Structural MRI correlates of amyotrophic lateral sclerosis progression

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ABSTRACT

Purpose: Amyotrophic lateral sclerosis (ALS) presents with varying degrees of brain degeneration that can extend beyond the corticospinal tract (CST). Furthermore, the clinical course and progression of ALS varies widely. Brain degeneration detected using structural magnetic resonance imaging (MRI) could reflect disease progression. **Subjects and methods:** On study registration, 3-Tesla volumetric MRI and diffusion tensor imaging scans were obtained at baseline in 38 healthy controls and 67 patients with sporadic ALS. Patients had Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALSFRS-R) scores of ≥ 36 and did not have the C9ORF72 repeat expansion. Six months later, changes in ALSFRS-R (delta-ALSFRS-R) scores were calculated and three groups of patients were extracted, namely, patients with slow progression with delta-ALSFRS-R scores ≤ 3 (n = 19), intermediate progression with delta-ALSFRS-R scores = 4, 5, and 6 (n = 36), and rapid progression with delta-ALSFRS-R scores ≥ 7 (n = 12). We analyzed voxel-based morphometry and tract-based spatial statistics among these subgroups and controls. **Results:** In comparison with controls, patients with ALS showed gray matter atrophy and decreased fractional anisotropy beyond the motor cortex and CST, especially in the frontotemporal lobes and basal ganglia. Moreover, the degree of change was highly

proportional to delta-ALSFRS-R at the 6-month assessment. **Conclusion:** A more rapid disease progression and poorer functional decline were associated with greater involvement of the extra-motor cortex and basal ganglia, suggesting that the spatial extent of brain involvement can be an indicator of the progression in ALS.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease involving degeneration of both upper and lower motor neurons, and is associated with varying degrees of extra-motor brain degeneration.[1-3] These findings suggest that motor neuron degeneration contributes to a wider progressive process that spreads through multiple brain regions, potentially explaining the clinical heterogeneity and pathological spectrum of ALS and frontotemporal dementia (FTD).[2-5] Moreover, transactive response DNA-binding protein 43 kDa (TDP-43) is the key common pathological hallmark of ALS and a subgroup of FTD cases.[5,6]

Advanced imaging techniques, such as volumetric brain magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI), provide insight into ALS pathophysiology. More specifically, volumetric brain MRI reveals subtle atrophic changes in brain structures, while DTI allows for the visualization of fibre tract involvement.

In terms of the brain structures involved in ALS, volumetric MRI has revealed involvement of both cortical and subcortical structures, including motor and extra-motor regions, such as the brain cortex and basal ganglia including the thalamus and caudate nucleus.[7,8] Moreover, DTI enables the assessment of white matter

(WM) integrity using common measures, such as fractional anisotropy (FA), mean diffusivity, and other parameters.[9,10] Indeed, DTI has already produced promising results in assessing widespread WM pathology in patients with ALS, most of which refer to changes in FA,[8,11-13] reflecting directional changes in water diffusivity and average diffusion in all directions.[9,10]

Symptoms in patients with ALS are progressive. However, advancement of the disease, as well as its course among individual patients, varies widely.[14-17] Further, the relationship between the extent of brain pathology and the progression of patients with ALS remains unclear. In the present study, we examined whether the extent of MRI-assessed brain damage was related to the progression of sporadic ALS phenotypes.

SUBJECTS AND METHODS

The subjects included 73 patients with sporadic ALS (45 males, 28 females; age, 60.9 ± 7.9 years) who had ALS Functional Rating Scale-Revised (ALSFRS-R) scores of ≥ 36 on an initial MRI scan, and 38 healthy controls (19 males, 19 females; age, 59.2 ± 8.4 years). None of the subjects included in this study had any medical or family history of neurodegenerative disorders. Moreover, none of the subjects

exhibited focal deep WM abnormalities with hyperintensities on T2-weighted MRI that were more severe than grade 2, based on the Fazekas Hyperintensity Rating System.[18] We confirmed that none of the patients with ALS had mutations in superoxide dismutase-1 (SOD1), TDP-43, fused in sarcoma (FUS), and TRK-fused gene (TFG), which are the most commonly occurring ALS mutations in the Japanese population. We also found no mutations chromosome 9, open reading frame 72 (C9ORF72) and found no mutations.[19] All patients with ALS satisfied the criteria for definite ALS using the El Escorial criteria.[20]

At registration, both patients with ALS and healthy controls underwent MRI scans. All patients participating in the present study were admitted for diagnosis at the Nagoya University Hospital, and their MRI scan was the first examination for diagnosis. Their cognitive scores were assessed using the Mini-Mental State Examination (MMSE) [21] for general cognitive assessment, and the Frontal Assessment Battery (FAB) [22] for frontal lobe cognitive function. We also assessed clinical and physical scores using the ALSFRS-R.[23] After 6 months, only ALSFRS-R scores were reassessed in the 67 patients with ALS, and changes in ALSFRS-R (delta-ALSFRS-R from diagnosis) were calculated based on direct observations [24]. Patients with ALS who had died within 6 months of registration (n =

6; the cause in all cases was respiratory failure) were excluded from the present study for several reasons. First, five of the patients had been transferred to another hospital or home doctor, and we could not directly assess their neurological or activities of daily living (ADL) condition at the point of death. Second, the ages of the patients with ALS who had died were significantly higher than those of other ALS groups and healthy controls (age has been reported to influence the progression of ALS [14,25]). Finally, only a small number of patients with ALS died. We divided the remaining 67 patients into the following three subgroups based on a previous study [26]: A) a slow progression group (delta-ALSFRS-R from diagnosis scores ≤ 3), B) an intermediate progression group (delta-ALSFRS-R scores from diagnosis = 4, 5, and 6); and C) a rapid progression group (delta-ALSFRS-R scores from diagnosis ≥ 7). Using this approach for disease progression classification, the backgrounds and characteristics of patients in the slow progression and rapid progression groups were almost equal, although the number of cases between groups varied. We also performed similar investigation for the delta-ALSFRS-R from onset.

Informed consent was obtained before subject participation. This study was approved by the ethics committee of Nagoya University Graduate School of Medicine. Patient registration and follow-up in the present study was carried out using the

Japanese Consortium for Amyotrophic Lateral Sclerosis (JaCALS) research system.

MRI protocol: Three-dimensional T1-weighted images, conventional MRI (T2-weighted and FLAIR images), and DTI data were acquired on a 3.0 Tesla scanner (Trio, Siemens, Munich, Germany). Structural T1 and T2/FLAIR images were reviewed to exclude potential abnormalities. For the T1-weighted images, 192 axial slices were obtained using a repetition time (TR) of 1,570 ms, an echo time (TE) of 2.15 ms, an inversion time of 800 ms, a flip angle of 15°, an acquisition matrix of 256 × 256, a reconstruction matrix of 256 × 256, a field of view (FOV) of 256 mm × 256 mm, an in-plane resolution of 1.0 × 1.0 mm^2, and a slice thickness of 1.0 mm, no gap. Diffusion-weighted images were obtained, employing optimal methods using a Stejskal–Tanner sequence with single shot spin echo-type, echo-planar imaging, a flip angle of 90°, and a TR of 7,800 ms, with a 32-channel phased-array head coil. The TE corresponding to the respective b-factor was 84 ms for 1,000 s/mm^2. Echo spacing was 0.69 ms, and the matrix size was 128 × 128 with a readout bandwidth of 1,776 Hz/pixel. Sixty-three axial slices, 2.0 mm thick with no interslice gaps, were used to image the entire brain with a FOV of 256 mm × 256 mm. A motion-probing gradient was applied to 12 orientations after acquisition of b = 0 images. The 128 × 128 data matrix was not interpolated.

Voxel-based morphometry analysis of gray matter: Three-dimensional T1-weighted images were analyzed using Statistical Parametric Mapping (SPM12; Wellcome Department of Imaging Neuroscience, London, UK; http://www.fil.ion.ucl.ac.uk/spm) [27] and VBM12 (Department of Psychiatry, University of Jena, Thuringia, Germany) running on Matlab (MathWorks, Natick, MA, USA) with Diffeomorphic Anatomical Registration using Exponentiated Lie algebra (DARTEL).[28] To facilitate an unbiased comparison among regions of interest in different patients, gray matter (GM) images were smoothed by convolving an 8-mm isotropic Gaussian kernel.

Diffusion tensor imaging analysis: DTI data was processed with the FSL 5.0.8 software package (www.fmrib.ox.ac.uk/fsl).[29] Pre-processing included eddy currents, motion correction, and brain-tissue extraction. After pre-processing, we concatenated diffusion tensor images into 13 (1b = 0 + 12b = 1,000) volumes and a diffusion tensor model was fitted at each voxel, generating FA maps. DTI group analyses included tract-based spatial statistics (TBSS). For group analyses, DTI sets were warped to the Montreal Neurological Institute (MNI) 152 template, available as standard T1 data in the FSL software package. FA maps were created using DTIFIT first, followed by alignment to a common target (FMRIB58_FA template). FA maps were calculated using the FSL diffusion toolbox and aligned to a 1*1*1 mm MNI 152 standard space.

We then created a mean FA skeleton with a threshold of FA > 0.2 and projected individual FA data onto this, as well as applying the same projection to the other maps.

Statistical analysis: For MRI group comparisons, the pre-processed data were analyzed using an analysis of covariance model; age and gender were considered nuisance variables. The statistical threshold for results was $p < 0.05$, corrected for multiple comparisons using family-wise error (FWE) in comparisons between patients with ALS and healthy controls. An uncorrected $p < 0.001$ was used for multiple comparisons among the subgroups of patients with ALS in GM volumes, and a corrected $p < 0.05$ was applied for multiple comparisons using threshold-free cluster enhancement (TFCE) [30] in TBSS using 5,000 permutations in the permutation test as implemented in the FSL submodule <Randomise>. Ordinary statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 20 (IBM, Armonk, NY, USA).

RESULTS

Demographic and clinical characteristics: The data of patients with ALS and healthy controls are summarized in Table 1. There were significant differences between baseline MMSE ($p = 0.002$) and FAB scores ($p < 0.001$) of patients with ALS and

those of controls; decreases in FAB scores were especially severe (Table 1). During baseline MRI scans, we observed no differences among the rapid (delta-ALSFRS-R from diagnosis scores ≥ 7; n = 12; 8 males, 4 females; age, 61.3 ± 7.7 years; onset form, limbs: n = 10, bulbar: n = 2), intermediate (delta-ALSFRS-R from diagnosis scores = 3, 4, and 5; n = 36; 19 males, 17 females; age, 60.4 ± 7.2 years; onset form, limbs: n = 27, bulbar: n = 9), and slow (delta-ALSFRS-R from diagnosis scores ≤ 3; n = 19; 13 males, 6 females; age, 60.4 ± 7.2 years; onset form, limbs: n = 14, bulbar: n = 5) progression groups in terms of age, gender ratio, onset form, and MMSE, FAB, and ALSFRS-R scores at patient registration. However, 6 months after the baseline MRI scan, there were significant differences in ALSFRS-R among the three groups (Figure 1A and 1B, and Table 2).

VBM results: There were lower cortical and subcortical GM volumes in patients with slow (Figure 2A), intermediate (Figure 2B), and rapid (Figure 2C) progression ALS compared with controls ($p < 0.05$, corrected for multiple comparisons using FWE), but the location of these changes differed. In the slow progression ALS group, these reductions were localized in the precentral knob of the motor cortex, and extra motor regions, such as the caudate head, medial frontal gyrus, thalamus, and cingulate gyrus. In the intermediate progression group, significant GM volume reductions appeared in

the frontotemporal lobes, a region not affected in the slow progression group. In the rapid progression group, GM volume reductions were more widespread and severe than in the other two ALS groups. The regions involved included the basal ganglia, particularly the caudate head and thalamus, and the dorsomedial frontal cortex, including the anterior cingulate cortex and the lateral part of the orbitofrontal cortex. Further reductions were seen in the inferior frontal gyrus, dorsolateral prefrontal cortex, insula, and temporal pole, in addition to motor cortex regions.

Direct comparisons between the slow and rapid progression groups revealed that the rapid progression group had significant GM atrophic changes in the caudate nucleus head, thalamus, insula, and dorsomedial frontal cortex, with an uncorrected statistical threshold of $p < 0.001$ (Figure 2D). However, these trends were not statistically significant after correction (FWE corrected at $p < 0.05$).

DTI results: Lower FA on TBSS analysis exhibited widespread areas in the slow (Figure 3A), intermediate (Figure 3B), and rapid (Figure 3C) progression groups compared with controls. The decreases in all three ALS groups were commonly observed beyond extra motor regions, including the corona radiata and internal capsule of the pyramidal tracts, specifically, the region surrounding the caudate nucleus, thalamus, and anterior horn of the lateral ventricle. The decreases were larger and more

widespread in subcortical regions of the dorsomedial frontal cortex and the lateral part of the orbitofrontal cortex, according to the patient's disease progression. Specifically, the rapid progression group showed more severe and widespread involvement than the slow progression group, with decreases extending into the WM of the insula, pars opercularis, the posterior temporal and occipital lobes, and regions around the basal ganglia, such as the nucleus head and thalamus (Figure 3D) ($p < 0.05$, corrected for multiple comparisons using TFCE).

A comparison of ALSFRS-R changes from onset to diagnosis and from diagnosis to 6 months after revealed that ALS patients in the rapid progression group showed significant sigmoid-like slope progression changes, but those in the slow and intermediate group showed changes that were relatively sequential (Supplemental Figure 1, A-C). Multiple linear regression models with age and sex as covariates in SPM or FSL showed no significant correlations between ALSFRS-R changes from onset to diagnosis or from diagnosis to 6 months after and GM volumes or FA changes in TBSS (uncorrected, $p < 0.001$). These results indicate that the extent of cerebral involvement influences ALS progression.

DISCUSSION

In the present study, we retrospectively investigated the association between the progression pattern of ALS and the brain degeneration that extends beyond the corticospinal tract, as assessed using structural MRI. Our assessments revealed the importance of both cortical and subcortical structures of the frontotemporal lobes in ALS (including the inferior and middle frontal, rectus, and superior temporal gyri), as well as components of the basal ganglia (the caudate nucleus and thalamus). Recently, both pathological and neuroradiological studies have suggested that ALS is associated with widespread involvement of the basal ganglia.[5,6] Previous studies have shown a correlation between the extent of microstructural abnormalities, including the basal ganglia beyond the extra-motor regions with TDP-43 inclusions, and clinically severe stages of ALS.[31,32]

ALS imaging studies have sought to correlate common clinical variables with various MRI measures. Regarding DTI measures, decreased FA in the CST [33,34] has been correlated with rates of disease progression.[11,33,35-37] In morphometric studies, GM density measures have been correlated with disability scores,[6,33,34] and widespread cortical thinning and GM volume loss beyond the motor cortex have been shown to be reflective of a declining clinical course and rapid disease progression.[34] Similarly, recent clinical studies have shown that some laboratory tests [38,39] and

clinical signs/factors [14-16] are correlated with disease progression in ALS.

Some longitudinal studies using MRI with field strengths of over 3T (providing a higher resolution and lower signal to noise ratio) revealed that DTI assessments offered more sensitive parameters than cortical thinning and GM volume.[12,40,41] However, DTI was not more sensitive than standard clinical outcome measures, such as the ALSFRS-R.[42-44] We suggest that these differences in sensitivity may produce the inconsistencies in correlations between these MRI parameters and clinical metrics that have thus far been observed because the pathological degeneration in ALS involves mixed upper and lower motor neurons in different spinal cord and brain regions.[45]

Our objective was to investigate whether structural MRI can be used as an indicator of progression in the disease course of sporadic ALS. In previous studies, the rate of ALS disease progression has been calculated as “ALSFRS-R full score - ALSFRS-R score at first visit / symptom duration”.[46,47] This delta ALSFRS-R from onset is one of the promising prognostic markers,[26] but has not been proven to be a prediction marker of progression in functional decline. Regarding the progression pattern, we identified the following four clusters of longitudinal functional decline among our cases 1) a rapid decline cluster (13%); 2) an intermediate decline cluster (24%); 3) a sigmoidal decline cluster (15%); and 3) a moderate decline cluster (48%).[16]

Furthermore, the identified trajectories were not exactly linear, but were curvilinear. Particularly, score of the "sigmoidal decline cluster" decreased slowly in the early course and became rapid along the way. Thus, delta-ALSFRS-R from onset and “delta-ALSFRS-R from diagnosis” will be different in some patients. Based on these backgrounds, we compared the relationship between widespread cerebral involvement and both delta-ALSFRS-R from onset and that from diagnosis, showing that ALS patients in the rapid progression group demonstrated significant sigmoidal curve changes in the progression slope.

Our study of genetically sporadic patients with ALS revealed grey matter changes in regions including the thalamus and caudate nucleus of the basal ganglia, which is consistent with previous studies.[41,48] Moreover, in the present study not only were changes observed in widespread brain regions beyond corticospinal tracts, including the thalamus and caudate nucleus of the basal ganglia, but also these changes correlated with ALS progression. These results strongly indicate that ALS is a widespread neurodegenerative disease that exerts its influence across motor regions. Based on our findings, we propose two underlying neuropathological mechanisms of ALS. First, both corticospinal tracts and direct regulator regions that exist around the tracts are related to basic and instrumental activities of daily living (ADL), which

mainly involve motor function. This type of motor function is based on more widespread neural networks and circuits, in contrast to previous reports.[49,50] Thus, we suppose that widespread brain changes across motor systems affect ADL functional outputs in ALS. Second, more widespread brain changes were found in accordance with the progression of ALS, regardless of the ADL condition, as ALSFRS-R scores were not qualitatively different at baseline MRI between the three patient subgroups. We suppose that the changes across motor regions were likely due to subclinical neurodegeneration, which would contribute to decline in ADL over the course of ALS.

The present study has several limitations. First, we examined only general cognitive functions via the MMSE and FAB, and were unable to conduct neuropsychological batteries focused on executive and memory functions, which can reveal subtle cognitive and behavioural changes. This assessment may be important because executive and memory functions are affected in frontotemporal dementia, and frontotemporal dementia is significantly related to shorter survival and faster progression in ALS.[47,51] This limitation may have concealed our ability to identify subtle clinical abnormalities. Additionally, we regrettably did not obtain upper motor neuron impairment scores that could represent upper motor neuron dysfunction,[52] which might more directly reflect brain degeneration in ALS. Patient fatigue imposed

limitations, therefore we examined MRI scans and MMSE/FAB batteries only at patient registration, and did not conduct follow-up MRI scans or cognitive tests after 6 months. Regarding MRI technical sectors, 12 axial slices were used in the DTI component of our study, which is a relatively small amount compared with other recent MRI imaging studies. However, our findings suggest that the progression patterns observed via VBM and DTI in patients with sporadic ALS may help with the clinical diagnosis of distinct disease subtypes. Longitudinal studies over longer periods of time and with a larger number of subjects are required to further clarify the clinical time course and distribution of both GM and WM pathologies in ALS.[12,39,40]

In the present study, we observed disruptions in the motor-frontal-subcortical areas in the form of decreased GM volume and reduced WM-FA, which was associated with impaired connections and disease progression. There was also a strong association between impaired connections identified via the anatomical degeneration analysis using VBM and DTI, indicating the usefulness of both approaches in characterizing the widespread effects of ALS on brain structure. Together, these findings suggest that the dysfunction of all fronto-striatal areas is not only likely associated with neuropsychological abnormalities, but also with the progression of ALS. The advancement of structural MRI analysis will offer a promising and useful tool to

diagnose and individually characterize the progression of ALS.

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FIGURE LEGENDS

Figure 1. Line graphs of (A) ALS change in each patient, with (B) medians and error-bars representing changes in ALSFRS-R scores between MRI scans at the time of registration and 6 months after registration in the slow progression group (delta-ALSFRS-R scores ≤ 3, n = 19) (blue colour), the intermediate progression group (delta-ALSFRS-R scores = 4, 5, and 6, n = 36) (black colour), and the rapid progression group (delta-ALSFRS-R scores ≥ 7, n = 12) (red colour).

ALS, amyotrophic lateral sclerosis; ALSFRS-R, ALS Functional Rating Scale-Revised; delta-ALSFRS-R scores, ALSFRS-R scores at the initial MRI scan minus those taken 6 months later.

Figure 2. Cortical and subcortical gray matter (GM) volume reduction at registration in the (A) slow, (B) intermediate, and (C) rapid ALS progression groups compared with controls ($p < 0.05$, corrected for multiple comparisons using family-wise error), and (D) between the slow and rapid progression groups ($p < 0.001$, uncorrected for multiple comparisons). These are 3D rendered, with the original T1 template averaged across the healthy controls normalized to MNI templates. Irrespective of the presence of rapid progression, patients with ALS commonly show significant GM reduction in

the dorsomedial frontal cortex, including the anterior cingulate cortex, lateral part of the orbitofrontal cortex (especially in the inferior frontal gyrus), dorsolateral prefrontal cortex, and temporal pole compared with controls. In cortical regions (i.e., medial prefrontal cortex, medial orbitofrontal cortex, and anterior insula) and in subcortical regions (i.e., basal ganglia and head of the caudate nucleus), GM reductions are observed in the rapid ALS progression group. More extensive reductions in the middle and inferior frontal gyri, inferior and middle temporal cortices, head of the caudate nucleus, and thalamus are clearly observed in direct comparisons between the slow and rapid progression groups.

ALS, amyotrophic lateral sclerosis; FWE, family-wise error; R, right; L, left.

Figure 3. Decreased fractional anisotropy (FA) on tract-based spatial statistics (TBSS) analysis at registration in the (A) slow, (B) intermediate, and (C) rapid ALS progression groups compared with controls, and (D) between the slow and rapid progression groups ($p < 0.05$, corrected for multiple comparisons using threshold-free cluster enhancement). The decreases in all three ALS groups are observed beyond extra motor regions, including the corona radiata and internal capsule of the pyramidal tracts, and especially surrounding the caudate nucleus, thalamus, and anterior horn of the

lateral ventricle. The decreases are larger and more widespread in subcortical regions of the dorsomedial frontal cortex and the lateral part of the orbitofrontal cortex, according to the patient's disease progression. Specifically, the rapid progression group show more severe and widespread involvement than the slow progression group, with decreases extending into the WM of the insula, pars opercularis, the posterior temporal and occipital lobes, and regions around the basal ganglia, such as the nucleus head and thalamus.

TFCE, threshold-free cluster enhancement; ALS, amyotrophic lateral sclerosis; R, right; L, left.

Table 1. All participant characteristics and cognitive test results at the initial MRI scan

	ALS (n = 73)	Controls (n = 38)	p values
Age (year)	60.9 ± 7.9	59.2 ± 8.4	NS
Sex (male / female)	45 / 28	19 / 19	NS
Disease duration (year)	1.6 ± 1.0	—	—
Education (year)	13.1 ± 2.2	14.6 ± 1.4	NS
ALSFRS-R	40.2 ± 3.3	48.0 ± 0.0	$p < 0.001$*
MMSE	28.1 ± 1.9	29.7 ± 0.4	$p = 0.002$*
FAB	15.6 ± 3.8	17.9 ± 0.2	$p < 0.001$*

*Mann-Whitney U test. ALS, amyotrophic lateral sclerosis; ALSFRS-R, revised amyotrophic lateral sclerosis functional rating scale; MMSE, mini-mental state examination; FAB, frontal assessment battery; NS, not significant.

Table 2. Changes in the conditions of ALS patients over 6 months and their cognitive test results

	(A) ΔALSFRS-R ≤ 3 slow progression	(B) ΔALSFRS-R = 4,5,6 intermediate progression	(C) ΔALSFRS-R ≥ 7 rapid progression	Death	p values
Age (year)	60.4 ± 7.2	61.8 ± 9.1	61.3 ± 7.7	*68.5 ± 8.5	p < 0.001†
Number (male / female)	19 (13 / 6)	36 (19 / 17)	12 (8 / 4)	6 (5 / 1)	–
Disease duration (year)	1.7 ± 0.7	1.6 ± 1.0	1.2 ± 0.4	1.3 ± 0.8	NS
Education (year)	13.4 ± 2.0	13.0 ± 2.8	14.0 ± 1.8	12.0 ± 0.0	NS
Limb-onset / Bulbar-onset	14 / 5	27 / 9	10 / 2	4 / 2	NS
ALSFRS-R (at registration of MRI scan)	41.4 ± 3.0	40.6 ± 3.2	41.1 ± 2.1	39.5 ± 1.8	NS
ALSFRS-R (after 6 months of registration)	39.7 ± 3.3	*35.4 ± 4.0	*30.9 ± 3.9	Death	p < 0.001†
Rate of ALSFRS-R changes per month (from onset to registration)	*0.293 ± 0.183	0.776 ± 0.418	0.947 ± 0.503	0.779 ± 0.450	p < 0.001†
Rate of ALSFRS-R changes per month (from registration to 6 months after)	0.271 ± 0.158	*0.898 ± 0.159	*1.708 ± 0.365	–	p < 0.001†
MMSE (at the registration of MRI scan)	28.5 ± 2.0	28.2 ± 1.6	28.7 ± 1.0	28.4 ± 5.8	NS
FAB (at the registration of MRI scan)	15.8 ± 1.5	15.4 ± 2.0	15.6 ± 1.3	15.1 ± 1.1	NS

†Kruskal-Wallis test. ALS, amyotrophic lateral sclerosis; ALSFRS-R, revised amyotrophic lateral sclerosis functional rating scale; ΔALSFRS-R, ALSFRS-R scores at the MRI scan - after 6 months of MRI scan; MMSE, mini-mental state examination; FAB, frontal assessment battery; NS, not significant.