^{1}H MRS Reveals Decreased Motor Cortex Glutathione in Patients with ALS

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Objectives

Oxidative stress has been implicated in both sporadic and familial forms of amyotrophic lateral sclerosis (ALS), suggesting an inadequate antioxidant defense system, of which glutathione (GSH) is the most abundant and important component. While GSH deficiency has been documented and associated with cell pathology and survival in preclinical models of ALS, direct in vivo evidence in patients' brain is lacking. In this study, we used ^{1}H MRS to measure and compare in vivo levels of GSH in the motor cortex of ALS patients and matched healthy volunteers (HV).

Methods

Subjects: This cross-sectional observational study enrolled 12 ALS patients, diagnosed according to El Escorial criteria, and 11 age-matched HV.

In vivo Brain GSH Measurements by ^{1}H MRS: All *in vivo* brain GSH spectra were recorded from a single 20x25x25mm^3 precentral gyrus voxel (**Fig. 1**) in the clinically most affected (or, in HV, the dominant) hemisphere, on a 3.0 T GE MR system, using the standard J-edited spin echo difference method and an 8-channel phased-array head coil. Briefly, volume-selective J-editing detection of GSH was accomplished by incorporating into the standard PRESS sequence a pair of frequency-selective "editing" pulses before and after the second 180^0 rf pulse flanked by spoiler gradients of opposite signs. Each frequency-selective editing pulse was applied at 4.56 ppm (the frequency of the GSH cysteinyl α protons) on alternate scans with TE/TR 68/1500 ms, resulting in alternated inversion of the GSH cysteinyl β doublet at 2.9 ppm by alternatively inhibiting and allowing its J-modulation. Subtracting two subspectra thus acquired in 15 min with 580 interleaved excitations yielded the desired GSH resonance at 2.9 ppm, while the much stronger overlapping tCr resonance -- a singlet that is not J-modulated -- is eliminated. The result of implementing this GSH editing method is shown in **Fig. 1**. GSH peak areas were derived by frequency-domain spectral fitting and expressed as ratios relative to the area of simultaneously acquired unsuppressed voxel tissue water (W). The following secondary metabolites were obtained from the same voxel using either J-editing or CT-PRESS and then also expressed as area ratios relative to W: NAA, Lactate, GABA, glutamate. Normality of the data was confirmed by Shapiro-Wilk tests and independent sample t-tests were performed to compare levels of GSH and those of the other metabolites in ALS patients and HV subjects. Potential age effects were assessed by ANCOVA with age as a covariate.

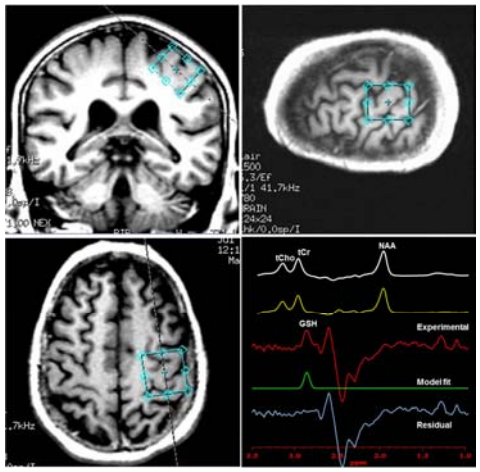

Figure 1: Voxel placement and sample measured and fitted GSH spectra

Results

Motor cortex GSH/W was significantly decreased in the ALS group ([1.2 ± 0.3] x10^{-3}) compared to HV ([1.6 ± 0.5] x10^{-3}) (t(21)=2.71, p=.001, **Fig. 2**). Also NAA/Cr was significantly decreased (p=.013, **Fig. 2**) in ALS patients (2.00 ± 0.13) compared to HV (2.25 ± 0.17). There were no significant differences between the groups for any other metabolite or age. Age had no significant effect on GSH/W in our sample (p=0.168).

Discussion

To our knowledge, this is the first study to show a significant cortical GSH deficit in vivo in patients with ALS, supporting a potential role for oxidative stress in the disorder, and suggesting the viability of exploring treatment strategies of using GSH-elevating compounds such N-acetylcysteine (NAC) for neuroprotection. Our finding of decreased NAA/Cr in ALS is consistent with prior MRS studies and with neurodegeneration in the disorder. Further studies are warranted to investigate MRS measurement of GSH and/or NAA as potential noninvasive biomarkers of the disorder and of therapeutic response monitoring.

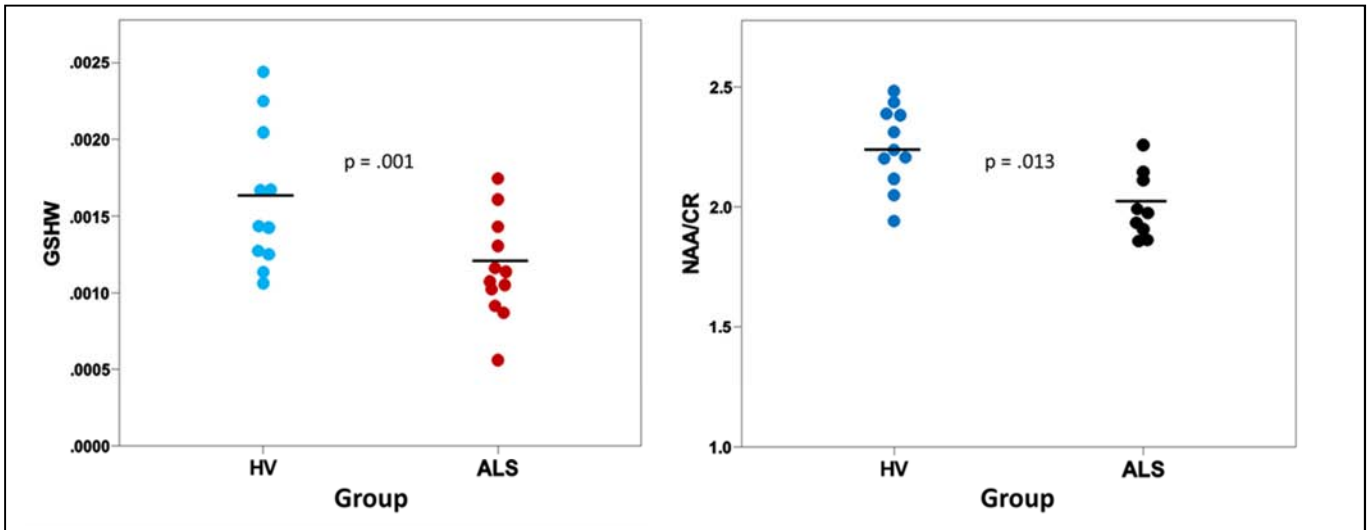

Figure 2: Motor cortex GSH/W and NAA/Cr are lower in ALS patients compared to healthy volunteers