NeuroImage: Clinical 11 (2016) 133–138

Contents lists available at ScienceDirect

NeuroImage: Clinical

journal homepage: www.elsevier.com/locate/ynicl

Profiles of aberrant white matter microstructure in fragile X syndrome

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ARTICLE INFO

Article history:
Received 24 July 2015
Received in revised form 9 January 2016
Accepted 12 January 2016
Available online 14 January 2016

Keywords:
Fragile X syndrome
White matter microstructure
Autism
FMR1 gene
Diffusion tensor imaging

ABSTRACT

Previous studies attempting to quantify white matter (WM) microstructure in individuals with fragile X syndrome (FXS) have produced inconsistent findings, most likely due to the various control groups employed, differing analysis methods, and failure to examine for potential motion artifact. In addition, analyses have heretofore lacked sufficient specificity to provide regional information. In this study, we used Automated Fiber-tract Quantification (AFQ) to identify specific regions of aberrant WM microstructure along WM tracts in patients with FXS that differed from controls who were matched on age, IQ and degree of autistic symptoms. Participants were 20 patients with FXS, aged 10 to 23 years, and 20 matched controls. Using Automated Fiber-tract Quantification (AFQ), we created Tract Profiles of fractional anisotropy and mean diffusivity along 18 major WM fascicles. We found that fractional anisotropy was significantly increased in the left and right inferior longitudinal fasciculus (ILF), right uncinate fasciculus, and left cingulum hippocampus in individuals with FXS compared to controls. Conversely, mean diffusivity was significantly decreased in the right ILF in patients with FXS compared to controls. Age was significantly negatively associated with MD values across both groups in 11 tracts. Taken together, these findings indicate that FXS results in abnormal WM microstructure in specific regions of the ILF and uncinate fasciculus, most likely caused by inefficient synaptic pruning as a result of decreased or absent Fragile X Mental Retardation Protein (FMRP). Longitudinal studies are needed to confirm these findings.

1. Introduction

FXS is the most common known inherited form of intellectual disability affecting approximately 1 in 3000 boys and 1 in 5000 girls worldwide (Hagerman, 2008). First described by Martin and Bell in 1943 as a "pedigree of mental defect showing sex linkage" (Martin and Bell, 1943), FXS is caused by mutations to the *FMR1* gene at locus 27.3 on the long arm of the X chromosome (Verkerk et al., 1991). Excessive methylation of the gene impairs production of Fragile X Mental Retardation Protein (FMRP), a key protein involved in synaptic plasticity and dendritic maturation in the brain (Greenough et al., 2001; Soden and Chen, 2010). As a result, individuals with FXS exhibit a specific profile of developmental and cognitive deficits (Reiss and Dant, 2003) including impairments in executive functioning, visual memory and perception, mental manipulation of visual–spatial relationships among objects, aberrant processing of arithmetical stimuli, as well as increased risk for autistic-like behaviors (e.g., social avoidance, communication impairments and repetitive behaviors) (Bennetto et al., 2001; Cornish et al., 2004; Dissanayake et al., 2009; Hall et al., 2006; Hall et al., 2009; Kaufmann et al., 2004; Mazzocco, 2001; Mazzocco et al., 2006; Murphy, 2009; Skinner et al., 2005; Sudhalter et al., 1990; Sullivan et al., 2007; Sullivan et al., 2006). However, data suggests that there are significant brain and behavioral differences between those diagnosed with FXS and those diagnosed with autism (Hall et al., 2009).

Over the past decade, studies employing diffusion tensor imaging (DTI) methods (Basser, 1995; Basser and Pierpaoli, 1996; Pierpaoli and Basser, 1996) have indicated that white-matter (WM) microstructure may be aberrant in individuals with FXS. For example, Barnea-Goraly and colleagues reported that fractional anisotropy (FA), which quantifies diffusion anisotropy, was significantly decreased in fronto-striatal pathways as well as in parietal sensory-motor tracts in 10 females with FXS, aged 13 to 22 years, compared to age-matched typically developing controls (Barnea-Goraly et al., 2003). In a more recent study, Villalon-Reina and colleagues (Villalon-Reina et al., 2013), reported that mean diffusivity (MD) — the average diffusion across all directions — was significantly increased in regions along the superior longitudinal fasciculus (SLF), inferior longitudinal fasciculus (ILF), and inferior fronto-occipital fasciculus (IFOF) in 18 girls with FXS, aged 7 to 14 years, compared to typically developing controls. Taken together, these studies suggest that FXS may be characterized by differences in tissue structure in long association WM tracts.

However, in studies in which patients with FXS are compared to neurotypical controls, it is unclear whether the differences in WM

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http://dx.doi.org/10.1016/j.nicl.2016.01.013

microstructure are specific to FXS, or simply related to differences in IQ and other cognitive and behavioral symptoms between the groups. One way to overcome this problem is to compare individuals with FXS to those who have similar IQs and levels of autistic symptoms, but who do not have FXS. In a recent study from our group (Green et al., 2015), for example, the WM microstructure of patients with FXS (25 females, 15 males) was compared to non-FXS individuals who had similar levels of IQ and autistic symptoms. Using Tract-Based Spatial Statistics (TBSS) (Smith et al., 2006), mean FA values in the inferior longitudinal, inferior fronto-occipital and uncinate fasciculi were found to be significantly *increased* in patients with FXS compared to controls. However, these analyses lacked sufficient specificity to provide information concerning whether WM microstructure is abnormal along the whole tract or at specific locations on a tract.

Given the inconsistent findings, here we refine our analyses and study design in three ways. First, we employed a more granular method of fiber-tract quantification — Automated Fiber-tract Quantification (AFQ) (Yeatman et al., 2012a) — to identify regions of aberrant WM microstructure in these patients. Second, we compared patients with FXS to a well-matched group of individuals with idiopathic developmental disability (but who did not have FXS) in order to rule out age, IQ, and degree of autistic symptoms as possible confounds. Finally, we examined the degree to which subject movement may have influenced our findings.

2. Materials and methods

2.1. Participants

All participants had taken part in a previous study investigating large-scale brain networks in patients with FXS (Hall et al., 2013). There was no overlap of the participants studied here with a previous DTI study from our group (Green et al.). Patients with FXS were included if they were aged between 10 and 23 years, had an IQ between 50 and 90 points on the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1999), and could demonstrate that they could remain immobile for 10 min while lying in the scanner. During recruitment, control participants were matched to children with FXS in terms of age (+/−3 years), IQ (+/−10 points) and severity of autistic symptoms (+/−5 points on the Social Communication Questionnaire [SCQ]) (Rutter et al., 2003). Individuals in both groups were excluded from the study if they were born preterm (<34 weeks), had low birth weight (<2000 g), showed evidence of a genetic condition other than FXS, exhibited sensory impairments, or had any serious medical or neurological condition that affected growth or development (e.g., seizure disorder, diabetes, congenital heart disease). Finally, individuals were excluded if they had materials in their body that would preclude an MRI scan (e.g., dental braces). Control participants were subsequently screened for FXS to confirm that they did not have FXS. All protocols were approved by the human subjects committee at Stanford University School of Medicine and all parents gave consent for their child to participate in the study.

All participants with FXS had a confirmed genetic diagnosis of FXS (i.e., >200 CGG repeats on the *FMR1* gene and evidence of aberrant methylation) as evidenced by standard Southern Blot techniques. Two male participants with FXS were mosaic (i.e., an additional unmethylated fragment was detected in the premutation range). Five control participants had an additional co-morbid diagnosis (2 ADHD, 1 PTSD and 2 ASD). As can be seen from Table 1, the two groups were well matched in terms of age, IQ, and degree of autistic symptomatology (Table 1). Nine (45%) participants with FXS and 6 (30%) controls were taking psychoactive medications. In the FXS group, medications included sertraline (2 participants), venlafaxine (1 participant), donepezil (1 participant), aripiprazole (1 participant), and methylphenidate (3 participants). In the control group, medications included methylphenidate (5 participants), aripiprazole (2 participants), imipramine (1 participant), and arbaclofen (1 participant). As expected, mean IQs were significantly higher in females with FXS ($M = 73.0$, $SD = 8.0$) than in males with FXS ($M = 59.75$, $SD = 9.0$) [($t(18) = 3.21$, $p = .006$)]. There were, however, no other differences between female and male participants with FXS on the other measures. Within the FXS group, age and IQ were not associated with scores on the SCQ.

Table 1
Demographic information.

Characteristic	FXS *N* (%)	Controls *N* (%)	*P*
Total	20	20	
Female	12 (60.0)	7 (35.0)	*NS*
Medications (any)	9 (45.0)	6 (30.0)	*NS*
	Mean (SD)	Mean (SD)	
Age, year	16.63 (4.69)	16.57 (3.90)	*NS*
FSIQ[a]	67.30 (10.54)	64.85 (11.03)	*NS*
Autistic symptoms[b]	8.90 (5.72)	11.15 (6.63)	*NS*

Abbreviations: FXS, fragile X syndrome; FSIQ, full scale IQ; VIQ, verbal IQ; PIQ, Performance IQ.
[a] Wechsler Abbreviated Scale of Intelligence (Wechsler, 1999).
[b] Social Communication Questionnaire (Rutter et al., 2003).

2.2. Diffusion weighted imaging acquisition and processing

Diffusion weighted imaging (DWI) data were acquired immediately following a blood oxygen level dependent (BOLD) resting-state MRI scan on a 3.0-T whole-body MRI scanner (GE Medical Systems, Milwaukee, WI) with the vendor-supplied 8-channel receive coil at Stanford University. A diffusion-weighted, single-shot, spin-echo, echoplanar imaging sequence (TE = 72.7 ms, TR = 5.7 s, FOV = 240 mm, matrix size = 128 × 128) was used to acquire 44 2.9 mm-thick slices in 23 different diffusion directions (b = 850) for a voxel size of 1.9 × 1.9 × 2.9 mm. The sequence was repeated four times and six non-diffusion weighted (b = 0) volumes were collected (total scan duration was 8 min). Eddy current distortions and subject motion in the diffusion weighted images were removed by a 14-parameter constrained non-linear co-registration based on the expected pattern of eddy-current distortions given the phase-encode direction of the acquired data (Rohde et al., 2004). Each diffusion-weighted image was registered to the mean of the (motion-corrected) non-diffusion-weighted (b = 0) images using a two-stage coarse-to-fine approach that maximized the normalized mutual information. The mean of the non-diffusion-weighted images was automatically aligned to the T1 image using a rigid body mutual information algorithm. All raw images from the diffusion sequence were resampled to 2-mm isotropic voxels by combining the motion correction, eddy-current correction, and anatomical alignment transforms into one omnibus transform and resampling the data using a trilinear interpolation algorithm based on code from SPM5 (Friston and Ashburner, 2004). An eddy-current intensity correction was applied to the diffusion-weighted images at the resampling stage (Rohde et al., 2004).

The rotation component of the omnibus coordinate transform was applied to the diffusion-weighting gradient directions to preserve their orientation with respect to the resampled diffusion images. The tensors were then fit using a robust least-squares algorithm designed to remove outliers from the tensor estimation step (Chang et al., 2005). We computed the eigenvalue decomposition of the diffusion tensor and the resulting eigenvalues were used to compute fractional anisotropy (FA) and mean diffusivity (MD) (Basser and Pierpaoli, 1996). All the custom image processing software are from the open-source mrDiffusion package, available for download from http://github.com/vistalab/vistasoft/.