Reduced magnetization transfer in gray and white matter in the developing *Fmr1* knockout mouse

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Introduction: Fragile x syndrome (FXS) is the most common form of inherited mental retardation and affects 1:5000 males (1), caused by silencing of the FMR1 gene (2). FXS patients exhibit behavioral and cognitive alterations including low IQ, hyperactivity, anxiety, and deficits in learning and memory (3). Much of the literature on cellular and molecular consequences of FXS comes from the commonly studied *Fmr1*$^{-/-}$ mouse model. Volumetric and metabolic changes were recently found during postnatal brain development in both FXS and the *Fmr1*$^{-/-}$ mouse by in vivo imaging and spectroscopy (4,5,6,7,8). Alterations in gray matter volume have been reported and overall white matter volume was increased in the developing FXS brain compared to control patients (6,7). Thus far, few studies have used MRI as a method to determine molecular pathology behind changes in brain volume during development and maturation. We hypothesized that magnetization transfer imaging (MTI) with biochemical correlation may improve our understanding of FXS pathology during the peak of myelination and synaptogenesis. In this study, we compared the brains of *Fmr1*$^{-/-}$ to age matched wild type (WT) mice during the peak of myelination at postnatal days (PND) of 18 and 21 and at PND 30 when the brain is structurally mature.

Methods:

Animals: Fmr1$^{-/-}$ mice (JAX B6.129P2-fmr1tmICgr mice; Jackson Laboratory, Bar Harbor, ME) from a C57Bl/6J background were obtained from our breeding colony at the University of Maryland, Baltimore. All protocols were approved by the Institutional Animal Care and Use Committee at University of Maryland, Baltimore. 6 male *Fmr1*$^{-/-}$ and WT mice were perfused at PND 18, 21 and 30. The mice were anesthetized with 4% isoflourane and then perfused through the left ventricle with 1X phosphate buffered saline and followed by 4% paraformaldehyde (PFA). The brain was stored in 4% PFA for at least two days, then scanned in a customized conical tube filled with Fluroinert (3M, St. Paul, MN) which provided a dark background for the brain images and facilitated segmentation. Brain tissue for protein assay was dissected in a 1mm thick brain matrix and immediately frozen in liquid nitrogen.

Ex vivo MRI: All experiments were performed on a BrukerBiospec 7.0 Tesla 30 cm horizontal bore scanner using Paravision 5.1 software (BrukerBiospin MRI, Ettlingen, Germany). A four-channel Bruker 1H surface array coil was used as the receiver and a Bruker 72-mm linear-volume coil as the transmitter. MTI was acquired from two FLASH T1 sequences: TE/TR 3.6/280 ms, 32 averages, slice thickness 0.5 mm, interslice thickness 0.5mm, 16 slices, with a matrix size 75 X 75 and field of view 1.5mm X 1.5 mm. MTI was facilitated by a 12.6 ms Gaussian RF pulse centered an offset of 5kHz from the center frequency. Magnetization transfer ratio (MTR) images were generated off-line.

Image processing: Regions of interest were drawn manually within Medical Image Processing, Analysis and Visualization tool (MIPAV v5.3.1, CIT; NIH, Bethesda, MD, USA) for MTI in both gray and white matter regions. Gray matter regions include thalamus, anterior and posterior cortex while white matter regions include the corpus callosum, internal and external capsule, and fimbria.

Protein assay: Relative myelin basic protein (MBP) concentration was determined by Western blot immuno-assay with anti-rat MBP antibody (abcam). Relative density of MBP was calculated from ratio of MBP to total protein from Ponceau protein stain.

Statistical analysis: MTR data was analyzed using a multivariate analysis of variance to compare values between genotypes. An analysis of variance was used to determine the effect of age on individual genotypes within the same regions. A student's T-test was used to determine the effect of genotype at each age.

Results: In the gray matter, significant reduction of MTR was observed (Fig 1A) in the thalamus [F(1,35)=12.77, p<0.005] and posterior cortex [F(1,35)=10.68, p<0.005] in the *Fmr1*$^{-/-}$ mouse at PND 18 and 21. Reduced MTR was also observed in white matter regions (Fig 1B) including the corpus callosum [F(1,35)=10.10, p<0.005], internal capsule [F(1,35)=11.35, p<0.005], external capsule [F(1,35)=12.77, p<0.01], and fimbria [F(1,35)=9.47, p<0.01] in the *Fmr1*$^{-/-}$ mice at PND 18 and 21. The effect of age was observed in all gray and white matter regions of *Fmr1*$^{-/-}$ mice, specifically between PND 18 and 21 compared to PND 30. Preliminary protein assay data demonstrated a trend toward reduced relative MBP concentration (n=2 at each age) in the cortical region at PND 18 but not 30 in *Fmr1*$^{-/-}$ mice compared to the WT (Fig 2).

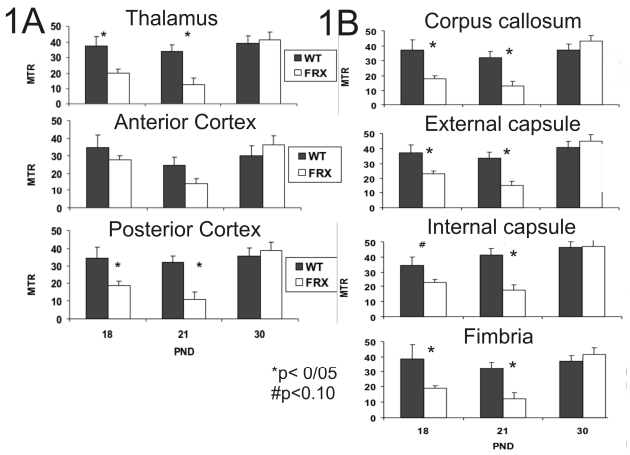

Fig 1: MTR of WT and *Fmr1*$^{-/-}$ mice at PND 18, 21 and 30 in gray (A) and white (B) matter regions.

Conclusion: The reduced MTR in white matter suggest that the trajectories associated with normal myelination are significantly different during brain development in the *Fmr1*$^{-/-}$ mouse compared to the WT. This may also suggest delayed myelin formation in the *Fmr1*$^{-/-}$ mice, and can be interpreted as a reduction in myelination at PND 18 and 21. Delayed myelination has already been shown in the cerebellum of developing *Fmr1*$^{-/-}$ mice by electron microscopy (9) but not in other brain regions by translational methods. In multiple sclerosis (MS), demyelination and axonal injury have been correlated with reduction of MTR in white matter (10), however, the pathology indicated by the reduction of MTR in gray matter is unknown. A recent study in MS patients showed reduced gray matter MTR also mirrored concurrent clinical progression, demonstrating MTR as a surrogate marker for progression of MS (11). Due to the cell complexity in gray matter, immunohistochemical assays are required to determine if the reduction of MTR is due to decreased protein concentration or enlarged liquid pool in the brain of *Fmr1*$^{-/-}$ mice. Preliminary MBP concentration suggests lowered protein concentration may lead to reduced MTR in the cortex.

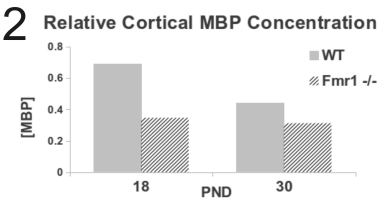

Fig 2: MBP concentration relative to total cortical protein from WT and *Fmr1*$^{-/-}$ mice at PND 18 and 30.

Funding partially supported by: FRAXA research foundation and NIH P01-HD016596-27

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