. *Neuroimage*. 2007; 37(2):387–393. [PubMed: 17583535]

Fig. 1. Group comparisons of the fractional anisotropy (FA), axial diffusivity, and radial diffusivity. (A - C) Middle cerebellar peduncle (MCP) (D - F) Right cingulum in the levels of the hippocampus. Asterisks indicate significant group difference indicated by a post-hoc test (Holm-Sidak test) ($P < 0.05$).

Fig. 2.

Significant reduction of the fractional anisotropy (FA) related to FXTAS identified by TBSS analysis. (A) Significant FA reduction in patients with FXTAS identified by HC vs. PFX+ in the MNI space (B) Significant FA reduction in patients with FXTAS identified by PFX- vs. PFX+. (C) Significant FA reduction correlated with FXTAS score. MCP = middle cerebellar peduncle

Fig. 3.

Inverted U-shaped relationships between CGG repeat size and axial and radial diffusivity in the middle cerebellar peduncle (MCP) among premutation carriers. The quadratic relationship was highly significant for axial diffusivity ($r^2 = 0.238$, $P = 0.001$) and radial diffusivity ($r^2 = 0.363$, $P < 0.001$).

Demographic data including molecular data for healthy control, premutation carriers affected with FXTAS, and unaffected premutation carriers

Table 1

	Healthy Control (HC) (n = 20)			Premutation with FXTAS (PEX+) (n = 35)			Premutation without FXTAS (PEX-) (n = 16)		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
Age ^a	60.2	9.2	40 - 79	65.5	7.57	47- 78	58.2	10.4	42 - 78
FSIQ ^b	118.1	15.1	90 - 142	103.1	17.1	67 - 136	119.8	18.8	83 - 152
PIQ ^b	114.8	14.2	87 - 134	99.9	18.3	64 - 128	121.5	18.3	91 - 155
VIQ ^c	117.3	15.1	84 - 144	106.2	14.5	79 - 135	115.1	18.4	78 - 142
FXTAS score		N.A.		3.00	1.03	2 - 5	0.31	0.48	0 - 1
CGG repeat	29.31	3.75	18 - 35	94.8	19.1	59 - 133	94.1	32.5	55 - 166
FMR1 mRNA	1.27	0.33	0.63 - 1.85	3.28	0.79	1.75 - 5.25	3.16	0.94	1.86 - 4.77

^aSignificant main effect of group ($F = 4.65$, $P = 0.012$).

^bData was not available in two HCs and two PEX+. FSIQ: $F = 7.23$, $P = 0.002$; PIQ: $F = 9.78$, $P = 0.0002$.

^cData was not available in two HCs and three PEX+. VIQ: $F = 3.51$, $P = 0.036$.

SD: standard deviation; FSIQ: WAIS III Full scale IQ; PIQ: Performance IQ; VIQ: Verbal IQ.

Table 2

Group comparisons of fractional anisotropy (FA), axial diffusivity, and radial diffusivity in tracts of interest

	FA			Axial Diffusivity			Radial Diffusivity		
	F	P		F	P		F	P	
Cerebellar and brainstem tracts									
MCP	7.53	0.003 ^a		23.29	<0.001 ^{x,y,z}		20.49	<0.001 ^{x,y,z}	
R. Superior cerebellar peduncle	6.01	0.010 ^a		2.17	0.160		4.87	0.019 ^x	
L. Superior cerebellar peduncle	5.63	0.011 ^a		4.40	0.030 ^x		7.12	0.004 ^{x,y}	
R. Inferior cerebellar peduncle	0.76	0.532		4.06	0.037 ^y		3.97	0.034 ^x	
L. Inferior cerebellar peduncle	1.35	0.378		3.44	0.059		5.22	0.015 ^x	
Pontine crossing tract	2.54	0.134		0.51	0.600		0.38	0.160	
R. Cerebral peduncle	10.20	<0.001 ^a		13.22	<0.001 ^x		20.01	<0.001 ^{x,y,z}	
L. Cerebral peduncle	4.72	0.023 ^a		17.26	<0.001 ^{x,y}		14.70	<0.001 ^{x,y}	
R. Corticospinal tract	2.72	0.124		4.73	0.025 ^x		6.95	0.017 ^x	
L. Corticospinal tract	1.02	0.447		5.20	0.019 ^x		4.19	0.029 ^x	
R. Medial lemniscus	1.55	0.317		0.54	0.624		1.16	0.338	
L. Medial lemniscus	1.02	0.480		0.58	0.640		1.84	0.188	
Limbic tracts									
Fornix	9.70	<0.001 ^{a,c}		7.26	0.009 ^x		8.35	0.002 ^x	
R. Cingulum in the levels of hippocampus	0.41	0.664		0.90	0.502		1.12	0.333	
L. Cingulum in the levels of hippocampus	0.72	0.523		2.29	0.156		3.57	0.044	
R. Fornix/Stria terminalis	11.00	<0.001 ^a		5.50	0.017 ^x		8.52	0.003 ^x	
L. Fornix/Stria terminalis	14.42	<0.001 ^{a,c}		8.64	0.002 ^x		12.27	<0.001 ^{x,z}	

a - c, x - z = Significant group difference identified by a post-hoc test (Holm-Sidak test, $P < 0.05$),

^a = HC > PFX+,

^b = HC > PFX-,

^c = PFX- > PFX+.

$x = \text{HC} < \text{PFX}^+,$ $y = \text{HC} < \text{PFX}^-,$ $z = \text{PFX}^- < \text{PFX}^+.$

$F = F$ value, $P = P$ value, $\text{HC} = \text{healthy control}$, $\text{PFX}^+ = \text{premutation carriers with FXTAS}$, $\text{PFX}^- = \text{premutation carriers without FXTAS}$, $\text{MCP} = \text{middle cerebellar peduncle}$, $L = \text{Left}$, $R = \text{Right}$

Table 3
Significant voxels in the cerebellar, brainstem, and limbic tracts identified by TBSS analysis

	HC > PFX+				PFX- > PFX+				Negative correlation with FXTAS Score							
	x	y	z	P	Volume (mm ³)	x	y	z	P	Volume (mm ³)	x	y	z	P	Volume (mm ³)	
Cerebellar and brainstem tracts																
MCP	33	-50	-40	0.002	1085				N.S.		32	-52	-43	0.033		71
R. Superior cerebellar peduncle	10	-51	-31	0.011	171				N.S.					N.S.		
L. Superior cerebellar peduncle	-6	-53	-31	0.010	190				N.S.					N.S.		
R. Inferior cerebellar peduncle	11	-45	-34	0.011	122				N.S.					N.S.		
L. Inferior cerebellar peduncle	-6	-54	-21	0.010	144				N.S.					N.S.		
Pontine crossing tract	7	-29	-30	0.019	31				N.S.					N.S.		
R.Cerebral peduncle	9	-7	-7	0.005	369				N.S.		15	-10	-6	0.022		123
L.Cerebral peduncle	-19	-21	-7	0.0002	253				N.S.		-18	-21	-5	0.007		93
R.Corticospinal tract	4	-20	-32	0.014	215				N.S.					N.S.		
L.Corticospinal tract	-5	-18	-24	0.012	51				N.S.					N.S.		
R. Medial lemniscus	7	-39	-28	0.011	110				N.S.					N.S.		
L. Medial lemniscus	-6	-39	-28	0.011	81				N.S.					N.S.		
Limbic tracts																
Fornix	2	-13	15	0.0002	78	1	-4	9	0.036	67	1	-5	9	0.01		87
R. Cingulum (hippocampal levels)	25	-29	-12	0.0004	85	13	-47	7	0.029	10	14	-45	7	0.0004		208
L. Cingulum (hippocampal levels)	-15	-46	1	0.0002	253	-14	-43	2	0.026	17	-11	-45	2	0.0004		221
R. Fornix/Stria terminalis	31	-26	-10	0.0002	119				N.S.		31	-22	-8	0.016		208
L. Fornix/Stria terminalis	-23	-35	2	0.0002	159	-25	-35	2	0.028	9	-28	-25	-10	0.004		143

HC = healthy control, PFX+ = premutation carriers with FXTAS, PFX- = premutation carriers without FXTAS, MCP = middle cerebellar peduncle, L = Left, R = Right, N.S. = No significant voxel was identified.