ORIGINAL ARTICLE

Gray Matter Volume Changes over the Whole Brain in the Bulbar- and Spinal-onset Amyotrophic Lateral Sclerosis: a Voxel-based Morphometry Study

Zhiye Chen^{1,2}, Mengqi Liu^{1,2}, Lin Ma^{1*}

¹Department of Radiology, Chinese PLA General Hospital, Beijing 100853, China

²Department of Radiology, Hainan Branch of Chinese PLA General Hospital, Sanya, Hainan 572013, China

Key words: amyotrophic lateral sclerosis; gray matter; magnetic resonance imaging; precentral gyrus; voxel-based morphometry

Objective To investigate cerebral structural signatures of the bulbar- and spinal-onset amyotrophic lateral sclerosis (ALS) using voxel-based morphometry on magnetic resonance imaging.

Methods The MR structural images of the brain were obtained from 65 ALS patients (15 bulbar-onset, 50 spinal-onset) and 65 normal controls (NC) on a 3.0T MRI system. Gray matter (GM) volume changes were investigated by voxel-based morphometry, and the distribution of the brain regions with volume changes was compared between ALS and normal controls, as well as between bulbar-onset and spinal-onset ALS based on Neuromorphometrics atlas.

Results On voxel-level the decreased volume of brain regions in ALS patients was located in the right precentral gyrus (rPrCGy) and right middle frontal gyrus compared with that in NC. The bulbar-onset ALS presented extra-motor cortex atrophy (fronto-temporal pattern), including left medial orbital gyrus, left inferior temporal gyrus and right middle temporal gyrus; the spinal-onset ALS suffered from motor cortex atrophy (rPrCGy dominance) and extra-motor cortex atrophy (fronto-temporal and extra-fronto-temporal pattern) compared with NC. The spinal-onset ALS featured by GM volume loss of left postcentral gyrus and bulbar-onset ALS featured by GM volume loss of left middle temporal gyrus compared with each other.

Conclusions The asymmetric GM atrophy of the motor cortex and extra-motor cortex represents the common MRI structural signatures of spinal-onset ALS, and sole extra-motor cortex atrophy represents the structural signatures of bulbar-onset ALS. The present study also demonstrated that the pattern of GM damage is likely to distribute wider in spinal-onset ALS than in bulbar-onset ALS.

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*Corresponding author E-mail: cjr.malin@vip.163.com

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AMYOTROPHIC lateral sclerosis (ALS) is a progressive neurodegenerative disease with selected both upper and lower motor neuron lesions.¹⁻² In ALS, many voxel-based morphometry (VBM) studies found that gray matter (GM) volume decreased widely, especially in motor cortex,³⁻⁴ frontal and temporal regions,⁴⁻⁶ corpus callosum,⁷ amygdala,⁸ caudate nucleus head.⁹ However, Mezzapesa *et al's* study showed that no volume reduction of primary motor cortices in ALS patients.¹⁰ Voxel-wise meta-analysis¹¹⁻¹² has revealed that ALS is a complex degenerative disease involving multi-systems besides motor system, and right precentral GM atrophy is a common finding and prominent feature of brain structural changes in ALS.¹³ However, GM changes over the whole brain were not comprehensively studied in bulbar-onset and spinal-onset ALS patients.

The aim of this study is to investigate brain structural imaging signatures in clinical subtypes of ALS. We hypothesized that some brain regions may intrinsically suffered from volume loss in the patients with ALS. To address this hypothesis, we obtained brain structure images of 65 patients with ALS (ALS group) and 65 normal controls (NC) from 3.0T MRI system. All the ALS patients were classified into bulbar-onset group (ALS-bulbar, $n=15$) and spinal-onset group (ALS-spinal, $n=50$) according to the initial onset location. We firstly identified the alteration of GM changes over the whole brain between the ALS group and the NCs, and then the GM volume analysis was performed among the ALS-bulbar group, ALS-spinal group and the control group to identify the pattern of different GM volume loss.

PATIENTS AND METHODS

Participants

This study was approved by the institutional ethic review board and written informed consents were obtained from all participants. Sixty-five patients, including 34 cases with definite diagnosis of ALS and 31 cases with suspicious diagnosis of ALS according to the revised El Escorial,¹⁴ were consecutively recruited from the Chinese PLA General Hospital from 2007 to 2010. The subjects were excluded for the following conditions: 1. history of cerebrovascular disease, long-standing hypertension, diabetes mellitus, inflammatory disease of the central nervous system and cranium trauma; 2. taking psychoactive drugs or hormone; 3. structural damage observed on the conventional MR images. All the

subjects were right handed. ALS functional rating score (ALSF_{RS})¹⁵ and Norris Scale¹⁶ were administered to all the patients for clinical rating their ALS symptoms, and mini-mental state examination (MMSE)¹⁷ was applied to evaluate the cognitive function of all subjects.

MRI examination and acquisition

MR structural images were obtained from a 3.0T MR imaging system (SIGNA EXCITE, GE Healthcare, Milwaukee, WI, USA) and a conventional eight-channel quadrature head coil. Three-dimensional T1-weighted fast spoiled gradient recalled echo (3D T1-FSPGR) sequence was used, and the parameters were as follows: TR (repetition time) = 6.3 ms, TE (echo time) = 2.8 ms, flip angle = 15°, FOV (field of view) = 24 cm × 24 cm, Matrix = 256 × 256, in-plane resolution of 0.9375 × 0.9375 mm², NEX (number of acquisition) = 1. Conventional T2-weighted images, T1-weighted images and diffusion weighted images were also obtained to exclude the patients with diseases other than ALS that inflecting brain morphometry. The imaging protocol was identical to each subject.

Data processing

All MR structural image data were processed using Statistical Parametric Mapping 12 (SPM 12) (<http://www.fil.ion.ucl.ac.uk/spm/>) running under MATLAB 7.6 (The Mathworks, Natick, MA, USA) to perform VBM.¹⁸ The preprocess of VBM was as follows: (1) setting the image origin at the anterior commissure (AC); (2) normalizing the individual structural images to the DARTEL (Diffeomorphic Anatomical Registration using Exponentiated Lie algebra) templates space; (3) segmenting the normalized images into GM, white matter and cerebrospinal fluid (CSF); (4) calculating the volume of 142 brain regions based on SPM Neuromorphometrics atlas; total GM volume (TGMV), total white matter volume (TWMV), and total CSF volume (TCSFV) were recorded. Total intracranial volume (TIV) was the sum of the volume of segmented brain tissue ($TIV = TGMV + TWMV + TCSFV$), and fraction of GMV (fGMV) was the ratio of TGMV to TIV ($fGMV = TGMV/TIV$); (5) smoothing the segmented GM images with a kernel of 8 mm FWHM (full width at half maxima) before statistical analysis.

Statistical analysis

VBM was performed using two-sample *t* test with TIV, age and sex as covariates by the factorial design specification tools of SPM. Significance was set if a *Puncorr*

value<0.001. The cluster size was listed as K value, and the difference of the cluster between two groups was represented by T value. The minimal number of contiguous voxels was set based on the expected voxels per cluster.

The quantitative data with normal distribution were presented as mean $\pm$ standard deviation, and the quantitative data with nonnormal distribution were presented as median $\pm$ interquartile range. The partial correlation analysis was applied between the clinical variables (ALSFRS, disease duration, Norris score, MMSE) and volume of abnormal brain regions in ALS patients. The group difference for the abnormal brain regions was compared using ANCOVA covarying with age and sex, and the Welch test was used because of variance non-homogeneity. The statistical analysis was performed using SPSS 19.0.

RESULTS

Demography and clinical scale test

The participants came from 10 provinces and included 63 sporadic cases and 2 familial cases. There were 28 female and 37 males in the ALS group, 29 females and 36 males in the NC group ($\chi^2=0.03$, $P=0.86$). The age of the two group were 49.63 ± 10.25 years old and 43.48 ± 12.22 years old ($t=1.98$, $P=0.002$). The MMSE of the ALS patients and NCs were comparable (both 28.00 ± 3.00 , $t=1.98$, $P=0.043$). For ALS patients, the average of disease duration, ALSFRS and Norris scores were 17.74 ± 21.46 months, 30.00 ± 7.00 (normal value 48) and 69.00 ± 28.50 (normal value 99), respectively.

GM volume changes over the whole brain of ALS patients

In patients with ALS, the decreased GM volume of brain regions, compared with NC, located in the right precentral gyrus (rPrcGy) [MNI coordinate (x, y, z): 39, -12, 53; K value: 624; T value: 4.38; P value: 0.000] and right middle frontal gyrus (rMidFroGy) [MNI coordinate (x, y, z): 42, 56, 11; K value: 150; T value: 3.88; P value: 0.000]. No increased GM volume of brain region in ALS was recognized (Fig. 1).

ANCOVA demonstrated the GM volume of the rPrcGy were significant different between the ALS and the NC groups ($P=0.03$). No significant difference of TGMV, TIV and fGMV was observed for the rMidFroGy between the ALS and NC group (all $P>0.05$) (Table 1).

Figure 1. Brain region with decreased gray matter (GM) volume in ALS group compared with NC group. Colored areas represent the involved brain areas: right precentral gyrus and middle frontal gyrus respectively. The value of the color bar represents T value.

Table 1. Volume comparisons of rPrcGy, rMidFroGy, TGMV, TIV and fGMV between the ALS and NC groups (10^{-3} ml, $n=65$)

Groups	Volume of rPrcGy		Volume of rMidFroGy	
	means±SD	95% <i>CI</i>	means±SD	95% <i>CI</i>
NCs	12.14±1.33	11.68-12.30	18.71±2.41	17.84-18.97
ALS	11.36±1.34	11.19-11.81	17.64±2.61	17.38-18.51
<i>F</i> value	4.76		1.26	
<i>P</i> value*	0.03		0.27	

Groups	TGMV		TIV		fGMV	
	means±SD	95% <i>CI</i>	means±SD	95% <i>CI</i>	means±SD	95% <i>CI</i>
NCs	610.75±56.74	589.83-617.29	1423.72±127.38	1385.11-1453.02	0.43±0.02	0.42-0.43
ALS	574.72±64.43	578.18-605.64	1393.96±144.74	1364.66-1432.57	0.42±0.02	0.41-0.43
<i>F</i> value	1.36		0.69		0.41	
<i>P</i> value*	0.25		0.41		0.52	

*analysis of covariance was performed for comparisons adjusted by age. NC: normal control; ALS: amyotrophic lateral sclerosis; rPreGy: right precentral gyrus; rMidFroGy: right middle frontal gyrus; TGMV: total gray matter volume; TIV: total intracranial volume; fGMV: fraction of gray matter volume (=TGMV/TIV).

GM volume changes between ALS-bulbar and NC

In patients with ALS-bulbar, compared with NCs, the decreased GM volume was located in the left medial orbital gyrus [MNI coordinate (x, y, z): -11, 41, -30; *K* value: 125; *T* value: 4.09; *P* value: 0.000], the left inferior temporal gyrus [MNI coordinate (x, y, z): -53, -32, -21; *K* value: 418; *T* value: 4.87; *P* value: 0.000] and the right middle temporal gyrus [MNI coordinate (x, y, z): 65, -32, -14; *K* value: 134; *T* value: 4.16; *P* value: 0.000] (Fig. 2). No increased GM volume in ALS-bulbar was recognized.

Among the above three regions with loss of GM, only the volume of the left inferior temporal gyrus in the ALS-bulbar was found to have significant difference from the NCs ($F=6.19$, $P=0.02$). The GMV, fGMV and TIV were not significantly different between the ALS-bulbar and NC groups (Table 2).

GM volume changes between ALS spinal and NC

Compared with the NC group, the decreased GM volume of brain regions in the ALS-spinal group were located in the rPrcGy, left medial precentral gyrus, left postcentral gyrus, bilateral superior frontal gyrus, right middle frontal gyrus, bilateral middle occipital gyrus, bilateral angular gyrus, right middle temporal gyrus, left thalamus proper (lThaPro), left central operculum, left supramarginal gyrus, left precuneus, left inferior frontal angular gyrus, left fusiform gyrus, and left superior parietal lobule (Table 3, Fig. 3).

Differences of GM volume alterations between ALS-spinal and ALS-bulbar

Compared with ALS-bulbar, the ALS-spinal presented decreased GM volume in the left postcentral gyrus [MNI coordinate (x, y, z): -65, -5, 29; *K* value: 335; *T*

Figure 2. Brain regions with decreased GM volume in bulbar-onset ALS patients compared with NC group. Colored areas represent the involved brain areas, including left medial orbital gyrus, left inferior temporal gyrus and right middle temporal gyrus group respectively. The value of the color bar represents *T* value.

Table 2. Volume comparisons of the brain regions with decreased GMV and TGMV, TIV, fGMV between the ALS-bulbar and NC groups (10^{-3} ml)

Groups	<i>n</i>	Volume of lMedOrbGy		Volume of lInfTemGy		Volume of rMidTemGy	
		means±SD	95% CI	means±SD	95% CI	means±SD	95% CI
NCs	65	4.40±0.57	4.08-4.64	10.00±1.00	9.39-10.59	14.14±1.97	13.09-14.76
bulbar-onset ALS	15	4.15±0.51	3.91-4.47	8.94±1.17	8.35-9.55	13.49±1.46	12.87-14.54
<i>F</i> value		0.70		6.19		0.14	
<i>P</i> value*		0.41		0.02		0.71	
Groups	<i>n</i>	TGMV		TIV		fGMV	
		means±SD	95% CI	means±SD	95% CI	means±SD	95% CI
NCs	65	610.75±56.74	589.83-617.29	1423.72±127.38	1385.11-1453.02	0.43±0.02	0.42-0.43
bulbar-onset ALS	15	577.18±65.12	554.32-614.58	1366.53±133.84	1309.04-1441.14	0.42±0.02	0.41-0.44
<i>F</i> value		0.07		0		0.32	
<i>P</i> value*		0.80		0.98		0.58	

*Analysis of covariance was performed for the group comparisons covarying with age. lMedOrbGy: left medial orbital gyrus; lInfTemGy: left inferior temporal gyrus; rMidTemGy: right middle temporal gyrus.

Table 3. Brain regions with decreased GM volume in patients with ALS-spinal compared with NCs

Anatomic region	MNI-space			K value	P value	T value
	X	Y	Z			
Left Thalamus Proper	-21	-21	12	928	0.000	5.58
Right Precentral Gyrus	48	-9	57	2753	0.000	5.46
Left Superior Frontal Gyrus	-18	54	6	4582	0.000	5.35
Left Postcentral Gyrus	-54	-8	33	4032	0.000	5.15
Right Middle Frontal Gyrus	42	53	9	1015	0.000	4.88
Left Central Operculum	-54	2	3	633	0.000	4.27
Right Superior Frontal Gyrus	23	54	21	351	0.000	4.24
Right Middle Occipital Gyrus	32	-75	41	215	0.000	4.15
Left Supramarginal Gyrus	-45	-48	24	141	0.000	4.14
Left Precuneus	-5	-65	27	1157	0.000	4.06
Left Middle Occipital Gyrus	-29	-80	15	293	0.000	4.00
Left Medial Precentral Gyrus	0	-18	75	220	0.000	3.97
Right Superior Frontal Gyrus	26	33	47	123	0.000	3.96
Right Middle Temporal Gyrus	60	-20	-8	274	0.000	3.95
Right Angular Gyrus	57	-48	18	166	0.000	3.87
Left Angular Gyrus	-48	-60	35	153	0.000	3.78
Left Inferior Frontal Angular Gyrus	-48	26	6	130	0.000	3.74
Left Fusiform Gyrus	-32	-33	-29	177	0.000	3.70
Left Superior Frontal Gyrus	-17	39	48	300	0.000	3.69
Left Superior Parietal Lobule	-26	-54	56	176	0.000	3.67

MNI: Montreal Neurological Institute; X, Y, Z: coordinates of the primary maximum of the cluster. ($T > 3.18$, *Puncorr* value < 0.001 , expected 122 voxels per cluster)

Figure 3. Brain regions with decreased GM volume in ALS-spinal patients compared with NC group. Multiple colored areas represent the involved brain regions, including bilateral precentral gyrus (right precentral gyrus dominance) and the other multiple brain regions. The value of the color bar represents T value.

value: 4.32; P value: 0.000]; meanwhile, the ALS-bulbar presented decreased GM volume in the left middle temporal gyrus (MNI coordinate (x, y, z): -59, -29, -18; K value: 281; T value: 4.07; P value: 0.000) compared with ALS-spinal (Fig. 4).

Correlation analysis between regional volume changes and the clinical features

In ALS patients, the volume of rPrcGy was positively correlated with ALSFRS ($r=0.299$, $P=0.017$). In ALS-bulbar patients, volume of the left medial orbital gyrus, left inferior temporal gyrus, right middle temporal gyrus was not found to correlate with any of clinical variables (disease duration, ALSFRS or Norris score). In ALS-spinal patients, there was a significant negative correlation between the volume of the lThaPro and the disease duration ($r=-0.383$, $P=0.007$), and no correlation was found between the volume of lThaPro and ALSFRS, or Norris score.

DISCUSSION

This study demonstrated that the rPreGy presented volume loss in the ALS patients. This result was consistent with the previous studies,¹¹⁻¹³ which suggested that the rPreGy atrophy may be a MRI structural signature for ALS, disregarding its clinical type. VBM analysis demonstrated that atrophy of rPreGy presented only in ALS-spinal patients, but not in

ALS-bulbar patients. Therefore, VBM technique could elucidate the different neuromechanism for the different ALS subtypes, and provide a valuable method to understand the structure changes of motor cortex in ALS. The finding of rPreGy atrophy on voxel-level might be explained by the decreased number and shape of cortical neurons¹⁹⁻²⁰ from the pathological viewpoints.

Further analysis in the clinical subtypes demonstrated that bulbar-onset and spinal-onset ALS presented different patterns of GM atrophy. For the bulbar-onset ALS, only extra-motor brain regions were involved, and the motor cortex was spared. The involved brain regions were located in frontal lobe (left medial orbital gyrus) and temporal lobe (left inferior temporal gyrus and right middle temporal gyrus). These findings may be associated with the poorer cognitive function in bulbar-onset ALS patients than those of spinal-onset subtype.²¹ Therefore, bulbar subtype may be considered as fronto-temporal pattern ALS, and the longitudinal observe will help to understand the association between ALS and fronto-temporal dementia.

However, the analysis of the whole brain region involvement for the bulbar-onset ALS patients showed that only left inferior temporal gyrus was significantly different from the controls. This finding suggested that the left inferior temporal gyrus was the key damaged brain region in bulbar-onset ALS patients.

In patients with spinal-onset ALS, our study demonstrated that motor and extra-motor brain regions were both involved compared with NCs. The involved motor brain regions included the rPreGy (2753 voxels) and left medial precentral gyrus (220 voxels), which was consistent with previous studies,^{4, 22-25} and could underlie the pathological characteristics²⁶⁻²⁷ and MRS findings²⁸ in ALS. However, some studies reported no atrophy of motor cortex,^{5, 10, 29} and some study found the metabolic changes took place in motor cortex of ALS patients, with no concomitant anatomical change.³⁰ The reasons for the inconsistency might be associated with heterogeneity course of pathologic process and the different clinical features, such as initial onset of the ALS patients in this study. The result of this study indicated that motor cortex atrophy was the MRI signature of spinal-onset ALS.

Extra-motor GM involvement was also demonstrated in this study, which could be considered as a substantial feature of ALS. The extra-motor GM damage involved bilateral frontal cortex, right temporal

Figure 4. Brain regions with altered volume of GM in the ALS-spinal and ALS-bulbar. Hot cluster in yellow, located in the left postcentral gyrus, represented the decreased volume in ALS-spinal compared with ALS-bulbar; cold cluster in green, located in the left middle temporal gyrus, represented decreased volume in ALS-bulbar compared with ALS-spinal.

cortex, as well as the other brain region where GM volume loss. Bilateral superior frontal gyrus presented significant GM loss, indicating that these brain regions may play a critical role in motor response inhibition³¹ and recovery of language function³² in ALS patients. Since left inferior frontal gyrus also plays a critical role in integrating individual word meanings to coherent sentence-level messages³³ and language production,³⁴ the volume loss of frontal gray matter we found in this study may help to understand the verbal fluency deficits of ALS.³⁵⁻³⁷

Regional atrophy of temporal lobe mainly located in the right middle temporal gyrus, which was partially consistent with previous studies.^{10, 24} The asymmetric atrophy in temporal lobe may be associated with heterogeneous pathological process and the cognitive state.

In this study we found that GM deficits distributed widely in the whole brain of spinal-onset ALS compared with bulbar-onset ALS. In clinical practice, the bulbar-onset ALS is less common than spinal-onset ALS;³⁸ however, 48% of ALS patients were identified with cognitive impairment,³⁹ and the abnormalities indicated fronto-temporal involvement. Our results suggest that widespread GM loss of extra-motor cortex such as frontal lobe and temporal lobe exists in spinal-onset ALS patients compared with bulbar-onset ALS patients. The extensive atrophy of extra-motor cortex also suggests that more upper motor neurons may be involved in spinal-onset ALS than bulbar-onset ALS patients. These findings may also reflect the heterogeneity of clinical onsets in ALS patients, and provide useful information to better understand the cognitive impairment in ALS.

VBM analysis demonstrated the differences existed between bulbar- and spinal-onset ALS. The volume of left postcentral gyrus in spinal-onset ALS and left middle temporal gyrus in bulbar-onset ALS were significantly different compared to each other. This finding indicates the intrinsic difference may exist between the clinical sub-types of ALS, and its clinical significance should be investigated in future.

The limits of this study included: (1) The samples presented heterogeneity, and the bulbar-onset ALS sample was relatively small; (2) Although MMSE was performed to exclude dementia, the mild cognitive impairment was not evaluated in detail.

To conclude, these findings supported that the atrophy of rPreGy might be used as a feasible imag-

ing biomarker for diagnosis of ALS. Extra-motor GM atrophy (fronto-temporal pattern) is a MRI structural signature in bulbar-onset ALS patients. Motor and extra-motor (fronto-temporal and extra-fronto-temporal pattern) GM atrophy were structural signatures on MRI in spinal-onset ALS patients. VBM is a sensitive and effective tool for brain structure evaluation in patients with ALS.

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