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Title: Association between brain and upper cervical spinal cord atrophy assessed by MRI and disease aggressiveness in amyotrophic lateral sclerosis

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ABSTRACT

Purpose

To study the relative contributions of brain and upper cervical spinal cord compartmental atrophy to disease aggressiveness in amyotrophic lateral sclerosis (ALS).

Methods

Twenty-nine ALS patients and 24 age- and gender-matched healthy controls (HC) were recruited. Disease duration and the Revised-ALS Functional Rating Scale (ALSFERS-R) at baseline, 3- and 6-months follow-up were assessed. Patients were clinically differentiated into fast ($n=13$) and slow ($n=16$) progressors according to their ALSFRS-R progression rate. Brain grey (GM) and white matter, brainstem sub-structures volumes and spinal cord cross-sectional area (SC-CSA) at C1-C2 vertebral levels were measured from a 3D-T1-weighted MRI.

Results

Fast progressors showed significant GM, medulla oblongata and SC atrophy compared to HC ($p<0.001$, $p=0.013$ and $p=0.008$) and significant GM atrophy compared to slow progressors ($p=0.008$).

GM volume correlated with the ALSFRS-R progression rate ($Rho/p=-0.487/0.007$), the ALSFRS-R at 3-months ($Rho/p=0.622/0.002$), and ALSFRS-R at 6-months ($Rho/p=0.407/0.039$). Medulla oblongata volume and SC-CSA correlated with the ALSFRS-R at 3-months ($Rho/p=0.510/0.015$ and $Rho/p=0.479/0.024$). MRI measures showed high performance to discriminate between fast and slow progressors.

Conclusion

Our study suggests an association between compartmental atrophy and disease aggressiveness. This result is consistent with the combination of upper and lower motor neuron degeneration as the main driver of disease worsening and severity in ALS. Our study highlights the potential of brain and spinal cord atrophy measured by MRI as biomarker of disease aggressiveness signature.

Keywords: Amyotrophic lateral sclerosis, MRI, atrophy, brain, spinal cord, disease aggressiveness

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a relentlessly progressive neurodegenerative disorder. Upper and lower motor neurons (UMN and LMN) degeneration was identified as a core feature of ALS since the earliest descriptions of the condition [1-3], accounting for the heterogeneity of clinical presentations [4]. Heterogeneous disease-progression rates impact prognosis and might affect treatment trial design and responsiveness to future treatments [5]. Biomarkers of disease aggressiveness signature are urgently needed to stratify patients into groups with homogeneous disease progression rates, facilitating testing new treatments and evaluating their short and mid-term efficiency [5].

The overwhelming majority of imaging studies in ALS remain cerebral, targeting UMN degenerative component in ALS [6-11]. Studies on the brainstem and spinal cord damage in ALS are scarce. Expanding the focus to the brainstem and spinal cord, disease-defining sites of ALS, provides a unique opportunity to capture both relative contributions to LMN and UMN degeneration [12-14].

Significant brain and spinal cord atrophy was evidenced in patients with ALS, predicting progression and survival [15-17]. However, the contribution of UMN and LMN degeneration to disease aggressiveness as well as the relationship between the two neurodegenerative components are far from being clarified [12,13,18].

The objective of this study was to characterize the relative contributions of UMN and LMN degeneration to disease aggressiveness by measuring brain and upper cervical spinal cord atrophy.

METHODS

Subjects

Twenty-nine patients with ALS (mean age \pm SD = 54 \pm 10 years of age, disease duration = 1.6 \pm 1.2 years; 9 females) and 24 age- and gender-matched healthy controls

(HC) (age = 51 ± 10 years of age, 11 females) were recruited. The exclusion criteria were the absence of current or past history of neurologic disease other than ALS, frontotemporal dementia, the presence of respiratory insufficiency, or substantial bulbar impairment incompatible with an MRI examination. Patients were clinically assessed immediately after the MRI and scored on the revised ALS Functional Rating Scale (ALSFRS-R) [19], and the ALSFRS-R progression rate, defined as $([48 - \text{ALSFRS-R}] / \text{disease duration in months})$. Patients were clinically differentiated into fast and slow progressors according to their ALSFRS-R progression rate [11]. A threshold of -0.5 point of ALSFRS-R per month was set to classify fast and slow progressors. ALSFRS-R was longitudinally assessed at 3 months for 22 patients and 6 months for 26 patients.

MRI acquisition

MRI acquisition was performed on a 3T Verio system (Siemens, Erlangen, Germany) using a 32-channel phased-array ^1H head coil (Siemens). ^1H -MRI protocol included a 3D T1-weighted (T1w) Magnetization-Prepared Rapid Acquisition Gradient-Echo (MPRAGE) sequence (TE/TR/TI = 3/2300/900 ms, FOV = 256 mm, 160 slices, voxel size = $1 \times 1 \times 1 \text{ mm}^3$, 3D geometric distortions correction, acquisition time = 5min19s. The FOV covered the brain and the upper cervical spinal cord level (Figure 1).

Data processing

Brain segmentation. T1w images were corrected for intensity non-uniformity (N4) [20]. Images were aligned to the AC-PC plane using a rigid registration to the Montreal Neurological Institute template (MNI152). Images were segmented into tissue types (grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF)) using the Computational Anatomy toolbox (CAT12: <http://www.neuro.uni-jena.de/cat/>) [21]. Deep GM sub-structures (accumbens, amygdala, caudate, hippocampus, thalamus, pallidum and putamen) were segmented using FMRIB's Integrated Registration and Segmentation Tool (FIRST-FSL) [22]. The brainstem sub-structures (midbrain, pons and medulla oblongata) and cerebellar GM were segmented using the spatially unbiased infratentorial for enhanced resolution toolbox (SUITer: <https://github.com/NeuroN-Lab/SUITer>) [23]. Brain volumes were normalized for head size using the intracranial volume (ICV) with $\text{ICV} = \text{GM} + \text{WM} + \text{CSF}$ volumes.

Spinal cord segmentation. 3D T1-weighted images were denoised using a Spatially adaptive Non-local Means Filter [24]. Images were aligned to the AC-PC plane using a rigid registration to a customized version of the MNI152 template in which we added 90 empty slices in the caudal part of the volume. Images were cropped to isolate the spinal cord from the brain. Spinal cord at C1-C2 vertebral levels was segmented using SCT-DeepSeg module of the Spinal Cord Toolbox (SCT) [25]. Spinal cord cross-sectional area (CSA) was computed by normalizing SC volume to the cord length [26,27].

Statistical Analysis

Statistical analysis was performed using SPSS, Version 23 (IBM) and JMP Pro 15.2.0 (SAS Institute Inc). The Shapiro-Wilk test was used to test for normality of demographical, clinical and MRI variables.

Group Comparisons. Differences in age, disease duration, and the ALSFRS-R score between groups were assessed using the Student's t-test or the Kruskal-Wallis when applicable. Differences in gender between groups were assessed using the χ^2 test. Differences in MRI outcomes were assessed using a general linear model.

Correlations with clinical disability scores. Pearson's or Spearman's correlation coefficients were used, when applicable, to evaluate correlations between MRI outcomes, and clinical data. MRI data included brain compartment volumes and spinal cord CSA. Clinical data included the ALSFRS-R and ALSFRS-R bulbar sub-scores the day of the MRI, the ALSFRS-R at 3 and 6 months and the ALSFRS-R progression rate.

Discrimination between ALS sub-groups. The performance of the MRI metrics in discriminating between fast and slow ALS progressors was assessed using receiver operating characteristic curve (ROC) analysis and the likelihood ratio test.

RESULTS

Demographical and clinical data of subjects are reported in **Table 1**. An example of a T1-weighted in an ALS patient as well as GM, deep GM, cerebellar GM, WM, brainstem sub-structures and spinal cord at C1-C2 vertebral levels segmentations is shown in **Figure 1**.

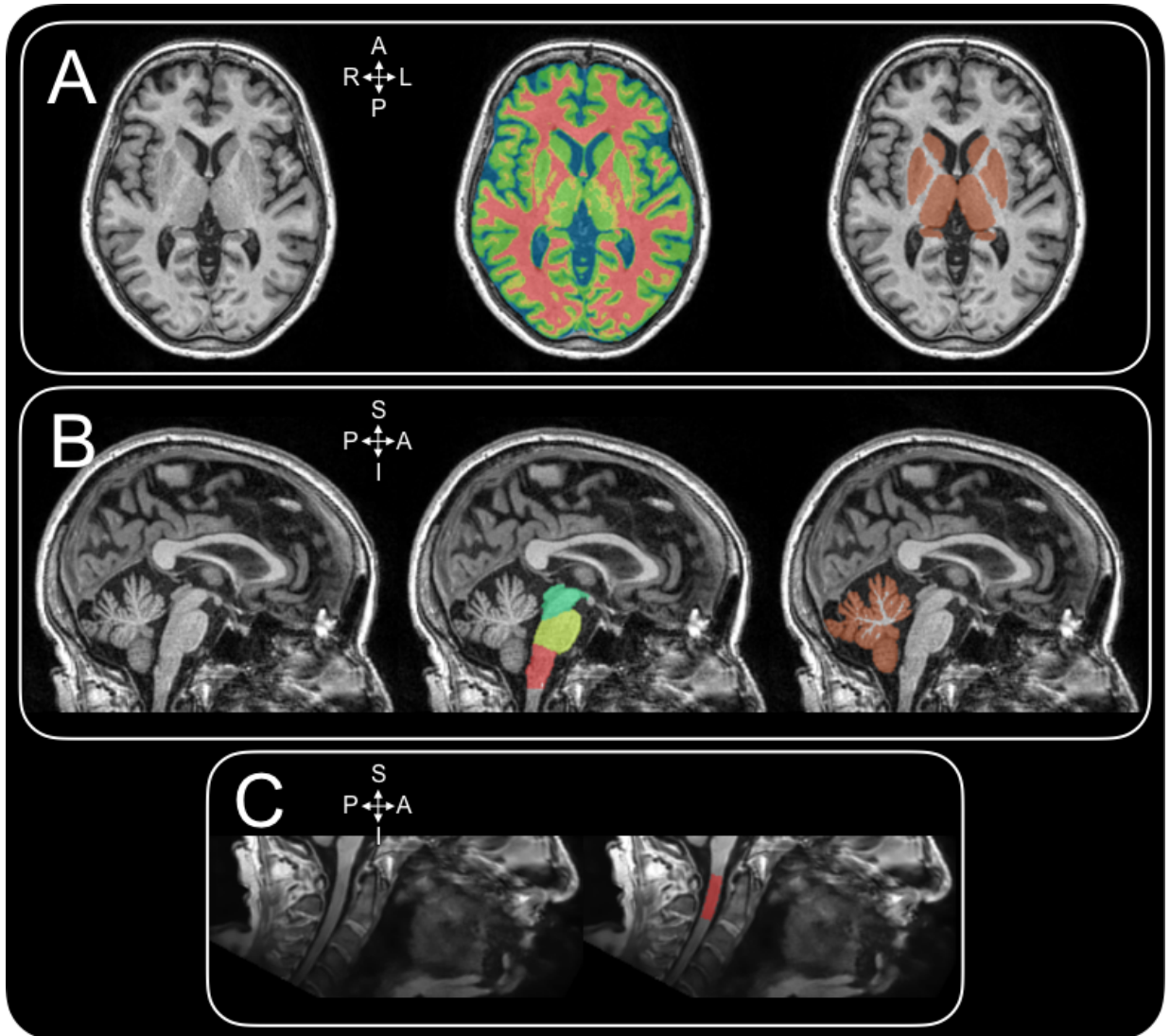


Figure 1. An example of a T1-weighted brain and spinal cord segmentations in an amyotrophic lateral sclerosis patient. (A) T1-weighted mid-axial slice and grey matter (green), white matter (red), cerebrospinal fluid (blue), and deep grey matter (orange) segmentations. (B) T1-weighted mid-sagittal slice and midbrain (green), pons (yellow), medulla oblongata (red), and cerebellar grey matter (orange) segmentations. (C) T1-weighted mid-sagittal slice and spinal cord at C1-C2 vertebral levels (red) segmentations. A, anterior; I, inferior, L, left; P, posterior; S, superior.

Groups comparison results for brain volumes and spinal cord CSA are shown in **Table 2**. **Figure 2** shows boxplots of GM, cerebellar GM and medulla oblongata volumes and spinal cord CSA in ALS patients, fast and slow progressors and HC.

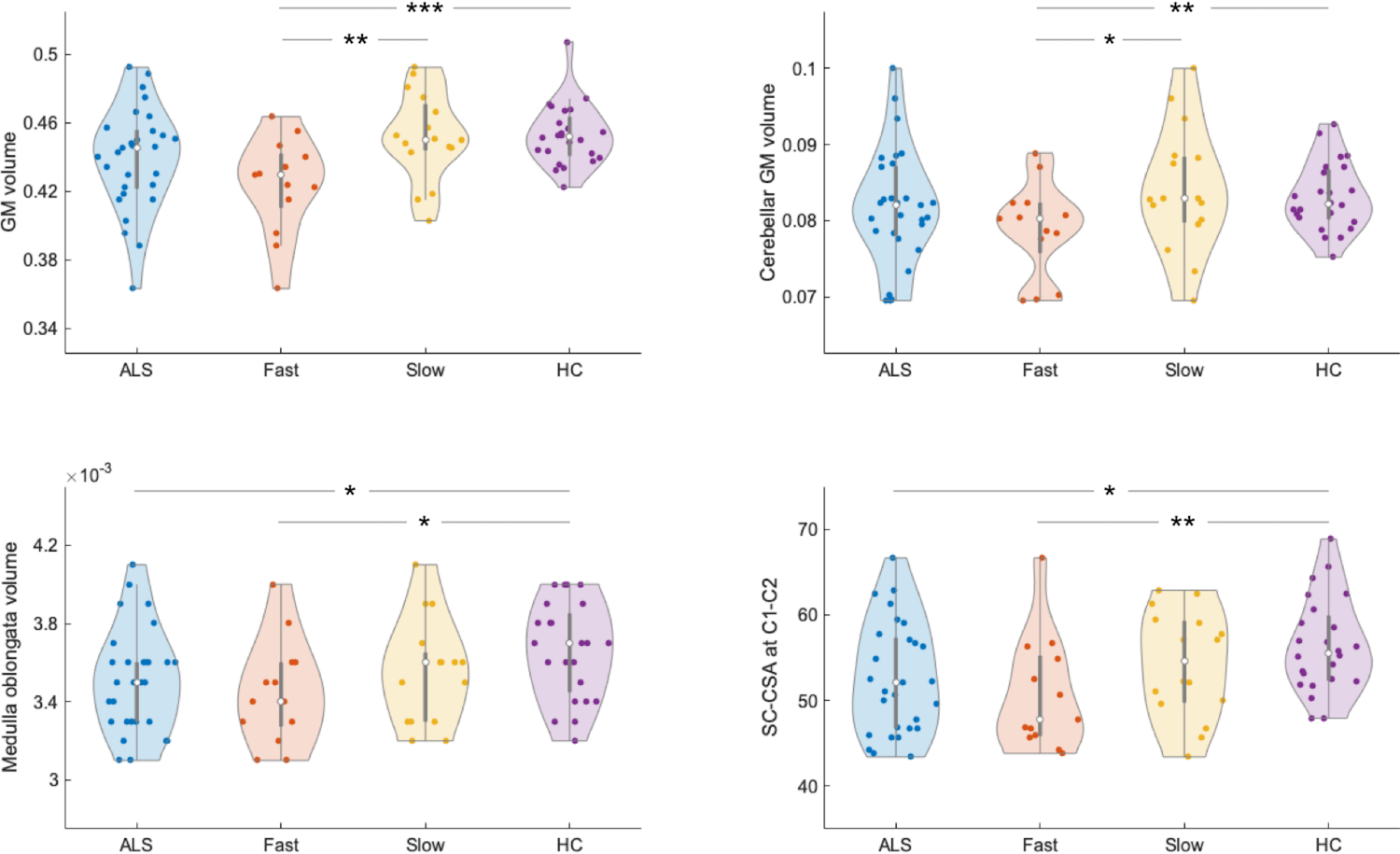


Figure 2. Violin plots reporting GM, cerebellar GM and medulla oblongata volumes and SC-CSA at C1-C2 vertebral levels in ALS patients, fast, slow ALS progressors and HC. Group differences were assessed using the general linear model. Levels of significance are indicated as: * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. ALS, amyotrophic lateral sclerosis; GM, grey matter; SC-CSA, spinal cord cross-sectional area.

ALS versus HC. ALS patients showed significantly lower deep GM and medulla oblongata volumes, and spinal cord CSA compared to HC ($p = 0.015$, $p = 0.042$ and $p = 0.030$, respectively). A trend of lower GM and cortical GM volumes in ALS patients

than in HC was found ($p = 0.058$ and $p = 0.068$, respectively). There were no significant differences between ALS patients and HC for cerebellar GM, WM, midbrain and pons volumes (all p -values > 0.05).

Fast progressors versus HC. Fast progressors showed significantly lower GM, cortical GM, cerebellar GM, deep GM, medulla oblongata volumes, and spinal cord CSA compared to HC ($p < 0.001$, $p = 0.001$, $p = 0.022$, $p = 0.049$, $p = 0.013$ and $p = 0.008$, respectively). There were no significant differences between ALS fast progressors and HC for WM, midbrain, and pons volumes (all p -values > 0.05).

Slow progressors versus HC. There were no significant differences between ALS slow progressors and HC for all MRI measures (all p -values > 0.05). However, a trend of lower deep GM volume in ALS slow progressors than in HC was found ($p = 0.053$).

Fast versus slow progressors. Fast progressors showed significantly lower GM and cerebral GM compared to slow progressors ($p = 0.008$ and $p = 0.009$, respectively). There were no significant differences between fast and slow progressors for cerebellar GM, deep GM, WM, midbrain, pons, medulla oblongata volumes, and spinal cord CSA (all p -values > 0.05).

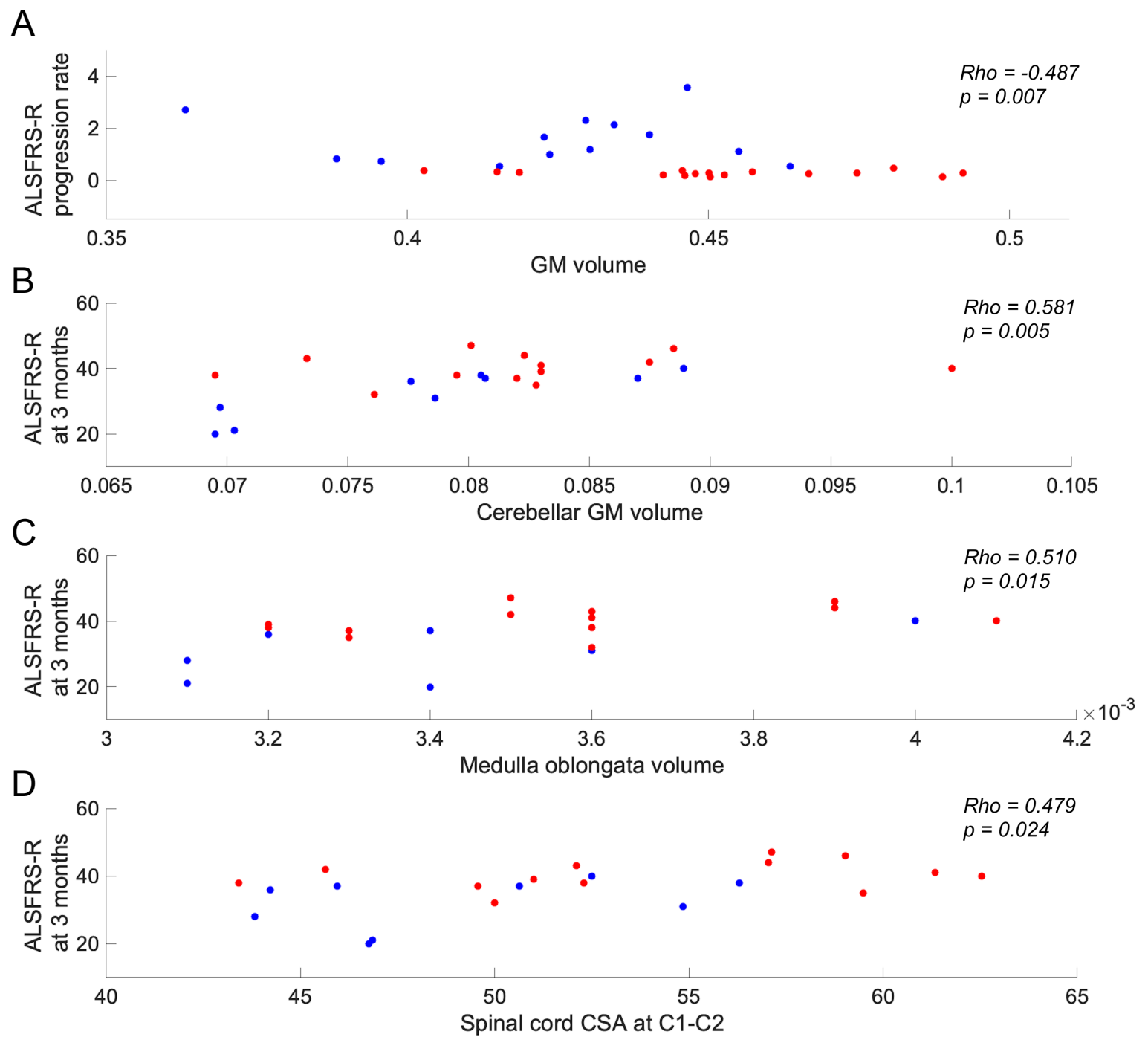


Figure 3. Correlations between MRI derived metrics and clinical scores from ALS patients. (A) Plots of GM volume versus ALSFRS-R progression rate. (B) Plots of cerebellar GM volume versus ALSFRS-R at 3 months. (C) Plots of medulla oblongata volume versus ALSFRS-R at 3 months. (D) Plots of spinal cord cross-sectional area at C1-C2 vertebral levels versus ALSFRS-R at 3 months. Blue and red dots represent fast and slow ALS progressors, respectively.

Correlations. Results of correlations are shown in Table 3 and illustrated in Figure 3. GM volume correlated with the ALSFRS-R progression rate ($Rho/p = -0.487/0.007$), the ALSFRS-R at 3 months ($Rho/p = 0.622/0.002$), and moderately correlated with ALSFRS-R at 6 months ($Rho/p = 0.407/0.039$). Cortical GM was moderately correlated with the ALSFRS-R progression rate ($Rho/p = -0.443/0.016$) and highly correlated with the ALSFRS-R at 3 months ($Rho/p = 0.457/0.005$). Cerebellar GM was moderately correlated with disease progression rate ($Rho/p = -0.390/0.037$), highly correlated with the ALSFRS at 3 months ($Rho/p = 0.581/0.005$) and moderately correlated with the ALSFRS-R at 6 months ($Rho/p = 0.401/0.042$). Medulla oblongata volume and spinal cord CSA moderately correlated with the ALSFRS-R at 3 months ($Rho/p = 0.510/0.015$ and $Rho/p = 0.479/0.024$, respectively). No correlations were found between the remaining MRI measures and clinical data, including the ALSFRS-R bulbar sub-score.

ROC analysis. ROC analysis showed that MRI measures (GM volume, medulla oblongata volume and spinal cord CSA) had a high area under curve (0.764), specificity (0.813), sensitivity (0.692) and accuracy (0.759) in discriminating between fast and slow ALS progressors (cut-off: GM volume = 0.439, medulla oblongata volume = 0.0035 and spinal cord CSA = 52.649). The likelihood ratio test showed that GM volume was the only significant discriminating parameter between fast and slow ALS progressors ($p = 0.020$). When computing ROC analysis using GM compartments (cortical, cerebellar and deep GM) volumes together with medulla oblongata volume and spinal cord CSA, the area under curve was 0.784, specificity was 0.563, sensitivity was 0.923 and accuracy was 0.724 in discriminating between fast and slow ALS progressors (cut-off: cortical GM volume = 0.3464, cerebellar GM volume = 0.0818, deep GM volume = 0.0111, medulla oblongata volume = 0.0035 and spinal cord CSA = 52.649). The likelihood ratio test showed that cortical GM volume was the only significant discriminating parameter between fast and slow ALS progressors ($p = 0.0499$).

DISCUSSION

The present study explored the contribution of compartmental atrophy including brain, brainstem and upper cervical SC to disease aggressiveness in ALS, and provided evidence of cerebral GM atrophy more pronounced in fast ALS progressors compared to lower progressors. Besides, GM volume was highly correlated with the ALSFRS-R progression rate and was associated with disease disability at 6 months and to a lesser extent, medulla oblongata and spinal cord atrophy were associated with disease disability at 3 months. Interestingly, brain and spinal cord measures were highly correlated with each other showing a continuum in the pathophysiological processes. Finally, ROC analysis suggested that MRI measures, particularly GM volume, enable good discrimination between fast and slow ALS.

Tissue atrophy in ALS is driven by the combination of UMN (brain, brainstem and spinal cord) and LMN (medulla oblongata and spinal cord) degeneration. Our study enabled, using a conventional MRI sequence, to explore UMN and LMN degenerative components in ALS by measuring brain, brainstem and upper cervical spinal cord compartmental atrophy. We found GM volume decreased in ALS patients compared to HC together with a significant atrophy of medulla oblongata and spinal cord in ALS patients. Our study suggests that brain GM atrophy could be an important marker of disease aggressiveness, as shown by the correlation between GM atrophy and ALSFRS-R progression rate. Interestingly, brain GM (cortical and cerebellar GM), medulla oblongata and spinal cord atrophy were associated with disease worsening at 3 months, and also at 6 months for cerebellar GM. Our study revealed thus an association between compartmental atrophy and disease aggressiveness. Volume reduction in the brainstem sub-structures was independent of bulbar clinical impairment in this study. This result can be biased by the limited number of subjects and the difficulty of including patients with significant bulbar impairment in MRI studies. A recently published study in a large dataset of ALS patients revealed that bulbar asymptomatic patients exhibit medulla oblongata atrophy [28].

Only fast ALS progressors showed significant brain and spinal cord atrophy when compared to HC, unlike slow ALS progressors. As in previous studies, our results suggest that fast progressors suffer from pronounced tissue damage compared to slow progressors in which alterations are less severe [8,11]. GM pathology (loss of LMN and UMN) has been suggested as the main driver of progression in ALS patients [9,29]. Our study highlights the potential of brain and spinal cord atrophy de-

rived from MRI as biomarker of disease aggressiveness signature, together with clinical functional scores.

Interestingly, our study shows that subcortical GM atrophy might also contribute to disease progression together with cortical GM. Compartmental analysis showed a combined contribution of cortical and subcortical GM atrophy to the overall ALS pathological process, which is in accordance with previous studies [7,30,31]. Structural, functional as well metabolic imaging studies pointed out cerebellar impairment in ALS [32]. Structural MRI studies showed the presence of cerebellar GM atrophy across ALS continuum [32,33]. Cerebellar pathology was detected up to a decade before the estimated symptoms onset in presymptomatic individuals carrying the C9orf72 mutation [34]. Senda et al. (2017) showed clusters of cerebellar atrophy specific to ALS fast and intermediate progression groups in comparison to ALS slow progression group [35].

Our results are in accordance with volumetric studies showing brainstem atrophy in ALS. Specifically, a recent study in a large cohort of ALS patients, showed considerable brainstem atrophy [12]. Volume reduction in ALS was dominated by medulla oblongata pathology at both baseline and 4 months follow-up. More importantly, the comparison with primary lateral sclerosis, a pure UMN disease, supports the contribution of UMN degeneration to brainstem volume reductions. Another study using vertex-wise analysis revealed that the ventral medulla oblongata and a small part of the ventral pons had significant atrophy in a cohort of ALS patients with a comparable size [13]. Together combined with a DTI analysis at the brainstem level, the authors suggested that degenerative processes in the brainstem may reflect disease severity in ALS [13]. Studies investigating upper cervical spinal cord in ALS showed significant atrophy at the level of C2-C3 vertebrae as well as at C2 and C1 vertebral levels computed from brain images [18,29,36]. Particularly, van der Burgh et al. (2019) showed upper cervical spinal cord atrophy in ALS and in primary muscular atrophy, suggesting that cervical spinal cord thinning is related to both UMN and LMN loss [18]. Large field of view head and neck imaging using state of art coils, enables quantifying brain and upper cervical spinal cord tissues atrophy [18,37,38], linking UMN and LMN degeneration as well as their impact on disease severity and progression [6-8,12,18].

Our results are partially discordant with two recently published studies, showing a significant difference in WM density between higher and lower disease aggressiveness groups, but not in cortical GM density nor cortical thickness [6,7]. This could be explained by different group comparisons methods (vertex/voxel comparisons using surface-based morphometry/voxel-based morphometry compared to our global approach using mean brain volumes). We also reported in a previous study, on the same cohort, widespread microstructural using DTI and ionic alterations using ^{23}Na -MRI in fast progressors, while slow progressors showed only restricted microstructure damage [11]. These MRI techniques were able to capture WM alterations that were not reflected by our global WM volumetric measurement.

Our study has several limitations. The main limitation is related to the restricted number of ALS patients, which prevented a better characterisation of ALS sub-groups and reduced the statistical power. The rapid progressive nature of ALS disease and its rarity prevented us from recruiting more patients within the 2-year inclusion period. Multicentric study would be of interest to increase the dataset size and to further investigate the link strength between compartmental atrophy and disease aggressiveness and worsening. Second, the T1w coverage restricted our analysis to the spinal cord at C1-C2 vertebral levels due to subjects variable head and neck size as well as the drop of MR signal of the most caudal segments of the volume. Besides, the standard resolution of our images was a limiting factor to investigate the compartmental spinal cord atrophy [39]. Large field of view head and neck imaging using state of art coils, that have recently become commercially available, would be of interest to quantify brain and several cervical spinal levels tissues atrophy [18]. Specific spinal cord acquisition, using high resolution sequences, would enable quantifying grey and white atrophy at cervical, thoracic and lumbar levels [39,40]. Finally, we regrettably did not investigate cognitive changes in our ALS cohort, which prevent us from linking GM degeneration, particularly cerebellar impairment, with cognitive decline [31,33].

CONCLUSION

Our study exploring brain and upper cervical spinal cord atrophy, by using conventional brain T1w-MRI, evidenced an association between compartmental atrophy and disease aggressiveness and worsening. Our study highlights the potential of brain

and spinal cord atrophy measured by MRI as biomarker of disease aggressiveness signature in ALS.

COMPETING INTERESTS

All authors certify that there is no financial interest related to this study.

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Table 1. Demographic and clinical data of study participants.

	ALS	Fast	Slow	HC	p-value
Number	29	13	16	24	-
Age (years)	54.3 ± 10.2	56 ± 9.9	52.9 ± 10.6	51 ± 10.7	0.244 ^{a+} , 0.152 ^{a#} , 0.586 ^{a*} , 0.388 ^{a=}
Gender	9F/20M	6F/7M	3F/13M	11F/13M	0.394 ^{b+} , 0.101 ^{b#} , 1.000 ^{b*} , 0.172 ^{b=}
Disease duration (months)	18.8 ± 14.5	19.4 ± 13.9	18.4 ± 15.4	-	0.660 ^{c=}
Site of onset					
Spinal	22 (6 UL, 16 LL)	10 (3 UL, 7 LL)	12 (3 UL, 9 LL)	-	-
Bulbar	7	3	4	-	-
Revised El Escorial criteria					
Definite	7	7	0	-	-
Probable	7	3	6	-	-
Probable laboratory supported	6	1	5	-	-
Possible	9	2	5	-	-
Disease progression rate	0.84 ± 0.87	1.54 ± 0.93	0.27 ± 0.09	-	0.001 ^{c=}
ALSFRS-R (/48)	38.72 ± 5.55	37.31 ± 4.81	40.46 ± 6.09	-	0.067 ^{c=}
ALSFRS-R 3-months (/48)	36.82 ± 6.98	32.00 ± 7.48	40.15 ± 4.30	-	0.006 ^{c=}
ALSFRS-R 6 months (/48)	33.38 ± 9.16	26.50 ± 10.56	37.68 ± 4.66	-	0.007 ^{c=}

Values are expressed in mean ± SD. -, not applicable; ALSFRS-R. revised ALS functional rating scale; DD. Disease duration; F. female; fast, fast progressors; LL. lower limb; M. male; slow, slow progressors; UL. upper limb.

^a Student's t-test; ^b Chi-squared test; ^c Kruskal-Wallis test

+ALS vs HC; #fast vs HC; *slow vs HC; =fast vs slow.

Table 2. Brain volumes and spinal cord cross-sectional area in ALS, fast and slow progressors and HC.

MRI measurement	ALS	Fast	Slow	HC	P-value ^{+,a,b,c,d}
Brain volumes					
GM	0.4393 ± 0.0297	0.4237 ± 0.0279	0.4520 ± 0.0254	0.4527 ± 0.0177	0.058, 5.10 ⁻⁴ *, 0.914, 0.008*
Cortex	0.3464 ± 0.0247	0.3337 ± 0.0239	0.3568 ± 0.0206	0.3575 ± 0.0173	0.068, 0.001*, 0.897, 0.009*
Cerebellum	0.0818 ± 0.0076	0.0789 ± 0.0061	0.0841 ± 0.0081	0.0831 ± 0.0044	0.449, 0.022*, 0.629, 0.069
Deep GM	0.0111 ± 0.0013	0.0110 ± 0.0012	0.0111 ± 0.0013	0.0120 ± 0.0015	0.015*, 0.049*, 0.053, 0.903
WM	0.3551 ± 0.0188	0.3571 ± 0.0198	0.3535 ± 0.0183	0.3611 ± 0.0153	0.213, 0.497, 0.161, 0.613
Midbrain	0.0068 ± 0.0003	0.0067 ± 0.0003	0.0068 ± 0.0003	0.0069 ± 0.0004	0.061, 0.078, 0.226, 0.413
Pons	0.0098 ± 0.0006	0.0097 ± 0.0007	0.0098 ± 0.0006	0.0100 ± 0.0006	0.273, 0.284, 0.485, 0.636
Medulla oblongata	0.0035 ± 0.0003	0.0034 ± 0.0003	0.0035 ± 0.0003	0.0037 ± 0.0002	0.042*, 0.013*, 0.299, 0.169
SC-CSA C1-C2	52.65 ± 6.51	50.68 ± 6.57	54.25 ± 6.21	56.40 ± 5.48	0.030*, 0.008*, 0.257, 0.145

⁺ General linear model. Significant values are marked with *. ^a ALS versus HC. ^b Fast versus HC. ^c Slow versus HC. ^d Fast versus Slow. ALS, amyotrophic lateral sclerosis; CSA, cross-sectional area; HC, healthy controls. ALS, amyotrophic lateral sclerosis; GM, grey matter; HC, healthy controls; SC-CSA C1-C2, spinal cord cross-sectional area at C1-C2 vertebral levels; WM, white matter.

Table 3. Correlations between MRI volumetric measurements and clinical disability scores.

MRI measurement	Disease			
	progression rate	ALSFRS-R	ALSFRS-R M3	ALSFRS-R M6
GM volume	-0.487 (0.007)*	0.166 (0.389)	0.622 (0.002)*	0.407 (0.039)*
Cortex	-0.443 (0.016)*	0.136 (0.481)	0.572 (0.005)*	0.371 (0.062)
Cerebellum	-0.390 (0.037)*	0.209 (0.277)	0.581 (0.005)*	0.401 (0.042)*
Medulla oblongata volume	-0.140 (0.469)	0.330 (0.080)	0.510 (0.015)*	0.366 (0.066)
SC-CSA C1-C2 (mm ²)	-0.286 (0.133)	0.278 (0.145)	0.479 (0.024)*	0.075 (0.717)

Significant values are marked with *. ALSFRS-R, revised amyotrophic lateral sclerosis functional rating scale; GM, grey matter; M3, 3-months follow-up; M6, 6-months follow-up; SC-CSA C1-C2, spinal cord cross-sectional area at C1-C2 vertebral levels.