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RESEARCH ARTICLE

Microglial responses partially mediate the effect of Aβ on cognition in Alzheimer's disease

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Abstract

INTRODUCTION: Microglial responses are an integral part of Alzheimer's disease (AD) pathology and are associated with amyloid beta (Aβ) deposition. This study aimed to investigate the effects of Aβ and microglial responses on global cognitive impairment.

METHODS: In this longitudinal study, 28 patients with mild cognitive impairment and 11 healthy controls underwent ^{11}C-PK11195 and ^{11}C-Pittsburgh compound B positron emission tomography (PET), structural magnetic resonance imaging scans, and global cognitive ratings at baseline and 2-year follow-up. Correlations between PET uptake and global cognition were assessed. Additionally, the mediation effect of the microglial response on the association between Aβ load and global cognition was assessed.

RESULTS: Aβ load and the microglial response were both independently detrimental to global cognitive performance at baseline; however, at 2-year follow-up the association between Aβ load and global cognitive ratings was partially mediated by the microglial response.

DISCUSSION: As AD progresses, the associated microglial response partially mediates the detrimental effect of aggregated Aβ on cognition.

KEYWORDS
Alzheimer's disease, amyloid beta, cognition, mild cognitive impairment, PK11195, position emission tomography, translocator protein

Highlights

- This was a longitudinal study of amyloid beta (Aβ), microglial responses, and global cognitive performance.
- Aβ and microglial responses both affect cognition in early Alzheimer's disease.
- Microglial response partially mediates the effect of Aβ on cognition in later stages.

Lasse S. Madsen and Rola Ismail contributed equally to this work.

1 | BACKGROUND

Alzheimer's disease (AD) is an irreversible neurodegenerative disorder, characterized clinically by progressive cognitive and functional impairment which leads to dementia and death. The characteristic brain cortical pathologies are the accumulation of extracellular amyloid beta (Aβ) fibrllar plaques followed by intracellular paired helical tau filaments in the form of neurofibrillary tau tangles. Both Aβ plaques and tau tangles can be surrounded by activated astrocytes and microglial cells, some of which have an inflammatory phenotype and release toxic cytokines.[1,2]

Cognitive impairment due to AD pathology progressively develops over several years before the onset of clinical dementia. Mild cognitive impairment (MCI) has been defined as a transitional syndrome between cognitive health and dementia. MCI patients experience cognitive deficits that are not severe enough to impair independent activities of daily living but are greater than those associated with normal aging.[3] The early MCI stage of AD is associated with increased levels of cortical Aβ and activated microglia, with the build-up of cortical tau tangles occurring later.[4]

Although Aβ plaques are believed to play a key role in triggering AD pathogenesis, the severity of cognitive impairment and cortical atrophy in dementia is more strongly associated with the tau tangle load.[5] Oligomers of Aβ have, however, been shown to be toxic to synapses. Whether Aβ aggregates themselves cause the neuronal damage seen in early AD or whether it is mediated by connected mechanisms such as toxic microglial activation remains a subject of debate.[6] Evidence suggests that the risk of patients with MCI converting to dementia is increased when elevated pro-inflammatory cytokines, such as tumor necrosis factor alpha and interleukin 1b, are detected in the blood.[7]

Human positron emission tomography (PET) imaging studies, detecting the 18 kDa translocator protein (TSPO), have shown a correlation between impairment of cognitive performance and elevated temporo-parietal levels of TSPO PET signals in patients with AD dementia.[8,9] Conversely, in early MCI patients with raised levels of cortical Aβ deposition, higher baseline levels of TSPO PET signal have been associated with slower cognitive decline.[10] In transgenic mouse models of AD, it has been demonstrated that activated microglia can act to reduce amyloid pathology and even improve memory.[11]

The exact quantitative interpretation of the TSPO PET signal remains under debate. While it has commonly been referred to as a marker of microglia activation based on rodent studies, recent studies have shown that in humans, the TSPO PET signal is more reflective of the density rather than the activation of microglia.[12] In this paper, we will refer to raised TSPO PET signals as reflecting microglial responses.

Because of the non-specific binding of TSPO to microglial phenotypes and the divergent results from human TSPO PET studies, the exact role of microglia in AD remains unclear. One major theory is that microglia may adopt either a protective or neurotoxic phenotype depending on the disease stage.[13,14] According to this theory, microglia initially have a protective phagocytic phenotype attempting to engulf Aβ fibrils in early MCI due to AD stages. This then fails and later, as tau tangles accumulate, a second peak of microglial response occurs with a detrimental inflammatory phenotype releasing cytokines. This may then drive disease progression alongside tau tangle formation, ultimately impairing cognition.[14–16]

However, the individual contributions of Aβ accumulation and microglial responses to cognitive impairment in early AD remain to be determined. The aim of this present study was to investigate the relationships among ratings of global cognition in MCI due to AD and cortical Aβ fibril deposition, tau tangle load, and levels of microglial response. This was achieved by studying MCI patients with raised levels of cortical Aβ over a 2-year follow-up period while assessing the level of Aβ, tau, and the microglial response with PET, and cognitive function with a battery of neuropsychological tests. We hypothesized that the association between the microglial response and cognitive decline would increase over a 2-year follow-up period, and that the microglial response would mediate the effect of fibrillar Aβ on cognitive impairment, especially in later disease stages.

2 | METHODS

2.1 | Subject inclusion

A total of 43 patients with MCI were recruited from national memory clinics in Denmark and by newspaper advertisements. Inclusion criteria were: (1) age between 50 and 85 years; (2) a history of memory decline for at least 6 months; (3) meeting the Petersen criteria for amnestic MCI,[3] that is, objective memory impairment and memory complaints but not demented and able to perform activities of daily living; (4) a Mini-Mental State Examination (MMSE) score > 24; and (5) no history of depression, stroke, or systemic diseases. Additionally, 23 healthy age-matched control subjects were recruited with the same inclusion criteria, except they had no memory or other cognitive complaints. Details of the subject recruitment are described in Parbo et al.[17] 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.

2.2 | Neuropsychological assessment

Patients were assessed with the Montreal Cognitive Assessment (MoCA), the MMSE, and the Clinical Dementia Rating Sum of Boxes (CDR-SB). Details of the full neuropsychological assessment are provided in Parbo et al.[17]

2.3 | Magnetic resonance imaging

Magnetic resonance imaging (MRI) was acquired on a 3T Siemens Skyra scanner using a 32-channel head coil. All subjects had a structural T1-weighted MP2RAGE[18] image (1 mm isotropic voxels, repetition time = 5 seconds, echo time = 2.98 seconds, inversion time

RESEARCH IN CONTEXT

1. **Systematic review**: The authors conducted a literature review on microglial responses, amyloid beta (Aβ), and their interaction with global cognitive ratings in early Alzheimer's disease (AD) using traditional search engines like PubMed. While the role of Aβ in AD development has been studied extensively, the interaction between Aβ and the associated microglial response and how they affect disease progression and cognitive decline remains uncertain.
2. **Interpretation**: This study shows that Aβ and the microglial response both have a direct detrimental effect on global cognitive performance in early AD. However, as the disease progresses, the effect of Aβ on global cognitive performance becomes partially mediated by the microglial response.
3. **Future directions**: Future studies should interrogate the relationship among the microglial response, Aβ, and tau deposition in a longitudinal design before symptom onset. A better understanding of the interactions among the different pathological aspects in the preclinical disease stages could help identify novel treatment strategies.

$(TI)_1$ = 0.7 seconds, TI_2 = 2.5 seconds, 4° and 5° flip angles, acquisition matrix 240 × 256 × 176). The T1-weighted images were first denoised and bias-field corrected and then transformed into Montreal Neurological Institute (MNI) space and skull stripped. The images were segmented into gray matter, white matter, and cerebrospinal fluid, and a probabilistic atlas was used to define specific structures including cerebellar, frontal, parietal, temporal, and occipital gray and white matter.[19]

2.4 | Positron emission tomography

PET data in list mode were acquired in 3D with a Siemens High-Resolution Research Tomograph. The level of fibrillar Aβ was measured using the ^{11}C-Pittsburgh compound B (^{11}C-PiB) tracer, the level of the microglial response was assessed using the TSPO marker ^{11}C-(R)-PK11195, and tau tangle load was assessed using ^{18}F-Flortaucipir (^{18}F-FTP).

While ^{11}C-PiB and ^{18}F-FTP bind directly to their target of interest, namely fibrillar Aβ plaques and phosphorylated tau (p-tau) tangles, ^{11}C-PK11195 binds to the TSPO (peripheral benzodiazepine receptor) located on the outer mitochondrial membrane within microglial cells. However, the TSPO signal is not specific to microglia and can be expressed by astrocytes and endothelial cells as well.[20] Recent studies indicate that the TSPO marker does not specifically represent the activation of microglia in humans, which has commonly been assumed, but is more reflective of the total density of inflammatory cells.[12] Hence, the exact biological interpretation of the ^{11}C-PK11195 signal is elusive and is referred to as the microglial response in this paper.

All PET images were attenuation corrected and reconstructed with an isotropic resolution of 2.5 mm. All PET tracers were delivered intravenously over 10 seconds followed by a 10 mL saline flush. A mean 391 MBq dose of ^{11}C-PiB was injected and dynamic PET data were acquired as five time frames (5 × 10 minutes) 40 to 90 minutes post-injection. A mean 390 MBq dose of ^{11}C-PK11195 was injected and dynamic PET was acquired and binned as 23 time frames (6 × 10 seconds, 2 × 30 seconds, 2 × 60 seconds 3 × 120 seconds, 10 × 300 seconds) starting 30 seconds post-injection. A mean 364 MBq dose of ^{18}F-FTP was injected and dynamic PET was acquired as 8 time frames (8 × 5 minutes) 80 to 120 minutes post-injection.

2.4.1 | PET processing

Individual PET images were co-registered to their corresponding T1-weighted MRI. The 60 to 90 minute ^{11}C-PiB PET and 80 to 100 minute ^{18}F-FTP PET images were motion corrected, averaged, and divided by the mean value within the cerebellar gray matter to produce parametric images of standardized uptake value ratios (SUVRs), which were subsequentially smoothed with a 3 mm full width half maximum (FWHM) 3D Gaussian filter. The ^{11}C-PK11195 PET images were processed as previously described.[17] Briefly, the images were smoothed with a 4 mm FWHM 3D Gaussian filter prior to the generation of parametric images of binding potential (BP) using a simplified reference tissue model.[21] Supervised cluster analysis with six tissue kinetic classes was used to identify voxels from each dynamic ^{11}C-PK11195 PET image, which provided a reference tissue input function representing normal gray matter kinetics.[22]

2.4.2 | β status

To identify MCI patients on the AD continuum, the cortical level of ^{11}C-PiB binding was assessed as the SUVR of a composite region comprising the association neocortex (excluding the primary visual cortex, the primary somatosensory cortex, the primary motor cortex, and medial temporal regions). A cut-off SUVR of 1.2 was used to define patients with raised levels of cortical fibrillar Aβ.[23]

2.5 | Cortical surface mapping

The parametric PET images were mapped onto individual cortical surfaces prior to the statistical analysis, including the evaluation of Aβ status. The cortical surfaces were extracted from the high-resolution T1-weighted MRI images using fast accurate cortex extraction.[24] The middle cortical layer was estimated as the surface between the white matter surface (white matter – gray matter interface) and the pial

surface (gray matter – cerebrospinal fluid interface). The surface of the middle cortical layer was then moved to each corresponding parametric PET image where the PET signal was sampled onto the surface to create a surface representation of the parametric image. All parametric surfaces were then moved to a common template in MNI space using a non-linear co-registration.[25] Finally, all parametric surfaces were smoothed using a 20 mm FWHM geodesic Gaussian kernel. Smoothing of the signal along the cortical surface, that is, in 2D, limits the influence of signal originating from outside the cortex and avoids smoothing of signal across sulci.[26]

2.6 | Statistical analyses

Statistical analyses were performed using Python 3.7. The 2-year differences in the global cognitive scores of the MoCA, MMSE, and CDR-SB were interrogated using a two-tailed paired Student *t* test. Similarly, the 2-year changes in ^{11}C-PiB PET, ^{11}C-PK11195, and ^{18}F-FTP PET were tested using a vertex-wise two-tailed paired *t* test. The differences in ^{11}C-PiB PET, ^{11}C-PK11195, and ^{18}F-FTP PET between the healthy controls and the MCI group at baseline were assessed using a vertex-wise two-tailed unpaired *t* test adjusted for age and sex. The results of the vertex-wise statistical tests were adjusted for multiple comparisons with a family-wise error rate correction ($\alpha = 0.05$) using cluster-extent–based thresholding with two levels of primary cluster-defining threshold; $P < 0.05$ and $P < 0.01$. The correlation between each PET scan and the global cognitive scores at baseline and 2-year follow-up was tested using a vertex-wise general linear model adjusted for age and sex. The results were corrected for multiple comparisons in the same way as the vertex-wise paired *t* test. Last, a mediation analysis was performed in overlapping cortical areas where both ^{11}C-PiB and ^{11}C-PK11195 uptake correlated inversely with the global cognitive scores ($P < 0.05$) to test if microglial activation mediated the negative effect of amyloid load on the global cognitive scores. For each hemisphere, the mean values of ^{11}C-PiB SUVR and ^{11}C-PK11195 BP in the composite overlapping region were used for the mediation analysis. Bootstrapping (1000 repetitions) was used to calculate confidence intervals and assess the statistical significance of the direct and the mediation effects.

3 | RESULTS

A total of 28 patients with MCI and raised cortical Aβ load completed ^{11}C-PiB PET and ^{11}C-PK11195 PET at both baseline and 2-year follow-up and were included in the present study. A subgroup of 17 patients also completed ^{18}F-FTP PET. Demographic characteristics of the included patients are presented in Table 1.

Healthy age-matched controls were included if they completed at least one PET scan, resulting in three different control groups: 11 controls completed ^{11}C-PiB (mean age: 68.9 [60; 77]), 9 controls completed ^{11}C-PK11195 (mean age: 67.9 [58; 80]), and 8 controls completed ^{18}F-FTP PET (mean age: 70.0 [59; 77]). Among the controls who completed ^{11}C-PiB, six individuals also completed ^{11}C-PK11195 PET and were thus included in both control groups.

TABLE 1 Characteristics of participants with mild cognitive impairment.

Parameter	Baseline	Follow-up	*p* value
N	28	–	–
Age (years)	71.1 [58; 83]	–	–
Education (years)	11.8 [7; 17]	–	–
Female/male	11/17	–	–
APOE ε4 carriers	15	–	–
MoCA	24.3 [16; 30]	22.2 [10; 30]	**0.004**
MMSE	27.0 [23; 30]	24.8 [17.0; 30.0]	**0.001**
CDR-SB	1.6 [0.0; 4.0]	3.5 [0.0; 10.0]	**<0.001**
Mean cortical ^{11}C-PiB SUVR	1.8 [1.2; 2.6]	1.9 [1.2; 3.1]	**0.001**
Mean cortical ^{11}C-PK11195 BP	0.06 [−0.06; 0.31]	0.05 [−0.1; 0.2]	0.326
Mean cortical ^{18}F-FTP SUVR[a]	1.2 [1.0; 1.6]	1.3 [1.0; 1.9]	**0.018**

Note: Results are presented with range in brackets. Bold indicates a statistically significant difference ($p < 0.05$).
Abbreviations: *APOE*, apolipoprotein E; BP, binding potential; CDR-SB, Clinical Dementia Rating Sum of Boxes; FTP, flortaucipir; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; PiB, Pittsburgh compound B; SUVR, standardized uptake value ratio.
[a]$N = 17$.

3.1 | Longitudinal changes in PET

The mean tracer uptakes for ^{11}C-PiB, ^{11}C-PK11195, and ^{18}F-FTP PET in the healthy control group as well as in the MCI group at baseline and 2-year follow-up are presented in Figure 1A. Statistically significant differences in tracer uptake between the healthy control group and the MCI group at baseline, as well as statistically significant changes over the 2-year follow-up period in the MCI group, are presented in Figure 1B. Compared to the healthy control group, the MCI group showed significantly increased baseline ^{11}C-PiB SUVR in the association cortex except for the medial temporal lobe and visual cortex. The MCI group also showed increased baseline levels of ^{11}C-PK11195 binding in temporal, occipital, and frontal association areas compared to the healthy control group. No significant difference in baseline ^{18}F-FTP uptake was detected between the two groups. Interrogating the 2-year longitudinal PET change in the MCI group, a significant increase in ^{11}C-PiB SUVR was observed in widespread cortical regions except the primary motor cortex and the medial temporal lobe. Furthermore, a significant decrease in ^{11}C-PK11195 BP compared to baseline was observed in the temporal and parietal lobe of the right hemisphere and the occipital and orbitofrontal cortex of the left hemisphere of the MCI cases; however, the level of ^{11}C-PK11195 BP at follow-up remained higher than that of the controls (Figure S1 in supporting information).

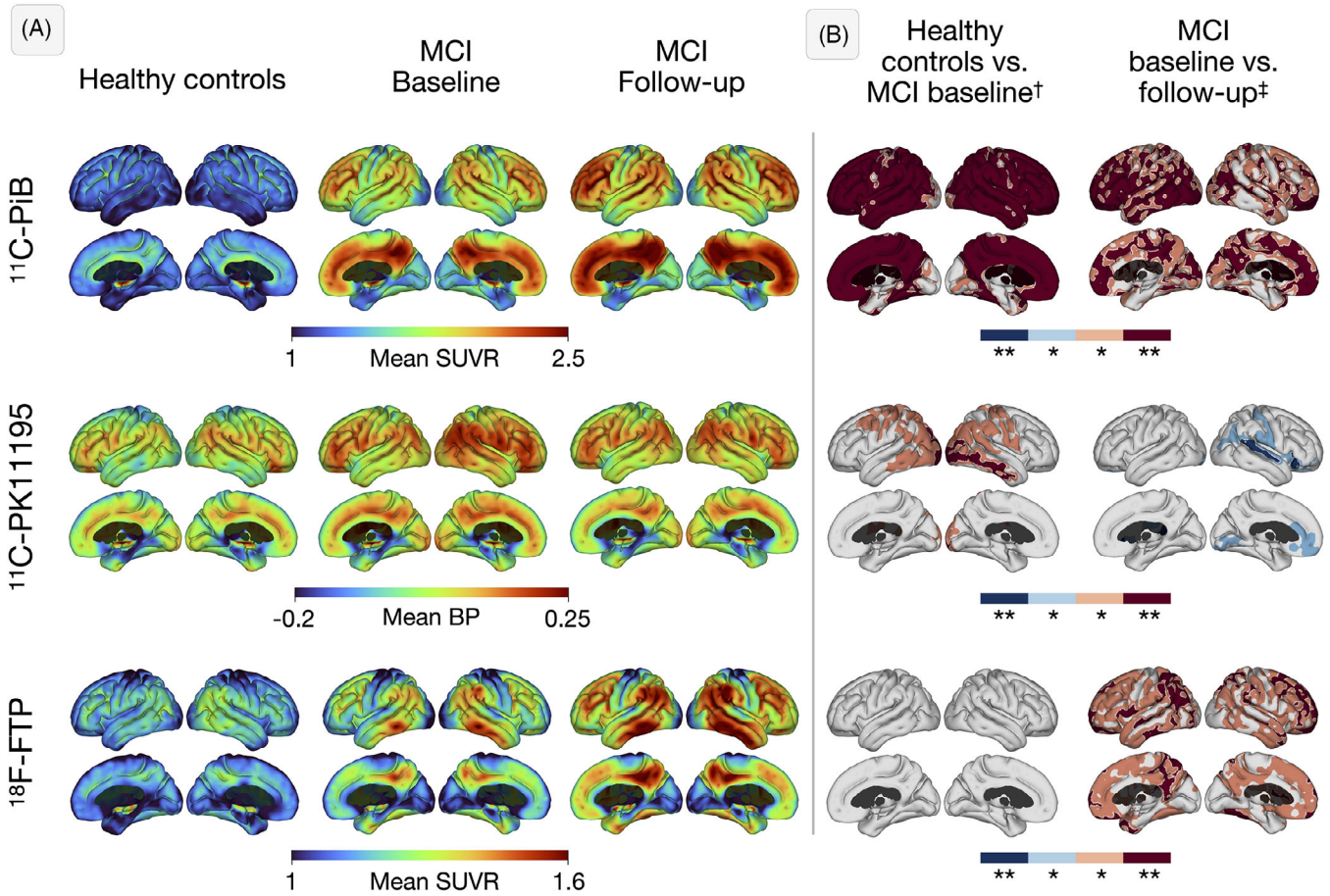

FIGURE 1 Mean PET uptake and group differences. A, Group means of ^{11}C-PiB SUVR, ^{11}C-PK11195 BP, and ^{18}F-FTP SUVR in healthy controls ($n = 11$, $n = 9$, and $n = 8$) and MCI patients with raised cortical amyloid beta load at baseline and 2-year follow-up ($n = 28$, $n = 28$, and $n = 17$). B, Group differences between healthy controls and baseline MCI (left panel), and 2-year longitudinal change in the MCI group (right panel). Positive t values (red colors) indicate a significantly higher uptake in the MCI group (left panel) and a significant increase in mean PET signal over the 2-year follow-up period, and vice versa for negative t values (blue colors). Statistical maps were family-wise error rate–corrected ($\alpha = 0.05$) using cluster-extent–based thresholding with two levels of primary cluster-defining threshold: $P < 0.05$ (*) and $P < 0.01$ (**). †Unpaired t test adjusted for age and sex, ‡Paired t test. BP, binding potential; FTP, flortaucipir; MCI, mild cognitive impairment; PET, positron emission tomography; PiB, Pittsburgh compound B; SUVR, standardized uptake value ratio

Last, a significant increase in ^{18}F-FTP SUVR was now observed in widespread cortical regions with peak clusters in the precuneus, parietal areas, and frontal areas. Detailed information of cluster location and individual differences are presented in Tables S1–S2 and Figures S2–S3 in supporting information.

3.2 | Correlations between PET and global cognition

The correlations between the MoCA scores and ^{11}C-PiB, ^{11}C-PK11195, and ^{18}F-FTP PET findings at baseline and 2-year follow-up are presented in Figure 2. The MoCA was selected as the main result as it is generally regarded to be the most sensitive scale for detecting and rating early changes in global cognition in MCI/AD.[27] A significant inverse correlation between ^{11}C-PiB SUVR and the MoCA was found in widespread cortical regions at both baseline and follow-up. Additionally, a significant inverse correlation was found between ^{11}C-PK11195 BP and the MoCA at baseline in the middle temporal lobe, the angular gyrus, and occipital areas, and at follow-up in the middle temporal and occipital areas. No correlation was detected between cortical ^{18}F-FTP SUVR and the MoCA scores at baseline; however, a significant inverse correlation was found in the left middle and inferior temporal gyrus and the left temporal pole at follow-up. Detailed information on cluster location and plots of the correlations are presented in Tables S3–S5 and Figure S4 in supporting information. The correlation between ^{11}C-PiB and ^{11}C-PK11195 and the MoCA showed similar results when only the 17 patients who completed ^{18}F-FTP were interrogated (Figure S5 in supporting information). The correlations between additional global cognitive ratings with the CDR-SB and MMSE, and ^{11}C-PiB and ^{11}C-PK11195 PET findings showed similar patterns of significant correlation (Figures S6 and S7 in supporting information), except the significant correlation between CDR-SB and ^{11}C-PiB PET was confined to occipital areas at baseline.

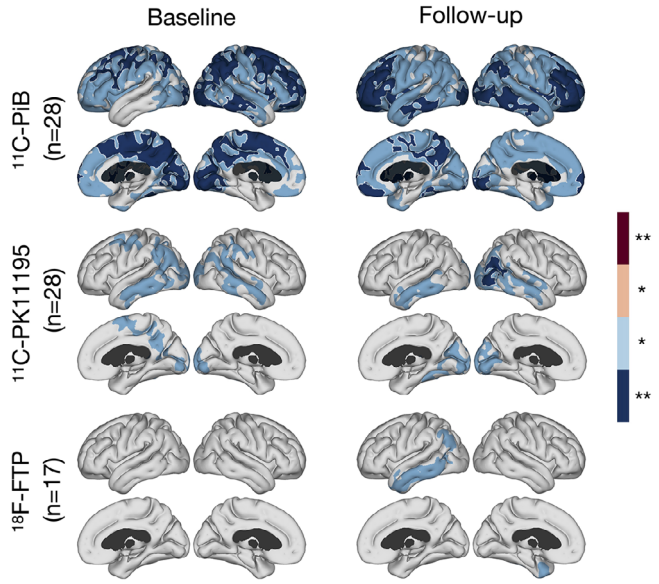

FIGURE 2 Correlation between ^{11}C-PiB ($n = 28$), ^{11}C-PK11195 ($n = 28$), and ^{18}F-FTP ($n = 17$) PET scans and the Montreal Cognitive Assessment. Baseline (left) and 2-year follow-up (right) correlations in patients with mild cognitive impairment and raised cortical amyloid beta load. Statistical tests were adjusted for age and sex and family-wise error rate–corrected ($\alpha = 0.05$) using cluster-extent–based thresholding with two levels of primary cluster-defining threshold: $P < 0.05$ (*) and $P < 0.01$ (**). Negative t values (blue colors) indicate a significant negative correlation. FTP, flortaucipir; PET, positron emission tomography; PiB, Pittsburgh compound B;

3.3 | Mediation of the inverse relationship between amyloid load and MoCA ratings by microglial activation

Results of the mediation analysis on the relationship between ^{11}C-PiB SUVR and the MoCA scores as mediated by ^{11}C-PK11195 BP are presented in Figure 3. At baseline, both ^{11}C-PiB and ^{11}C-PK11195 PET signals inversely correlated with the MoCA in temporal, parietal, and occipital regions of both hemispheres ($P < 0.05$). In this overlapping composite region, the effect of ^{11}C-PiB uptake on the MoCA was partially mediated by ^{11}C-PK11195 signal (28% and 29% of total effect in left and right hemisphere, respectively); however, the mediation effect of ^{11}C-PK11195 binding did not reach statistical significance. At the 2-year follow-up, a similar overlapping region was seen, although this was confined to occipital and temporal areas. Here, the mediation effect of ^{11}C-PK11195 binding was larger (35% and 48% of the total effect in the left and right hemispheres, respectively) and statistically significant ($P < 0.05$). In all overlapping areas, the levels of ^{11}C-PiB uptake correlated positively with the levels of ^{11}C-PK11195 binding ($P < 0.05$).

Similar findings of a larger mediation effect of ^{11}C-PK11195 binding on amyloid burden at follow-up were observed in the mediation analysis with the CDR-SB and MMSE (Figures S8 and S9 in supporting information).

4 | DISCUSSION

In this study, we evaluated the longitudinal changes in cortical levels of Aβ and tau aggregates and microglial responses with PET at baseline and at a 2-year follow-up in 28 patients with MCI and raised levels of cortical Aβ. Additionally, the PET baseline levels were compared to those of healthy age-matched controls. Furthermore, we investigated the association between global cognition and PET findings at the two visits. The MCI group showed significantly increased levels of Aβ and the microglial response at baseline compared to the controls, while no significant load of tau tangles was detected. The elevated levels of Aβ deposition were observed throughout the association cortex, while the microglial response was elevated in temporal, parietal, and occipital areas. Over the 2-year follow-up period a widespread significant increase was observed in Aβ deposition and a significant tau tangle signal could now be detected. At the same time a significant decrease in extent of microglial responses was observed in temporal areas, although the overall level of microglial responses remained elevated compared to controls.

We found widespread areas of significant inverse correlation between levels of Aβ deposition and MoCA scores at both visits. We also found areas of significant inverse correlation between levels of microglial responses and MoCA scores at both visits in temporal, parietal, and occipital areas. A significant inverse correlation between tau tangle levels and cognitive scores was found at the 2-year follow-up situated in the left temporal lobe.

To investigate whether the level of microglial responses mediated the negative effect of Aβ deposition on global cognitive performance in these early MCI due to AD cases, a mediation analysis was performed interrogating those cortical areas where the global cognitive scores inversely correlated with both Aβ load and levels of the microglial response. Overlapping areas were identified in regions across the temporal, parietal, and occipital lobes at baseline and in temporal and occipital regions at follow-up. At baseline, the levels of the microglial response showed a modest but non-significant mediation effect on the association between elevated Aβ and lower global cognitive scores. Over the 2-year follow-up period, however, the mediation effect of the microglial response became more pronounced and reached statistical significance. This suggests that microglial responses partially mediate the inverse association between Aβ load and impaired performance on the global cognitive ratings; however, given the debated interpretation of TSPO-PET, it is not possible to determine the level of activated inflammatory cells or other microglial phenotypes. Even so, the results collectively suggest that during the early stages of AD, cognitive decline is directly affected by accumulated aggregating Aβ while the effect of microglial response on cognitive decline is small and occurs largely independently. However, as the disease progresses, a mediation effect of the microglial response on the relationship between Aβ and cognition becomes more pronounced and significant.

Although microglial response has been widely acknowledged as a significant pathological element of AD, there has been an ongoing debate regarding the role in neurodegeneration and cognitive impair-

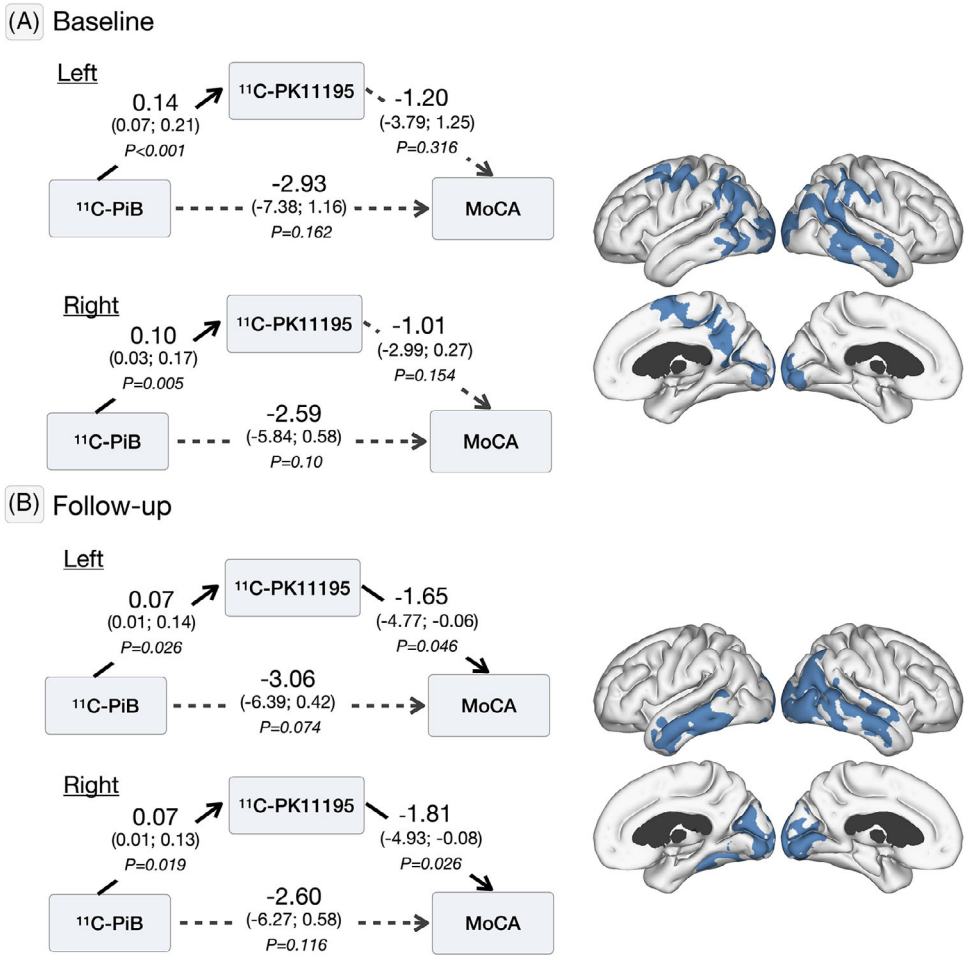

FIGURE 3 Mediation analyses results. Mediation analyses for the relationship between ^{11}C-PiB PET and the MoCA as mediated by ^{11}C-PK11195 PET in 28 patients with mild cognitive impairment and raised cortical amyloid beta load. The mediation analyses were adjusted for age and sex. A, Baseline mediation results in cortical areas where both ^{11}C-PiB PET and ^{11}C-PK11195 PET negatively correlated with the MoCA scores at baseline ($P < 0.05$). Overlapping regions are presented on the right. B, Two-year follow-up mediation results in cortical areas where both ^{11}C-PiB PET and ^{11}C-PK11195 PET negatively correlated with the MoCA scores at follow-up ($P < 0.05$). Overlapping regions are presented on the right. Regression coefficients are presented with 95% confidence intervals. Solid lines indicate a statistically significant effect ($P < 0.05$). FTP, flortaucipir; MCI, mild cognitive impairment; PET, positron emission tomography; MoCA, Montreal Cognitive Assessment; PiB, Pittsburgh compound B

ment. This controversy arises from seemingly contradictory findings that microglia may be either protective or detrimental to neurons depending on the phenotype adopted by the cells.[11] Increasing evidence now suggests that microglia, a key component of intrinsic neuroinflammation, may have diverse phenotypes that change with disease progression.[28] The morphology and behavior of microglia depend on the intensity and duration of the pathological environment in which they reside. Initially, microglia may be homeostatic or phagocytic, clearing extra-cellular protein aggregates and remodeling synapses, but with chronic exposure to pathology, they can become pro-inflammatory and exert a detrimental effect by releasing harmful cytokines.[11] This has led to a dual-peak hypothesis of microglial responses in AD,[15,16,28] which suggests that an initial peak of protective microglia occurs as extracellular Aβ starts to accumulate. However, as the disease progresses and intraneuronal p-tau tangles start to accumulate, chronic activation causes the microglia to gradually adopt a detrimental pro-inflammatory state. In the present study, we saw an increased extent of the microglial response at baseline compared to controls; however, the extent decreased over the 2-year follow-up period as Aβ plaques failed to clear. This is in agreement with previous longitudinal studies of MCI cases[15] and potentially supports a dual-peak hypothesis of microglial response during AD progression. In clinically established dementia cases due to AD, Kreisl et al., using ^{11}C-PBR28 PET, showed a steady increase in the microglia response over time as cognitive performance declined.[9]

We found areas where increased levels of microglial response were associated with worse cognition at baseline in our MCI cohort. This suggests that detrimental microglial phenotypes were present in some brain areas even at this early disease stage. We found no protective effect of the microglial response at baseline, contrary to what might be expected according to the dual-peak hypothesis. However, some individual MCI cases showed elevated baseline tau tangle levels and tau

toxicity which may have masked any protective effects of the microglial response in our MCI cohort. Further investigations in individuals with presymptomatic AD are warranted to determine the role of microglia on cognition before excessive Aβ aggregation and formation of tau tangles has occurred.

The role of Aβ plaques per se on neurodegeneration and cognitive impairment has also been debated.[29] Longitudinal studies have shown that both healthy individuals and early MCI cases with cerebral Aβ plaques develop worse cognitive performance over time compared to individuals without brain Aβ.[30,31] Additionally, soluble oligomers of Aβ have been shown to cause synaptic dysfunction in vitro and their levels correlate with the extent of synaptic loss at *post mortem* and levels of *ante mortem* cognitive impairment.[32,33] In contrast, the density of Aβ plaques has been reported to correlate poorly with the severity of dementia in patients with established AD.[33–36] Additionally, studies of healthy elderly individuals have found significantly elevated levels of cerebral Aβ deposition which were not associated with any overt cognitive impairment.[37,38] It has been demonstrated that the Aβ plaque load reaches a plateau once the patients advance to clinical dementia, despite the ongoing neurodegeneration and continuing cognitive decline.[39] Increases in intraneuronal Aβ are thought to initiate the pathological process of AD and, when a critical level is reached, extracellular aggregates are released, and cortical microglial responses and p-tau tangle accumulation are triggered. As the disease progresses, these other pathological mechanisms become the primary cause of neurodegeneration and cognitive impairment.[36,40] Intrinsic neuroinflammation has been suggested to link Aβ and tau aggregation by triggering tau hyperphosphorylation.[41,42] This may explain why some elderly individuals show cerebral Aβ plaques without cognitive impairment; cortical microglia may not have yet released cytokines triggering tau phosphorylation so the pathological cascade leading to AD dementia has not been initiated.[43] The results from our present study support the notion that Aβ is a key factor in early disease progression; however, as the disease develops, the extracellular toxicity of Aβ aggregates becomes more indirect and partially mediated by microglia while plaque formation could act to reduce aggregated Aβ toxicity. It is important to note that current in vivo imaging of Aβ, such as ^{11}C-PiB PET, only reflects the levels of insoluble Aβ fibrils. Insoluble plaques are the largest but probably least toxic assemblies of Aβ though they may act as a reservoir for oligomeric Aβ. In fact, an inverse relationship between the size of Aβ aggregates and toxicity has been found after a critical mass has been reached.[6] How the level of Aβ plaques correlate with the level of smaller and more toxic forms of Aβ remains to be established. Underlying effects of soluble Aβ aggregates on synaptic degeneration and cognition cannot be identified with current in vivo imaging.

A limitation of the current study is the relatively low number of participants, which reduced its statistical power. This is especially true for tau PET as only 17 participants completed baseline and follow-up tau scans due to delays in accessing ^{18}F-FTP. Consequently, investigations of the mediating effect of microglial responses on the association between tau and cognitive performance were not feasible. Additionally, we used a first-generation TSPO PET tracer, ^{11}C-PK11195, as a marker of microglial responses. This tracer has a relatively low signal-to-noise ratio compared to newer second-generation tracers and cannot discriminate between different microglial phenotypes.[44] However, ^{11}C-PK11195 has the advantage that its binding is only weakly influenced by TSPO polymorphisms, which becomes more problematic when using second-generation TSPO tracers. Even so, TSPO polymorphisms could have a mild influence on the ^{11}C-PK11195 binding in our cohort, but we were unable to control for this as genotyping was not performed. Finally, a threshold of 1.2 SUVR in the composite region of ^{11}C-PiB was selected to define MCI due to AD—this threshold has recently been pathologically validated.[23] Selecting a higher threshold would increase the certainty of attributing the cognitive decline to AD among included patients; however, it might also result in us overlooking some early cases.

In conclusion, we found evidence that cortical Aβ fibrillar load was independently associated with a detrimental cognitive performance in the early stages of MCI due to AD; however, as the disease progressed, this association became weaker and was partially mediated by the microglial response. Our early MCI subjects showed mildly elevated cortical tau tangle load at baseline, but this only became significant after the 2-year follow-up period. As cortical tau tangles start to accumulate, the direct negative effect of Aβ load on cognitive decline likely diminishes and becomes in part mediated by a detrimental microglial response. Further studies into the mechanisms of these associations are needed to further explore this.

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CONSENT STATEMENT

All participants signed an informed written consent at enrolment in the study.

CONFLICT OF INTEREST STATEMENT

The authors report no competing interests. Author disclosures are available in the supporting information.

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