

# Amyloid- and FDG-PET imaging in amyotrophic lateral sclerosis

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## Abstract

**Purpose** We aimed to study brain metabolism and presence of beta-amyloid deposits using positron emission tomography (PET) in patients with amyotrophic lateral sclerosis (ALS).

**Methods** This prospective cross-sectional study included 18 patients with definite or probable ALS according to the revised El Escorial diagnostic criteria, and 24 healthy controls. Patients underwent neurological and neuropsychological assessments, PET with <sup>18</sup>F-fluorodeoxyglucose (FDG), and amyloid-PET with <sup>18</sup>F-florbetaben.

**Results** Patients with ALS showed hypometabolism in the frontal area and hypermetabolism in the cerebellum compared to healthy controls. Four patients (22 %) displayed cognitive impairment and decreased metabolism in the frontal area extending bilaterally to the parietal regions, and increased metabolism in the posterior area of the cerebellum. In patients with no cognitive impairment, metabolism was lower in the left superior frontal gyrus and higher in the anterior and posterior lobes of the cerebellum. In the individual analysis, six patients (35 %) displayed more anterior involvement with hypometabolism affecting the superior frontal, medial, and

inferior gyri; six patients (35 %) exhibited a more posterior pattern with hypometabolism in the precentral and postcentral gyri and in the superior and inferior parietal lobules; two patients (11 %) showed a mixed pattern; and three patients (17 %) showed no alterations in brain metabolism. Three (16 %) showed increased <sup>18</sup>F-florbetaben uptake compared to controls.

**Conclusions** We have identified two main patterns of brain metabolism with an association to cognitive status. Only a subgroup of patients showed an increased uptake of the amyloid tracer. Our results suggest that ALS is heterogeneous from a clinical, metabolic, and molecular standpoint.

**Keywords** Amyloid · Amyotrophic lateral sclerosis · Brain metabolism · Positron emission tomography · Cognitive impairment · Alzheimer's disease

## Introduction

Amyotrophic lateral sclerosis (ALS) is a disease with highly heterogeneous clinical and pathological traits. Post-mortem studies have shown that ALS affects a number of areas and structures in the brain, causing not only motor neuron degeneration, but also a wide range of alterations in extra-motor areas such as the dorsolateral prefrontal cortex, anterior cingulate, hippocampus, dentate gyrus, parietal lobe, substantia nigra, cerebellum, amygdala, and basal ganglia [1–5].

Positron emission tomography (PET) with <sup>18</sup>F-fluorodeoxyglucose (FDG) is used to study brain metabolism as an indicator of synaptic dysfunction and neurodegeneration in neurodegenerative disorders. Frontal hypometabolism is the most frequent finding in <sup>18</sup>F-FDG PET imaging in patients with ALS [6]. However, there is no consensus in the literature about the involvement of other areas or structures, such as the

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dorsolateral prefrontal cortex, the lateral and medial premotor cortex, the insula, the occipital cortex, the anterior temporal lobe, and the parietal lobe. It has been suggested that the different regions involved may be related to subtypes of the disorder (clinical, genetic, etc.) [6–11].

In recent years, cognitive alterations have also been reported among some patients. In fact, ALS has been associated with certain forms of frontotemporal dementia (FTD) [12–14], and some have suggested a link between ALS and Alzheimer's disease (AD) [15]. In rodent models, a connection between amyloid precursor protein and ALS was observed, and an amyloid-like cascade has been suggested to explain certain aspects of the pathophysiology of ALS [16, 17]. Recent histological studies report amyloid plaques in 35–50 % of patients with ALS, even in patients without dementia or aphasia [4, 15]. Since these post-mortem studies are conducted in terminal stages of the disease, it may be difficult to interpret the pathophysiological role of beta-amyloid deposits in ALS.

Amyloid tracers enable *in vivo* studies of amyloid deposits; the validity of these studies is based on the correlation between PET imaging findings and histological results [18, 19]. To our knowledge, there are no published amyloid-PET studies in patients with ALS, and only three case reports of patients with ALS and dementia who were evaluated using amyloid-PET (PiB-PET in all cases) have been published to date [20–22].

Our purpose was to study brain metabolism and beta-amyloid deposition using  $^{18}\text{F}$ -FDG PET and  $^{18}\text{F}$ -florbetaben in a cohort of patients with ALS.

## Material and methods

### Study design and population

We conducted a prospective cross-sectional descriptive study in a cohort of 18 patients diagnosed with ALS (definite, probable, or probable laboratory-supported) according to the revised El Escorial diagnostic criteria [23]. Patients were recruited from the ALS unit of a university hospital. From 67 ALS patients on active follow-up in our unit, 24 were excluded due to advanced clinical status or criteria of “possible ALS”, and 25 cases rejected to participate.

The sample included 18 patients: 12 women and six men. Mean age at the time of inclusion in the study was  $57.58 \pm 11.16$  years. Mean age at symptom onset was  $55.44 \pm 12.20$  years, and mean progression time from symptom onset to inclusion in the study was  $29.39 \pm 27.94$  months. Onset was spinal in 13 patients and bulbar in the remaining five. At the time of the study, 15 patients displayed motor weakness in the limbs, nine had bulbar symptoms, five required non-invasive ventilation, and one required gastrostomy

feeding. The mean ALSFRS-R score was  $35.06 \pm 7.58$  (Table 1). All patients were in treatment with riluzole and underwent a neurological examination, a neuropsychological assessment, and an amyloid-PET study. Medical histories were completed based on the information reported by both patients and their companions.

The control group included 24 healthy individuals. Controls had no neurological disorders or systemic diseases with the potential to cause neurological impairment. Mean age in the 24 controls was  $60.50 \pm 8.15$  years; all controls completed cognitive tests and  $^{18}\text{F}$ -FDG PET studies, which yielded apparently normal results. Seven controls (mean age  $59.14 \pm 8.57$  years) also underwent amyloid-PET studies. There were no age- or sex-related differences between controls and patients.

Subjects were recruited between January and July 2015. The study was approved by the Clinical Research Ethics Committee at our hospital. All participants signed informed consent forms.

### Clinical and cognitive assessment

Medical histories were recorded for of all subjects. Additionally, all participants underwent a neurological examination and neuropsychological assessment, including the Mini-Mental State Examination (MMSE) [24], the Spanish-language version of Addenbrooke's Cognitive Examination III (ACE-III) [25], memory span (forward and backward digit spans) [26], visuospatial span (Corsi block-tapping test) [26], Trail Making Test [27, 28], Rey-Osterrieth Complex Figure Test [29, 30], Free and Cued Selective Reminding Test [31, 32], Visual Object and Space Perception Battery (object decision, progressive silhouettes, position discrimination, and number location subtests) [33], Stroop Color-Word Interference Test [34, 35], letter verbal fluency (words beginning with ‘p’ in 1 min), category fluency test (names of animals in 1 min) [36, 37], and Tower of London (Drexel Edition) [38]. We used normative data from NEURONORMA, a normative data project conducted in our setting and similar to MOANS in the United States [39]. Participants also completed action fluency tests [40, 41], picture-sentence matching tasks, semantic association tests, the Hayling Test [42], Edinburgh Cognitive and Behavioural ALS Screen (ECAS) [43], Cambridge Behavioural Inventory revised (CBI-R) [44], and Anosognosia Questionnaire for Dementia [45]. We followed the DSM-IV diagnostic criteria for dementia [46]. The revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) was used to assess level of disability [47]. Five patients failed to complete all neuropsychological tests due to physical inability (severe tetraparesis/quadruplegia and/or severe dysarthria/anarthria).

In ALS patients, mean MMSE score was  $26.94 \pm 3.74$  and mean ACE-III score was  $83.44 \pm 15.38$ . Fourteen patients with

**Table 1** Clinical and demographic characteristics of the ALS patients

Cases	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	ALS (N=18)
Age (years)	39	54	63	64	47	46	66	47	68	61	71	54	52	63	74	60	39	74	57.89±11.16
Sex	F	F	F	F	F	F	F	M	F	F	M	M	F	M	M	M	F	F	66 % female (n=12)
Age at onset (years)	35	52	62	63	45	38	63	45	66	58	70	53	46	61	72	58	37	74	55.44±12.20
Progression time (months)	55	19	7	9	18	120	27	28	26	29	12	16	72	14	31	21	18	7	29.39±27.94
Time from symptom onset to diagnosis (months)	6	7	7	8	13	48	1	5	9	15	9	4	60	14	13	12	10	7	13.78±15.22
Type of onset	S	S	S	B	S	S	S	S	S	S	B	S	S	B	S	S	S	B	72 % spinal (n=13)
Family history of ALS	NO	NO	NO	NO	NO	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	YES	NO	YES	n=5 (27 %)
Family history of AD	NO	YES	NO	YES	NO	NO	NO	NO	YES	NO	NO	YES	NO	NO	NO	NO	YES	NO	n=5 (27 %)
ALSFRS-R	24	43	33	47	37	36	43	36	41	37	39	27	23	37	29	20	38	41	35.06±7.58
Limb motor weakness	YES	YES	YES	NO	YES	YES	YES	YES	YES	YES	YES	YES	YES	NO	YES	YES	YES	NO	n=15 (83 %)
Bulbar symptoms	YES	NO	YES	YES	YES	NO	NO	NO	NO	NO	YES	NO	YES	YES	NO	YES	NO	YES	n=9 (50 %)
Cognitive impairment	NO	YES <sup>a</sup>	NO	NO	NO	NO	NO	YES <sup>b</sup>	NO	NO	YES <sup>c</sup>	YES <sup>b</sup>	n=4 (22 %)						
ACE-III	82	97	59	83	94	100	85	91	87	92	79	82	96	77	86	82	95	35	83.44±15.38

F female; M male; S spinal; B bulbar

<sup>a</sup> Executive dysfunction and hippocampal memory impairment

<sup>b</sup> Dementia

<sup>c</sup> Executive dysfunction

ALS displayed normal cognitive function for their age and education level, while four presented cognitive impairment. Of these four, two did not meet the criteria for dementia (one with executive dysfunction, one with executive dysfunction associated with hippocampal memory impairment), whereas the other two did (global deficit in all evaluated cognitive areas) (Table 1).

## Positron emission tomography

### Image acquisition

The clinical-cognitive assessment and imaging studies were conducted with an interval of less than 2 months between them. PET images were acquired using a PET-computed tomography (CT) Siemens Biograph True Point platform including a 6-slice detector with a latest-generation PET scanner featuring a lutetium oxyorthosilicate crystal array. CT parameters for both <sup>18</sup>F-FDG and <sup>18</sup>F-Florbetaben PET-CT studies were: 130 kVp, 40 mAs/rotation, and one rotation. Slice thickness was 3 mm, the reconstruction interval was 1.5 mm, and pitch was 0.75. Iterative 3D image reconstruction was performed with two iterations and 21 subsets. We used a 30 cm field of view and a Gaussian filter with full width at half maximum of 4 mm. FDG-PET and amyloid-PET studies were

conducted on different days, but with an interval of less than 2 months between them.

FDG-PET studies were performed in 17 patients. Patients fasted a minimum of 6 h before undergoing the FDG-PET scan. <sup>18</sup>F-FDG (mean dose of 185 MBq) was administered intravenously 30 min before image acquisition. During this time, patients rested in a dark room with their eyes closed. Static PET images were acquired for 15 min, at a single bed position.

We used <sup>18</sup>F-florbetaben for the amyloid-PET study. Participants were given this radiotracer (mean dose of 300 MBq) as a slow intravenous injection (6 s/mL), followed by a 10 mL saline flush (0.9 % sodium chloride, 9 mg/mL). Static PET images at a single bed position were acquired for a period of 20 min starting at 90 min from the <sup>18</sup>F-florbetaben injection.

### Processing and statistical analysis of <sup>18</sup>F-FDG PET images

Images were preprocessed using Statistical Parametric Mapping software version 8 (SPM8) (The Wellcome Trust Centre for Neuroimaging, Institute of Neurology, University College of London). Images were transformed from DICOM to NifTI-1 format using MRIConvert version 2.0 (Lewis Center for Neuroimaging, University of Oregon). Images were normalised to Montreal Neurological Institute (MNI)

space using an FDG-PET template [48], and subsequently smoothed with a 12-mm full width at half maximum Gaussian kernel. The study groups were compared using the *t*-test for two independent samples. Analyses were conducted by group (for example, ALS group vs control group) and individually (each patient vs the control group, i.e. single-subject analysis) using proportional scaling [49]. Age and sex were added to the model as covariates. Uncorrected *p* values <0.001 were considered statistically significant and an extent threshold > 50 voxels was used. We used an uncorrected *p* value <0.005 and an extent threshold > 50 voxels for the individual analysis. MNI coordinates were converted to Talairach coordinates using the conversion applet included in Bioimage Suite 2.0 [50]. Additional data about voxel-based brain mapping analysis results are available in Online Resource 1.

### Processing and statistical analysis of <sup>18</sup>F-florbetaben-PET images

Images were preprocessed using SPM8. Indirect spatial normalisation to MNI was performed with a template created at our centre based on magnetic resonance (MR) images from a random sample of patients. We used controls, patients with ALS, and patients with AD to create the template. A region of interest (ROI) analysis of the Automated Anatomical Labeling Atlas (AAL) was conducted using MarsBaR software [51]. We used grey matter ROIs exclusively to avoid the effect of normal uptake in healthy white matter. The whole cerebellum and the pons served as regions of reference, and we calculated the standard uptake value relative (SUVR) from each brain region and each of the regions of reference (SUVR<sub>c</sub>, cerebellum; SUVR<sub>p</sub>, pons). The mean and standard deviation (SD) for each ROI were calculated based on the uptake displayed by the control group. We then estimated *z* scores for each region in each of the patients with ALS (single-subject analysis). Uptake in each ROI was assigned a *z* score, and presence of amyloid deposits was defined as a *z* score > 2 in at least 2 ROIs using both the cerebellum and the pons as regions of reference. Additionally, a composite SUVR<sub>c</sub> for AD patients (AD-SUVR<sub>c</sub>) was calculated based on the following regions: medial orbitofrontal area, anterior and posterior cingulate, superior parietal lobule, precuneus, and inferior temporal area. These regions are considered to have the greatest discriminant ability for diagnosing AD [52].

### Statistical analysis

Data were analysed using IBM® SPSS statistical software, version 20 [53]. The descriptive analysis was performed using either absolute frequencies and percentages [n (%)] or means ± SD. Given the sample size, non-parametric tests

were used for comparison of clinical and demographic characteristics between groups. Qualitative variables were compared using the Fisher exact test. To compare means between two groups we used the Mann–Whitney *U* test. *p* values ≤ 0.05 were considered statistically significant and led to rejection of the null hypothesis.

## Results

### FDG-PET

#### *Analysis by group*

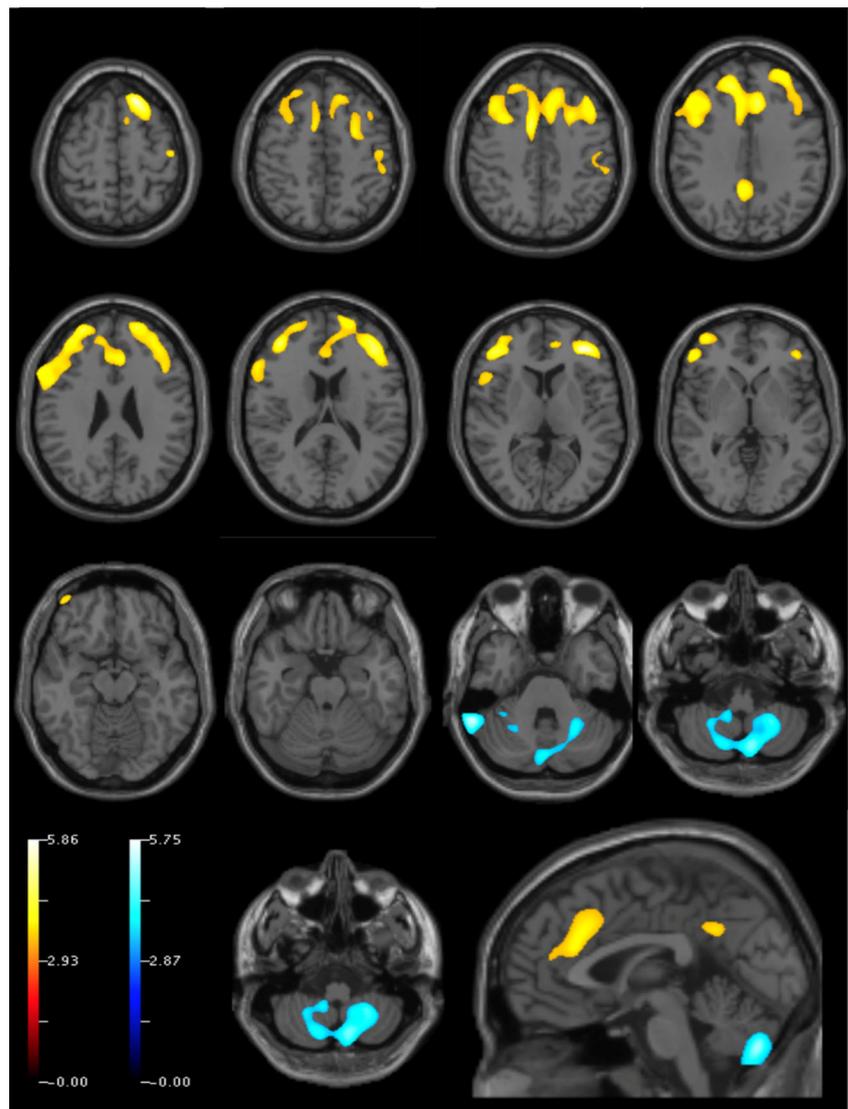
Compared to controls, patients with ALS (*n* = 17) displayed bilateral frontal hypometabolism in the superior, middle, and medial frontal gyri bilaterally; bilateral anterior cingulate, left precentral gyrus; right posterior cingulate; and left inferior parietal lobule. The ALS group also showed hypermetabolic areas in the cerebellum bilaterally (Fig. 1). Frontal hypometabolism in the superior, middle, and medial frontal gyri, anterior cingulate, thalamus (bilateral), left precentral gyrus, and left inferior parietal lobule was observed in ALS patients with cognitive impairment (*n* = 4). Metabolism was increased in the cerebellum (posterior lobe) and fusiform and middle occipital gyri (Fig. 2) in these patients. On the other hand, ALS patients with no cognitive impairment (*n* = 13) displayed hypometabolism in the left superior frontal gyrus and hypermetabolism in the anterior and posterior lobes of the cerebellum (Fig. 3).

#### *Individual analysis*

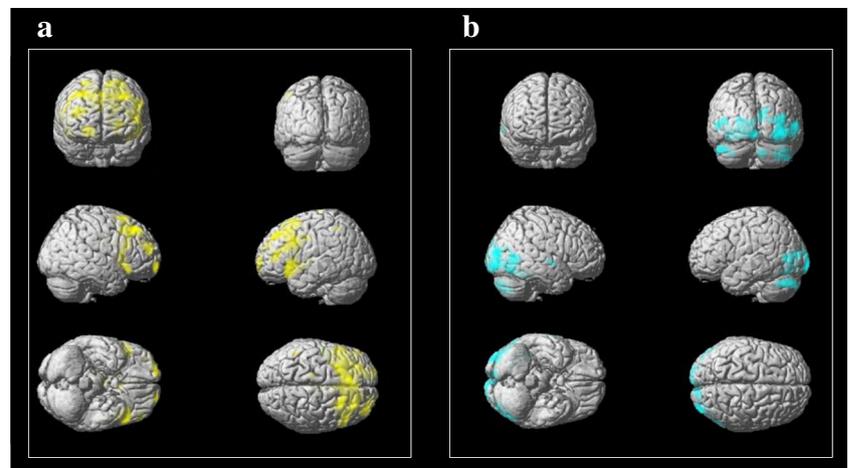
Each ALS patient was compared to the control group. Figure 4 shows the regions with lower metabolism in each case. Two different patterns were identified: predominantly anterior hypometabolism (*n* = 6) and predominantly posterior hypometabolism (*n* = 6). Two patients displayed a mixed pattern and three showed no alterations in brain metabolism. The patients with predominantly anterior hypometabolism showed metabolic alterations mainly in the superior, middle, and inferior frontal gyri, while patients with the predominantly posterior pattern displayed hypometabolism mostly in the precentral and postcentral gyri, precuneus, superior and inferior parietal lobules, and cingulate gyrus.

Table 2 shows the clinical and demographic characteristics of the groups with predominantly anterior and predominantly posterior hypometabolism in <sup>18</sup>F-FDG PET images. We observed a trend to a higher prevalence of cognitive deficit in the group with the predominantly anterior pattern (50 vs. 0 %; *p* = 0.09).

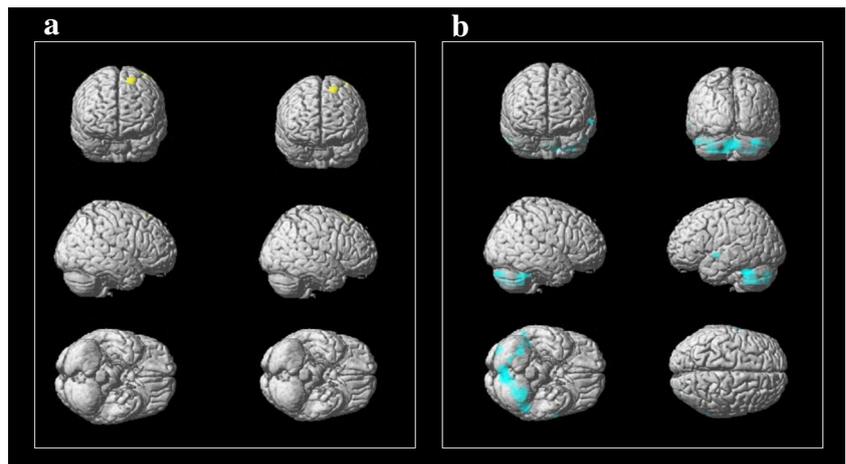
**Fig. 1** MRI template showing hypometabolic (*yellow*) and hypermetabolic (*blue*) regions in ALS. A two-sample *t*-test with an uncorrected *p* value < 0.001 and an extent threshold of 50 voxels was used



**Fig. 2** MRI template showing hypometabolic (*yellow*) and hypermetabolic (*blue*) regions in ALS patients with cognitive impairment compared to healthy controls. A two-sample *t* test with an uncorrected *p* value < 0.001 and an extent threshold of 50 voxels was used



**Fig. 3** MRI template showing hypometabolic (*yellow*) and hypermetabolic (*blue*) regions in ALS patients with no cognitive impairment compared to healthy controls. A two-sample *t* test with an uncorrected *p* value < 0.001 and an extent threshold of 50 voxels was used



### Amyloid-PET

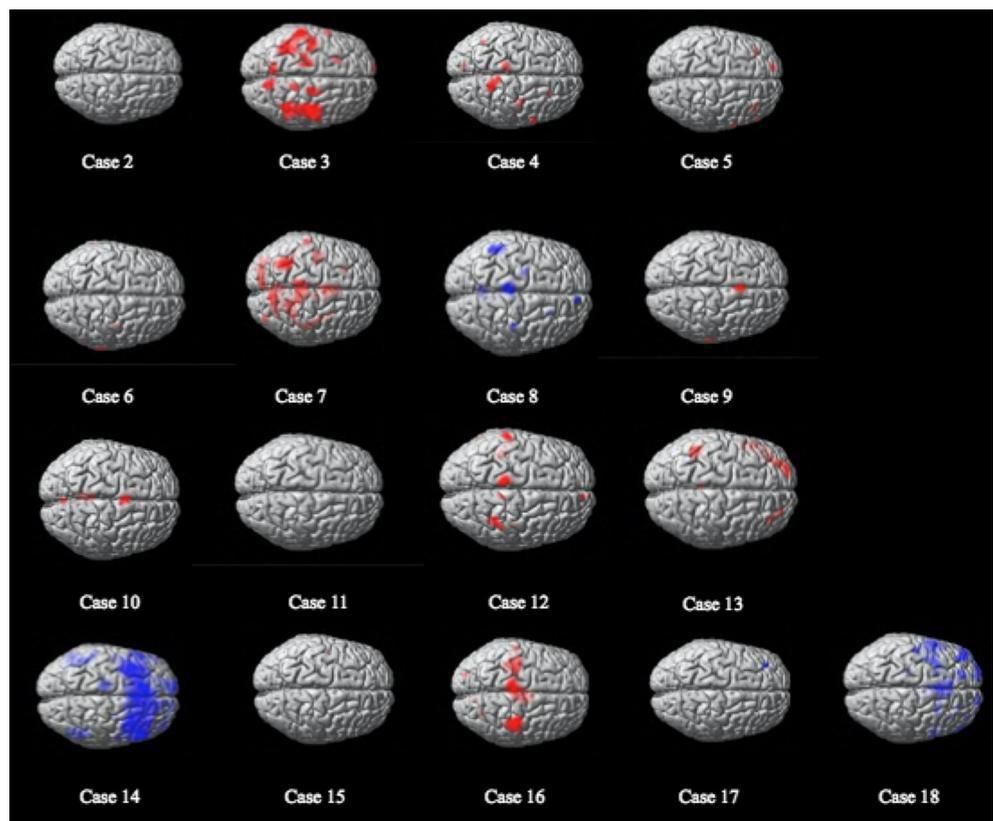
#### Analysis by group

We compared the SUVR for different brain regions between ALS group and controls using the Mann–Whitney *U* test and found no statistically significant differences. Likewise, composite AD-SUVRc was  $1.29 \pm 0.07$  in the ALS group and  $1.31 \pm 0.08$  in the control group ( $p = 0.856$ ).

#### Individual analysis

Following the previously established criterion for determining the presence of amyloid deposits from a single-subject analysis, we found three patients showing more pronounced tracer uptake than the control group’s in several regions: middle and superior frontal gyri, lingual gyrus, precentral gyrus, supplementary motor area, inferior and middle temporal gyri, parahippocampal gyrus, straight gyrus, hippocampus,

**Fig. 4** Hypometabolic regions from each patient compared to healthy controls. In *blue*, cases with cognitive impairment; in *red*, those with no cognitive impairment. A two-sample *t*-test with an uncorrected *p* value < 0.005 and an extent threshold of 50 voxels was used



**Table 2** Clinical and demographic characteristics of patients by  $^{18}\text{F}$ -FDG uptake pattern (anterior or posterior)

	Anterior pattern ( $n=6$ )	Posterior pattern ( $n=6$ )	Mann Whitney $U$	$p$ value
Age (years)	57.17 ± 13.37	61.33 ± 4.17	15.5	0.69
Sex (female)	$n=5$ (83 %)	$n=4$ (66 %)	0.4*	1.00
Age at onset (years)	54.83 ± 14.30	59.5 ± 3.93	–	–
Progression time (months)	25.83 ± 23.44	18.17 ± 9.13	–	–
Time from symptom onset to diagnosis (months)	18.83 ± 20.33	7.83 ± 5.11	9.5	0.18
Spinal onset	$n=4$ (66 %)	$n=4$ (66 %)	–	–
Family history of ALS	$n=1$ (16 %)	$n=2$ (33 %)	–	–
Family history of AD	$n=2$ (33 %)	$n=2$ (33 %)	–	–
ALSFRS-R	36.17 ± 6.70	34.50 ± 10.03	16.0	0.81
Limb motor weakness	$n=4$ (66 %)	$n=5$ (83 %)	–	–
Bulbar symptoms	$n=4$ (66 %)	$n=3$ (50 %)	–	–
Ventilatory support	$n=2$ (33 %)	$n=1$ (16 %)	–	–
PEG	$n=0$ (0 %)	$n=1$ (16 %)	–	–
Cognitive impairment	$n=3$ (50 %)	$n=0$ (0 %)	4.0*	0.09
Amyloid-PET showing increased uptake	$n=2$ (33 %)	$n=0$ (0 %)	–	–

\*: Chi-square test

fusiform gyrus, and cuneus. Table 3 compares the clinical and demographic characteristics of the group displaying increased  $^{18}\text{F}$ -florbetaben uptake and the group showing normal uptake.

The patients with increased  $^{18}\text{F}$ -florbetaben uptake showed reduced metabolism in the right middle frontal gyrus compared to controls. Patients with normal uptake displayed

**Table 3** Clinical and demographic characteristics in ALS subgroups with and without increased  $^{18}\text{F}$ -florbetaben uptake

	ALS with amyloid deposits ( $n=3$ )	ALS with no amyloid deposits ( $n=15$ )	Mann Whitney $U$	$p$ value
Age (years)	56.33 ± 10.69	58.20 ± 11.59	21.0	0.91
Sex (female)	$n=3$ (100 %)	$n=9$ (60 %)	1.8*	0.51
Age at onset (years)	54.33 ± 10.69	55.67 ± 12.81	–	–
Progression time (months)	21 ± 4.35	31.07 ± 30.45	21.5	0.91
Time from symptom onset to diagnosis (months)	9.67 ± 3.05	14.60 ± 16.60	–	–
Spinal onset	$n=3$ (100 %)	$n=10$ (66 %)	–	–
Family history of ALS	$n=0$ (0 %)	$n=5$ (33 %)	1.3*	0.52
Family history of AD	$n=2$ (66 %)	$n=3$ (20 %)	2.7*	0.17
ALSFRS-R	40.33 ± 3.05	34 ± 7.82	10.0	0.16
Limb motor weakness	$n=3$ (100 %)	$n=12$ (80 %)	–	–
Bulbar symptoms	$n=1$ (33 %)	$n=8$ (53 %)	–	–
Ventilatory support	$n=0$ (0 %)	$n=5$ (33 %)	–	–
PEG	$n=0$ (0 %)	$n=1$ (66 %)	–	–
Cognitive impairment	$n=0$ (0 %)	$n=4$ (26 %)	–	–
ACE-III	92.67 ± 5.13	81.60 ± 16.18	–	–

\*: Chi-square test

reduced metabolism in the superior and middle frontal gyri, precentral and postcentral gyri, and bilaterally in the anterior cingulate. There were no ALS patients in a single-subject analysis with a composite AD-SUV<sub>Rc</sub> score 1.5 standard deviations above the mean values for the control group.

## Discussion

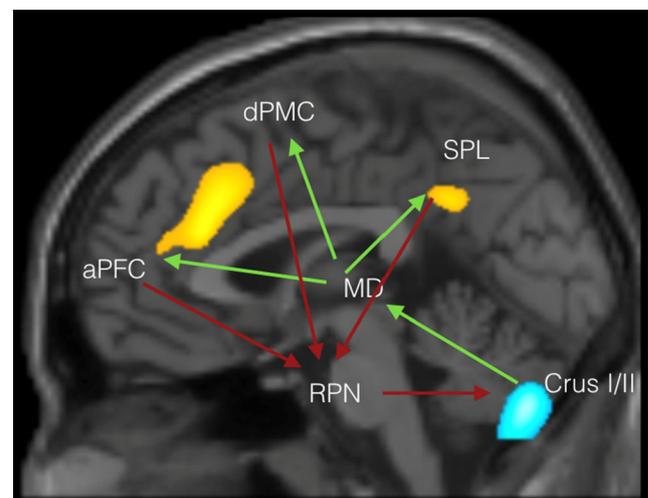
This study analysed the <sup>18</sup>F-FDG and amyloid-PET findings in a cohort of patients with ALS. We identified two main patterns of brain metabolism, which might be associated to some differences in clinical profiles, as has also been recently reported [11]. Furthermore, we detected an increased uptake of amyloid-tracer <sup>18</sup>F-florbetaben in a subgroup of patients. Evaluating brain metabolism as a marker of neurodegeneration and synaptic dysfunction *in vivo*, as well as examining deposition of the proteins, such as amyloid, involved in the pathophysiology of neurodegenerative diseases, significantly contributes to our knowledge of those diseases.

Previous studies with <sup>18</sup>F-FDG PET have demonstrated that ALS patients show lower metabolism than controls in prefrontal regions and several other areas. In this regard, different studies have pointed to the temporal, parietal, and occipital lobes, insula, putamen, globus pallidus, and thalamus [8–10]. Our group analysis confirmed frontal hypometabolism as the main finding in patients with ALS. However, the individual analysis showed relatively heterogeneous brain metabolism patterns with several different affected areas in the frontal, parietal, and temporal lobes. We attempted to distinguish between two patterns according to whether hypometabolism was predominantly anterior (frontal lobe) or posterior (precentral and postcentral gyri and extending to the parietal lobes). No differences were found in progression time or clinical severity between these patterns (as shown in Table 2), which suggests that neither of them evolves to become the other. Nevertheless, the cases with a predominantly anterior pattern displayed cognitive impairment more frequently (50 %) than the group with a predominantly posterior pattern (0 %). Likewise, extensive frontal hypometabolism was observed in the two patients with the most pronounced cognitive impairment and meeting criteria for dementia. This suggests that frontal hypometabolism and its extension to other areas is linked to the degree of cognitive impairment in patients with ALS [11]. <sup>18</sup>F-FDG PET findings may therefore constitute a marker of cognitive impairment in cases showing hypometabolism in anterior regions, especially if it is extensive, although further studies with a larger sample size are necessary to confirm this issue. This is relevant, considering that cognitive assessment is of limited utility in advanced stages of the disease, although other factors such as cognitive reserve should also be taken into account [54]. The presence of different metabolic patterns

may be explained by the clinical, genetic, and prognostic heterogeneity of ALS [55]. In this regard, several studies have revealed different <sup>18</sup>F-FDG PET patterns in patients with ALS and FTD depending on causal mutations and clinical progression [56–58].

Another interesting finding from our study was brain hypermetabolism in patients with ALS, described in previous studies as a phenomenon mainly affecting the cerebellum and mesencephalon [10, 11]. Some researchers suggest that increased metabolism may be secondary to astrogliosis [10, 11, 59]. Our sample also displayed increased metabolism in the cerebellum, along with interesting topographic differences: hypermetabolism in the posterior lobe of the cerebellum in patients with cognitive impairment; and hypermetabolism in the anterior and posterior lobes (although more marked in the anterior lobe) in those with no cognitive impairment. Given that the anterior lobe of the cerebellum is mainly involved in motor function and the posterior lobe in cognitive function [60–63], our findings may support the assumption that hypermetabolism may be caused by a compensatory mechanism secondary to neuronal degeneration in functionally related regions. In Fig. 5, the regions with a lower and higher metabolism in our ALS patients are shown together with the main connections between the cerebellum and the neocortex. This supports the idea of a functional compensation as the explanation of the hypermetabolism observed in ALS.

Amyloid-PET imaging found that three patients (16.66 %) displayed greater <sup>18</sup>F-florbetaben uptake than controls in multiple regions, which suggests presence of amyloid deposits. To our knowledge, no prior studies have addressed using



**Fig. 5** Main cerebellar areas involved in cognitive function. Feedforward tracts (pons), in red, and feedback tracts (thalamus), in green, which connect the cerebellum and the cerebral cortex. Hypometabolic (yellow) and hypermetabolic (blue) areas in the cerebellum. *dPMC* dorsal premotor cortex, *MD* mediodorsal thalamic nuclei, *aPFC* anterior prefrontal cortex, *SPL* superior parietal lobule, *RPN* rostral pontine nuclei

amyloid-PET to evaluate ALS, except for individual case reports [20–22] describing two patients with ALS/dementia associated with amyloid deposits. One patient exhibited amyloid deposition in the frontal lobe, parietal lobe, both lateral temporal lobes, posterior cingulate gyrus, and precuneus, and the other patient in the prefrontal, posterior cingulate, and parietal cortices. Both displayed hypometabolism in  $^{18}\text{F}$ -FDG PET, one of them in both parietal lobes and the left temporolateral cortex, and the other in the left temporal and prefrontal cortices. These previously reported cases suggest that AD may be a comorbidity in patients with ALS, rather than implying that beta-amyloid could contribute to ALS pathophysiology.

The hypothesis that the amyloid protein plays a role in ALS is based on the high rate of amyloid deposits detected in these patients by post-mortem studies (35–50 % according to the main studies) [4, 15]. This percentage is higher than the prevalence rate of cerebral  $\beta$ -amyloidosis reported by studies in healthy volunteers [64]. Known pathogenic mechanisms of beta-amyloid include accumulation of aggregates, inflammatory cascade, neuronal oxidative damage, tau protein alterations, neurofibrillary tangle formation, synaptic defects, and neurotransmitter depletion [65]. Many of these events are common to a number of neurodegenerative diseases, such as ALS. Amyloid accumulation may not only be the cause, but also the consequence of some of those diseases. This study included patients in a non-end-stage of the disease, thus providing information about the amyloid deposition in early phases of the disease.

In our study, the percentage of increase in amyloid deposits is lower than those reported by series of histological studies, suggesting that amyloid accumulates at a late stage of the disease. In addition, amyloid deposits in ALS and AD are located in different areas, and there are no metabolic alterations in the areas linked to AD; these findings seem to indicate that these ALS cases are not preclinical AD. This observation, in turn, confirms that amyloid-PET is a useful tool for differential diagnosis of AD and other neurodegenerative diseases, especially when used to analyse the areas most affected by AD (posterior cingulate, precuneus, etc.) [52]. Likewise, the fact that a subgroup of patients displayed amyloid deposits at relatively early stages of the disease seems to indicate a connection between amyloid and ALS. Similarly, amyloid deposits are not very likely to be related to age, considering that 72.22 % of our patients were younger than 65, and that the patients displaying increased  $^{18}\text{F}$ -florbetaben uptake were 47, 54, and 68 years old. In population studies of healthy subjects evaluated with PiB-PET, the frequency of amyloid deposits in this age range is much lower and deposition is limited to the regions most linked to AD [64].

On the other hand, although amyloid tracer findings have been validated by several autopsy studies demonstrating the correlation between increased uptake and increased amyloid deposition, they may be less specific than expected. For

example, amyloid tracers show non-specific uptake by normal white matter [66] and may bind to plaques or fibrils produced by other non-amyloid aggregates involved in neurodegenerative diseases [67].

Our study has a few limitations. Because its design called for thorough cognitive assessment and neuroimaging studies, we mainly included patients in relatively early clinical stages. Likewise, its cross-sectional design did not allow us to draw conclusions as to whether the metabolic patterns described or the subgroup of patients showing increased  $^{18}\text{F}$ -florbetaben uptake might be associated with different outcomes. Another limitation of our study concerns the analysis of PET images. We used two regions of reference for amyloid-PET images in order to avoid the effect caused by using only one region. It would be interesting to conduct longitudinal studies or even obtain histological confirmation in terminal stages in order to compare PET findings with the results from patient follow-up. We scaled  $^{18}\text{F}$ -FDG PET images to the global mean, since some regions (cerebellum, pons, etc., used in other studies on neurodegenerative diseases such as AD and FTD) may show metabolic alterations in patients with ALS because they participate in motor control. Given that most of our patients were in early stages, we feel that scaling to the mean should not have a significant impact on the main results [68].

In conclusion, our study reports findings from a cohort of patients assessed using  $^{18}\text{F}$ -FDG and  $^{18}\text{F}$ -florbetaben. Patients with ALS showed, in comparison to healthy controls, a lower metabolism, mainly in frontal lobe, anterior cingulate and parietal lobe, and higher metabolism in cerebellum. Using single-subject analysis, we have identified two main patterns of brain metabolism: one with predominantly anterior hypometabolism (especially in the superior, middle, and inferior frontal gyri), which is more often associated with cognitive impairment, and a second with more posterior hypometabolism (precentral and postcentral gyri, precuneus, parietal lobules). Only a low percentage of patients displayed abnormal tracer uptake in amyloid-PET images, and this finding did not seem to correlate with presence of AD. These results suggest that ALS presents heterogeneous clinical, metabolic, and molecular characteristics. While FDG-PET and amyloid-PET may help identify disease subgroups, further studies should analyze the connection between these findings and the prognosis and progression of patients with ALS.

#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflicts of interest.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments. Informed consent was obtained from all individual participants included in the study.

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