Alterations in $\alpha 4\beta 2$ nicotinic receptors in cognitive decline in Alzheimer’s aetiopathology

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Nicotinic acetylcholine receptor subtype $\alpha 4\beta 2$ is considered important in the regulation of attention and memory, and cholinergic degeneration is known as one pathophysiology of Alzheimer’s disease. Brain amyloid-$\beta$ protein deposition is also a key pathological marker of Alzheimer’s disease. Recent amyloid-$\beta$ imaging has shown many cognitively normal subjects with amyloid-$\beta$ deposits, indicating a missing link between amyloid-$\beta$ deposition and cognitive decline. To date, the relationship between the $\alpha 4\beta 2$ nicotinic acetylcholine receptor and amyloid-$\beta$ burden has not been elucidated in vivo. In this study we investigated the relation between $\alpha 4\beta 2$ nicotinic acetylcholine receptor availability in the brain, cognitive functions and amyloid-$\beta$ burden in 20 non-smoking patients with Alzheimer’s disease at an early stage and 25 age-matched non-smoking healthy elderly adults by measuring levels of $\alpha 4\beta 2$ nicotinic acetylcholine receptor binding estimated from a simplified ratio method (BPRI) and Logan plot-based amyloid-$\beta$ accumulation (BPND) using positron emission tomography with $\alpha 4\beta 2$ nicotinic acetylcholine receptor tracer $^{18}$F-2FA-85380 and $^{11}$C-Pittsburgh compound B. The levels of tracer binding were compared with clinical measures for various brain functions (general cognition, episodic and spatial memory, execution, judgement, emotion) using regions of interest and statistical parametric mapping analyses. Between-group statistical parametric mapping analysis showed a significant reduction in $^{18}$F-2FA-85380 BPRI in the cholinergic projection region in patients with Alzheimer’s disease with a variety of $^{11}$C-Pittsburgh compound B accumulation. Spearman rank correlation analyses showed positive correlations of $^{18}$F-2FA-85380 BPRI values in the medial frontal cortex and nucleus basalis magnocellularis region with scores of the Frontal Assessment Battery (a test battery for executive functions and judgement) in the Alzheimer’s disease group ($P < 0.05$ corrected for multiple comparison), and also positive correlations of the prefrontal and superior parietal $^{18}$F-2FA-85380 BPRI values with the Frontal Assessment Battery score in the normal group ($P < 0.05$ corrected for multiple comparison). These positive correlations indicated an in vivo $\alpha 4\beta 2$ nicotinic acetylcholine receptor role in those specific functions that may be different from memory. Both region of interest-based and voxelwise regression analyses showed a negative correlation between frontal $^{11}$C-Pittsburgh compound B BPND and $^{18}$F-2FA-85380 BPRI values in the medial frontal cortex and nucleus basalis magnocellularis region.
in patients with Alzheimer’s disease (P < 0.05 corrected for multiple comparison). These findings suggest that an impairment of the cholinergic \(\alpha_4\beta_2\) nicotinic acetylcholine receptor system with the greater amount of amyloid deposition in the system plays an important role in the pathophysiology of Alzheimer’s disease.

Keywords: \(\alpha_4\beta_2\) nicotinic receptor; cognitive decline; amyloid deposition; Alzheimer’s disease; positron emission tomography

Abbreviation: PiB = Pittsburgh compound B

Introduction

Changes in the cholinergic system during ageing and in Alzheimer’s disease have been documented in many studies that focus on alterations in functional components of the system such as acetylcholine synthesis and degradation enzymes, vesicular transporters, muscarinic and nicotinic receptors and neurotrophic factors (Perry et al., 1977; Hefti et al., 1985; Phillips et al., 1991; Davis et al., 1999). These post-mortem and in vivo studies on patients with mild cognitive impairment and early-stage Alzheimer’s disease indicated enzymatic reduction seen only at the advanced stage. Because acetylcholine plays an important role in functional and structural remodelling of cortical circuits that operate in complicated cognitive processing in adulthood (Berger-Sweeney, 2003), it is desirable to depict changes in the cholinergic function early during cognitive declines in vivo. Use of in vivo techniques such as PET allows us to visualize nicotinic acetylcholine receptors lost early in the disease (Kadir et al., 2007), indicating that alteration in nicotinic acetylcholine receptors is of pathophysiological and therapeutic interest. Indeed, it has been shown that the nicotinic acetylcholine receptor system plays a crucial role in modulating attention and enhancing cognitive performance (Roelfsema, 2011) and that the \(\alpha_4\beta_2\) subtype of nicotinic acetylcholine receptors are involved in many cognitive-behavioural actions (Picciotto et al., 2000). Among the nicotinic acetylcholine receptor subtypes, the \(\alpha_4\beta_2\) heteromer is the most abundant and has a high affinity for agonists including nicotine (Nordberg, 1992). In human studies, \(\alpha_4\beta_2\) nicotinic acetylcholine receptor-mediated agonists act on working memory and attentional performance (Dubel et al., 2007; Loughead et al., 2010), and hence marked reduction in \(\alpha_4\beta_2\) nicotinic acetylcholine receptor availability is present in the post-mortem Alzheimer’s disease brain (Court et al., 2001). Thus, measuring nicotinic acetylcholine receptor availability in vivo may help understand the role of nicotinic acetylcholine receptors in elderly subjects and/or dementia patients on the molecular basis.

In addition to cholinergic dysfunction, amyloid-\(\beta\) protein is a characteristic pathological hallmark of Alzheimer’s disease. A recent development in in vivo PET techniques is amyloid imaging with \(^{11}\)C-Pittsburgh compound B (PiB) tracer, which enables us to elucidate the role of the missing link in the living human subjects. A recent post-mortem study showed that elevation in \(^{11}\)H-PiB accumulation and decreased choline acetyltransferase activity were associated with cognitive deterioration (Ikonomovic et al., 2011).

The purpose of the present study was to investigate whether changes in \(\alpha_4\beta_2\) nicotinic acetylcholine receptors and amyloid-\(\beta\) burden would exist in a cholinergic vulnerability fashion in the living brains of cognitively normal elderly subjects and patients with early-stage Alzheimer’s disease by measuring \(^{18}\)F-2FA-85380 binding potential and \(^{11}\)C-PiB uptake using PET.

Materials and methods

Participants

Twenty-five non-smoking, healthy elderly subjects (10 males and 15 females; mean age 62.2 ± 12.5 years) and 20 drug-naïve, non-smoking patients with Alzheimer’s disease at an early-to-moderate severity stage (nine males and 11 females; mean age 64.7 ± 9.1 years) participated in this study (Table 1). No subjects had any family history of psychiatric or neurological diseases. Subjects were excluded if they showed the presence of significant white matter microvascular changes on MRI. No participants took any nootropic drugs including acetylcholine esterase inhibitors. The Ethics Committee of Hamamatsu
Table 1  Demographic features of participants

<table>
<thead>
<tr>
<th>Measures</th>
<th>Normal</th>
<th>Alzheimer's disease</th>
<th>P-value</th>
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<tbody>
<tr>
<td>Total number</td>
<td>25</td>
<td>20</td>
<td>0.621a</td>
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<tr>
<td>Male/female</td>
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<tr>
<td>Age (years)</td>
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<td>64.7 (9.1)</td>
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<tr>
<td>Education (years)</td>
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<td>13.2 (2.3)</td>
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<td>&lt;0.001</td>
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<tr>
<td>MMSE</td>
<td>28.0 (1.7)</td>
<td>19.9 (4.9)*</td>
<td>&lt;0.001</td>
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<tr>
<td>Cubic copying test</td>
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<td></td>
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<tr>
<td>Point of connection</td>
<td>8 (0)b</td>
<td>3.4 (2.1)*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plane-drawing errors</td>
<td>0 (0)c</td>
<td>4.5 (2.4)*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Frontal assessment battery</td>
<td>14.7 (2.3)</td>
<td>7.6 (2.5)*</td>
<td>&lt;0.001</td>
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<td>Rivermead Behavioural</td>
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<td>4.9 (4.1)*</td>
<td>&lt;0.001</td>
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<tr>
<td>Memory Test</td>
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<tr>
<td>WMS-R (delayed logical subst)</td>
<td>17.7 (5.7)</td>
<td>2.8 (3.7)*</td>
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<td>Zung Self-rating</td>
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<td>Depression Scale</td>
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</tbody>
</table>

Data are expressed as mean (SD). *P < 0.05, unpaired t-test.

a Pearson’s Chi-square test.

b Eight points represents a perfect score.

c Zero represents a perfect score.

CDR = Clinical Dementia Rating; MMSE = Mini-Mental State Examination; WMS-R = Wechsler Memory Scale revised.

University and Hamamatsu Medical Centre approved the study, and written informed consent was obtained from all participants before enrolment. As shown in Table 1, several neuropsychological tests were conducted at entry to evaluate various cognitive functions; the Mini-Mental State Examination for general cognition, Frontal Assessment Battery for frontal lobe function or executive function, Rivermead Behavioural Memory Test for everyday memory performance, delayed logical memory of Wechsler Memory Scale for verbal, episodic memory, and the Zung self-rating depression scale for mood.

Magnetic resonance imaging data acquisition

All participants underwent a 3D MRI scan immediately before PET measurements. Magnetic resonance images were obtained using a static magnet (0.3 T MRP7000AD; Hitachi) with the following acquisition parameters: 3D mode sampling, repetition time/echo time (200/23), 148 flip angle, 2 mm slice thickness with no gap, and 256 x 256 matrices (x = 0.938 mm, y = 0.938 mm, z = 1.2 mm) (Ouchi et al., 1998).

Positron emission tomography data acquisition

We used a high-resolution brain PET scanner (SHR12000; Hamamatsu Photonics K.K.) (Ouchi et al., 1999). After head fixation using a thermoplastic face mask, a 10-min transmission scan was performed in all participants. In the complete scan protocol, 14 subjects (six males and eight females) among 25 normal participants and three additional patients with Alzheimer’s disease (one male and two females) underwent a 4-h-long PET measurement that consisted of dynamic scans for the first 120 min (12 × 10 s, 18 × 60 s, and 20 × 300 s), which began immediately after tracer injection, a 30-min waiting period followed by a second 30-min scanning session (3 × 10 min) performed 210 min after the start of PET, which was similar to but a shorter measurement protocol than the previous report (Meyer et al., 2009). The dose of 18F-2FA-85380 injected was 4 MBq/kg per scan. Although the fixation of the head of an examinee was temporarily released during the waiting period, the use of a thermoplastic face mask and a 3D laser ensured the head remained fixed in the same position during the series of PET scans (Ouchi et al., 1999). Arterial blood samples were collected from the brachial artery at designated times (25 time points) during PET measurements to assess plasma radioactivity. Additional sampling was conducted for the metabolite correction of 18F-2FA-85380 in plasma (Gallezot et al., 2005). After validation of using the simplified method as a substitute for the complete scan (see below), the remaining 11 healthy elderly subjects and patients with Alzheimer’s disease underwent the second scan only (simplified scan). Some of these participants were scanned under the continuous scan protocol described above without arterial blood sampling.

As for amyloid imaging, 70-min-duration PET scans (time frames: 12 × 10, 18 × 60, and 10 × 300 s) initiated just after an injection of 5 MBq 11C-PiB/kg without blood sampling were performed within a week after or before the 18F-2FA-85380 scan.

Evaluation of 18F-2FA-85380 binding potential

Using PMOD software (PMOD technologies) and data from the complete scan method, the distribution volume and then binding potential (BP) of 18F-2FA-85380 were calculated on the basis of invasive Logan plot analysis with a metabolite-corrected arterial plasma input function (Chefer et al., 2003; Meyer et al., 2009) that assumed that the corpus callosum was a reference region devoid of nictinic acetylcholine receptor (Brody et al., 2006; Mukhin et al., 2008). Because the complete scan protocol was time-consuming and less practical in a clinical setting, we semiquantified the ratio of 18F-2FA-85380 radioactivity in a target tissue to 18F-2FA-85380 radioactivity in the corpus callosum (ratio index of binding potential; BPi(t)) without blood sampling in order to simplify the quantification of 18F-2FA-85380 BP (simplified method). The irregular regions of interest were determined in the following brain regions: cerebellum, amygdala, nucleus basalis magnocellularis, putamen and thalamus, medial frontal, dorsal frontal, temporal and parietal cortices on MRI images using an MRI atlas (Mai et al., 1997), the procedure of which was able to minimize the partial volume effect. Specifically, according to the human nucleus basalis magnocellularis on MRI (Zaborszky et al., 2008), the mean volume of nucleus basalis magnocellularis is 23–80 mm3 and the gravity centre of the nucleus basalis magnocellularis in the anatomical MNI space is as follows; x = 6–18, y = −2–2, z = −6–−2. The present region of interest on the nucleus basalis magnocellularis is, therefore, determined on the area covering this reported region, i.e. using the 4 × 4 × 4.8 mm depth value. This means that the target of the nucleus basalis magnocellularis region was determined onto the area beneath the anterior commissure at the nucleus accumbens on MRI (Zaborszky et al., 2008). The regions of interest placed on MRI images were automatically transferred onto the corresponding parametric BPi(t) images using image processing software (Dr View, Asahi Kasei Co.) on a SUN workstation (Ultrasp, SUN Microsystems) as described elsewhere (Ouchi et al., 1999, 2009). The parametric BPi(t) images were analysed two ways with region of interest and voxelwise statistical parametric mapping (SPM8, Wellcome Department of Cognitive Neurology, London, UK) analyses.
Evaluation of amyloid-β accumulation

Parametric images of 11C-PiB uptake in the brain were generated as a unit of BPND by using Logan plot analysis (Logan et al., 1994; Yokokura et al., 2011). The purpose of the present amyloid-β imaging was to indicate the degree of the amyloid-β pathology occurring in individual brains. The 11C-PiB BPND parametric images were normalized to the standardized Montreal Neurological Institute (MNI) template using SPM8. Instead of using MarsBar regions of interest (MARSeille Boîte À Région d’Intérêt (Brett et al., 2002)), as previously used in our study that dealt with multiple PET tracers images (Kikuchi et al., 2011), we set volumes of interest with a 5 mm radius on the T1-weighted MRI images (Brodmann area 11 and 24) and medial parietal precuneus regions (Brodmann area 7) bilaterally in the 11C-PiB BPND images and estimated the volume of interest values as indices of amyloid-β burden in the medial prefrontal and medial parietal cortices, respectively (Fig. 2B). These regions were chosen because amyloid-β deposition is known to occur predominantly in these areas in normal ageing and dementia (Buckner et al., 2005; Sperling et al., 2009; Kikuchi et al., 2011).

Statistical analysis

In region of interest-based analyses, a simple regression analysis was first performed to compare quantified binding potential values with BPND values in various (receptor-rich and receptor-sparse) brain regions in order to determine whether the simplified method for binding potential calculation could be an adequate alternative to the complete scan method. The level of significance was set at \( P < 0.05 \). Comparisons between dependent variables (18F-2FA-85380 BPND levels from nine regions of interest) and independent variables (clinical scores) were conducted using Spearman’s rank correlation, and significance was defined as \( P < 0.00138 \) because of multiple comparisons (nine regions of interest). These analyses were conducted using SAS (version 9.0, SAS Institute Inc.). For the purpose of examining the relationship between 18F-2FA-85380 binding and amyloid-β burden, regression analysis was performed to compare regional 18F-2FA-85380 BPND levels with medial prefrontal and parietal 11C-PiB BPND values in Alzheimer’s disease and normal healthy groups. In this correlation analysis, the explanatory variables were 11C-PiB BPND data obtained from the medial prefrontal and medial parietal volume of interests on spatially-normalized PET images as shown in the Fig. 2B.

Statistical parametric mapping was used in voxel-wise analyses. According to the previous report (Meyer et al., 1999), we used the T1-weighted MRI images for 18F-2FA-85380 PET data normalization because not all 18F-2FA-85380 scans provided early-phase accumulation data due to the simplified scan protocol. Individual MRI images were first co-registered to 18F-2FA-85380 PET data, and the co-registered MRI images were spatially normalized to the T1-weighted MRI template using the algorithm provided with SPM8. Then, using the generated normalization parameters, 18F-2FA-85380 BPND parametric images of each individual were normalized to the MNI space. In the 11C-PiB data normalization, K1 images of 11C-PiB and PET template in SPM8 were used for spatial normalization. The normalized images were smoothed with an isotropic Gaussian kernel of 6 mm full-width at half-maximum. Voxel-wise multiple regression analyses were performed to compare 18F-2FA-85380 BPND levels with clinical variables and the 11C-PiB index. Using the factorial statistical parametric mapping model, a one-way ANOVA was performed to determine differences in 18F-2FA-85380 BPND levels between groups with gender, age and education as confounding factors. The level of significance was set at \( P < 0.001 \) uncorrected for peak height with a cluster size larger than 100 because the regions of focus were known a priori as areas with cholinergic innervation (Gallezot et al., 2005).

Results

Semiquantification of 18F-2FA-85380 binding

As shown in Fig. 1, the levels of 18F-2FA-85380 BPND, estimated by the simplified scan method, were positively correlated with the value of 18F-2FA-85380 BP, quantitatively estimated using obtained arterial and tissue time-activity curves, in receptor-rich (thalamus, \( r = 0.967, P < 0.0001, y = 0.612x + 0.358 \)) and receptor-sparse regions (frontal \( r = 0.941, P < 0.0001, y = 0.713x + 0.015 \), temporal, \( r = 0.965, P < 0.0001, y = 0.598x – 0.018 \), Spearman’s rank correlation).

Amyloid-β deposition in the brain

Statistical parametric mapping analysis showed significant elevation of 11C-PiB BPND globally in the Alzheimer’s disease brain, with prefrontal and precuneus dominance (Fig. 2A) as reported elsewhere (Buckner et al., 2005). In these regions, as shown in Fig. 2B, the value of volumes of interest set on the normalized brain were used as an indicator of the degree of amyloid pathology occurring in the brain, which are similar to the method using a MarsBar tool in statistical parametric mapping in our previous study (Kikuchi et al., 2011) (e.g. precuneus volumes of interest: 2.61 ± 0.32, precuneus MarsBar: 2.44 ± 0.40). The region of interest analysis showed significant elevation of amyloid accumulation (11C-PiB BPND) in the entire brain compared with the normal counterpart (Fig. 2C). Compatible with our previous report (Yokokura et al., 2011), we failed to find significant correlations between 11C-PiB BPND and clinical scores, e.g. correlation coefficients (\( r \)) between Frontal Assessment Battery scores and 11C-PiB BPND in nucleus basalis magnocellularis, medial frontal cortex and precuneus are 0.12, 0.18 and 0.11, respectively.

Reduction in 18F-2FA-85380 binding in Alzheimer’s disease

As shown in Fig. 3, the 18F-2FA-85380 BPND level was apparently lower in a patient with Alzheimer’s disease (Fig. 3B) compared to a healthy subject (Fig. 3A). The changes in tissue time activity curves seen in both receptor-dense (thalamus) and receptor-sparse (frontal) regions upheld this finding. In contrast, the curves in the corpus callosum were similar to each other, indicating that the corpus callosum was adequately regarded as the reference region, as reported elsewhere (Meyer et al., 2009).

Voxelwise and region of interest-based between-group analyses showed widespread reduction in 18F-2FA-85380 BPND level over the cholinergic projection regions (thalamus, caudate, prefrontal cortex, medial and lateral temporal cortices) (Fig. 4A and Table 2). Interestingly, the nucleus basalis magnocellularis, a major cholinergic origin, was among significantly affected regions, which was in line with the evidence that α4β2 nicotinic
acetylcholine receptors are also rich in the nucleus basalis magnocellularis (Azam et al., 2003).

## Regional $^{18}$F-2FA-85380 binding and cognitive functions

Spearman rank correlation test showed that, in cognitively normal older subjects, there were significant correlations between Frontal Assessment Battery score and $^{18}$F-2FA-85380 $\text{BP}_{\text{RI}}$ levels in the dorsal prefrontal ($\rho = 0.718, P < 0.001$), medial prefrontal ($\rho = 0.686, P < 0.001$), and superior medial parietal ($\rho = 0.597, P < 0.003$) cortices (Fig. 5A). There was a tendency of correlation in the nucleus basalis magnocellularis ($\rho = 0.563$), thalamus ($\rho = 0.543$), and temporal cortex ($\rho = 0.512$). Statistical parametric mapping correlation analysis supported this finding (Fig. 5B and Table 3). No other clinical variables are relevant to $^{18}$F-2FA-85380 binding in any regions.

In the Alzheimer’s disease group (Fig. 5C and D), the statistics showed positive correlations of Frontal Assessment Battery points with $^{18}$F-2FA-85380 $\text{BP}_{\text{RI}}$ levels in the nucleus basalis magnocellularis ($\rho = 0.668, P < 0.003$) and medial prefrontal area ($\rho = 0.721, P < 0.001$), suggesting that lower frontal function might be still under the influence of $\alpha 4\beta 2$ nicotinic acetylcholine receptors function in patients with early-stage Alzheimer’s disease.

### Regional $^{18}$F-2FA-85380 binding and $^{11}$C-PiB deposition

Statistical parametric mapping correlation analysis depicted significant brain regions in which $^{18}$F-2FA-85380 $\text{BP}_{\text{RI}}$ levels were
negatively correlated with the $^{11}$C-PiB $B_{\text{ND}}$ in the Alzheimer’s disease group (Fig. 6A and B, Table 4). Region of interest-based correlation coefficients in each brain region are shown in Table 5. Such a significant association failed to be found in the healthy group possibly due to the limited degree of $^{11}$C-PiB accumulation in the group. Interestingly, as shown in the scattergrams (Fig. 6C), we found significantly negative correlations of $^{18}$F-2FA-85380 $B_{\text{R1}}$ levels in the nucleus basalis magnocellularis region and medial frontal cortex with $^{11}$C-PiB $B_{\text{ND}}$ of the medial prefrontal cortex, but not with $^{11}$C-PiB $B_{\text{ND}}$ of the medial parietal cortex, although there was a tendency of negative correlation between $^{18}$F-2FA-85380 $B_{\text{R1}}$ and medial parietal $^{11}$C-PiB $B_{\text{ND}}$.

**Discussion**

We have shown, for the first time, that $^{18}$F-2FA-85380 $B_{\text{R1}}$ values in the medial frontal cortex and nucleus basalis magnocellularis region, significantly lower in Alzheimer’s disease than normal control subjects, were negatively correlated with the values of $^{11}$C-PiB $B_{\text{ND}}$ in the medial prefrontal cortex in the Alzheimer’s disease group. This negative correlation, however, was not found against $^{11}$C-PiB $B_{\text{ND}}$ in the medial parietal cortex (precuneus). Considering the evidence that amyloid-β accumulation depicted by $^{11}$C-PiB is highlighted most in the precuneus in the Alzheimer’s disease brain (Ikonomovic et al., 2011), greater deposits of amyloid-β would not always give the most deleterious effect on the cholinergic terminals. Rather as reported previously, even small amyloid-β deposition would be detrimental in the vulnerable area such as nucleus basalis magnocellularis and medial prefrontal cholinergic regions that were manifest in the present study. The presence of this cholinergic vulnerability may partly explain why many healthy elderly people are found cognitively normal despite a great amount of $^{11}$C-PiB accumulated globally in their brains (Jack et al., 2009; Villain et al., 2012).

In the present study, we targeted changes in the α4β2 nicotinic acetylcholine receptor system in consideration of cognitive decline under the amyloid pathology in vivo. As the α4β2 nicotinic acetylcholine receptor is the most abundant nicotinic acetylcholine receptor subtype in the human brain (Gotti et al., 2006), it is...
Figure 3. MRI-PET fusion parametric images of $^{18}$F-2FA-85380 $B_{P_{RI}}$ in a normal elderly subject (NC, A) and a patient with Alzheimer’s disease (AD, B). There is a marked decrease in $^{18}$F-2FA-85380 binding in widespread areas of the Alzheimer’s disease brain. The colour bar indicates a level of binding ratio. Scattergrams show the time activity curves of $^{18}$F-2FA-85380 for each region in the healthy control subject (A) and a patient with Alzheimer’s disease (B).

Figure 4. Between-group analysis on $^{18}$F-2FA-85380 binding. Statistical parametric mapping demonstrates a significant reduction in $^{18}$F-2FA-85380 in the frontal and temporal regions (A, $P < 0.0001$ uncorrected). Region of interest analysis also showed an extensive reduction in $^{18}$F-2FA-85380 binding in the Alzheimer’s disease group (B, $P < 0.05$ corrected). NMB = nucleus basalis magnocellularis.
particularly important for in vivo imaging to assess executive and memory functions in humans, irrespective of normal and disease conditions. Indeed, in the present study, we showed significant association between the $^{18}$F-2FA-85380 availability in the brain and frontal lobe functions by the Frontal Assessment Battery test in healthy elderly subjects and patients with Alzheimer’s disease. To quantify the $^{18}$F-2FA-85380 binding, ideally this tracer should be followed for a relatively long period (4-h in theory). However, as reported previously (Brody et al., 2006), the use of the corpus callosum as a reference region (Fig. 3) and the current simplified measurement allowed us to obtain a significantly positive correlation between quantitatively estimated binding potential value and semiquantitatively calculated BP$_{RI}$ value. This confirmed the validity of using simplified measurement on the binding of $^{18}$F-2FA-85380 on a larger scale, extending to the study of elderly and/or cognitively impaired individuals. Indeed, as shown in Fig. 3, it is apparently easy to differentiate a patient with Alzheimer’s disease from a normal elderly subject by the appearance of time-activity curves or the parametric images of BP$_{RI}$.

Between-group analysis made this differentiation further remarkable as shown in Fig. 4. Previous ex vivo studies showing a correlation between the loss of nicotine-binding sites in the frontal cortex and the grade of dementia in patients with Parkinson’s dementia (Rinne et al., 1991) or with Mini-Mental State Examination scores in patients with Alzheimer’s disease (Whitehouse and Kalaria, 1995) indicate that an impairment of the nicotinic acetylcholine receptor binding sites is closely linked with cognitive deficit. In mice lacking the β2 subunits, executive function-related cognitive performance is deteriorated (Picciotto et al., 1995; Shoaib et al., 2002) despite the fact that general spatial memory tested in the water-maze task is not affected (Zoli et al., 1999), suggesting that the loss of α4β2 nicotinic acetylcholine receptors would not affect memory but executive function (Granot et al., 2003). Consistent with these reports, we found a significant correlation between $^{18}$F-2FA-85380 BP$_{RI}$ in the prefrontal and precuneus cortices in healthy subjects and scores of Frontal Assessment Battery for testing higher cognitive and executive functions, not memory-task scores in the present study (Fig. 5A). In a recent animal experiment, administration of a selective α4β2 nicotinic acetylcholine receptor agonist facilitated attentional performance under demanding conditions by evoking sharper cholinergic transients on cue detection in performing animals (Howe et al., 2010). In humans, an α4β2 partial agonist was shown to increase working memory-related brain activity (Loughhead et al., 2010). Thus, cholinergic activation through α4β2 nicotinic acetylcholine receptors is considered important in activation of cognitive functions. This contention may be applicable under the pathological condition since the decreased levels of $^{18}$F-2FA-85380 BP$_{RI}$ in the prefrontal cortex and nucleus basalis magnocellularis in Alzheimer’s disease were shown to correlate with the Frontal Assessment Battery scores (Fig. 5C).

A contribution of regionally different α4β2 nicotinic acetylcholine receptor availability to cognition was intriguing, i.e. the regions having a significant association with $^{18}$F-2FA-85380 BP$_{RI}$ alteration corresponded largely with the cholinergic projection regions originating from the nucleus basalis magnocellularis. It is known that whereas the nucleus basalis magnocellularis sends cholinergic fibres mainly to the cerebral cortex, the pontomesencephalotegmental neurons project to the thalamus, striatum and cerebellospinal region (Woolf et al., 1989). A comparison of gene expression profiles in these two projection systems showed that ageing elevates metabolic activity in cholinergic neurons occurring to a much greater degree in the basal forebrain than in the brainstem (pontine system) (Baskerville et al., 2008). As such, regarding changes in higher cognitive function, the nucleus basalis magnocellularis projection system may be more significant than the pontine cholinergic system. Indeed, whereas the $^{18}$F-2FA-85380 BP$_{RI}$ in the thalamus was significantly lower in Alzheimer’s disease in the present study, the $^{18}$F-2FA-85380 BP$_{RI}$ levels in the nucleus basalis magnocellularis projection region were associated with cognitive changes. This finding was supported by a series of pharmacological animal studies showing a special role of cholinergic signalling in the medial prefrontal region in attention and cognition (McGaughy et al., 2002; Hahn et al., 2003; Parikh et al., 2007). The α4β2 nicotinic acetylcholine receptor in the medial prefrontal cortex is specifically implicated because selective agonists of α4β2 nicotinic acetylcholine receptors in this region can enhance attention and attention-dependent cognitive performance effectively (Buccafusco et al., 1995; Poter et al., 1999). A recent experiment in transgenic mice with nicotinic acetylcholine receptor β2-subunit deletions has shown that restoration of α4β2 nicotinic acetylcholine receptors in the prefrontal cortex can reverse attentional

### Table 2 Statistical parametric mapping results of lower $^{18}$F-2FA-85380 binding in the Alzheimer’s disease group than in the healthy group

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Brodmann area</th>
<th>L/R</th>
<th>Coordinate (x,y,z)</th>
<th>Z-score</th>
<th>Cluster size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Middle temporal gyrus</td>
<td>21</td>
<td>L</td>
<td>−52</td>
<td>6</td>
<td>−28</td>
</tr>
<tr>
<td>Thalamus</td>
<td>R</td>
<td>8</td>
<td>−16</td>
<td>4</td>
<td>4.95</td>
</tr>
<tr>
<td>NBM</td>
<td>R</td>
<td>−4</td>
<td>0</td>
<td>−4</td>
<td>4.91</td>
</tr>
<tr>
<td>Superior temporal gyrus</td>
<td>38</td>
<td>R</td>
<td>42</td>
<td>16</td>
<td>−36</td>
</tr>
<tr>
<td>Inferior frontal gyrus</td>
<td>47</td>
<td>R</td>
<td>40</td>
<td>14</td>
<td>−14</td>
</tr>
<tr>
<td>Middle frontal gyrus</td>
<td>10</td>
<td>L</td>
<td>−26</td>
<td>46</td>
<td>8</td>
</tr>
<tr>
<td>Superior temporal gyrus</td>
<td>22</td>
<td>R</td>
<td>58</td>
<td>−8</td>
<td>2</td>
</tr>
<tr>
<td>Superior frontal gyrus</td>
<td>6</td>
<td>L</td>
<td>−18</td>
<td>16</td>
<td>65</td>
</tr>
<tr>
<td>Superior frontal gyrus</td>
<td>10</td>
<td>R</td>
<td>20</td>
<td>70</td>
<td>−8</td>
</tr>
</tbody>
</table>

Statistical significance was assumed at an individual voxel level of $P<0.0001$, uncorrected, $k>100$ voxels.

†Talairach brain atlas. L/R = left or right side of the brain; NBM = magnocellular basal nucleus.

[57x163](Zoli et al., 2013: 136; 3004–3017 | 3011)
deficits (Guillem et al., 2011). Thus, cholinergic vulnerability with selective impairment of \(\alpha_4\beta_2\) nicotinic acetylcholine receptors may be a key neurophysiological feature to understand the mechanism of cognitive decline in ageing and dementia.

The negative correlation of prefrontal \(^{11}\text{C}\)-PiB accumulation with \(^{18}\text{F}\)-2FA-85380 \(B_P\text{RI}\) in the nucleus basalis magnocellularis and medial prefrontal cortex along with corresponding Frontal Assessment Battery changes in patients with Alzheimer’s disease in the present study is the first to show the interrelation among amyloid-\(\beta\) deposition, \(\alpha_4\beta_2\) nicotinic acetylcholine receptors dysfunction and cognitive decline in Alzheimer’s disease. It was reported that total amyloid level did not correlate with disease progression in patients with histologically proven Alzheimer’s disease (Lue et al., 1999; McLean et al., 1999), and that in patients with clinically diagnosed Alzheimer’s disease there was no significant association between \(^{11}\text{C}\)-PiB uptake and cognitive deterioration or clinical severity (Rowe et al., 2007; Yokokura et al., 2011) or only a weak association (Pike et al., 2007). As the \(^{11}\text{C}\)-PiB accumulation may reach a plateau at the level of dementia with moderate clinical severity (Grimmer et al., 2009), it is likely that the degree of cognitive deterioration depends on how much the nicotinic acetylcholine receptors are impaired. Mounting evidence that \(^{11}\text{C}\)-PiB deposition affects cognitive performance in normal ageing (Pike et al., 2007; Rentz et al., 2010; Kikuchi et al., 2011) emphasizes that \(^{11}\text{C}\)-PiB uptake can be an index for reduced cognitive reserve during the preclinical state of
Table 3  Statistical parametric mapping results on correlations between α4β2 receptor and Frontal Assessment Battery scores

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Brodmann area</th>
<th>L/R</th>
<th>Coordinate (x,y,z)</th>
<th>Z-score</th>
<th>Cluster size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive correlation in the normal group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle frontal gyrus</td>
<td>8</td>
<td>L</td>
<td>±40</td>
<td>8</td>
<td>44</td>
</tr>
<tr>
<td>Superior frontal gyrus</td>
<td>8</td>
<td>L</td>
<td>±20</td>
<td>44</td>
<td>50</td>
</tr>
<tr>
<td>Precuneus</td>
<td>7</td>
<td>R</td>
<td>4</td>
<td>±54</td>
<td>54</td>
</tr>
<tr>
<td>Positive correlation in the Alzheimer’s disease group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superior temporal gyrus</td>
<td>38</td>
<td>L</td>
<td>±14</td>
<td>24</td>
<td>±28</td>
</tr>
<tr>
<td>Inferior frontal gyrus</td>
<td>46</td>
<td>L</td>
<td>±46</td>
<td>22</td>
<td>8</td>
</tr>
<tr>
<td>Superior frontal gyrus</td>
<td>10</td>
<td>R</td>
<td>10</td>
<td>±64</td>
<td>20</td>
</tr>
</tbody>
</table>

Statistical significance was assumed at an individual voxel level of P < 0.001, uncorrected, k > 100 voxels.

*Talairach brain atlas. L/R = left or right side of the brain; NBM = nucleus basalis magnocellularis.

Figure 6  Correlation between 18F-2FA-85380 binding and 11C-PiB uptake. Glass brains and sectioned images show that the medial frontal and nucleus basalis magnocellularis (NBM) 18F-2FA-85380 BPRI levels were negatively correlated with the 11C-PiB BPND in the medial prefrontal cortex not with the BPND in the medial parietal cortex (k > 100 contiguous voxels; P < 0.001 uncorrected) (A and B, see Table 4). Dotted lines represent 95% confidence intervals of the fitted lines in the scattergrams (C).
amyloid pathology. Our results may go further to the speculation that amyloid pathology-related impairment of the α4β2 nicotinic acetylcholine receptors system plays a critical role in the aetiology of preclinical and early-stage Alzheimer’s disease. The reason for the present correlation seen on the right side was unclear, but there might be right-sided vulnerability in the nicotinic cholinergic system among the memory-deteriorating patients examined in the present study because recent memories preferentially involve the right prefrontal cortex in Alzheimer’s disease (Eustache et al., 2004). Although the mechanism of amyloid-β-related nicotinic acetylcholine receptor loss remains unclear, a hypothesis of soluble amyloid-β being a main culprit has received much attention. Amyloid-β[1–42] would damage the function of α4β2 nicotinic acetylcholine receptors by suppressing cholinergic signalling (Wu et al., 2004), and human APP mutation Tg2576 mice show decreased density of nicotinic acetylcholine receptors before significant plaque load occurs (Apelt et al., 2002). Albeit a different subtype of nicotinic acetylcholine receptor, amyloid-β seems to affect α7 nicotinic acetylcholine receptors by binding to them directly, with a high affinity (Wang et al., 2000; Nagele et al., 2002). In humans, increased soluble amyloid-β levels in the Alzheimer’s disease cerebral cortices are highly correlated with disease severity (Lue et al., 1999; McLean et al., 1999). As 11C-PiB is unable to separate insoluble from soluble amyloid-β (Klunk et al., 2004), we do not currently know how much soluble amyloid-β contributes to the α4β2 nicotinic acetylcholine receptors damage in vivo. However, the present observations may support the contention that soluble amyloid-β (oligomer) plays a crucial role in α4β2 nicotinic acetylcholine receptor dysfunction that would lead to cognitive deterioration in patients with early-stage Alzheimer’s disease.

A caveat of this study might be the selection of candidates. The α4β2 nicotinic acetylcholine receptor is upregulated by nicotine exposure through changes in cell-surface trafficking, functional activity and the transition to a high-affinity state (Marks et al., 1983; Vallejo et al., 2005). This increased binding of 18F-2FA-85380 is true in smokers (Brody et al., 2006; Mukhin et al., 2008; Wullner et al., 2008). Thus, a mixture of an Alzheimer’s disease patient who smokes or has once smoked would have affected the present result. Another possible limitation is an inability for a direct comparison between 18F-2FA-85380 and 11C-PiB accumulations in exactly the same brain regions. This was inevitable because no precise matching of two 18F-2FA-85380 and 11C-PiB images was realistic using original PET images. In the present study, the 11C-PiB deposition in the medial prefrontal cortex, not in the parietal cortex, correlated negatively with levels of 18F-2FA-85380 BPND in the nucleus basalis magnocellularis projection region, suggesting that amyloid-β deposition in the nucleus basalis magnocellularis cholinergic innervation regions might be more significant than amyloid pathology occurring in other regions in deterioration of cognitive function. However, we cannot exclude the possibility of effect of amyloid burden outside the medical frontal cortex because there was a tendency of correlation in the prefrontal cortex because there was a tendency of correlation in the prefrontal cortex. Despite this, it is accepted that the level of 11C-PiB uptake in

### Table 4 Statistical parametric mapping results on correlations between α4β2 receptor and amyloid-β deposition in Alzheimer’s disease

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Brodmann area</th>
<th>L/R</th>
<th>Coordinate (x,y,z)</th>
<th>Z-score</th>
<th>Cluster size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior cingulate gyrus</td>
<td>32</td>
<td>R</td>
<td>6,38</td>
<td>20</td>
<td>4.32</td>
</tr>
<tr>
<td>Caudate-NBM</td>
<td>18</td>
<td>R</td>
<td>20</td>
<td>2</td>
<td>3.91</td>
</tr>
<tr>
<td>Superior frontal gyrus</td>
<td>10</td>
<td>R</td>
<td>10,60</td>
<td>16</td>
<td>3.82</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>4</td>
<td>R</td>
<td>-6</td>
<td>-2</td>
<td>3.72</td>
</tr>
</tbody>
</table>

Statistical significance was assumed at an individual voxel level of P < 0.001, uncorrected, k > 100 voxels.

†Talairach brain atlas. L/R = left or right side of the brain; NBM = nucleus basalis magnocellularis.

### Table 5 Regions-of-interest based correlation coefficients between α4β2 receptor and Aβ deposition in Alzheimer’s disease

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Medial frontal amyloid-β</th>
<th>Medial parietal amyloid-β</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right</td>
<td>Left</td>
</tr>
<tr>
<td>Amygdala</td>
<td>-0.41 (3.71)*</td>
<td>-0.36 (2.71)</td>
</tr>
<tr>
<td>Nucleus basalis magnocellularis</td>
<td>-0.66 (14.4)*</td>
<td>-0.49 (5.43)</td>
</tr>
<tr>
<td>Caudate</td>
<td>-0.37 (2.10)</td>
<td>-0.35 (1.64)</td>
</tr>
<tr>
<td>Putamen</td>
<td>-0.56 (8.01)</td>
<td>-0.48 (5.31)</td>
</tr>
<tr>
<td>Thalamus</td>
<td>-0.37 (2.85)</td>
<td>-0.35 (2.45)</td>
</tr>
<tr>
<td>Frontal cortex</td>
<td>-0.67 (15.5)*</td>
<td>-0.51 (5.57)</td>
</tr>
<tr>
<td>Temporal cortex</td>
<td>-0.63 (12.1)</td>
<td>-0.62 (10.8)</td>
</tr>
<tr>
<td>Parietal cortex</td>
<td>-0.56 (7.96)</td>
<td>-0.50 (6.01)</td>
</tr>
<tr>
<td>Medial prefrontal cortex</td>
<td>-0.74 (22.5)*</td>
<td>-0.59 (8.75)</td>
</tr>
</tbody>
</table>

Data are expressed as r-values of correlation coefficient (F-value). *P < 0.05 corrected for multiple comparison.
the individual brain is generally evaluated as a mean global neocortical value above a certain threshold, not as a singular region of interest or voxel value (Villain et al., 2012). Furthermore, it is difficult to compare the binding of these two tracers on a voxel basis due to sparse accumulation of tracers in the neocortex.

In conclusion, the correlation of α4β2 nicotinic acetylcholine receptor reduction with amyloid-β deposition was detected in the nucleus basalis magnocellularis cholinergic projection region, being an in vivo neuropathological feature of Alzheimer’s disease. Consistent with this cholinergic vulnerability, α4β2 nicotinic acetylcholine receptor availability in the prefrontal cortex (originating from the nucleus basalis magnocellularis) was associated with executive cognitive function (Frontal Assessment Battery score) in both healthy elderly subjects and patients with early-stage Alzheimer’s disease. The results suggest that cognitive deterioration may depend more on how much the α4β2 nicotinic acetylcholine receptors in the nucleus basalis magnocellularis system is affected than on how much the amyloid pathology exists in the living brain. To confirm this, a larger study with a variety of patients with Alzheimer’s disease at early-to-advanced stages of severity would be necessary.

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**References**


