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Abstract

Objective The present study investigated the relationship between amyloid deposition and glucose metabolism using Pittsburgh compound B (11C-PIB) and fluorodeoxyglucose (18F-FDG) positron emission tomography (PET) in patients with Alzheimer’s disease (AD) and amnestic mild cognitive impairment (aMCI) and assessed the apolipoprotein E (ApoE) ε4 allele to explore the correlation between aMCI and AD. Methods Amyloid load in the brain and cerebral glucose metabolism were determined and ApoE genotypes were analyzed in patients with AD (N = 14), aMCI (N = 10), and healthy controls (N = 5). Results The mean 11C-PIB standardized uptake value ratio (SUVR) was higher in inferior parietal lobe, lateral temporal cortex, frontal cortex, posterior cingulate cortex and precuneus, occipital lobe, and striatum in AD patients compared with controls (P < 0.05). 11C-PIB binding levels in aMCI patients were bimodal. No significant difference in the 11C-PIB SUVR was found between the 11C-PIB + aMCI subgroup and AD group (P > 0.05). 18F-FDG PET revealed hypometabolism in bilateral parietal lobes, temporal lobe, and precuneus in 3 of 5 11C-PIB + aMCI subjects, including two of them with ApoE ε4 allele converted to AD, and hypometabolism in the bilateral frontal lobe and anterior cingulate in 3 of 5 11C-PIB - aMCI subjects. Conclusions 11C-PIB PET is a powerful tool for screening aMCI with AD pathology. The aMCI patients with AD pathology who presented hypometabolism in the parietal lobe, lateral temporal cortex, precuneus and with ApoE ε4 allele are more likely to convert to clinical AD dementia.

【摘要】目的 应用11C-PIB PET 和18F-FDG PET 显像诊断阿尔茨海默病和遗忘型轻度认知损害患者β-淀粉样蛋白(AB)沉积与葡萄糖代谢之间的关系, 联合载脂蛋白E(ApoE)基因型进一步探讨遗忘型轻度认知损害与阿尔茨海默病的相关性。方法 利用PET显像对阿尔茨海默病(14例) 、遗忘型轻度认知损害(10例) 和正常对照者(5例) 脑组织Aβ沉积和葡萄糖代谢变化进行分析,采用聚合酶链反应-限制性片段长度多态性方法对ApoE基因型进行分析。结果 阿尔
Introduction

Alzheimer's disease (AD) is an age-related neurodegenerative disorder that results in a progressive loss of cognitive function. It is characterized by the accumulation of amyloid-beta (Aβ) peptide into amyloid plaques in extracellular brain parenchyma and intraneuronal neurofibrillary tangles (NFTs) caused by the abnormal phosphorylation of tau protein [1]. Amyloid deposits and tangles are necessary for the postmortem diagnosis of AD [2].

Positron emission tomography with 18F-fluorodeoxyglucose (18F-FDG PET) highlights the differential distribution of pathology in dementing disorders and has been used to study neurodegenerative diseases for over two decades. AD causes hypometabolism predominantly in posterior regions, including posterior temporoparietal association cortex and posterior cingulate cortex [3]. Frontotemporal dementia (FTD) causes hypometabolism predominantly in anterior regions, including frontal lobes, anterior temporal cortex and anterior cingulate cortex [4]. 18F-FDG PET studies with small samples of patients with mild cognitive impairment (MCI) showed that parietotemporal and posterior cingal hypometabolism may characterize patients who later convert to AD [5-6]. Brain FDG retention is a nonspecific indicator of metabolism that can be deranged for a variety of reasons (e.g., ischemia or inflammation) and may be irrelevant or only indirectly related to any AD-related process in certain individuals.

The PET tracer N-methyl[11C]2-([4'-methylaminophenyl]-6-hydroxy-benzothiazole, better known as Pittsburgh compound B (11C-PIB), has been used to detect amyloid deposition in vivo. Previous 11C-PIB PET studies reported quantitative increases in 11C-PIB uptake, reflecting greater amyloid burden, in AD and MCI patients compared with controls [7-10]. In AD, 11C-PIB uptake is particularly evident in the frontal, parietotemporal, and posterior cingulate cortices, consistent with the known distribution of amyloid plaques [11-17]. Less work has been done with 11C-PIB in patients with MCI, which is associated with an increased likelihood of converting to AD [18]. 11C-PIB studies in small samples suggested that approximately two-thirds of patients with amnestic MCI (aMCI) show 11C-PIB retention similar to AD, whereas one-third of patients are within the healthy control range [7,8,14-16]. In MCI, amyloid-positive 11C-PIB PET may indicate an increased likelihood of converting to AD [17].

The most common genetic variant associated with late-onset AD is apolipoprotein E (ApoE) ε4 allele [18-21]. The presence of the ε4 allele confers a significantly higher likelihood of developing AD. ApoE genotypes are also associated with AD biomarkers, with the presence of the ApoE ε4 allele associated with greater amyloid deposition [20,22] and alterations in brain function and glucose metabolism [23,24] in patients with MCI and AD and cognitively healthy older adults.

The present study investigated the relationship between plaque deposition and glucose metabolism using 11C-PIB PET and 18F-FDG PET in AD and aMCI patients and assessed the presence of the ApoE ε4 allele to explore the correlation between aMCI and AD.

Methods

Subjects

A total of 14 AD patients and 10 aMCI patients were randomly recruited at Tianjin Huanhu Hospital, Tianjin, China, between April 2012 and October 2012. All of the subjects underwent an extensive diagnosis and behavioral assessment by trained neurologists. The diagnosis of AD was made according to the criteria of National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) [25]. The diagnosis of dementia was
based on the criteria of Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) [25]. No familial cases of AD were included in this study. To avoid the inclusion of vascular dementia cases, we excluded patients who scored > 2 points on the Hachinski Ischemic Scale [28]. Amnestic MCI was diagnosed using Petersen criteria [29], which require subjective memory complaints and serial reaction time scores for either immediate or delayed recall > 1.50 standard deviation (SD) below age- and education-adjusted norms in the absence of impairment in activities of daily living.

All of the controls were required to have a Mini-Mental State Examination (MMSE) score ≥ 28, serial reaction time total and delayed recall scores within 1 SD of age-adjusted norms, and no current diagnosis of any DSM-IV Axis I psychiatric disorder, neurological disorder or acute medical illness. A family history of dementia was not an exclusion criterion. Subjects who received Warfarin or had any contraindication to undergoing MRI or PET were excluded from the study. Controls were group-matched to patients with regard to age and sex.

### MRI

MR images were acquired using a 3.0T Siemens Trio a Tim MR scanner. Coronal T1WI was acquired using a three-dimensional spoiled gradient recalled echo (3D-SPGR) sequence [repetition time (TR) = 11 ms, echo time (TE) = 4.94 ms, flip angle (FA) = 20°, 1 mm slice thickness (zero gap), 160 slices, field of view (FOV) = 230 mm × 230 mm]. All of the images were reconstructed to a size of 256 × 256 with an isotropic resolution of 1 mm × 1 mm × 1 mm.

### PET imaging

Head movement was minimized using a polyurethane immobilizer molded around the head. The PET images were acquired on a GE Discovery LS PET/CT scanner in the three-dimensional scanning mode, yielding 35 slices with 4.25 mm thickness that covered the entire brain. 11C-PIB PET scans were acquired during 90-min dynamic PET acquisition (a total of 34 frames: 4 × 15 s, 8 × 30 s, 9 × 60 s, 2 × 180 s, 8 × 300 s, 3 × 600 s). 11C-PIB was administered into an antecubital vein as a bolus injection, with a mean dose of 370-555 MBq. Then those images were reconstructed to a 128 × 128 matrix (2.50 mm × 2.50 mm pixel size).

The 18F-FDG study was conducted 1 h after the 11C-PIB PET scan using the same scanner, scanning mode, positioning and reconstruction matrix. The subjects received an intravenous injection of 250 MBq 18F-FDG and remained in a darkened, quiet room. A 10-min static PET emission scan was performed 60 min after the 18F-FDG injection.

### A. Quantification of 11C-PIB uptake

The uptake of 11C-PIB was quantified at the voxel level using the region-to-cerebellum ratio, which was identical to the standardized uptake value ratio (SUVR). This simplified quantification enabled the utilization of a short 30-min image acquisition.

### B. Automated region-of-interest analysis

Standardized region of interest (ROI) were defined on the MRI template image that represented brain anatomy in accordance with the Montreal Neurological Institute (MNI). We merged and pooled subsets from the original Automated Anatomic Labeling (AAL) atlas to form the following ROIs: middle frontal gyrus (MFG), medial prefrontal cortex (MPFC), lateral temporal cortex (LTC), hippocampus and parahippocampus (HF +), inferior parietal lobe (IP), posterior cingulate cortex and precuneus (PCCPre), striatum, thalamus, occipital lobe (OL), superior temporal gyrus (STG), and supplementary motor area (SMA).

### C. Image preprocessing

The preprocessing of the 11C-PIB imaging data was performed using Statistical Parametric Mapping 8 (SPM8) software and MATLAB 2010b for Windows (Mathworks, Natick, MA, USA). First, 11C-PIB integral images (data corrected for radioactive decay summed from 60 to 90 min post-injection) were created from the dynamic PET images (frames 32 to 34) and coregistered to the subject's MR images. Second, the MR images were segmented into three classes (gray matter, white matter and cerebrospinal fluid) in SPM8 using 16 non-linear iterations and 7 × 9 × 7 basis functions. Third, the PET images and gray matter MR images were normalized using a T1-weighted basis function in SPM8 using 16 non-linear iterations and 7 × 9 × 7 basis functions. The application of a 0.50 threshold to the gray matter probability map created a gray matter probability map in MNI space. The gray matter probability map was then coregistered to the AAL template, and the PET counts were extracted from the gray matter probability map and ROIs. The mean values for all of the regions were calculated from the integral 11C-PIB image. Target-to-cerebellum ratios were subsequently calculated for 11 bilateral regions.

### D. 18F-FDG PET image and statistical analysis

Spatial preprocessing and statistical analyses of 18F-FDG PET images in all of the subjects were also performed using SPM8 software and MATLAB 2010b for Windows. We compared cerebral glucose metabolism...
in the AD group with the control group. We also compared cerebral glucose metabolism between each aMCI subject and the control group. First, 11C-FDG PET images were converted to ANALYZE format and then normalized to the MNI standard proportional stereotaxic space. Second, an isotropic 10 mm full-width half-maximum Gaussian spatial smoothing filter was applied to the image. Third, all of the comparisons of brain metabolism were performed on a voxel-by-voxel basis using a two-sample t-test. Statistical significance was determined using an extent threshold of 50 voxels. Regions that reached an uncorrected P value of less than 0.001 were considered statistically significant.

### ApoE genotyping

Genomic DNA was extracted from total blood, and the ApoE gene was amplified by polymerase chain reaction (PCR) using reaction conditions modified from Wenham et al. [32]. The two primers were 5'-TCCAAGGAG - GTGCAGGCGGCCGA - 3' (upstream) and 5'-ACAGAATTCCGCC - CCGGCTTGATACGTGCCA - 3' (downstream). Briefly, each amplification contained 200 ng of genomic DNA, 25 pmol of primer, 2.50 μl of 10% dimethyl sulfoxide, and 0.50 units of Taq DNA polymerase in a final volume of 25 μl. In the thermal reactor, initial denaturation occurred at 94 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 65°C for 1 min, extension at 72 °C for 1 min, and final extension at 72 °C for 10 min. The amplification product (20 μl) was then digested with 5 units CfoI for at least 3 h at 37 °C. The samples were then subjected to electrophoresis for 2 h at 200 V on 12% native polyacrylamide gel. The genotypes were determined by the size of the DNA fragments present, viewed, and photographed under ultraviolet light after staining with 0.5 μg/ml ethidium bromide. We determined all of the genotypes without knowledge of the patient’s control status.

### Follow-up

The subjects with aMCI were clinically followed for 1-1.50 years after their baseline 11C-PIB PET scan. During this follow-up period, no change was found in their diagnosis, and they still fulfilled the criteria for MCI (nonconverters), or their symptoms and clinical performance declined to the extent that they fulfilled the NINCDS-ADRDA criteria [30] for a diagnosis of probable AD (converters).

### Statistical analyses

Descriptive statistics were used to make comparisons on the demographic and clinical variables for the 3 groups of subjects (control, aMCI and AD) and the 11C-PIB + aMCI and 11C-PIB-aMCI subgroups. Independent Student’s t-tests were used to determine the differences in the 11C-PIB SUVR and 11C-FDG SPM between the AD group and control group. Analysis of variance (ANOVA) was used to compare differences in radiotracer uptake within the different brain regions among 4 groups (AD, 11C-PIB + aMCI, 11C-PIB-aMCI and control). Significant main effects in the ANOVA were followed by two-tailed t-tests for post hoc pairwise comparisons. Values of $P < 0.05$ were considered statistically significant.

### Ethics

Detailed informed written consent was obtained from all subjects and their relatives. The study was approved by Tianjin Huanhu Hospital and Tianjin Medical University Ethics Committees. The procedures were done in accordance with the ethical standards of Committee on Human Experimentation of Tianjin Huanhu Hospital and Tianjin Medical University.

### Results

#### Clinical data

The control, aMCI and AD groups were compared with each other regarding to demographic and cognitive characteristics of subjects in each group (Table 1). These subjects in 3 groups differed in cognitive performance on MMSE and Montreal Cognitive Assessment (MoCA), in which AD patients scored
worse than MCI patients, and MCI patients scored worse than normal control subjects. The Activities of Daily Living (ADL) score was significantly higher in AD subjects than that in control and MCI subjects (P < 0.05), but the ADL score did not differ between the MCI and control groups.

**11C-PIB PET**

Voxel-based automatic quantitative analysis was used to determine the mean 11C-PIB SUVR in AD, 11C-PIB + aMCI, 11C-PIB - aMCI, and control groups (Figure 1). The mean 11C-PIB SUVR was higher in the IP, LTC, MFG, MPFC, PCCPre, OL, SMA, and striatum in the AD group than in control group (range, 1.80-2.10 and 1.10-1.20; P < 0.05). 11C-PIB binding levels in the aMCI group were bimodal. Using 1.60 as a negative cutoff value for 11C-PIB, the aMCI group could be divided into 11C-PIB + aMCI (N = 5) and 11C-PIB - aMCI (N = 5) subgroups. No significant difference in the 11C-PIB SUVR was found between the 11C-PIB + aMCI subgroup and AD group (P > 0.05) or between the 11C-PIB - aMCI subgroup and control group (P > 0.05) in all of the ROIs (Figure 2).

**18F-FDG PET**

18F-FDG SPM showed hypometabolism in the bilateral IP, posterolateral portions of the temporal lobe, PCCPre, and frontal lobe in AD subjects. In aMCI subjects, hypometabolism was relatively mild compared with the AD group. Three of 5 11C-PIB + aMCI subjects exhibited hypometabolism in the bilateral parietal lobes, lateral temporal cortex, and PCCPre. Three of 5 11C-PIB - aMCI subjects exhibited hypometabolism in the bilateral frontal lobes and anterior cingulate cortex. The remaining 4 subjects had normal 18F-FDG PET (Figure 3).

**Regional analyses of 11C-PIB and 18F-FDG**

The SPM and ROI analyses showed Aβ deposition and hypometabolism in the same regions in the AD group. In the 11C-PIB + aMCI group, high Aβ deposition and 18F-FDG hypometabolism were consistently found in parietal regions, including the PCCPre, and lateral temporal cortex, but inconsistency was observed in the frontal lobe, with high amyloid deposition but
Comparison between $^{11}$C-PIB + aMCI and $^{11}$C-PIB - aMCI subgroups

The $^{11}$C-PIB + aMCI and $^{11}$C-PIB - aMCI subgroups were not different with regard to age, age at onset, gender, and education (Table 2). Although the mean MMSE and MoCA scores were higher in the aMCI subgroup than in the aMCI + subgroup, no statistically significant difference was observed. The ratio of $ApoE_4$ allele carriers was higher in the $^{11}$C-PIB + aMCI group (3/5) than in the $^{11}$C-PIB - aMCI group (0/5, $P<0.01$).

Follow-up in aMCI group

During a 12-18 month follow-up period after their baseline $^{11}$C-PIB PET scans, 2 of the 10 subjects with aMCI (20%) clinically converted to AD. Both of these converters were $^{11}$C-PIB-positive, with bilateral parietal lobes, lateral temporal cortex, and PCCPre hypometabolism at baseline, making the percentage conversion rate in the $^{11}$C-PIB + aMCI subgroup 40% over this same follow-up period. Two converters were $ApoE_4$ carriers. One $^{11}$C-PIB - aMCI subject with bilateral frontal lobes and anterior cingulate cortex hypometabolism converted to FTD.

Discussion

The $^{11}$C-PIB SUVR was $>1.60$ and higher in the IP, LTC, MFG, MPFG, PCCPre, OL, SMA and striatum in AD patients compared with the control group but not in the hippocampal region, which is consistent with the literature $^{[7,10,30]}$. These findings support the relative regional distribution of $A\beta$ deposition reported in other $^{11}$C-PIB studies that also found increased prefrontal, parietal, and precuneus uptake $^{[7,10]}$. The results are also consistent with autopsy data that showed that $A\beta$ deposition in early AD is greater in the frontal and parietal cortices than in the hippocampus $^{[31]}$. $^{11}$C-PIB binding levels in the aMCI group were variable and bimodal. Based on the cutoff value used in the present study, 50% of the MCI patients had low $^{11}$C-PIB binding rates, similar to controls, and 50% exhibited no differences with AD patients in all of the ROIs. One possible explanation for these data is that $^{11}$C-PIB - MCI subjects who have been followed in the clinic for more than 1 year without converting to AD dementia would be less likely to have AD brain pathology than MCI patients who are $^{11}$C-PIB - positive in the initial evaluation.

Control subjects with high $^{11}$C-PIB retention were not present in our sample. In contrast, a previous
study reported high $^{11}$C-PIB retention in approximately 20% of healthy elderly control subjects \cite{22}. Different criteria used to select control subjects may partly account for these differences across studies. In the present study, impairment on neuropsychological tests was a strict exclusion criterion for healthy control subjects, in contrast to the use of CDR of global cognitive/functional ability in some studies, which may have allowed for the inclusion of control subjects with mild neuropsychological deficits \cite{32}. Importantly, the present study had relatively small control samples and the age of the control less than 65 years.

Initial follow-up studies suggested that increased $^{11}$C - PIB retention is associated with an increased likelihood that healthy controls will convert to MCI and MCI patients will convert to AD \cite{16,33}. In a recent study, $^{11}$C - PIB - positive patients with MCI were more likely to convert to AD than $^{11}$C - PIB - negative patients, and faster converters had higher $^{11}$C - PIB retention levels at baseline than slower converters \cite{17}. In patients diagnosed with AD, no increase \cite{34} or a small increase in $^{11}$C - PIB retention may be found during the follow-up.

Figure 3 $^{18}$F - FDG SPMs. Hypometabolism in the bilateral inferior parietal lobes, posterolateral portions of the temporal lobe, posterior cingulate cortex and precuneus, and frontal lobe in AD subjects (Panel 3a). Hypometabolism in the bilateral parietal lobes, lateral portions of temporal lobe, and posterior cingulate cortex and precuneus in $^{11}$C - PIB + aMCI subjects (Panel 3b). Hypometabolism in bilateral frontal lobes and anterior cingulate cortex in $^{11}$C - PIB - aMCI subjects (Panel 3c). Normal $^{18}$F - FDG metabolism in aMCI subjects (Panel 3d) and control subjects (Panel 3e).
peroid \cite{14, 35-36}. These results indicate that once the stage of established AD is reached, Aβ deposition in most regions has plateaued. In our 18C-PIB-positive aMCI subgroup, the 18C-PIB SUVRs were consistent with AD in the frontal, parietal and occipital lobes. One possibility is that Aβ deposition plateaued in these regions, and these patients may be more likely to convert to AD. At the 1-year follow-up, the MMSE and MoCA scores declined significantly in 2 18C-PIB-positive aMCI patients, who converted to the clinical AD stage.

18F-FDG hypometabolism differed between the AD and control groups in bilateral PCCPre, IP, posterolateral portions of the temporal lobes and frontal lobe but not in the hippocampus or parahippocampal gyrus. These findings are consistent with the literature on metabolic deficits in the parietal and posterior cingulate that distinguish AD from control subjects \cite{1}. In this sample, however, no significant differences were found in medial temporal regions. Some other reports indicated that metabolic deficits in both the parietal and temporal lobes distinguish AD from control subjects \cite{2}. Metabolic deficits in AD gradually worsen throughout the course of the disease. More advanced stages of the disease usually involve prefrontal association areas, and primary cortices may eventually be affected. Six of our AD subjects were already in the moderate stage of the disease with frontal lobe involvement. Three of 5 18C-PIB-positive aMCI subjects exhibited hypometabolism in the bilateral parietal lobe, lateral temporal cortex, and PCCPre. Previous 18F-FDG PET studies that used small samples of MCI patients reported that hypometabolism in the parietotemporal and posterior cingulate may characterize future converters to AD \cite{3, 4}. These 18C-PIB-positive patients with hypometabolism may have a greater probability of converting to AD dementia. Another study that used a small sample suggested that regional decreases in the regional cerebral metabolic rate of glucose in the parietal and posterior cingulate may be superior to 18C-PIB in distinguishing MCI from control subjects \cite{5}. In our sample, however, 3 of 5 18C-PIB- aMCI subjects exhibited hypometabolism in the bilateral frontal lobes and anterior cingulate but not in the parietal and posterior cingulate. These patterns of hypometabolism are consistent with FTD \cite{2}. In our 1-year follow-up, one of the subjects converted to FTD. One possibility is that these patients would convert to FTD or another dementia but not AD dementia. However, this needs to be clarified in longitudinal studies.

In our aMCI group, the 18C-PIB-positive subgroup had more ApoE ε4 carriers than the 18C-PIB-negative subgroup and control group, consistent with previous studies that suggested that the presence of the ApoE ε4 allele is associated with increased 18C-PIB retention in AD \cite{6, 7}. Postmortem studies have reported correlations between the presence of the ApoE ε4 allele and higher Aβ burden in the brains of patients with sporadic AD \cite{8, 9}. Okello et al \cite{10} found an association between ApoE ε4 status in PIB-positive subjects with MCI and the rate of clinical conversion to AD, and all of the faster converters were ApoE ε4 carriers. In the present study, two 18C-PIB+aMCI converted patients were ApoE ε4 carriers, also suggesting that the ApoE ε4 allele accelerated the conversion from aMCI to AD.

One limitation of the present study was its cross-sectional design. Follow-up data to examine the prognostic implications of the baseline PET findings are clearly needed. The sample size in the present study was also relatively small. A study with a larger patient sample would be of great interest to determine the aMCI patterns that are more likely to convert to AD dementia.

**Conclusion**

18C-PIB PET is a powerful tool to screen aMCI with AD pathology. The aMCI patients with AD pathology in the present study who exhibited hypometabolism in the parietal lobe, lateral temporal cortex and precuneus in 18F-FDG PET and were ApoE ε4 allele carriers were more likely to convert to clinical AD dementia. These PET techniques combined with ApoE genotyping...
provide complementary information to strongly distinguish diagnostic groups in cross-sectional comparisons, but longitudinal studies are still required.

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**Disclosure**

No authors report any conflict of interest.

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