Structural Analysis of Axonal Degeneration by MRI diffusion in an ALS Mice Model

Gatto, R.G. (Anatomy and Cell Biology, University of Illinois at Chicago), Amin, M., Mareci, T.H. (Biochemistry and Molecular Biology, University of Florida) and Magin, R.L. (Bioengineering, University of Illinois at Chicago)

Introduction:

Amyotrophic lateral Sclerosis (ALS) is characterized by the progressive degeneration and death of motor neurons. Our previous studies [1] suggested that axonal degeneration occurs early in the development of ALS. To visualize these events, we crossed the G93A-SOD1 mice with a yellow fluorescent protein (YFP) reporter mouse. Thus, the aim of this study is to determine if early changes in ALS spinal cord (SC) white matter (WM) can be visualized using ultra high field (UHF) MRI diffusion and validated using comprehensive histological methods.

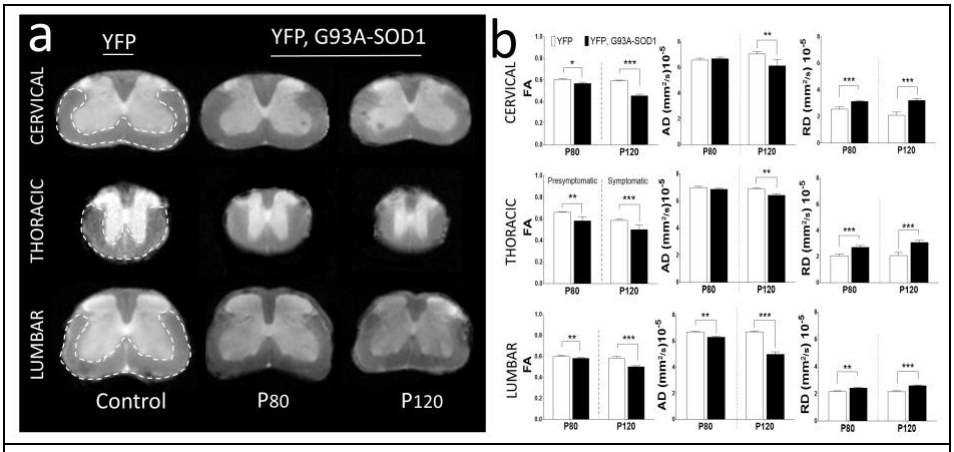

Fig.1: a – T2-weighted MRI anatomical images (0.1x0.1x0.3 mm^3 voxels) from spinal cords from mice with ALS. **b** - Changes in fractional anisotropy (FA), axial diffusion (AD) and radial diffusion (RD) can be observed at symptomatic (P120) and presymptomatic stages of the disease (P80) in the ALS mice.

Experimental:

Samples: Spinal cords (SC) were obtained from mice at 80 and 120 days of age (P80 and P120 groups) following an approved UIC Animal Care protocol (#14-162). *MRI studies*: Paraformaldehyde-fixed SC from control mice, YFP (n=4), and from ALS mice, YFP,G93A-SOD1 (n=4) at the presymptomatic (P80) and symptomatic (P120) stages were placed in individual 5 mm NMR tubes (New Era, NJ) and immersed in Fluorinert silicone oil. Scanning was achieved using a 17.6 T, 8.9 cm bore Bruker MRI scanner with 5 mm and 25 mm Quad Transceiver coils. Measurements were obtained by using a spin-echo diffusion weighted sequence with the following acquisition parameters: TR = 4000 ms, TE = 28 ms, NEX = 2, FOV = 20×20 mm^2, slice thickness = 0.3 mm, matrix size = 133x133 image resolution = 100x100x300 μm^3 and b = 700, 2500 s/mm^2). We used 12 and 64 directions of diffusion gradients, DWI acquisition time was 6 hours for b = 700, and 19 hours for b = 2500. *Histological analysis*: SCs were processed for confocal fluorescence microscopy following standard procedures. Anatomical images and ROIs were centered in cervical, thoracic and lumbar regions. DTI Studio and ImageJ software were used for data post-processing.

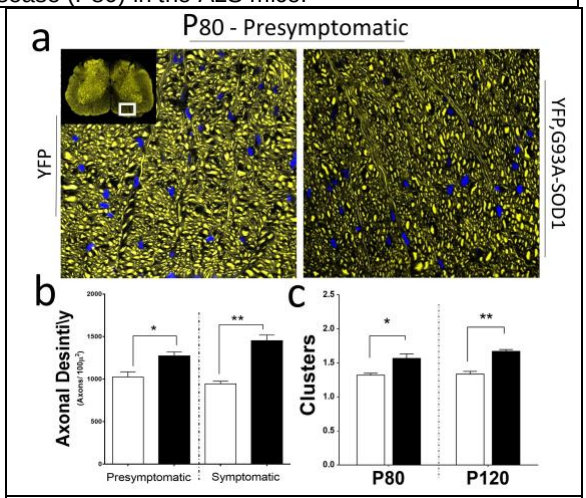

Fig.2: a, b – Histological sections from WM ROIs in lumbar segments demonstrate an early increase in axonal density in the WM SC of ALS mice, validating a significant increase in WM voxel-based fiber populations clusters at P80, calculated by UHF-MRI diffusion (**c**).

Results and Discussion:

Significant changes ($p < 0.05$) at P80 in Fractal Anisotropy (FA) (6,7 % decrease and axial diffusivity (AD) (6.8 % decrease) and radial diffusivity (RD) (14.2% increase) values were observed in lumbar WM of the YFP, G93A-SOD1 mice (ALS mice) at the presymptomatic stage (P80) **[Fig.1]**. In addition, MRI diffusion and connectome analysis demonstrated an increase in the number of fiber clusters mirroring the increase of axonal density validated by the histological techniques **[Fig2]**.

Conclusions:

Our studies demonstrate that axonal degeneration in ALS can be detected by non-invasive ultra-high field MRI diffusion techniques at very early stages of the disease. Specifically, the structural alterations visualized in the ALS fluorescent mice model validate our MRI findings. Thus, the combination of these two imaging modalities (Ultra-high field MRI and optical confocal microscopy) connect the observed MRI signals with the underlying subcellular changes in spinal WM in ALS and hence, may provide clinicians with a means to detect this disease at earlier stages.

Acknowledgements: This work was possible by funds of the Magnet Lab Visiting Scientist program at NSF-AMRIS [VSP #278/ P17430] and the Chicago Biomedical Consortium [KC Award #085740]. **References:** Gatto, RG et al (2017). Brain Research, Nov 23.