Diffusion Tensor Imaging demonstrated significant axonal damage in Fmr1 knock out mice

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Introduction:
Volumetric and metabolic changes were recently found during postnatal brain development in Fragile X syndrome (FXS) and its Fmr1 KO mouse model by in vivo imaging and spectroscopy.1-8,9,10 Diffusion tensor imaging (DTI) has also shown increase of tract density in the striatum that correlates with lower IQ in FXS children. However, the link between brain structure and metabolism still remains largely unknown during development of FXS and Fmr1 KO mice. The study of the Fmr1 KO mice compared to wild type (WT) controls using DTI with histological correlates can improve our understanding of FXS pathology in a time point during the critical period of myelination and synaptogenesis.

Materials and Methods:
Animals: Fmr1 KO mice (JAX B6.129P2-fmr1ItmICgr mice; Jackson Laboratory, Bar Harbor, ME) from a C57Bl/6j background were obtained 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. Five Fmr1 KO mice and 6 WT (JAX C57Bl/6j) brains were used for each group. On day 30 the mice were anesthetized with 4% isoflurane 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 Fluorinert (3M, St. Paul, MN) that decreased background. Afterward the brains were transferred to 30% sucrose Utitil use for histology.

ex vivo MRI: All experiments were performed on a BrukerBiopsic 7.0 Tesla 30 cm horizontal bore scanner using Paravision 5.1 software (BrukerBiopsic 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. DTI images were acquired with the 4-shot segmented spin echo echo-planar-imaging sequence in the axial plane. 30 diffusion directions were applied at b=700 s/mm², 2100 s/mm², and 4000 s/mm². Five images at b=0 s/mm² were acquired. The field of view was 1.88 x 1.50cm², with matrix resolution 64 x 64, TR/TE 6000/27.7msec, slice thickness 1mm, 12 slices and one average.

Imaging processing: Regions of interest were drawn manually with FSLview (Analysis Group, MRIB, Oxford, UK) including the hippocampus (HP), striatum (ST), thalamus (TH), prelimbic cortex (PLC), anterior cortex (ACx), and posterior cortex (PCx) in gray matter. Additionally, white matter regions studied were: corpus callosum (CC), external capsule (EC), and internal capsule (IC). Mean, axial and radial diffusion values were then extracted from each of the regions.

Histology: The fixed brain was frozen in OCT and sectioned on a cryostat. Black gold II (Millipore) was used for myelin staining according to manufacturer's instructions and sections were visualized on a Nikon Eclipse 90i microscope (bright field).

Results:
Whole brain analysis of Fmr1 KO mice compared to WT at PND 30 revealed a significant reduction in axial diffusivity (AD) (p = 0.03) and a trend of decrease in mean and radial diffusivity (MD and RD, respectively) (p = 0.06). Mean diffusivity (MD) was significantly reduced in Fmr1 KO mice compared to WT in the ST and IC (p < 0.05) and decreasing trends were observed in HP, TH, PLC, ACx, PCx, CC and EC (p < 0.10) (Fig 1A). AD in Fmr1 KO mice compared to WT was significantly decreased in the HP, TH, PLC, EC and IC (p < 0.05) and decreasing trends in ACx, PCx, CC and IC (p < 0.10) (Fig 1B). RD in Fmr1 KO mice compared to WT was significantly decreased in ST and IC (p < 0.05), with decreasing trends in HP, TH, ACx, PCx, CC, and EC (p < 0.05) (Fig 1C). Preliminary histology show decrease in myelin tracts in the CC of Fmr1 KO (Fig 2A) compared to WT (Fig 2B) at PND 30.

Figure 1: Diffusion values of WT mice (dark bar) compared to Fmr1 KO mice (light bar) at PND 30. A) mean diffusivity, B) axial diffusivity, C) radial diffusivity. *p<0.05, #p<0.10. Standard error bars are shown.

Discussion and Conclusion:
The diffusion tensor findings in Fmr1 KO mice at PND 30 is consistent with accepted Fmr1 KO and FXS phenotypes, including deficits in axonal and dendritic spine morphology. Also in neurons, a lack of fragile x mental retardation protein (FMRP) leads to decreased synaptic development and plasticity, which can affect learning and memory. Increased axonal branching was reported previously in the brain of the drosophila FXS model.10 Such increased branching may be possible in the Fmr1 KO mouse brain which results in the reduction of both AD and RD in white and gray matter. Axonal degeneration may also be a contributor of decreasing AD in many brain regions of Fmr1 KO mice. Axonal degeneration could be related to a delayed or inappropriate development during myelination and synaptogenesis of the Fmr1 KO mouse brain at PND 30. In the absence of myelin, axons are known to degrade and the decreased myelin staining (Fig 2) could indicate potential axonal degeneration. Song et al.11 have already shown that a decrease of AD could lead to axonal degeneration in a demyelinating mouse model, however our results are not consistent with demyelination in Fmr1 KO brain. The decrease in RD could be result of increased swelling in some brain regions. This is also consistent with clinical and preclinical findings of increased brain regions found during development of both FXS and Fmr1 KO mice.12,13 Altered osmolyte concentration can also contribute to swelling. Our lab previously found increase of the osmolyte taurine in the hippocampus of Fmr1 KO mice at 30 days of age, but also a decrease of myo-inositol, both of which can contribute to changes in osmolarity in the brain.14 While confirming previous anatomical studies, this study provides detailed insights into microstructural changes in the brain for the pathophysiologically changes in the FXS and Fmr1 KO phenotype, markers of which can be used for evaluating novel therapies.

References: