Longitudinal Correlation of T2 and Motor Neuron Loss in the SOD1G93A Mouse Mode of Amyotrophic Lateral Sclerosis

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Background:

Amyotrophic lateral sclerosis (ALS) is a devastating neurological disease characterized by motor neuron loss eventually leading to paralysis and death. A mouse model transgenically overexpressing the familial ALS SOD1^{G93A} mutation phenocopies many aspects of the human disease. Previous studies have confirmed the loss of motor neurons in the brain stem of mice with this mutation at late time points [1]. T_2 changes and vacuole formation have been shown to follow a similar time course [2]. In this study, we define the relationship between T_2 values and motor neuron density longitudinally in the SOD1^{G93A} transgenic mouse line with corresponding MRI and histology at each time point. This longitudinal characterization will further validate T_2 MRI as a valuable tool to characterize neuronal loss and facilitate the use of T_2 MRI as a preclinical readout of potential therapies for neurodegenerative disease.

Methods:

Animal Model: Animal procedures were approved by the institutional AAALAC-accredited review board. Twenty seven SOD1^{G93A} pos and 5 SOD1^{G93A} neg mice to serve as a control were divided into 7 age matched groups to be imaged and taken down for histology at 14 day intervals. Mice were anaesthetized with 1.5% isoflurane and maintained at 37 C for imaging.

Imaging: MRI data were obtained on a Varian 9.4T system with a 30 mm transmit/receive volume coil. Sixteen contiguous axial slices were obtained of the spin–spin relaxation time (T_2) using a multi echo multi slice (MEMS) sequence with parameters: TR 4000 ms, 8 echo images, TE 10ms, slice thickness 0.8mm, FOV 25.6 mm x 25.6 mm, matrix size 256 x 128, NEX 4. For data analysis, a set of ROIs were defined that encompassed the facial nucleus, nucleus trigeminus and nucleus hypoglossus.

Histology: Mice were sacrificed 24-48 hours after their final MRI. Twenty μm thick sections of brainstem were stained using a standard Nissl protocol. Neurons were manually counted in each region.

Results:

In the brainstem of SOD1^{G93A} mice, longitudinal T_2 maps showed hyperintense regions and a quantifiable increase in T_2 beginning by 84 days of age in the facial nucleus, nucleus trigeminus and nucleus hypoglossus and continuing until late stage disease at 143 days of age (fig 1). T_2 changes from baseline in the SOD1^{G93A} pos mice reach significance ($p<0.001$) by 84 days of age in the facial nucleus (fig 2), nucleus trigeminus and nucleus hypoglossus (data not shown). A subset of mice for each time point were euthanized shortly after imaging for Nissl staining to assess for motor neuron counts. Histology confirmed a decrease in motor neurons at 84 days and an increase in vacuolization (fig 3). Using the same mice for T_2 quantification of the hyperintense regions seen in the MRI and histological counting of motor neurons in those regions, we demonstrated that in SOD1^{G93A} positive mice, T_2 values and neuron count are correlated in the facial nucleus region (R^2 = 0.66, $p<0.0001$, fig 4). Additionally, the nucleus trigeminus and nucleus hypoglossus regions were analyzed and demonstrated a similar correlation (data not shown).

Conclusion:

In the SOD1^{G93A} mouse model of ALS, we demonstrated that T_2 values obtained for the facial nucleus, nucleus trigeminus and nucleus hypoglossus are inversely correlated with motor neuron count in these nuclei. In addition, longitudinal imaging enabled us to detect significant change in T_2 between SOD1^{G93A} pos and control mice at 84 days of age. The T_2 and histological correlation data will enable treatment studies to assess both delay of onset of motor neuron loss as well as motor neuron rescue at late time points using a single cohort of animals.

***Figure 1*: T_2 maps and corresponding Nissl staining in the facial nucleus of the same mice A) SOD1^{G93A} neg control mouse B) SOD1^{G93A} pos mouse at 84 days of age C) SOD1^{G93A} pos mouse at 143 days of age.**

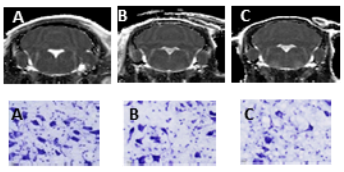


***Figure 2:* Changes in T_2 (ms) in the facial nucleus region of SOD1^{G93A} pos and neg mice relative to baseline.**

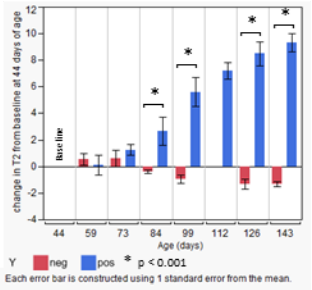


***Figure 3:* Age dependent changes in T_2 (ms) and neurons in the facial nucleus region of SOD1^{G93A} pos mice.**

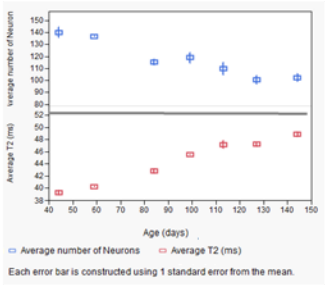
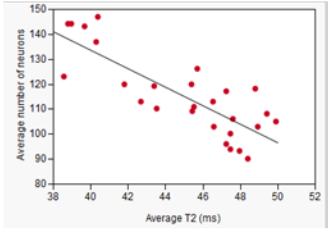


***Figure 4*: Correlation of T_2 (ms) and neurons in the facial nucleus region of SOD1^{G93A} pos mice. R^2 = 0.66, $p<0.0001$**



[1] Zang DW, et al Magnetic resonance imaging reveals neuronal degeneration in the brainstem of the superoxide dismutase1 $^{G93A\ G1H}$ transgenic mouse model of amyotrophic lateral sclerosis. *European Journal of Neuroscience.* 2004; 20: 1745–1751.

[2] Bucher S, et al Vacuolization correlates with spin–spin relaxation time in motor brainstem nuclei and behavioural tests in the transgenic G93A-SOD1 mouse model of ALS. *European Journal of Neuroscience.* 2007; 26: 1895–1901.