

Regional dynamics of β -amyloid deposition in healthy elderly, Mild Cognitive Impairment and Alzheimer's disease: a voxelwise PiB-PET longitudinal study

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Keywords: Alzheimer's Disease, Mild Cognitive Impairment, Normal Aging, β -amyloid imaging, longitudinal imaging, Pittsburgh compound B, positron emission tomography.

Counts:

Title – number of characters: 151

Text – number of words: 6293

Abstract

Introduction: β -amyloid ($A\beta$) deposition in Alzheimer's disease (AD) 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, only a few longitudinal PiB-PET studies have tested this hypothesis with conflicting results. The purpose of this work is to further our understanding of the dynamics of $A\beta$ deposition in a large longitudinal cohort.

Methods: 32 AD patients, 49 mild cognitive impairment (MCI) subjects and 103 healthy controls underwent two PiB-PET scans 18 months apart. For each participant, a parametric map of PiB-PET rate of change was created ((Follow-up scan-Baseline scan)/Follow-up Duration) and entered in a voxelwise 3-way ANCOVA, with clinical status (healthy controls, MCI or AD), disease progression (clinical conversion from healthy controls to MCI or AD, or from MCI to AD) and PiB status (PiB+ vs. PiB-) as independent factors.

Results: Only a significant effect of the PiB status was found: both PiB+ and PiB- showed a significant increase in $A\beta$ deposition, with this increase being significantly higher in PiB+. This finding suggests either that PiB- individuals have slower rates of $A\beta$ accumulation than PiB+, or that the proportion of individuals showing significant increase in $A\beta$ deposition, termed "PiB Accumulators", is higher within the PiB+ than within the PiB-. The bimodal distribution of the individual rates of neocortical $A\beta$ accumulation observed support the existence of "PiB Non-Accumulators" and "PiB Accumulators", and different clustering analyses led to a consistent threshold to separate these two subgroups (+0.014 $SUVR_{pons}/year$ - +0.022 $SUVR_{pons}/year$). The voxelwise 3-way ANCOVA was thus re-computed with the "PiB Accumulators" only and the results were almost unchanged, with PiB+ showing higher accumulation than PiB-. Finally, a significant negative correlation was found between PiB rate of change and PiB baseline burden but only in the PiB+ group ($r=-0.24$; $p = 0.025$).

Discussion: Higher rates of A β deposition are associated with higher A β burden suggesting that A β deposition does not reach a plateau when cognitive impairments manifests but is instead an on-going process present even at the AD stage. A β accumulation also seems to slow down at the latest stages of the process, i.e. in participants with the highest amyloid burden. Furthermore, this study identified the existence of “PiB Accumulators” and “PiB Non-Accumulators”, notably within the PiB- group, which may be a relevant concept for future studies.

INTRODUCTION

Alzheimer's disease affects around 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). β -amyloid ($A\beta$) is one of the major neuropathological hallmarks of Alzheimer's disease (AD) together with neurofibrillary tangles and neuronal/synaptic loss (Duyckaerts et al., 2009). The leading hypothesis driving current pathophysiology and therapeutic investigations is that excess $A\beta$ 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; Engler et al., 2006; Ingelsson et al., 2004), it has been proposed that $A\beta$ deposits occur while individuals are still cognitively normal and then reach a plateau when cognitive decline occurs (Aisen et al., 2010; Ewers et al., 2011; Frisoni et al., 2010; Jack et al., 2010b; Perrin et al., 2009; Petersen, 2010; Sperling et al., 2011a; Weiner et al., 2010). Based on this, it has become crucial to identify subjects at the early stages of $A\beta$ 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 $A\beta$ deposition with ^{11}C -Pittsburgh compound B (PiB) combined to positron emission tomography (PET) (Klunk et al., 2004). Nonetheless, conflicting results have emerged from longitudinal PiB studies describing the dynamics of $A\beta$ deposits using ^{11}C -PiB. Indeed, both at the earliest stages of $A\beta$ deposition (i.e. in healthy elderly) and at the latest stages of AD course (i.e. in AD dementia), a high and significant increase in $A\beta$ deposition was found in some studies (Grimmer et al., 2010; Jack et al., 2009; Kadir et al., 2012; Koivunen et al., 2011; Rinne et al., 2010; Sojkova et al., 2011b; Villemagne et al., 2011) while others reported low or null changes in $A\beta$ burden (Engler et al., 2006; Jagust et al., 2010; Kadir et al., 2008, 2011; Scheinin et al., 2009).

Beyond these inconsistencies regarding the dynamics itself of the global neocortical A β accumulation, the topography of this dynamics remains unclear. Indeed, regional analyses have reported conflicting results in AD patients, with some studies showing no obvious regional differences (Engler et al., 2006; Grimmer et al., 2010; Jack et al., 2009) while others reported significant A 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 mild cognitive impairment (MCI) subjects, Koivunen et al. (2011) and Kadir et al. (2012) recently highlighted a significant A 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 orbitofrontal and dorsolateral 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, the sample size, the proportion of participants with high or low A β burden in each group, etc. Besides, except for one (Koivunen et al., 2011), all these previous studies have used a region-of-interest (ROI) approach where *a priori* defined regions of varying size are sampled and changes in parts of these ROIs or in other regions could be diluted or missed.

The aim of this study was thus to assess the regional dynamics of A β deposition in the brain across different cognitive stages (from normal to demented) through a voxelwise approach allowing to explore the whole brain without an *a priori* regional constraint. For this purpose, we used a large longitudinal cohort allowing to account for the major factors thought to

influence A β deposition: the baseline clinical status (healthy elderly, MCI and AD patients), the disease progression (converters to MCI or AD versus non-converters) and the PiB status (PiB+ vs. PiB-).

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). Thirty-two AD and 49 MCI participants were recruited from the Austin Health Memory Disorders Clinic and 103 healthy controls were recruited by advertisement and from the Melbourne Healthy Aging 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. The AD patients 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 standard deviations (SD) of the published norms for their age group on neuropsychological tests. All participants were re-evaluated after a 20.3 (\pm 3.6) months follow-up period. At each visit, participants were classified as healthy controls, MCI, or AD 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

MCI, and 10 AD subjects after a 39.7 (\pm 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 A β deposition (see Supplementary Material).

Neuroimaging data acquisition

Magnetic Resonance Imaging

All subjects underwent a clinical magnetic resonance imaging (MRI) for screening and subsequent co-registration with the PET images. A fluid attenuated inversion recovery 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 (MR) sequences. Only a subsample of 11 AD, 15 MCI and 74 healthy controls underwent both PiB-PET and T1-MRI at baseline and follow-up using the same scanners and MR sequences (see Supplementary Table 1 for demographics). For this subsample sagittal T1-weighted MR images were acquired on a Siemens (Erlangen, Germany) Magnetom Trio with Tim 3.0T scanner (Brain Research Institute, Melbourne, Australia) using a standard 3D-magnetization prepared rapid gradient echo sequence with in-plane resolution 1 \times 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 \times 256 and 160 slices.

PET

All PET acquisitions were performed with the same PET scanner (Philips [Amsterdam, Netherlands] Allegro PET camera) in a single PET center (Austin Hospital, Melbourne, Australia). Each participant received ~370MBq PiB intravenously over 1 minute. A 30-minute acquisition (6 \times 5-minute frames) in 3-dimensional (3D) mode starting 40 minutes after injection of PiB was performed with a resolution of 5.0 \times 5.0 \times 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 $2.0 \times 2.0 \times 2.0 \text{ mm}^3$ (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 analyzed using Statistical Parametric Mapping 8 (SPM8; Wellcome Trust Centre for Neuroimaging, London, UK). For each participant, the two PiB-PET scans were first rigidly coregistered using the “Realign: Estimate & Reslice” SPM8 procedure. This coregistration 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 (the customized PiB-PET template was created using the Template-O-Matic toolbox [(Wilke et al., 2008)], and the subsample who underwent both PiB-PET and T1-MRI at baseline and follow-up allowing to use the normalisation parameters from the MRI to normalize corresponding PiB images; see Supplementary Material for details) via the “Normalise: Estimate” SPM procedure. These spatial normalization parameters determined from each mean image were then applied to the two corresponding coregistered PiB-PET scans using the “Normalise: Write” SPM8 function. The two coregistered 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 (see Supplementary Material). The individual mean global neocortical PiB value—expressed as neocortex-to-pons ratios ($\text{SUVR}_{\text{pons}}$)—were obtained from the resulting masked normalized PiB-PET images and used to classify participants as either high A β burden (PiB+) or low A 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 mm (x y z) full width at half maximum (FWHM). For each participant a PiB annual rate of change image was created using SPM “Image Calculator” and the following formula
$$\frac{\text{Follow - up Scan} - \text{Baseline Scan}}{\text{Follow - up Duration (years)}}$$
. Finally PiB rate of change parametric maps were smoothed using an isotropic Gaussian filter of 10.3 mm FWHM for a final total smooth of 14.0×14.0×14.0 mm FWHM. 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. For the sake of 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 AD, MCI and controls. 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. Because, within the AD group, there was no possible converter and only one AD patient was found to be PiB- (see Results), 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 controls 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 changes between the three clinical groups when controlling for the PiB status).

Further analyses were then conducted to support the interpretation of the main analyses (see below in the ‘Results’ and ‘Supplementary Material’ sections). Voxelwise analyses were performed using SPM8 software and “standard” statistical analyses were conducted with the Statistica software (Statsoft, Tulsa, OK).

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 mild cognitive impairment (Petersen et al., 1999) and 103 healthy controls did not suffer from any neurological, psychiatric or metabolic disorder (see Table 1 for details). At the end of the 20-month follow-up period, out of the 49 MCI patients, 17 progressed to probable AD (=converters), 2 converted to non-AD dementia (1 fronto-temporal dementia and 1 dementia with Lewy bodies), 27 remained clinically stable and 3 were re-classified as controls (=non-converters) (Table 2). Amongst the 103 healthy controls, one progressed to probable AD, 5 to MCI and 97 remained clinically stable over the 20-month follow-up period (Table 2).

Only one AD patient was classified as PiB-. Amongst MCI, all converters and 14/27 non-converters were classified as PiB+, while the 2 MCI who converted to another dementia and the 3 MCI who were deemed cognitively normal at follow-up were PiB-. Amongst the healthy controls, the subject who progressed to AD, 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 PiB rate of change

The simple effects of the clinical status assessed in a separate model are illustrated in Figure 1. A significant PiB accumulation (i.e. positive PiB rate of change) was found in AD patients 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 MCI patients. Finally, in healthy controls 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 AD patients compared to 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 controls 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 (Figure 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 (Figure 2). Areas of significantly positive rate of change were also found in the PiB- group in the temporal poles, orbito-frontal cortex and insula (Figure 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 (see Supplementary Material for details).

Evidence for a PiB 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 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 SUVR_{pons}/year and the other around +0.03 PiB SUVR_{pons}/year (Figure 3), with an inflexion between these two peaks around +0.02 PiB SUVR_{pons}/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 SUVR_{pons}/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 (see Supplementary Material).

Second, 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 SUVR_{pons} 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 SUVR_{pons} threshold, i.e. with a mean global

neocortical PiB rate of change around 0.0 PiB SUVR_{pons}/year in PiB- participants and around +0.02 PiB SUVR_{pons}/year in the PiB+ group (Figure 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 SUVR_{pons}, which was confirmed by a significant negative linear correlation in this group (Pearson $r=-0.24$; $p = 0.025$) (Figure 4). This suggests that, within the PiB+ individuals, PiB accumulation decreases in individuals with the highest baseline PiB SUVR_{pons} values.

PiB “Accumulators” vs. PiB “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 above +0.022 PiB SUVR_{pons}/year (highest threshold obtained from clustering analyses) and below +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 to the PiB- group, with 58.6% (17/32) of the AD patients, 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- AD patient was classified as an Accumulator) (Table 3). Most of the participants classified as undefined PiB accumulation status were PiB+ MCI participants (Table 3). To assess whether the PiB accumulation status significantly differed between converters and non-converters, a 2-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 HC) 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 2-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+ vs. PiB-.

Voxelwise pattern of PiB rate of change amongst Accumulators

To assess whether the greater rate of PiB accumulation within the PiB+ compared to 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 AD and MCI groups). Group differences revealed a significantly higher regional PiB rate of change in AD patients versus healthy controls within the lateral temporal cortex, the occipital lobe and the dorso-lateral and medial parts of the prefrontal cortex. AD patients also showed a higher regional PiB increase than MCI mainly in the lateral temporal cortex, whereas MCI patients 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 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 (Figure 5). The 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. Within 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 (Figure 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 temporo-parietal junction, the posterior cingulate cortex and the occipital lobe (Figure 5).

DISCUSSION

This study demonstrates that the dynamics of A β accumulation within the brain are not related to baseline clinical status, nor to disease progression, but are associated with the global neocortical A β burden with higher accumulation in PiB+ than in PiB- individuals. Though lower than that found in PiB+, A β accumulation was yet found to be significant in PiB- participants. The present study actually reveals the existence of two clearly identifiable subgroups: “PiB Accumulators” and “PiB Non Accumulators”. “PiB Accumulators” were found to be more numerous in the PiB+ group than in the PiB- group (~50% vs. ~20%) which partly accounted for the effect of the PiB status (PiB+ > PiB-) on the regional PiB rate of change, though this effect remained when assessed within the PiB Accumulators only. Besides, voxelwise analyses performed in Accumulators revealed that the accumulation was significant within the whole brain. The temporo-parietal junction and the posterior cingulate cortex were the areas of highest PiB accumulation within the PiB- Accumulators participants, while the rate of PiB accumulation was higher within the posterior cingulate cortex, the medial, orbital and dorso-lateral prefrontal cortex, the anterior insula and the lateral and inferior temporal cortex in PiB+ Accumulators participants (Figure 5). Finally we observed a negative correlation between the baseline global neocortical PiB uptake and the global neocortical PiB rate of change within the PiB+ group, which is consistent with the concept of a saturable process of A β deposition as the PiB retention reaches highest values (Figure 4).

Consistent with previous studies we observed a significant A β accumulation over time in AD and MCI participants (Grimmer et al., 2010; Jack et al., 2009; Kadir et al., 2012; Koivunen et al., 2011; Rinne et al., 2010; Villemagne et al., 2011). Also a highly significant A β accumulation was found in healthy controls in line with previous studies with the largest

healthy control samples (Jack et al., 2009; Sojkova et al., 2011b; Villemagne et al., 2011). The regional pattern of PiB rate of change in the AD group described here matches previous reports (Grimmer et al., 2010; Rinne et al., 2010; Scheinin et al., 2009) as well as our previous study (Villemagne et al., 2011). It is interesting to note that the regional between-group differences mainly concerned the lateral and inferior temporal cortex, with higher PiB rate of change in the AD patients versus the MCI or the healthy controls (Figure 1). Indeed, the PiB retention in this region has recently been highlighted as an independent contributor to episodic memory decline in MCI and healthy controls (Chételat et al., 2011b). Thus, the specific involvement of the lateral and inferior temporal cortex in terms of A β deposition, amplified when the global A β burden reaches the PiB status threshold, might be responsible for the specific episodic memory impairment observed in the disease.

This work also demonstrates that the effect of the clinical status or conversion on the regional PiB rate of change is mediated by the baseline global A β burden (neocortical PiB SUVR_{pons}). Thus, the total A β burden is itself a risk factor for AD and in turn drives the current A β accumulation rate, but the clinical status itself does not influence directly the rate of A β deposition. Finally, the results presented here are consistent with the recent work by Koivunen and colleagues (Koivunen et al., 2011) who did not observe a significant difference in PiB retention rate of change between MCI converters and non-converters. Given its complexity, the effect of ApoE4 on the rate of A β accumulation and atrophy is not addressed here, as it is the focus of another manuscript that examines the aforementioned interactions in detail (Chételat et al., 2011a).

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 A β in plaques (Jack et al., 2009; Jagust et al., 2010; McLean et al., 1999; Mukaetova-Ladinska et al., 2000; Nelson et al., 2009; Petersen et al., 2006; Scheinin et al., 2009; Villemagne et al., 2011). It has been proposed that the poor clinical-A β plaques relationship could be in line with the amyloid cascade hypothesis (Hardy and Selkoe, 2002) that argues that A β is the earliest pathological feature of Alzheimer's pathology and would possibly lead to a time-lag between A β accumulation and symptomatology (Tiraboschi et al., 2004; Villemagne et al., 2008). Therefore, according to that hypothesis, a snapshot of the course of AD pathology over a short period of time, as performed here over 20 months, would be expected to be independent of clinical progression. Besides the voxelwise analyses revealed that the temporo-parietal junction and the precuneus were the areas of earliest A β accumulation and could thus be used as markers of early amyloidosis (Figure 5). On the other hand, A 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 A β deposition in participants with high A β burden (Figure 5). The validity of these biomarkers remains to be established, especially by comparison to other well-described longitudinal imaging biomarkers such as ¹⁸FDG-PET or structural T1-MRI (Drago et al., 2011; Frisoni et al., 2010; Schill and Fox, 2007). Note that the voxelwise approach proved superior to the region-of-interest approach to detect regional variations since we could identify here significant regional changes in PiB retention within all clinical groups as well as regional differences between these groups that were overlooked using a region-of-interest approach (Villemagne et al., 2011).

One of the main findings of this study is that significant A β accumulation within the brain could be also detected in PiB- healthy controls, a result that was not reported before and revealed by the voxelwise approach (Villemagne et al., 2011). This significant accumulation

in PiB- actually appears to be mainly driven by a subgroup termed as PiB Accumulators. A bi-modal distribution of rates of PiB accumulation revealed this distinction between Accumulators and Non-Accumulators and was then confirmed and validated by a series of converging arguments, especially the confirmation of the PiB accumulation status in 95.6% of the subsample of Accumulators that had a third PiB-PET at 40-month follow-up.

PiB- healthy controls Accumulators did not differ from the PiB- healthy controls Non-Accumulators in baseline demographic variables or neuropsychological assessments. In addition, over the short 20-month follow-up, the PiB accumulation status was not related to clinical progression to AD 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 A β is a self-aggregating protein. Therefore subjects with a significant higher A β burden are more likely to continue this pathological process of A β accumulation than subjects with no or low A 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 underline 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 to 60% of PiB+ 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 A 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 AD) did not show a significant PiB accumulation over time.

It is important to note that a bimodal distribution of PiB rate of change values (Figure 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 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 intra-subject misregistration compared to the pons measurement.

Besides, results show a negative correlation between the baseline global neocortical PiB $\text{SUVR}_{\text{pons}}$ and the global neocortical PiB rate of change within the PiB+, which is consistent with the concept of a saturable process of A β deposition as the PiB $\text{SUVR}_{\text{pons}}$ reaches highest values (Figure 4). Nonetheless, contrary to previous assumptions (Aisen et al., 2010; Ewers et al., 2011; Frisoni et al., 2010; Jack et al., 2010b; Perrin et al., 2009; Petersen, 2010; Sperling et al., 2011a; Weiner et al., 2010), A β accumulation was found to be significantly ongoing at the dementia stage of Alzheimer's disease (Figures 1 and 4). It should be noted that despite these general group patterns, the rate of A β deposition was highly variable from one subject to another, for instance there was no A β accumulation in a PiB+ healthy control with a global neocortical PiB $\text{SUVR}_{\text{pons}}$ of +0.90 while it was on-going in a AD patient with a global neocortical PiB $\text{SUVR}_{\text{pons}}$ of +1.2 (Figure 4).

As a whole, the dynamics of A β deposition are unlikely to be constant and are probably more consistent with a sigmoid curve, but with a different timing as previously proposed (Aisen et al., 2010; Ewers et al., 2011; Frisoni et al., 2010; Jack et al., 2010b; Perrin et al., 2009; Petersen, 2010; Sperling et al., 2011a; Weiner et al., 2010) (Figures 4 and 6). Indeed, PiB accumulation was found to be significantly higher in PiB+ compared to PiB- individuals. Even if A β accumulation slows down when the highest A β burden 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 (Figures 4 and 6). Anyhow, using the median PiB rate of change observed in the PiB- Accumulators (+0.030 SUVR_{pons}/year), it would take about 7 years for a PiB- subject to reach the PiB+ status threshold (from a baseline +0.50 SUVR_{pons} to +0.71 SUVR_{pons}), and about 7.5 additional years for a PiB+ accumulator (+0.041 SUVR_{pons}/year) to reach the mean AD neocortical PiB burden (from a baseline +0.71 SUVR_{pons} to +1.02 SUVR_{pons}) (Figure 6). Nonetheless these durations are rough estimations obtained from basic calculation 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 A β deposition.

To our knowledge, this is the first study to identify these earliest stages of A 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 thus useful to further elucidate *in vivo* the mechanisms that trigger A β deposition.

This sensitivity in detecting early A 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 A β tracer. Indeed, PiB binds to fibrillar A β , found in both cored and non-cored plaques (Ikonomovic et al., 2008), while other A β tracers only bind to cored plaques (Kudo et al., 2007) thus precluding their ability to detect early A β deposition. Further studies with the

newly developed F-18 A β tracers (e.g. forbetaben, forbetapir, flutemetamol) are thus necessary to confirm these findings.

Consistently 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 (Jack et al., 2009; Jagust et al., 2010; McLean et al., 1999; Mukaetova-Ladinska et al., 2000; Nelson et al., 2009; Petersen et al., 2006; Scheinin et al., 2009; Villemagne et al., 2011). Nonetheless our estimations suggest that the hypothetical 14.5 years time-lag between the occurrence of detectable A 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 A β deposition and cognition. In other words, it remains to be demonstrated that the observed earliest stages of A 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 A β deposition on clinical symptoms in healthy controls (Ch  telat et al., 2012; Jack et al., 2010a; Morris et al., 2009; Okello et al., 2009; Resnick et al., 2010), 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 AD (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 to develop AD 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 A β deposition. First, the degree of regional retention of PiB is highly correlated with the regional concentration of A β , as reported at autopsy (Bacskai et al., 2007;

Burack et al., 2010; Ikonovic et al., 2008; Kadir et al., 2011; Leinonen et al., 2008; Sojkova et al., 2011a; Villemagne et al., 2009). Second, it is interesting to note that for PiB-participants (global neocortical baseline PiB = 0.5-0.71 SUVR_{pons}), the identified threshold for Accumulators is mostly above the 3.5% test-retest variability measure ($> +0.022$ SUVR_{pons}/year $\Leftrightarrow > +3.1 - +4.4\%$ /year) reported for this dataset (Villemagne et al., 2011). Moreover, the long term consistency of the Accumulation status (see Supplementary Material) as well as the consistent threshold for PiB Accumulation across individuals ($+0.014$ SUVR_{pons}/year - $+0.022$ SUVR_{pons}/year) further support the robustness of these findings.

In conclusion, ongoing A β deposition was detected in all clinical groups, being significantly higher in the AD group. Nonetheless, this significant clinical group effect actually proved to be completely mediated by the baseline A β burden when this variable was taken into account, i.e. higher rates of A β deposition were associated with higher A β burden. Moreover, significant rates of A β deposition could be detected in a distinct group (“Accumulators”) even in non-demented individuals with low A β burden, but were somewhat slower than in those with high A β 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 to the long term prediction of who is at risk of developing the disease. Besides, we also observed that A β accumulation slows down when the highest A β burden are reached which is consistent with the concept that A β deposition is a saturable process. Finally, and in contrast to a region-of-interest analysis, a voxelwise approach has allowed to determine that the temporo-parietal junction and the precuneus could be used as a marker of early A β deposition while the lateral and inferior temporal cortex could be useful for monitoring A β deposition in the late and

symptomatic stages. Longer longitudinal follow up studies are warranted to further validate these results.

ACKNOWLEDGEMENTS

The study was partially supported by the Servier Institute (NV and BG), the Inserm-Liliane Bettencourt MD-PhD Program (NV), the Commonwealth Scientific Industrial Research Organization Preventative Health Flagship Program through the Australian Imaging, Biomarkers, and Lifestyle flagship study of aging (AIBL), the Austin Hospital Medical Research Foundation, Neurosciences Victoria, and the University of Melbourne. The funding sources had no input into the design of this study, the analysis of data, or writing of the manuscript. We thank Dr M. Woodward, Dr J. Merory, Dr P. Drysdale, Dr G. Chan, Dr K. Young, Dr S. Gong, Dr G. Savage, Dr P. Maruff, Dr P. Yates, Dr D. Darby, Dr A. Bahar-Fuchs, T. Cowie, F. Lamb, J. Robertson, T. Petts, T. Saunder, J. Sagona, and J. Bradley, the Brain Research Institute and the AIBL Study Group (<http://www.aibl.csiro.au/>) for their assistance with this study.

REFERENCES

- Aisen PS, Petersen RC, Donohue MC, Gamst A, Raman R, Thomas RG, et al. Clinical Core of the Alzheimer's Disease Neuroimaging Initiative: progress and plans. *Alzheimers Dement* 2010; 6: 239-246.
- Aizenstein HJ, Nebes RD, Saxton JA, Price JC, Mathis CA, Tsopelas ND, et al. Frequent amyloid deposition without significant cognitive impairment among the elderly. *Arch Neurol* 2008; 65: 1509-1517.
- Alzheimer's Disease International. World Alzheimer Report 2009. 2009.
- Alzheimer's Disease International. World Alzheimer Report 2010. 2010.
- Andreasen N, Hesse C, Davidsson P, Minthon L, Wallin A, Winblad B, et al. Cerebrospinal fluid beta-amyloid(1-42) in Alzheimer disease: differences between early- and late-onset Alzheimer disease and stability during the course of disease. *Arch. Neurol* 1999; 56: 673-680.
- Bacskai BJ, Frosch MP, Freeman SH, Raymond SB, Augustinack JC, Johnson KA, et al. Molecular imaging with Pittsburgh Compound B confirmed at autopsy: a case report. *Arch. Neurol.* 2007; 64: 431-434.
- Burack MA, Hartlein J, Flores HP, Taylor-Reinwald L, Perlmutter JS, Cairns NJ. In vivo amyloid imaging in autopsy-confirmed Parkinson disease with dementia. *Neurology* 2010; 74: 77-84.
- Chételat G, Villain N, Villemagne VL, Ames D, Ellis KA, Head R, et al. The effects of ApoE4 on the rate of atrophy and ABeta deposition in asymptomatic elderly. *Alzheimers.Dement.* 2011a; 7 Supp: S208.
- Chételat G, Villemagne VL, Pike KE, Ellis KA, Bourgeat P, Jones G, et al. Independent contribution of temporal \beta-amyloid deposition to memory decline in the pre-dementia phase of Alzheimer's disease. *Brain* 2011b; 134: 798-807.
- Chételat G, Villemagne VL, Villain N, Jones G, Ellis KA, Ames D, et al. Accelerated cortical atrophy in cognitively normal elderly with high β -amyloid deposition. *Neurology* 2012; 78: 477-484.
- Drago V, Babiloni C, Bartrés-Faz D, Caroli A, Bosch B, Hensch T, et al. Disease tracking markers for Alzheimer's disease at the prodromal (MCI) stage. *J. Alzheimers Dis.* 2011; 26 Suppl 3: 159-199.
- Duyckaerts C, Delatour B, Potier MC. Classification and basic pathology of Alzheimer disease. *Acta Neuropathol.* 2009; 118: 5-36.
- Engler H, Forsberg A, Almkvist O, Blomquist G, Larsson E, Savitcheva I, et al. Two-year follow-up of amyloid deposition in patients with Alzheimer's disease. *Brain* 2006; 129: 2856-2866.
- Ewers M, Sperling RA, Klunk WE, Weiner MW, Hampel H. Neuroimaging markers for the prediction and early diagnosis of Alzheimer's disease dementia. *Trends Neurosci* 2011

Frisoni GB, Fox NC, Jack CR, Scheltens P, Thompson PM. The clinical use of structural MRI in Alzheimer disease. *Nat Rev Neurol* 2010; 6: 67-77.

Grimmer T, Tholen S, Yousefi BH, Alexopoulos P, Förchler A, Förstl H, et al. Progression of cerebral amyloid load is associated with the apolipoprotein E ϵ 4 genotype in Alzheimer's disease. *Biol. Psychiatry* 2010; 68: 879-884.

Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* 2002; 297: 353–356.

Ikonomic MD, Klunk WE, Abrahamson EE, Mathis CA, Price JC, Tsopelas ND, et al. Post-mortem correlates of in vivo PiB-PET amyloid imaging in a typical case of Alzheimer's disease. *Brain* 2008; 131: 1630–1645.

Ingelsson M, Fukumoto H, Newell KL, Growdon JH, Hedley-Whyte ET, Frosch MP, et al. Early A β accumulation and progressive synaptic loss, gliosis, and tangle formation in AD brain. *Neurology* 2004; 62: 925-931.

Jack CR Jr, Wiste HJ, Vemuri P, Weigand SD, Senjem ML, Zeng G, et al. Brain beta-amyloid measures and magnetic resonance imaging atrophy both predict time-to-progression from mild cognitive impairment to Alzheimer's disease. *Brain* 2010a; 133: 3336–3348.

Jack CR, Knopman DS, Jagust WJ, Shaw LM, Aisen PS, Weiner MW, et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurol* 2010; 9: 119-128.

Jack CR, Lowe VJ, Weigand SD, Wiste HJ, Senjem ML, Knopman DS, et al. Serial PIB and MRI in normal, mild cognitive impairment and Alzheimer's disease: implications for sequence of pathological events in Alzheimer's disease. *Brain* 2009; 132: 1355-1365.

Jagust WJ, Bandy D, Chen K, Foster NL, Landau SM, Mathis CA, et al. The Alzheimer's Disease Neuroimaging Initiative positron emission tomography core. *Alzheimers Dement* 2010; 6: 221-229.

Kadir A, Almkvist O, Forsberg A, Wall A, Engler H, Långström B, et al. Dynamic changes in PET amyloid and FDG imaging at different stages of Alzheimer's disease. *Neurobiol. Aging* 2012; 33: 198.e1–198.e14.

Kadir A, Andreasen N, Almkvist O, Wall A, Forsberg A, Engler H, et al. Effect of phenserine treatment on brain functional activity and amyloid in Alzheimer's disease. *Ann. Neurol* 2008; 63: 621-631.

Kadir A, Marutle A, Gonzalez D, Schöll M, Almkvist O, Mousavi M, et al. Positron emission tomography imaging and clinical progression in relation to molecular pathology in the first Pittsburgh Compound B positron emission tomography patient with Alzheimer's disease. *Brain* 2011; 134: 301-317.

Karran E, Mercken M, De Strooper B. The amyloid cascade hypothesis for Alzheimer's disease: an appraisal for the development of therapeutics. *Nat Rev Drug Discov* 2011; 10: 698–712.

Klunk WE, Engler H, Nordberg A, Wang Y, Blomqvist G, Holt DP, et al. Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. *Ann.Neurol.* 2004; 55: 306–319.

Koivunen J, Scheinin N, Virta JR, Aalto S, Vahlberg T, Någren K, et al. Amyloid PET imaging in patients with mild cognitive impairment: a 2-year follow-up study. *Neurology* 2011; 76: 1085-1090.

Kudo Y, Okamura N, Furumoto S, Tashiro M, Furukawa K, Maruyama M, et al. 2-(2-[2-Dimethylaminothiazol-5-yl]ethenyl)-6- (2-[fluoro]ethoxy)benzoxazole: a novel PET agent for in vivo detection of dense amyloid plaques in Alzheimer's disease patients. *J. Nucl. Med.* 2007; 48: 553–561.

Leinonen V, Alafuzoff I, Aalto S, Suotunen T, Savolainen S, Någren K, et al. Assessment of beta-amyloid in a frontal cortical brain biopsy specimen and by positron emission tomography with carbon 11-labeled Pittsburgh Compound B. *Arch. Neurol.* 2008; 65: 1304–1309.

McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 1984; 34: 939–944.

McLean CA, Cherny RA, Fraser FW, Fuller SJ, Smith MJ, Beyreuther K, et al. Soluble pool of Abeta amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. *Ann.Neurol.* 1999; 46: 860–866.

Mormino EC, Brandel MG, Madison CM, Rabinovici GD, Marks S, Baker SL, et al. Not quite PIB-positive, not quite PIB-negative: slight PIB elevations in elderly normal control subjects are biologically relevant. *Neuroimage* 2012; 59: 1152–1160.

Morris JC, Roe CM, Grant EA, Head D, Storandt M, Goate AM, et al. Pittsburgh compound B imaging and prediction of progression from cognitive normality to symptomatic Alzheimer disease. *Arch. Neurol.* 2009; 66: 1469–1475.

Mukaetova-Ladinska EB, Garcia-Siera F, Hurt J, Gertz HJ, Xuereb JH, Hills R, et al. Staging of cytoskeletal and beta-amyloid changes in human isocortex reveals biphasic synaptic protein response during progression of Alzheimer's disease. *Am. J. Pathol.* 2000; 157: 623–636.

Nelson PT, Braak H, Markesbery WR. Neuropathology and cognitive impairment in Alzheimer disease: a complex but coherent relationship. *J Neuropathol.Exp.Neurol* 2009; 68: 1–14.

Okello A, Koivunen J, Edison P, Archer HA, Turkheimer FE, Någren K, et al. Conversion of amyloid positive and negative MCI to AD over 3 years: an 11C-PIB PET study. *Neurology* 2009; 73: 754–760.

Perrin RJ, Fagan AM, Holtzman DM. Multimodal techniques for diagnosis and prognosis of Alzheimer's disease. *Nature* 2009; 461: 916-922.

Petersen RC, Parisi JE, Dickson DW, Johnson KA, Knopman DS, Boeve BF, et al. Neuropathologic features of amnesic mild cognitive impairment. *Arch. Neurol.* 2006; 63: 665–672.

Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. Mild cognitive impairment: clinical characterization and outcome. *Arch Neurol* 1999; 56: 303–308.

Petersen RC. Alzheimer's disease: progress in prediction. *Lancet Neurol* 2010; 9: 4-5.

Resnick SM, Sojkova J, Zhou Y, An Y, Ye W, Holt DP, et al. Longitudinal cognitive decline is associated with fibrillar amyloid-beta measured by [11C]PiB. *Neurology* 2010; 74: 807–815.

Rinne JO, Brooks DJ, Rossor MN, Fox NC, Bullock R, Klunk WE, et al. 11C-PiB PET assessment of change in fibrillar amyloid-beta load in patients with Alzheimer's disease treated with bapineuzumab: a phase 2, double-blind, placebo-controlled, ascending-dose study. *Lancet Neurol* 2010; 9: 363-372.

Rowe CC, Ellis KA, Rimajova M, Bourgeat P, Pike KE, Jones G, et al. Amyloid imaging results from the Australian Imaging, Biomarkers and Lifestyle (AIBL) study of aging. *Neurobiol. Aging* 2010; 31: 1275-1283.

Scahill RI, Fox NC. Longitudinal imaging in dementia. *Br J Radiol* 2007; 80 Spec No 2: S92–98.

Scheinin NM, Aalto S, Koikkalainen J, Lötjönen J, Karrasch M, Kemppainen N, et al. Follow-up of [11C]PIB uptake and brain volume in patients with Alzheimer disease and controls. *Neurology* 2009; 73: 1186-1192.

Sojkova J, Driscoll I, Iacono D, Zhou Y, Codispoti K-E, Kraut MA, et al. In vivo fibrillar beta-amyloid detected using [11C]PiB positron emission tomography and neuropathologic assessment in older adults. *Arch. Neurol.* 2011a; 68: 232–240.

Sojkova J, Zhou Y, An Y, Kraut MA, Ferrucci L, Wong DF, et al. Longitudinal patterns of β -amyloid deposition in nondemented older adults. *Arch. Neurol* 2011b; 68: 644-649.

Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, et al. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 2011a; 7: 280–292.

Sperling RA, Jack CR Jr, Aisen PS. Testing the right target and right drug at the right stage. *Sci Transl Med* 2011b; 3: 111cm33.

Talairach J, Tournoux P. Co-Planar Stereotaxic Atlas of the Human Brain. New York: Thieme; 1988.

Tiraboschi P, Hansen LA, Thal LJ, Corey-Bloom J. The importance of neuritic plaques and tangles to the development and evolution of AD. *Neurology* 2004; 62: 1984–1989.

Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, et al. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage*. 2002; 15: 273–289.

Villemagne VL, Fodero-Tavoletti MT, Pike KE, Cappai R, Masters CL, Rowe CC. The ART of loss: Abeta imaging in the evaluation of Alzheimer's disease and other dementias. *Mol.Neurobiol* 2008; 38: 1–15.

Villemagne VL, McLean CA, Reardon K, Boyd A, Lewis V, Klug G, et al. 11C-PiB PET studies in typical sporadic Creutzfeldt-Jakob disease. *J. Neurol. Neurosurg. Psychiatr.* 2009; 80: 998–1001.

Villemagne VL, Pike KE, Chételat G, Ellis KA, Mulligan RS, Bourgeat P, et al. Longitudinal assessment of A β and cognition in aging and Alzheimer disease. *Ann. Neurol* 2011; 69: 181-192.

Weiner MW, Aisen PS, Jack CR Jr, Jagust WJ, Trojanowski JQ, Shaw L, et al. The Alzheimer's disease neuroimaging initiative: progress report and future plans. *Alzheimers Dement* 2010; 6: 202-211.e7.

Wilke M, Holland SK, Altaye M, Gaser C. Template-O-Matic: a toolbox for creating customized pediatric templates. *Neuroimage* 2008; 41: 903-913.