Increased in vivo glial activation in patients with amyotrophic lateral sclerosis: Assessed with \([^{11}C]-PBR28\)

Nicole R. Zürcher\textsuperscript{a}, Marco L. Loggia\textsuperscript{a}, Robert Lawson\textsuperscript{b}, Daniel B. Chonde\textsuperscript{a}, David Izquierdo-Garcia\textsuperscript{a}, Julia E. Yasek\textsuperscript{b}, Oluwaseun Akeju\textsuperscript{c}, Ciprian Catana\textsuperscript{a}, Bruce R. Rosen\textsuperscript{a}, Merit E. Cudkowicz\textsuperscript{b}, Jacob M. Hooker\textsuperscript{**,**,A, Nazem Atassi*\textsuperscript{b}}

\textsuperscript{a}A. A. Martinos Center for Biomedical Imaging, Department of Radiology, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA, USA
\textsuperscript{b}Neurological Clinical Research Institute (NCRI), Department of Neurology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA
\textsuperscript{c}Department of Anesthesiology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder in which upper and lower motor neurons degenerate leading to progressive muscle weakness, respiratory failure and death often within 2–5 years (Ferraiuolo et al., 2011). Riluzole, the only FDA-approved treatment provides a modest survival benefit (Lacomblez et al., 1996), but there are no available treatments that prevent or stop disease progression and current animal models have not yet successfully predicted treatment response in people (Atassi et al., 2012; Ferraiuolo et al., 2011). A substantial body of evidence implicates the neuroimmune system and specifically activated microglia in ALS pathophysiology (Appel et al., 2011). In post mortem studies increased activated microglia are correlated with increased upper motor neuron symptoms and faster disease progression (Brettschneider et al., 2012). Despite years of research, the fundamental question of whether the immune response observed in ALS is primary or secondary, beneficial or harmful, or a combination of both, has not yet been clearly answered.

Given the disconnection between mouse models and human disease, it is critical to develop methods to examine disease biology in vivo in patients with ALS. With positron emission tomography (PET) a radiotracer is used to visualize and quantify molecular interactions with high sensitivity. Several PET radiotracers have been developed to image activated microglia and most provide contrast by binding the 18 kDa translocator protein (TSPO), formerly known as the peripheral benzodiazepine receptor (PBR), which is highly increased in vivo inflammation in individuals with amyotrophic lateral sclerosis using \([^{11}C]-PBR28\) positron emission tomography. Ten patients with amyotrophic lateral sclerosis (seven males, three females, 38–68 years) and ten age- and \([^{11}C]-PBR28\) binding affinity-matched healthy volunteers (six males, four females, 33–65 years) completed a positron emission tomography scan. Standardized uptake values were calculated from 60 to 90 min post-injection and normalized to whole brain mean. Voxel-wise analysis showed increased binding in the motor cortices and corticospinal tracts in patients with amyotrophic lateral sclerosis compared to healthy controls \((p_{FWE} < 0.05)\). Region of interest analysis revealed increased \([^{11}C]-PBR28\) binding in the precentral gyrus in patients \((\text{normalized standardized uptake value} = 1.15)\) compared to controls \((1.03, p < 0.05)\). In patients those values were positively correlated with upper motor neuron burden scores \((r = 0.69, p < 0.05)\), and negatively correlated with the amyotrophic lateral sclerosis functional rating scale \((r = -0.66, p < 0.05)\).

Increased in vivo glial activation in motor cortices, that correlates with phenotype, complements previous histopathological reports. Further studies will determine the role of \([^{11}C]-PBR28\) as a marker of treatments that target inflammation.
expressed in activated microglia and astrocytes (Brown et al., 2007; Lavisse et al., 2012). The first application of TSPO PET imaging in patients with ALS confirmed widespread microglial activation (Turner et al., 2004). This pioneering study conducted with the radioligand \(^{11}C\)-(R)-PK11195 showed increased binding in the motor cortex, pons, dorsolateral prefrontal cortex and thalamus in a group of ALS patients. Older generation TSPO radioligands such as \(^{11}C\)-(R)-PK11195 suffered from high levels of non-specific binding and poor signal-to-background ratio (Kreisl et al., 2010). Increased TSPO expression, assessed using the radioligand \(^{18}F\)-DPA-714, was subsequently reported in the primary motor cortex, supplementary motor area as well as temporal cortex of patients with ALS, thereby providing additional support for a role for inflammatory processes in ALS (Corcia et al., 2012).

The radioligand \(^{11}C\)-PBR28, developed at the National Institute of Mental Health, was shown to exhibit 80 times more specific binding compared to \(^{11}C\)-(R)-PK11195 in rhesus macaques (Kreisl et al., 2010). The aim of this proof-of-concept study was to investigate \(^{11}C\)-PBR28 binding in a group of individuals with ALS compared to a matched group including for TSPO polymorphism of healthy controls and to investigate whether the \(^{11}C\)-PBR28 radiotracer could better subcategorize ALS patients based on the anatomical regions with the highest disease burden.

2. Materials and methods

The study was conducted at the Athinoula A. Martinos Center for Biomedical Imaging at Massachusetts General Hospital. The protocol was approved by the Institutional Review Board and the Radioactive Drug Research Committee. All participants provided written informed consent according to the Declaration of Helsinki.

2.1. Participants

Fourteen ALS patients were initially screened for the study. To meet inclusion criteria, participants had to fulfill the revised EL Escorial criteria (Brooks et al., 2000) for possible, probable, probable laboratory-supported or definite ALS, not have any signs of frontotemporal dementia and could not be taking any anti-inflammatory or immunosuppressant medications or benzodiazepines. None of the patients had a familial history of ALS.

\(^{11}C\)-PBR28, along with all second-generation TSPO radiotracers to date, has differential binding affinity to TSPO depending on an Ala147Thr polymorphism in the TSPO gene with Ala/Ala leading to high-,

To investigate whether \(^{11}C\)-PBR28 binding correlated with ALS disease severity, Spearman’s \(r\) was used to investigate for the presence with 0 representing no reflex involvement, and 45 maximal abnormal UMNB. VC measures respiratory functioning and is expressed as a percentage out of 100%, with 100 being normal and scores below 100 indicating decreased lung capacity. The 10 participants with ALS were compared to 10 healthy controls matched for age and TSPO binding affinity.

2.2. Radiotracer synthesis and data acquisition

\(^{11}C\)-PBR28 was produced in-house using a procedure modified from the literature (Imaizumi et al., 2007). Briefly, the desmethyl precursor (1.0 mg in 100 µL) was loaded into a 5 mL stainless steel loop for reaction with CH₃I using the Wilson captive solvent method (Wilson et al., 2000). \(^{11}C\)-PBR28 was purified by reversed-phase chromatography and reformulated by solid-phase extraction in 10% ethanol/saline and then aseptically filtered. The radioligand was injected as slow intravenous bolus, with a median administered dose of 419.49 MBq for patients with ALS and 419.08 MBq for controls. PET data were acquired over 90 min and stored in list-mode format.

Participants were scanned on a Siemens magnetic resonance (MR)/PET scanner consisting of a dedicated brain avalanche photodiode-based PET scanner operating in the bore of a 3 T whole-body MR scanner, and an 8-channel head coil was used. This combined MR/PET scanner allowed the simultaneous acquisition of MR and PET data (Catana et al., 2010). An anatomical scan, a multi-echo MP-RAGE (TR = 2530, TE 1–4 = 1.64, 3.5, 5.36, 7.22 ms, flip angle = 7°, voxel size = 1 mm isotropic) was acquired at the beginning of the scan.

2.3. Data analysis

After acquisition, PET images were reconstructed using the Ordinary Poisson Ordered Subset Expectation Maximization 3D algorithm from prompt coincidences, with corrections for normalization, dead time, isotope decay, photon attenuation and expected random and scatter coincidences. Attenuation correction maps were created using MR-based methods (Izquierdo-Garcia et al., 2014). SUV images were created for radioactivity in the field of view 60–90 min post-radioligand injection. To account for motion that may have occurred between MP-RAGE acquisition and the 60–90 min post-injection time point corresponding to the PET frame of interest, SUV_{60–90 min} was generated in a two-step procedure. First, a SUV_{60–90 min} image was created for each subject using an attenuation correction map computed from the native MP-RAGE. Subsequently, a new attenuation map was created based on the registration of the native MP-RAGE with the SUV_{60–90 min} image obtained in this first reconstruction using FreeSurfer’s spmregister. A final SUV_{60–90 min} was then reconstructed based on this new attenuation correction image well registered with the 60–90 min PET data. Individual SUV_{60–90 min} images were then registered to MNI (Montreal Neurological Institute) space, spatially smoothed (6 mm full width at half maximum), and intensity-normalized to a mean of 1 (SUV_{60–90 min}) in order to account for differences in global signal across subjects, as previously done for \(^{11}C\)-PBR28 (Loggia et al., 2015). SUV_{60–90 min} in MNI was then fed into a voxelwise between-group analysis. FSL’s randomize was used to perform a permutation-based nonparametric two-sample unequal-t test (n permutation = 10,000, 5 mm variance smoothing) with TSPO genotype added as a nuisance regressor. Threshold-free cluster enhancement (TFCE) was applied and p values were family-wise error rate (FWE) corrected \((p_{FWE} < 0.05)\) (Nichols and Holmes, 2002).

An a priori region of interest for the left and right precentral gyri was selected for each subject using FreeSurfer’s automated parcellation. Two-tailed Mann–Whitney was used to assess between-group differences. To investigate whether \(^{11}C\)-PBR28 binding correlated with ALS disease severity, Spearman’s \(r\) was used to investigate for the presence
of correlation between SUVR_{60-90 min} of the a priori precentral gyrus ROI and UMNB scores, ALSFRS-R, and disease duration.

3. Results

3.1. Study participants

Ten ALS patients (7 males, 3 females, 6 high- and 4 mixed-affinity binders), with a mean age of 53.2 years (SD = 10.8, range 38–68) successfully completed scanning and were compared to healthy controls matched for binding affinity and age (6 males, 4 females, 6 high- and 4 mixed-affinity). Healthy controls were on average 51.1 years old (SD = 11.0, range 33–65). See Table 1.

3.2. Individual data in patients with ALS and group means

Visual inspection of SUVR_{60-90 min} images revealed regional increase in the precentral gyrus in patients with ALS with limb-onset disease. See Fig. 1A for individual data projected onto the MNI template and Fig. 1B for means for the ALS group, including comparisons between limb- and bulbar-onset patients, and the control group. Patients with limb-onset weakness showed increased binding in the precentral gyrus and patients with bulbar-onset weakness showed increased binding in the brainstem (Fig. 1B, top two rows).

3.3. Whole brain between-group analysis

The unpaired t-test conducted on the whole brain static SUVR_{60-90 min} image showed that the ALS group has increased \[^{11}\text{C}\]-PBR28 binding in the bilateral motor cortices, including the primary motor cortex (M1) and supplementary motor area, as well as in the upper region of the corticospinal tract _priori_ image showed that the ALS group has increased binding in the bilateral motor cortices, including the primary motor cortex (M1) and supplementary motor area, as well as in the upper region of the corticospinal tract in patients with ALS. This finding is consistent with the first microglial PET study in ALS patients (Turner et al., 2004), as well as histopathological studies reporting increased activated microglia near degenerating motor neurons (Brettschneider et al., 2012; Henkel et al., 2004; Kawamata et al., 1992), suggesting that \[^{11}\text{C}\]-PBR28 PET is a robust candidate as an _in vivo_ biomarker of inflammation in ALS. This is the first study conducted in patients with ALS using a second-generation TSPO radioligand, while controlling for TSPO binding affinity genotype. In accordance with our findings, previous studies investigating TSPO binding reported increased binding in motor cortices (Corcia et al., 2012; Turner et al., 2004). Compared to previous tracers, \[^{11}\text{C}\]-PBR28 SUVR images provide higher contrast in regions of activated glia, which represents a considerable advantage when evaluating neuroinflammation in individual patients.

Apart from clinical signs, there are no reliable biomarkers of upper motor neuron dysfunction in ALS. \[^{11}\text{C}\]-PBR28 PET could represent a marker of upper motor neuron injury that can complement electromyography as an indicator of lower motor neuron injury. Notably, the individual scores from the UMNB scale and ALSFRS-R were correlated with \[^{11}\text{C}\]-PBR28 binding, suggesting a clinical relevance of brain

### Table 1

<table>
<thead>
<tr>
<th>Subject</th>
<th>TSPO genotype</th>
<th>Age</th>
<th>Sex</th>
<th>Site of onset</th>
<th>Disease duration (months)</th>
<th>VC</th>
<th>ALSFRS-R</th>
<th>UMNB</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALS-1</td>
<td>Ala/Ala</td>
<td>53</td>
<td>M</td>
<td>Left lower limb</td>
<td>26</td>
<td>77</td>
<td>100</td>
<td>32</td>
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<tr>
<td>ALS-2</td>
<td>Ala/Ala</td>
<td>48</td>
<td>M</td>
<td>Left upper limb</td>
<td>22</td>
<td>100</td>
<td>31</td>
<td>25</td>
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<tr>
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<td>Ala/Ala</td>
<td>38</td>
<td>F</td>
<td>Left upper limb</td>
<td>9</td>
<td>106</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>ALS-4</td>
<td>Ala/Thr</td>
<td>68</td>
<td>M</td>
<td>Right &amp; left lower limbs</td>
<td>21</td>
<td>66</td>
<td>41</td>
<td>21</td>
</tr>
<tr>
<td>ALS-5</td>
<td>Ala/Thr</td>
<td>55</td>
<td>M</td>
<td>Right upper limb</td>
<td>16</td>
<td>93</td>
<td>36</td>
<td>28</td>
</tr>
<tr>
<td>ALS-6</td>
<td>Ala/Thr</td>
<td>48</td>
<td>M</td>
<td>Right lower limb</td>
<td>11</td>
<td>89</td>
<td>42</td>
<td>34</td>
</tr>
<tr>
<td>ALS-7</td>
<td>Ala/Thr</td>
<td>39</td>
<td>M</td>
<td>Right upper limb</td>
<td>57</td>
<td>76</td>
<td>30</td>
<td>33</td>
</tr>
<tr>
<td>ALS-8</td>
<td>Ala/Ala</td>
<td>51</td>
<td>M</td>
<td>Bulbar</td>
<td>11</td>
<td>76</td>
<td>45</td>
<td>17</td>
</tr>
<tr>
<td>ALS-9</td>
<td>Ala/Ala</td>
<td>66</td>
<td>F</td>
<td>Bulbar</td>
<td>34</td>
<td>78</td>
<td>35</td>
<td>28</td>
</tr>
<tr>
<td>ALS-10</td>
<td>Ala/Ala</td>
<td>66</td>
<td>F</td>
<td>Bulbar</td>
<td>13</td>
<td>60</td>
<td>37</td>
<td>25</td>
</tr>
<tr>
<td>Mean for ALS</td>
<td>60% Ala/Ala</td>
<td>53.2 ± 10.8</td>
<td>70% M</td>
<td>70% Limb</td>
<td>22.0 ± 14.6</td>
<td>82.1 ± 14.6</td>
<td>36.4 ± 4.9</td>
<td>28.5 ± 6.8</td>
</tr>
<tr>
<td>CTRL-1</td>
<td>Ala/Ala</td>
<td>59</td>
<td>M</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
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</tr>
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<td>M</td>
<td>N/A</td>
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<td>N/A</td>
<td>N/A</td>
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<tr>
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<td>M</td>
<td>N/A</td>
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<td>N/A</td>
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<tr>
<td>CTRL-4</td>
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<td>F</td>
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<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>CTRL-5</td>
<td>Ala/Ala</td>
<td>58</td>
<td>F</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>CTRL-6</td>
<td>Ala/Ala</td>
<td>52</td>
<td>F</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>CTRL-7</td>
<td>Ala/Thr</td>
<td>60</td>
<td>M</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
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</tr>
<tr>
<td>CTRL-8</td>
<td>Ala/Thr</td>
<td>43</td>
<td>M</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>CTRL-9</td>
<td>Ala/Thr</td>
<td>34</td>
<td>M</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
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<td>CTRL-10</td>
<td>Ala/Thr</td>
<td>56</td>
<td>F</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Mean for CTRL</td>
<td>60% Ala/Ala</td>
<td>51.1 ± 11.0</td>
<td>60% M</td>
<td>N/A</td>
<td>82.1 ± 14.6</td>
<td>36.4 ± 4.9</td>
<td>28.5 ± 6.8</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Compared to controls, individuals with ALS exhibited significantly increased binding in the bilateral precentral gyri, a priori identified region of interest. ALS (median, range): 1.15, 1.05–1.30, controls: 1.03, 0.99–1.18, p < 0.05 (Fig. 2). In ALS patients, SUVR_{60-90 min} of the right precentral gyrus was positively correlated with UMNB scores, r = 0.69, p < 0.05 (Fig. 3A) and negatively correlated with functional status measured by ALSFRS-R, r = −0.66, p < 0.05 (Fig. 3B). Disease duration did not correlate with SUVR_{60-90 min} in the precentral gyrus.

An exploratory comparison of individuals with limb vs. bulbar onset ALS showed that individuals with limb onset (N = 7) had a significantly higher SUVR_{60-90 min} than individuals with ALS with bulbar onset (N = 3) in the precentral gyrus (p < 0.05).

4. Discussion

Our study demonstrates increased _in vivo_ \[^{11}\text{C}\]-PBR28 binding in the motor cortices and corticospinal tract in patients with ALS. This finding is consistent with the first microglial PET study in ALS patients (Turner et al., 2004), as well as histopathological studies reporting increased activated microglia near degenerating motor neurons (Brettschneider et al., 2012; Henkel et al., 2004; Kawamata et al., 1992), suggesting that \[^{11}\text{C}\]-PBR28 PET is a robust candidate as an _in vivo_ biomarker of inflammation in ALS. This is the first study conducted in patients with ALS using a second-generation TSPO radioligand, while controlling for TSPO binding affinity genotype. In accordance with our findings, previous studies investigating TSPO binding reported increases in motor cortices (Corcia et al., 2012; Turner et al., 2004). Compared to previous tracers, \[^{11}\text{C}\]-PBR28 SUVR images provide higher contrast in regions of activated glia, which represents a considerable advantage when evaluating neuroinflammation in individual patients.
inflammation measured in vivo. Specifically, the UMNB score was positively correlated with microglial PET tracer uptake in the motor cortex using $[^{11}C]$-PK11195 (Turner et al., 2004). Additionally, our exploratory analysis showed that individuals with bulbar-onset did not show the increased binding in the motor cortex to the extent that it was observed in patients with limb-onset ALS. On the other hand, individuals with bulbar-onset ALS showed regional increases in PBR28 uptake in the brainstem. This suggests that $[^{11}C]$-PBR28 binding has a strong anatomical relevance to ALS clinical phenotype (Fig. 1B). The increase in PBR28 uptake in the brainstem could represent inflammation around the lower motor neurons of the cranial nerves as all three bulbar-onset subjects had evidence of lower motor neuron dysfunction on examination. This emphasizes the importance of exploring PBR28 PET imaging in the spinal cord to better characterize this observation. On the other hand,
tions between [11C]-PBR28 binding in the primary motor cortex and ALS disease severity assessed using UMNB scores and SUVR60.

Increased binding in the motor cortex as shown by a positive correlation between UMNB and ALSFRS-R were observed. A. Patients with higher UMNB show increased PBR28 binding in the motor cortex compared to healthy controls, *p < 0.05.

The main limitation of our study is the relatively small sample size. However, both the two previously published PET studies in ALS using [11C]-PBR28 PET imaging may provide important contributions to the fundamental question of immune system involvement in ALS by allowing a mechanistic investigation of the role of activated microglia. The study of individuals who are pre-symptomatic, but at high risk of developing the disease, such as superoxide dismutase-1 (SOD1) or C9orf72 gene carriers, may also provide important insights.

In conclusion, [11C]-PBR28 PET imaging may provide important contributions to the fundamental question of immune system involvement in ALS by allowing a mechanistic investigation of the role of activated microglia. The study of individuals who are pre-symptomatic, but at high risk of developing the disease, such as superoxide dismutase-1 (SOD1) or C9orf72 gene carriers, may also provide important insights.

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