

Microglial activation and amyloid deposition in mild cognitive impairment

A PET study

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ABSTRACT

Background: Activated microglia may play a role in the pathogenesis of Alzheimer disease (AD) as they cluster around beta-amyloid ($A\beta$) plaques. They are, therefore, a potential therapeutic target in both AD and its prodrome amnesic mild cognitive impairment (MCI).

Objective: To characterize in vivo with ^{11}C -(R)-PK11195 and ^{11}C -PIB PET the distribution of microglial activation and amyloid deposition in patients with amnesic MCI.

Methods: Fourteen subjects with MCI had ^{11}C -(R)-PK11195 and ^{11}C -PIB PET with psychometric tests.

Results: Seven out of 14 (50%) patients with MCI had increased cortical ^{11}C -PIB retention ($p < 0.001$) while 5 out of 13 (38%) subjects with MCI showed increased ^{11}C -(R)-PK11195 uptake. The MCI subgroup with increased ^{11}C -PIB retention also showed increased cortical ^{11}C -(R)-PK11195 binding ($p < 0.036$) though this increase only remained significant in frontal cortex after a correction for multiple comparisons. There was no correlation between regional levels of ^{11}C -(R)-PK11195 and ^{11}C -PIB binding in individual patients with MCI: only three of the five MCI cases with increased ^{11}C -(R)-PK11195 binding had increased levels of ^{11}C -PIB retention.

Conclusions: Our findings indicate that, while amyloid deposition and microglial activation can be detected in vivo in around 50% of patients with mild cognitive impairment (MCI), these pathologies can occur independently. The detection of microglial activation in patients with MCI suggests that anti-inflammatory therapies may be relevant to the prevention of AD. *Neurology*® 2009;72:56-62

GLOSSARY

$A\beta$ = beta-amyloid; **AD** = Alzheimer disease; **BP** = binding potential; **MCI** = mild cognitive impairment; **MMSE** = Mini-Mental State Examination; **PBBS** = peripheral benzodiazepine binding site; **ROI** = region of interest; **SD** = standard deviation; **SRTM** = simplified reference tissue model.

There has been increasing interest in the early identification of subjects who have memory impairment beyond that expected with normal aging, but who do not fulfill criteria for dementia. These cases have been labeled as having amnesic mild cognitive impairment (MCI).¹

The positron emitting radiotracer ^{11}C -PIB (Pittsburgh compound B) is a neutral derivative of thioflavin-T with nanomolar affinity for fibrillar amyloid in the AD brain.² Using ^{11}C -PIB (*N*-methyl- ^{11}C] 2-(4'-methylaminophenyl)-6-hydroxybenzothiazole) with PET it has been possible to localize and quantify $A\beta$ plaque load in vivo. Several PET studies have reported increased cortical ^{11}C -PIB uptake in 90% of patients with probable AD³⁻⁸ while around 50% of patients with amnesic MCI also have significantly raised uptake.^{5,8-10}

Although the formation of $A\beta$ and neurofibrillary tangles are key features in AD pathogenesis, activated microglia are thought to be important and have been shown to cluster around sites of $A\beta$ in human postmortem brain slices and in transgenic mouse models of AD.^{11,12} The PET radiotracer

Supplemental data at
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Table 1 Demographic details of patients with MCI

	MCI: total	MCI: AD-range PIB	MCI: norm-range PIB	AD	¹¹ C-PIB controls	¹¹ C-(R)-PK11195 controls
Total no.	14	7	7	22	14	10
Mean age, $\bar{y} \pm SD$	66.6 \pm 9.6	69.1 \pm 6.4	64 \pm 11.9	64.9 \pm 6.4	63.9 \pm 5.3	60.2 \pm 9.3
Gender: women/total	5/14	3/7	2/7	10/22	6/14	4/10
Mean duration of symptoms, $\bar{y} \pm SD$	4.1 \pm 2.1	3.4 \pm 0.8	4.7 \pm 2.9	5.5 \pm 3.8	—	—
Mean MMSE \pm SD	27.7 \pm 1.5	28.1 \pm 1.4	27 \pm 2.2	21.5 \pm 3.6	29.8 \pm 0.43	29.9 \pm 0.32

MCI = mild cognitive impairment; AD = Alzheimer disease; norm-range = normal-range; MMSE = Mini-Mental State Examination; SD = standard deviation.

¹¹C-(R)-PK11195 (1-[2-chlorophenyl]-N-methyl-N-[1-methyl-propyl]-3-isoquinoline carboxamide) is a ligand that is specific for the peripheral benzodiazepine binding site (PBBS), a receptor abnormally expressed by the mitochondria of activated microglia.¹³ Increased binding of ¹¹C-(R)-PK11195 has been reported in AD,^{14,15} and other neurodegenerative disorders including frontotemporal dementia¹⁶ and Parkinson disease,¹⁷ indicating that microglia activation is a nonselective response to neuronal damage. The precise role of microglial activation and its relationship to A β remains controversial. Microglia phagocytose dead cells, remodel synapses, and remove A β fibrils^{18,19} but also have damaging effects, releasing cytokines and nitric oxide.²⁰ Less is known about their relevance to MCI. Using the PET radiotracers ¹¹C-PIB and ¹¹C-(R)-PK11195 we sought to characterize and compare in vivo the patterns of amyloid deposition and microglial activation in a group of subjects with MCI.

METHODS Subjects with MCI. Fourteen subjects (mean age 66.6 years; SD 9.6) fulfilling Petersen's criteria for amnesic MCI¹ participated in the study (table 1). All subjects underwent both ¹¹C-PIB and ¹¹C-(R)-PK11195 PET on separate occasions (mean interval between scans 6.4 \pm 5.4 weeks). Subjects were recruited from the Hammersmith Hospitals NHS Trust (UK), the National Hospital for Neurology and Neurosurgery (UK), St. Margaret's Hospital (UK), and Victoria Hospital (UK). All patients had a comprehensive assessment including neurologic examination, neuropsychological testing, and MRI. Patients with significant white matter disease in the view of an experienced radiologist were excluded from the study. Ethical approval was granted by the Hammersmith and Queen Charlotte's Hospitals ethics committee. All patients gave informed written consent prior to their participation.

Subjects with AD and healthy controls. PET data from 22 subjects (mean age 64.9 years; SD 6.4) meeting the National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association²¹

criteria for the diagnosis of probable AD were used for comparison with our MCI group. All 22 AD subjects had ¹¹C-PIB (PIB) PET and, of these, 15 (mean age 63.7 years; SD 6.0) additionally had ¹¹C-(R)-PK11195 (PK) PET (mean scan interval 3.1 \pm 2.8 weeks). PIB PET data from 14 controls (mean age 63.9 years; SD 5.3) and PK PET data from 10 controls (mean age 60.2 years; SD 9.3) were also used for comparison with the MCI group. Eighteen of the 22 patients with AD and 11 of the 14 controls have had their PIB PET data previously reported.⁷ PK PET data for 11 of the 15 patients with AD and 8 of the 10 controls have now been accepted for publication. We have included these reference data so that our novel MCI PET data may be interpreted in context.

MRI scanning. All subjects underwent volumetric T1-weighted MRI for the purpose of structure-function coregistration with PET and T2-weighted MRI to exclude structural lesions.

PET scanning. Permission to administer ¹¹C-PIB and ¹¹C-(R)-PK11195 was obtained from the Administration of Radioactive Substances Advisory Committee of the United Kingdom. ¹¹C-PIB and ¹¹C-(R)-PK11195 were manufactured and supplied by GE Healthcare, Hammersmith Hospital, UK.

¹¹C-PIB PET. All subjects were scanned using a Siemens ECAT EXACT HR+ camera in three-dimensional acquisition mode using a scanning protocol described previously.⁷ Subjects received 366 \pm 26.8 MBq ¹¹C-PIB, injected IV.

Image analysis: ¹¹C-PIB PET. Target region: cerebellar cortex ratio images of PIB retention were generated as follows: 10–90 minute (total) and 60–90 minute (late) summation images were created in Matlab. Each MRI was segmented into gray, white matter, and CSF images using Statistical Parametric Mapping software (SPM2; Wellcome Department of Imaging Neuroscience, University College London, UK). The probabilistic gray matter image was binarized by thresholding at 50% probability. Both summation PET images were then coregistered to individual subjects' MRIs which were used as a template to spatially normalize those PET images and binarized MRI probability gray matter image into Montreal Neurological Institute space. Using Analyze software (Mayo Clinic, MN), we convolved the binarized gray matter image with an in-house probabilistic brain atlas.²² This creates an individualized anatomic atlas for each subject in standard space which is used as a template to sample a priori designated regions of interest (ROIs). At this stage, ¹¹C-PIB activity in the reference region (cerebellar cortex) is sampled. Finally, the late summation images were normalized to the mean cerebellar cortical uptake value over this 60–90-

Table 2 Mean PIB retention values in AD, MCI, and healthy controls

	AD (n = 22)	MCI (n = 14)	MCI: AD-range (n = 7)	MCI: normal-range (n = 7)	HC (n = 14)
Ant cingulate	2.23 ± 0.42	1.64 ± 0.56	2.14 ± 0.33*	1.15 ± 0.06	1.17 ± 0.12
Post cingulate	2.22 ± 0.40	1.67 ± 0.59	2.19 ± 0.34*	1.16 ± 0.09	1.20 ± 0.07
Frontal	2.06 ± 0.37	1.54 ± 0.49	1.95 ± 0.34*	1.12 ± 0.02	1.10 ± 0.07
Temporal	1.86 ± 0.34	1.45 ± 0.43	1.78 ± 0.39*	1.11 ± 0.04	1.12 ± 0.03
Parietal	2.02 ± 0.37	1.51 ± 0.50	1.92 ± 0.41*	1.11 ± 0.04	1.11 ± 0.05
Whole brain	1.96 ± 0.34	1.49 ± 0.46	1.86 ± 0.37*	1.13 ± 0.03	1.12 ± 0.05

Values = mean ± SD.

* $p < 0.001$ for MCI: AD-range vs controls.

AD = Alzheimer disease; MCI = mild cognitive impairment; HC = healthy control; Ant cingulate = anterior cingulate; Post cingulate = posterior cingulate.

minute period, generating the late ratio image. We used a late scan reference region approach because it provides a robust measure of total: nonspecific PIB relative volumes of distribution in target brain regions without the need for arterial sampling. The cerebellum was chosen as the reference region as it is relatively free of fibrillar plaques, reflected by its lack of staining with Congo red and thioflavins.²³

ROI analysis. We quantified PIB retention in the anterior cingulate, posterior cingulate, frontal, temporal, and parietal cortex. Additionally, whole brain retention was quantified, although not considered as a region when performing statistical analysis.

¹¹C-(R)-PK11195 PET. All subjects were scanned using an ECAT EXACT HR ++ (CTI/Siemens, Knoxville, TN) PET Tomograph (three-dimensional mode) which has a total axial field of view of 23.4 cm. Subjects received 299 ± 16 MBq ¹¹C-(R)-PK11195 injected IV and scanning was performed in list mode and the dynamic images rebinned over 60 minutes as 18 time frames.

Image analysis: ¹¹C-(R)-PK11195 PET. Parametric images of PK binding potential (BP) were generated by a simplified reference tissue model (SRTM), using RPM software written in Matlab.²⁴ Unlike fibrillar amyloid plaques, activated microglia are distributed throughout all brain regions in AD, including the cerebellum,²⁵ potentially making the selection of a suitable reference region for nonspecific signal difficult. We, therefore, used a supervised clustering technique to derive a nonspecific uptake tissue reference input function in all groups studied. This technique produces BP estimates that are reproducible and comparable to those derived from plasma input.^{26,27} In summary, the method uses six predefined kinetic classes to extract as a reference region a cluster of gray matter voxels in each individual that exhibits the kinetic behavior closest to that of gray matter in a population of healthy controls. The supervised clustering algorithm avoids regions with specific binding such as association cortex in AD and large vessels, and the resulting cluster of voxels is clear of unwanted spurious signals arising from areas of the brain known to have a rich density of PBBS receptors such as the meninges.

The resulting parametric PK BP images and 60-minute summation images were then coregistered to each subject's MRI. The 60-minute summation image contains blood flow dependent signal, providing good anatomic detail, enabling accurate coregistration. Spatial normalization and creation of gray matter object maps were performed by the same methods described for

analysis of PIB images, and PK BPs quantified in the same brain regions described earlier.

Statistical analysis. Statistical tests were performed using SPSS for Windows 14.0 statistical software. Repeated measures analysis of variance was applied to test for global differences in PIB and PK binding between MCI groups and controls, using the Greenhouse Geisser factor to correct for variance heterogeneity across regions. Univariate analysis of variance was used to detect significant differences in regional PIB and PK binding between groups, using age as a covariate and the *p* plot method to control for multiple comparisons.²⁸

Because of the heterogeneity of PK binding within the two MCI subgroups (those with increased AD-range and normal control-range PIB binding), PK BPs in subjects with MCI are also described on an individual basis. We considered regional PK binding to be raised if ≥ 2 SD greater than the control mean BP for that region.

Pearson's rank correlation coefficient was used to interrogate the correlation between PIB and PK binding in subjects with MCI. Spearman's rank correlation coefficient was used to assess the correlation between these PET measures and Mini-Mental State Examination (MMSE) scores and duration of symptoms.

RESULTS ¹¹C-PIB retention. Visual inspection of the late PIB ratio images showed increased uptake in 7 of 14 (50%) subjects with MCI. Quantitative analysis revealed PIB uptake ratio values comparable to our AD group, with twofold increased uptake in cingulate and frontal regions. These seven patients with MCI were therefore considered as a subgroup, having mean AD-range PIB binding which was greater than control mean binding in all cortical ROIs ($p < 0.001$). The remaining seven subjects with MCI had normal levels and patterns of cortical PIB uptake, with no mean binding difference in this normal-range subgroup compared to that of controls ($p = 0.69$) (table 2). Two of our 22 subjects with AD had regional ¹¹C-PIB uptake within control-range.⁷ One control (64-year-old man) had increased PIB retention (>2 SD greater than control mean) in the anterior cingulate, frontal, and temporal cortex (see discussion).

¹¹C-(R)-PK11195 binding. Coregistration of the PK BP image with MRI in one subject with MCI who had normal PIB uptake (55-year-old man) was unsatisfactory. He was, therefore, excluded from further PK PET analysis. Repeated measures analysis of variance showed a significant difference ($p = 0.036$) between mean PK BPs in AD-PIB range patients with MCI and controls. AD-PIB range patients with MCI had significantly increased mean PK binding in the anterior cingulate, posterior cingulate, and frontal cortex. However, after correction for multiple comparisons, only frontal cortex PK uptake remained significantly raised. There was no difference between mean regional PK binding in the AD-PIB range MCI and AD groups ($p = 0.31$), or between normal-PIB range MCI and controls

Table 3 Region of interest ¹¹C-(R)-PK11195 binding potential values in AD, MCI, and controls

	AD (n = 15)	MCI (n = 13)	MCI: AD-range (n = 7)	MCI: norm-range* (n = 6)	Controls (n = 10)	p Value
Ant cingulate	0.46 ± 0.16	0.48 ± 0.16	0.53 ± 0.19	0.43 ± 0.12	0.41 ± 0.09	0.03
Post cingulate	0.51 ± 0.18	0.54 ± 0.15	0.59 ± 0.16	0.47 ± 0.12	0.45 ± 0.10	0.03
Frontal	0.43 ± 0.11	0.44 ± 0.15	0.48 ± 0.16	0.39 ± 0.14	0.35 ± 0.07	0.02*
Temporal	0.43 ± 0.08	0.43 ± 0.14	0.46 ± 0.13	0.39 ± 0.14	0.37 ± 0.07	0.14
Parietal	0.38 ± 0.11	0.41 ± 0.14	0.43 ± 0.14	0.39 ± 0.16	0.35 ± 0.08	0.10
Whole brain	0.43 ± 0.10	0.44 ± 0.14	0.47 ± 0.14	0.40 ± 0.14	0.37 ± 0.07	0.06

Values = mean ± SD.

*One MCI: normal-range PIB patient excluded (PK PET coregistration unsatisfactory).

*The p value is for MCI: AD-range vs controls. p Value significant after correction for multiple comparisons.

MCI = mild cognitive impairment; MCI: AD-range = mild cognitive impairment: Alzheimer's disease range PIB; MCI: norm-range = mild cognitive impairment: normal range PIB; Ant cingulate = anterior cingulate; Post cingulate = posterior cingulate.

($p = 0.67$). Grouped mean PK BPs in the subject groups are shown in table 3.

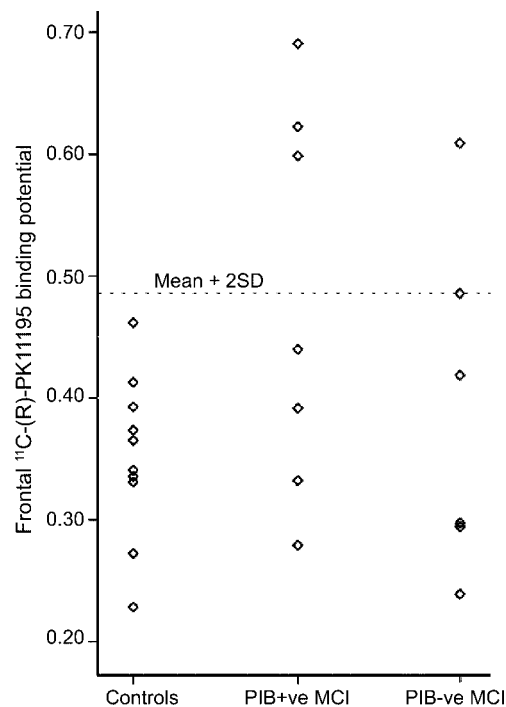
Individually, PK binding was increased in 5 of 13 (38%) subjects with MCI, three with AD-range and two with normal-range PIB binding (table e-1 on the *Neurology*[®] Web site at www.neurology.org). PK BP values in the MCI subgroups compared to that of controls in the frontal cortex are displayed in figure 1. PET images showing PK binding in patients with MCI are displayed in figure 2.

Correlations. We found no correlation between individual regional PK binding and PIB retention in our MCI groups. Additionally, no correlation was found between duration of symptoms or MMSE scores with either PIB retention or PK binding in our AD-PIB range subgroup. However, in the PIB-negative subgroup, lower MMSE scores correlated with higher ¹¹C-(R)-PK11195 binding in the anterior cingulate (Spearman rho, $\rho = -0.812$, $p < 0.05$) and temporal cortex (Spearman rho, $\rho = -0.812$, $p < 0.05$).

Follow-up. Five PIB-positive subjects with MCI have been followed for 24–36 months after their baseline PIB PET scan and 3 (60%) of these have clinically converted to AD. Three PIB-negative MCI subjects have also been followed up for 2–3 years and none have converted to AD, although they continue to fulfill the criteria for a diagnosis of MCI. These three include the two MCI subjects with regionally increased PK binding but normal PIB uptake.

DISCUSSION In this combined ¹¹C-PIB and ¹¹C-(R)-PK11195 PET study, we found that 7 (50%) of our 14 subjects with amnesic MCI had evidence of increased amyloid deposition. This is consistent with neuropathologic studies which report A β deposition in a significant proportion of subjects with MCI,^{29,30}

Figure 1 Scatterplot showing ¹¹C-(R)-PK11195 binding potentials in frontal cortex of controls, and mild cognitive impairment (MCI) subgroups; PIB-positive (+ve) and PIB-negative (-ve) MCI



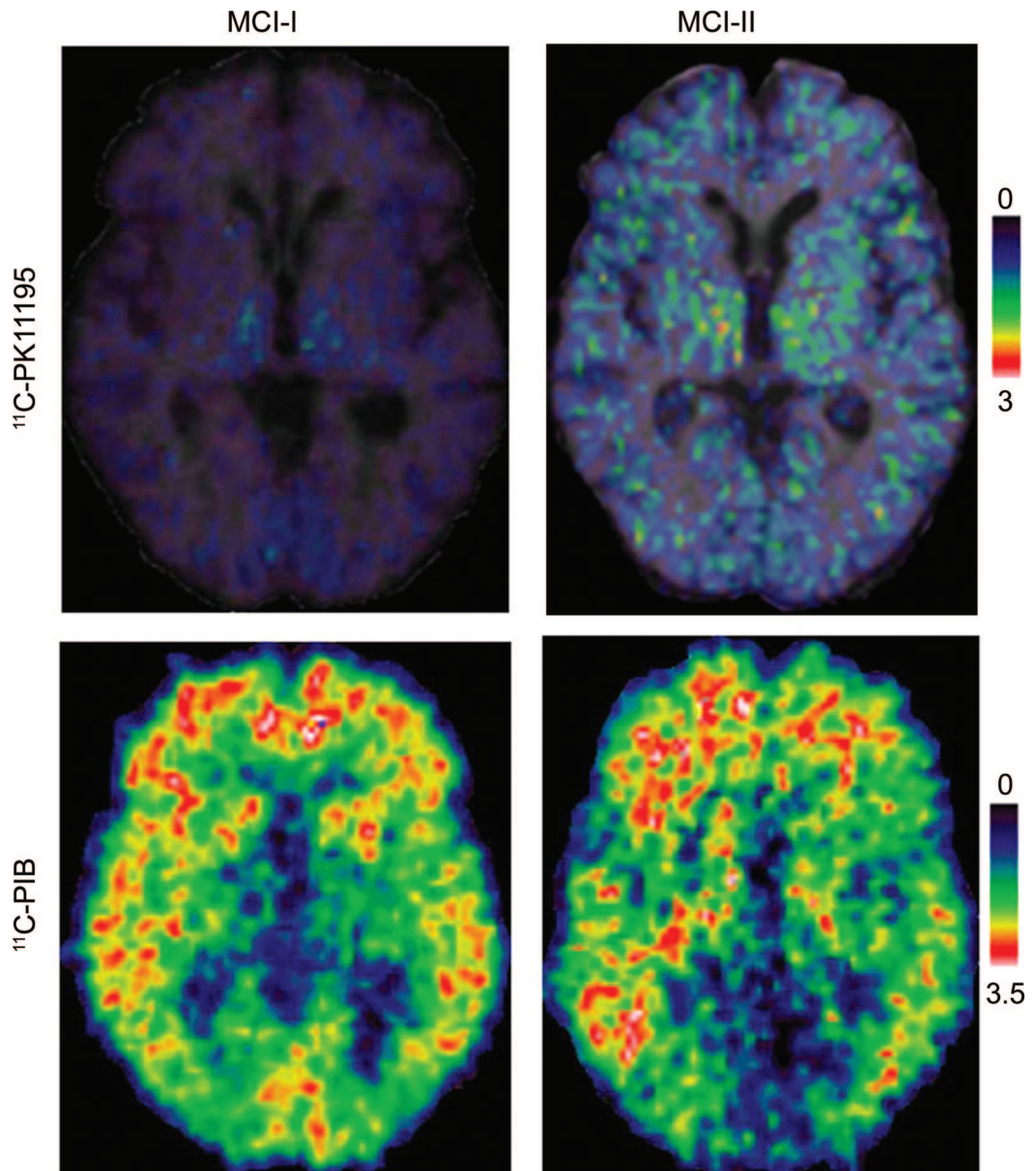
The dotted horizontal line shows controls' mean + 2SD.

and with the concept that amyloid deposition occurs early in the clinical evolution to AD. During a 2–3-year follow-up, three out of five of our PIB-positive but no PIB negative patients with MCI have converted to clinically probable AD. A longitudinal follow-up of the MCI cohort is ongoing.

Only one of 14 healthy controls showed increased PIB retention; this targeted the anterior cingulate and frontal and temporal cortices. Increased amyloid deposition has been reported in 10% of elderly healthy adults³¹ and its prevalence increases with age. Our relatively young control group (mean age 63.9 years; SD 5.3) might account for the low prevalence of significant PIB retention in our controls.

Compared to controls, our patients with MCI with increased PIB retention had significantly higher mean levels of cortical PK binding, although, after correction for multiple comparisons, only frontal PK uptake remained significantly raised, probably reflecting the low power of our small series. We detected regional increases in PK binding in our patients with MCI less frequently than raised PIB binding and there are a number of possible explanations for this. First, the specific signal associated with ¹¹C-(R)-PK11195 uptake is low compared with ¹¹C-PIB uptake, in part due to the lower density of acti-

Figure 2 ^{11}C -(R)-PK11195 binding potentials and corresponding PIB ratio images in two patients with PIB-positive mild cognitive impairment (MCI)



MCI-I has normal PK binding and MCI-II has increased PK binding.

vated microglia compared with amyloid plaques, as seen in AD,³² and also the higher background non-specific signal seen with PK compared to that seen with ^{11}C -PIB. This may have resulted in false negative findings in some patients. When delineating ROIs, we used a probabilistic atlas that takes into account variations in individual brain structures and atrophy. However, a limitation of this study is that we did not formally perform partial volume correction. This also may have resulted in an underestimation of cortical PK binding and false negative findings. Against this, we detected increased binding in the anterior and posterior cingulate, relatively small structures compared to our other interrogated ROIs. Finally, evidence exists for multiple factors

apart from $A\beta$ that may influence reactivity of microglia within brain, for example ApoE4 status³³ and region-specific differences in the reactivity of microglial populations.³⁴ We did not perform apolipoprotein E genotyping in our subjects. Different ApoE alleles influence amyloid load and so future PET studies may allow the interaction between ApoE genotype, amyloid deposition, and microglial activation to be explored in vivo.

An early ^{11}C -PK11195 PET study in patients with AD with a lower sensitivity PET camera failed to detect increased binding compared to controls.³⁵ However, there are a number of important methodologic differences between that and the present study. First, they used a cerebellar reference region where specific binding

can be found. This may have led to apparent absent binding in other brain areas. Second, their control group consisted predominantly of patients with small cerebral gliomas which can bind PK. Third, they used racemic PK11195 whereas we used the active (R)-enantiomer of PK11195 which has been shown to bind PK binding sites with nanomolar affinity.³⁶ More recently, SPECT¹⁵ and PET¹⁴ studies in patients with AD demonstrated significant increases in cortical PK binding, confirming this tracer is capable of detecting microglial activation in vivo. The PET study¹⁴ included one patient with isolated memory impairment who demonstrated increased binding in subregions of the temporal lobe, although ¹⁸F-FDG PET was normal at this time, suggesting that microglial activation can occur before metabolic deficits become evident, a viewpoint that is backed up by a histopathologic study in patients with AD.³⁷ Here, microglial activation along with A β and congophilic deposits were found in the middle frontal gyrus in the absence of neurofibrillary tangles. These observations and our finding of comparable levels of PK binding in our PIB-positive MCI and AD group provide a rationale for assessing microglial activation in patients with MCI, a significant proportion of whom already have amyloid deposition at the time of clinical presentation.

We found no correlation between individual levels of amyloid deposition, as measured by ¹¹C-PIB PET, and microglial activation, as assessed by ¹¹C-(R)-PK11195 PET. This is in contrast to immunohistochemistry studies which have found activated microglial cells clustered at sites of neuritic plaques. However, it has also been demonstrated that not all amyloid plaques are associated with surrounding microglia,³⁸ again implying that multiple factors may be involved in microglial activation as a response to amyloid pathology. Additionally, we found no correlation between MMSE scores or disease duration with PIB retention and PK binding in our PIB-positive subgroup. It is likely, however, that MMSE and other neuropsychometry measures in these patients are influenced by additional pathology such as the presence or absence of neurofibrillary tangles. The recent development of higher affinity PET radioligands which bind to the PBBS, such as ¹¹C-DAA1106³⁹ and ¹¹C-vinpocetine,⁴⁰ may provide greater sensitivity for assessing and quantifying microglial activation in cognitive impairment and allow one to further explore the relationship between microglial activation, fibrillar amyloid, and cognition. The follow-up of PIB-positive patients with MCI with in vivo PBBS markers will enable a better understanding of the time course of microglial activation and its significance as a predictor of clinical progression to AD, and provide the opportunity to

monitor the pathophysiologic effects of anti-inflammatory agents in vivo.

Finally, two subjects with MCI with increased PK binding had normal PIB uptake. The first patient (MCI-5) was a 70-year-old woman who had small focal increases in PK binding in the frontal and parietal cortices. Since her symptoms began 5 years ago she has shown no progression in her symptoms, supported by annual clinical, neuropsychological, and neuroimaging assessments. While these do not suggest a neurodegenerative cause for her memory impairment, her diagnosis, and the cause for the PK findings, remains unclear. Continued follow-up will be important in assessing its significance. The second patient (MCI-3), who had increased PK binding in all cortical regions of interest, was a 74-year-old man with a history of memory impairment dating back at least 5 years. This was associated with ventricular enlargement on magnetic resonance neuroimaging but annual clinical and neuropsychological assessments showed no convincing progression over this time. Subsequently he suffered a subacute deterioration in his cognition associated with increasing ventricular enlargement. A ventriculoperitoneal shunt was inserted which led to an improvement in his symptoms. An underlying neurodegenerative condition, again, seems unlikely given the long period of stability in his symptoms. A more definitive diagnosis has not yet been made and it remains to be seen whether shunting remains beneficial over the long term.

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