Anatomical Phenotyping of the Knockout Mouse Model of Fragile X Syndrome

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Introduction - Mutations of the FMR-1 (Fragile X Mental Retardation 1) gene cause a genetic condition know as Fragile X Syndrome (FXS). FXS is the most common inherited cause of mental retardation. The Fragile X knockout mouse (FX KO) is the most widely used animal model of FXS (1). Anatomical phenotyping in the mouse brain using Magnetic Resonance Imaging (MRI) has been shown in a number of mutants to be quite useful in determining specific volumetric changes (2). The purpose of this study was to assess anatomical changes between FX KO and wild type (WT) mice.

Methods - *Specimen Preparation* - Fourteen male C57/B6 fixed mice brains were examined at post-natal day 30 (P30, 7 wild-type and 7 FX KO). The mice were anesthetized and intracardially perfused. Following perfusion, the heads were removed along with the skin, lower jaw, ears and the cartilaginous nose tip. The remaining skull structures containing the brain were placed in 4% PFA and 2mM Prohance (a Gadolinium contrast agent) overnight and then transferred to PBS, 0.02% sodium azide, and 2mM Prohance for at least 7 days prior to the MRI acquisition. *MRI Acquisition* - A multi-channel 7.0 Tesla MRI scanner (Varian Inc., Palo Alto, CA) with a 6-cm inner bore diameter insert gradient set (max gradient strength 100 G/cm) was used to acquire anatomical images of brains within skulls. Three custom-built solenoid coils were used to image three brains in parallel. Parameters used in the anatomical conventional MRI scans were optimized for grey/white matter contrast: a T2- weighted, 3-D fast spin-echo sequence, with a TR of 325 ms, and TEs of 10 ms per echo for 6 echos, four averages, field-of-view of 14 × 14 × 25 mm^3 and matrix size = 432 × 432 × 780 giving an image with 0.032 mm isotropic voxels. Total imaging time was ~11 h. *Data Analysis* - To visualize and compare volumetric changes, the brains were registered together resulting in deformation fields for each individual brain, which were used to calculate individual volumes from the segmented population average. From this data the volume of 62 different structures (3) can be assessed for all 14 brains. At P30, there is still a fair amount of developmental variability in the mouse brain, and therefore the regional volume measurements were measured as a percentage of the overall brain size.

Results and Discussion - Significant differences were found in 3 regions, the arbor vita of the cerebellum, the striatum, and the cerebral cortex of the parieto-temporal lobe (Table 1). Significant volume changes in humans have also been associated with these three regions (4). The caudate nucleus, which is part of the striatum, has shown to increase in FXS in humans (4), which is contrary to what has been shown here, where a decrease in total striatal volume was found. The most significant difference, in the current work, was the arbor vita of the cerebellum, p=0.0002, false discovery rate (FDR) = 1.5%. Since the cerebellum has been closely linked with FXS in both the human and the mouse (5), the cerebellum was examined on a voxel by voxel basis to determine where the changes were localized (Figure 1). The significant decreases in the cerebellum seem to be located in specific nuclei such as the dentate nucleus (yellow arrows) and the nucleus interpositus (red arrows), which are both associated with movement. Volume and T2 hyper-intensity changes in the cerebellar nuclei are seen in Fragile X associated Tremor and Ataxia Syndrome, which is also a genetic mutation linked to the FMR-1 gene (6), and may be related to the changes seen here.

Conlcusions – Although changes in the striatum seem to be in the opposite direction of what has been shown in human there is a definite volume change in that region associated with the FMR-1 gene. This study confirms the connection between the cerebellum and Fragile X syndrome that has been shown previously (5), and furthermore this study reports specific volumetric changes within the cerebellum, which warrant histological investigation to determine the specific causes for the volume change.

References – 1) Dutch-Belgian Fragile X Consortium. *Cell* 78: 28-33, 1994, 2) Nieman et al. *Physiol Genomics* 24: 154-162 (2006), 3) Dorr et al. *Neuroimage* 42:60-69 (2008), 4) Gothelf et al. Ann. *Neurology* 63:40-51 (2008), 5) Huber, et al. *TRENDS in Neurosciences* 29:183-185 (2006), 6) Brunberg et al. *AJNR* 23:1757-1766 (2002).

Table 1 – Volume measurements (%) for significantly different regions in the FX knockout mouse and the wild-type.

Region	FX-KO	WT	p-value	FDR
Arbor Vita - Cerebellum	1.98±0.03	2.04±0.03	0.0002	0.02
Parieto Temporal Lobe	17.78±0.26	17.36±0.33	0.0077	0.16
Striatum	4.40±0.12	4.56±0.09	0.0076	0.16

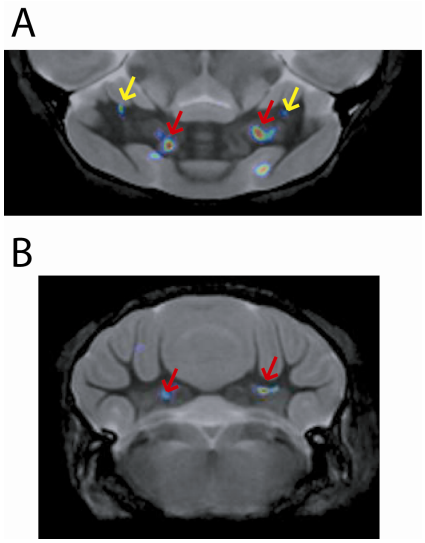

Figure 1 – Significant volume decreases in the cerebellum of the FX KO mouse when compared to the WT. A) Axial and B) Coronal. These regions have a FDR of < 10%. Red arrows indicate significant decreases in the nucleus interpositus, and yellow arrows the dentate nucleus.