Longitudinal Correlation of T2 and Motor Neuron Loss in the SOD1G93A Mouse Model 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 SOD1G93A 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. T2 changes and vacuole formation have been shown to follow a similar time course. In this study, we define the relationship between T2 values and motor neuron density longitudinally in the SOD1G93A transgenic mouse line with corresponding MRI and histology at each time point. This longitudinal characterization will further validate T2 MRI as a valuable tool to characterize neuronal loss and facilitate the use of T2 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 SOD1G93A pos and 5 SOD1G93A 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 anesthetized 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 (T2) using a multi echo multi slice (MEMS) sequence with parameters: TR 4000 ms, 8 echo images, TE 10 ms, slice thickness 0.8 mm, 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 SOD1G93A mice, longitudinal T2 maps showed hyperintense regions and a quantifiable increase in T2 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). T2 changes from baseline in the SOD1G93A 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 T2 quantification of the hyperintense regions seen in the MRI and histological counting of motor neurons in those regions, we demonstrated that in SOD1G93A positive mice, T2 values and neuron count are correlated in the facial nucleus region (R2 = 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 SOD1G93A mouse model of ALS, we demonstrated that T2 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 T2 between SOD1G93A pos and control mice at 84 days of age. The T2 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: T2 maps and corresponding Nissl staining in the facial nucleus of the same mice. A) SOD1G93A neg control mouse B) SOD1G93A pos mouse at 84 days of age C) SOD1G93A pos mouse at 143 days of age.

Figure 2: Changes in T2 (ms) in the facial nucleus region of SOD1G93A pos and neg mice relative to baseline.

Figure 3: Age dependent changes in T2 (ms) and neurons in the facial nucleus region of SOD1G93A pos mice.

Figure 4: Correlation of T2 (ms) and neurons in the facial nucleus region of SOD1G93A pos mice. R2 = 0.66, p<0.0001.

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