Diffusion Tensor Imaging Studies Support Pre-symptomatic Degeneration of Selective Axonal Fibers in a Mouse Model of Amyotrophic Lateral Sclerosis (Lou Gehrig's disease).

Rodolfo Gatto[1], Weiguo Li[2], Ehsan Tavassoli[1], Andrea Buenaventura[1], William Hendrickson[3], Gerardo Morfini[1], and Richard Magin[2]
[1]Department of Anatomy and Cell Biology, University of Illinois at Chicago, Chicago, Illinois, United States, [2]Department of Bioengineering, University of Illinois at Chicago, Chicago, Illinois, United States, [3]Research Resource Center, University of Illinois at Chicago, Chicago, Illinois, United States

Target audience: Radiologists working on human neurodegenerative diseases, particularly on amyotrophic lateral sclerosis (Lou Gehrig's disease).

Purpose: Amyotrophic lateral Sclerosis (ALS) is characterized by progressive degeneration and eventual death of motor neurons. A large fraction of familial, inheritable forms of ALS (FALS) results from mutations in the gene encoding Superoxide Dismutase 1 (SOD1). The discovery of SOD1 mutations in association with FALS facilitated the development of animal models that illuminated early pathogenic events and mechanisms. Among several mutant SOD1 mouse models generated, G93A-SOD1 represent the best-characterized one. The remarkable similarity of clinical phenotype observed between G93A-SOD1 mice and human ALS made this model a benchmark for pre-clinical screening of ALS therapies [1,2].

Extensive pathological evidence in G93A-SOD1 mice indicates that dying back degeneration of axons represents an early and critical pathogenic event in ALS [4]. Despite this knowledge, few studies have addressed alterations in axonal connectivity in ALS using non-invasive imaging methods. As a step towards this end, we performed correlative diffusion tensor imaging (DTI) and histological studies in white (WM) and grey (GM) matter of spinal cords obtained from pre-symptomatic G93A-SOD1 mice and wild-type (control, WT-SDO1) mice.

Methods: Animal tissues were obtained in accordance with institutional Animal Care and Use Committee regulations.

MRI studies: Paraformaldehide-fixed spinal cords (SCs) from WT-SOD1 (n=3) and G93A-SOD1 (n=3) mice were placed in 5 mm NMR tubes (New Era, NJ) and immersed in immersion oil. MRI scanning was performed using a 9.4 T 31 cm bore Agilent MRI scanner (Santa Clara, CA) with a 39mm Quad Transceiver mouse coil. Careful manual shimming was performed before DTI measurement. DTI measurements were obtained by using a spin-echo diffusion weighted sequence with the following acquisition parameters: TR = 1 s, TE = 38 ms, δ/Δ = 4/20 ms, NEX = 2, FOV = 32 mm × 32 mm, matrix size = 512 × 512, and b = 1000 s/mm2). 12 directions of diffusion gradients, plus two images with no diffusion gradients were used.

Histological Analysis: After MRI scanning, SCs were processed for immuno-histochemistry as in our previous studies [3]. Axonal fiber morphology and integrity were evaluated using antibodies recognizing phosphorylated (SMI-31) and dephosphorylated (SMI-32) epitopes of neurofilament heavy chains (NFs), major protein components of the axonal cytoskeleton. Antibodies against Myelin Basic Protein (MBP) were used to assess myelin integrity, whereas morphological alterations in GM of the ventral horn were assessed using antibodies against NeuN and the cholinergic neuronal marker ChAT.

Data Analysis: Image post-processing was performed offline using DTI Studio (Freeware, Johns Hopkins University). For mean fractional anisotropy (mFA), axial diffusivity (AD), radial diffusivity (RD) and mean diffusivity (MD) analysis, four regions of interest (ROIs) were manually selected and centered within WM corresponding to posterior (PF), lateral (LF) and anterior (AF) funiculi, as well as in GM corresponding to the ventral horn. Representative tissue sections were obtained at cervical, thoracic and lumbar levels of the SC **[Fig1A]**. Tractographic studies were further performed using Trackvis software (Freeware, Univ. of Massachusetts) across these regions. Data was obtained following a boundary of 15 degrees in fiber angle.

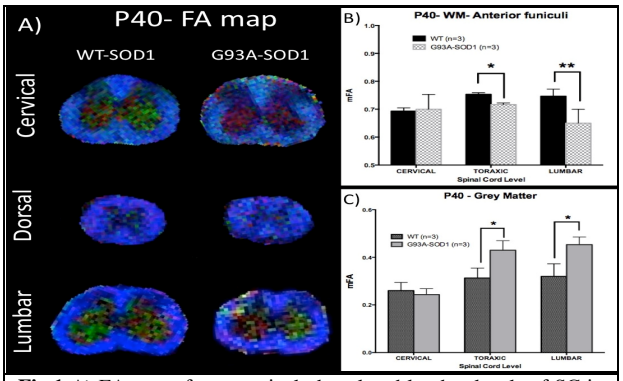

Fig.1 A) FA maps from cervical, dorsal and lumbar levels of SC in the WT-SOD1 and G93A-SOD1. B) Mean fractional anisotropy (mFA values from the Anterior funiculi (AF) of the WM. C) Mean fractional anisotropy (mFA) levels in the grey matter (GM) across different levels of the SC.

Results: Compared to WT-SOD1 mice, a significant decrease in mFA values was observed within the AF of G93A-SOD1 mice at both thoracic and lumbar levels **[Fig1B]**. Moreover, AD diffusion at the lumbar level was increased in the G93A-SOD1 mice when compared to WT-SOD1 mice. G93A-SOD1 mice also displayed increased mFA values in GM, particularly at the thoracic and lumbar levels **[Fig.1C]**. In parallel to these changes in GM, decreasing levels of AD, RD and MD were observed in thoracic and lumbar GM regions of G93A-SOD1 mice, compared to WT-SOD1 mice. Immuno-histochemistry data from GM supported these findings, further providing a structural basis for the changes in mFA, AD, RD, and MD above. Consistent with the dying-back-pattern of degeneration characteristic of ALS, tractography-based data obtained from the AF, LF and in the PF at the lumbar region further revealed a reduction in the mean length of axonal fibers of G93A-SOD1, compared to WT-SOD1 mice (PF length WT *vs*. G93A-SOD1, $p<0.02$).

Discussion and Conclusions: Taken together, results from our studies are consistent with variable degrees of axonal degeneration within different SC areas of G93A-SOD1 mice, where axons in the AF region being the most affected. Significantly, mFA changes followed a distal to proximal progression, consistent with the dying back pattern of motor neuron degeneration observed in ALS [5]. In terms of GM, an increment in the mFA and restricted diffusion coefficients were observed, probably due to early biochemical and structural alterations in neuronal cell bodies **[Fig. 2]**. By tractography analysis, a significant reduction in fiber length was found in the PF **[Data not shown]**.

The establishment of non-invasive methods to evaluate axonal degeneration in G93A-SOD1 mice will help address the effectiveness of therapeutic strategies aimed to preserve axonal connectivity in this ALS model.

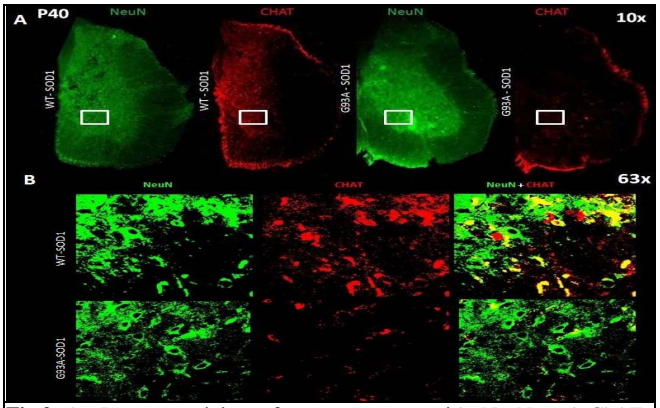

Fig.2 A) Immuno-staining of motor-neurons with NeuN and ChAT antibody in the lumbar spinal cord of the P40 WT-SOD1 and G93A-SOD1 mice. B) Magnification of NeuN and ChAT staining in the GM

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