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Amygdala pathology in amyotrophic lateral sclerosis and primary lateral sclerosis

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ABSTRACT

Temporal lobe studies in motor neuron disease overwhelmingly focus on white matter alterations and cortical grey matter atrophy. Reports on amygdala involvement are conflicting and the amygdala is typically evaluated as single structure despite consisting of several functionally and cytologically distinct nuclei. A prospective, single-centre, neuroimaging study was undertaken to comprehensively characterise amygdala pathology in 100 genetically-stratified ALS patients, 33 patients with PLS and 117 healthy controls. The amygdala was segmented into groups of nuclei using a Bayesian parcellation algorithm based on a probabilistic atlas and shape deformations were additionally assessed by vertex analyses. The accessory basal nucleus (p = .021) and the cortical nucleus (p = .022) showed significant volume reductions in *C9orf72* negative ALS patients compared to controls. The lateral nucleus (p = .043) and the cortico-amygdaloid transition (p = .024) were preferentially affected in *C9orf72* hexanucleotide carriers. A trend of total volume reduction was identified in *C9orf72* positive ALS patients (p = .055) which was also captured in inferior-medial shape deformations on vertex analyses. Our findings highlight that the amygdala is affected in ALS and our study demonstrates the selective involvement of specific nuclei as opposed to global atrophy. The genotype-specific patterns of amygdala involvement identified by this study are consistent with the growing literature of extra-motor clinical features. Mesial temporal lobe pathology in ALS is not limited to hippocampal pathology but, as a key hub of the limbic system, the amygdala is also affected in ALS.

1. Introduction

While ALS is primarily associated with motor cortex [1,2] and spinal cord degeneration [3,4], it has gradually been recognised as a multi-system disorder with widespread frontotemporal [5,6], extra-pyramidal [7], cerebellar involvement [8,9] and a variety of extra-motor manifestations [10].

Imaging studies in ALS have consistently captured some degree of temporal lobe pathology, but significant inconsistencies exist due to differences in imaging methods, sample sizes, recruitment strategies, inclusion criteria and patient stratification [11]. Early temporal lobe studies in ALS primarily focused on cortical grey matter involvement [12] before white matter alterations were also gradually characterised [13,14]. Dedicated hippocampus studies have been recently published both in ALS [15,16] and PLS [17,18] confirming that mesial temporal lobe pathology in an important feature of the motor neuron disease spectrum. The amygdala however is surprisingly understudied in ALS in vivo, despite reports of deficits in social cognition [19,20], memory

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Glossary

AAA	Anterior amygdaloid area
ABN	Accessory basal nucleus
ALS	Amyotrophic lateral sclerosis
ALS	Amyotrophic Lateral Sclerosis
ALS C9+	*C9orf72* positive ALS
ALS C9-	*C9orf72* negative ALS
ANCOVA	Analysis of covariance
BG	Basal ganglia
BN	Basal nucleus
C9orf72	chromosome 9 open reading frame 72
CAT	Cortico-amygdaloid transition
CST	Corticospinal tract
CN	Cortical nucleus
DTI	diffusion tensor imaging
ECAS	Edinburgh Cognitive ALS Screen
EMM	Estimated marginal mean
EPI	Echo-planar imaging
FTD	Frontotemporal dementia
FOV	Field-of-view
FSL	FMRIB Software Library
FWE	Familywise error
GM	Grey matter
HC	Healthy control
HSP	hereditary spastic paraplegia
IR-SPGR	Inversion Recovery prepared Spoiled Gradient Recalled echo
LMN	Lower motor neuron
LN	Lateral nucleus
M	Mean
MN	Medial nucleus
MND	Motor neuron disease
MNI152	Montreal Neurological Institute 152 standard space
MR	magnetic resonance
NODDI	neurite orientation dispersion and density imaging
PLS	Primary lateral sclerosis
PMA	Progressive muscular atrophy
PN	Paralaminar nucleus
pTDP-43	phosphorylated 43 kDa TAR DNA- binding protein
RE	Repeat expansion
SBMA	Spinal and bulbar muscular atrophy / Kennedy's disease
SD	standard deviation
SE	standard error
SMA	Spinal muscular atrophy
T1W	T1-weighted imaging
TE	Echo time
TFCE	threshold-free cluster enhancement
TI	Inversion time
TIV	Total Intracranial Volume
ToM	Theory of Mind
TR	repetition time
UMN	Upper motor neuron

impairment [21], behavioural changes [22,23], and evidence of amygdala pathology on post mortem examination [24–26]. The amygdala is a key structure of the limbic system which mediates a number of cognitive and behavioural functions and has direct connections to brain regions affected in ALS such as the thalamus [27], hypothalamus [28], and the accumbens nucleus [29]. It plays a key role in memory modulation, reward processing, emotional learning, and the regulation of aggression, fear, and anxiety.

Dedicated amygdala studies are not only sparse in the ALS imaging literature but their findings are strikingly inconsistent. Some of the inconsistency may be explained by differences in imaging methodology and patient selection, but the most likely cause of the inconsistency is that the amygdala is typically evaluated as a single structure in ALS. Metabolic, diffusivity, morphometric, volumetric and vertex-based approaches have previously been utilised to characterise overall amygdala pathology in vivo [13,17,29–39] but the involvement of specific nuclei has not been systematically assessed to date. The obvious risk of assessing the amygdala as a single structure is that pathology in affected and unaffected regions is likely to be averaged. Evidence from histopathology studies suggests that amygdala pathology in ALS in not global, but selectively involves specific nuclei [24,25,40,41]. Many of the seminal histology reports on selective amygdala pathology in ALS however predate the discovery of *C9orf72* GGGGCC hexanucleotide repeats.

Accordingly, our objective was the systematic evaluation of amygdala pathology in vivo in a large cohort of genetically and phenotypically characterised motor neuron disease patients using both measures of the entire amygdala and evaluating alterations in specific nuclei. Based on the available histopathology literature we hypothesised that selective nuclear degeneration may be captured in the amygdala of ALS patients using high-resolution structural imaging, in-depth genetic and clinical profiling and conservative structural analysis pipelines. Based on the clinical phenotype of GGGGCC hexanucleotide carriers [42,43] we additionally hypothesised that genotype-specific amygdalar signatures may be captured.

2. Methods

2.1. Ethics statement

This study was approved by the institutional ethics committee (Beaumont Hospital, Dublin, Ireland), in accordance with the 1964 Helsinki declaration and its later amendments. All participants provided informed consent prior to inclusion. Recruitment, data management, consent forms and information leaflets were specifically approved by the institutional ethics committee.

2.2. Participants

ALS patients had ‘probable’ or ‘definite’ ALS according to the El Escorial criteria [44] and PLS patients were diagnosed based on the Gordon criteria [45]. To aid the interpretation of the radiological profile of the patient groups, healthy controls were also included in this study. Exclusion criteria for all participants included known cerebrovascular events, head injuries, neoplastic conditions, neuroinflammatory conditions and inability to undergo MRI scanning due to implanted metallic devices such as pacemakers, dental plates or baclofen pumps. A total of 5 patients and 7 controls could not be included due to claustrophobia, orthopnoea or unexpected incidental intracranial findings. The final analysis included data from 100 ALS patients, 33 PLS patients and 117 healthy controls. The demographic and clinical profile of each participant was carefully recorded; motor disability was appraised by the revised ALS functional rating scale (ALSFRS-r) [46], the Edinburgh Cognitive ALS Screen (ECAS) [47] was administered for cognitive screening using population-based normative values [48] and co-morbid FTD was established based on the revised Strong criteria [49]. The healthy controls of the study had no known neurological or psychiatric conditions, previous head injuries or a family history of neurodegenerative conditions. Healthy controls were unrelated to the participating patients.

2.3. Neuroimaging methods

MRI data were acquired in a dedicated research facility on a 3 Tesla Philips Achieva system using an 8-channel receive-only head coil. T_1-weighted images were acquired with a 3D Inversion Recovery prepared Spoiled Gradient Recalled echo (IR-SPGR) pulse sequence with a field-of-view (FOV): 256 × 256 × 160 mm, spatial resolution: 1 mm^3, TR/TE = 8.5/3.9 ms, TI =1060 ms, flip angle = 8°, SENSE factor = 1.5.

2.4. TIV volumes

For the comparisons of amygdala volumes, total intracranial volume (TIV) was used as a covariate in addition to age, gender and education. TIV was calculated for each participant by adding grey matter, white matter and cerebrospinal fluid volume estimates. Subsequent to skull-removal [50] and quality control, each participant's T1-weighted image was linearly aligned to MNI152 standard space using FMRIB's Linear Image Registration Tool (FLIRT) [51]. FMRIB's Automated Segmentation Tool (FAST) [52] was utilised for tissue-type segmentation which accounts for spatial intensity variations. The inverse of the determinant of the affine registration matrix was calculated and multiplied by the size of the template for tissue-type volume estimations.

2.5. Amygdala segmentation

A Bayesian inference was used to segment the amygdala into 9 sub-regions using a probabilistic atlas developed based on histological data in the FreeSurfer analysis suite [53,54] (Fig. 1.). The amygdala was parcellated into the following nuclei in each hemisphere: Lateral nucleus (LN), Basal nucleus (BN), Accessory basal nucleus (ABN), Anterior amygdaloid area (AAA), Central nucleus (CN), Medial nucleus (MN), Cortical nucleus (CN), Cortico-amygdaloid transition (CAT), and Paralaminar nucleus (PN). The raw volume estimates of the above nuclei were averaged between left and right. “Total amygdala volume” was defined as the mean of the left and right total amygdala volume estimates.

2.6. Vertex analyses

Patterns of amygdala atrophy were further evaluated using vertex analyses. Focal amygdala shape deformations were explored using FMRIB's subcortical segmentation and registration tool FIRST [55]. An average amygdala shape was generated from all participants. Vertex locations of each subject were projected on the surface of the average amygdala shape template as scalar values, positive value being outside the surface and negative values inside. Group differences were explored using permutation based non-parametric inference as implemented by FMRIB's ‘RANDOMISE’ module [56]. Design matrices included de-meaned total intracranial volume, age, education, and gender as covariates [56].

2.7. Genetics

All patients were tested for pathogenic GGGGCC hexanucleotide repeat expansions by repeat-primed PCR using the Applied Biosystems (Foster City, CA, USA) 3130xl Genetic Analyser and visualised using GeneMapper version 4.0. More than 30 hexanucleotide repeats were considered pathological. Additionally, ALS and PLS patients were tested for a panel of motor neuron disease associated mutations including *SOD1, TARDBP, FUS, OPTN, ATXN2, VCP, ANG, ALS2, SETX, SPG11,*

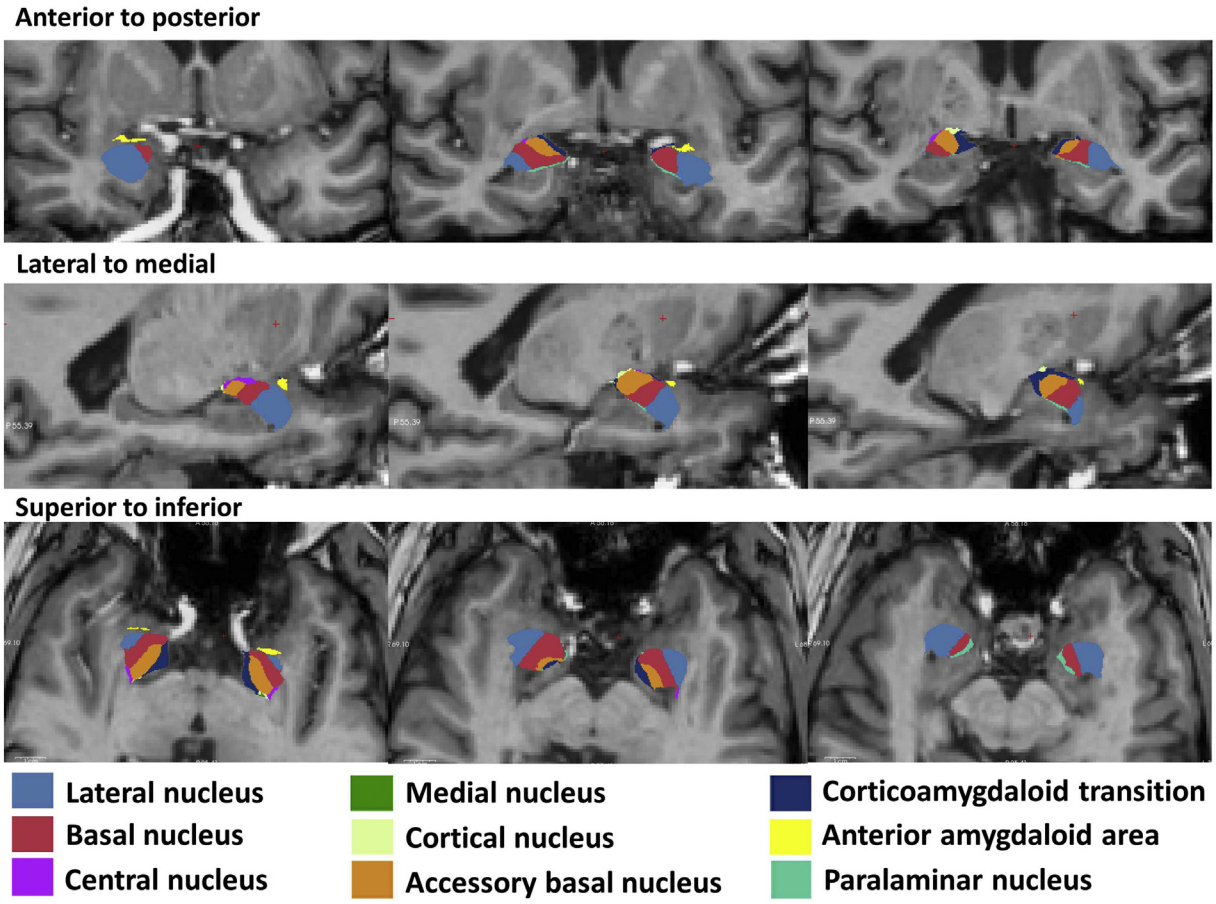

Fig. 1. Atlas-based segmentation of the amygdala; Lateral nucleus (LN), Basal nucleus (BN), Accessory basal nucleus (ABN), Anterior amygdaloid area (AAA), Central nucleus (CN), Medial nucleus (MN), Cortical nucleus (CN), Cortico-amygdaloid transition (CAT), Paralaminar nucleus (PN).

VAPB, FIG. 4, UBQLN2, SIGMAR1, CHMP2B, PFN1, ERBB4, HNRNPA1, MATR3, CHCHD10, UNC13A, DAO, DCTN1, NEFH, PRPH, SQSTM1, TAF15, SPAST, ELP3, LMNB1, SARM1, C21orf2, NEK1, FUS, CHMP2B, GRN, MAPT, PSEN1, PSEN2, TBK1. Sixty-seven ALS patients underwent whole genome sequencing [57] and 33 ALS patients underwent target next –generation targeted sequencing. [58] All 33 PLS patients underwent whole genome sequencing. In addition to *C9orf72* testing and genetic screening for established ALS-causing mutations, PLS patients were also screened for 70 genes implicated in hereditary spastic paraplegia (HSP) [59,60].

2.8. Statistical analysis

Volumetric and demographic data were interpreted using IBM SPSS v. 20. Assumptions of normality were examined using the Kolmogorov-Smirnov test. Skewness and kurtosis were assessed separately for each study group. Since all variables followed a normal distribution, parametric statistics were applied. Group differences (HC, ALS-C9+, ALS-C9-, PLS) in demographic variables were examined using multivariate analysis of variance (MANOVA) followed by post-hoc comparisons (age, education) and chi-square test (gender, handedness, site of onset). Group differences in amygdala nuclei volumes were examined using multivariate analysis of covariance (MANCOVA) with total intracranial volume (TIV), age, gender and education as covariates followed by effect size calculations. $p < .05$ was considered significant in post-hoc comparisons following Bonferroni corrections for multiple comparisons to reduce Type I error. The association between symptom duration and nuclear volumes was examined with partial correlation, using age, gender, education and TIV as covariates.

3. Results

The four study groups were matched for age, gender, education and handedness (Table 1.) ALS patients were categorised based on their *C9orf72* hexanucleotide expansion status. All ALS patients and PLS patients tested negative for other established mutations associated with ALS.

3.1. Volumetric profile

Differences in total amygdala volumes did not reach statistical significance following corrections for multiple comparisons, but an important trend of volume reduction was detected in *C9orf72* positive ALS patients compared to healthy controls ($p_{cor} = 0.055$). In *C9orf72* negative ALS patients, accessory basal nucleus ($p_{cor} = 0.021$) and the cortical nucleus ($p_{cor} = 0.022$) volumes were significantly lower than in healthy controls. A trend of volume reduction was also observed in the anterior amygdaloid area ($p_{cor} = 0.057$). *C9orf72* negative patients also had significantly lower cortical nucleus ($p_{cor} = 0.034$) and anterior amygdaloid area ($p_{cor} = 0.042$) volumes compared to PLS patients. *C9orf72* hexanucleotide carriers exhibited preferential volume reductions in the lateral nucleus ($p_{cor} = 0.043$), the cortico-amygdaloid transition area ($p_{cor} = 0.024$) and a trend of volume reduction in the accessory basal nucleus ($p_{cor} = 0.057$) with reference to healthy controls. PLS patients did not show overall amygdala atrophy or volume reductions in specific nuclei. (Table 2, Fig. 2.) Furthermore, no direct association was identified between symptom duration and nuclear volumes in any of the patient groups.

3.2. Vertex changes

Surface projected shape deformation only reached significance in the *C9orf72* positive ALS versus healthy control contrast, highlighting a unilateral pattern of inferior medial atrophy in the left amygdala (Fig. 3.).

4. Discussion

Our findings highlight the selective degeneration of amygdalar nuclei as opposed to global amygdala pathology in ALS. Furthermore, our data suggest genotype-specific patterns of amygdala pathology.

Global amygdala involvement in ALS has been observed qualitatively [61] and investigated in dedicated cross-sectional [31,33,34] and longitudinal imaging studies [36,38]. Imaging modalities of existing amygdala studies in ALS include PET [32,62], volumetry [29,33,34], fMRI [35], voxel-based morphometry [30,39], ROI morphometry [33], vertex analyses [29,33], diffusion tensor imaging [29,38] and the assessment of peri-amygdalar white matter alterations [13]. The findings of these reports are strikingly inconsistent; some studies did not detect significant amygdala atrophy [29,33,36,39] while others identified considerable pathological changes [32,34,35,38]. Interestingly, some studies found no volume reductions, but identified focal shape deformation in the same cohort [33], which highlights the differing detection sensitivity of the various methodological approaches. A PET study identified a cluster of relative hypermetabolism in the medial temporal lobe including the amygdala which was putatively interpreted as astrocytosis or microglial activation [32]. The majority of ALS imaging studies which comment on the amygdala are cross-sectional and the few longitudinal studies arrive to different conclusions. Some studies detect progressive amygdala atrophy over time [38], while others don't detect progressive change longitudinally [36]. Patient selection and the choice of image analysis are the two most likely factors in the inconsistency. ALS is a clinically heterogeneous condition with considerable differences in motor disability and neuropsychological performance. Compared to other neurodegenerative conditions, imaging studies of ALS typically include relatively small patient samples [63] and patients tend to only tolerate MRI scanning for a limited period of time after the diagnosis which leads to a considerable selection bias. Small patient samples coupled with clinical heterogeneity are likely to contribute to the inconsistency of mesial temporal lobe findings in ALS. Clinical heterogeneity in motor neuron disease (MND) is multidimensional and include variance in relative upper motor neuron /

Table 1
The clinical and demographic profile of study participants: *C9orf72* positive ALS patients (ALS C9+), *C9orf72* negative ALS patients (ALS C9-), primary lateral sclerosis patients (PLS), and healthy controls (HC).

Study group	*ALS C9+ n = 12*	*ALS C9- n = 88*	*PLS n = 33*	*HC n = 117*	*p value*
Age (years)	57.25 (16.45)	60.18 (10.31)	60.48 (10.49)	57.38 (11.91)	0.263
Gender (Male)	6 (50%)	56 (63.6%)	19 (57.6%)	56 (47.9%)	0.598
Education (years)	12.50 (3.21)	13.58 (3.17)	12.88 (3.38)	14.31 (3.29)	0.053
Handedness (right)	9 (75%)	81 (92%)	29 (87.9%)	109 (93.2%)	0.185
Onset (Bulbar)	2 (16.66%)	16 (18.18%)	1 (3.03%)	n/a	0.102
Onset (Limb)	10 (83.33%)	72 (81.81%)	32 (96.96%)	n/a	0.102
ALSFRS-R	36.08 (7.585)	36.69 (7.495)	34.36 (5.337)	n/a	0.272
Cognitive impairment on ECAS	7 (58.3%)	19 (21.5%)	8 (24.2%)	n/a	0.023
Comorbid FTD as per the Strong criteria	8 (66.66%)	7 (7.95%)	1 (3.03%)	n/a	< 0.01

Table 2
Estimated marginal means (Mean ± S.E.) are adjusted for age (58.77), gender (1.45), education (13.78) and TIV (1,429,036.83).

	Study group				Post-hoc comparisons						
	ALS C9+	ALS C9-	PLS	HC	ALS C9+ vs HC	ALS C9- vs HC	PLS vs HC	ALS C9+ vs ALS C9-	ALS C9+ vs PLS	ALS C9- vs PLS	Effect size
Whole amygdala	1672.88 ± 50.03	1754.97 ± 18.50	1806.97 ± 30.15	1811.23 ± 16.12	**0.055**[t]	0.144	1.000	0.753	0.134	0.847	$\eta^2p = 0.043$
Lateral nucleus	632.39 ± 18.34	669.41 ± 6.78	678.01 ± 11.05	684.74 ± 5.91	**0.043**	0.555	1.000	0.358	0.203	1.000	$\eta^2p = 0.035$
Basal nucleus	428.78 ± 13.74	449.06 ± 5.08	462.37 ± 8.28	462.62 ± 4.43	0.120	0.284	1.000	1.000	0.222	1.000	$\eta^2p = 0.034$
Accessory basal nucleus	251.83 ± 9.04	263.48 ± 3.34	275.43 ± 5.45	276.68 ± 2.91	**0.021**	1.000	1.000	1.000	0.156	0.371	$\eta^2p = 0.054$
Anterior amygdaloid area	52.50 ± 2.11	54.09 ± 0.78	58.13 ± 1.27	56.82 ± 0.68	0.317	**0.057**[t]	1.000	1.000	0.137	**0.042**	$\eta^2p = 0.051$
Central nucleus	45.86 ± 2.07	45.70 ± 0.77	47.77 ± 1.25	47.17 ± 0.67	1.000	0.922	1.000	1.000	1.000	0.946	$\eta^2p = 0.013$
Medial nucleus	20.96 ± 1.35	19.93 ± 0.50	22.34 ± 0.82	21.07 ± 0.44	1.000	0.547	1.000	1.000	1.000	0.074	$\eta^2p = 0.028$
Cortical nucleus	24.95 ± 1.13	25.06 ± 0.42	27.27 ± 0.68	26.70 ± 0.36	0.856	**0.022**	1.000	1.000	0.470	**0.034**	$\eta^2p = 0.051$
Cortico-amygdaloid transition	165.04 ± 5.95	176.66 ± 2.20	183.45 ± 3.58	183.26 ± 1.92	**0.024**	0.155	1.000	0.410	**0.050**	0.635	$\eta^2p = 0.049$
Paralaminar nucleus	50.56 ± 1.51	51.56 ± 0.56	52.12 ± 0.91	52.19 ± 0.49	1.000	1.000	1.000	1.000	1.000	1.000	$\eta^2p = 0.006$

Bold *p*-values are significant at $p < .05$ following Bonferroni correction for multiple comparisons. Partial η^2 effect size is interpreted as small ($\eta^2p = 0.01$), medium ($\eta^2p = 0.06$) and large ($\eta^2p = 0.14$). [t] indicates a statistical trend of $0.05 < p_{cor} < 0.06$.

lower motor neuron (LMN) involvement, differing motor disability profiles, varying progression rates, inconsistencies in neuropsychological manifestations and considerable differences in survival [64]. While clinical heterogeneity is regarded as a barrier to drug development by some [65] and a key contributor to diagnostic delay by others [66], it highlights the need for individualised management strategies and interventions tailored to individual clinical, genetic and radiological profiles. To circumvent disease heterogeneity and sample size limitations, extra-motor studies in ALS typically use one of the two main stratification strategies [67,68]; patient categorisation based on neuropsychological profile [33,39] or based on genotype [29]. Our findings illustrate the importance of patient stratification based on objective criteria which in our case allows the description of genotype-specific signatures. Our findings also showcase the limitation of measuring total volumes in a cytologically and functionally heterogeneous structure; total volumes were not significantly different between the study groups but the volumes of individual nuclei were.

Our cohort of *C9orf72* negative ALS patients exhibited focal changes in the accessory basal nucleus, cortical nucleus and anterior amygdaloid area compared to healthy controls. The accessory basal nucleus forms the connection between the central and lateral nuclei [69,70], and its activation has been shown to subdue high-anxiety states and fear-related freezing [70,71]. The cortical nucleus of the amygdala mediates ingestive, defensive and reproductive behaviours and is also thought to be involved in the processing of pheromonal information [72]. The corticomedial nuclei are involved in olfaction and appetite regulation. The relevance of characterising the extra-motor profile of *C9orf72* negative sporadic ALS patients is that these patients represent the majority of patients presenting to ALS clinics. Following the discovery of GGGGCC repeats, there was a notion that frontotemporal changes are unique to hexanucleotide repeat carriers and C9orf72 negative ALS patients may represent a relatively pure or 'classical' motor variant of ALS with limited extra-motor involvement [43]. This concept has been gradually challenged by the detection of considerable temporal, frontal and subcortical pathology in *C9orf72* negative cohorts [37,73]. Our findings also indicate that mesial temporal lobe involvement is not unique to the *C9orf72*.

Degeneration of the amygdala has been previously linked to *C9orf72* repeat expansions based on both post mortem [74] and imaging observations [75]. Presymptomatic studies of *C9orf72* hexanucleotide repeat carriers seldom comment specifically on amygdala involvement [76] despite description of extensive temporal lobe changes long before symptom manifestation [42,77]. We have identified a statistical trend of total amygdala volume reduction in hexanucleotide repeat carriers compared to controls ($p = .055$) and significant shape deformation. In our study, *C9orf72* hexanucleotide carriers exhibited preferential volume reductions in the lateral nucleus, cortico-amygdaloid transition area and accessory basal nucleus. The lateral, basal and accessory basal nuclei all form part of the basolateral group of nuclei which has reciprocal connections to sensory association areas of the cortex and direct projections to the thalamus [78]. The lateral nucleus has an established role in fear-related processes, a key component of Pavlovian fear-conditioning [79], involved in short-term [80], and long-term fear-memory [81–83]. The corticoamygdaloid transition zone is a zone of confluence between the medial parvicellular basal nucleus, paralaminar nucleus, and the sulcal periamygdaloid cortex and provides the main input to the lateral core subdivision [84]. Our findings indicate relatively divergent amygdalar profiles in hexanucleotide repeat carriers and sporadic ALS patients. The only shared area affected in both patient groups irrespective of *C9orf72* hexanucleotide status is the accessory basal nucleus. The characterisation of *C9orf72*-associated amygdalar changes in ALS complements previous reports of unique radiological features, progression patterns, and clinical manifestations associated with the genotype [43,85,86].

PLS patients showed no volume reductions in any of the amygdalar nuclei. PLS is still widely regarded as 'pure' motor system disorder

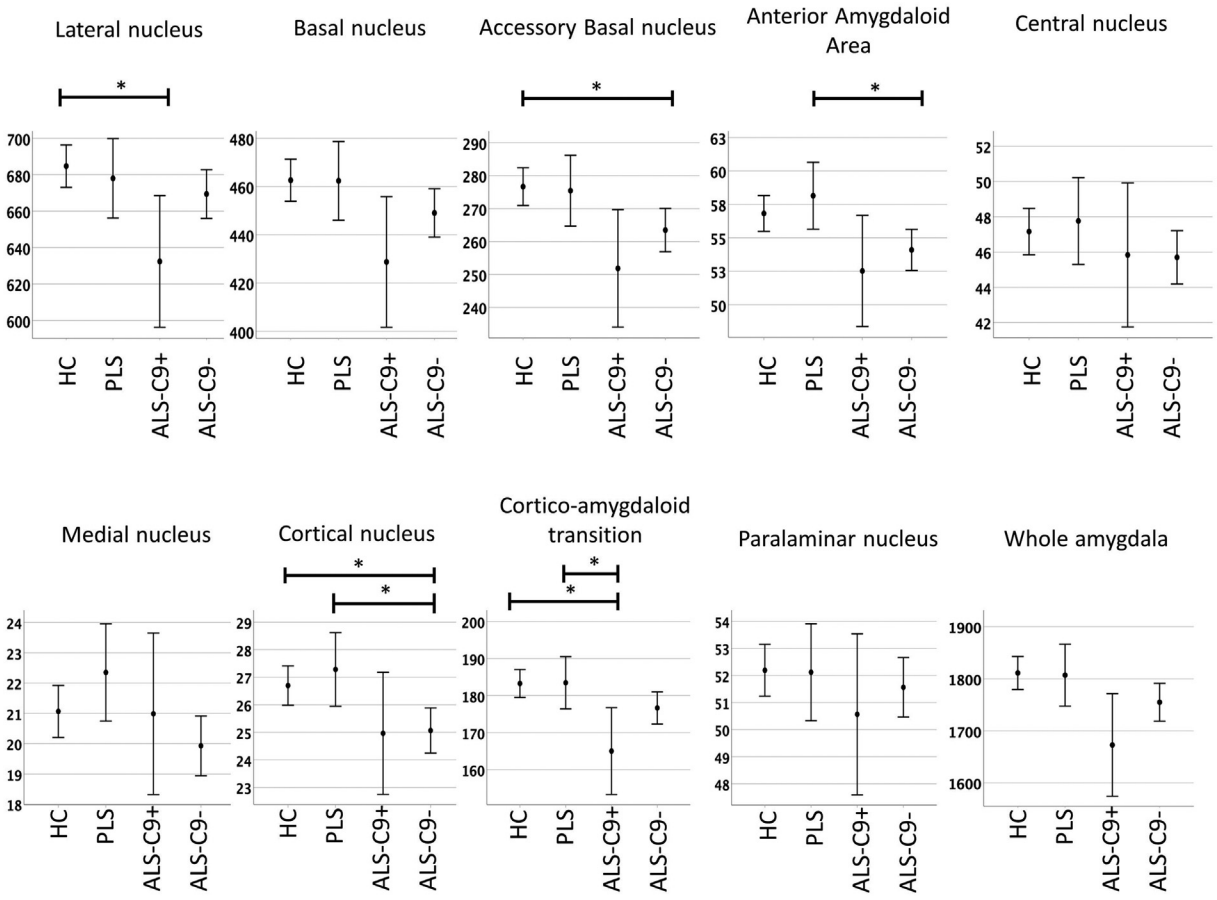

Fig. 2. The volumetric profile of the amygdala in healthy controls (HC), primary lateral sclerosis (PLS), *C9orf72* positive ALS (ALS-C9 +), *C9orf72* negative ALS (ALS-C9-) based on estimated marginal means adjusted for age, gender, education and total intracranial volumes. Error bars represent 95% confidence intervals.
* indicates statistically significant intergroup differences following corrections for multiple comparisons and adjustments for demographic variables.

despite recent reports of cognitive and behavioural deficits [87–89]. PLS is considered a separate entity by some and the UMN-dominant extreme of the ALS spectrum by others [90]. From a clinical perspective, PLS has a number of unique features and a relatively stereotyped disease trajectory which justify the assessment of cerebral pathology as a distinct group separate from ALS. PLS patients typically have longer survival than ALS patients, present with relatively symmetric limb spasticity, and despite frequent bulbar involvement, they rarely require feeding tube placement or respiratory support. Given the predominance of upper motor neuron signs on clinical assessment, PLS imaging studies typically focus on precentral gyrus pathology. From a radiological perspective PLS is classically associated with motor cortex atrophy [2,59,91], corpus callosum [92,93] and corticospinal tract degeneration [94–96], but more recently, brainstem [97], temporal lobe [59], cerebellar [59] and subcortical grey matter changes have also been described [17,18]. Consistent with the expanding literature of extra-motor changes in PLS white matter alterations have been described in the superior and inferior longitudinal fasciculi, fornix, thalamic radiations, and parietal lobes based on diffusivity metrics [98,99]. Emerging evidence of neuropsychological deficits [87,88] provide the rational for the targeted assessment of temporal lobe changes in PLS.

Post mortem studies consistently describe amygdala changes in amyotrophic lateral sclerosis [100–103]. Histopathological changes in the amygdala include focal TDP-43 burden [101,102,104,105], neuronal loss [24,40,104,106–108] and gliosis [106,107,109,110]. Pathological TDP-43 accumulation in the anteromedial portions of the temporal lobe is regarded as stage 4 of the recently proposed pathological staging system [111]. Amygdalar changes have also been linked to clinical stages on imaging. Peri-amygdalar white matter alterations were identified in patients with stage 2 disease based on the King's staging system [13]. Focal amygdalar changes have been previously described in ALS with the preferential involvement of the basolateral nuclei showing heavy gliosis [25] and neuronal loss [24]. With few exceptions [112], the majority of large ALS imaging studies lack post mortem assessment which precludes the histological validation of ante mortem imaging findings and the appraisal of the sensitivity profile of specific imaging techniques. From a biological perspective, there is considerable concordance among post mortem, clinical and imaging studies in ALS that interconnected brain regions exhibit concomitant degeneration [113–115]. This is commonly interpreted as evidence of connectivity-based [114], trans-synaptic [116] or prion-like [117] disease propagation. The amygdalar changes identified in this study are consistent with this notion, as other components of the limbic system and key projections of the amygdala such as the hypothalamus, dorsomedial thalamus, facial nerve nuclei, and the nucleus accumbens are also established foci of ALS pathology [15,29].

The post mortem literature of PLS is sparse compared to the number of studies published in ALS. The commonest post mortem findings in PLS include Betz cell depletion in the motor cortex, demyelination and the degeneration of the corticospinal tracts [118–123]. Extra-motor involvement has also been described in PLS [124–127], but many of these reports include clinically diverse patients. Very few post mortem studies specifically highlight amygdala pathology in primary lateral sclerosis [104,128]. Our imaging study did not identify amygdala atrophy in PLS which is in contrast to our findings in the ALS cohort and supports the conceptualisation of PLS as a separate entity, distinct from ALS [90].

Neuropsychological deficits in ALS are thought to be dominated by

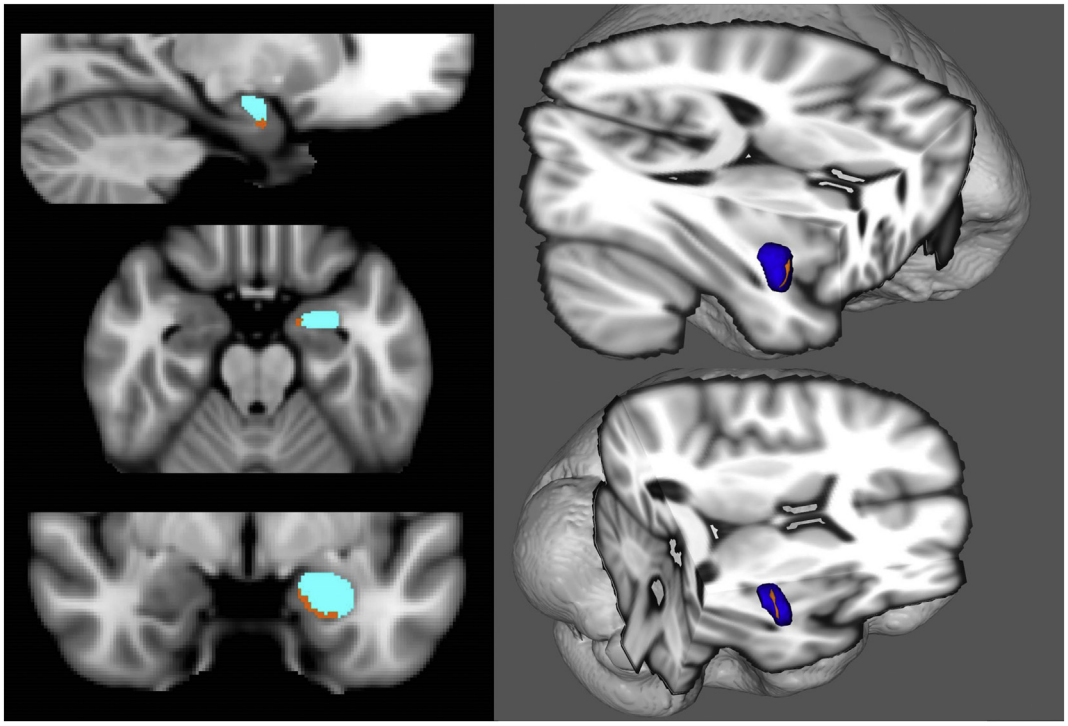

Fig. 3. Shape deformation in *C9orf72* positive ALS patients compared to healthy controls (HC) based on vertex analyses at $p < .05$ corrected for age, gender, education and total intracranial volumes. Blue colour indicates the 3 dimensional amygdalal mesh and orange highlights surface projected atrophy at $p < .05$ FWE. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

executive dysfunction [10] and behavioural impairment [23], but memory impairment and deficits in social cognition are increasingly recognised [19,20,129,130]. Amygdala-mediated limbic dysfunction has been specifically suggested in ALS based on impaired recognition of threat in fear-inducing situations [131]. Altered cerebral activation to emotional stimuli has also been repeatedly reported in ALS [132,133]. Abnormal anxiety-mediated amygdala activation patterns were previously described in ALS based on emotional processing paradigms [35]. The neural underpinnings of Theory of Mind (ToM) deficits have also been investigated by resting-state fMRI studies in ALS and recent data suggest a divergent longitudinal course in limb-onset and bulbar onset patients [134]. Anxiety is seldom studied specifically in ALS, but a recent large study which included 159 patients demonstrated the important quality-of-life ramification of anxiety in ALS [135]. Impaired emotional regulation is hugely relevant clinically as it may impact on engagement with multidisciplinary management, decisions to participate in clinical trials and adherence to therapy. Reduced fear or anxiety may affect safety awareness, usage of assistive devices and compliance with and rehabilitation efforts [136]. Deficits in social cognition may affect caregiver burden [137]. ALS patients make a series of emotional adjustments as they face difficult decisions around finances, continuation of employment, feeding tube placement, and ventilation all of which require the careful consideration of individual preferences, caregiver views, and personal values [138]. Subtle autonomic [139,140], sensory [141], and olfactory [142,143] dysfunction have also been documented in ALS [144,145] as well as changes in appetite [146] implicating the involvement of amygdala-mediated circuits. Cerebral sexual dimorphism is well established based on neuroimaging data form healthy cohorts [147,148], but also in ALS [149,150]. Developmental gender differences are thought to be particularly important with regards to amygdala volumes [151–153] which are often linked to gender-specific stress response profiles [135,154]. In our study we did not identify gender-specific volumetric traits, but the emerging literature of gene-gender interactions in ALS [149,155,156] underline the importance of examining gender effects in neuroimaging studies.

This study is not without limitations. The size of our *C9orf72* positive ALS cohort is relatively small to make conclusive observations about genotype-specific amygdalar signatures. We have no post mortem data to validate the selective degeneration of amygdalar nuclei observed in vivo. Our study is cross-sectional and merely provides a snapshot of structural degeneration instead of characterising the longitudinal trajectory of accruing amygdala pathology over time. A multi-time point longitudinal study design with presymptomatic mutation carriers would provide important additional insights [157,158]. The assessment of additional metabolic, functional or diffusivity metrics would have complemented our structural findings [159–162]. Notwithstanding these limitations, our study confirms considerable amygdala degeneration in ALS and highlights the importance of screening for limbic system-mediated cognitive functions. Our findings showcase the importance of evaluating non-motor brain regions in ALS radiologically and demonstrate that routine T1-weighted sequences can be utilised for the detailed characterisation of mesial temporal lobe structures. The heterogeneity of extra-motor involvement across motor neuron disease phenotypes (ALS/PLS) and genotypes (sporadic/C9orf72) underscores the importance of individualised management strategies. Our findings need to be validated in larger cohorts, possibly pooled from multiple centres and replicated in longitudinal studies.

5. Conclusions

Amygdala pathology is a consistent feature of both sporadic and *C9orf72*-associated ALS. The selective pathology of amygdalar nuclei detected in vivo is consistent with previous post mortem reports. The assessment of total amygdala volumes and shape deformations is insufficient to capture focal alterations in the structure. The involvement of the amygdala in ALS is likely to contribute to the heterogeneity of extra-motor manifestations including deficits in social cognition, emotional processing and memory impairment. Our findings confirm that mesial temporal lobe pathology is not unique to the *C9orf72* genotype

and underscore the importance of also screening for neuropsychological deficits in sporadic patients.

Potential conflicts of interest

None declared.

Authors' contribution

Conceptualisation and design of the study: RC, FC, EF, OH, PB. Analysis of radiological data: RC, FC, EF, SLHS, MCMcK, KMC, EK, PB. Analysis of genetic data; MAD, JCH, AV, RMcL. Clinical profiling: RC, EF, SLHS, NP, SH, CD, OH, PB. Drafting the manuscript, statistics, generation of figures: RC, FC, EF, SLHS, MCMcK, EK, PB. Revising the manuscript for intellectual content: RC, FC, EF, SLHS, MCMcK, RMcL, EK, OH, PB. Approval of the manuscript: all authors.

Declaration of Competing Interest

None.

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