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Brain inflammation accompanies amyloid in a majority of mild cognitive impairment cases due to Alzheimer’s disease

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Title:
Brain inflammation accompanies amyloid in a majority of mild cognitive impairment cases due to Alzheimer’s disease.

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Abstract:
Subjects with amnestic mild cognitive impairment (MCI) associated with cortical beta-amyloid (Aβ) have a greatly increased risk of progressing to Alzheimer’s disease. We hypothesised that neuroinflammation occurs early in Alzheimer’s disease and would be present in most Aβ positive MCI cases. $^{11}$C-PiB (PiB) and $^{11}$C-(R)-PK11195 (PK11195) PET was used to determine the Aβ load and detect the extent of neuroinflammation (microglial activation) in 42 MCI cases. 12 age-matched healthy controls (HC) had PiB PET and 10 HC had PK11195 PET for comparison. PiB-positivity was defined as target-to-cerebellar ratio above 1.5 within a composite cortical volume-of-interest (VOI). Supervised cluster analysis was used to generate parametric maps of PK11195 binding potential (BP). Levels of PK11195 BP were measured in a composite cortical VOI and at a voxel level. Twenty-six of 42 (62%) MCI cases showed a raised cortical PiB RATIO compared to HC. Seventeen (65%) of these 26 PiB-positive MCI cases showed clusters of increased cortical microglial activation accompanying the amyloid. There was a positive correlation between levels of PiB RATIO and PK11195 BP at a voxel level within subregions of frontal, parietal and temporal cortices. PK11195 PET reveals increased inflammation in a majority of Aβ positive MCI cases, its cortical distribution overlapping that of Aβ deposition.

Abbreviations:
Aβ = beta-amyloid; BP = binding potential; BPM = biological parametric mapping; CDR = Clinical Dementia Rating; HC = healthy control; GM = grey matter; MBq = megabecquerel; MCI = mild cognitive impairment; MMSE = mini-mental state examination; SPM = statistical parametric mapping; VOI = volume-of-interest.
Introduction

Alzheimer’s disease pathology is characterised by abnormal aggregation of the proteins beta-amyloid (Aβ) and hyperphosphorylated tau (Braak and Braak, 1991). The Aβ fibrils form extracellular beta-sheeted plaques while hyperphosphorylated tau aggregates intracellularly as neurofibrillary tangles (NFTs) composed of insoluble paired helical filaments (PHF) of tau containing both 3- and 4-tubulin site repeats. In the last decade positron emission tomography (PET) radiotracers have become available to image in vivo aggregated Aβ (Klunk et al., 2004) and PHF-tau protein (Xia et al., 2013; Chien et al., 2013). Brain inflammation in the form of microglial activation, its intrinsic immune defence, is also a component of Alzheimer’s disease and it has been suggested that it could drive the neurodegenerative processes via cytokine release which promotes tau hyperphosphorylation (Maphis et al., 2015). However, initially microglial activation may play a protective role in prodromal Alzheimer’s disease by clearing amyloid, remodelling connections and releasing growth factors (Hamelin et al., 2016). The exact role of the microglial activation in dementias remains uncertain, as does the timing of its response relative to the deposition of Aβ and hyperphosphorylated tau. Activated microglia express translocator protein 18kDa (TSPO) on the outer membrane of their mitochondria. TSPO has an isoquinoline site which binds 11C-(R)-PK11195. Varying extents and levels of microglial activation have been reported using TSPO PET imaging in groups of patients with clinically probable Alzheimer’s disease and cases with amnestic mild cognitive impairment (MCI) (Stefaniak and O’Brien, 2015). Recent PET studies have reported both raised and absent baseline TSPO binding in MCI cases (Okello et al., 2009) (Fan et al., 2015) (Hamelin et al., 2016) (Kreisl et al., 2013). Where inflammation is present in Alzheimer’s disease it can be seen in areas with high Aβ deposition such as frontal cortex and anterior cingulate and with high NFTs density such as medial temporal cortex and hippocampus (Fan et al., 2015).

Subjects with mild cognitive impairment (MCI) have an increased risk of dementia and the amnestic subtype is most likely to progress to Alzheimer’s disease (Petersen, 2004). The presence of biomarkers such as hippocampal atrophy, low Aβ42 in cerebrospinal fluid (CSF), and positive Aβ PET increases the likelihood the MCI being due to Alzheimer’s pathology (Albert et al., 2011). Identifying early stages of Alzheimer’s disease is of interest, as potential disease-modifying drugs are likely to have the greatest impact if administered in the early or preclinical stages of Alzheimer’s disease. Anti-inflammatory drugs have been suggested as a way of modifying Alzheimer’s disease progression (Heneka et al., 2015).
In this study, we hypothesised that amnestic MCI cases with PET evidence of cortical Aβ deposition would also show cortical microglial activation detectable with $^{11}$C-(R)-PK11195 PET compared with MCI cases without raised cortical Aβ.

**Materials and methods**

**Study subjects**

MCI subjects were recruited from Dementia/Memory clinics in Jutland and Funen, Denmark, and by newspaper advertisements. Subjects were included if they presented with a history of declining memory function over a minimum of 6 months, preferably corroborated by an informant and in the absence of a history of recreational drug use, sedative medication, depression, stroke or systemic diseases. Further inclusion criteria were: 1) Age 50-85 years; 2) ≥7 years of education or good working history; 3) Meets Petersen criteria (Petersen, 2004) for amnestic MCI (no strict memory score cut-off was used); 4) An informant was available who had frequent contact with the subject and could accompany the subject to clinic visits or be available to talk on the telephone about the subject’s memory and complete the interview for Clinical Dementia Rating (CDR); 5) Modified Hachinski Ischemic Scale score ≤ 4; 6) MMSE score 24-30; 7) Geriatric Depression Scale (GDS-15) score ≤ 6; 8) An MRI examination that excluded MCI arising from structural causes.

Exclusion criteria were: 1) Significant neurologic or psychiatric diseases; 2) history of alcohol and/or recreational drug abuse within 2 years; 3) contraindications to MRI; 4) significant reductions in serum B12, red cell folate or thyroid function; 5) use of medication with known anticholinergic effects (which could impair memory) within the last 3 months or a drug that could impair cognition. Age-matched healthy controls (HC) were recruited by newspaper advertisements and screened for neurological diseases. The same inclusion/exclusion criteria as MCI were applied, except that HC had no complaints of memory decline.

The Central Denmark Region Committees on Health Research Ethics approved the study in accordance with the declaration of Helsinki. All participants signed an informed written consent at enrolment in the study.
MRI
Magnetic resonance imaging (MRI) was performed on a Skyra 3 Tesla system (Siemens, Erlangen, Germany). A MP2RAGE (Magnetization Prepared Rapid Gradient-Echo with two gradient echo images) (Marques et al., 2010) sequence was used for co-registration of MRI with PET, normalisation into standard space, and generation of grey matter (GM) masks. An experienced neuroradiologist visually evaluated all the MRIs.

PET
All PET scans were acquired on a High Resolution Research Tomograph (ECAT HRRT; CTI/Siemens, Knoxville, TN, USA). A 6-minute transmission scan was performed prior to each PET emission scan to enable attenuation correction of emission data. Images were reconstructed with a 3D-OSEM (ordered subset expectation maximum) with 10 iterations and 16 subsets. Point-spread function (PSF) reconstruction was applied to minimise partial volume effects, improve image quality, contrast and quantitative accuracy and achieve a reconstructed resolution of 2.5 mm. Images were not partial volume corrected.

Amyloid imaging (PiB PET):
A mean dose of 391 MBq (SD=63) 11C-PiB (Pittsburgh compound B, N-methyl-[11C]2-(4-methylaminophenyl)-6-hydroxybenzothiazole) (PiB) was injected intravenously over 10 seconds, followed by a 10 mL saline flush. Subjects rested for 30 minutes after injection before installation in scanner. PET was acquired for 50 minutes in list mode at 40-90 minutes post injection (p.i.). Image data were subsequently re-binned into 5 frames of 10 minutes each.

TSPO imaging (11C-(R)-PK11195 PET):
A mean dose of 390 MBq (SD=47) 11C-(R)-PK11195 (1-[2-chlorophenyl]-N-methyl-N-[1-methylpropyl]-3-isoquinoline carboxamide) (PK11195) was injected intravenously over 10 seconds, followed by a 10 mL saline flush. Emission scans were initiated with a 30 second “background” frame before injection of PK11195. The total dynamic scan time was 60.5 minutes (list mode). Frames were re-binned as: 1x 30 seconds ‘background’, 6x 10s, 2x 30s, 2x 60s, 3x 120s, 10x 300s.

Image analysis
MRI volumes were segmented into grey (GM) and white (WM) matter images and CSF using MINC software (http://en.wikibooks.org/wiki/MINC). The GM masks were convolved with a probabilistic atlas (Hammers et al., 2003) to “individualise” subject VOIs to their GM. The composite VOI was a weighted average of 5 bilateral regions (inferolateral parietal cortex, inferior
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frontal gyri, middle and inferior temporal gyri, posterior cingulate gyrus and parahippocampal gyrus). Within this composite VOI we compared levels of PK11195 BP_{ND} in PiB-positive MCI cases to those of PiB-negative MCI cases and HC subjects. An average GM mask from MCI and healthy subjects was used for explicit masking with Statistical Parametric Mapping 8 (SPM8; Wellcome Trust Centre for Neuroimaging).

\[ ^{11} \text{C-PiB RATIO} \]

PiB images of each individual were co-registered to their T1 MR images, and then the transformation matrix from the individuals T1 space to MNI space were applied to the PET images using MINC tools. The spatially normalised PiB images were summed from 60-90 minutes, and voxel signals divided by the mean signal from the individual’s cerebellar GM VOI to generate PiB RATIO images (Edison et al., 2007). Images were not smoothed before extraction of measurements from the composite cortical VOI, to minimise spill-in/spill-out. PiB-positivity was defined from the bimodal MCI distribution as a composite cortical PiB RATIO>1.5. PiB RATIO images were smoothed with a 6 mm FWHM Gaussian filter prior to SPM and BPM analyses.

PK11195 binding potential maps

After smoothing the dynamic PET images with a 4 mm full width at half maximum (FWHM) three-dimensional Gaussian filter, parametric maps of binding potential (BP_{ND}) were generated at a voxel level using the Simplified Reference Tissue Model (SRTM) (Lammertsma and Hume, 1996) implemented in Matlab. As all anatomical regions in the brain can show specific PK11195 binding in Alzheimer’s disease, a Supervised Cluster Analysis with 6-classes (Turkheimer et al., 2007) (SVCA6) was used to localise a cluster of voxels from the dynamic images of each MCI case which provided a reference tissue input function representing normal grey matter kinetics. The PK11195 images were spatially normalised into MNI space in the same manner as described for the PiB images. PK11195 images were smoothed with a 6 mm FWHM Gaussian filter prior to SPM and BPM analyses.

Statistical Parametric Mapping and Biological Parametric Mapping

Clusters of increased PK11195 binding are scattered and so a VOI approach based on Brodmann regions of the brain will include both voxels with raised and normal PK11195 signal. Alongside a pre-defined VOI approach, parametric maps of PK11195 BP_{ND} were interrogated with SPM to detect clusters of voxels with significantly increased PK11195 BP_{ND} in the PiB-positive MCI group.
compared to the HC group. The amplitude of increased microglial activation in these clusters of increased PK11195 BP<sub>ND</sub> was then quantified, thus avoiding dilution by partial volume effects from surrounding normal voxels - a problem with anatomically based VOIs. SPMs comparing PiB-positive MCI > HC, PiB-positive > PiB-negative MCI, and PiB-negative MCI > HC were generated within a mask defined by the voxels where an ANOVA (uncorrected p<0.001) showed a significant mean difference between these three groups (Friston et al., 2006). This map was subsequently thresholded at p<0.001 to identify the cluster size corresponding to an FWE corrected cluster level p-value<0.05. This cluster extent was then used as a threshold to construct the final SPMs.

Biological Parametric Mapping (Casanova et al., 2007) (BPM toolbox running in SPM5) was used to detect voxels where there was a positive correlation between individual z-scores of PiB RATIO and PK11195 BP<sub>ND</sub> in PiB-positive MCI cases. The z-score maps were generated for each PiB-positive MCI case using mean and standard deviation (SD) values from PiB RATIO and PK11195 BP<sub>ND</sub> maps of healthy controls (PiB n=10 HC, PK11195 n=10 HC).

Statistical analysis
Data were analysed using STATA version 13.1 (StataCorp LP, Texas, USA). The Wilcoxon rank-sum (Mann-Whitney) test was used for between group comparisons of CDR sum of box scores. P-values < 0.05 were considered statistically significant. A one-way ANOVA was employed to determine whether cortical levels of PK11195 BP<sub>ND</sub> in the VOIs were different between PiB-positive MCI, PiB-negative MCI, and HC cohorts. Tukey post-hoc tests were used for between group comparisons of means across the three groups (Tukey's HSD pairwise comparisons; UCLA Academic Technology Services).

Results
The MCI cohort comprised 42 subjects (mean age 70 years) who had both PiB and PK11195 PET. Twelve healthy controls (HC) had PiB PET (mean age 69) and 10 healthy controls had PK11195 PET (mean age 68) (see table 1 for further details). Seven controls had both PiB and PK11195 PET. Four MCI cases were excluded due to incidental findings (a meningioma, an arterial aneurism, previous stroke, and an arteriovenous malformation). One healthy control was found to have a meningioma and was subsequently excluded.

Twenty-six (62%) of the 42 MCI cases had a composite cortical PiB RATIO>1.5 and were categorised as PiB-positive. Two (17%) of the 12 controls were also PiB-positive (Fig. 1) and so represented outliers. The percentage of MCI cases that were PiB-positive differed depending on the
source for recruitment: Nineteen (79%) of 24 MCI cases referred from Dementia Clinics were PiB-positive compared with only 7 (39%) of 18 MCI cases recruited via advertisements. The mean CDR sum of boxes differed across the cohorts of PiB-positive and PiB-negative MCI cases (1.9 versus 1.0; \( p=0.022 \)), and between clinic- versus advertisement-derived samples (2.0 versus 1.0, \( p=0.0015 \)).

Compared to healthy controls, the 26 PiB-positive MCI cases showed a mean 1.36 fold increase in composite VOI PK11195 BP\(_{\text{ND}}\) (0.13 ± 0.086 vs. 0.055 ± 0.064 (mean ± SD)) (Fig. 2). Mean PK11195 BP\(_{\text{ND}}\) was significantly different across the three groups as determined by a one-way ANOVA (\( F(2,49)=8.83, p=0.0005 \)). A Tukey post-hoc test revealed that mean PK11195 BP\(_{\text{ND}}\) was significantly higher in PiB-positive MCI compared to the controls \( (p=0.037) \), and compared to PiB-negative MCI \( (p=0.001) \). However, there was no significant difference in mean PK11195 BP\(_{\text{ND}}\) between the PiB-negative MCI cases and controls \( (p=0.71) \).

To obtain a truer estimate of the amplitude of focal PK11195 BP\(_{\text{ND}}\) rises in PiB-positive MCI cases, we measured the PK11195 BP\(_{\text{ND}}\) within the clusters of voxels of raised BP\(_{\text{ND}}\) localised by statistical parametric mapping when interrogating the 26 PiB-positive MCI cases versus 10 HC (Fig. 3-C). SPM identified clusters of significantly raised PK11195 BP\(_{\text{ND}}\) in 65% of PiB-positive MCI individuals (Fig. 4). Because of differences in mean age and CDR sum of boxes between the PiB-positive and PiB–negative MCI group (table 1), ANCOVA was performed to extract the variance in PK11195 BP\(_{\text{ND}}\) at a voxel level arising due to these factors. The age and CDR sum of boxes corrected SPMs and uncorrected SPMs showed similar distributions of voxels with significantly raised PK11195 BP\(_{\text{ND}}\) for the PiB-positive MCI cases.

BPM was used to localise voxels where individual levels of PiB RATIO and PK11195 BP\(_{\text{ND}}\) had a positive correlation in the group of 26 PiB-positive MCI cases. This approach detected clusters of correlation in subregions of frontal, temporal and parietal cortices (Fig. 5-A) which differed from the areas of raised PK11195 binding identified in PiB-positive MCI with SPM in that the emphasis was more posterior (Fig. 3-B and 3-C).
Discussion

This report provides evidence that neuroinflammation is a component of the neurodegenerative pathology in a majority of Aβ positive MCI, the cases who are most likely to progress to Alzheimer’s dementia. We detected microglial activation in two-thirds of our PiB-positive MCI cases, however, other studies have failed to detect baseline microglial activation in MCI (Klunk et al., 2009) (Kreisl et al., 2013). Klunk et al. supported the viewpoint that microglia activation is an early feature in Alzheimer’s disease, but concluded that their ¹¹C-(R)-PK11195 PET study lacked the sensitivity to detect it. Our ability to demonstrate a high prevalence of microglial activation in prodromal Alzheimer’s disease could reflect the combined use of a high sensitivity HRRT scanner, a more sensitive SVCA6 kinetic modelling approach, and a larger sample size.

Nine (35%) of our PiB-positive MCI subjects had PK11195 BPₙₚ values within the upper normal range. It is, therefore, possible to have amyloid deposition without significant neuroinflammation and these MCI cases may in the future progress less rapidly than those with both amyloid plaques and microglial activation evident on PET scanning. However, our cases will have been scanned at different time points of their disease trajectory and, if inflammation declines as MCI progresses – as suggested in a recent review (Calsolaro and Edison, 2016) and by findings from a recent longitudinal study using ¹⁸F-DPA714 PET (Hamelin et al., 2016), this could also account for negative findings in a minority of our cases. To gain more knowledge concerning this we will follow our MCI cohort for minimum of 2 years and re-scan them.

The PiB-negative MCI cases all had PK11195 BPₙₚ values within the HC range and it is still unclear whether such cases have neurodegenerative pathology and will progress to dementia on follow-up. PiB-negative MCI cases represent a heterogeneous group and their memory problems can arise from non-Alzheimer pathologies such as frontotemporal dementia or vascular disease or non-degenerative conditions such as stress, depression, or sleep deprivation, even though we have tried to exclude these. Interestingly, two of our normal controls were PiB-positive outliers and they showed no evidence of raised microglial activation. This would favour inflammation arising as a secondary event to Aβ aggregation.

BPM localised a significant correlation between levels of amyloid and levels of inflammation in some brain clusters. These clusters differed from those detected by a between group SPM comparing MCI cases with controls. This is because BPM identifies voxels where inflammation and amyloid levels are correlated as opposed to voxels where there is a mean increase in inflammation due to any cause. The BPM findings link amyloid deposition and inflammation, particularly in more posterior brain regions. However, we cannot exclude that other pathologies
may be influencing inflammation with a different distribution to amyloid. A study correlating tau deposition with inflammation is in progress.

Interestingly, the prevalence of PiB-positivity was 79% in the MCI cases derived from memory clinics, but only 39% in advertisement-derived MCI cases. Along with the high prevalence of PiB-positivity, the clinic sample also showed a higher mean CDR sum of boxes compared to MCI cases recruited by advertisements. This is in line with prior studies (Farias et al., 2009) and may be relevant to subsequent progression. As this study was designed to investigate the relationship between Aβ load and levels of microglial activation (PiB and PK11195 PET) within a group of PiB-positive MCI cases, we believe that mixing MCI cases from different sources is not a problem with regard to interpreting the relationship between Alzheimer pathologies in our cases.

The conservative PiB RATIO cut-off at 1.5 was defined from the bimodal distribution of PiB uptake in our MCI cases and chosen to ensure we identified a prodromal Alzheimer’s disease group with a significant level of cortical amyloid. Additionally, the use of a HRRT scanner provides images with higher sensitivity than the conventional PET-CT cameras usually employed in PiB PET findings. Use of the HRRT may also provide a greater specific cortical signal due to reduced spill-in to cerebellar grey matter, resulting in higher ratios measured within cerebral cortical regions.

In summary, this study provides supportive evidence that neuroinflammation is a component of the neurodegenerative pathology in a majority of MCI cases due to Alzheimer’s disease. PK11195 PET imaging of our amnestic MCI cohort showed neuroinflammation in 65% of Aβ positive MCI (prodromal Alzheimer’s disease) subjects but we found no evidence of inflammation in the Aβ negative MCI subjects and healthy controls. The distribution of neuroinflammation in MCI mirrored the distribution of Aβ in the frontal, temporal and parietal cortices. However, the temporal order of these pathologies still needs to be further investigated and it will probably require longitudinal PET studies of high-risk normal subjects. Our MCI cohort will be followed for a minimum of 2 years and rescanned to determine whether neuroinflammation declines with progression towards Alzheimer’s disease. On occasion raised Aβ can be detected in MCI and normal subjects without evidence of neuroinflammation. Follow-up of such subjects may determine whether they have a more benign syndrome than MCI subjects with both Aβ deposition and neuroinflammation present.
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References


Table 1: Subject details.

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Table 1: Subject details. *Two-sample t-test p<0.05 for PIB-positive MCI > PIB-negative MCI.
*Two-sample t-test p<0.05 for PIB-positive MCI < Controls. *n=12 PIB controls. 5n=10 PK11195 controls. CDR=clinical dementia rating; MBq=megabecquerel; MCI=mild cognitive impairment; MMSE=mini-mental state examination; PIB=Pittsburgh compound B; SD=standard deviation.
Figure 1: Plot of 11C-PiB RATIO in composite cortical VOI. Twenty-six (62%) of MCI cases were above the 1.5 cut-off line, and hence classified as PiB-positive MCI. The two PiB-positive HC are marked with the letters ‘A’ and ‘B’ for identification in figure 2.
Figure 2: Plot of PK11195 BPND in composite cortical VOI over groups of PiB-positive and PiB-negative MCI, and HC. One-way ANOVA test and post-hoc pairwise comparisons demonstrated significant group differences (see details in results section). Solid lines are group means; dotted lines indicate ±1SD; HC marked with the letters ‘A’ and ‘B’ correspond to the two PiB-positive HC marked in figure 1.
Figure 3: Statistical parametric mapping of $^{11}$C-PK11195 BPND. A: The ANOVA with a F-contrast display clusters of voxels with any difference between the three groups (PiB-positive MCI, PiB-negative MCI and HC), uncorrected $p<0.001$. B-D: Two-sample t-tests between PiB-positive and PiB-negative MCI, PiB-positive MCI and HC, and between PiB-negative MCI and HC, the displayed clusters are of significant size (FWE cluster-level $p<0.05$, with a cluster defining threshold of $p<0.001$).

179x179mm (300 x 300 DPI)
Figure 4: Plot of PK11195 BPND in resulting clusters from SPM analysis (Fig. 3-C). Seventeen (65\%) of 26 PiB-positive MCI cases had PK11195 binding levels >2SD above mean of HC. PiB-negative MCI subjects are measured within the same resulting clusters seen in fig. 3-C, and added to the plot. Short solid lines are group means; dotted lines indicate ±1SD; solid horizontal line at y=0.117 marks mean + 2SD of the HC group.
Figure 5: Biological Parametric Mapping (BPM) of PiB RATIO and PK11195 BPND. A: Clusters of voxels with a positive correlation between PiB RATIO and PK11195 BPND within 26 PiB-positive MCI cases. Voxel-level uncorrected p<0.001, cluster-level corrected at p<0.05. B and C: Two clusters are picked for extraction of individual measures of the 26 PiB-positive MCI cases (B, right inferior frontal gyrus; C, left superior temporal gyrus).

179x179mm (300 x 300 DPI)