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Imaging of glia activation in people with primary lateral sclerosis

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ABSTRACT

Background: Glia activation is thought to contribute to neuronal damage in several neurodegenerative diseases based on preclinical and human post-mortem studies, but its role in primary lateral sclerosis (PLS) is unknown.

Objectives: To localize and measure glia activation in people with PLS compared to healthy controls (HC).

Methods: Ten participants with PLS and ten age-matched HCs underwent simultaneous magnetic resonance (MR) and proton emission tomography (PET). The radiotracer [11C]-PBR28 was used to obtain PET-based measures of 18 kDa translocator protein (TSPO) expression, a marker of activated glial cells. MR techniques included a structural sequence to measure cortical thickness and diffusion tensor imaging (DTI) to assess white matter integrity.

Results: PET data showed increased [11C]-PBR28 uptake in anatomically-relevant motor regions which co-localized with areas of regional gray matter atrophy and decreased subcortical fractional anisotropy.

Conclusions: This study supports a link between glia activation and neuronal degeneration in PLS, and suggests that these disease mechanisms can be measured in vivo in PLS. Future studies are needed to determine the longitudinal changes of these imaging measures and to clarify if MR-PET with [11C]-PBR28 can be used as a biomarker for drug development in the context of clinical trials for PLS.

1. Introduction

Glia activation has been implicated in the pathogenesis of several neurodegenerative diseases based on both pre-clinical and human post-mortem studies. For instance, glia activation has been detected in animal models and human CNS autopsy tissue in Alzheimer's disease (AD) (Yin et al., 2017; Griciuc et al., 2013; Kreisl et al., 2016), amyotrophic lateral sclerosis (ALS) (Alexianu et al., 2001; Brett Schneider et al., 2012), multiple sclerosis (MS) (Brett Schneider et al., 2012; Franciosi et al., 2012) and Huntington's disease (HD) (Franciosi et al., 2012; Sapp et al., 2001). Work in mouse models of ALS suggest that glia activation contributes to disease progression and neurotoxicity (Boilee et al., 2006; Beers et al., 2006; Zhao et al., 2004). ALS is a form of motor neuron disease (MND) where both the upper and lower motor neurons are progressively lost whereas primary lateral sclerosis (PLS) is an MND that predominantly affects the neurons that arise from the motor cortex (Upper Motor Neurons, UMNs) (Statland et al., 2015). The role of glia activation in PLS is unknown.

Glia activation can be tracked in vivo by using PET radiotracers such as [11C]-PBR28 that bind to the 18 kDa translocator protein (TSPO). [11C]-PBR28 uptake is low in healthy brain tissue, but is increased in the setting of microglia activation and reactive astrocytosis (Lavisse et al., 2015). Further, PET can be combined with magnetic resonance (MR) techniques to assess white matter integrity (diffusion tensor imaging or DTI) and neuronal loss (morphometric analyses). This type of multimodal neuroimaging can deliver a snapshot of complex disease mechanisms in vivo and may represent a novel tool to track disease severity, progression, and response to candidate treatments. Indeed, there is growing interest in multimodal neuroimaging across neurologic disease as shown by recent proof-of-concept studies suggesting that MR-
PET can be used to identify glia activation and its relationship to neuronal loss and structural abnormalities in AD, (Kreisl et al., 2013; Kreidl et al., 2013), ALS (Zurcher et al., 2015; Alshikho et al., 2016; Turner et al., 2004; Corcia et al., 2012), HD (Politis et al., 2011; Tai et al., 2007), and in populations with a history of concussion or moderate-to-severe traumatic brain injury (Coughlin et al., 2015; Coughlin et al., 2017; Ramlaokhansingh et al., 2011).

While the course of PLS is slower and the prognosis is more favorable than ALS, it still leads to progressive disability including difficulty walking, difficulty performing activities of daily living, dysarthria, and dysphagia. There are no available treatments that slow down PLS disease progression. Further, there are no animal models of PLS, no available biomarkers, and patients with PLS are usually excluded from ALS clinical trials because it is unclear if they share the same pathophysiology. Elucidating in vivo disease mechanisms is clearly an unmet need for this orphan disease. Here we employed MR-PET to test the hypothesis that people with PLS have increased glia activation in the motor regions, and that glia activation co-localizes and correlates with cortical thinning (suggestive of neuronal loss) and white matter dysfunction. The hypothesis is anchored on autopsy reports from PLS patients showing glia proliferation surrounding areas of motor neuron loss in the motor cortices (Beal and Richardson, 1981; Hudson et al., 1993; Pringle et al., 1992).

2. Materials and methods

2.1. Study participants

Ten individuals with PLS (7 men, 3 women; mean age [SD] in years, 62 [8.5]) and ten age-matched healthy controls (5 men, 5 women; mean age [SD] in years, 54 [11.3]) were included in the study. To meet inclusion criteria, PLS participants had to meet Pringle’s criteria for the diagnosis of PLS (Pringle et al., 1992). Diagnosis was confirmed at enrollment by a physician with sub-specialty training in Neuromuscular Medicine and expertise in PLS. Since the Ala147Thr polymorphism in the TSPO gene imparts a trimodal pattern of binding affinity to second generation TSPO ligands such as [11C]-PBR28 (Owen et al., 2012), the participants in both groups were genotyped for this polymorphism at screening in order to exclude low affinity binders (Thr/Thr), and to match the proportion of high- (Ala/Ala) and mixed-affinity binders (Ala/Thr) across groups (4 and 6, respectively in both groups). The study was approved by the Partners Human Research Committee and the Radioactive Drug Research Committee. All participants provided written informed consent.

2.2. Clinical assessments

Clinical assessment included the revised ALS functional rating scale (ALSFRS-R) (Cedarbaum et al., 1999) and the upper motor neuron burden scale (UMNB) (Zurcher et al., 2015). The ALSFRS-R is a 12-question ordinal rating scale (ratings 0–4) that is widely used to measure functional status in patients with motor neuron disease. The scale ranges from 48 (normal level of functioning) to zero, with lower scores indicating increased disability. The scale evaluates function in four domains: gross and fine motor function, bulbar function, and breathing. Vital capacity was assessed using a portable spirometer and expressed as percent of predicted for age, gender and height. Demographic information and clinical assessments are summarized in Table 1.

2.3. MR-PET imaging

All participants had simultaneous MR-PET imaging using the radiotracer [11C]-PBR28. [11C]-PBR28 was produced in-house as previously described (Imazumii et al., 2007). The radiotracer was administered as a slow intravenous bolus. Mean [SD] administered dose of [11C]-PBR28 was 495 MBq (66.47) for PLS participants and 450 MBq

### Table 1

Demographic and clinical characteristics of study participants.

|             | PLS          | HC          |
|-------------|--------------|-------------|
| Number of participants | 10           | 10          |
| Male/female | 7/3          | 5/5         |
| Age (years)  | 44.3–70.3    | 31.7–65.3   |
| Age (years, mean ± SD) | 61.8 ± 8.54 | 54.03 ± 11.3 |
| TSPO genotype | Ala/Ala (high affinity binding) | 4/10       |
|             | Ala/Thr (mixed affinity binding) | 6/10       |
| Disease duration (months) | 140.1 ± 56.3 |
| Range of disease duration (months) | 62.3–216.6 |
| Limb onset  | 8/10         |             |
| ALSFRS-R (mean ± SD) | 31.9 ± 7.06 |
| UMNB (mean ± SD) | 31.7 ± 3.13 |
| Vital capacity (mean ± SD) | 83.1% ± 16.06 |

Abbreviations: Ala = alanine; ALSFRS-R = amyotrophic lateral sclerosis functional rating scale revised; HC = healthy control; PLS = primary lateral sclerosis; SD = standard deviation; Thr = threonine; TSPO = translocator protein; UMNB = upper motor neuron burden scale. Vital capacity is expressed as percent of predicted for age, sex and height.

[53] for HC, not significant.

MR-PET images were acquired simultaneously from all participants using a Siemens 3T Magnetom Tim Trio scanner (Siemens Erlangen, Germany) with PET insert. This scanner is equipped with an 8-channel head coil. PET data was acquired for 90 min after radio-tracer injection. MR data included T1-weighted 3D multi-echo magnetization prepared rapid acquisition gradient echo (MEMPRAGE) and diffusion weighted imaging (DWI). The diffusion data was obtained at b = 3000 s/mm² using a single-shot, spin-echo and echo-planar imaging (EPI) sequence with twice refocused spin echo diffusion preparation (Q-ball imaging). Each set of diffusion data included sixty diffusion images and eight non-collinear directions. Acquisition parameters for MR are available online (eTable 1 in the Supplement).

2.4. MR-PET data analyses

FreeSurfer version 6.0 tools were used for T1 image pre-processing including reconstruction, parcellation of cortical surfaces, and segmentation of sub-cortical regions. FreeSurfer was used to perform surface-based analyses (SBA). The FMRIB software library (FSL, v.5.0.9; Oxford, UK) was used to create diffusion maps for fractional anisotropy (FA) and diffusivities (mean MD, axial AD, and radial RD). Preprocessing steps of diffusion data including motion evaluation were conducted as previously reported (Alshikho et al., 2016). [11C]-PBR28 PET data was reconstructed as previously described (Zurcher et al., 2015). Standardized uptake value (SUV) normalized to whole brain mean (SUVr) from 60 to 90 min post time of injection were used to assess TSPO expression (SUVR60–90 min). Finally, the individual SUVR60–90 min images were registered to the standard MNI template (Montreal Neurological Institute MNI152) to conduct between-group analyses.

2.5. Statistical analyses

2.5.1. Voxel-wise SUVR analysis

SUVR60–90 min images in MNI152 standard space were fed into a voxel-wise between-groups analysis. Non-parametric permutation inference was performed using 5000 permutations and threshold-free cluster enhancement (TFCE). The resultant statistical maps were family-wise error (FWE) adjusted (pFWE < 0.05) to correct for multiple comparisons. General linear model (GLM) was used in the voxel-wise analyses and covariates were added to adjust for the effect of age, sex and [11C]-PBR28 binding affinity.
2.5.2. Surface-based analyses (SBAs)

SBAs were employed to investigate the difference between groups in cortical thickness and $[^{11}C]$-PBR28 uptake. The analyses were conducted in the pial surface (which follows the border between gray matter and cerebrospinal fluid), as well as in sub-cortical white matter surface (which follows the border between white and gray matter). For SBA analysis of cortical thickness, age and sex were entered as covariates while for SBA of PET data, age, sex and $[^{11}C]$-PBR28 binding affinity were entered as covariates.

Cortical thickness processed in FreeSurfer version 6.0 and smoothed at full width at half maximum (FWHM) = 6 mm was then fed into SBA. The resultant statistical maps were cluster-wise corrected for multiple comparisons using Monte Carlo simulation.

SBA of SUVR was performed using PET Surfer, a set of tools within FreeSurfer version 6.0 that include correction of partial volume effects. The resultant SUVR images resampled onto brain surface were then smoothed at (FWHM = 6 mm) and fed into a GLM analysis to study on surface the difference in $[^{11}C]$-PBR28 uptake between groups. A projection factor (projfrac) was used in SBA to project the SUVR maps onto the brain surface. The default value for projfrac is between (0 and 1). Zero represents the border line between white and pial. The negative values of the projfrac (e.g. projfrac = −2) means (2 × thickness) from the white surface into the white matter. To define which layer of the brain has the maximum $[^{11}C]$-PBR28 uptake difference between groups, multiple values of projfrac (+ 0.5, 0, −0.5, −1, −1.5, −2) were tested in a separate SBA analysis. The resultant SUVR images, which were resampled onto the brain surface, were then organized by group, concatenated together, smoothed at (FWHM = 6 mm), and fed into a GLM analysis to study the difference in $[^{11}C]$-PBR28 uptake between groups on the surface. Monte Carlo simulation was used to correct for multiple comparisons.

2.5.3. Tract-based spatial statistics (TBSS) analysis

We performed statistical analysis to study differences in FA values between PLS and controls. In this analysis, FA maps were fed into non-parametric permutation inference. Data were permuted (n = 5000), threshold free cluster enhancement (TFCE) method was applied, and the p values were FWE adjusted at p = 0.05 to correct for multiple comparisons. The anatomical locations were determined by using the JHU White-Matter Tractography and JHU ICBM-DTI-81 White-Matter Labels atlas (Wakana et al., 2007; Hua et al., 2008). GLM was used in TBSS to adjust FA values for the effect of age.

2.5.4. Region of Interest (ROI) analysis

The a priori ROI was defined to encompass the precentral and paracentral gyri bilaterally (motor region) based on FreeSurfer cortical reconstruction of the standard template MN152. MR-PET measures ($[^{11}C]$-PBR28 SUVR$_{60-90}$ min, FA, MD, AD, and RD) were then computed within this ROI and corrected by age. Statistical analyses were performed using JMP pro 13.0.0 (SAS Institute Inc., Cary, NC, 1989–2014). Between-groups differences were tested using non-parametric Wilcoxon test. Spearman correlation coefficient was used to investigate the relationship between $[^{11}C]$-PBR28 uptake and FA within the ROI and clinical measures (ALSFRS-R, UMBB, vital capacity, and disease duration). Effect size (Cohen d and 95% confidence interval (CI)) was calculated based on differences between groups.

3. Results

3.1. Mean group $[^{11}C]$-PBR28 uptake and voxel-wise analysis for between-group differences

Whole brain voxel-wise analysis showed increased $[^{11}C]$-PBR28 uptake in the motor regions in individuals with PLS compared to healthy controls in both hemispheres (Fig. 1 A,B,C,E). There were no regions for which PLS showed less $[^{11}C]$-PBR28 uptake than healthy controls. There were no significant right-to-left differences in clinical presentation as measured by UMN Burden scores in this cohort.

3.2. Tract-based spatial statistics (TBSS) analysis

TBSS analysis revealed decreased FA in PLS compared to controls, even after adjusting for age (Fig. 1D), in the left and right corticospinal tracts, left and right superior longitudinal fasciculus and in the body of the corpus callosum (all $p_{\text{FWE}} < 0.05$).

3.3. Surface-based (SBA) analyses

SBA of cortical thickness revealed cortical thinning in PLS compared to HC in the precentral gyri ($p < 0.01$) (Fig. 2A) which colocalized with regions of increased $[^{11}C]$-PBR28 uptake in the subcortical white matter of the motor regions (Fig. 2B).

3.4. Region of interest (ROI) analyses

The anatomical boundaries of the ROI used in this study are shown in Fig. 3A and include the precentral and paracentral gyri bilaterally. In this region, mean $[^{11}C]$-PBR28 uptake and radial diffusivity were significantly higher in PLS than HC ($p < 0.05$) while FA values were significantly lower ($p < 0.05$) (Fig. 3B).

Table 2 includes differences in RD, AD and MD between groups.

$[^{11}C]$-PBR28 uptake in the brains of people with PLS showed no significant correlations with FA ($r = +0.17; p = 0.62$), cortical thickness ($r = −0.38; p = 0.28$), ALSFRS-R scores ($r = +0.33; p = 0.35$), UMBB scores ($r = +0.26; p = 0.46$), vital capacity ($r = +0.26; p = 0.47$), or disease duration ($r = −0.52; p = 0.12$). FA significantly correlated with ALSFRS-R total score ($r = +0.85; p = 0.002$) but not with UMBB ($r = −0.04; p = 0.89$), vital capacity ($r = +0.37; p = 0.29$), or disease duration ($r = −0.32; p = 0.36$).

4. Discussion

Our study demonstrates increased in vivo glia activation in the motor regions in people with PLS, as measured by $[^{11}C]$-PBR28 PET imaging. $[^{11}C]$-PBR28 binds to TSPO, whose expression is dramatically upregulated in activated glial cells including microglia and astrocytes (Lavisse et al., 2015; Rupprecht et al., 2010). This report represents the first step in the development of $[^{11}C]$-PBR28 PET as an in vivo PLS biomarker, an approach that is aligned with efforts that are unfolding in other neurologic diseases (Coughlin et al., 2017; Brier et al., 2016; Passamonti et al., 2017; Gomperts et al., 2016; Herranz et al., 2016). The translational potential of these findings is that $[^{11}C]$-PBR28 PET signal could be further developed as a biomarker to support proof-of-molecule trials of compounds that target glia activation in PLS.

Our findings are consistent with autopsy reports from PLS patients showing glia proliferation surrounding areas of motor neuron loss in the motor cortices (Beal and Richardson, 1981; Hudson et al., 1993; Pringle et al., 1992). Increased glia activation overlapped with areas of cortical thinning and white matter abnormalities. Thus, MR-PET can be used to simultaneously capture in vivo molecular and structural changes that underlie PLS disease biology. Cortical thinning and white matter abnormalities have been reported previously in PLS (Iwata et al., 2011; Kwan et al., 2012; Canu et al., 2013; Butman and Floeter, 2007; Tartaglia et al., 2009). Here, we confirm these findings and show colocalization with a molecular marker of glia activation.

There is growing evidence that glia activation and phenotypic changes occur during disease progression of motor neuron disease and that microglia exert both neuroprotective and neurotoxic effects at various stages of the disease (Boillee et al., 2006; Beers et al., 2006; Zhao et al., 2004; Appel et al., 2011; Liao et al., 2012). Other glial cells, such as reactive astrocytes, are also proliferated in motor neuron disease (Vargas and Johnson, 2010). While these glia changes denote the
presence of neuroinflammation in the brain, it remains debated whether neuroinflammation drives the initial pathogenesis (Boillee et al., 2006) or occurs as a consequence of neurodegeneration, or both, in motor neuron diseases in general and PLS in particular. \([^{11}C]\)-PBR28 is a radiotracer that can be used to visualize and measure neuroinflammation. However, \([^{11}C]\)-PBR28 is not specific for microglia or their phenotypes. Thus, development of PET radioligands that are specific for microglia phenotypes is an unmet need and would greatly enhance the impact of this type of studies on our understanding of disease pathophysiology.

This study has additional limitations including the relatively small sample size, though previously published PET studies in other neurodegenerative diseases have shown disease-related changes with comparable sample sizes (Kreisl et al., 2016; Kreisl et al., 2013; Zürcher et al., 2015; Alshikho et al., 2016; Turner et al., 2004; Corcia et al., 2012; Politis et al., 2011; Tai et al., 2007; Coughlin et al., 2015; Coughlin et al., 2017). Given the small sample size, our findings should be considered as preliminary and warrant replication in larger cohorts. In addition, information about how glia activation evolves over time and whether these changes might be predictive of future disease progression is not available. Lastly, a potential limitation of the study is the use of SUVR to represent \([^{11}C]\)-PBR28 binding without performing arterial sampling for kinetic modeling. The approach used here has been used successfully in previous studies (Zürcher et al., 2015; Alshikho et al., 2016; Herranz et al., 2016; Loggia et al., 2015; Nair et al., 2016) and is strengthened by recent data showing good correlation between SUVR and DVR computed using radiometabolite-corrected arterial input function (Herranz et al., 2016).

The area of highest \([^{11}C]\)-PBR28 uptake in PLS was located in the subcortical white matter underlying the motor cortex and was therefore
partially outside the *a priori* ROI based on our previous ALS studies, where maximal $^{[11C]}$-PBR28 uptake was found in the cortex of the precentral and paracentral gyri (Zurcher et al., 2015; Alshikho et al., 2016). For this reason, the results of the ROI analyses may not describe the full extent of glia activation in PLS. Our study was instrumental in localizing the area of highest $^{[11C]}$-PBR28 uptake in PLS and will guide future projects to more accurately measure the extent of $^{[11C]}$-PBR28 uptake in PLS. $^{[11C]}$-PBR28 is a molecular marker of neuro-inflammation while FA is measure of white matter integrity and the two may serve different roles as PLS biomarkers. Thus, $^{[11C]}$-PBR28 may represent a dynamic measure of target engagement of compounds that affect neuroinflammatory pathways. Larger, longitudinal studies are urgently needed to establish whether $^{[11C]}$-PBR28 may be used as a pharmacodynamic biomarker in the context of PLS clinical trials.

Contrary to our results in ALS (Zurcher et al., 2015; Alshikho et al., 2016), we did not observe a correlation between $^{[11C]}$-PBR28 uptake and available clinical outcomes such as UMN and the ALSFRS-R. The reasons for these findings are unclear and may relate to a ceiling effect of the UMN when used to assess function in PLS. UMN is measured by grading deep tendon reflexes and may have an insufficient dynamic range in PLS where reflexes are broadly elevated. The ALSFRS-R was developed and validated to measure function in ALS (Cedarbaum et al., 1999), where disability is driven not only by upper but also by lower motor neuron dysfunction. Preserved lower motor neuron function in PLS may underlie the lack of correlation between MR-PET measures and ALSFRS-R scores. Of note, no PLS-specific functional rating scale is available at the present time. Alternatively, these findings may reflect a different relationship between glia activation and neurodegeneration in PLS when compared to ALS. It should be noted that due to established PLS diagnostic criteria that require to wait a few years before diagnosing PLS (3 to 4 years depending on whether Pringle (Pringle et al., 1992) or Gordon (Gordon et al., 2006) criteria are used), our cohort comprised individuals whose mean disease duration was about 11 years, well beyond the 2-year disease duration of participants in our previous ALS study (Zurcher et al., 2015; Alshikho et al., 2016). Thus, the lack of an association between imaging metrics and clinical measures may be due to the late observation time, when clinical changes may no longer be associated with some of the mechanisms we investigated with imaging. Future studies are needed to determine the time course of $^{[11C]}$-PBR28 uptake early after onset of UMN-predominant disease.

In conclusion, we demonstrated that multimodal MR-PET can localize and quantify glia activation *in vivo* in patients with PLS. We propose that this approach may inform clinical drug development by providing a platform to screen candidate therapeutics that target glia activation. Large, multi-center, longitudinal studies are needed to validate MR-PET with $^{[11C]}$-PBR28 as a biomarker for PLS.

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**Author contributions**

Dr. Paganoni had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Dr. Paganoni, Dr. Zürcher, Dr. Loggia, Dr. Rosen, Dr. Cudkowicz, Dr. Hooker, Dr. Atassi.

Acquisition, analysis, or interpretation of data: All authors.

Drafting of the manuscript: Dr. Paganoni.

Critical revision of the manuscript for important intellectual content: All authors.

Statistical analysis: James Chan.
Obtained funding: Dr. Atassi, Dr. Paganoni.
Administrative, technical, or material support: Dr. Zürcher, Dr. Loggia.
Study supervision: Dr. Paganoni, Dr. Zürcher, Dr. Loggia, Dr. Rosen, Dr. Cudkowicz, Dr. Hooker, Dr. Atassi.

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Fig. 3. Region of Interest (ROI) analyses.
Panel A: Representation of the ROI overlaid onto MNI152-1 mm standard space, and shown at MNI coordinates x = −10, y = −20, and z = +55. The ROI includes the bilateral precentral and paracentral gyri. Contribution from gray and subcortical white matter to the ROI is indicated in maroon and purple, respectively.
Panel B: Box plots showing [11C]-PBR28 uptake, FA and RD values in PLS (orange circles) and HC (blue circles) within the ROI. The horizontal white line in each box plot represents the median (the box contains median, 25th, and 75th percentiles). The asterisk denotes significant group differences, at p < 0.05. The scale of RD values is divided by 1000. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
ROI analysis: between group comparisons of MR-PET measures.

| Measures             | HC       | PLS      | p value (Wilcoxon) | Cohen d (95% CI) |
|----------------------|----------|----------|--------------------|-----------------|
| [11C]-PBR28 SUVR60−90 min | 0.97 (0.03) | 1.07 (0.07) | 0.0013a            | 1.86 (0.98 to 1.05) |
| FA                   | 0.22 (0.04) | 0.15 (0.03) | 0.0097a            | 1.94 (0.16 to 0.20) |
| RD                   | 0.55 (0.1)  | 0.67 (0.08) | 0.035a             | 1.32 (0.56 to 0.68) |
| MD                   | 0.62 (0.14) | 0.72 (0.08) | 0.18               | 0.87 (0.62 to 0.74) |
| AD                   | 0.76 (0.18) | 0.82 (0.09) | 0.73               | 0.42 (0.73 to 0.86) |

Values are presented as means (standard deviation).
Diffusivity values (RD, MD, AD) were adjusted to 0 decimals (multiplied by 1000).
Abbreviations: AD: axial diffusivity; CI: confidence interval; FA: fractional anisotropy; MD: mean diffusivity; RD: radial diffusivity; SUVR: standardized uptake value ratio.

a Significant difference between the groups.
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