Diffusion MRI of the spinal cord allows in vivo early detection and monitoring of GM and WM degeneration in a murine ALS model

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TARGET AUDIENCE: Pre-clinical MRI researchers, Physicists, Neurologists, Biologists involved in research on amyotrophic lateral sclerosis.

PURPOSE. Amyotrophic lateral sclerosis (ALS) is a fatal disease characterized by selective degeneration of motor neurons in the motor cortex, brainstem and spinal cord. Its diagnosis is based on clinical and electrophysiological criteria. Diffusion-weighted magnetic resonance imaging, and in particular diffusion tensor imaging (DTI[1]), might be helpful to assess motor neuron degeneration in patients non-invasively and to stratify ALS patients[2-7]. The purpose of this study was to examine the potential of diffusion parameters derived from in vivo DTI, such as fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD) in the detection of white matter (WM) and gray matter (GM) degeneration in the lumbar spinal cord of a murine ALS model, to verify whether DTI abnormalities correlate with disease severity and progression.

METHODS. DTI experiments were carried on a 7T scanner (Bruker BioSpec 70/30) in 7 transgenic G93A-SOD1 (B6SJL-Tg(SOD1*G93A)1Gur) mice carrying a high-copy number of mutant human allele SOD1 and 7 transgenic WT-SOD1 (B6SJL-Tg(SOD1)2Gur/J) mice at 5 time points: 9, 10, 12, 15 and 17 weeks of age. The DTI protocol was a single-shot EPI sequence with the following parameters: 12 directions of the diffusion-encoding gradients, b = 1200 s/mm^2, δ = 4 ms, Δ = 11 ms, TE = 23.3 ms, TR = 2100 ms, 5 volumes without diffusion weighting, 4 averages, 15 repetitions. Eight contiguous axial slices of the spinal cord at the lumbar level with a slice thickness of 0.8 mm, an in-plane resolution of 0.109 x 0.078 mm^2 and a FOV of 1.4 x 1 cm^2, were obtained. The acquisition time was about 30 minutes. All the images were corrected for motion and distortions using FLIRT[8]. Fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD) were estimated using a homemade code in MatLab. Eight regions of interest (ROIs) were manually outlined in ventral (vWM), left/right ventrolateral (vll/vlrWM), left/right dorso-lateral (dll/dlrWM) and dorsal (dWM) WM, ventral (vGM) and dorsal (dGM) GM, as shown in Figure 1. The difference in diffusion parameters between G93A-SOD1 and WT-SOD1 groups in each ROI at each time point was evaluated by a Mann-Whitney's non-parametric test with a significance level of $p < 0.05$. Histological analysis was performed on lumbar spinal cord semi-thin sections (1.5µm) from two male G93A-SOD1 and WT-SOD1 mice at 7, 8, 10, 12, 15, and 18 weeks of age. The number of α-motor neurons was calculated on one ventral horn area of lumbar spinal cord tissue sections. Transmission electron microscopy (EM) was performed in lumbar spinal cord ultra-thin sections (0.08µm) from two male G93A-SOD1 and WT-SOD1 mice at the same weeks of age.

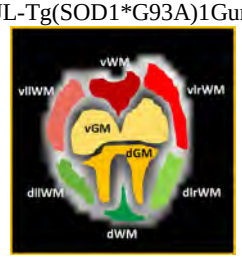

Figure 1. The ROIs considered for the analysis of DTI parameters, overlaid on an FA map.

RESULTS. In G93A-SOD1 mice, FA values were significantly reduced in all WM ROIs since 15 weeks, with the only exception of dWM, where they were reduced only at 17 weeks of age. MD values were significantly reduced since 10 weeks in vllWM and vlrWM, since 12 weeks in vWM, dllWM and dlrWM, since 15 weeks in dWM, but only in vWM at 17 weeks. AD values were significantly reduced since 10 weeks in vllWM and vlrWM, since 12 weeks in the other WM ROIs. RD values were significantly reduced only at 12 weeks in vllWM and vlrWM, at 17 weeks in vlrWM and dlrWM. In GM, FA values were significantly reduced since 15 weeks in vGM and at 17 weeks in dGM; diffusivities were significantly reduced since 12 weeks of age (Figure 2).

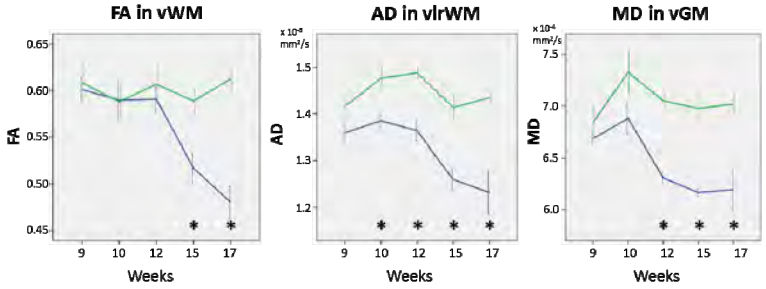

Figure 2. Mean values of representative DTI parameters, obtained in G93A-SOD1 (blue) and WT-SOD1 mice (green) over time. Asterisks mark significant differences.

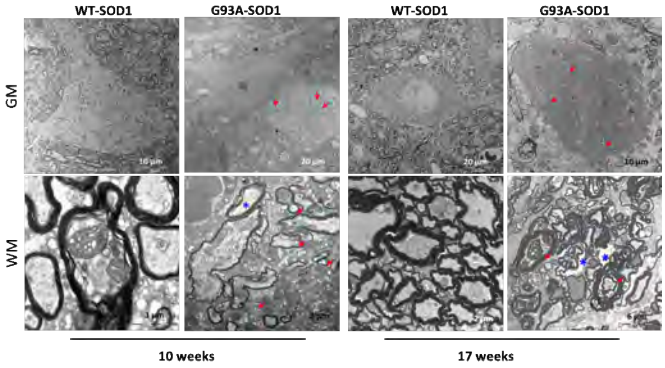

Figure 3. Representative electron microscopy in GM (top) and WM (bottom) of G93A-SOD1 and WT-SOD1 mice at 10 and 17 weeks of age. Red arrows highlight vacuoles, whereas blue asterisks show degenerated axons with disorganized myelin sheets.

Histological analysis of the spinal cord tissues highlighted the reduction of the number of motor neurons since 12 weeks. EM analysis showed vacuolization and axonal degeneration in G93A-SOD1 mice compared to controls since 10 weeks (Figure 3).

DISCUSSION AND CONCLUSION. The overall results of our dMRI analysis highlighted spinal cord microstructural alterations in an ALS mouse model since 10 weeks of age, earlier than in all previous MRI studies[9-10]. AD showed the best sensitivity to pathology in WM; MD detected very early alterations as well, but less reproducible at later time points. In GM all the diffusivity parameters provided significant differences since 12 weeks of age. The dMRI results were confirmed by histological analysis and electron microscopy. In conclusion, our study proposes dMRI parameters, and in particular AD in WM and MD in GM, as non-invasive biomarkers for early detection and monitoring of neurodegeneration in mouse spinal cord.

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