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Decrease in N-acetylaspartate/creatine ratio in the motor area and the frontal lobe in amyotrophic lateral sclerosis

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Abstract We studied whether N-acetylaspartate (NAA), a neuronal marker, is reduced in the brain of 14 patients with clinically definite amyotrophic lateral sclerosis (ALS) and whether NAA levels in the motor area and frontal lobe correlate with the clinical features, including frontal lobe function. We also studied 14 normal controls were evaluated. We obtained peak integrals in ^{1}H magnetic resonance spectroscopy (MRS) for NAA, creatine (Cr), and choline-containing compounds (Cho). Severity of the disease was determined using the manual muscle strength test, and the Norris limb and bulbar scales. In the patients, the NAA/Cr ratio was reduced in the motor area and frontal lobe, while the Cho/Cr ratio was normal throughout the brain. There were significant correlations between the NAA/Cr ratio in the motor area and the Norris limb scale ($r = 0.50$; $P < 0.01$) and between the NAA/Cr ratio in the frontal lobe and the number of categories achieved in the Wisconsin Card Sorting test ($r = 0.71$; $P < 0.05$), implying frontal lobe dysfunction. These correlations suggest that a reduced NAA/Cr ratio is a marker of cortical neuronal loss and dysfunction in ALS.

Keywords Motor neurone disease · N-acetylaspartate · Magnetic resonance spectroscopy

Introduction

Amyotrophic lateral sclerosis (ALS) is a motor neurone disease (MND) characterised by progressive degeneration of upper and lower motor neurones. Cortical involvement in patients with ALS has been well known, but correlations between cortical dysfunction and clinical features are uncertain. Cognitive function in ALS has recently been a focus of attention [1, 2, 3, 4, 5, 6] and some workers have differentiated cases of ALS with dementia from those of classic ALS. However, even patients with classic ALS have been shown to develop cognitive, especially frontal lobe dysfunction [7].

Magnetic resonance spectroscopy (MRS) enables us to investigate neuronal metabolism in vivo and studies on patients with ALS have been reported [8, 9, 10, 11, 12, 13]. However, correlations between biochemical markers and neuronal function have not been clarified [9, 14, 15]. Our primary objective was therefore to see whether levels of N-acetylaspartate (NAA), a neuronal marker [16, 17], are reduced in ALS, and whether the levels in the motor area and frontal lobe correlate with clinical features.

Subjects and methods

We studied 14 patients with clinically definite ALS by El Escorial criteria [18] two with bulbar and twelve with limb onset: eight men, six women, mean age 65.8 ± 9.9 years, range 48–72 years, mean duration of disease 2.58 ± 1.7 years, range 1–6 years, plus 14 age- and sex- matched normal controls without neurological deficits (eight men six women, mean age 59.3 ± 12.8 years, range 44–72 years), using ^{1}H MRS. No of patient had taken riluzole. All subjects gave informed consent in written form, according to our institutional guidelines.

All patients were neurologically examined and the severity of disease was assessed using the manual muscle strength test (full

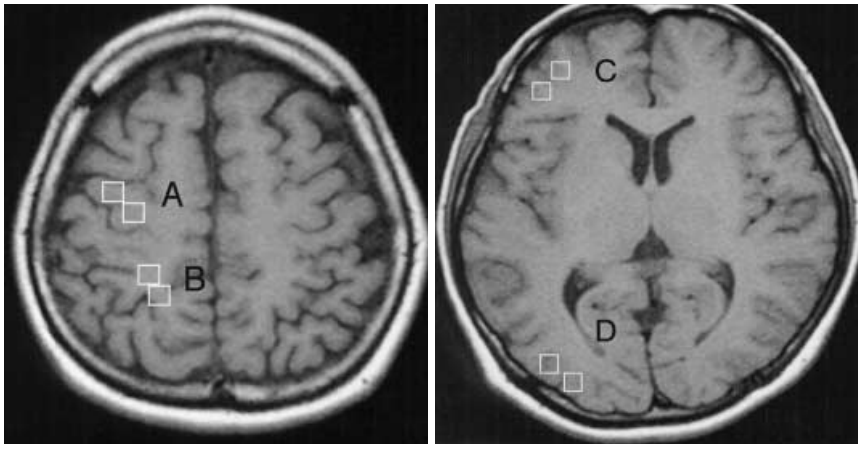

Fig. 1 Anatomical location of the volumes of interest (VOI): on T1-weighted axial images parallel to the orbitomeatal line, cubic 2 ml VOI were placed in the motor area, frontal lobe (including Brodmann's areas 6, 8 and 46), the parietal lobe (including areas 39 and 40) and in the occipital lobe (including areas 18 and 19), taking care to avoid partial-volume effects caused by parenchymal atrophy and to fit the VOI entirely within the same neuroanatomical structure

score 110) [19], and the Norris limb (full score 63), and bulbar (full score 39) scales [20]. Cognitive function was screened by the Mini Mental State Examination (MMSE) [21]. To assess frontal lobe function, we used the Wisconsin Card Sorting Test (WCST) [22]; these tests have been standardised as Japanese versions [6]. In order to exclude the possibility that neuropsychological abnormalities in the patients were caused by associated depression or anxiety, patients and controls were rated with Zung's self-rating depression scale (SRS) [23].

MRI and ^{1}H MRS studies were performed with a 1.5 tesla system, using a standard quadrature head coil. A neuroradiologist (Y.W.) who had no knowledge of the subject's diagnosis selected 2 ml volumes of interest (VOI) in the motor area, in the frontal lobe, including Brodmann's areas 6, 8 and 46 by, in the parietal lobe including areas 39 and 40 and in the occipital lobe, including areas 18 and 19 with care to avoid partial-volume effects due to parenchymal atrophy and to fit the VOI entirely within the same neuroanatomical structures (Fig. 1). We located two VOI in each of the motor area, frontal, parietal and occipital lobes to minimise the location effect. Only when we found no difference between two VOI in each group, did we use the mean value. After localised shimming of the VOI, ^{1}H-MRS was performed using a stimulated echo acquisition mode sequence for volume selection with a chemical-shift selective presequence for water suppression. Acquisition parameters were repetition time (TR) of 1500 ms, echo time (TE) of 140 ms, with 1024 acquisition points, and 256 acquisitions. The procedure including preparation, MRI, and MRS lasted approximately 30 min. After acquisition, the MRS data were transferred to a workstation and processed using a SA/GE software. The process included zero-filling to 2048 data-points, 3 Hz line-broadening and fast Fourier transformation (FFT). After zero-order phase correction and cubic baseline correction, Lorenzian fit for the peak area, determined by the Marquardt-Levenberg method [24] was used to measure the areas of spectra from each VOI. We measured NAA, Creatine (Cr) and choline-containing compounds (Cho) and calculated the metabolite signal-intensity ratios (NAA/Cr or Cho/Cr) for each VOI. Nonparametric statistics, the Kruskal-Wallis and Mann-Whitney tests were used to assess differences in ratios between patients and controls. Spearman's rank correlation was used to test a possible linear relationship between the clinical rating scores and the ratios. All statistical analyses were carried out by a statistics software, using a microcomputer. Statistical significance was defined as $P < 0.05$.

Results

There was no correlation between NAA/Cr ratios and age in patients or controls. The patients showed lower rations than the controls in the motor area (1.69 ± 0.14 vs 2.23 ± 0.26, $P < 0.01$) and frontal lobe (1.74 ± 0.232 vs 2.27 ± 0.15, $P < 0.01$) (Fig. 2), but not in the parietal (2.05 ± 0.19 vs 2.19 ± 0.24) or occipital (1.99 ± 0.15 vs 2.02 ± 0.19) lobes. There was no significant difference in Cho/Cr ratio between patients and controls in the motor area (1.03 ± 0.21 vs 1.08 ± 0.26), or the frontal (1.12 ± 0.16 vs 1.05 ± 0.15), parietal (1.09 ± 0.15 vs 1.19 ± 0.14) or and occipital (1.05 ± 0.20 vs 1.04 ± 0.16) lobes.

The patients had lower scores than the controls on the manual muscle strength test (75.7 ± 6.6 vs 110 ± 0.0, $P < 0.001$), and Norris limb (51.0 ± 4.5 vs 63 ± 0.0, $P < 0.001$), and bulbar (28.6 ± 3.4 vs 39 ± 0.0, $P < 0.001$) scales. There was a significant correlation between the NAA/Cr ratio in the motor area and the Norris limb scale ($r = 0.50$;$P < 0.01$) (Fig. 3), consistent with the neuronal loss. There was no significant difference between patients and controls concerning in total MMSE score (28.8 ± 1.2 vs 29.4 ± 0.91). However, the patients with ALS had lower scores on the WCST (2.64 ± 1.7 vs 5.86 ± 0.73, $P < 0.001$), and there was a significant correlation between the NAA/Cr ratio in the frontal lobe and the WCST score ($r = 0.71$;$P < 0.05$) (Fig. 4).

Discussion

Degeneration of upper motor neurones in the cerebral cortex is a fundamental pathological change in ALS [25, 26, 27, 28, 29]. However, there have been controversies concerning reduction of NAA in the motor area and frontal lobe in this disease. In spinal cords obtained at autopsy, the NAA level, measured by high-performance liquid chromatography (HPLC) was 40% lower in pa-

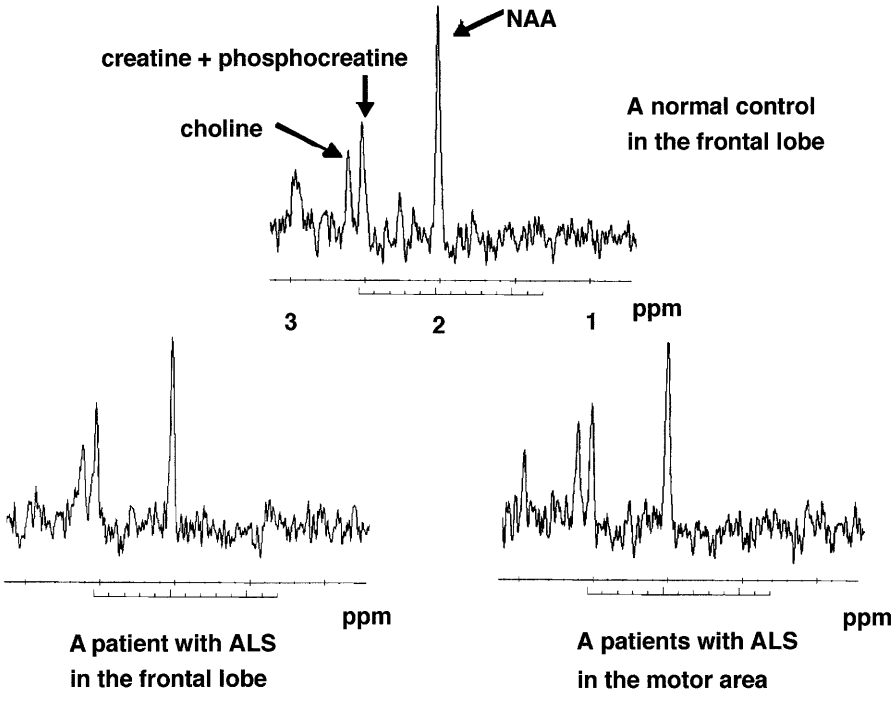

Fig. 2 Sample of spectra from a control subject and a patient

tients with ALS than in controls [30], but there was no change in the levels in the frontal lobe or motor area [31]. In contrast to these postmortem findings, in vivo ^{1}H-MRS studies [9, 10, 11, 12, 32] have suggested a decrease in the NAA level in the motor area. The reason for this discrepancy is not clear. However, low NAA/Cr ratios have been observed in vivo in various neurological disorders and appear to result from a decrease in the volume of neurones per unit volume of the brain or in the concentration of NAA within neurones, caused by metabolic dysfunction [2, 33]. Either of these mechanisms may be related to the clinical deficits in neurological disorders such as ALS.

We showed a decrease in the NAA/Cr ratio in the motor area and frontal lobe in patients with clinically definite ALS, and a significant correlation between this decrease and clinical features. A correlation between a decrease in the NAA concentration in the motor area and muscle weakness has been reported previously [8, 9, 10, 11]. Kalra et al. [22] reported recovery of NAA in corticomotor neurones in patients with ALS after administration of riluzole, a glutamate antagonist, suggesting that a low NAA concentration could be caused by metabolic dysfunction. Strong et al. [34] showed that patients with a bulbar onset showed greater impairment in neuropsychological tests than those with a limb onset, and a decrease in the NAA/Cr ratio in the cingulate gyrus. These are the reports of a correlation between a decreased NAA/Cr ratio in the frontal lobe and frontal lobe dysfunction. Our study patients with a limb onset

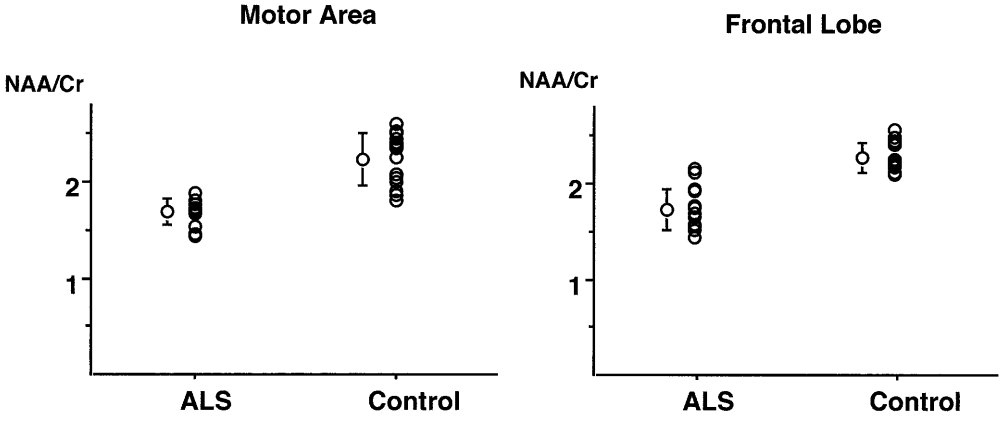

Fig. 3 N-acetylaspartate/creatine (NAA/Cr) ratios in the motor area and the frontal lobe. The ratio was lower in the patients in the motor area (1.69 ± 0.14 vs 2.23 ± 0.26, $P < 0.01$) and frontal lobe (1.74 ± 0.232 vs 2.27 ± 0.15, $P < 0.01$)

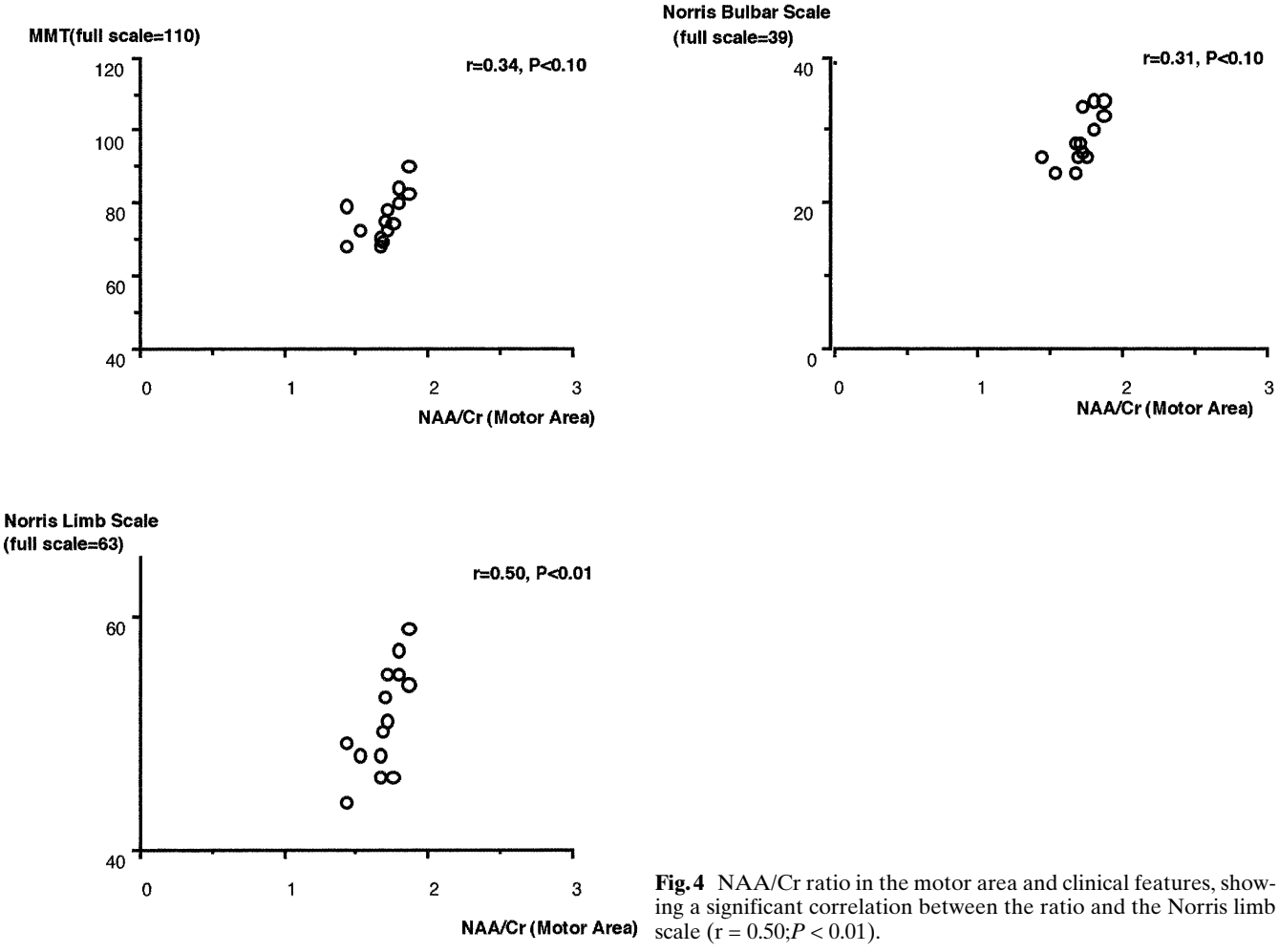

Fig. 4 NAA/Cr ratio in the motor area and clinical features, showing a significant correlation between the ratio and the Norris limb scale ($r = 0.50$; $P < 0.01$).

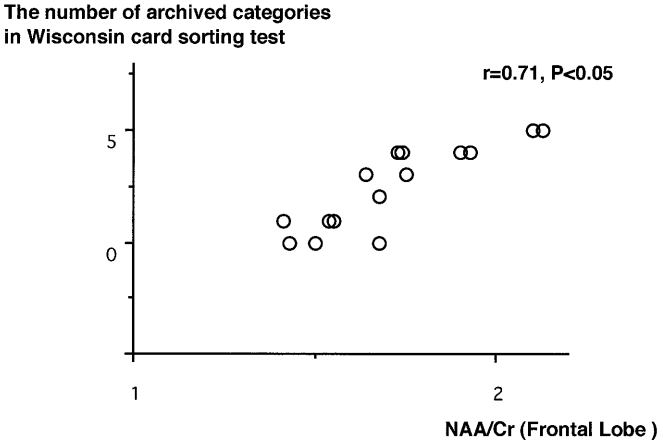

Fig. 5 NAA/Cr ratio in the frontal lobe and the Wisconsin card-sorting test, showing significant correlation between the and the test score ($r = 0.71$; $P < 0.05$)

also showed a decreased NAA/Cr ratio in the frontal lobe and frontal dysfunction. There are no pathological changes outside the motor area of the cerebrum in ALS, and cognitive impairment in these patients has been controversial. Previous reports, including some from our group, supported the presence of cognitive impairment using neuropsychological examination or neuroimaging [4, 5, 6, 7]. The results presented here confirm these reports and demonstrate a correlation between cognitive impairment and frontal lobe dysfunction in patients with ALS. They support the notion that NAA levels as shown by ^{1}H MRS might be useful as an objective, quantitative measure of neuronal degeneration in the motor area and frontal lobe in patients with ALS who present with clinical features including cognitive dysfunction.

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