Neuroinflammation in Huntington’s Disease: New Insights with 11C-PBR28 PET/MRI

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ABSTRACT: Huntington’s disease is a devastating neurodegenerative genetic disorder that causes progressive motor dysfunction, emotional disturbances, and cognitive impairment. Unfortunately, there is no treatment to cure or slow the progression of the disease. Neuroinflammation is one hallmark of Huntington’s disease, and modulation of neuroinflammation has been suggested as a potential target for therapeutic intervention. The relationship between neuroinflammation markers and the disease pathology is still poorly understood. To improve our understanding of neuroinflammation in Huntington’s disease, we measured translocator protein (TSPO) expression using 11C-PBR28 and simultaneous PET/MRI. Standardized-uptake-value ratios, normalized by whole brain uptake, were calculated for data acquired 60–90 min after radiotracer administration. We identified distinct patterns of regional neuroinflammation (as defined by TSPO overexpression relative to a control group) in the basal ganglia of Huntington’s disease patients. These patterns were observed at the individual level in all patients, with region of interest analysis confirming significant differences between patients and the control group in the putamen and the pallidum. Additionally, we observed further distinct regional and subregional signatures, which may provide insights into phenotypical variability. For example, in certain Huntington’s disease patients, we observed in vivo elevation of the level of TSPO binding in subnuclei in the thalamus and brainstem that have been previously associated with visual function, motor function, and motor coordination. Our main result is an objective score, based solely on 11C-PBR28 measurements, that correlates well with Huntington’s disease progression. Our results suggest 11C-PBR28 might prove useful in clinical trials evaluating therapies targeting neuroinflammation.

KEYWORDS: Huntington’s disease, 11C-PBR28, PET/MRI, translocator protein (18 kDa), TSPO, neuroinflammation, glia

INTRODUCTION

Huntington’s disease is a devastating fully penetrant autosomal dominant neurological disorder characterized by progressive motor dysfunction, emotional disturbances, dementia, and weight loss.1 The gene responsible for the disease, first cloned in 1993,2 encodes a large, highly conserved protein of unknown function known as huntingtin (htt). In individuals with Huntington’s disease, a polymorphic trinucleotide repeat sequence (CAGn) near the 5’ end of the gene is expanded beyond the normal repeat range, leading to translation of an expanded polyglutamine sequence in the protein.3 Proteolysis of mutant htt, whereby abnormal and toxic N-terminal fragments of htt are released, plays a central role in the pathogenesis of this disease.3–5 These toxic htt fragments set in motion a complicated cascade of both damaging and compensatory molecular processes and genetic programs, including neuroinflammation. These events ultimately lead to increasingly fragile, atrophic, dysfunctional neurons susceptible to a variety of stressors such as oxidative injury, excitotoxic stress, disordered neurophysiology, proapoptotic signals, malfunctioning proteolysis, and energy depletion, all of which might play roles in neuronal death.6

Neuroinflammation has been suggested as an important early pathological process in Huntington’s disease. It is mediated through the activation of glia and results in the formation of...
Evidence of progressive neurodegeneration, neuroinflammation, and volume loss during the course of disease has also been observed in vivo with PET and MR imaging. MRI studies have provided extensive evidence of global brain atrophy in Huntington’s disease, with marked decreases in volume in the striatum and particularly in the caudate nucleus. Most PET studies have focused on the investigation of regional glucose metabolism using FDG and have revealed significant hypometabolism in cortical regions and in the caudate and putamen of Huntington’s disease patients. For a recent review on the current status of PET imaging in Huntington’s disease, see ref 15.

A small number of PET studies have investigated regional neuroinflammation using \(^{11}C\text{-}\text{PKI11195}\), a tracer that binds to the 18 kDa translocator protein (TSPO). This protein is expressed at very low levels in the healthy CNS but becomes dramatically upregulated by reactive microglia and astrocytes in the context of neuroinflammation. As such, PET imaging of TSPO can localize neuroinflammation. Previous PET studies with \(^{11}\text{C}\text{-}\text{PKI11195}\) demonstrated evidence of glial activation in the striatum (caudate and putamen), thalamus, and cortical regions in both symptomatic and presymptomatic Huntington’s disease. Those studies demonstrated that the degree of glial activation in the striatum correlates with the severity of disease, based on Unified Huntington’s Disease Rating Scale motor scores, and that the TSPO signal also correlates with striatal dopamine D2 receptor dysfunction as measured by \(^{11}\text{C}\text{-}\text{raclopride}\). However, although those studies found statistically significant differences at the group level between Huntington’s disease patients and controls, they showed some overlap in the distribution of the data points so that specific individuals could not be classified on the basis of \(^{11}\text{C}\text{-}\text{PKI11195}\) imaging alone. Unfortunately, \(^{11}\text{C}\text{-}\text{PKI11195}\) suffers from high levels of nonspecific binding and a poor signal-to-background ratio, which hampers its ability to look at the individual level and within individual brain regions. Second-generation TSPO-targeted tracers such as \(^{11}\text{C}\text{-}\text{PBR28}\) overcome some of those limitations and have the potential to provide more insight into the role of neuroinflammation in Huntington’s disease. However, these radiotracers, including \(^{11}\text{C}\text{-}\text{PBR28}\), exhibit large interindividual variability in binding affinity in humans, some of which is predicted by TSPO Ala147Thr polymorphism and must be accounted for in cross-sectional studies.

Currently, there are no treatments available to cure or slow Huntington’s disease progression. Because glial activation is present early in the course of the disease and can be damaging and promote neurodegeneration, it may provide an important target for therapeutic intervention. Although there are treatments targeting inflammatory pathways, there is no robust way to determine whether a treatment acutely modulates glial activation, particularly in premanifest or early manifest Huntington’s disease subjects. Markers of neuroinflammatory

Figure 1. (A) Axial views of \(^{11}\text{C}\text{-}\text{PBR28}\) SUVR60–90 of three representative controls (1–3), one premanifest Huntington’s disease gene carrier (7), and all seven Huntington’s disease patients (8–14) imaged in our study. Data were projected onto MNI standard space, spatially smoothed, and overlaid onto MR images for common visualization across subjects. Distinct patterns of uptake were observed in patients compared to controls, with increased regional \(^{11}\text{C}\text{-}\text{PBR28}\) uptake mainly in the putamen and the pallidum. Strikingly, this pattern was observed at the individual level in all patients. In addition, all subjects showed \(^{11}\text{C}\text{-}\text{PBR28}\) uptake in brain regions such as the thalamus and brainstem. (B) Average images for the groups of controls and Huntington’s disease patients.
treatment response would therefore be invaluable for determining which anti-inflammatory treatments merit later phase efficacy studies.

The goal of this study was to evaluate for the first time $^{11}$C-PBR28, a second-generation TSPO ligand, in Huntington’s disease. A secondary goal was to determine whether heterogeneity in $^{11}$C-PBR28 uptake might be useful in follow-up studies for understanding variability in symptoms and disease progression or, ultimately, in response to anti-inflammatory treatment.

## RESULTS

**Visual Evaluation of $^{11}$C-PBR28 PET Data.** Through a visual evaluation of $^{11}$C-PBR28 SUVR60–90 PET data, we found distinct patterns of uptake in Huntington’s disease patients compared to controls, with patients showing increased regional
glial activation levels in the putamen and the pallidum. Strikingly, this pattern of uptake was observed at the individual level in all Huntington’s disease subjects. In addition, all scanned subjects, both controls and patients, showed uptake in other brain regions, such as the thalamus and brainstem. Figure 1A displays individual SUVR data for individual subjects projected onto MNI space, and Figure 1B the average images for the groups of controls and Huntington’s disease patients. An interactive exploration of a high-resolution version of these images is available at neurovault.org (Neuroinflammation in Huntington’s Disease: New Insights with \textsuperscript{11}C-PBR28 PET/MRI. http://neurovault.org/collections/GHXGLWPB/).

**Quantitative Evaluation of \textsuperscript{11}C-PBR28 PET Data. Region of Interest Analysis.** Region of interest analysis confirmed that the regional distribution of the \textsuperscript{11}C-PBR28 signal was different for controls and Huntington’s disease patients (see Figure 2). In the pallidum and putamen, controls have \textsuperscript{11}C-PBR28 signal distributions centered at values lower than those of the premanifest Huntington’s disease gene carrier and the patients. The difference between the median distributions for controls and Huntington’s disease patients (excluding the premanifest gene carrier) is statistically significant for the putamen and the pallidum ($p < 0.01$ and $p < 0.06$, respectively, under a permutation test). In contrast, in the caudate and the thalamus, there are not statistically significant differences between the two groups ($p > 0.4$). Similar results hold for the mean distributions (see https://doi.org/10.5281/zenodo.1174364 for details).

**Principal Component Analysis.** We applied PCA to the regional \textsuperscript{11}C-PBR28 SUVR 60–90 distributions shown in Figure 2. Our results show that the three best combinations of features (the first three PCA axes) account for 90% of the observed variance. The reason for that is that the activity distributions shown in Figure 2 (or, ultimately, Figure 1) have numerous commonalities and, therefore, redundant information. By eliminating the redundancies, one can “compress” the information conveyed by the data in a much smaller set of features.

A key finding is that the component along the first PCA axis is enough to classify our subjects according to their disease status (see Figure 3A). Controls have negative values; the premanifest and patient 8 have negative values but closer to zero, and the rest of the Huntington’s disease patients have all positive values. The first and second component axes for all subjects are shown in Figure 3B. Figure 3C can be read as a table to extract the corresponding component along each of the first three PCA axes. For example, to extract the score shown in Figure 3A, one should multiply the value of each of the features for the subject (e.g., the median value of the \textsuperscript{11}C-PBR28 SUVR 60–90 in the putamen) by the value of that feature in the first vertical panel in Figure 3C ($≈0.23$ for the median in the putamen) and add them all into a single number.

It is important to emphasize that no information about the status of the disease in the subjects is used in our analysis, the only two assumptions made being the choice of features to describe each of the regions and the choice of the regions themselves.

**Correlations with Caudate Volume.** The caudate volume, adjusted for intracranial volume, shows a clear correlation with the score obtained from our PCA (see Figure 4). The data were fitted using linear regression (adjusted $R^2 = 0.68$). The slope of the regression line is negative, $−0.81 \pm 0.15$, with a 95% confidence interval between $−1.138$ and $−0.477$, indicating that higher values of our score correspond to more atrophied cAudates. The caudate volume was standardized so the whole sample has an average equal to zero and a standard deviation equal to one to facilitate comparisons (this does not change the $p$ values for the fit).\textsuperscript{26} All Huntington’s disease subjects show some degree of atrophy in the caudate, with a more advanced stage associated with a larger volume loss: the premanifest gene carrier (7) and patient 8 show a slightly reduced caudate volume compared to controls; all other Huntington’s disease patients have a more marked volume loss (mean caudate volume adjusted for intracranial volume in patients, $3.2 \pm 0.6$; in controls, $4.9 \pm 0.4$; statistically significant different with $p < 0.001$). It is important to note that the measurement of the caudate volume and our score from the PCA are independent measurements: the first is obtained from MR data while the second is obtained from PET data.

Similar although weaker correlations are observed for pallidum, putamen, and thalamus regions with negatives slopes of $−0.77 \pm 0.17$, $−0.76 \pm 0.17$, and $−0.73 \pm 0.18$ and adjusted $R^2$ values of 0.61, 0.6, and 0.55, respectively.

**Heterogeneous Distribution of the \textsuperscript{11}C-PBR28 Signal in the Thalamus and Brainstem of Huntington’s Disease Patients.** We observed a markedly heterogeneous \textsuperscript{11}C-PBR28 signal distribution in the thalamus and the brainstem of some Huntington’s disease patients. This heterogeneity explains why the thalamic \textsuperscript{11}C-PBR28 signal distributions shown in Figure 2 are so wide.

In an exploratory analysis, the areas with increased uptake in the thalamus were refined to the subnucleus level using a three-dimensional atlas, normalized to MNI space, that represents the anatomy of the thalamus and subthalamic structures and was generated from multiple histological data.\textsuperscript{27} We followed two approaches. First, the most affected thalamic subnuclei were visually identified through the superimposition of the atlas onto the PET data. Second, for each subject, we measured the \textsuperscript{11}C-PBR28 signal distribution in every subnucleus.

![Figure 4. The first PCA component is correlated with the caudate volume. The measurement of the caudate volume is consistent with the score obtained from our PCA. We show the caudate volume adjusted for intracranial volume (y axis) as a function of the component along the first PCA axis (x axis) for all subjects. The caudate volume is further standardized so the whole sample has an average equal to zero and a standard deviation equal to one. The solid line is a linear fit, and the inner dashed lines enclose the 95% confidence interval for the parameters of the fit. The fact that the slope is negative means that the score obtained from our PCA is higher for subjects with a greater volume loss in the caudate. The outer dashed lines enclose the 95% prediction interval for the data.](image-url)
With the first approach, the subnuclei that showed the most notable increase in $^{11}$C-PBR28 uptake were identified as the ventral anterior nucleus in patient 11 and as the ventral lateral posterior in patient 12. These individual examples are shown in Figure 5. In addition, in patients 8 and 13, we observed a marked uptake in an area approximating the red nucleus in the brainstem. The delineation of these subnuclei does not match perfectly the area of the increased signal, and we referenced here those that showed the highest percentage of overlap.

The second approach confirmed increased uptake in the subnuclei that were visually pinpointed as $^{11}$C-PBR28 avid by the first approach and further identified other patients presenting similar patterns of uptake. For example, the ventral lateral nucleus was noted to show increased uptake in patients 10–13. In addition, this analysis unveiled increased uptake in the pulvinar nuclei of most patients compared to controls, a pattern that had not been noted visually. Figure 6 shows, for each subject, the three subnuclei where the differences between controls and patients were more apparent: the left ventral anterior, ventral lateral, and pulvinar nuclei. Interestingly, their right counterparts show no differences across subjects.

**DISCUSSION**

We performed a pilot study of glial activation with simultaneous PET/MRI and $^{11}$C-PBR28, a second-generation ligand that measures TSPO expression, to evaluate in vivo regional neuroinflammation in Huntington's disease. Our main finding is a distinct pattern of $^{11}$C-PBR28 binding observed at the individual level in all Huntington’s disease patients. We are able to extract a quantitative score, based solely on $^{11}$C-PBR28 measurements, that correlates well with measurements of brain atrophy. In addition, we observed subregional heterogeneity that may provide mechanistic insights into disease.

As previously shown in prior PET studies with the first-generation ligand $^{11}$C-(R)-PK11195,18–21 we found significantly increased levels of $^{11}$C-PBR28 binding in the putamen and pallidum of Huntington’s disease patients compared to controls (Figures 1 and 2). We also found evidence of increased levels of binding in basal ganglia structures in our presymptomatic subject, suggesting that neuroinflammation is an early pathological process that may be associated with subclinical progression of the disease. One key difference with respect to prior studies, however, is that we were able to detect alterations in TSPO expression in single individuals.

The caudate is known to be one of the brain regions that show atrophy earliest in the course of the disease.12,25 We observed atrophy in the caudate of most of our patients but, however, did not find significant differences in $^{11}$C-PBR28 binding compared to controls. This could potentially be due to an underestimation of the quantitative PET values in the caudate of Huntington’s disease patients due to partial volume effects, which are more severe in patients due to the atrophy.

Our work demonstrates that $^{11}$C-PBR28 provides a very consistent signal across subjects belonging to the same group, allowing a reliable classification based on imaging alone. By performing a PCA, we are able to obtain an objective, quantitative score that classifies our subjects consistently as controls or patients. Interestingly, the premanifest subject and a patient at a very early stage are classified according to our PCA results midway between controls and the rest of the patients. These findings suggest that $^{11}$C-PBR28 may provide an in vivo tool for monitoring the progression and severity of neuroinflammation in Huntington’s disease, including the prodromal phase.

It is important to stress again that the quantitative score obtained from the PCA uses information from several regions and makes no assumptions about their relative importance, painting a richer picture of the extent of neuroinflammation in our subjects. In addition, the PCA algorithm treats all subjects as a single group without any information about the diagnosis to guide group selection. We find it is remarkable that a strong correlation between the score obtained from the PCA and the caudate volume emerges very clearly, suggesting greater neuroinflammation as a function of disease severity. TSPO measurements might offer advantages over measurements of brain atrophy, as successful treatments might reduce neuroinflammation faster than any observable change in brain volume. Therefore, measurements of TSPO expression using $^{11}$C-PBR28 PET/MR might have the potential to assess target engagement at the individual level on a time scale more relevant to clinical research. Longitudinal studies will be needed to elucidate whether immune modulation is important in Huntington’s disease.

Because of the high signal-to-background ratio of $^{11}$C-PBR28, we were also able to study the heterogeneity of uptake in the thalamus. Although prior studies have been inconsistent with respect to thalamic atrophy in early stages of Huntington’s disease,30 post-mortem studies have demonstrated loss of thalamic neurons.31 In our group of patients, we observed in vivo increased levels of TSPO binding in certain structures within the thalamus and brainstem associated with motor function, motor coordination, and visual function. In particular, some patients showed increased levels of binding in the ventral lateral posterior nucleus, the ventral anterior nucleus, and the pulvinar nucleus. Interestingly, the ventral lateral posterior nucleus and the ventral anterior nucleus form the motor functional division in...
the thalamus and are major sources of reciprocal thalamocortical projections to motor and premotor cortex.32 Both areas show early regional degeneration in Huntington’s disease.33 The pulvinar projects to secondary visual areas and to association areas in the parietotemporal cortex. It contributes to visual perception and eye movements, likely related to its important role in visual attention, which are disrupted early in Huntington’s disease.34 We also found increased uptake in the red nucleus, an area that is important for motor coordination. These preliminary observations may be useful in prospective studies characterizing the heterogeneity of patient symptoms.

This pilot study has some limitations, the most relevant of which is the small sample size. For example, although we observed increased levels of TSPO binding in thalamic and brainstem nuclei related to motor function and coordination of a few patients, a larger cohort would be needed to determine whether they are clinically meaningful in terms of correlation with motor dysfunction severity. Another limitation is that longitudinal studies including presymptomatic Huntington’s disease gene carriers would be needed to better assess the potential of 11C-PBR28 PET imaging to evaluate disease onset and progression.

In conclusion, 11C-PBR28 PET provides a high signal-to-background ratio for assessing neuroinflammation in early stage Huntington’s disease patients. This in turn allows the investigation of regional heterogeneity at the individual level, which may provide insights into glial activation of brain regions related to specific symptoms. We anticipate that approaches building on the one presented here will be useful for the evaluation of disease progression and for the assessment of the efficacy of anti-inflammatory and neuroprotective agents in future clinical trials.

**METHODS**

**Study Participants.** Seven patients with early symptomatic Huntington’s disease (two female and five male, ages of 56.0 ± 8.4), one premanifest Huntington’s disease gene carrier (male, age 38), and six healthy controls (four female and two male, ages of 57.8 ± 8.1) participated in this pilot study at the Massachusetts General Hospital. The study protocols were approved by the Partners Human Research Committee and by the Radioactive Drug Research Committee. According to the Declaration of Helsinki, procedures were explained and consent was obtained. A general medical history was obtained from each subject, including age, medications, and total functional capacity (TFC), which assesses the capacity to work, handle finances, perform domestic chores and self-care tasks, and live independently22 (see Table 1).

**DNA Polymorphism Genotyping.** Blood was collected by venipuncture for genotyping for the rs6971 polymorphism (Ala147Thr) in the TSPO gene with Ala/Ala leading to high-affinity binding, Ala/Thr to mixed-affinity binding, and Thr/Thr to low-affinity binding.25,35 Polymorphism rs6971 was genotyped variously using a TaqMan assay on demand C_2512565_20. Allele T147 was linked to Vic, and allele A147 was linked to FAM. Polymerase chain reactions (PCRs) were performed in a 96-well microtite-plate on a GeneAmp PCR System 9700. After PCR amplification, the end point plate was read and allele calling performed using an ABI 7900 HT and the corresponding SDS software. Individuals with genotype Ala147/Ala147 were classified as high-affinity binders, those with genotype Ala147/Thr147 as mixed-affinity binders, and those with genotype Thr147/Thr147 as low-affinity binders. This binding affinity difference can be detected by 11C-PBR28 standardized-uptake-value (SUV) measurements36 and needs to be
Table 1. Fourteen Subjects Who Were Enrolled in This Study*  

<table>
<thead>
<tr>
<th>subject ID</th>
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<th>TSPO genotype</th>
<th>TFC score</th>
<th>molar activity (MBq/nmol) at time of injection</th>
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<tr>
<td>1</td>
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<td>Ala/Ala</td>
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<tr>
<td>2</td>
<td>control</td>
<td>Ala/Thr</td>
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<tr>
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<td>control</td>
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<td>Ala/Ala</td>
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<tr>
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Six healthy controls, one pre-manifest Huntington’s disease carrier, and seven early symptomatic Huntington’s disease patients. TSPO polymorphisms with Ala/Ala lead to high-affinity \(^{11}\text{C}\)-PBR28 binding and those with Ala/Thr to mixed-affinity \(^{11}\text{C}\)-PBR28 binding. Huntington’s disease patients are classified based on a subjective neurological examination using the total functional capacity (TFC) score. TFC assesses the capacity to work, handle finances, perform domestic chores and self-care tasks, and live independently and ranges from 13 (normal) to 0 (severe disability).

controlled for in cross-sectional study designs. Only high-affinity binders (HABs) and mixed-affinity binders (MABs) were enrolled in this study.

**Ligand Preparation.** \(^{11}\text{C}\)-PBR28 was produced in house, using a procedure modified from the literature. Briefly, the desmethyl precursor (1.0 mg in 100 \(\mu\)L) was loaded into a 5 \(\mu\)L stainless steel loop for reaction with \(\text{CH}_2\text{I}\) using the Wilson captive solvent method. \(^{11}\text{C}\)-PBR28 was purified by reversed phase chromatography and reformulated by solid phase extraction in a 10% ethanol/saline mixture and then aseptically filtered.

**Image Acquisition and Processing.** PET/MRI Data Acquisition. All subjects underwent a PET/MRI scan using \(^{11}\text{C}\)-PBR28 as a PET tracer. Scans were performed on a clinical whole-body Siemens Biograph mMR scanner, which allows simultaneous acquisition of PET and MR images. A mean dose of 499 ± 59 MBq of \(^{11}\text{C}\)-PBR28 was administered intravenously as a slow bolus injection (see the specific activity at the time of injection in Table 1). Scanning began 30 min after tracer injection, and PET data were acquired for 60 min and stored in list-mode format. At the same time as the PET scan, a series of MR sequences were acquired. An anatomical MP-RAGE scan (TR 2530 ms, TE 2.15 ms, flip angle of 7°) was included for the purpose of anatomical localization, exclusion of incidental pathology, and generation of attenuation correction maps.

**MR Image Processing.** The MP-RAGE scan data were used for automated volumetric segmentation of cortical and subcortical brain structures, which was performed with the Freesurfer image analysis suite, documented and freely available for download online (version 5.3, http://surfer.nmr.mgh.harvard.edu/). The MP-RAGE data were used to segment and classify brain volumes as described previously. Regions of interest (ROIs) were generated and used for sampling of PET images and for determination of regional volumes. These methods have provided reliable measurements of cortical thickness and brain volumes, comparable to manual measurements. The technical details of these procedures are described in prior publications.

**PET Image Processing.** PET data were divided into 12 5-min frames and reconstructed with OP-OSEM 3D (3 iterations, 21 subsets; 344 × 344 image matrix, with 2.1 mm in-plane pixel size and 2.0 mm slice thickness; 4 mm Gaussian filter). Photon attenuation was estimated from MR MP-RAGE data and corrected using attenuation maps calculated though a combination of intensity- and prior-based tissue segmentation and atlas registration. Basic motion correction was applied through frame-by-frame realignment of the reconstructed data using Statistical Parametric Mapping (SPM8) software (Wellcome Trust Centre for Neuroimaging, University College London, London, U.K., http://www.fil.ion.ucl.ac.uk/spm/software/spm8/).

Standardized-uptake-value (SUV) images were calculated for data acquired 60–90 min post-tracer injection by averaging the last six 5-min frames. Individual SUV60–90 min images were normalized by the whole brain uptake (SUVR60–90 min) o account for differences in the global signal across subjects, including differences due to rs9671 polymorphism, as previously validated for other \(^{11}\text{C}\)-PBR28 studies. In addition, for visualization purposes only, individual SUV60–90 min images were registered to standard Montreal Neurological Institute (MNI) space using FSL (version 4.1.9, http://fsl.fmrib.ox.ac.uk/) and spatially smoothed (6 mm full width at half-maximum).

**Image and Statistical Analysis.** Regions of interest (ROIs) were defined on the individual MP-RAGE images, as described above. Individualized ROIs were subsequently used to segment PET images and obtain regional SUV values in four a priori selected ROIs: the caudate, putamen, pallidum, and thalamus. For each patient, left and right hemisphere ROIs were combined and analyzed together.

Principal component analysis (PCA) was used to obtain a blinded classification of disease based on imaging, using a few statistical quantities to describe each subject. PCA is a multivariate statistical method that allows researchers to reduce the number of features used to describe a statistical distribution while minimizing the loss of information. The goal was to derive a quantitative score that summarized the complex landscape of neuroinflammation observed in our PET images. Our program was as follows. First, we summarized each of the region of interest histograms for each subject by a set of descriptive statistical features (average, median, first and third quartiles, and maximum and minimum values). Second, we used the PCA algorithm to extract what combinations of those features account for most of the observed differences (the statistical variance). Each feature was standardized across subjects, so that its average is zero and its standard deviation is one, to account for the different scales of the different features.

The correlation between the component along the first PCA axis and the caudate volume was investigated using linear regression. The caudate volume was adjusted for intracranial volume to facilitate intrasubject comparisons and was further standardized so the whole sample has an average equal to zero and a standard deviation equal to one.

Image analysis was performed using a combination of tools, including SciPy, scikit-learn, nilearn, and nibabel. The source code and image data are publicly available under open licenses (http://neurovault.org/collections/GHXGLWPB/ and https://doi.org/10.5281/zenodo.1174364).

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**Author Contributions**

H.D.R. and J.M.H. conceived the study. C.L. and I.G. analyzed the data and wrote the manuscript. All authors discussed the results and implications and commented on the manuscript at all stages. All authors have given approval to the final version of the manuscript.

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Notes
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■ ABBREVIATIONS
SUVR, standardized-uptake-value ratio; PCA, principal component analysis

■ ADDITIONAL NOTES
“This strong correlation between the caudate volume and the first principal component seems to support our choice of features as discussed in footnote 14. To describe the unknown regional SUVR distributions in a general manner, we have chosen a set of features with some intrinsic redundancy. For example, some degree of correlation is expected between the mean and median. Redundant features influence the PCA outcome, which will assign more of the explained variance to the combination of a variable and its redundancies. This, however, does not affect the separability of the data, in the sense that separable data will remain so even after redundancies are removed. This means that the main conclusion extracted from the analysis leading to Figure 4 remains valid: a measurement extracted from the PET image can be used to separate controls from Huntington’s disease subjects. Such measurement can be expressed as a combination of very few (possibly one) of the principal components.

■ REFERENCES


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