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Gliial activation colocalizes with structural abnormalities in amyotrophic lateral sclerosis

ABSTRACT
Objective: In this cross-sectional study, we aimed to evaluate brain structural abnormalities in relation to gliial activation in the same cohort of participants.

Methods: Ten individuals with amyotrophic lateral sclerosis (ALS) and 10 matched healthy controls underwent brain imaging using integrated MR/PET and the radioligand [11C]-PBR28. Diagnosis history and clinical assessments including Upper Motor Neuron Burden Scale (UMNB) were obtained from patients with ALS. Diffusion tensor imaging (DTI) analyses including tract-based spatial statistics and tractography were applied. DTI metrics including fractional anisotropy (FA) and diffusivities (mean, axial, and radial) were measured in regions of interest. Cortical thickness was assessed using surface-based analysis. The locations of structural changes, measured by DTI and the areas of cortical thinning, were compared to regional gliial activation measured by relative [11C]-PBR28 uptake.

Results: In this cohort of individuals with ALS, reduced FA and cortical thinning colocalized with regions demonstrating higher radioligand binding. [11C]-PBR28 binding in the left motor cortex was correlated with FA (r = −0.68, p < 0.05) and cortical thickness (r = −0.75, p < 0.05). UMNB was correlated with gliial activation (r = +0.75, p < 0.05), FA (r = −0.77, p < 0.05), and cortical thickness (r = −0.75, p < 0.05) in the motor cortex.

Conclusions: Increased uptake of the glial marker [11C]-PBR28 colocalizes with changes in FA and cortical thinning. This suggests a link between disease mechanisms (gliosis and inflammation) and structural changes (cortical thinning and white and gray matter changes). In this multimodal neuroimaging work, we provide an in vivo model to investigate the pathogenesis of ALS.

GLOSSARY
AD = axial diffusivity; ALS = amyotrophic lateral sclerosis; ALSFRS-R = ALS Functional Rating Scale-Revised; CST = corticospinal tract; DTI = diffusion tensor imaging; FA = fractional anisotropy; FDR = false discovery rate; FSL = FMRIB’s linear image registration tool; FWE = family-wise error; HC = healthy controls; MD = mean diffusivity; MR = magnetic resonance; MRSI = magnetic resonance spectroscopic imaging; PCG = precentral gyrus; RD = radial diffusivity; RDP = rate of disease progression; ROI = region of interest; SUV = standardized uptake value; SUVR = standardized uptake value ratio; TBSS = tract-based spatial statistical analysis; TSPO = translocator protein; UMNB = Upper Motor Neuron Burden Scale.

The pathophysiology of amyotrophic lateral sclerosis (ALS) is poorly understood, but the immune system has increasingly been implicated in the pathogenesis of the disease. Activated microglia are increased in postmortem brain and spinal cord tissue from people with ALS, and the number of activated microglia correlates with the rate of disease progression. A strong proinflammatory signature in ALS is found from gene expression profiling of postmortem spinal cord tissue. In addition, proinflammatory cytokines are increased in CSF. Studies in the widely used SOD1*G93A transgenic ALS mouse model showed activated microglia near the motor

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neurons before the onset of weakness and the inflammatory response in these mice correlates with disease progression.5

[11C]-PBR28 is a PET radiotracer that selectively binds to the translocator protein (TSPO). TSPO is upregulated in activated microglia and reactive astrocytes.6,7 We previously reported increased glial activation measured by [11C]-PBR28 uptake in the motor cortices in people with ALS.8

We hypothesize that glial activation measured by [11C]-PBR28 binding is anatomically correlated with microstructural tissue damage including subcortical white matter changes and cortical thinning. Our hypothesis is anchored on ALS postmortem and mouse model pathology showing active microglia and astroglisosis surrounding degenerating motor neurons.5,9

This study demonstrates the value of simultaneous MR/PET in vivo imaging techniques in measuring structural and molecular changes in patients with ALS compared to healthy controls (HC).

METHODS Standard protocol approvals, registrations, and patient consents. This study was approved by the local institutional review board and the Radioactive Drug Research Committee. All participants provided written informed consent for study participation.

Study participants. In this study, we investigated a cohort of previously reported participants.5 The participants are 10 individuals diagnosed with ALS and 10 HC matching in age, sex, and [11C]-PBR28 binding affinity. Demographic information and clinical assessment are summarized in table 1. [11C]-PBR28 binding affinity studies using postmortem human brain tissue revealed substantial variation (low, mixed, high) in binding affinity of [11C]-PBR28 for TSPO.6 This variation is due to a single nucleotide polymorphism (rs6971) in the gene encoding TSPO, which causes a substitution of threonine and alanine at position 147 (Ala147Thr). All participants were tested for this polymorphism and groups were matched for the number of medium and high affinity binders.

Participants with ALS were diagnosed with possible, probable laboratory-supported, or definite ALS, according to modified El Escorial criteria.11 None of the ALS participants had clinical signs of frontotemporal dementia and none of the study participants were taking benzodiazepines, immunosuppressive, or anti-inflammatory medications.

ALS evaluation. The Upper Motor Neuron Burden Scale (UMNB) was obtained from all participants with ALS. This scale measures the following deep tendon reflexes: biceps, brachioradialis, triceps, knee jerk, ankle jerk (scores 0–4); and pathologic reflexes: Hoffmann, Babinski, and jaw jerk (present, 1; or absent, 0). All reflexes are assessed bilaterally except for jaw jerk, producing a scale ranging between 0 and 45. Higher scores indicate a greater extent of upper motor neuron dysfunction. The Revised ALS Functional Rating Scale (ALSFRS-R) was administered to all participants with ALS.12 ALSFRS-R is a quantitative measure of ALS-related physical and functional deficits and ranges from 0 to 48, with lower scores representing more disability.

[11C]-PBR28 radiotracer administration. [11C]-PBR28 was produced in-house as described previously.9 The radioligand was injected as slow IV bolus, with a mean administered dose of 430 MBq ± 34 for participants with ALS and 424 MBq ± 42 for HC.

MR/PET data acquisition. Imaging data were collected at the Martinos Center for Biomedical Imaging at the Massachusetts General Hospital, using a Siemens 3T Magnetom Tim Trio scanner (Siemens, Erlangen, Germany). This magnetic resonance (MR) scanner is equipped with an 8-channel head coil and a dedicated brain avalanche photodiode-based PET scanner operating in the bore of the 3T whole-body MR scanner. MRI scans included diffusion-weighted imaging and a T1-weighted 3D multiecho magnetization-prepared rapid acquisition gradient echo. The acquisition parameters are listed in table e-1 at Neurology.org. A multislice single-shot echo planar spin echo pulse sequence was employed to obtain measurements at a diffusion weighting of b = 3,000 s/mm2 and the diffusion-encoding directions were distributed uniformly over the surface of a sphere using electrostatic repulsion. Images were acquired using a single-shot, spin echo, and echo planar imaging sequence with twice refocused spin echo diffusion preparation (Q-ball imaging). Each series included 60 images acquired with diffusion weighting and 8 noncollinear directions. PET data and structural scans were acquired simultaneously for all 20 participants and diffusion data were acquired for all but 4 HC participants due to scanning time limitations.

MR/PET data preprocessing. PET images were reconstructed as previously described.9 The images of standardized uptake value (SUV) were created for radioactivity in the field of view (60–90 minutes post radioligand injection. These images were normalized to the whole brain mean (SUV ratio [SUVR]) 60–90 minutes as previously reported.13,14 Preprocessing of raw diffusion data was performed using FMRIB software library (FSL; Oxford, UK) version 5.0.7.15 The following measures were applied to determine whether diffusion tensor imaging (DTI) scans acquired were of sufficient quality15: percentage of slices with signal dropout score and motion parameters as well as signal to noise ratio evaluation for every scan. Cortical and subcortical segmentation of the T1 images was carried out in Freesurfer version 5.3.

MR/PET data analyses. Diffusion analyses and correlation with [11C]-PBR28 SUVR. Tract-based spatial statistical analysis (TBSS). TBSS, part of FSL,16 was applied to study white matter changes in ALS. The fractional anisotropy (FA) and non-FA maps (mean diffusivity [MD], axial diffusivity [AD], and radial diffusivity [RD]) were fed consecutively into the TBSS processing pipeline.17 Voxelwise analyses were performed by FSL randomise12 using threshold-free cluster enhancement16 and 5,000 permutations. The resultant statistical maps were family-wise error (FWE) adjusted and thresholded to pFWE < 0.05. The anatomical locations were determined by using the JHU White-Matter Tractography Atlas.18 In addition, age was included as a covariate in these analyses, to control for the potential confounding effect of this factor.

Precentral gyrus (PCG) region of interest (ROI) analyses. The predetermined ROIs for the left and right PCG for both the gray matter and the underlying white matter were derived from the automatic T1 segmentation in Freesurfer.20 Those ROIs were used to estimate FA, diffusivities, and [11C]-PBR28 SUVR. All
Table 1  Demographic data, amyotrophic lateral sclerosis (ALS) history, and clinical assessment

| Characteristics                  | ALS          | Healthy controls |
|----------------------------------|--------------|------------------|
| Demographics                     |              |                  |
| No. participants                 | 10           | 10               |
| Male/female                      | 7/3          | 6/4              |
| Age, y, mean ± SD                | 38±68        | 33±65            |
| Age, y, mean ± SD                | 53.2±10.75   | 51.1±11.01       |
| Handedness (left/right/unknown)  | 1/9          | 1/8/1            |
| PBR genotype                     |              |                  |
| ALA/ALA (high)                   | 6/10         | 6/10             |
| ALA/THR (mixed)                  | 4/10         | 4/10             |
| ALS history and clinical assessment |          |                  |
| Disease duration, mo, mean ± SD  | 22±14.5      |                  |
| Site of ALS onset                |              |                  |
| Limb                             | 7/10         |                  |
| Bulbar                           | 3/10         |                  |
| ALSFRS-R, mean ± SD              | 36.4±4.9     |                  |
| UMNB, mean ± SD                  | 28.5±6.3     |                  |
| Vital capacity, mean ± SD        | 82.1±14.6    |                  |

Abbreviations: ALA = alanine; ALSFRS-R = Amyotrophic Lateral Sclerosis Functional Rating Scale- Revised; THR = threonine; UMNB = Upper Motor Neuron Burden Scale.

statistical analyses were performed using JMP pro 12.0.1 (SAS Institute Inc., Cary, NC). Between-group differences were tested using independent samples t test. Effect size (Cohen d and 95% confidence interval) was calculated based on differences between groups. Spearman correlation coefficients were calculated.

DTI-derived corticospinal tract (CST) tractography. Depending on TBSS findings and in order to obtain a mask for the upper part of CST, our other ROI, local probabilistic tractography of CST was carried out using FSL tools. We used masks from the output of T1 segmentation in Freesurfer as seed and target. The seed was located in the cortical parcellate of the PCG and the target mask at the level of the basal ganglia. FMRIB’s linear image registration tool (FLIRT) was used to linearly transform each FA and non-FA map to T1 and the reverse matrix was applied to move each mask (seed and target) from T1 to diffusion space. BedpostX/ProbTrackX algorithm was run with its default parameters: 2-fiber model per voxel, 5,000 probabilistic streamlines for each tract with a fixed separation distance of 0.5 mm between successive points to generate the underlying white matter connectivity data.21 In order to measure [14C]-PBR28 SUVR within CST, the CST probabilistic distribution map (derived from ProbTrackX and thresholded for values >0.20 to constrain the resultant pathway to white matter voxels) was used as an ROI. The analysis was conducted in MNI152 standard space. FLIRT and FMRIB’s nonlinear image registration tool were used to transform each SUVR map to MNI152 and FLIRT was used to linearly transform each DTI map to MNI152. Each CST ROI was also transformed into MNI152.

Correlation between DTI metrics and [14C]-PBR28 SUVR in PCG. Spearman analyses were conducted to investigate the relationship between DTI metrics and [14C]-PBR28 SUVR within the gray and white matter parcellates of the left and right PCG. The significance threshold was set at (p < 0.05).

Correlation between corticospinal tract and [14C]-PBR28 SUVR. Whole brain surface-based morphometric analysis. The thickness of the motor cortex was measured using Freesurfer v.5.3.21 Surface-based cortical thickness comparison between ALS and HC was performed in Qdec V1.5, a statistical tool part of Freesurfer. False discovery rate (FDR) was applied to correct the results for multiple comparisons (p < 0.05).

Correlation between cortical thickness and [14C]-PBR28 uptake in PCG. Spearman correlation was carried out to investigate the relationship between cortical thickness and [14C]-PBR28 SUVR in PCG.

Correlations between clinical and DTI metrics, cortical thickness, and [14C]-PBR28 uptake. Spearman correlations were employed to evaluate the relationship between UMNB and DTI metrics, UMNB and cortical thickness, and UMNB and [14C]-PBR28 SUVR within the bilateral PCG (right and left PCG).

Correlations among rate of disease progression (RDP), [14C]-PBR28 uptake in the motor cortex, cortical thickness, and DTI metrics. RDP was measured using the following formula:

\[ RDP = \frac{\text{total ALSFRS} \ (48) - \text{ALSFRS at time of scan}}{\text{disease duration at time of scan}} \]

Spearman analysis was used to test the relationship between RDP and MR/PET measures.

RESULTS  Diffusion analyses and correlation with [14C]-PBR28 SUVR. TBSS whole brain white matter skeleton. TBSS revealed widespread decreased FA values (p_FWE < 0.05) in ALS compared to HC in multiple white matter tracts (table e-2). By far, the largest reduction in FA (smaller p value) was observed along the upper part of the left CST (figure 1A, table e-1), which anatomically colocalized with the most increased [14C]-PBR28 uptake in ALS compared to HC (figure 1B, table 2). Feeding non-FA images into TBSS yielded no difference between the groups. Including age as a covariate led to the same findings for both FA and non-FA images as when age was not included.

ROI-PCG gray and white matter. ROI analysis in the cortical parcellate (gray matter) of the left PCG showed an 18% decrease in FA and a 10% increase in [14C]-PBR28 SUVR in ALS compared to HC (figure 1C). ROI analysis of the white matter parcellate of the left PCG showed 13% decreased FA and 16% increased [14C]-PBR28 SUVR in the ALS group compared to HC (all, p < 0.05) (table 2).

Neither FA nor [14C]-PBR28 SUVR values were different between the groups in the right PCG (gray and white matter) (table 2).

RD was higher in the left PCG (gray and white matter) in ALS compared to HC (p < 0.05), but not the right PCG (table 2).

DTI-derived CST tractography. Local tractography analysis revealed decreased FA values (p < 0.05) and increased diffusivities within the left and right CST.
Figure 1: [14C]-PBR28 uptake colocalizes with reduced fractional anisotropy (FA) in the motor cortex.

A. Decreased FA in ALS vs HC

- Decreased FA in ALS compared to HC. Using the probabilistic distribution map of CST as ROI, [14C]-PBR28 SUVR was higher ($p < 0.05$) in the left but not the right CST in ALS (table e-3).

B. Increased [14C]-PBR28 uptake in ALS vs HC

- Increased [14C]-PBR28 uptake in ALS compared to HC. Correlation between cortical thickness and [14C]-PBR28 uptake in ALS in the white matter parcellates of PCG for both hemispheres or in the reconstructed CST for both hemispheres.

C. Precentral gyrus

- Precentral gyrus (PCG) in ALS. FWE = family-wise error; SUVR = standardized uptake value ratio.

D. Spearman $r = 0.68 (p < 0.05)$

- Spearman correlation between cortical thickness and [14C]-PBR28 uptake in the PCG. Correlation between cortical thickness and [14C]-PBR28 uptake was $r = -0.75 (p = 0.011)$ in the left PCG and $r = +0.03 (p = 0.93)$ in the right PCG in ALS (figure 2D).

DISCUSSION

We found anatomical links between molecular changes (expression of the glial marker TSPO) and structural anomalies (decreased FA, increased diffusivity and cortical thinning) in patients with ALS compared to HC. We previously provided in vivo evidence for glial activation in the motor cortices and subcortical white matter in ALS using [14C]-PBR28 PET. Here, we report altered white and gray matter integrity in the same regions of increased glial activation, in the same cohort of participants. Specifically, increased [14C]-PBR28 uptake in the motor cortex (gray matter) in participants with ALS was associated with cortical thinning and loss of tissue integrity as indicated by decreased FA and increased diffusivity. All 3 measures including
Our results indicate a mechanistic link between tissue integrity and glial activation in people with sporadic ALS. In neuroimaging studies, combining multiple advanced neuroimaging modalities has contributed to a better understanding of disease mechanism in ALS. For example, using DTI and MR spectroscopic imaging (MRSI) to evaluate structural and neurochemical characteristics of brain in ALS has demonstrated in vivo evidence of neuroinflammation, gliosis, and degeneration of the motor cortices and CST. Although MRSI metabolites (e.g., myo-inositol) are not specific for neuroinflammation or gliosis, the relationship between these measures and the metrics of DTI is interesting. On the other hand, MRI measures and the molecular markers of inflammation assessed with PET have been reported in few cases.

Several factors can contribute to the laterality of our results, as well as those reported by groups. These factors include different phenotypes of study populations and the thresholds applied to the statistical maps. When examining the data using lower statistical thresholds, it becomes apparent that both hemispheres are affected. Therefore, it is possible that statistical threshold may be partly responsible for the observed laterality. On the other hand, the colocalization we saw in this study between different imaging modalities strengthens the relevance of these results and demonstrates the power of multimodal neuroimaging approaches.
Figure 2  

$[^{11}C]$ PBR28 uptake colocalizes with cortical thinning

(A) Statistical map of cortical thickness differences between patients with amyotrophic lateral sclerosis (ALS) and healthy controls (HC) shows cortical thinning in the left precentral gyrus (PCG) in ALS. The results are FDR corrected. (B) Projection onto the surface of voxel-wise whole brain analysis for 10 patients with ALS and 10 HC shows brain regions of higher $[^{11}C]$ PBR28 binding in ALS compared to controls ($p_{FWE} < 0.05$). (C) Boxplot representation (box contains median, 25th, and 75th percentiles) of cortical thickness in the left and right PCG. The left orange box shows cortical thickness in ALS and the right blue box in HC. The horizontal white line in each box represents the median. (D) Scatterplot shows the correlation between cortical thickness and $[^{11}C]$ PBR28 standardized uptake value ratio (SUVR) as measured in the left PCG in ALS. (E) Scatterplot shows the correlation between cortical thickness and radial diffusivity in the left PCG in ALS. $FWE = $ family-wise error.

Figure 3  

Correlations with Upper Motor Neuron Burden Scale (UMNB)

(A) Correlation between UMNB and fractional anisotropy (FA) within the bilateral precentral gyrus (PCG). (B) Correlation between UMNB and bilateral PCG thickness. (C) Correlation between UMNB and standardized uptake value ratio within the bilateral PCG. ALS = amyotrophic lateral sclerosis.

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between increases in CNS inflammation and decreases in tissue integrity.

Cortical thinning and decreases in FA and increased diffusivity have been reported previously in ALS cross-sectional studies. Here, we were able to replicate these findings, and in addition, show colocalization with a molecular marker of glial activation. One can speculate that we are observing both cause and effect of neurodegeneration. In other words, when cortical motor neurons degenerate, they lose their intrinsic axons, which may trigger regional reactive glial activation, and vice versa. The proinflammatory mediators expressed by activated glia can lead to axonal loss and neurodegeneration.

It is debated whether glial activation is protective or toxic in neurodegeneration; however, prior studies in ALS mouse models suggest that inflammation and glial activation play an important role in accelerating disease progression.

In this study, the structural and molecular changes of neuroimaging analyses were strongly correlated with clinical signs of upper motor neuron dysfunction, demonstrating the clinical relevance of our findings. On the other hand, the rate of disease progression did not correlate with the imaging findings. It is possible that the relationship between the anatomical changes and disease progression is nonlinear or the sample size is too small to show statistical significance. Future longitudinal studies with and without therapeutic interventions are needed to address the temporal sequence of events, including changes in FA within gray and white matter regions, inflammation, cortical thinning, and clinical disease progression.

Small sample size is the major limitation of this study. We consider our results as exciting preliminary data that need further replication in larger studies. At the same time, our data demonstrate concordance across multiple imaging modalities that solidifies the validity of our findings within this small cohort.

Our findings, although preliminary, could still have direct implications on clinical drug development for ALS and other neurodegenerative disorders. Historically, most phase II clinical trials that assess preliminary efficacy in ALS have been significantly underpowered because of the use of clinical measures such as ALSFRS-R as primary trial outcomes. This phenotypical evidence of target engagement.

Future longitudinal studies with and without therapeutic interventions are needed to address the temporal sequence of events, including changes in FA within gray and white matter regions, inflammation, cortical thinning, and clinical disease progression.

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DISCLOSURE

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