Amygdala Abnormalities Are Related to Anxiety in Patients With Sporadic Amyotrophic Lateral Sclerosis

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Abstract

To explore selective atrophy patterns and resting-state functional connection (FC) alterations in the amygdala at different stages of amyotrophic lateral sclerosis (ALS), and to determine any correlations between amygdala abnormalities and neuropsychiatric symptoms. We used the King's clinical staging system for ALS to divide 83 consecutive patients with ALS into comparable subgroups at different disease stages. We investigated the pattern of selective amygdala subnucleus atrophy and analysed amygdala-based whole-brain FC analysis in the patients and 94 healthy controls (HCs). Cognitive and emotional functions were also evaluated using a neuropsychological test battery. There were no significant differences between King's stage 1 ALS patients and HCs for any amygdala subnucleus volumes. Compared with HCs, King's stage 2 patients had significantly lower left accessory basal nucleus and cortico-amygdaloid transition volumes after Bonferroni correction. Furthermore, after Bonferroni correction, King's stage 3 patients demonstrated significant reductions in most subnucleus volumes as well as global amygdala volume compared with HCs. Notably, amygdala-based resting-state FC was unaltered in ALS patients until King's stage 3. Specific subnucleus volumes were significantly associated with Mini-Mental State Examination scores and Hamilton Anxiety Rating Scale scores in ALS patients. In conclusions, our study provides a comprehensive profile of amygdala abnormalities in ALS patients. The pattern of amygdala abnormalities in ALS patients differed across King's clinical disease stages, and our findings suggest that amygdala abnormalities are an important feature of patients with ALS. Moreover, amygdala volume may play an important role in anxiety and cognitive dysfunction in ALS patients.

Introduction

Amyotrophic lateral sclerosis (ALS) is a rare neurodegenerative disease with both clinical and hereditary heterogeneity [1]. The aetiology of ALS remains unknown; however, interactions between genetic and environmental factors likely underpin disease susceptibility [2]. ALS is likely derived from cortical influences and the onset of ALS appears to involve a multistep process, with a long pre-symptomatic period [3, 4]. In most patients with sporadic ALS, the main protein identified in cytoplasmic inclusions is phosphorylated 43 kDa transactive response DNA-binding protein (TDP-43) [3, 5, 6]. ALS is currently considered a multisystemic disorder, and approximately 30% of patients present with varying degrees of anxiety and depressive symptoms [7, 8].

The amygdala consists of multiple cytoarchitectonically defined subnuclei and is a key area for processing fear and stress that can orchestrate a complex set of emotional and behavioural responses [9–11]. A growing body of research indicates the important role of amygdala volume and its functional network in mood disorders, including those of emotional processing, sensory information regulation, and modulation of motivational behaviour [12–14]. Furthermore, the amygdala has been implicated in multiple neurodegenerative disorders [15–19]. Such as, amygdala atrophy occurs in all forms of frontotemporal dementia (FTD), and atrophy in specific subnuclei is related to difficulties recognizing facial emotional expressions in FTD patients [15, 18]. Moreover, Carey et al. reported that amygdala

volume and resting-state functional connectivity (FC) abnormalities are associated with the severity of anxiety in Parkinson's disease (PD) [16].

Previous neuroimaging studies of amygdala volume in patients with ALS have had largely contradictory results, with reports of no changes, smaller volumes, or volumetric reductions restricted to specific amygdala subnuclei compared with healthy participants [20–25]. In neuropathologic studies, TDP-43 pathology has been suggested to be divisible into four stages in ALS (3 Braak stages), which begin focally and then spread persistently in sequential, regional patterns. The pathology typically originates from the motor cortex and disseminates to the prefrontal cortex, thalamus, and finally, the hippocampus [26]. Thus, volumes loss in the amygdala, which is a key structure of the limbic system and sits adjacent to the hippocampus, is not likely to occur in relatively early stages of ALS [3]. In the present study, we hypothesised that volume alterations in the amygdala, either globally or in specific subnuclei, would emerge in advanced stages in patients with ALS. We also proposed that discrepancies among previous studies are likely to have been caused by averaging the biophysical indices of different patients at affected and unaffected disease stages.

Moreover, Passamonti et al. used functional magnetic resonance imaging (fMRI) to demonstrate that ALS patients have altered left amygdala–prefrontal cortex connectivity in emotional processing tasks compared with healthy subjects [17]. An increasing number of studies have shown that resting-state fMRI (rsfMRI), in which subjects do not perform any specific task throughout the scan, is an excellent tool for probing brain networks [12, 14]. Previous studies have reported that rsfMRI can reliably describe the resting-state functional network in patients with ALS and other neurodegenerative conditions [16, 23]. However, it remains unknown whether amygdala-based resting-state FC is altered in patients with ALS.

Thus, the present study had three aims. First, we used the well-validated King's clinical staging system to divide ALS patients into homologous subgroups, to explore amygdala subnucleus atrophy patterns at different disease stages using *in vivo* structural MRI in a relatively large sample of Chinese patients with ALS [27]. Second, we used seed-based whole-brain connectivity analysis to examine amygdala resting-state FC alterations in HCs and in patients with ALS during different disease stages. Third, it is well known that the amygdala is essential for cognition and emotional processing [9–11]. Amygdala abnormalities are likely to be involved in the mechanisms underlying cognitive deficits, anxiety and depressive symptoms in older people and individuals with multiple neuropsychiatric disorders; however, few studies have focused on this topic in ALS [16–19]. Thus, we aimed to explore the relationship between amygdala abnormalities and cognitive and emotional symptoms in patients with ALS.

Methods

Participants

All newly diagnosed patients with ALS between November 2019 and December 2020 were consecutively included. All patients met the revised El Escorial criteria for possible, probable, or definite ALS [5].

Additionally, all patients presented with progressive disability during a 3-month outpatient or telephone follow-up visit. The exclusion criteria for ALS patients were as follows: 1) family history of ALS; 2) inability to complete an MRI scan; 3] FTD, which we chose to exclude because FTD is uncommon (4.7%) in Chinese patients with sporadic ALS [28, 29]; 4) comorbidity of other neurological or psychiatric disorders; and 5) refusal to participate. The 30 Rascovsky criteria were used to diagnose FTD [30]. In addition, 94 age-matched healthy controls (HCs) were also recruited from community and were subjected to the same exclusion criteria as the ALS patients.

We recorded the demographic and clinical information of all participants, including age, sex, education, family history of neurological disease, comorbid conditions, site of symptom onset, and disease duration. The revised ALS Functional Rating Scale (ALSFRS-R) was used to assess disease severity [29]. Depression and anxiety were quantified using the Hamilton Depression Rating Scale (HDRS) and Hamilton Anxiety Rating Scale (HARS), respectively [28].

All participants also completed a neuropsychological test battery to screen for cognitive and behavioural features [28, 29, 31]. Briefly, the screening battery included the Mini-Mental State Examination (MMSE), Frontal Assessment Battery (FAB), Boston Naming Test (BNT), and Auditory Verbal Learning Test (AVLT). Behavioural symptoms were also assessed in patients with ALS through an interview with the informant, and were quantified using the Frontal Behavioral Inventory.

Standard protocol approvals, registrations, and patient consents

This study was approved by the Research Ethics Committee of the School of Medicine, Shandong University. Participant information was only collected after all patients and HCs had been made aware of the purpose of the study and provided their written informed consent.

ALS staging

During clinical screening, the King's clinical staging system was used to evaluate clinical staging [27]. Stages 1–3 relate to the body regions that are involved (bulbar, upper limbs, and lower limbs, respectively), while stage 4 is defined by the need for nutritional or respiratory support. The King's staging system might be closely linked to anatomical spread [27, 32]. We opted not to include stage 4 patients in the final analysis for the following reasons: 1) the naming of stage 4 milestones may be problematic, and ALS patients have less homogeneity in stage 4 compared with the other three stages; and 2) only three patients in the present cohort were classified as King's stage 4 because of all included patients with ALS in the present study were newly diagnosed [5, 27, 32].

MRI acquisition

All MRI data were obtained on a 3.0 T magnetic resonance system (Philips Medical System Ingenia scanner) with dStream head coil. During the scan, all subjects were asked to be quiet, remain supine, and refrain from any conscious thinking. Structural images of the whole brain were scanned using a three-

dimensional (3D) fast spoiled gradient-echo sequence: repetition time (TR) = 6.7 ms, echo time (TE) = 3.0 ms, matrix = 68×68 , voxel size = $1\text{mm} \times 1\text{mm} \times 1\text{mm}$, field of view (FOV) = $240\text{ mm} \times 240\text{ mm}$, slice thickness = 1.0 mm, no slice gap, and a total of 180 slices. FLAIR data were scanned using TR = 7000 ms, Flip Angle 90° , TE = 125 ms, acquisition matrix = 272×176 , and slice thickness 6 mm. The rsfMRI images were obtained using an echoplanar imaging sequence with the following parameters: TR = 2000 ms, TE = 30 ms, FOV = $230\text{mm} \times 230\text{mm} \times 144\text{mm}$, matrix = 68×68 , voxel size = $3.5\text{mm} \times 3.5\text{mm} \times 4\text{mm}$, slice thickness = 4 mm, no slice gap, and a total of 32 slices and 240 phases.

Amygdala volume

In the present study, amygdala subnuclei were automatically segmented and measured using FreeSurfer version 7.1.1 (<http://surfer.nmr.mgh.harvard.edu>). A package available in FreeSurfer 7.1.1 was able to automatically segment the amygdala subnuclei [33]. Using this algorithm, the amygdala was accurately segmented into the following subnuclei: anterior amygdaloid area (AAA), accessory basal nucleus (ABN), basal nucleus (BN), cortico-amygdaloid transition (CAT), central nucleus (CeN), cortical nucleus (CoN), lateral nucleus (LN), medial nucleus (MN), and paralaminar nucleus (PN) (Fig. 1). Moreover, total intracranial volume (TIV) was calculated for each subject for further analysis as a covariate. The procedure, which included motion correction, intensity normalisation, automated topology corrections, and the automatic segmentation of grey matter (GM) regions, has been documented in detail elsewhere [20].

Resting-stage fMRI data pre-processing and seed-based whole brain connectivity analysis

The rsfMRI data pre-processing was conducted using SPM12 (www.fil.ion.ucl.ac.uk/spm/software/spm12) and DPABI (Data Processing and Analysis for Resting-State Brain Imaging) tools. The brain extraction, motion correction and denoising procedures have been documented in detail in our previous studies [34, 35]. FreeSurfer-generated white matter and cerebrospinal fluid (CSF) signals were aligned with rsfMRI data using Advanced Normalization Tools (ANTs), and the mean time series within these regions were extracted using the FMRIB software library (<http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/>). A regression of all other voxels' time series on eight nuisance variables was then performed, which included the time series of WM and CSF as well as the six motion parameters. Six participants (three ALS patients and three HCs) were excluded from the present study because of head motion exceeding 3mm. A seed-based whole-brain analysis was used to explore whole brain resting-state FC originating from the bilateral amygdala, respectively. Resting-state FC analyses were conducted following the method of 12 Cullen and colleagues [12]. FreeSurfer-based left and right amygdala regions of interest were registered to the pre-processed rsfMRI data using ANTs, and the mean time series of voxels in these regions were extracted. These time series were used as primary regressors in separate (left and right) general linear model analyses of all other voxel time series, which resulted in amygdala-based whole-brain resting-state FC maps for each participant, and the correlation coefficients were then transformed into Fisher's Z values. Data were smoothed using a full-width-at-half-maximum

Gaussian kernel of 6 mm and then normalized to Montreal Neurological Institute space for the group analyses.

Statistical analysis

Clinical data analysis

Continuous variables are reported as the mean and standard deviation, and categorical variables are reported as the frequency and proportion. Student's *t*-tests or analysis of variance were used to compare continuous variables (with Mann–Whitney *U* tests if necessary). Categorical variables were compared using chi-squared tests. Post hoc *t*-tests were performed to identify pairwise group differences. Values of $p < 0.05$ indicated significance. All statistical analyses were performed using SPSS version 20.0 (IBM Corp., Armonk, NY).

MRI data analysis

Analysis of variance (ANOVA) models was constructed to investigate differences in the structural volumes and resting-state FC maps between groups. We used age, sex, and TIV (for GM volumes) as covariates. To identify pairwise group differences, further *post hoc t*-tests were performed between groups. After Bonferroni correction, $p < 0.05$ was taken as significant. Partial correlations were performed between the imaging metrics and the clinical data, controlling for age, sex, and TIV. To avoid type II errors, the partial correlations were restricted to imaging metrics that were significantly different between patients with ALS and HCs. Values of $p < 0.05$ were recognised as significant.

Results

Demographic and clinical information

Finally, eighty-three consecutive ALS patients and 94 HCs were included in the present study. All participants except for two patients with ALS (who were unable to perform cognitive tests because of serious physical disability) completed the clinical screening, MRI acquisition, and cognitive, behavioural, anxiety, and depression assessments. The MMSE and FAB scores were lower in ALS patients than in HCs ($p < 0.05$). In contrast, the HDRS and HARS scores were higher in ALS patients than in HCs ($p < 0.05$). The demographic and clinical information for ALS patients and HCs is shown in Table 1.

Table 1
Demographic and clinical features of patients with ALS and HCs

	Patients with ALS [n = 83]	HCs [n = 94]	P-value
Age [years]	56.9 ± 11.4	55.2 ± 6.8	0.22
Men/Women [n]	47/36	35/59	0.01
Education	9.5 ± 3.6	10.3 ± 3.8	0.14
ALS duration [month]	11.9 ± 7.9	-	-
Bulbar ALS onset n, [%]	18 [21.7]	-	-
ALSFRS-R score	41.4 ± 3.2	-	-
Riluzole, n [%]	7 [8.4]	-	-
King's clinical stage [stages 1/2/3], %	26.5%, 53.0%, 20.5%	-	-
MMSE	26.7 ± 2.9	28.3 ± 1.9	<0.01
FAB	14.8 ± 2.1	17.2 ± 0.7	<0.01
FBI	1.2 ± 1.4	-	-
BNT	24.1 ± 4.0	24.9 ± 4.1	0.13
AVLT, short delayed [5min]	7.1 ± 3.1	7.7 ± 3.3	0.29
AVLT, long delayed [20min]	6.6 ± 3.3	7.4 ± 2.8	0.06
HARS	8.3 ± 4.4	2.6 ± 3.7	<0.01
HDRS	11.4 ± 6.9	3.4 ± 3.8	<0.01
Abbreviations: ALS = amyotrophic lateral sclerosis; HC = healthy control; ALSFRS-R = ALS Functional Rating Scale-Revised; MMSE = Mini-Mental State Examination; FAB = Frontal Assessment Battery; FBI = Frontal Behavioral Inventory; BNT = Boston Naming Test; AVLT = Auditory Verbal Learning Test; HARS = Hamilton Anxiety Rating Scale; HDRS = Hamilton Depression Rating Scale.			

Comparisons between patients with ALS at different King's stages

Based on the involved body regions, patients with ALS were divided into their corresponding King's clinical stages at clinical screening. There were no significant differences between the three patient subgroups for most data. However, there were significant differences among the three patient subgroups in ALSFRS-R scores. *Post hoc* analysis revealed that, compared with ALS patients at King's stage 1 and 2, ALSFRS-R scores were lower in patients at King's stage 3. Moreover, compared with ALS patients at King's

stage 1, ALSFRS-R scores were lower in patients at King's stage 2. Demographic and clinical information for each disease stage group is shown in Table 2.

Table 2
Demographic and clinical information for each disease stage subgroup

	Stage 1 [n = 22]	Stage 2 [n = 44]	Stage 3 [n = 17]	F or χ^2	P-value
Age [years]	53.7 $\pm$ 12.8	56.5 $\pm$ 10.7	62.1 $\pm$ 10.3	2.73	0.07
Men/Women [n]	13/9	27/17	7/10	2.10	0.35
Education	9.7 $\pm$ 3.4	9.2 $\pm$ 3.6	9.7 $\pm$ 4.0	0.21	0.81
ALS duration [month]	9.0 $\pm$ 4.1	12.8 $\pm$ 9.1	13.1 $\pm$ 7.4	1.96	0.15
Bulbar ALS onset n, [%]	5 [22.8]	10 [22.8]	3 [17.6]	0.16	0.92
ALSFRS-R score	44.6 $\pm$ 1.4	41.2 $\pm$ 2.4	37.4 $\pm$ 3.3	41.73	<0.01
MMSE	27.4 $\pm$ 2.5	26.6 $\pm$ 2.6	26.2 $\pm$ 4.0	0.91	0.40
FAB	15.2 $\pm$ 1.2	14.9 $\pm$ 2.2	14.0 $\pm$ 2.5	1.87	0.16
BNT	25.5 $\pm$ 4.7	23.6 $\pm$ 3.7	23.8 $\pm$ 3.6	1.72	0.18
AVLT, short delayed	7.8 $\pm$ 3.4	6.9 $\pm$ 2.8	7.0 $\pm$ 3.2	0.57	0.56
AVLT, long delayed	7.1 $\pm$ 3.6	6.2 $\pm$ 3.1	6.7 $\pm$ 3.2	0.58	0.56
HARS	8.4 $\pm$ 3.9	7.9 $\pm$ 4.6	9.5 $\pm$ 4.5	0.88	0.42
HDRS	12.0 $\pm$ 9.2	11.1 $\pm$ 6.1	11.8 $\pm$ 6.9	0.15	0.86
Abbreviations: ALS = amyotrophic lateral sclerosis; HC = healthy control; ALSFRS-R = ALS Functional Rating Scale-Revised; MMSE = Mini-Mental State Examination; FAB = Frontal Assessment Battery; BNT = Boston Naming Test; AVLT = Auditory Verbal Learning Test; HARS = Hamilton Anxiety Rating Scale; HDRS = Hamilton Depression Rating Scale.					

Amygdala volumes

Compared with HCs, ALS patients had significant atrophy in the bilateral LN, BN, ABN, AAA, CoN, CAT, and global amygdala, and in the right PN, after Bonferroni correction. There were no significant differences between patients with ALS and HCs in the bilateral CeN and MN or left PN volumes. Amygdala profiles for HCs and ALS patients are presented in Fig. 2.

There were also significant differences in the bilateral LN, BN, ABN, AAA, CoN, CAT, and global amygdala volumes, as well as in the right PN volumes, between the King's stage patient subgroups and HCs. *Post hoc* analysis revealed that there were no significant differences between King's stage 1 patients and HCs in any amygdala subnucleus volumes. However, compared with HCs, King's stage 2 patients had

significantly lower left ABN and CAT volumes, while King’s stage 3 patients had significantly lower bilateral LN, BN, AAA, ABN, CoN, CAT, and global amygdala volumes, as well as right PN volumes, after Bonferroni correction. Compared with ALS patients at King’s stage 1, patients at King’s stage 3 had significant atrophy of the right ABN. Moreover, compared with ALS patients at King’s stage 2, patients at King’s stage 3 had significant atrophy of the right LN, BN, ABN, CAT, PN, and global amygdala. The amygdala profiles of HCs and ALS patients at each disease stage are presented in Fig. 3.

Seed-based whole-brain FC analysis

Compared with HCs, there were no significant alterations in resting-state FC in ALS patients after Bonferroni correction. However, there were significant differences in right amygdala–cuneus connectivity between the King’s stage patient subgroups and HCs. *Post hoc* analysis revealed that, compared with ALS patients at King’s stage 1 or 2 and HCs, right amygdala–cuneus connectivity was significantly higher in ALS patients at King’s stage 3 after Bonferroni correction (Fig. 4 and Table 3).

Table 3
Regions with greater amygdala functional connectivity in group analysis

Brain region	Voxels	MNI Coordinates of Peak Voxel [x, y, z]	Peak z Value
King’s stage 3 > King’s stage 1 or 2 and HCs			
Right Cuneus	112	15, -75, 27	17.03
Abbreviations: MNI = Montreal Neurological Institute; HCs = healthy controls.			

Correlation analyses

In patients with ALS, there were significant associations between MMSE scores and left ABN ($r = 0.278$; $p = 0.02$), left AAA ($r = 0.249$; $p = 0.03$), left CoN ($r = 0.276$; $p = 0.02$), left CAT ($r = 0.285$; $p = 0.01$), left global amygdala ($r = 0.253$; $p = 0.03$), right LN ($r = 0.304$; $p < 0.01$), right BN ($r = 0.281$; $p = 0.02$), right ABN ($r = 0.279$; $p = 0.02$), right AAA ($r = 0.359$; $p < 0.01$), right CAT ($r = 0.235$; $p = 0.04$), and right global amygdala ($r = 0.297$; $p = 0.01$). Moreover, HARS scores were significantly correlated with left CoN ($r = -0.318$; $p = 0.01$), left ABN ($r = -0.245$; $p = 0.03$), right CoN ($r = -0.246$; $p = 0.03$), right CAT ($r = -0.292$; $p = 0.01$), and right ABN ($r = -0.240$; $p = 0.04$). There were no correlations between amygdala subnucleus volumes, FAB scores, and HDRS scores in the patients with ALS.

Discussion

In a relatively large cohort of ALS patients, we revealed that the pattern of amygdala abnormalities in ALS patients was substantially different among patients at different King’s clinical disease stages. In the present study, amygdala subnucleus volumes were unaltered at King’s stage 1. However, ALS patients at

King's stage 2 had significantly reduced left ABN and CAT volumes compared with HCs. Importantly, global amygdala atrophy and abnormal amygdala-based resting-state FC alterations were detected only in patients at King's stage 3. Moreover, amygdala atrophy was associated with global cognition, and reduced volumes of specific subnuclei were independently correlated with anxiety, but not depression, in patients with ALS after controlling for age, sex, and TIV. Thus, amygdala abnormalities may play a more important role in emotional and cognitive impairments in ALS than was previously thought [20, 21].

Numerous neuroimaging studies have been conducted to examine the incidence of amygdala atrophy in ALS patients; however, these studies have generated inconsistent results [20–25]. Some previous studies, using either shape or volume analyses, reported that the amygdala did not differ significantly between ALS patients and HCs, which is consistent with our findings in patients with ALS at King's Stage 1 [22, 25]. Consistent with the pattern observed in our patients with ALS at King's Stage 2, Finegan and colleagues reported that, compared with HCs, ALS patients had reduced left amygdala volumes, whereas right amygdala volume remained unaffected in a group of advanced-stage ALS patients [with mean ALSFRS-R scores of 36.6] [21]. Moreover, using voxel-based morphometry analysis of grey matter structures, Menke et al. reported progressive reductions in bilateral amygdala volumes in patients with ALS during a longer period of follow-up, which is consistent with our findings in patients with ALS at King's stage 3 [23]. Additionally, Pinkhardt et al. reported that a group of patients with definite ALS without dementia trended to have reduced amygdala volumes [24]. However, none of these studies analysed amygdala subnuclei volumes. Recently, Chipika et al. reported significantly reduced ABN and CoN volumes in a large cohort of patients with ALS compared with healthy participants, which is similar to our findings of the ALS patients at King's stage 2 in the present study, and they suggested selective atrophy of amygdala subnuclei is a consistent feature of patients with ALS [20]. Our findings support their viewpoint that amygdala subnuclei may be nonuniformly affected by ALS pathology, and we further suggest that the inconsistencies among previous studies might largely result from averaged biophysical indices from affected and unaffected stages' patients, because the pattern of amygdala atrophy in patients with ALS differs substantially at different disease stages. However, none of the previous studies used the well-validated King's clinical staging system to divide ALS patients into homologous subgroups at different disease stages.

In the present study, right amygdala–cuneus connectivity was significantly increased in ALS patients at King's stage 3. Similarly, in an ^{18}F fluorodeoxyglucose positron emission tomography study, Laere et al. reported that patients with ALS have clusters of relative hypermetabolism in the amygdala [36]. Using echo-planar spectroscopic imaging, Verma et al. demonstrated that the N-acetylaspartate/creatine ratio [a biomarker of neuronal integrity] is significantly lower in the right cuneus of patients with ALS [37]. The cuneus is a hub of the visual association cortex and may play an important role in visual information processing [37, 38]. Moreover, the cuneus also seems to participate in multisensory information integration and cognitive processes [37, 38]. Using ^{11}C -flumazenil positron emission tomography, Wicks et al. reported that decreased ^{11}C -flumazenil binding in the cuneus is related to confrontation naming impairment in patients with ALS [39]. Thus, the abnormal amygdala–cuneus resting-state FC in the

present study may represent a compensatory change in response to structural damage in patients with ALS [23]. Consistent with our findings, Menke et al. recently also reported a mixed picture of widespread grey matter volume decreases and resting-state FC increases in patients with ALS over 2 years of follow-up, which is compatible with compensatory responses [23]. However, these findings need to be confirmed by further studies.

Another key finding of the present study was that amygdala atrophy was significantly related to anxiety [but not depression] and global cognitive deficits in ALS patients. The amygdala is an important hub of the limbic system and plays a pivotal role in cognitive and emotional processing. However, few studies have focused on the associations between amygdala abnormalities and neuropsychiatric symptoms that occur over the course of ALS [9, 22, 25]. The ABN has a cortical-like profile and forms a connection between LN and CeN, and its outputs project to several anxiety-related brain regions [11, 40, 41]. ABN activation can suppress high-anxiety states and fear-related freezing, whereas inhibition of the ABN increases anxiety and freezing [10, 11, 41]. The CoN can mediate aversive, defensive, and reproductive responses [40, 42]. Thus, it is not surprising that smaller ABN and CoN volumes were associated with anxiety in patients with ALS in the current study, although further studies are needed to investigate causality. Similar to our findings, Vriend et al. reported smaller left amygdala volumes in anxious PD patients, and suggested that amygdala volume was related to anxiety [19]. Furthermore, España et al. reported that intraneuronal β -amyloid accumulation in the amygdala may enhance anxiety and fear in an AD mouse model [43]. Previous studies have also demonstrated that an accumulation of AD pathology in the amygdala is detrimental to cognition in preclinical AD patients, and that amygdala atrophy may be an early marker of AD [44–46]. Moreover, Bouchard et al. reported that a smaller amygdala is associated with cognitive deficits in PD [47]. 48 Ahveninen et al. demonstrated that amygdala volumes are significantly reduced in patients with Huntington's disease, and are associated with global cognition [48]. Recent studies have suggested that cognitive impairments might worsen across King's stages in patients with ALS, and may also correlate with pathological TDP-43 accumulation in corresponding cortical regions [5, 49]. Thus, our findings provide important evidence to support these studies, and further highlight that cognitive competency is not completely dependent on cortical integrity, but that subcortical abnormalities may also play a role in cognitive impairments in patients with ALS.

Overall, our findings suggest that amygdala abnormalities are an important feature of ALS, and that smaller amygdala volume is related to anxiety in these patients. Because medication currently available for ALS patients have limited efficacy, physicians should focus on improving prognosis and quality of life [2]. Importantly, if our findings are confirmed, they indicate that patient's anxiety cannot be entirely attributed to receiving such a devastating diagnosis; the neurodegenerative process of ALS also seems to be involved [7, 8]. It may be possible to improve anxiety in ALS patients using both pharmacological and non-pharmacological approaches [50]. These approaches might alleviate disease progression and improve patient's quality of life, and may become a key component of individualised therapy for ALS patients [50]. However, although ALS patients experience negative effects from anxiety, it remains under-recognised in clinical practice [8]. We therefore propose that the identification and management of anxiety in ALS should be given more attention.

Inevitably, the present study had several limitations. First, this study used a cross-sectional design, which prevented the establishment of causality between amygdala abnormalities, anxiety, and cognitive deficits. Thus, causality remains to be validated in further studies. Second, all included patients with ALS in the present study were newly diagnosed, and only three patients were classified as King's stage 4. Thus, our study had a complete lack of patients with King's stage 4 (nutritional or respiratory failure); however, ALS patients have less homogeneity in stage 4 compared with the other three stages, and it is commonly difficult for these patients to complete an MRI scan [27, 32]. Moreover, in this consecutive cohort, although there were no significant differences between the three patient subgroups in age and we used age, sex, and TIV as covariates, ALS patients at King stage 3 were (on average) 8.4 years older than patients at King stage 1, which is consistent with some previous studies [5, 51]. Similarly, in a population-based study, Manera et al. reported three regions were functionally involved in 196 patients with ALS (18.5%) at diagnosis, and 180 patients (91.8%) were older than 60 years [51]. The onset of ALS appears to involve a multistep process, and aging seem to be one of the processes and may accelerate the neurodegeneration of ALS. However, these findings need to be discussed by further studies. Third, we only used the MMSE, BNT, AVLT, and FAB to screen cognitive function in the present study, and we did not use ALS-specific tests, for example, Edinburgh cognitive and behavioural ALS screen [5]. However, these methods are included in our ongoing work, and only two patients with ALS were unable to complete the cognitive assessments in our study. Fourth, our results were also susceptible to selection bias, because the ALS patients who visit our centre commonly have a relatively short disease course (we are the largest ALS centre in the Shandong province). Thus, our findings need to be confirmed by population-based studies. Fifth, in the present study, we did not use any amygdala subnucleus as region of interest to explore resting-state FC alterations because of their relatively small size. Finally, we did not perform any genetic testing. However, the ALS patients included in this study were sporadic cases, and very few sporadic ALS patients in China carry known genetic mutations [52].

In conclusion, our study provides a comprehensive profile of amygdala abnormalities in ALS patients. The pattern of amygdala abnormalities in these patients differed greatly across King's clinical disease stages; our findings suggest that amygdala abnormalities are an important feature in patients with ALS at relatively advanced stages. Moreover, specific amygdala subnucleus atrophy may play an important role in anxiety and cognitive impairment in these patients.

Declarations

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Competing interests

The authors declared none conflicts of interest.

Authors' Roles

SWL: study concept, data acquisition, interpretation of the results, writing the first version of the manuscript. YYZ: study concept, interpretation of the results, revising the manuscript. QGR: study concept, interpretation of the results, revising the manuscript. CZY: study concept, ALS diagnosis, FTD diagnosis, interpretation of the results, writing the final version of the manuscript. GLG and SYZ: Image preprocessing, revising the manuscript. XTM: statistical analyses. NZ, YL, YS, BZ, YY, XLY, KS, PFL and YY: data acquisition, revising the manuscript. LL, TJD and YQZ: revising the manuscript. DZ, WQW and RZ: EMG study, revising the manuscript. WL, PYS and XSM: ALS diagnosis, FTD diagnosis, revising the manuscript.

Statistical analysis

Statistical Analysis conducted by Dr. Kai Shao, PhD, Department of Clinical Laboratory, Qilu Hospital of Shandong University (Qingdao), Qingdao, China

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Figures

Figure 1

Atlas-based segmentation of the amygdala. A. Coronal; B. Sagittal; C. Axial. Abbreviations: AAA = Anterior amygdaloid area, ABN = Accessory basal nucleus, BN = Basal nucleus, CAT = Cortico-amygdaloid transition, CeN = Central nucleus, CoN = Cortical nucleus, LN = Lateral nucleus, MN = Medial nucleus, and PN = Paralamina nucleus.

Figure 2

Amygdala subnucleus profiles for patients with ALS and HCs. Abbreviations: AAA = Anterior amygdaloid area, ABN = Accessory basal nucleus, BN = Basal nucleus, CAT = Cortico-amygdaloid transition, CeN = Central nucleus, CoN = Cortical nucleus, LN = Lateral nucleus, MN = Medial nucleus, and PN = Paralaminar nucleus. * $p \leq 0.05$; ** $p \leq 0.01$.

Figure 3

Amygdala subnucleus profiles for patients with ALS at each disease stage and HCs. Abbreviations: AAA = Anterior amygdaloid area, ABN = Accessory basal nucleus, BN = Basal nucleus, CAT = Cortico-amygdaloid transition, CeN = Central nucleus, CoN = Cortical nucleus, LN = Lateral nucleus, MN = Medial nucleus, and PN = Paralaminar nucleus. * $p \leq 0.05$; ** $p \leq 0.01$.

Figure 4

Amygdala-based whole-brain functional connectivity analysis results. Compared with ALS patients at King's stage 1 or 2 and HCs, right amygdala–cuneus connectivity was significantly greater in ALS patients at King's stage 3 after Bonferroni correction.

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