

High PIB Retention in Alzheimer's Disease is an Early Event with Complex Relationship with CSF Biomarkers and Functional Parameters

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Abstract: *Background:* New *in vivo* amyloid PET imaging tracers, such as ¹¹C-PIB, provide possibilities to deeper understand the underlying pathological processes in Alzheimer's disease (AD). In this study we investigated how ¹¹C-PIB retention is related to cerebral glucose metabolism, episodic memory and CSF biomarkers.

Method: Thirty-seven patients with mild AD and 21 patients with mild cognitive impairment (MCI) underwent PET examinations with the amyloid tracer ¹¹C-PIB, ¹⁸F-FDG for measurement of regional cerebral metabolic rate of glucose (rCMRglc), assessment of episodic memory and assay of cerebral spinal fluid (CSF) levels of amyloid- β (A β ₁₋₄₂), total tau and phosphorylated tau respectively. Analyses were performed using Statistical Parametric Mapping (SPM) and regions of interest (ROIs).

Results: Pooled data from AD and MCI patients showed strong correlations between ¹¹C-PIB retention, levels of CSF biomarkers (especially A β ₁₋₄₂), rCMRglc and episodic memory. Analysis of the MCI group alone revealed significant correlations between ¹¹C-PIB retention and CSF biomarkers and between CSF biomarkers and episodic memory respectively. A strong correlation was observed in the AD group between rCMRglc and episodic memory as well as a significant correlation between ¹¹C-PIB retention and rCMRglc in some cortical regions. Regional differences were observed as sign for changes in temporal patterns across brain regions.

Conclusions: A complex pattern was observed between pathological and functional markers with respect to disease stage (MCI versus AD) and brain regions. Regional differences over time were evident during disease progression. ¹¹C-PIB PET and CSF A β ₁₋₄₂ allowed detection of prodromal stages of AD. Amyloid imaging is useful for early diagnosis and evaluation of new therapeutic interventions in AD.

Keywords: Amyloid, CSF biomarkers, ¹¹C-PIB-PET, ¹⁸F-FDG-PET, AD, MCI, cognition.

INTRODUCTION

Extracellular deposits of amyloid- β (A β) in senile plaques and intracellular neurofibrillary tangles of hyperphosphorylated tau protein are considered to represent the pathological hallmarks of Alzheimer's disease (AD). Amyloid deposition starts many years before clinical symptoms of dementia are evident [1]. Studies of brain tissue taken at autopsy have however shown inconclusive results regarding the amount of amyloid deposition in AD brain tissue, and its relationship to cognitive decline [2] and stage of disease [3].

Amyloid imaging offers possibilities to further understand the influence of amyloid deposition on functional changes during development of AD. Positron emission to-

mography (PET) studies with the amyloid tracer ¹¹C-PIB (*N*-methyl [¹¹C]2-(4'-methylaminophenyl)-6-hydroxy-benzothiazole) have shown clear differences in PIB retention between AD patients and healthy controls [4-10]. Low ¹¹C-PIB retention has mainly been reported in patients with frontotemporal lobe dementia [9, 11-13] and Parkinson's disease [14-16] while patients with Lewy body dementia have shown high ¹¹C-PIB retention [9, 16]. The high ¹¹C-PIB retention in Lewy body dementia is most probably due to amyloid binding rather than binding to alpha synuclein [17]. Increased ¹¹C-PIB retention has been detected in patients with mild cognitive impairment (MCI) compared to controls [18, 19] and especially in MCI patients that later convert to AD [20]. A 2 year follow-up study with ¹¹C-PIB in mild AD patients showed, at a group level, unchanged ¹¹C-PIB retention while deterioration was observed in cerebral glucose metabolism (CMRglc) and cognition [21]. Similarly Jack *et al.* [22] recently reported unchanged PIB retention at 1 year follow-up scans in AD and MCI patients while increased brain volumes [21]. Several studies have revealed high ¹¹C-PIB retention in

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cognitively normal elderly subjects [5, 9, 23] and especially in those thought to have a high cognitive reserve [24-26]. These observations stress the question regarding the role of amyloid (A β) in the pathophysiology of AD.

Studies of CSF biomarkers have shown low levels of A β_{1-42} and high levels of total and hyperphosphorylated tau (tTau and pTau) in MCI patients who convert to AD [27-30]. Levels of CSF biomarkers have similar to ¹¹C-PIB retention been shown to be relatively stable over the course of the AD [31, 32]. ¹¹C-PIB retention has been observed to negatively correlate to levels of CSF A β_{1-42} in demented and non-demented subjects [33].

Results concerning cerebral glucose hypometabolism in MCI patients are still inconclusive [34], while a progressive decline in CMRglc over time is observed in AD patients [35]. Episodic memory is typically the first affected cognitive domain in AD [36, 37] but there is also progressive decline in other domains such as verbal and spatial cognition, executive function and complex attention [38, 39]. Data suggest that decline in CMRglc precedes cognitive decline and development of AD [40].

The aim of the present study was to investigate by PET relationships between ¹¹C-PIB retention, CMRglc measured by ¹⁸F-FDG and levels of CSF biomarkers (A β_{1-42} , tTau and pTau), and episodic memory during a continuum of the disease process from MCI to AD. These investigations were performed in order to understand the interrelationships between pathological and functional processes with respect to time course and regional involvement in AD.

METHODS

Participants

Thirty-seven patients with predominantly mild AD and 21 patients with MCI were included in this study. The pa-

tients were all recruited from the Department of Geriatric Medicine, Karolinska University Hospital Huddinge, Stockholm, Sweden, where they had undergone comprehensive assessment of memory and cognition according to a standard procedures at the clinic which have been described before [20]. The diagnosis of AD was performed according to the criteria of the National Institute of Neurological and Communication Disorders, Alzheimer's Disease and related disorders Association (NINCDS-ADRDA) [41]. The diagnosis of MCI was carried out according to modified Petersen criteria [42, 36].

The demographic data for the AD and MCI patients are presented in Table 1. The AD patients comprised of 17 males and 20 females, with a mean age of 67.5 \pm 9.2 (SD) (range 51-83) and a mean MMSE score of 23.7 \pm 4.0 (range 9-29). The duration of disease varied between 1-8 years. Thirteen patients were on treatment with cholinesterase inhibitors at the time of PET scanning and the rest started treatment after their PET investigation. The MCI patients consisted of 8 males and 13 females with a mean age of 63.3 \pm 7.8 (range 50-78) and a mean MMSE score of 28.2 \pm 1.0 (range 25-30). None of them were on cholinesterase inhibitor treatment and they have been clinically followed at the PET scanning. Twenty-five out of 37 AD patients (68%) and 14 out of 21 MCI patients (67%) were carriers of at least one APOE e4 allele. Seven of the 21 MCI patients converted to AD within 8.1 \pm 0.5 months (2-16 months) after their PET scans (MCI-c) while 14 patients remained as MCI after 45.5 \pm 8.5 months of clinical follow-up (non-converting MCI, MCI-nc). No significant difference in age was observed between the AD and MCI patients (p >0.05). Data have been represented earlier for AD [7, 21, 43] and for MCI patients [20].

All patients enrolled in the study gave written consent to participate. The Ethics Committees of the Karolinska Insti-

Table 1. Demographic and Clinical Data of the AD Patients, PIB Positive MCI Patients (PIB-) and PIB Positive MCI Patients (PIB+)

	AD	MCI PIB-	MCI PIB+
N	37	10	11
Age (years)	67.5 \pm 9.2	63.3 \pm 8.2	63.4 \pm 7.9
Gender M/F	17/20	4/6	4/7
ApoE ϵ 4-carriers	25	5	9
MMSE	23.7 \pm 4.0*** ^{xy}	28.7 \pm 1.1	27.8 \pm 1.5
RAVLinl	30.2 \pm 10.9***	46.7 \pm 8.0 [#]	36.7 \pm 6.3
RAVLret	3.3 \pm 3.4*** ^y	10.2 \pm 4.2 [#]	6.4 \pm 3.0
A β_{1-42} (pg/mL)	380 \pm 115***	737 \pm 153 ^{###}	440 \pm 66
tTau (pg/mL)	615 \pm 357*	306 \pm 146	443 \pm 222
pTau (pg/mL)	88 \pm 31*	53 \pm 19	71 \pm 34

Table 1. Data shown as mean \pm SD; MMSE = Mini-Mental-State Examination; RAVLinl/ret= Rey auditory verbal learning/retention; A β_{1-42} = CSF amyloid- β_{1-42} ; tTau = CSF total Tau protein; pTau = CSF phosphorylated Tau protein. *Significant difference between AD and MCI PiB- (p <0.05, ** p <0.01, *** p <0.001). ^ySignificant difference between AD and MCI PIB+ (^x p <0.05, ^{xy} p <0.01, ^{xyy} p <0.001). [#]Significant difference between MCI PIB- and MCI PIB+ ([#] p <0.05, ^{##} p <0.01, ^{###} p <0.001).

Student's t -test, two-tailed, unequal variance.

tute, Stockholm and Uppsala University, Uppsala, and the Isotope Committee at Uppsala Academic Hospital, Uppsala, Sweden, have approved the study.

Positron emission Tomography (PET)

All AD and MCI patients underwent PET examinations with ^{11}C -PIB and ^{18}F -FDG at Uppsala PET centre/Uppsala Imanet AB in Uppsala, Sweden. Production of ^{18}F -FDG and ^{11}C -PIB, tracer doses, and scanner protocol for transmissions, emissions and reconstructions have been described in detail previously [7].

Quantitative data on ^{11}C -PIB retention and the cerebral metabolic rate of glucose (CMRglc) were generated as described in detail previously giving Patlak data for CMRglc and late scan ratio data for ^{11}C -PIB retention [20, 21]. In order to take global differences between subjects into account, the CMRglc data was normalized to that of the pons [44].

The set of 10 regions of interest (ROIs) used included the frontal, parietal and temporal cortices divided right and left, posterior cingulate, striatum, primary visual cortex and thalamus as described in detail before [7, 45]. Magnetic resonance imaging scans were not available for all patients and were therefore not used in delineation of ROIs, limiting the possibility to study small areas such as the hippocampus separately. No partial volume correction was performed.

Statistical Parametric Mapping Analysis of PET data

Statistical parametric mapping (SPM2) (The Wellcome Department of Behavioral Neurology, London, UK) together with MATLAB 7.1 (The Mathworks) were used for voxel-based analyses. The PET images were normalized into a standard stereotactic space [46, 47]. The images were then smoothed by means of an isotropic Gaussian filter (12-mm full width at half-maximum). Patlak images of regional CMRglc (rCMRglc) were stereotactically normalized with each individual image as reference. In the statistical analysis voxels were normalized to the global level of rCMRglc. Early summation ^{11}C -PIB PET images were used to calculate the warping parameters for spatial normalization of the late scan ratio images, as has been done in a previous study concerning ^{11}C -PIB PET [48]. No global normalization was applied in analysis of ^{11}C -PIB retention as a result of the fact that the data were already normalized to the reference region in the cerebellum.

SPM analyses were performed using pooled data from AD and MCI patients. Analysis of covariance between the PET measurements, concentrations of CSF biomarkers and the results of episodic memory tests were performed by entering a regressor of respective covariates. Voxels were considered significant at a threshold of $p < 0.001$, uncorrected. Clusters were considered significant at a threshold of $p < 0.05$, corrected for multiple comparisons.

Neuropsychological Assessments

All AD and MCI patients underwent extensive neuropsychological testing as described earlier [20]. Episodic memory was evaluated by means of total learning in Rey Auditory Verbal Learning (RAVLTot) and Retention (RAVLret) [49].

Data on episodic memory was available for all subjects but not all performed all sub-tests. RAVLTot and RAVLret test data was available for 26 AD patients and for MCI patients for 20 and 21 patients respectively.

Cerebrospinal Fluid Measurements

Cerebrospinal fluid (CSF) was obtained by lumbar puncture performed in non-fasting subjects between 8-11 am. The CSF samples were stored at -80 C until analysis. Measurement of total tau (tTau) phosphorylated tau (pTau) and amyloid- β_{1-42} ($\text{A}\beta_{1-42}$) in CSF was performed using a sandwich enzyme-linked immunosorbent assays (ELISA)[50-52]. Data on CSF biomarkers was available: for AD patients on $\text{A}\beta_{1-42}$, tTau and pTau for 29, 30 and 19 patients respectively. For the MCI subjects $\text{A}\beta_{1-42}$, tTau and pTau were available for 16, 18 and 15 patients respectively.

Statistical Analysis

All statistical analyses were performed using STATISTICA software (data analysis software system version 8.0. www.statsoft.com). Correlation analyses of ROI data were conducted using Pearson's product moment correlation coefficient r . Data from the AD and MCI groups were analysed as pooled data as well as separately. Pair-wise analyses were chosen to relate the different parameters to one another directly and to extrapolate the regional differences, as well as to be able to carry out analysis on a voxel level. No correction for multiple comparisons was performed in ROI based analyses because of the exploratory nature of the study. Therefore the interpretations are limited to examination of patterns in relationships between parameters and single correlations should be evaluated with caution as a result of the risk of type one errors.

RESULTS

^{11}C -PIB Retention and Cerebral Glucose Metabolism

Fig. (1) show mean values of ^{11}C -PIB retention in seven brain regions of AD and MCI patients. Thirty-two of the 37 AD patients showed high ^{11}C -PIB retention, calculated as described before [20]. Five AD patients showed low ^{11}C -PIB retention, three of these AD patients with mild cognitive problems have been described before [7] and also found to have low ^{11}C -PIB retention at follow-up [21]. The other two AD patients around 80 years of age showed typical progressive clinical AD. Of these AD patient, one was a ApoE e2/4 carrier who showed increased cortical PIB retention at repeated PET scan.

Eleven of the 21 MCI patients showed high ^{11}C -PIB retention. Seven MCI patients who later converted to AD (MCI-c) showed high cortical levels of ^{11}C -PIB retention while additional four MCI patients with high ^{11}C -PIB retention remained as MCI patients (MCI-nc) at clinical follow-up.

Fig. (2) illustrates ^{11}C -PIB retention and ^{18}F -FDG uptake in a ^{11}C -PIB negative (PIB-) MCI patient with low ^{11}C -PIB retention and unimpaired CMRglc, a ^{11}C -PIB positive (PIB+) MCI patient with high ^{11}C -PIB retention and relatively unimpaired CMRglc, and an AD patient with high ^{11}C -PIB retention and a clear reduction of CMRglc in the parietal

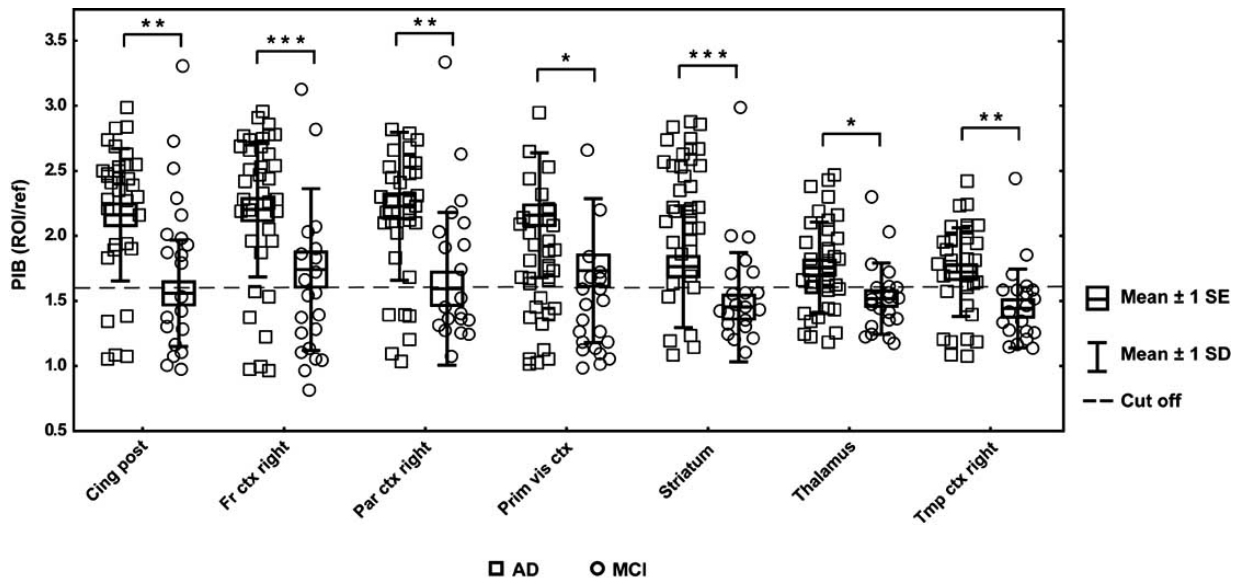


Fig. (1). ¹¹C-PIB retention in AD and MCI patients, showing both individual values as well as mean, standard error of the mean (SE), and standard deviation (SD). *Significant difference between AD and MCI (**p*<0.05, ***p*<0.01, ****p*<0.001)

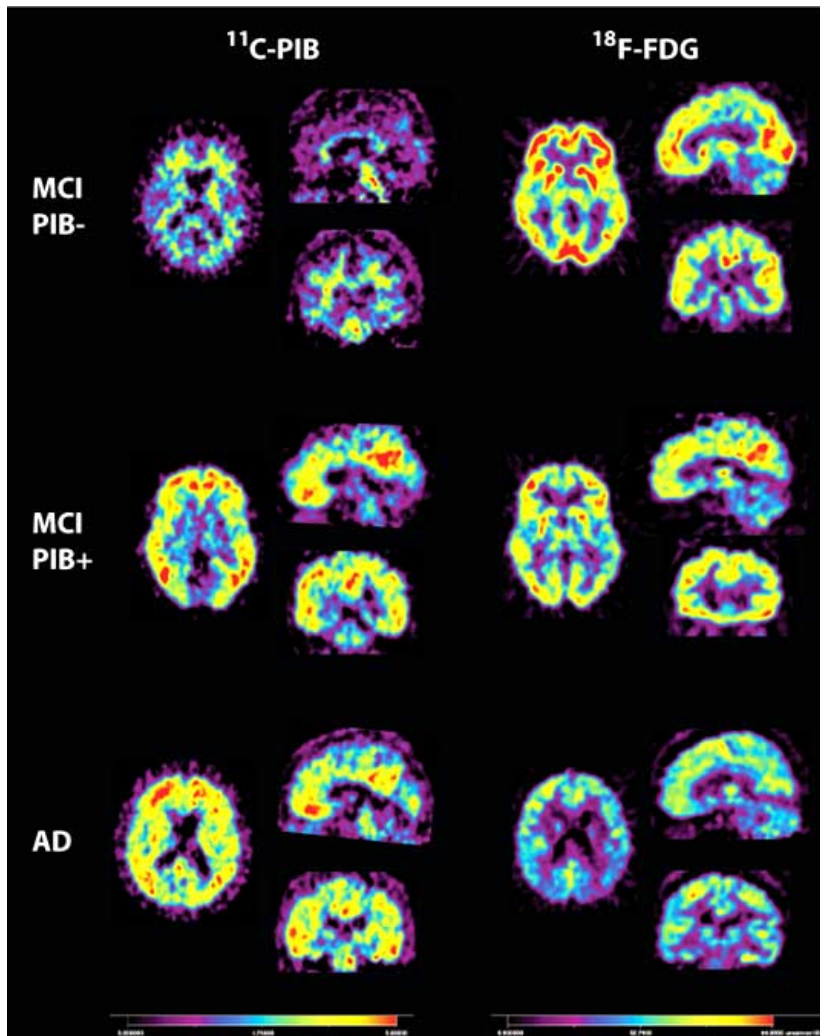


Fig. (2). Examples of ¹¹C-PIB retention and rCMRglc in one PIB negative (PIB-) MCI patient with unimpaired rCMRglc, one PIB positive (PIB+) MCI patient with relatively unimpaired CMRglc and one AD patient with clear impaired rCMRglc. Images showing both transaxial, coronal and sagittal slices.

cortex. These are individual patients exemplifying the group findings, although there are variances in the groups, as evident in the correlation data.

Significant negative correlations between ^{11}C -PIB retention and rCMRglc using pooled data of AD and MCI patients were observed in 5 ROIs, posterior cingulate, right and left frontal, and parietal cortices (Fig. 3A; $p < 0.05$). No significant correlation ($p < 0.05$) was observed in the striatum, primary visual cortex, thalamus, and left and right temporal cortices.

When data from the AD patients were analysed separately a significant correlation was observed between ^{11}C -PIB retention and rCMRglc in the parietal cortices and the posterior cingulate (Fig. 3A; $p > 0.05$).

No significant correlation between ^{11}C -PIB retention and rCMRglc was found in the MCI group in any of the 10 brain areas included in the analysis (Fig. 3A; $p > 0.05$).

^{11}C -PIB Retention and CSF Biomarkers

Correlation analysis with pooled ROI data for ^{11}C -PIB retention in both AD and MCI patients showed significant negative correlation between CSF concentrations of $\text{A}\beta_{1-42}$, and positive correlations between tTau and pTau respectively versus ^{11}C -PIB retention in almost all cortical and subcortical brain regions with only a few exceptions (Fig. 3B; $p < 0.05$). The ^{11}C -PIB retention in the thalamus did not correlate to any of the three CSF biomarkers ($p > 0.05$). ^{11}C -PIB retention in primary visual cortex and left temporal cortex did not reach statistical significance when correlated to pTau values in CSF ($p > 0.05$).

Similarly, negative correlations between ^{11}C -PIB retention and $\text{A}\beta_{1-42}$, and positive correlations between tTau and pTau levels in CSF were found in almost all cortical and subcortical brain regions (Fig. 3B; $p < 0.05$) when the data of the MCI patients was analysed separately. The ^{11}C -PIB retention in thalamus did not show significant correlation to any of the CSF biomarkers ($p > 0.05$). Neither did ^{11}C -PIB retention in primary visual cortex reach significant correlation to the values of pTau ($p > 0.05$).

No significant correlations were observed between ^{11}C -PIB retention and the CSF biomarkers when the AD patients were analysed separately (Fig. 3B; $p > 0.05$).

Analyses of covariance between ^{11}C -PIB retention and concentrations of CSF biomarkers were also performed using SPM2. Clusters with significant negative covariance between ^{11}C -PIB retention and levels of $\text{A}\beta_{1-42}$ were found in the parietal and temporal areas, the posterior cingulate, the frontal cortex, as well as the primary visual cortex (Fig. 4A; Table 2A) when pooled data from both AD and MCI patients was used. No significant clusters were found when analysing covariance between ^{11}C -PIB retention and levels of tTau or pTau (data not shown).

^{11}C -PIB Retention and Episodic Memory

Pooled ROI data from AD and MCI patients showed significant negative correlations between ^{11}C -PIB retention in almost all cortical and subcortical brain regions and scores in episodic memory tests (Fig. 3C; $p < 0.05$), with only the ex-

ception of ^{11}C -PIB retention in the thalamus versus RAVLret ($p > 0.05$).

Solely weak negative correlations were found between ^{11}C -PIB retention and RAVLtot scores in the right and left frontal cortices as well as in the posterior cingulate in AD patients (Fig. 3C; $p < 0.05$).

Similarly, only a few weak correlations were observed between ^{11}C -PIB retention in the left frontal and temporal cortices versus RAVLtot and ^{11}C -PIB retention in the left temporal cortex versus RAVLret (Fig. 3C; $p < 0.05$) for the MCI group separately.

Covariance between ^{11}C -PIB retention and episodic memory was investigated using SPM and including pooled data from AD patients and MCI patients. Significant negative covariance was found between ^{11}C -PIB retention in most parts of the cortex and RAVLtot test scores (Fig. 4B, Table 2B). The same pattern of covariance was found between ^{11}C -PIB retention and RAVLret scores using SPM analysis (data not shown).

Cerebral Glucose Metabolism and Episodic Memory

Significant positive correlations were observed for the AD and MCI patients between the rCMRglc ROI data in all 10 cortical and subcortical brain areas and the two tests of episodic memory (RAVLtot and RAVLret) (Fig. 3D; $p < 0.05$).

The AD patients showed significant, but in some cases weak, positive correlations between RAVLtot test versus rCMRglc in all cortical and subcortical brain regions (Fig. 3D; $p < 0.05$). Significant correlations were observed between RAVLret and rCMRglc in the posterior cingulate, left frontal, left parietal, left temporal and primary visual cortices, as well as striatum and thalamus ($p < 0.05$).

No significant correlation was observed between rCMRglc and the two tests of episodic memory in the MCI group (Fig. 3D; $p > 0.05$).

Analysis of covariance between rCMRglc and episodic memory, using SPM2, showed significant clusters in both the parietal cortex and posterior cingulate as regards RAVLtot results (Fig. 4C, Table 2C), while RAVLret results showed significant positive covariance only with rCMRglc in the posterior cingulate (data not shown).

CSF Biomarkers and Episodic Memory

Significant correlations between $\text{A}\beta_{1-42}$ versus RAVLtot ($p < 0.01$) and versus RAVLret ($p < 0.05$) and between tTau versus RAVLtot ($p < 0.05$) and versus RAVLret ($p < 0.05$) were observed in AD and MCI patients when analysed together. No significant correlations were found between the concentrations of pTau versus RAVLtot or RAVLret results ($p > 0.05$).

No significant correlations were observed in the AD group between CSF biomarkers and tests of episodic memory ($p > 0.05$). Significant correlation was found between CSF levels of $\text{A}\beta_{1-42}$ and test scores of RAVLret in MCI patients ($p < 0.05$).

Table 2A. Statistics from SPM Analysis Related to Fig. 3A, Showing Clusters with Statistically Significant Negative Covariance between ^{11}C -PIB Binding and Levels of $\text{A}\beta_{1-42}$ in CSF. $p = 0.001$ (Uncorrected)

Cluster level		Voxel level			x, y, z	Anatomical area
Cluster size (k_E)	$p_{\text{corrected}}$	T	Z_E	$P_{\text{uncorrected}}$		
24614	0.000	4.78	4.25	0.000	60 -38 -28	(Right temporal lobe)
		4.47	4.02	0.000	66 -60 10	(Right parietal lobe)
		4.43	3.99	0.000	2 -62 40	Right parietal lobe, precuneus (close to posterior cingulate)
4050	0.023	3.98	3.64	0.000	-32 56 16	Left frontal lobe, superior gyrus
		3.89	3.57	0.000	-12 66 -6	Left frontal lobe, medial gyrus, BA 10
		3.86	3.55	0.000	30 64 10	Right frontal lobe, middle gyrus

Height threshold: $T = 3.30$, $p = 0.001$ (0.270)Extent threshold: $k = 150$ voxels, $p = 0.750$ (0.211)

BA = Brodmann area

Table 2B. Statistics from SPM Analysis Related to Fig. 3B, Showing Clusters with Statistically Significant Negative Covariance Between ^{11}C -PIB Retention and Episodic Memory Measured by RAVLtot Tests. $p = 0.001$ (Uncorrected)

Cluster level		Voxel level			x, y, z	Anatomical area
Cluster size (k_E)	$p_{\text{corrected}}$	T	Z_E	$P_{\text{uncorrected}}$		
64795	0.000	4.93	4.37	0.000	-22 64 -4	Left frontal lobe, superior gyrus, BA 10
		4.86	4.32	0.000	28 38 48	(Right frontal lobe)
		4.76	4.25	0.000	32 62 -8	Right frontal lobe, superior gyrus

Height threshold: $T = 3.29$, $p = 0.001$ (0.312)Extent threshold: $k = 150$ voxels, $p = 0.716$ (0.235)

BA = Brodmann area

Table 2C. Statistics from SPM Analysis Related to Fig. 3C, Showing Clusters with Statistically Significant Positive Covariance between rCMRglc and Episodic Memory Measured by RAVLinl Tests. $p = 0.001$ (Uncorrected)

Cluster level		Voxel level			x, y, z	Anatomical area
Cluster size (k_E)	$p_{\text{corrected}}$	T	Z_E	$P_{\text{uncorrected}}$		
3115	0.000	6.68	5.50	0.000	4 -44 36	Right limbic lobe, cingulated gyrus, BA 31
	0.000	5.20	4.56	0.000	-46 -76 38	(Left parietal lobe)
		4.73	4.22	0.000	-32 -52 40	Left parietal lobe, sub-gyral
	0.021	4.32	3.92	0.000	42 -74 46	(Right parietal lobe)
	0.101	4.24	3.85	0.000	-44 24 34	Left frontal lobe, precentral gyrus, BA 9
		3.98	3.65	0.000	-44 16 42	Left frontal lobe, middle gyrus

Height threshold: $T = 3.29$, $p = 0.001$ (0.954)Extent threshold: $k = 150$ voxels, $p = 0.161$ (0.390)

BA = Brodmann area

DISCUSSION

There has been a rapid development in the field of amyloid imaging in AD since the first ^{11}C -PIB scans were performed in AD patients in 2002 in Sweden [53]. Seven amy-

loid PET tracers have so far been tested in patients and among them ^{11}C -PIB has been used in the largest number of patients [54]. A crucial question is whether the new technique, that PET imaging of amyloid represents, can provide

deeper insight into the disease processes of AD and thereby help to develop new diagnostic tools as well as drug targets? Cognitive testing together with the emerging use, at least in academic clinics of structural imaging (MRI), functional imaging (SPECT, PET) and assay of biomarkers in CSF has provided evidence of early changes in the brain during disease progression in cases of AD [55,56,]. New diagnostic criteria for the diagnosis of AD was recently suggested by Dubois *et al.* [57] with the aim of identifying AD at an earlier stage than is currently possible. The aim of the present study was to compare pathological markers (^{11}C -PIB PET and CSF biomarkers) with functional parameters (^{18}F -FDG PET and episodic memory) in AD and MCI patients. The conversion rate of MCI patients to AD was considerable (33%) but comparable to the annual conversion rate reported by others [58- 60].

Brain Pathology Versus Brain Dysfunction

As summarized in Table 3 the findings of the present study show relationships, ranging from weak (+) to strong (+++) and dissociations (0), between pathological (^{11}C -PIB retention, CSF biomarkers) and functional parameters (^{18}F -FDG, cognition) respectively. A strong relationship was observed between ^{11}C -PIB retention in brain and CSF $\text{A}\beta_{1-42}$ at prodromal stage of AD, as demonstrated by findings in the MCI group, but absent at the clinical stage of AD. The functional parameters, rCMRglc and episodic memory on the other hand, showed strong correlation at the clinical stage of AD, while not at MCI. These relationships and dissociations between pathological and functional parameters are discussed in detail below.

Data analyses were performed both using ROI and SPM analyses and the differences between findings might be the result of higher sensitivity in ROI analysis, which is less vulnerable to large variances in the material compared with SPM analysis. Nevertheless, the statistical inferences in SPM analysis are achieved by using thresholds of uncorrected p -levels at the voxel level and are corrected for multiple comparisons at the cluster level. These estimates reflect the certainty of significant findings in the relationships observed supporting the crude findings in ROI analysis based on pairwise correlations not corrected for multiple comparisons.

^{11}C -PIB Retention and CSF Biomarkers

The ^{11}C -PIB PET retention, showed a strong correlation primarily with levels of CSF $\text{A}\beta_{1-42}$ but also to some extent with levels of tTau and pTau, when using pooled data from AD and MCI patients or data from MCI patients alone (Table 2). The relationship was lost when the data from AD patients was analysed separately. These results are in agreement with those in earlier studies on non-demented subjects and demented patients [33] and MCI patients alone [20]. Koivunen *et al.* [61] however did not observe any correlation between ^{11}C -PIB retention CSF biomarkers in MCI patients. An interesting question is whether increase in amyloid brain deposition is measurable by PET before decrease of amyloid in CSF? In the present study some patients showed high ^{11}C -PIB retention in the brain but normal CSF $\text{A}\beta_{1-42}$ levels (Fig. 3). Similar findings were recently reported by Koivunen *et al.* [61]. These data lend support to the assumption

that abnormal ^{11}C -PIB retention occurs prior abnormal $\text{A}\beta$ levels are found in CSF. CSF biomarkers have been reported to be stable when MCI patients are converting to AD [62]. Some critical comments have been made concerning the selectivity of CSF biomarkers in AD versus other dementia disorders [56].

Analysis by means of SPM clearly showed covariance between ^{11}C -PIB retention in the parietal and temporal cortices and CSF $\text{A}\beta_{1-42}$, while no significant relationship was observed between ^{11}C -PIB retention and the concentrations of CSF tTau or pTau. Although a relatively stronger relationship between ^{11}C -PIB retention and CSF $\text{A}\beta_{1-42}$, compared with tTau and pTau would be expected there might a reduced power in the PIB/tau analysis due to fewer available samples of tTau and pTau.

The findings in the present study suggest a strong relationship between $\text{A}\beta$ fibrillar deposits, measured as ^{11}C -PIB retention in the brain and CSF $\text{A}\beta_{1-42}$ levels at prodromal stages of AD. The variability of the parameters is decreased and the correlation between them is diminished at clinical stages of AD. Further longitudinal studies in MCI patients are needed to elucidate the time course in the regional retention of ^{11}C -PIB retention and at which stage the plateau as reflection of maximal amyloid load might be reached. No significant correlation was observed between cerebral ^{11}C -PIB retention in the thalamus and levels of CSF biomarkers suggesting that the pathological changes in this brain region probably have a different course compared to other brain areas.

^{11}C -PIB Retention and Cerebral Glucose Metabolism

The significant correlation between ^{11}C -PIB retention and rCMRglc metabolism in the frontal and parietal cortices and posterior cingulate in AD plus MCI patients is in agreement with previous findings in AD patients and healthy controls [4, 7, 21]. Others have failed to observe this relationship [63], suggesting that it might be weak and dependent on the heterogeneity of the material and a greater variability in these areas severely affected in AD and showing continuous decline in rCMRglc during disease progression [34]. No significant relationship between ^{11}C -PIB retention and rCMRglc was observed in MCI patients, supporting the hypothesis that $\text{A}\beta$ pathology precedes functional changes [64, 65]. A similar regional topographical change has been observed for brain atrophy and rCMRglc impairment while the ^{11}C -PIB retention showed a more extensive distribution [66]. Autopsy AD brain studies have confirmed a significant correlation between ^3H -PIB binding and levels of $\text{A}\beta_{1-40}$ and $\text{A}\beta_{1-42}$ [67, 68].

Amyloid Load and Episodic Memory

Episodic memory performance showed covariance with ^{11}C -PIB retention in most cortical regions in AD+MCI patients. A much stronger and more regional focal relationship was observed between episodic memory and rCMRglc (Table 3).

Interestingly enough, a significant correlation was observed between episodic memory and levels of CSF biomarkers in MCI patients but weaker than for ^{11}C -PIB reten-

Table 3. Summary of Correlative Results Between Pathological and Functional Parameters

Patient group	PIB vs rCMRglc	PIB vs CSF Biomarkers	PIB vs Ep mem	FDG vs Ep mem	CSF biomarkers vs Ep mem
AD+MCI	+++ a)	+++ c)	+++ e)	+++	++
AD	++ b)	0	(+) f)	+++ h)	0
MCI	0	+++ d)	0 g)	0	+ A β

a) no correlation in striatum, visual cortex, thalamus or temporal cortex

b) correlation only in posterior cingulate and parietal cortex

c) no correlation in thalamus

d) no correlation in thalamus

e) no correlation in thalamus

f) solely with RAVLret in frontal cortex, and right parietal cortex

g) solely in temporal cortex left

h) except in right parietal cortex, and right temporal cortex versus RAVLret

tion versus CSF biomarkers (Table 3). A significant correlation between ^{11}C -PIB retention and episodic memory has earlier been shown in AD patients [4, 21] as well as in MCI patients [20]. Pike *et al.*[69] demonstrated a relationship between ^{11}C -PIB retention and episodic memory in a mixed population of AD and MCI patients and in MCI patients separately, but not in AD patients. ^{11}C -PIB retention has also been related to clinical severity in cases of AD [70] and to cognitive decline in non-demented aging subjects [25].

Amyloid Retention and ApoE Genotype

The proportion of ApoE e4 carrier was high in the present study, 67 % and 66 % for AD and MCI group respectively but similar to what has been reported recently in other studies [71,72]. We were not able to observe any significant correlation between ApoE genotype and PIB retention. PIB retention has recently been reported to be associated with APOE e4 gene dose in healthy individuals with normal cognitive function [73] as well as in AD patients [74]

CONCLUSIONS

High retention of ^{11}C -PIB and low levels A β_{1-42} in CSF are detectable in prodromal stages of AD. These early pathological changes in amyloid are followed by brain regional declines in CMRglc and in episodic memory during the clinical manifestations of AD. Amyloid imaging is promising for early detection of AD and for deeper understanding of the underlying pathological disease processes in AD. Further studies are needed to understand the regional time course of different amyloid processes in brain especially in prodromal AD and how amyloid imaging could be used for evaluation of new drug intervention in AD.

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DISCLOSURE

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ABBREVIATIONS

CBF = Cerebral blood flow

^{18}F -FDG = [^{18}F]-2-deoxy-d-glucose

MMSE = Mini-Mental State Examination

^{11}C -PIB = *N*-methyl [^{11}C] 2-(4'-methylaminophenyl)-6-hydroxy-benzothiazole

rCMRglc = Regional cerebral metabolic rate of glucose

ROIs = Regions of interest

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