Sensitivity of ultra-high field MRS to Recovery from Neurodegeneration in a Conditional Mouse Model: A multi-modal investigation with Histology, Behavioral Testing and Quantitative PCR

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Target audience: MR spectroscopists, neurologists, neuroscientists.

Purpose: Spinocerebellar ataxia type 1 (SCA1) is a hereditary, polyglutamine-induced neurodegenerative disorder that results in loss of motor coordination primarily caused by cerebellar Purkinje cell dysfunction and loss. The sensitivity of ultra-high field ^{1}H MRS to reversal of neurodegeneration was recently demonstrated by using a conditional transgenic mouse model of SCA1[1]. In this model the cerebellar pathology and ataxic phenotype are reversible by suppressing transgene expression with doxycycline[2]. Here we determined **1)** if MRS reflects the extent of disease reversal by correlating MRS measures to histology and residual transgene expression as assessed by qPCR and **2)** the relative sensitivities of MRS and the standard motor behavioral testing (Rotarod) to disease reversal in a comprehensive multi-modal investigation.

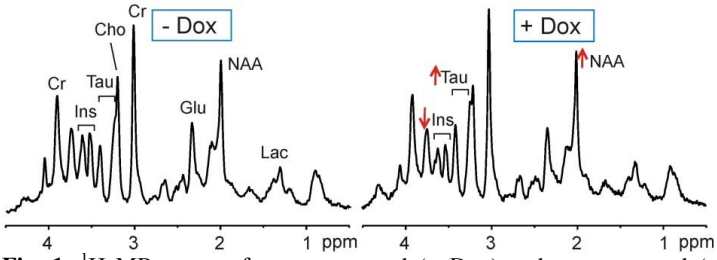

Fig. 1: ^{1}H MR spectra from one treated (+ Dox) and one untreated (- Dox) conditional SCA1 mouse at 24 weeks. Cr: creatine; Cho: choline; Tau: taurine; Glu: glutamate; NAA: N-acetylaspartate; Ins: *myo*-inositol, Lac: lactate. The changes in NAA, Ins and Tau with treatment are marked with arrows.

Methods: Conditional SCA1 mice (N=28) and wild type controls (background strain tTA, N=14) were scanned at 9.4 tesla under 1.5 - 2% isoflurane anesthesia at ages 12 and 24 weeks with a quadrature surface coil. Half of the SCA1 mice were treated with doxycycline from 12 to 24 weeks. Spectra from the cerebellum (5 - 7μL VOI) were acquired with a short echo (TE = 15 ms) localization by adiabatic selective refocusing (LASER) sequence[3]. Metabolites were quantified with LCModel[4] using unsuppressed water as reference. Reliable concentrations were selected based on Cramér-Rao lower bounds (CRLB) criteria[1]. Motor ability of the mice was assessed using an accelerating Rotarod apparatus on 4 consecutive days[2], one of which coincided with the second and last MR scan. After the last MR scan/Rotarod assessment, brains were harvested and cerebellum bisected. Half of the cerebellum was used for histology (with hematoxylin-and-eosin staining) and the other half for residual ataxin-1 transgene expression (with qPCR). Statistical significance of group differences was assessed using the two-tailed, unpaired student's t-test.

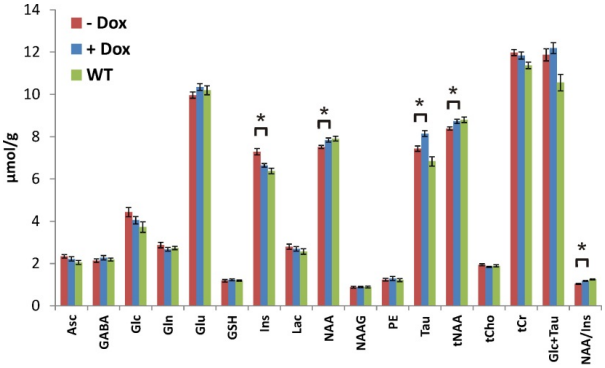

Fig. 2. Average (± SEM) metabolite concentrations at 24 weeks in treated (+ Dox) and untreated (- Dox) SCA1 mice and wild type controls (N = 14 in each group). Changes between treated and untreated mice are shown (*), $p \leq 0.01$ in all cases.

Results: Consistent with prior observations[1], MRS-detected neurochemicals were sensitive to disease reversal by transgene suppression (Fig. 1). Specifically, significant differences were detected in the neuronal marker NAA, the putative glial marker *myo*-inositol (Ins) and the osmolyte taurine between the doxycycline treated vs. untreated groups post-treatment (Fig. 2). The NAA/Ins ratio correlated significantly with the molecular layer thickness ($p < 0.005$) and residual transgene expression ($p < 0.001$), indicating that this measure accurately reflects the underlying pathology and extent of disease reversal (Fig. 3). While NAA/Ins was highly significantly different between the treated vs. untreated groups ($p = 0.0001$), similar to the invasive measures (molecular layer thickness $p < 0.0001$; relative residual transgene expression $p < 0.0001$), the Rotarod assessment only showed a trend for treatment effect due to the large variance between mice (mean latency to fall on Day 4 = 197 ± 23 (SEM) sec in dox-treated group vs. 142 ± 21 sec in the untreated group, $p = 0.08$).

Conclusion: Ultra-high field MRS accurately reflects the extent of recovery from neurodegeneration and is more sensitive than behavioral measures in detecting treatment effects, thereby showing great potential utility in pre-clinical and clinical treatment trials.

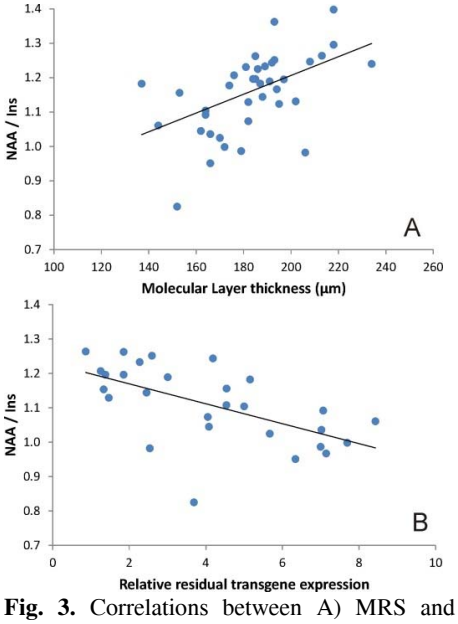

Fig. 3. Correlations between A) MRS and histology, b) MRS and qPCR.

References: 1. Oz et al, Exp Neurol, 232: 290, 2011. **2.** Zu et al, J Neurosci, 24: 8853, 2004. **3.** Garwood and DelaBarre, J Magn Reson, 153: 155, 2001. **4.** Provencher SW, MRM, 30: 672, 1993. *Supported by NIH grants R01 NS070815, P41 RR008079, P41 EB015894, P30 NS076408, and the W.M. KECK Foundation.*