Diffusion tensor imaging for assessment of brain structural abnormalities in the Mucopolysaccharidosis VII murine model

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Introduction: Mucopolysaccharidosis VII (MPS VII, Sly syndrome), is one of the family of lysosomal storage diseases (LSDs) that results from deficiency of the lysosomal enzyme β-glucuronidase[1]. Progressive lysosomal storage and distension of the cells is a hallmark of the pathological changes in MPS VII. The glucuronidase deficient MPS VII mouse model closely reflects the human disease and has been well characterized as it exhibits severe peripheral organ abnormalities, skeletal deformities, and cataracts, auditory, behavioral and neurological deficits, neurodegeneration, as well as early death, similar to humans with MPS VII[2-4]. We have earlier reported morphological abnormalities in MPS VII mice using high resolution MRI[5]. The purpose of the current study was to evaluate the sensitivity of diffusion tensor imaging (DTI) in detecting cellular abnormalities in this model since lysosomal accumulation in cells is likely to affect water mobility that can affect water diffusion anisotropy.

Materials and Methods: Age/sex match 22 week old MPS VII (*GusB -/-)* mice (n=6) on a C57BL/6 background and wild-type littermates (n=5) were included in this study.

In-vivo DTI: In-vivo MRI was performed on 4.7T, 50cm horizontal bore magnet (Varian, Palo Alto, CA) equipped with a 12cm, 250mT/m gradient set. A 20mm i.d. quadrature birdcage coil (M2M, Cleveland, OH) was used for signal transmit and receive. A diffusion weighted spin echo sequence was used to acquire 2D multi-slice images with diffusion weighting along six directions optimally selected for anisotropy measurement[6] using a b-value=996.713mm^2/s; TR=2s; TE=33ms; matrix size=128x128, FOV=20mm, averages= 2; 15 axial slices; thickness= 0.8mm; interleave slice orientation; acquisition time = 2 hours.

Ex-vivo DTI: After in-vivo imaging, the mice were anesthetized with an intraperitoneal injection of ketamine. The animals were perfused with 4% paraformaldehyde and the brains were extracted. After fixation, the brains were placed in a plastic tube with proton-free solution (Fomblin, Ausimount, Thorofare USA). Ex-vivo high resolution DTI experiments were carried out on Varian 9.4T, 8.9cm vertical bore magnet (Varian, Palo Alto, CA) using a 20 mm inner diameter loop-gap transmit receive resonator coil. DTI images were acquired using a 3D multi-echo pulsed-gradient spin echo sequence[7] by using: TR=800ms; TE=29.50ms; FOV=17mm×8.5mm×10mm; acquisition matrix size=136×68×80; number of acquisitions=6 and b-value=902mm^2/s. The diffusion-weighted images were acquired with diffusion weighting in 6 non-collinear directions in a total acquisition time of 13.19 hrs per brain sample. After the ex-vivo scan brains were stored at 4^{o}C in 4% formaldehyde solution till the time for histology.

Histology: 4 brain samples (2 MPS VII and 2 wild-type) were processed for histology. The brain samples were cut into 2mm thick axial blocks with 5-6 blocks per brain. All tissue blocks were processed for paraffin-wax embedding and were cut in a series of 5μm thick axial sections. Staining was performed according to standard methods, using hematoxylin and eosin (H&E) for morphology and Luxol fast blue (LFB), a Kluver-Barrera method[8] for myelin staining. Stained slides were scanned and digitally converted into images using a Leica dissecting microscope.

Data quantification: In-vivo and ex-vivo DTI data were processed to images and saved in DTI studio format using custom software developed in the IDL programming environment. DTI studio was used for region of interest (ROI) analysis to measure fractional anisotropy (FA) and mean diffusivity (MD×10^{-3}mm^2/s). A single ROI was placed in mid axial slice of the color coded map on the corpus callosum (CC) and bilaterally on the cerebral cortex (Cereb), hippocampus (Hippo), caudate putamen (CPu), external and internal capsule (EC and IC, Fig.1), based on the mouse brain atlas[9].

Results: Independent t-test was performed between MPS VII and wild type mice. We observed reduced FA (Fig.2) and high MD values in various different regions of the brain in MPS VII mice. Significantly decreased FA from the CC was found in MPS VII mice both in-vivo (p=0.02) as well as ex-vivo (p=0.05). Significantly decreased FA was also observed from the EC of MPS VII mice both in-vivo (p=0.03) and ex-vivo (p=0.02). Similarly, the in vivo images demonstrated significantly decreased FA (p=0.03) from the Hippo of MPS VII mice. The LFB stains from the CC are shown in Figure 1, which demonstrate reduced myelin staining and less densely packed cells in MPS VII brains.

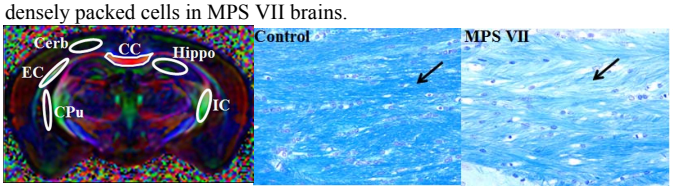

Fig. 1: Placement of ROI's in different regions of the brain. LFB stain from CC showing myelination in control and MPS VII affected mice.

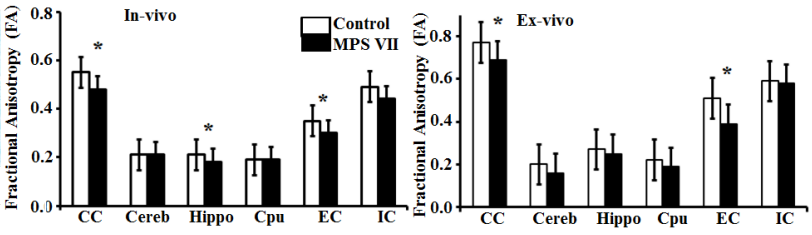

Fig. 2. FA values from different regions of the brain from In-vivo and ex vivo DTI. * p < 0.05

Discussion We demonstrate the potential of DTI in delineating microstructural brain abnormalities in MPS VII mice using DTI. Significant reductions in FA from CC and EC in these animals suggest decrease size/number of axon as well as disrupted myelination in the WM regions. Reduced myelination was confirmed by histology as LFB staining demonstrated less dense WM fibers in MPS VII mice. Earlier histological studies have also reported gliosis, loss of axons, and myelin in the brain of MPS patients and animal models[10]. It has been also reported that abnormal myelination; demyelination and Wallerian degeneration occurs in different animal models of LSDs[11]**.** The current study demonstrates that these abnormalities can be non-invasively detected using DTI. Future studies will include longitudinal DTI studies to monitor and assess enzyme replacement therapy response in this model.

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