Regional dynamics of amyloid-β deposition in healthy elderly, mild cognitive impairment and Alzheimer’s disease: a voxelwise PiB–PET longitudinal study

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Amyloid-β deposition in Alzheimer’s disease is thought to start while individuals are still cognitively unimpaired and it is hypothesized that after an early phase of fast accumulation, a plateau is reached by the time of cognitive decline. However, few longitudinal Pittsburgh compound B-positron emission tomography studies have tested this hypothesis, and with conflicting results. The purpose of this work is to further our understanding of the dynamics of amyloid-β deposition in a large longitudinal cohort. A total of 32 patients with Alzheimer’s disease, 49 subjects with mild cognitive impairment and 103 healthy controls underwent two Pittsburgh compound B-positron emission tomography scans 18 months apart. For each participant, a parametric
Introduction

Alzheimer’s disease affects ~0.5% of the world’s total population (35.5 million) with total estimated worldwide costs of US$604 billion in 2010 (Alzheimer’s Disease International, 2009, 2010). Amyloid-β is one of the major neuropathological hallmarks of Alzheimer’s disease together with neurofibrillary tangles and neuronal/synaptic loss (Duyckaerts et al., 2009). The leading hypothesis driving current pathophysiology and therapeutic investigations is that excess amyloid-β initiates a cascade of events that result in neuronal/synaptic death and cognitive decline (Hardy and Selkoe, 2002). According to that hypothesis and based on former observations (Andreasen et al., 1999; Ingelsson et al., 2004; Engler et al., 2006), it has been proposed that amyloid-β deposits occur while individuals are still cognitively normal and then reach a plateau when cognitive decline occurs (Perrin et al., 2009; Asen et al., 2010; Frisoni et al., 2010; Jack et al., 2010a; Petersen, 2010; Weiner et al., 2010; Ewers et al., 2011; Sperling et al., 2011a). Based on this, it has become crucial to identify subjects at the early stages of amyloid-β deposition because they are the most likely to benefit from disease-specific therapeutics (Karran et al., 2011; Sperling et al., 2011b). Thanks to the emergence of new radiotracers, it is now possible to visualize in vivo amyloid-β deposition with [11C]-Pittsburgh compound B (PiB) combined with positron emission tomography (PiB-PET) (Klunk et al., 2004). Nonetheless, conflicting results have emerged from longitudinal PiB studies describing the dynamics of amyloid-β deposition using 11C-PiB. Indeed, both at the earliest stages of amyloid-β deposition (i.e. in healthy elderly) and at the latest stages of Alzheimer’s disease course (i.e. in Alzheimer’s disease dementia), a high and significant increase in amyloid-β deposition was found in some studies (Jack et al., 2009; Grimmer et al., 2010; Rinne et al., 2010; Koivunen et al., 2011; Sojkova et al., 2011b; Villemagne et al., 2011; Kadir et al., 2012) while others reported low or null changes in amyloid-β burden (Engler et al., 2006; Kadir et al., 2008, 2011; Scheinin et al., 2009; Jagust et al., 2010).

Beyond these inconsistencies regarding the dynamic itself of the global neocortical amyloid-β accumulation, the topography of this dynamic remains unclear. Indeed, regional analyses have reported conflicting results in patients with Alzheimer’s disease, with some studies showing no obvious regional differences (Engler et al., 2006; Jack et al., 2009; Grimmer et al., 2010) while others reported significant amyloid-β accumulation within the medial prefrontal cortex only (Scheinin et al., 2009), or within the frontal, temporal, parietal and cingulate cortices (Rinne et al., 2010; Villemagne et al., 2011). In subjects with mild cognitive impairment (MCI), Koivunen et al. (2011) and Kadir et al. (2012) recently highlighted a significant amyloid-β accumulation within the frontal, cingulate, temporal and parietal cortices. Note that interestingly, Koivunen et al. (2011) only described this regional pattern in MCI non-converters, while no significant increase was found in MCI converters, though the direct comparison between...
both groups did not reveal any significant difference. Finally, one study has reported a significant increase in PiB retention within the orbito-frontal and dorso-lateral prefrontal cortices of healthy controls with high PiB retention (PiB+) after 38-month follow-up (Villemagne et al., 2011), while another study described significant changes in almost all the neocortex (Sojkova et al., 2011b).

Several methodological differences could account for the discrepancies between these studies, such as the follow-up duration, sample size or the proportion of participants with high or low amyloid-β burden in each group, etc. Except for one (Koivunen et al., 2011), all these previous studies have used a region of interest approach where a priori defined regions of varying size are sampled and changes in parts of these regions of interest or in other regions could be diluted or missed.

The aim of this study was to assess the regional dynamics of amyloid-β deposition in the brain across different cognitive stages (from normal to demented) through a voxelwise approach allowing us to explore the whole brain without an a priori regional constraint. For this purpose, we used a large longitudinal cohort allowing us to account for the major factors thought to influence amyloid-β deposition: the baseline clinical status (in the healthy elderly, patients with MCI and Alzheimer’s disease), the disease progression (converters to MCI or Alzheimer’s disease versus non-converters) and the PiB status (PiB+ versus PiB–).

Subjects and methods

Participants

Approval for the study was obtained from the Austin Health Human Research Ethics Committee. All participants gave written informed consent to the study after detailed information was provided to them. The participants were partly the same as those reported in Villemagne et al. (2011). In total 32 participants with Alzheimer’s disease and 49 with MCI were recruited from the Austin Health Memory Disorders Clinic and 103 healthy controls were recruited by advertisement and from the Melbourne Healthy Ageing Study (see Villemagne et al. (2011) for details). Participants underwent medical, neurological, neuroradiological and neuropsychological examinations that included the Mini-Mental State Examination, Clinical Dementia Rating, California Verbal Learning Test–Second Edition (CVLT-II), Rey Complex Figure Test (RCFT), 30-item Boston Naming Test, Digit Span, category fluency, letter fluency, Digit Symbol-Coding, Stroop and Hospital Depression and Anxiety Scale. All participants were screened for mental disorder, substance abuse, head trauma and significant MRI or biological abnormality. Patients with Alzheimer’s disease were selected according to National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer’s Disease and Related Disorders Association criteria for probable Alzheimer disease (McKhann et al., 1984) whereas all participants in the MCI group met the Petersen’s criteria of subjective and objective cognitive difficulties in the absence of significant functional loss (Petersen et al., 1999). All healthy controls performed within <1.5 SD of the published norms for their age group on neuropsychological tests. All participants were re-evaluated after a 20.3 ± 3.6-month follow-up period. At each visit, participants were classified as healthy controls, MCI or Alzheimer’s disease by consensus between a neurologist and a neuropsychologist blind to PiB status. Only participants who had PiB–PET examinations at baseline and follow-up were included in the present study. A third PiB–PET scan was obtained in 33 healthy controls, 12 subjects with MCI and 10 with Alzheimer’s disease after 39.7 ± 3.6 months follow-up. These longer term follow-up data were used to confirm the validity of the classification based on the rates of amyloid-β deposition (Supplementary material).

Neuroimaging data acquisition

Magnetic resonance imaging

All subjects underwent a clinical MRI for screening and subsequent co-registration with the PET images. A FLAIR sequence was obtained for exclusion of subjects with cortical stroke. Baseline and follow-up MRI images were frequently acquired in different scanners and using different magnetic resonance sequences. A subsample of 11 patients with Alzheimer’s disease, 15 with MCI and 74 healthy controls underwent both PiB–PET and T1-MRI at baseline and follow-up using the same scanners and magnetic resonance sequences (see Supplementary Table 1 for demographics). For this subsample sagittal T1-weighted magnetic resonance images were acquired on a Siemens Magnetom Trio with Tim 3.0T scanner (Brain Research Institute) using a standard 3D-MP-RAGE sequence with in-plane resolution 1 × 1 mm², slice thickness 1.2 mm, repetition time/echo time/inversion time = 2300/2.98/900 ms, flip angle 9° and field of view 240 × 256 and 160 slices.

Positron emission tomography

All PET acquisitions were performed with the same PET scanner (Philips Allegro PET camera) in a single PET centre (Austin Hospital, Melbourne, Australia). Each participant received ~370 MBq PiB intravenously over 1 min. A 30-min acquisition (6 × 5-min frames) in 3D mode starting 40 min after injection of PiB was performed with a resolution of 5.0 × 5.0 × 6.5 mm³ (x y z). A transmission scan was performed for attenuation correction. PET images were reconstructed using a 3D RAMLA algorithm using a voxel size of 2.0 × 2.0 × 2.0 mm³ (x y z). Summed images for the 40–70 min time frame were used in this study.

Neuroimaging data processing

PiB–PET data sets were analysed using Statistical Parametric Mapping 8 (SPM8; Wellcome Trust Centre for Neuroimaging). For each participant, the two PiB–PET scans were first rigidly co-registered using the ‘Realign: Estimate and Reslice’ SPM8 procedure. This co-registration procedure also created a mean image that was then used to estimate the spatial normalization parameters to a customized PiB–PET template in the Montreal Neurological Institute reference space via the ‘Normalise: Estimate’ SPM procedure. The customized PiB–PET template was created using the Template-O-Matic toolbox (Wilke et al., 2008) and the subsample of patients who underwent both PiB–PET and T1-MRI at baseline and follow-up allowed us to use the normalization parameters from the MRI to normalize corresponding PiB images (Supplementary material). These spatial normalization parameters determined from each mean image were then applied to the two corresponding co-registered PiB–PET scans using the ‘Normalise: Write’ SPM8 function. The two co-registered normalized PiB–PET images were quantitatively normalized using the pons as the reference region (note that the pons was chosen over the cerebellar grey matter due to reduced variability in the paired voxelwise statistical analyses; data not shown) and masked using the grey matter partition of a customized MRI template obtained from the same subsample with both PiB and MRI, thresholded at 0.4 (Supplementary material). The
The individual mean global neocortical PiB value—expressed as neocortex-to-pons ratios (standardized uptake value ratio (SUVR), normalized)—were obtained from the resulting masked normalized PiB-PET images and used to classify participants as either high amyloid-β burden (PiB +) or low amyloid-β burden (PiB−) using a cut-off of 0.71, determined through a cluster analysis in the healthy controls. Images were then slightly smoothed using a Gaussian filter of 6.5 × 6.5 × 5.0 mm³ (x, y, z) full-width at half-maximum. For each participant a PiB annual rate of change image was created using SPM Image Calculator and the following formula: 

\[ \frac{\text{Baseline Scan} - \text{Follow-up Scan}}{\text{Follow-up Duration (years)}} \] 

Finally, PiB rate of change parametric maps were smoothed using an isotropic Gaussian filter of 10.3 mm full-width at half-maximum for a final total smooth of 14 × 14 × 14 mm³ full-width at half-maximum. These maps were then used for all voxelwise analyses.

**Statistical analyses**

PiB rate of change maps were entered in different voxelwise ANCOVAs using the SPM8 software and the ‘Flexible Factorial’ procedure. Age, gender and education were controlled for in all statistical analyses. In the interest for comparison with previous studies, we first assessed the simple effects of the clinical status and disease progression in separate models without considering the PiB status. The PiB rate of change maps were thus first compared between Alzheimer’s disease, MCI and control groups. Then, PiB rate of change maps were compared between converters and non-converters, within the MCI and within the healthy controls.

Then, the factors were considered together in the models to assess their independent effects. Since there were no possible converters within the Alzheimer’s disease group and only one patient with Alzheimer’s disease was found to be PiB− (see ‘Results’ section), the effects of the clinical status, disease progression and PiB status could not be tested in a single statistical factorial model with the three clinical groups. Thus, the effects of the three factors were first assessed within the MCI and healthy control groups only, using an ANCOVA on the PiB rate of change parametric maps, with the clinical status, disease progression and PiB status as categorical factors. A second model was performed with all three clinical groups but only considering the PiB+ individuals (to compare the PiB rate of change between the three clinical groups when controlling for the PiB status).

Further analyses were then conducted to support the interpretation of the main analyses (refer to the ‘Results’ section and Supplementary material). Voxelwise analyses were performed using SPM8 software and standard statistical analyses were conducted with the Statistica software (Statsoft).

SPM-T maps of all previously described analyses were thresholded using a P uncorrected < 0.001 and a k = 50 mm³. Anatomical localization was based on the superimposition of the SPM-T maps onto the customized MRI template and identification of the localization using the AAL software and anatomical atlases (Talairach and Tournoux, 1988; Tzourio-Mazoyer et al., 2002). The findings were rendered using the publicly available Anatomist/BrainVISA and MRICron software (www.brainvisa.info; http://www.sph.sc.edu/comd/rorden/mricron/).

**Results**

**Participants**

Demographics and neuropsychological scores for each group are reported in Table 1. At baseline, 32 of the 184 participants fulfilled the diagnostic criteria for probable Alzheimer’s disease (McKhann et al., 1984), 49 fulfilled the clinical criteria for MCI (Petersen et al., 1999) and 103 healthy controls did not suffer from any neurological, psychiatric or metabolic disorder (Table 1). At the end of the 20-month follow-up period, out of the 49 patients with MCI, 17 progressed to probable Alzheimer’s disease (termed ‘converters’), two converted to non-Alzheimer’s disease dementia (one fronto-temporal dementia and one dementia with Lewy bodies), 27 remained clinically stable and three were reclassified as controls (termed ‘non-converters’) (Table 2). Among the 103 healthy controls, one progressed to probable Alzheimer’s disease, five to MCI and 97 remained clinically stable over the 20-month follow-up period (Table 2).

Only one patient with Alzheimer’s disease was classified as PiB−. Among patients with MCI, all converters and 14/27 non-converters were classified as PiB+, while the two patients with MCI who converted to another dementia and the three with MCI who were deemed cognitively normal at follow-up were PiB−. Amongst the healthy controls, the subject who progressed to Alzheimer’s disease, 4/5 participants who progressed to MCI and 27/97 non-converters healthy controls were classified as PiB+. The PiB status did not change in any of the participants when the follow-up neocortical PiB values were used instead of the baseline neocortical PiB values.

**Voxelwise pattern of Pittsburgh compound B rate of change**

The simple effects of the clinical status assessed in a separate model are illustrated in Fig. 1. A significant PiB accumulation (i.e. positive PiB rate of change) was found in patients with Alzheimer’s disease within the lateral temporal cortex, inferior parietal lobe, dorso-lateral and orbito-medial regions of the prefrontal cortex, posterior cingulate cortex, insula and occipital lobe. The pattern of significant PiB accumulation was similar though less marked within the patients with MCI. Finally, in healthy controls.

**Table 1** Demographics and clinical characteristics of the longitudinal PiB-PET sample

<table>
<thead>
<tr>
<th></th>
<th>Alzheimer’s disease</th>
<th>MCI</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>32</td>
<td>49</td>
<td>103</td>
</tr>
<tr>
<td>Age (years)</td>
<td>72.6 ± 9.0</td>
<td>74.4 ± 7.3</td>
<td>73.6 ± 7.3</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>16/16</td>
<td>28/21</td>
<td>48/55</td>
</tr>
<tr>
<td>Education (years)</td>
<td>11.9 ± 3.5</td>
<td>12.3 ± 4.1</td>
<td>13.2 ± 3.5</td>
</tr>
<tr>
<td>Mini-Mental State</td>
<td>22.2 ± 4.4</td>
<td>26.8 ± 2.4</td>
<td>28.8 ± 1.2</td>
</tr>
<tr>
<td>Examination baseline</td>
<td>Memory Z-score</td>
<td>−3.28 ± 0.56</td>
<td>−2.14 ± 1.00</td>
</tr>
<tr>
<td></td>
<td>Non-memory Z-score</td>
<td>−2.33 ± 1.50</td>
<td>−0.88 ± 0.86</td>
</tr>
<tr>
<td></td>
<td>Follow-up duration (years)</td>
<td>1.75 ± 0.45</td>
<td>1.68 ± 0.23</td>
</tr>
</tbody>
</table>

Memory and non-memory Z-scores are composite neuropsychological scores, obtained from extensive neuropsychological testing (Villemagne et al., 2011).
a significant positive regional PiB rate of change was observed within the lateral temporal cortex, dorso-lateral and orbital parts of the prefrontal cortex, insula and occipital lobe. Group differences revealed a significantly higher regional PiB rate of change in patients with Alzheimer’s disease compared with MCI or healthy controls within the lateral temporal cortex, the occipital lobe and the dorso-lateral and medial parts of the prefrontal cortex. There were no significant differences in any other group comparisons.

The ANCOVA with the three factors (clinical status, disease progression and PiB status) performed in MCI and healthy control groups revealed no significant main effect of clinical status and of disease progression on the regional PiB rate of change and no interaction between both factors. This lack of a significant main effect of the clinical status was also observed in the ANCOVA performed in the three clinical groups with PiB+ individuals only. By contrast, a main effect of the PiB status was found, with significantly higher PiB rate of change in PiB+ versus PiB− individuals in several cortical regions including the posterior cingulate cortex, precuneus, medial and dorso-lateral frontal cortex and temporo-parietal cortex (Fig. 2). There was no interaction between the PiB status and the other factors. Within the PiB+ group, the PiB rate of change was significantly positive within the whole brain, predominantly in the lateral temporal cortex, lateral and medial parietal cortex, dorso-lateral and orbito-medial frontal cortex, posterior cingulate cortex and occipital lobe (Fig. 2). Areas of significantly positive rate of change were also found in the PiB− group in the temporal poles, orbito-frontal cortex and insula (Fig. 2). The lack of association with disease progression was performed in the three clinical groups with PiB+ individuals only. By contrast, a main effect of the PiB status was found, with significantly higher PiB rate of change in PiB+ versus PiB− individuals in several cortical regions including the posterior cingulate cortex, precuneus, medial and dorso-lateral frontal cortex and temporo-parietal cortex (Fig. 2). There was no interaction between the PiB status and the other factors. Within the PiB+ group, the PiB rate of change was significantly positive within the whole brain, predominantly in the lateral temporal cortex, lateral and medial parietal cortex, dorso-lateral and orbito-medial frontal cortex, posterior cingulate cortex and occipital lobe (Fig. 2). Areas of significantly positive rate of change were also found in the PiB− group in the temporal poles, orbito-frontal cortex and insula (Fig. 2). The lack of association with disease progression was
further strengthened by the lack of significant relationship between PiB rate of change and depression and anxiety scores (Supplementary material).

**Evidence for a Pittsburgh compound B accumulation status**

The finding of significant regional PiB accumulation within the PiB− group raised the question of whether all PiB− individuals are starting to accumulate and will eventually turn to PiB+ or whether they reflect a significant accumulation only in some of these PiB− individuals (termed ‘accumulators’) who will most likely progress to PiB+. To address this question the distribution of the individual values of global PiB rate of change in healthy controls was first assessed, where a normal distribution would suggest an accumulation in every participant while a bimodal distribution would support the concept of two distinct groups with different accumulation status. The Shapiro–Wilk test revealed that the distribution was significantly different from a normal distribution (P < 0.05) and the histogram showed that the distribution was bimodal with one mode around 0.0 PiB SUVRpons/year and the other around +0.03 PiB SUVRpons/year (Fig. 3), with an inflexion between these two peaks around +0.02 PiB SUVRpons/year. Clustering analyses performed within different groups (within all PiB− participants, within the PiB− healthy controls, within all healthy controls and with all participants), revealed similar thresholds (ranging from +0.014 to +0.022 PiB SUVRpons/year). The consistency of the classification was finally checked using a subsample that underwent a third PiB PET scan after a 40-month follow-up (Supplementary material).

Secondly, the global neocortical PiB rates of change of all participants were plotted according to their baseline global neocortical PiB value in order to test whether the difference in regional PiB rate of change between PiB+ and PiB− reflects a continuous phenomenon from a single group (i.e. a continuous increase of the PiB rate of change as the baseline PiB increases) or a dichotomous pattern with two subgroups (i.e. a biphasic curve around the baseline +0.71 PiB SUVRpons/threshold, reflecting a difference in proportion of accumulators and non-accumulators between PiB+ and PiB−). This plot showed a biphasic pattern around the baseline +0.71 PiB SUVRpons threshold, i.e. with a mean global neocortical PiB rate of change around 0.0 PiB SUVRpons/year in PiB− participants and around +0.02 PiB SUVRpons/year in the
PiB + group (Fig. 4). Furthermore, this plot also revealed that the mean PiB rate of change in the PiB + group tended to be lower in higher baseline PiB SUVRpons, which was confirmed by a significant negative linear correlation in this group (Pearson $r = -0.24$; $P = 0.025$) (Fig. 4). This suggests that, within the PiB + individuals, PiB accumulation decreases in individuals with the highest baseline PiB SUVRpons values.

**Pittsburgh compound B accumulators versus Pittsburgh compound B non-accumulators**

Further analyses were then performed based on the evidence of the existence of PiB accumulators and PiB non-accumulators. Based on the clustering analyses in different subgroups (see above), a threshold of global neocortical PiB rate of change $> +0.022$ PiB SUVRpons/year (highest threshold obtained from clustering analyses) and $< +0.014$ (lowest threshold obtained from clustering analyses), respectively, were used to discriminate accumulators from non-accumulators. The few participants with intermediate PiB rate of change values (i.e. between $+0.014$ and $+0.022$) were classified in a third subgroup of ‘undefined’ PiB accumulation status (Mormino et al., 2012). The proportion of PiB accumulators was higher within the PiB + group compared with the PiB– group, with 58.6% (17/32) of the patients with Alzheimer’s disease, 35.0% (7/31) of the MCI participants and 50.0% (16/32) of the healthy controls within the PiB + group against 22.2% (4/18) for the MCI participants and 28.6% (19/71) for the healthy controls within the PiB– group (the PiB– patient with Alzheimer’s disease was classified as an accumulator) (Table 3). To assess whether the PiB accumulation status significantly differed between converters and non-converters, a two-way ANCOVA was performed with disease progression as the dependent variable and clinical status and PiB accumulation status as categorical factors (age, sex and education as covariates). This analysis only revealed a significant main effect of the clinical status (i.e. more converters in the MCI than in the healthy controls) but no effect of the PiB accumulation status ($P = 0.60$). Finally to assess the effects of the clinical group and the PiB baseline status on the PiB accumulation status, a two-way ANCOVA was performed including all MCI and healthy controls with the PiB accumulation status as a dependent variable and the PiB status and the clinical status as categorical factors (age, sex and education as covariates). This analysis showed that the proportion of accumulators was significantly higher in PiB + versus PiB–.

**Voxelwise pattern of Pittsburgh compound B rate of change amongst accumulators**

To assess whether the greater rate of PiB accumulation within the PiB + compared with the PiB– participants we first observed was due to the higher proportion of accumulators within the PiB +, the voxelwise analyses described above were repeated within the accumulators only. The results of the one-way voxelwise ANCOVA revealed significantly positive PiB rates of change within the whole grey matter in each of the three groups (except for the hippocampus in the Alzheimer’s disease and MCI groups). Group differences revealed a significantly higher regional PiB rate of change in patients with Alzheimer’s disease versus healthy controls within the lateral temporal cortex, the occipital lobe and the dorso-lateral and medial parts of the prefrontal cortex. Patients with Alzheimer’s
disease also showed a higher regional PiB increase than those with MCI mainly in the lateral temporal cortex, whereas patients with MCI showed a higher regional PiB increase than healthy controls within the medial prefrontal cortex. No other group difference was found.

The ANCOVA with the three factors (clinical status, disease progression and PiB status) performed in patients with MCI and healthy controls still revealed no significant interaction and no effect of the clinical status or disease progression on the regional PiB rate of change and a significant main effect of the PiB status in similar though more confined brain areas, including the posterior cingulate cortex, the precuneus, the medial and dorso-lateral frontal cortex, the right temporal pole, the right inferior temporal gyrus and the left middle temporal gyrus (Fig. 5). Amongst the PiB− accumulators, the regional PiB rate of change was significantly higher than zero within the whole brain with maximal values within the lateral temporal cortex, the lateral and medial parietal cortex, the dorso-lateral and orbito-medial frontal cortex, the posterior cingulate cortex and the occipital lobe (Fig. 5).

**Discussion**

This study demonstrates that the dynamics of amyloid-β accumulation within the brain are not related to baseline clinical status or to disease progression, but are associated with the global neocortical amyloid-β burden with higher accumulation in PiB+ than in PiB− individuals. Though lower than that found in PiB+, amyloid-β accumulation was yet found to be significant in PiB− participants. The present study actually reveals the existence of two
Table 3  Demographics and disease progression of the longitudinal PiB-PET sample according to the PiB accumulation status (accumulators versus non-accumulators)

<table>
<thead>
<tr>
<th>Alzheimer’s disease</th>
<th>MCI</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PIB−</strong></td>
<td><strong>PIB+</strong></td>
<td><strong>PIB−</strong></td>
</tr>
<tr>
<td>Accumulators</td>
<td>Accumulators</td>
<td>Non-accumulators</td>
</tr>
<tr>
<td>Conversion to AD or MCI</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>Age (years)</td>
<td>80.3</td>
<td>74.8</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>0/1</td>
<td>11/6</td>
</tr>
<tr>
<td>Education (years)</td>
<td>6</td>
<td>12.3</td>
</tr>
<tr>
<td>Mini-Mental State Examination baseline (SUVR)</td>
<td>22</td>
<td>22.9</td>
</tr>
<tr>
<td>Neocortical PiB Baseline (SUVR_{0})</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Neocortical PiB rate of change (SUVR_{year})</td>
<td>+0.06</td>
<td>+0.05</td>
</tr>
</tbody>
</table>

**AD** = Alzheimer’s disease; **HC** = healthy controls.
The pathological–clinical discrepancy observed here between PiB rate of change and both clinical status and disease progression in PiB + and PiB− groups is consistent with previous findings that observed a better correlation between cognitive disorders or dementia stages and neurofibrillary tangles or synapse loss than with insoluble amyloid-β in plaques (McLean et al., 1999; Mukaetova-Ladinska et al., 2000; Petersen et al., 2006; Jack et al., 2009; Nelson et al., 2009; Scheinin et al., 2009; Jagust et al., 2010; Villemagne et al., 2011). It has been proposed that the poor clinical–amyloid-β plaques relationship could be in line with the amyloid cascade hypothesis (Hardy and Selkoe, 2002), which argues that amyloid-β is the earliest pathological feature of Alzheimer’s pathology and would possibly lead to a time-lag between amyloid-β accumulation and symptomatology (Tiraboschi et al., 2004; Villemagne et al., 2011). It has been proposed that the poor clinical–amyloid-β plaques relationship could be in line with the amyloid cascade hypothesis (Hardy and Selkoe, 2002), which argues that amyloid-β is the earliest pathological feature of Alzheimer’s pathology and would possibly lead to a time-lag between amyloid-β accumulation and symptomatology (Tiraboschi et al., 2004; Villemagne et al., 2011). Therefore, according to that hypothesis, a snapshot of the course of Alzheimer’s disease pathology over a short period of time, as performed here over 20 months, would be expected to be independent of clinical progression.

The voxelwise analyses revealed that the temporo-parietal junction and the precuneus were the areas of earliest amyloid-β accumulation and could thus be used as markers of early amyloidosis (Fig. 5). Further, amyloid-β accumulation was highest within the lateral and inferior temporal cortex at the latest stages of amyloidosis and might therefore prove to be useful for monitoring amyloid-β deposition in participants with high amyloid-β burden (Fig. 5). The validity of these biomarkers remains to be established, especially by comparison with other well-described longitudinal imaging biomarkers such as [18]FDG-PET or structural T1-MRI (Scailhill and Fox, 2007; Frisoni et al., 2010; Drago et al., 2011). Note that the voxelwise approach proved superior to the region of interest approach to detect regional variations since we could identify significant regional changes in PiB–PET rate of change images in accumulators thresholded at $P < 0.001$ uncorrected ($k > 50$ voxels). Note that the other main effects and interactions were not significant. PiB + = high amyloid burden; PiB− = low amyloid burden.

Figure 5 Voxelwise ANCOVA on the PiB–PET rate of change images in accumulators using clinical status, clinical conversion and PiB status as categorical predictors. Top: Mean values of PiB–PET rate of change in accumulators across the mean follow-up period of 20 months according to each PiB status group (PiB+, PiB−) and rendered onto a 3D surface using the Anatomist/BrainVISA software (www.brainvisa.info). Bottom: SPM glass brain render and slice renders of the main effect of the PiB status on PiB–PET rate of change images in accumulators thresholded at $P < 0.001$ uncorrected ($k > 50$ voxels). Note that the other main effects and interactions were not significant. PiB + = high amyloid burden; PiB− = low amyloid burden.
related to clinical progression to Alzheimer’s disease or MCI. The same findings were observed for the PiB+ group.

The prevalence of accumulators was higher amongst the PiB+ participants (~50%) than in the PiB– group (~20%), consistent with the notion that amyloid-β is a self-aggregating protein. Therefore subjects with a significantly higher amyloid-β burden are more likely to continue this pathological process of amyloid-β accumulation than subjects with no or low amyloid-β. The absolute numbers are likely to be impaired by the noise of the measurement and should thus be interpreted with caution. Nonetheless it is interesting to emphasize that the proportion of accumulators in the PiB– groups (20–30%) is similar to the previously described proportion of healthy controls with a high PiB retention (Aizenstein et al., 2008; Rowe et al., 2010). On the other hand only 50–60% of PiB+ subjects showed increases in PiB retention over 20 months. Whether this lack of accumulation in 40–50% of PiB+ participants is temporary or permanent remains to be established, but it might also suggest that amyloid-β deposition can saturate, slow down or interrupt during the course of amyloidosis. This is supported by the fact that a sizeable percentage of PiB+ MCI (a clinical stage considered to be prodromal Alzheimer’s disease) did not show a significant PiB accumulation over time.

It is important to note that a bimodal distribution of PiB rate of change values (Fig. 3) and more significant statistical thresholds for the voxelwise results were observed when the pons was used as the reference region for scaling rather than the cerebellum. Indeed, when using the cerebellum, the findings were highly similar but less significant due to increased variability of the results (data not shown). This scaling issue is likely to be specific to longitudinal analyses since cross-sectional differences between groups were similar using these two reference regions (data not shown). It is possible that the cerebellum would be more sensitive to subtle intrasubject misregistration compared to the pons measurement.

Results show a negative correlation between the baseline global neocortical PiB SUVRpons and the global neocortical PiB rate of change within the PiB+, which is consistent with the concept of a saturable process of amyloid-β deposition as the PiB SUVRpons reaches highest values (Fig. 4). Nonetheless, contrary to previous assumptions (Perrin et al., 2009; Aisen et al., 2010; Frisoni et al., 2010; Jack et al., 2010a; Petersen, 2010; Weiner et al., 2010; Ewers et al., 2011; Sperling et al., 2011a) amyloid-β accumulation was found to be significantly ongoing at the dementia stage of Alzheimer’s disease (Figs 1 and 4). It should be noted that despite these general group patterns, the rate of amyloid-β deposition was highly variable from one subject to another, for instance there was no amyloid-β accumulation in a PiB+ healthy control with a global neocortical PiB SUVRpons of +0.90 while it was ongoing in a patient with Alzheimer’s disease with a global neocortical PiB SUVRpons of +1.2 (Fig. 4).

As a whole, the dynamics of amyloid-β deposition are unlikely to be constant and are probably more consistent with a sigmoid curve, but with a different timing as previously proposed (Perrin et al., 2009; Aisen et al., 2010; Frisoni et al., 2010; Jack et al., 2010a; Petersen, 2010; Weiner et al., 2010; Ewers et al., 2011; Sperling et al., 2011a) (Figs 4 and 6). Indeed, PiB accumulation was found to be significantly higher in PiB+ compared with PiB– individuals. Even if amyloid-β accumulation slows down when the highest amyloid-β burdens are reached, a threshold for the stopping of the PiB rate of change cannot be derived from the available data. Furthermore, this threshold might be idiosyncratic, differing from one person to another, which might explain the significant overlap of global neocortical PiB baseline values and global neocortical PiB rate of change values between the clinical groups (Figs 4 and 6). Anyhow, using the median PiB rate of change observed in the PiB– accumulators (+0.030 SUVRpons/year), it would take ~7 years for a PiB– subject to reach the PiB+ status threshold (from a baseline +0.50 SUVRpons to +0.71 SUVRpons) and ~7.5 additional years for a PiB+ accumulator (+0.041 SUVRpons/year) to reach the mean Alzheimer’s disease neocortical PiB burden (from a baseline +0.71 SUVRpons to +1.02 SUVRpons) (Fig. 6). Nonetheless these durations are rough estimations obtained from basic calculations that did not take into account all the parameters necessary for a precise estimation such as the idiosyncratic slow down of PiB accumulation as stated earlier. Therefore, longitudinal measures with additional time points and longer follow-up durations will allow a better characterization of the time course of amyloid-β deposition.

To our knowledge, this is the first study to identify these earliest stages of amyloid-β deposition in the brain in otherwise healthy controls (PiB– accumulators). The PiB accumulation status offers an interesting biological marker of early amyloidosis and could be useful to further elucidate in vivo the mechanisms that trigger amyloid-β deposition.

This sensitivity in detecting early amyloid-β deposits may be attributable to both image processing, since it was not observed using a region of interest approach (Villemagne et al., 2011) and amyloid-β tracer. Indeed, PiB binds to fibrillar amyloid-β, found in both cored and non-cored plaques (Ikonomovic et al., 2008), while other amyloid-β tracers only bind to cored plaques (Kudo et al., 2007) thus precluding their ability to detect early amyloid-β deposition. Further studies with the newly developed F-18 amyloid-β tracers (e.g. forbetaben, flurbiprofen, flutemotanol) are thus necessary to confirm these findings.

Consistent with the regional PiB rate of change, PiB accumulation status was not related to clinical status or disease progression. As discussed earlier, this finding is consistent with the amyloid-clinical discrepancy observed in cross-sectional or short-term longitudinal studies (McLean et al., 1999; Mukaeova-Ladinska et al., 2000; Petersen et al., 2006; Jack et al., 2009; Nelson et al., 2009; Scheinin et al., 2009; Jagust et al., 2010; Villemagne et al., 2011). Nonetheless our estimations suggest that the hypothetical 14.5 years time-lag between the occurrence of detectable amyloid-β deposition and dementia is far beyond the 20-month follow-up of this study. Therefore, only longer longitudinal follow-up will help elucidate the true nature of the relationship between amyloid-β deposition and cognition. In other words, it remains to be demonstrated that the observed earliest stages of amyloid-β deposition in healthy controls are indeed a clinically relevant risk factor for Alzheimer’s disease.

An increasing number of reports are providing indirect evidence for a possible long-term effect of amyloid-β deposition on clinical symptoms in healthy controls (Morris et al., 2009; Okello et al., 2009; Jack et al., 2010b; Resnick et al., 2010; Chételat et al., 2012) further supporting the growing consensus that anti-amyloid...
therapy might need to be given early in the course of the disease to successfully prevent the development of Alzheimer’s disease (Karran et al., 2011; Sperling et al., 2011b). In line with this assumption, our results might be a crucial finding to discriminate between subjects that are at high risk of developing Alzheimer’s disease from those who are not, several years before the manifestation of the clinical phenotype.

A potential limiting factor of this study could be the sensitivity of PiB–PET to detect longitudinal changes in amyloid-\(\beta\) deposition. First, the degree of regional retention of PiB is highly correlated with the regional concentration of amyloid-\(\beta\), as reported at autopsy (Bacskai et al., 2007; Ikonomovic et al., 2008; Leinonen et al., 2008; Vilmagne et al., 2009; Burack et al., 2010; Kadir et al., 2011; Sojkova et al., 2011a). Secondly, it is interesting to note that for PiB– participants (global neocortical baseline PiB = 0.5–0.71 SUVRpons), the identified threshold for accumulators is mostly above the 3.5% test–retest variability measure (\(> +0.022\) SUVRpons/year \(\leftrightarrow +3.1\) to \(+4.4\% /\text{year}\)) reported for this data set (Vilmagne et al., 2011). Moreover, the long term consistency of the accumulation status (Supplementary material) as well as the consistent threshold for PiB accumulation across individuals (\(+0.014\) SUVRpons/year \(\leftrightarrow +0.022\) SUVRpons/year) further support the robustness of these findings.

In conclusion, ongoing amyloid-\(\beta\) deposition was detected in all clinical groups, being significantly higher in the Alzheimer’s disease group. Nonetheless, this significant clinical group effect actually proved to be completely mediated by the baseline amyloid-\(\beta\) burden when this variable was taken into account, i.e. higher rates of amyloid-\(\beta\) deposition were associated with higher amyloid-\(\beta\) burden. Moreover, significant rates of amyloid-\(\beta\) deposition could be detected in a distinct group (accumulators) even in non-demented individuals with low amyloid-\(\beta\) burden, but were somewhat slower than in those with high amyloid-\(\beta\) burden in the brain. The identification of accumulators and non-accumulators offers an interesting biological marker of early amyloidosis and despite weak amyloid-clinical relationship over 20 months, might prove to be relevant in the long-term prediction of who is at risk of developing the disease. We also observed that amyloid-\(\beta\) accumulation slows down when the highest amyloid-\(\beta\) burden is reached, which is consistent with the concept that amyloid-\(\beta\) deposition is a saturable process. Finally, and in contrast to a region of interest analysis, a voxelwise approach has allowed us to determine that the temporo-parietal junction and the precuneus could be used as markers of early amyloid-\(\beta\) deposition while the lateral and inferior temporal cortex could be useful for monitoring amyloid-\(\beta\) deposition in the late and symptomatic stages. Longer longitudinal follow-up studies are warranted to further validate these results.

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Supplementary material
Supplementary material is available at Brain online.

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