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Early detection and longitudinal changes in amyotrophic lateral sclerosis by ^1H MRSI

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Abstract

Objective—To determine 1) the reproducibility of metabolite measurements by ^1H MRS in the motor cortex; 2) the extent to which ^1H MRS imaging (MRSI) detects abnormal concentrations of *N*-acetylaspartate (NAA)-, choline (Cho)-, and creatine (Cre)-containing compounds in early stages of ALS; and 3) the metabolite changes over time in ALS.

Methods—Sixteen patients with definite or probable ALS, 12 with possible or suspected ALS, and 12 healthy controls underwent structural MRI and multislice ^1H MRSI. ^1H MRSI data were coregistered with tissue-segmented MRI data to obtain concentrations of NAA, Cre, and Cho in the left and right motor cortex and in gray matter and white matter of nonmotor regions in the brain.

Results—The interclass correlation coefficient of NAA was 0.53 in the motor cortex tissue and 0.83 in nonmotor cortex tissue. When cross-sectional data for patients were compared with those for controls, the NAA/(Cre + Cho) ratio in the motor cortex region was significantly reduced, primarily due to increases in Cre and Cho and a decrease in NAA concentrations. A similar, although not significant, trend of increased Cho and Cre and reduced NAA levels was also observed for patients with possible or suspected ALS. Furthermore, in longitudinal studies, decreases in NAA, Cre, and Cho concentrations were detected in motor cortex but not in nonmotor regions in ALS.

Conclusion—Metabolite changes measured by ^1H MRSI may provide a surrogate marker of ALS that can aid detection of early disease and monitor progression and treatment response.

ALS is a neurodegenerative disorder that causes rapid loss of motor neurons in the brain and spinal cord leading to paralysis and death. Diagnosis is based solely on clinical data, and there is no definitive diagnostic test for ALS and no surrogate marker for directly measuring disease progression. Therefore, an objective and quantitative method would be extremely helpful to evaluate viability and functioning of upper motor neurons in ALS, identify individuals at an early stage of the disease, and monitor responses to treatment.

Structural MRI studies of ALS have revealed little evidence of brain atrophy and other findings, including T2-weighted MRI signal changes that were inconsistent.¹ Proton MRS (^1H MRS) measures levels of *N*-acetylaspartate (NAA), a compound that is localized in high concentration in neurons and their processes but is virtually absent in nonneuronal tissue, including glial

cells.^{2,3} Thus, NAA is considered a neuron-specific marker that could provide an index of neuronal density. In addition, reversible changes in NAA levels measured by ¹H MRS have been reported in ALS following treatment trials⁴ as well as in temporal lobe epilepsy after surgery,⁵ suggesting that NAA concentrations reflect neuronal metabolism in addition to neuron density. Therefore, MRS measurements of NAA may provide a sensitive indicator of upper motor neuron degeneration that can be used as an early marker of ALS and to monitor progression and effects of treatment.

In addition to NAA, ¹H MRS measures resonances from choline (Cho)- and creatine (Cre)-containing compounds in the brain. Cre represents a combination of creatine and phosphocreatine, a putative marker of gliosis,^{3,6} and Cho is thought to be a marker associated with membrane phospholipids.⁷

In most previous MRS studies of ALS,^{4,8-20} sampling of metabolite signals from the motor cortex was limited, because measurements were restricted to a rectangular region within the brain that was sufficiently distant from the skull to avoid interferences with an intense signal from extracranial lipids. Furthermore, because the selected regions of interest were generally large, including unavoidably some white matter and nonmotor tissue, results from these studies may have been skewed to the extent that white matter and nonmotor regions contributed to the metabolite signal from the motor cortex. With use of a multiplanar ¹H MRS imaging (MRSI) technique that accomplishes sampling of larger regions in the brain (including the surface cortex), significant reductions in NAA/(Cre + Cho) ratios in the motor cortex and posterior internal capsule in the brain during ALS and normal ratio levels in nonmotor brain regions have been reported.²¹ Subsequently, quantitative reevaluation of these ¹H MRSI data²² showed that reduced NAA/(Cre + Cho) ratios in the motor cortex regions in the brain during ALS were primarily due to diminished NAA levels (as expected), while reductions in the posterior internal capsule were due to increased Cho levels (not expected). However, contributions from gray and white matter to the metabolite signal were not distinguished in this previous study, which complicated interpretation of the results.

The major goals of this study were as follows: 1) to determine reproducibility of ¹H MRSI measurements in the motor cortex; 2) to determine the extent to which ¹H MRSI detects changes in NAA, Cre, and Cho levels specifically in the gray matter of the motor cortex during possible or suspected as well as probable or definite ALS; and 3) to measure metabolite changes over time in different gray and white matter regions in the brain during ALS.

Subjects and methods

Subjects

The Committee on Human Research at the University of California at San Francisco approved the study, and all subjects provided written informed consent before examination by MRI and MRS. Sixteen patients with definite or probable ALS (as defined by the diagnostic criteria of El Escorial World Federation of Neurology²³), 12 with possible or suspected ALS (8 women and 20 men; mean age, 57.7 years), and 12 healthy controls (4 women and 8 men; mean age, 49.7 years) underwent MRI and multislice ¹H MRSI (1.5-Tesla Magnetom VISION system, Siemens, Erlangen, Germany). Patients were studied at 1 month and again every 3 months after initial scanning.

Acquisition of MRI and MRSI scans

Proton-density and T2-weighted MR images were acquired by using a turbo spin-echo sequence (echo time, 14/85 ms; repetition time, 7,000 ms; 3-mm slice thickness; 1.0 × 1.0 mm² inplane resolution; acquisition time [AT], 4 minutes) covering the entire brain and oriented along the anterior–posterior commissure (AC-PC) line. A volumetric T1-weighted

gradient echo MRI scan of the entire brain was acquired (echo time, 4 ms; repetition time, 10 ms; flip angle, 15°; $1.0 \times 1.0 \text{ mm}^2$ in-plane resolution; AT, 7 minutes) that was angled perpendicular to the AC-PC line with a 1.5-mm effective slice thickness. Together, proton density and T2- and T1-weighted MR images were used for tissue segmentation (see below).

Multislice ^1H MRSI data, obtained with use of a spin-echo sequence, were acquired from three axially oblique multislice planes with a 15-mm thickness that were positioned as shown in figure 1. One slice was at the level of the internal capsule, and two slices were supraventricular with coverage through motor cortical regions and centrum semiovale. To perform longitudinal studies on the exact same brain regions, positions of the ^1H MRSI slices were determined for each subject relative to an imaginary line that connects the AC-PC, as seen on high-resolution scout MRI scans. In the initial study, the distance between each slice and the AC-PC line was measured and recorded, and in subsequent studies, the ^1H MRSI slices were placed in the exact same positions relative to the AC-PC line.

^1H MRSI parameters

The ^1H MRSI parameters were as follows: repetition time/echo time, 1,800/70 ms; 36 phase-encoding steps in each direction with circular sampling; $260 \times 260\text{-mm}^2$ field of view providing $7.5 \times 7.5 \text{ mm}^2$ nominal in-plane resolution; and AT, 30 minutes. A slice-selective inversion pulse was applied 165 ms before the first excitation to provide inversion–recovery nulling of lipid resonances. Chemical shift-selective²⁴ water suppression was accomplished by using three frequency-selective radio frequency pulses.

MRI and MRSI processing

MRI segmentation into the primary tissue categories of gray matter, white matter, and CSF was performed by an automated procedure, as described previously with minor modifications.²⁵ In addition, an experienced operator marked the left and right motor cortex on MRI scans as well as hemispheric, rolandic, and sylvian fissures, yielding four gray matter subcategories of left/right motor and frontal/parietal cortex. The motor cortex was defined as the first gyrus anterior to the central sulcus. The temporal lobe was not categorized, because the ^1H MRSI slices did not cover this region, and white matter was not further categorized into lobes, because of missing anatomic landmarks.

Processing of the ^1H MRSI data has been described previously²⁶ and included k-space zero filling to 64^2 points, Fourier reconstruction, selective k-space extrapolation,²⁷ and fully automated spectral fitting of the peak areas of NAA, Cre, and Cho, based on a parametric model spectrum and a nonparametric baseline model.²⁸ Quality control was assured by rejecting spectra with $<4 \text{ Hz}$ or $>12 \text{ Hz}$ line width at half peak height or those with variations of the peak location of more than $\pm 0.05 \text{ ppm}$. This resulted in rejection of approximately 4% of spectra. No patients were excluded from any of the analyses. Finally, the peak areas were corrected for receiver gain and expressed relative to the intensity of the median ventricular CSF level for each subject, as measured from proton density MRI. All metabolite concentrations are expressed in arbitrary units.

MRI and MRSI co-analysis and linear regression

Because MRSI provides many voxels from different locations in the brain, one approach for separation of gray and white matter contributions to the metabolite signal is the use of linear regression to evaluate the relationship between metabolite changes and gray and white matter variations in the voxels. This approach is possible when MRI is employed to estimate the amount of gray and white matter contained in the MRSI voxels. The principle concept in estimating metabolite concentrations with use of linear regression has been previously described in some detail²⁶. In this study, a similar concept was applied for estimations of NAA,

Cre, and Cho concentrations in gray matter of the left or right frontal or parietal lobes and white matter. Finally, these estimations of metabolite concentrations were then used to account for nonmotor cortex impurities in MRSI voxels within the motor cortex and to calculate motor cortex concentrations of NAA, Cre, and Cho. The most affected motor cortex (determined by the upper motor neuron score and other clinical data) was used in all data analyses. To determine the method that produced the least variability in motor cortex data, we compared our results by using volume-corrected metabolite values, volume-corrected metabolite values corrected for instrumental parameters, and volume-corrected metabolite values corrected for instrumental parameters and nonmotor cortex contribution. Simple volume-corrected metabolite values corrected for instrumental parameters only had the least variability (lowest SD) in motor cortex data, and these values are used throughout.

Reproducibility of NAA measurements in the motor cortex was tested by studying seven controls and seven patients with ALS twice within a 2-week period; reproducibility was expressed as an interclass correlation coefficient (ICC), where ICC is defined as (between-subject variance)/(total variance). Total variance is defined as (between-subject variance) + (within-subject variance) + (random error variance).

Statistical analysis

Between-group comparisons were analyzed by using two-tailed *t* tests.

Results

Measurement variability

The ICC for NAA was 0.53 in the motor cortex and 0.83 in nonmotor cortex tissue (0.88, frontal lobe; 0.73, parietal lobe). The ICC for Cre was 0.62 in the motor cortex and 0.79 in nonmotor cortex tissue (0.85, frontal lobe; 0.70, parietal lobe). The ICC for Cho was 0.71 in the motor cortex and 0.90 in the nonmotor cortex (0.88, frontal lobe; 0.86, parietal lobe).

Comparison of patients with controls

Table 1 shows metabolite concentrations and ratios in the motor cortex. There were a number of significant differences between the metabolites in the most affected motor cortex for the subjects with probable or definite ALS and those for the controls; NAA/Cre, NAA/Cho, and NAA/(Cre + Cho) ratios were significantly decreased. The Cho level was significantly increased. The NAA level was lower, and the Cre level was higher; however, these changes were not significant. For the subjects with possible or suspected ALS, all of the absolute values of the metabolites and the metabolite ratios trended in the same direction as for the subjects with probable or definite ALS, but these changes were not significant. In the least affected motor cortex, similar changes were found.

We compared gray and white matter in both hemispheres between the patients and controls and found no significant differences in nonmotor cortex brain tissue. The purpose of this analysis was to show that changes we were observing were seen only in motor regions in the brain during ALS and were absent from nonmotor regions in the brain during ALS. We also compared the right and left motor cortex of controls and found no asymmetry of motor cortex in controls. However, we found right or left hemisphere differences in Cre and Cho levels between controls and patients, but no asymmetry of NAA was found. Previously, other researchers reported right or left differences,²⁹ while others reported no asymmetry³⁰ in the brain. Further work is needed to verify these results.

Longitudinal changes in NAA levels in the motor cortex

Table 2 shows results for all 9 subjects with ALS (6, possible or suspected; 3, probable or definite) from whom a minimum of three sequential measurements were determined. For the most affected motor cortex, NAA/Cre and NAA/(Cre + Cho) ratios decreased significantly after 1 month. After 3 months, absolute values of NAA, Cre, and Cho decreased significantly. The metabolite ratios changed to a smaller extent, and these changes were not significant. For the least affected motor cortex, no significant changes were found over 3 months. Figure 2 shows significant decreases in NAA, Cre, and Cho levels with time.

Discussion

There were four major findings of our study. First, the ICC of NAA, Cre, and Cho in the motor cortex were 0.53 to 0.71. Second, when cross-sectional data for patients with ALS were compared with those for controls, the NAA/(Cre + Cho) ratio was significantly decreased, and this change was due to both decreases in the NAA level and increases in Cre and Cho levels. Moreover, the changes in Cre and Cho levels were greater than the changes in the NAA level. Third, nonsignificant trends for metabolite changes were detected for patients with possible or suspected ALS (whose levels of diagnostic certainty were insufficient for a definite or probable ALS diagnosis). However, the changes for these subjects with possible or suspected ALS are small in magnitude and overlap with those for controls; therefore, it is unlikely that a single ^1H MRSI study with the current techniques would be useful for early detection of disease. Fourth, longitudinal decreases in NAA, Cre, and Cho levels in the motor cortex (but not in nonmotor regions) were found for patients with ALS. Taken together, these results support the use of metabolite changes measured by MRSI as surrogate markers of disease progression.

The first finding was good test–retest reliability, a key property if ^1H MRSI is to be developed further for diagnosis or staging of ALS. ICC of NAA, Cre, and Cho were high in the frontal and parietal cortex, although in the motor cortex ICC were high for Cre and Cho and relatively low for NAA. Other MRSI studies, determining reproducibility of NAA measurement, reported ICC of between 0.62³¹ and 0.91.³² However, these ICC were derived from measurements in large brain regions, such as the frontal or parietal lobe, where voxel positioning is not a problem. In contrast, voxel positioning is much more critical when sampling the motor cortex, which may have contributed to lower reproducibility of NAA measurements.

The second finding was significantly decreased metabolite ratios in the motor cortex of brains in patients with probable or definite ALS, as well as trends of decreased NAA and increased Cre and Cho levels from cross-sectional data. The reduced NAA/(Cre + Cho) ratio confirms several previous observations. Furthermore, several studies demonstrated diminished NAA concentrations in ALS,^{16,22} although this study found only a trend of reduced NAA level. We further found that patients with probable or definite ALS had lower NAA concentrations than did patients with possible or suspected ALS, as expected. In addition, we found a greater increase in Cho and Cre levels in patients with probable or definite ALS than in those with possible or suspected ALS. Cross-sectional analyses revealing increased Cho levels in motor fiber regions but not in the motor cortex have been previously reported.^{14,22} Our interpretation of increased Cre and Cho concentrations in the motor cortex is probably due to increased gliosis and membrane phospholipid breakdown. The significant changes reported for the least affected motor cortex demonstrate that disease activity is present throughout the motor cortex. For subjects with probable or definite ALS, the decrease in NAA level in the least affected motor cortex compared with that for controls was more significant than the decrease in NAA level in the most affected motor cortex compared with that for controls. Subsequently, all of the NAA ratios were also more significantly decreased in the least affected motor cortex for patients with ALS than for controls. These measurable changes in the least affected motor cortex demonstrate the usefulness MRSI data in the detection of early disease.

The third finding was nonsignificant trends for metabolite changes for patients with possible or suspected ALS that were in the same direction as those found for the patients with probable or definite ALS. As expected, the magnitude of these trends for patients with possible or suspected ALS was less than the changes for patients with probable or definite ALS. The lack of significance is most likely due to a combination of a small magnitude of change and a small number of subjects in this study. These results suggest that ^1H MRSI may have limited value to detect modest metabolite changes at an early stage of the disease.

The fourth major finding was longitudinal metabolite changes in the motor cortex of patients with ALS. Significant decreases in NAA, Cre, and Cho concentrations in the most affected motor cortex were observed over time, and the most significant change was found for Cho. The levels in the least affected motor cortex did not change significantly over the 3 months, but changes were in the same direction as those for the most affected motor cortex. The progressive decrease in the NAA level was expected, because of the well-known relentless motor neuron loss in ALS, and is consistent with findings from another longitudinal MRS study of ALS.¹⁴ Furthermore, because NAA was atrophy corrected, progressive NAA loss suggests a disproportionate greater loss of neuronal than glial cells. Progressive neurodegeneration would also be expected to account for longitudinal decreases in Cre and Cho levels. However, we cannot easily explain the apparently conflicting finding of increased Cre and Cho levels in the motor cortex of patients with ALS (when compared with controls in a cross-sectional analysis) with the progressive longitudinal decrease in Cre and Cho concentrations in the motor cortex of patients with ALS.

One possibility is that Cre and Cho levels are increased in the early stages of ALS (substantiated by increased Cre and Cho concentrations at the initial examination of all patients with ALS), followed by reductions in these metabolites as the disease progresses further. This is evident from the lack of significant changes in Cre and Cho levels after month 1, followed by a dramatic decrease after month 3. The effect from outliers was minimal because the SD remained <15% of the reported values. An increased Cho level has been previously interpreted to represent degradation of membrane phospholipids.⁷ An increased Cre level has been thought to represent glial cell proliferation.^{3,6} These processes may be increased in the early stages of ALS, leading to our findings of increased Cre and Cho concentrations at the initial examination, followed by progressive decreases. Therefore, it is possible that although Cre and Cho levels are increased with respect to those for controls at the initial examination, they begin to decrease as the disease progresses. Additional research is needed to elucidate these findings.

There were several limitations to this study. First, the sample size was relatively small, especially the number of patients with suspected or possible ALS. Second, the coarse spatial resolution of ^1H MRSI made it difficult to obtain spectra from volumes of interest that were not made up entirely of motor cortex tissue. However, we had a model to account for and minimize this effect. Possibly, the use of higher-field ^1H MRSI systems will provide higher spatial resolution.¹² One limitation of the reliability studies was that the two studies were performed with an interval of up to 2 weeks. Because of the length of the studies, approximately 1 hour and 15 minutes, it was not practical to subject the patients with ALS to scanning again with a shorter interval. Therefore, the ICC does not simply reflect the measurement noise but may also reflect scanner drift and metabolite variability within each subject during the interscan interval. Finally, no effort was made to calculate propagation of error introduced by various corrections; thus, the standard errors and 95% confidence intervals of the final metabolite concentration estimates might be underestimated. The accuracy of these measurements was also not determined by comparing them with findings of brain tissue metabolite assays. Future improvements to reduce lipid contamination should facilitate ^1H MRSI with short echo times so that additional metabolites can be detected.

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Figure 1. Position of multislice volumes of interest (A) in a study of early detection of and longitudinal changes in ALS by ^1H MRS imaging. Motor cortex (arrows) shown in top (B) and middle (C) slices; bottom slice (D).

Figure 2.
Longitudinal changes in N-acetylaspartate (NAA), creatine, and choline levels in the motor cortex of patients with ALS. Squares = most affected; triangles = least affected.

Table 1
Comparison of motor cortex metabolites and ratios for patients with ALS and controls

Metabolite or metabolite ratio	Mean value $\pm$ SD (%)		
	Controls (n = 12)	Patients with positive or suspected ALS (n = 12)	Patients with possible or definite ALS (n = 16)
Most affected motor cortex			
NAA	17.2 $\pm$ 1.7	17.7 $\pm$ 1.4 (2.9)	17.1 $\pm$ 2.5 (−0.9)
Cre	7.7 $\pm$ 1.0	8.0 $\pm$ 1.2 (3.9)	8.2 $\pm$ 1.0 (7.2)
Cho	4.4 $\pm$ 0.8	4.7 $\pm$ 0.8 (7.7)	5.1 $\pm$ 0.6 (16.5)*
NAA/Cre	2.14 $\pm$ 0.21	2.07 $\pm$ 0.22 (−3.1)	1.98 $\pm$ (−7.5)*
NAA/Cho	3.76 $\pm$ 0.60	3.60 $\pm$ 0.60 (−4.1)	3.18 $\pm$ (−15.5) [†]
NAA/(Cre + Cho)	1.35 $\pm$ 0.15	1.30 $\pm$ 0.15 (−3.5)	1.21 $\pm$ (−10.6) [‡]
Least affected motor cortex			
NAA	17.2 $\pm$ 1.7	17.0 $\pm$ 1.9 (−1.5)	15.9 $\pm$ 1.5 (−7.5)*
Cre	7.7 $\pm$ 1.0	7.7 $\pm$ 1.0 (0.4)	8.0 $\pm$ 1.0 (3.6)
Cho	4.4 $\pm$ 0.8	4.5 $\pm$ 1.1 (2.67)	4.8 $\pm$ 0.7 (9.9)
NAA/Cre	2.14 $\pm$ 0.21	3.76 $\pm$ 0.30 (−1.2)	1.89 $\pm$ 0.15 (−11.5) [‡]
NAA/Cho	3.76 $\pm$ 0.60	3.55 $\pm$ 0.54 (−5.4)	3.76 $\pm$ 0.36 (−16.2) [‡]
NAA/(Cre + Cho)	1.35 $\pm$ 0.15	1.31 $\pm$ 0.16 (−2.9)	1.17 $\pm$ 0.09 (−13.4)#

The most affected motor cortex was used for patients with ALS, and the average of the left and right motor cortex was used for controls. The percent change is compared with that for controls.

NAA = *N*-acetylaspartate; Cre = creatine; Cho = choline.

* $p < 0.05$ (two-tailed *t*-test).

[†] $p < 0.01$ (two-tailed *t*-test).

[‡] $p < 0.005$ (two-tailed *t*-test).

Table 2
Longitudinal changes in motor cortex metabolites for nine patients with ALS who were studied at 0, 1, and 3 months

Metabolite or metabolite ratio	0 mo	1 mo	3 mo
Most affected motor cortex			
NAA	17.7 ± 2.6	16.4 ± 2.2 (−7.2)	15.1 ± 1.3 (−14.5)*
Cre	8.3 ± 0.8	8.2 ± 1.0 (−0.1)	7.3 ± 0.7 (−11.8)*
Cho	5.0 ± 0.6	5.1 ± 0.6 (1.1)	4.1 ± 0.6 (−18.8)*
NAA/Cre	2.1 ± 0.2	1.9 ± 0.1 (−7.1) [†]	2.0 ± 0.1 (−2.7)
NAA/Cho	3.4 ± 0.4	3.1 ± 0.2 (−8.2)	3.5 ± 0.4 (4.7)
NAA/(Cre + Cho)	1.3 ± 0.1	1.2 ± 0.1 (−7.4) [†]	1.3 ± 0.1 (0.1)
Least affected motor cortex			
NAA	16.2 ± 2.4	16.5 ± 2.0 (1.6)	16.2 ± 2.1 (−0.3)
Cre	8.0 ± 1.1	7.9 ± 1.2 (−1.3)	7.7 ± 0.6 (−2.9)
Cho	4.5 ± 0.7	4.8 ± 0.7 (6.2)	4.4 ± 0.4 (−2.5)
NAA/Cre	2.0 ± 0.2	2.0 ± 0.2 (3.3)	2.0 ± 0.2 (2.4)
NAA/Cho	3.3 ± 0.4	3.3 ± 0.4 (−0.9)	3.4 ± 0.5 (2.1)
NAA/(Cre + Cho)	1.2 ± 0.1	1.2 ± 0.1 (1.6)	1.2 ± 0.1 (2.4)

The most affected motor cortex was used. The percent change is compared with that at month 0.

Values are expressed as mean ± SD (%).

NAA = *N*-acetylaspartate; Cre = creatine; Cho = choline.

* $p \leq 0.005$ (two-tailed test).

[†] $p \leq 0.05$ (two-tailed test).