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RESEARCH ARTICLE

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Amygdala abnormalities across disease stages in patients with sporadic amyotrophic lateral sclerosis

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Abstract

To examine selective atrophy patterns and resting-state functional connectivity (FC) alterations in the amygdala at different stages of amyotrophic lateral sclerosis (ALS), and to explore 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 explored the pattern of selective amygdala subnucleus atrophy and amygdala-based whole-brain FC alteration in these patients and 94 healthy controls (HCs). Cognitive and emotional functions were also evaluated using a neuropsychological test battery. There were no significant differences between ALS patients at King's stage 1 and HCs for any amygdala subnucleus volumes. Compared with HCs, ALS patients at King's stage 2 had significantly lower left accessory basal nucleus and cortico-amygdaloid transition volumes. Furthermore, ALS patients at King's stage 3 demonstrated significant reductions in most amygdala subnucleus volumes and global amygdala volumes compared with HCs. Notably, amygdala-cuneus FC was

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increased in ALS patients at 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 greatly across King's clinical disease stages, and amygdala abnormalities are an important feature of patients with ALS at relatively advanced stages. Moreover, our findings suggest that amygdala volume may play an important role in anxiety and cognitive dysfunction in ALS patients.

KEYWORDS
amygdala, amyotrophic lateral sclerosis, anxiety

1 | INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a rare neurodegenerative disease with both clinical and hereditary heterogeneity (Swinnen & Robberecht, 2014; Westeneng et al., 2021). The aetiology of ALS remains unknown; however, interactions between genetic and environmental factors likely underpin disease susceptibility (Taylor et al., 2016). ALS is likely derived from cortical influences and the onset of ALS appears to involve a multistep process, with a long presymptomatic period (Al-Chalabi et al., 2014; Braak et al., 2013; Eisen et al., 2017). In most patients with sporadic ALS, the main protein identified in cytoplasmic inclusions is phosphorylated 43 kDa transactive response DNA-binding protein (TDP-43; Braak et al., 2013; Chiò et al., 2019; Kassubek et al., 2014). ALS is currently considered a multisystemic disorder with widespread brain involvement (Braak et al., 2013; Chiò et al., 2019). Moreover, approximately 30% of patients with ALS present with varying degrees of anxiety and depressive symptoms (Heidari et al., 2021; Kurt et al., 2007).

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 (Gothard, 2020; Pessoa & Adolphs, 2010; Roozendaal et al., 2009). A growing body of research indicates amygdala abnormalities are an important feature of many neurodegenerative disorders, and may underlie multiple behavioural symptoms that involved emotional processing, sensory information regulation and modulation of motivational behaviour (Cullen et al., 2014; He et al., 2019; Tang et al., 2013). Such as, amygdala atrophy may occur in all forms of frontotemporal dementia (FTD), and atrophy in specific subnuclei is related to difficulties recognizing facial emotional expressions in FTD patients (Bocchetta et al., 2019; Takeda et al., 2017). 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; Carey et al., 2021).

Notably, neuroimaging studies of amygdala abnormalities in patients with ALS have been largely contradictory results to date, with reports of no changes, smaller volumes, or volumetric reductions restricted to specific amygdala subnuclei compared with healthy participants (Chipika et al., 2020; Finegan et al., 2019; Machts et al., 2015; Menke et al., 2017; Pinkhardt et al., 2006; Tae et al., 2020; Westeneng et al., 2015). In a neuropathologic study, Geser et al. reported that pathological TDP-43 has been detected in amygdala in nearly half of patients with sporadic ALS (Geser et al., 2008). Furthermore, TDP-43 pathology has been suggested to be divisible into four stages in ALS by Braak and colleagues (Braak stages), which begin focally and then spread persistently in sequential and regional patterns that typically originate from the motor cortex and disseminate to the prefrontal cortex, thalamus, and finally, the hippocampus (Brettschneider et al., 2013). Thus, volumes loss in the amygdala, which is a key structure of the limbic system and adjacent to the hippocampus, is not likely to occur in relatively early stages of ALS and may be nonuniformly affected in ALS patients (Braak et al., 2013). In the present study, we hypothesized that volume alterations in the amygdala, either globally or in specific subnuclei, would emerge in advanced stages in patients with ALS. We also suggested that discrepancies among previous studies are likely to have been caused by averaging the biophysical indices of different patients at affected (advanced) and unaffected (early) 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 (Passamonti et al., 2013). 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 a more excellent tool for probing brain functional activity (Cullen et al., 2014; Tang et al., 2013). Previous studies have reported that rsfMRI can reliably describe the resting-state functional alterations in patients with ALS and other neurodegenerative conditions that can provide pivotal information on the nature of these diseases (Carey et al., 2021; Menke et al., 2017). 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 different disease stage 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 (Roche et al., 2012). Second, we also examined amygdala resting-state FC alterations in patients with ALS across different disease stages using seed-based whole-brain FC analysis. Third, we aimed to explore the relationship between amygdala abnormalities and cognitive and emotional symptoms in patients with ALS.

2 | METHODS

2.1 | Participants

Patients with ALS included in this study need to meet the revised El Escorial criteria for possible, probable, or definite ALS (Chiò et al., 2019). 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 (Cui, Cui, Gao, et al., 2015; Cui, Cui, Liu, et al., 2015; Liu et al., 2018); (4) comorbidity of other neurological or psychiatric disorders; and (5) refusal to participate. The Rascovsky criteria were used to diagnose FTD (Rascovsky et al., 2011). Between November 2019 and December 2020, 83 newly diagnosed patients with ALS were included. All patients presented with progressive disability during a 6-month outpatient or telephone follow-up visit. 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. In the present study, all HCs were recruited through advertising, leaflets and community bulletin boards.

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 (Liu et al., 2018). Depression and anxiety were quantified using the Hamilton Depression Rating Scale (HDRS) and Hamilton Anxiety Rating Scale (HARS), respectively (Cui, Cui, Gao, et al., 2015).

All participants also completed a neuropsychological test battery to screen for cognitive and behavioural features (Cui, Cui, Liu, et al., 2015; Liu et al., 2018; Pan et al., 2020). 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 caregiver, and were quantified using the Frontal Behavioral Inventory.

2.2 | 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.

2.3 | ALS staging

During clinical screening, the King's clinical staging system was used to evaluate clinical staging (Roche et al., 2012). 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. Recent studies have shown that the motor and cognitive component might worsen in parallel across King's stages in patients with ALS (Chiò et al., 2019; Gregory et al., 2020). Moreover, cognitive impairments may correlate with pathological TDP-43 accumulation in corresponding cortical regions in ALS patients (Gregory et al., 2020). Thus, the King's staging system might be closely linked to anatomical spread (Canosa et al., 2021; Consonni et al., 2020; Roche et al., 2012). Because of all included patients with ALS in the present study were newly diagnosed, only three patients were classified as King's stage 4, and it is commonly difficult for these patients to complete an MRI scan. Therefore, in the present study, we opted not to include patients at stage 4 in the final analysis.

2.4 | 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 mm × 1 mm × 1 mm, field of view (FOV) = 240 mm × 240 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 echo-planar imaging sequence with the following parameters: TR = 2000 ms, TE = 30 ms, FOV = 230 mm × 230 mm × 144 mm, matrix = 68 × 68, voxel size = 3.5 mm × 3.5 mm × 4 mm, slice thickness = 4 mm, no slice gap and a total of 32 slices and 240 phases.

2.5 | 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 (Saygin et al., 2017). 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) (Figure 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,

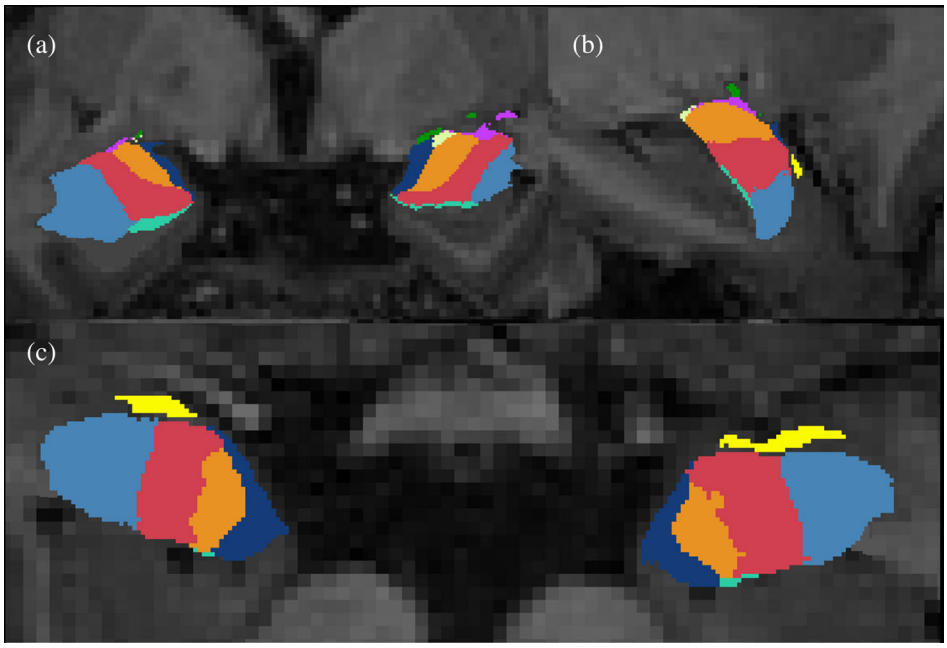

FIGURE 1 Atlas-based segmentation of the amygdala. (a) Coronal; (b) sagittal; (c) axial. AAA, anterior amygdaloid area; ABN, accessory basal nucleus; BN, basal nucleus; CAT, cortico-amygdaloid transition; CeN, central nucleus; CoN, cortical nucleus; LN, lateral nucleus; MN, medial nucleus; PN, paralaminar nucleus

automated topology corrections and the automatic segmentation of grey matter (GM) regions, has been documented in detail elsewhere (Chipika et al., 2020).

2.6 | 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 (Reid et al., 2017; Song et al., 2020). Briefly, the first 10 volumes of each functional time series of each subject were removed to reach signal equilibrium. The remaining time-series were then band-pass filtered (0.01–0.08 Hz). 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 (Friston et al., 1995). To minimize the potential effect of head motion on FC, six participants (three ALS patients and three HCs) were excluded from further analysis due to a maximum displacement in any orthogonal directions greater than 3 mm or a maximum head rotation greater than 3.0°. 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 Cullen and colleagues (Cullen et al., 2014). 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.

2.7 | Statistical analysis

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

2.7.1 | 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 $p < .05$ indicated significance. All statistical analyses were performed using SPSS version 20.0 (IBM Corp., Armonk, NY).

2.7.2 | MRI data analysis

Analysis of variance (ANOVA) models was constructed to investigate differences in the imaging metrics (structural volumes and resting-

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

	Patients with ALS ($n = 83$)	HCs ($n = 94$)	p
Age (years)	56.9 ± 11.4	55.2 ± 6.8	.22
Men/women (*n*)	47/36	35/59	.01
Education (years)	9.5 ± 3.6	10.3 ± 3.8	.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	<.01
FAB	14.8 ± 2.1	17.2 ± 0.7	<.01
FBI	1.2 ± 1.4	–	–
BNT	24.1 ± 4.0	24.9 ± 4.1	.13
AVLT, short delayed (5 min)	7.1 ± 3.1	7.7 ± 3.3	.29
AVLT, long delayed (20 min)	6.6 ± 3.3	7.4 ± 2.8	.06
HARS	8.3 ± 4.4	2.6 ± 3.7	<.01
HDRS	11.4 ± 6.9	3.4 ± 3.8	<.01

Abbreviations: ALFRS-R, ALS Functional Rating Scale-Revised; ALS, amyotrophic lateral sclerosis; AVLT, Auditory Verbal Learning Test; BNT, Boston Naming Test; FAB, Frontal Assessment Battery; FBI, Frontal Behavioral Inventory; HARS, Hamilton Anxiety Rating Scale; HC, healthy control; HDRS, Hamilton Depression Rating Scale; MMSE, Mini-Mental State Examination.

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, $p < .05$ was taken as significant. Partial correlations were performed between the imaging metrics and the clinical data, controlling for age, sex, education (years) and TIV. To avoid type II errors, the partial correlation analyses in this study were restricted to imaging metrics that were significantly different between patients with ALS and HCs. Values of $p < .05$ (uncorrected) were recognised as significant.

3 | RESULTS

3.1 | Demographic and clinical information

Finally, 83 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. There were no significant differences between ALS patients and HCs for BNT and AVLT (short and long delayed) scores, and the MMSE and FAB scores were lower in ALS patients than in HCs ($p < .05$). In contrast, the HDRS and HARS scores were higher in ALS patients than in HCs ($p < .05$). The demographic and clinical information for ALS patients and HCs is shown in Table 1.

3.2 | 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 stages 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.

3.3 | 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 Figure 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

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
Age (years)	53.7 ± 12.8	56.5 ± 10.7	62.1 ± 10.3	2.73	.07
Men/women (n)	13/9	27/17	7/10	2.10	.35
Education (years)	9.7 ± 3.4	9.2 ± 3.6	9.7 ± 4.0	0.21	.81
ALS duration (month)	9.0 ± 4.1	12.8 ± 9.1	13.1 ± 7.4	1.96	.15
Bulbar ALS onset, n (%)	5 (22.8)	10 (22.8)	3 (17.6)	0.16	.92
ALSFRS-R score	44.6 ± 1.4	41.2 ± 2.4	37.4 ± 3.3	41.73	<.01
MMSE	27.4 ± 2.5	26.6 ± 2.6	26.2 ± 4.0	0.91	.40
FAB	15.2 ± 1.2	14.9 ± 2.2	14.0 ± 2.5	1.87	.16
BNT	25.5 ± 4.7	23.6 ± 3.7	23.8 ± 3.6	1.72	.18
AVLT, short delayed	7.8 ± 3.4	6.9 ± 2.8	7.0 ± 3.2	0.57	.56
AVLT, long delayed	7.1 ± 3.6	6.2 ± 3.1	6.7 ± 3.2	0.58	.56
HARS	8.4 ± 3.9	7.9 ± 4.6	9.5 ± 4.5	0.88	.42
HDRS	12.0 ± 9.2	11.1 ± 6.1	11.8 ± 6.9	0.15	.86

Abbreviations: ALFRS-R, ALS Functional Rating Scale-Revised; ALS, amyotrophic lateral sclerosis; AVLT, Auditory Verbal Learning Test; BNT, Boston Naming Test; FAB, Frontal Assessment Battery; HARS, Hamilton Anxiety Rating Scale; HC, healthy control; HDRS, Hamilton Depression Rating Scale; MMSE, Mini-Mental State Examination.

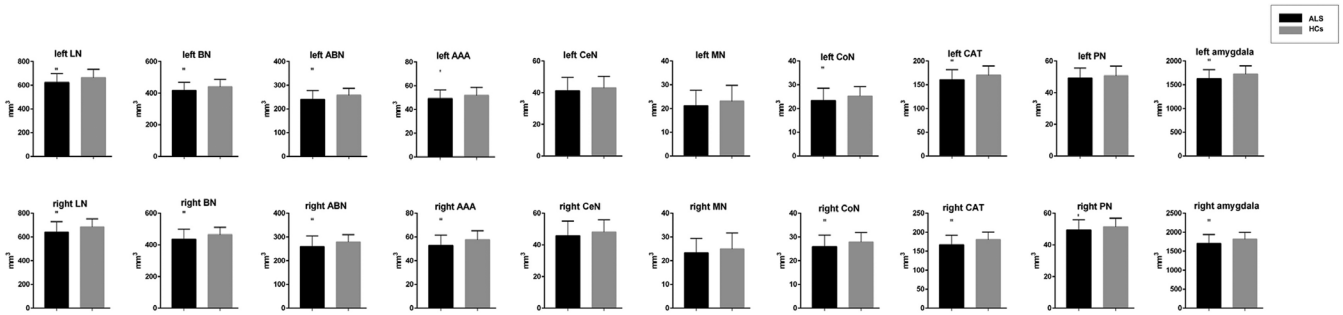

FIGURE 2 Amygdala subnucleus profiles for patients with ALS and HCs. AAA, anterior amygdaloid area; ABN, accessory basal nucleus; BN, basal nucleus; CAT, cortico-amygdaloid transition; CeN, central nucleus; CoN, cortical nucleus; LN, lateral nucleus; MN, mdial nucleus; PN, paralaminar nucleus. *p < .05; **p < .01

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 Figure 3.

3.4 | 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 (Figure 4 and Table 3).

3.5 | Correlation analyses

In patients with ALS, there were significant associations between MMSE scores and left ABN ($r = .240$; $p = .04$), left CoN ($r = .243$; $p = .03$), left CAT ($r = .255$; $p = .03$), left global amygdala ($r = .234$; $p = .04$), right LN ($r = .309$; $p < .01$), right BN ($r = .282$; $p = .01$), right ABN ($r = .266$; $p = .02$), right AAA ($r = .340$; $p < .01$), right CAT ($r = .226$; $p = .04$) and right global amygdala ($r = .296$; $p = .01$). Moreover, HARS scores were significantly correlated with left CoN ($r = -.312$; $p < .01$), left ABN ($r = -.234$; $p = .04$), right CoN ($r = -.232$; $p = .04$), right CAT ($r = -.272$; $p = .02$) and right ABN ($r = -.238$; $p = .04$). There were no correlations between amygdala subnucleus volumes, FAB scores, BNT scores, AVLT (short and long delayed) scores and HDRS scores in the patients with ALS.

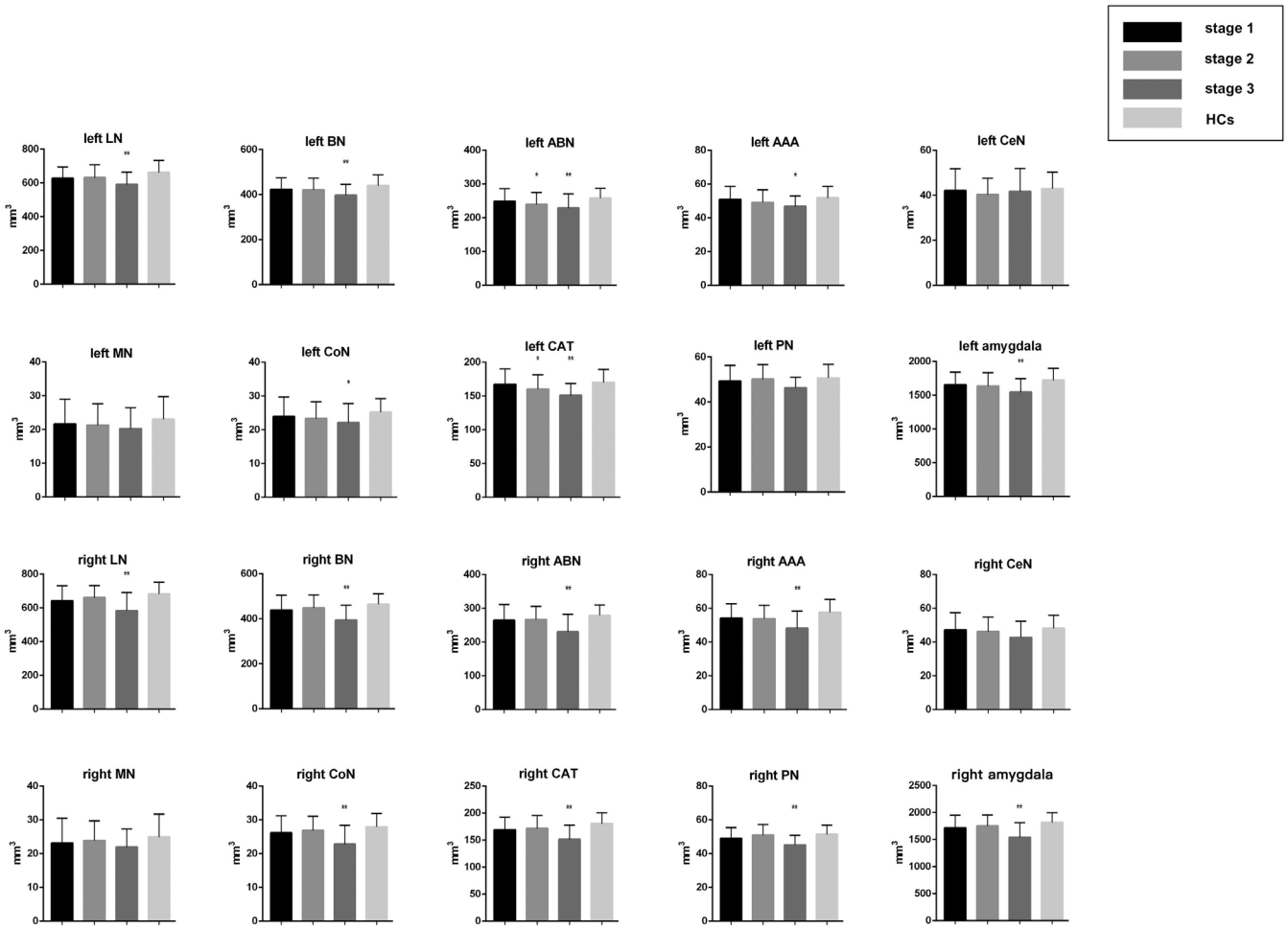

FIGURE 3 Amygdala subnucleus profiles for patients with ALS at each disease stage and HCs. AAA, anterior amygdaloid area; ABN, accessory basal nucleus; BN, basal nucleus; CAT, cortico-amygdaloid transition; CeN, central nucleus; CoN, cortical nucleus; LN, lateral nucleus; MN, medial nucleus; PN, paralaminar nucleus. *$p < .05$; **$p < .01$

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

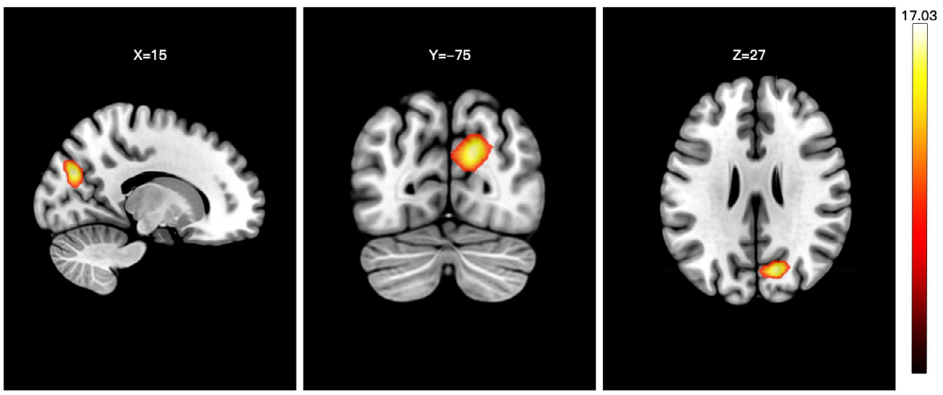

TABLE 3 Regions with greater amygdala functional connectivity in group analysis

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: HCs, healthy controls; MNI, Montreal Neurological Institute.

4 | 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. Amygdala volumes were unaltered in ALS patients 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 amygdala-based resting-state FC alterations were emerged in ALS patients at King's stage 3. Moreover, amygdala atrophy was negatively associated with global cognition, and reduced volumes of specific subnuclei were negatively correlated with anxiety in patients with ALS after controlling for age, sex and TIV. Thus, we suggested that amygdala abnormalities are an important feature in patients with ALS at relatively advanced stages and may play an important role in emotional and cognitive impairments in ALS (Chipika et al., 2020; Finegan et al., 2019).

Numerous neuroimaging studies have been conducted to examine the incidence of amygdala atrophy in ALS patients; however, these studies have generated inconsistent results (Chipika et al., 2020; Finegan et al., 2019; Machts et al., 2015; Menke et al., 2017; Pinkhardt et al., 2006; Tae et al., 2020; Westeneng et al., 2015). 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 (Machts et al., 2015; Tae et al., 2020; Westeneng et al., 2015). 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; Finegan et al., 2019). 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 (Menke et al., 2017). Additionally, Pinkhardt et al. reported that a group of patients with definite ALS without dementia trended to have reduced amygdala volumes (Pinkhardt et al., 2006). Specifically, these studies did not further analyse amygdala subnuclei volumes (Finegan et al., 2019; Machts et al., 2015; Menke et al., 2017; Pinkhardt et al., 2006; Tae et al., 2020; Westeneng et al., 2015). 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 (Chipika et al., 2020). In neuropathologic studies, pathological TDP-43 can be detected in amygdala in nearly half of patients with sporadic ALS, and TDP-43 pathology has been suggested to be divisible into four stages in ALS that typically originate from the motor cortex and disseminate to the prefrontal cortex, thalamus, and finally, the hippocampus (Brettschneider et al., 2013; Geser et al., 2008). Thus, our findings further suggested that amygdala atrophy is likely a consistent feature of patients with ALS at advanced stages. Moreover, none of the previous studies used the well-validated King's clinical staging system to divide ALS patients into different disease stages (Chipika et al., 2020; Finegan et al., 2019; Machts et al., 2015; Menke et al., 2017; Pinkhardt et al., 2006; Tae et al., 2020). Our findings suggested that amygdala atrophy may be nonuniformly affected in patients with ALS, and thus, the inconsistencies result among previous studies might largely result from the distinct patterns of amygdala atrophy in patients with ALS at different disease stages.

In the present study, right amygdala–cuneus connectivity was significantly increased in ALS patients at King's stage 3. In an ^{18}fluorodeoxyglucose positron emission tomography study, Van Laere et al. (2014) reported that patients with ALS have clusters of relative hypermetabolism in the amygdala. Using echo-planar spectroscopic imaging, Verma et al. (2013) demonstrated that the *N*-acetylaspartate/creatine ratio (a biomarker of neuronal integrity) is significantly lower in the right cuneus of patients with ALS. The cuneus is a hub of the visual association cortex and may play an important role in visual information processing (Parise et al., 2014; Verma et al., 2013). Moreover, the cuneus also seems to participate in multisensory information integration and cognitive processes (Parise et al., 2014; Verma et al., 2013). Using ^{11}C-flumazenil positron emission tomography, Wicks et al. (2008) reported that decreased ^{11}C-flumazenil binding in the cuneus is related to confrontation naming impairment in patients with ALS. 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 (Menke et al., 2017). Consistent with our findings, Menke et al. (2017) 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. However, seven ALS patients used riluzole in this study, and thus, our findings regarding resting-state FC alterations in the amygdala of ALS patients need to be interpreted prudently and confirmed by further studies.

Another key finding of the present study was that amygdala atrophy was significantly related to anxiety 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 (Gothard, 2020; Machts et al., 2015; Tae et al., 2020). Moreover, 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 (Chiò et al., 2019; Gregory et al., 2020). Thus, our findings may provide complementary evidence for these studies, and suggest 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.

In the present study, FAB scores were lower in patients with ALS, whereas the FBI scores were relatively normal. Frontal executive function is commonly evaluated using the FAB in studies on patients

with ALS, and the FBI is a caregiver questionnaire (Cui, Cui, Gao, et al., 2015). All the included ALS patients in the present study were newly diagnosed and had a relatively short disease course; therefore, during this phase, caregivers were more likely to focus on motor symptoms and overlook abnormal behaviours. However, this viewpoint should be confirmed by future population studies. Similar to our findings, Wei et al. (2016) reported that the NPI score was 2.0 and the FAB score was 16.0 in ALS patients. Moreover, Cui, Cui, Liu, et al. (2015) also reported that the FBI negative score was 1.0 and the FBI disinhibition score was 0.1 in ALS patients.

Inevitably, the present study had several limitations. First, this study used a cross-sectional design, and without longitudinal assessment, it might be possible that different ALS subgroups have been compared which would have been already different when they were assessed at the same disease stage. We would like to explore longitudinal alterations of the amygdala in ALS patients at each stage separately in our future studies. Moreover, the causal relationship among amygdala abnormalities, anxiety and cognitive deficits also needs to be validated by longitudinal studies in the future. Second, all included patients with ALS in the present study were newly diagnosed, and only three patients were classified as King's stage 4. Moreover, it is commonly difficult for these patients to complete an MRI scan (Consonni et al., 2020; Roche et al., 2012). Thus, our study had a complete lack of patients with King's stage 4 (nutritional or respiratory failure). In addition, 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 (Chiò et al., 2019; Manera et al. 2020). Similarly, in a population-based study, Manera et al. (2020) 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. Moreover, ALS patients and HCs were not sex matched. In the present study, all HCs were continuously recruited from the community. Recently, Lvet al. (2019) explored whether cognitive impairment was associated with mortality among community-dwelling older Chinese people and found that 44.9% (5264/11,732) of subjects were men. 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 (Chiò et al., 2019). However, only two patients with ALS were unable to complete the cognitive assessments in the present study. Fourth, similar to previous studies, multiple comparisons were not performed for correlation analyses between imaging metrics and cognitive measures (Ahveninen et al., 2018; Christidi et al. 2019). Fifth, in the present study, we only included the consecutive and newly diagnosed ALS patients. Similar to previous studies, this study had a disproportionate number of subjects in stage 2 compared to stages 1 and 3 (Christidi et al., 2019; Manera et al., 2020). Our results were also susceptible to selection bias. Thus, although the epidemiological features of ALS patients in this study are consistent with those of a recent national population-based study that may thus strengthen the validity and credibility of our results, our findings still need to be confirmed by population-based studies (Xu et al., 2020). Sixth, 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. Seventh, in the present study, all HCs were recruited from the community through advertising, leaflets and community bulletin boards, and might increase risk of selection bias within the HCs. 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 (Liu et al., 2016).

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 patients with ALS.

AUTHOR CONTRIBUTIONS

Shuangwu Liu: study concept, data acquisition, interpretation of the results, and writing the first version of the manuscript. Yuying Zhao: study concept, interpretation of the results, and revising the manuscript. Qingguo Ren: study concept, interpretation of the results, and revising the manuscript. Chuanzhu Yan: study concept, ALS diagnosis, FTD diagnosis, interpretation of the results, and writing the final version of the manuscript. Ying Yuan, Kai Shao, and Pengfei Lin: data acquisition and revising the manuscript. Ling Li, Tingjun Dai, and Yongqing Zhang: revising the manuscript. Dong Zhang: EMG study and revising the manuscript. Wei Li, Peiyan Shan, and Xiangshui Meng: ALS diagnosis, FTD diagnosis, and revising the manuscript.

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CONFLICT OF INTEREST

The authors declared none conflicts of interest.

DATA AVAILABILITY STATEMENT

The anonymized data presented in this article are available at the request of a qualified investigator, after review by the corresponding author. Final approval will be granted by the Research Ethics Committee of the School of Medicine, Shandong University.

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