Increased metabolic vulnerability in early-onset Alzheimer’s disease is not related to amyloid burden

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Patients with early age-of-onset Alzheimer’s disease show more rapid progression, more generalized cognitive deficits and greater cortical atrophy and hypometabolism compared to late-onset patients at a similar disease stage. The biological mechanisms that underlie these differences are not well understood. The purpose of this study was to examine in vivo whether metabolic differences between early-onset and late-onset Alzheimer’s disease are associated with differences in the distribution and burden of fibrillar amyloid-β. Patients meeting criteria for probable Alzheimer’s disease (National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association criteria) were divided based on estimated age at first symptom (less than or greater than 65 years) into early-onset (n = 21, mean age-at-onset 55.2 ± 5.9 years) and late-onset (n = 18, 72.0 ± 4.7 years) groups matched for disease duration and severity. Patients underwent positron emission tomography with the amyloid-β-ligand [11C]-labelled Pittsburgh compound-B and the glucose analogue [18F]-labelled fluorodeoxyglucose. A group of cognitively normal controls (n = 30, mean age 73.7 ± 6.4) was studied for comparison. [11C]-labelled Pittsburgh compound-B images were analysed using Logan graphical analysis (cerebellar reference) and [18F]-labelled fluorodeoxyglucose images were normalized to mean activity in the pons. Group differences in tracer uptake were assessed on a voxel-wise basis using statistical parametric mapping, and by comparing mean values in regions of interest. To account for brain atrophy, analyses were repeated after applying partial volume correction to positron emission tomography data. Compared to normal controls, both early-onset and late-onset Alzheimer’s disease patient groups showed increased [11C]-labelled Pittsburgh compound-B uptake throughout frontal, parietal and lateral temporal cortices and striatum on voxel-wise and region of interest comparisons (P < 0.05). However, there were no significant differences in regional or global [11C]-labelled Pittsburgh compound-B binding between early-onset and late-onset patients. In contrast, early-onset patients showed significantly lower glucose metabolism than late-onset patients in precuneus/posterior cingulate, lateral temporo-parietal and occipital cortices (voxel-wise and region of interest comparisons, P < 0.05). Similar results were found for [11C]-labelled Pittsburgh...
compound-B and [18F]-labelled fluorodeoxyglucose using atrophy-corrected data. Age-at-onset correlated positively with glucose metabolism in precuneus, lateral parietal and occipital regions of interest (controlling for age, education and Mini Mental State Exam, \( P < 0.05 \)), while no correlations were found between age-at-onset and [11C]-labelled Pittsburgh compound-B binding. In summary, a comparable burden of fibrillar amyloid-\( \beta \) was associated with greater posterior cortical hypometabolism in early-onset Alzheimer’s disease. Our data are consistent with a model in which both early amyloid-\( \beta \) accumulation and increased vulnerability to amyloid-\( \beta \) pathology play critical roles in the pathogenesis of Alzheimer’s disease in young patients.

Keywords: Alzheimer’s disease; age of onset; amyloid-\( \beta \); [18F]-labelled fluorodeoxyglucose; [11C]-labelled Pittsburgh compound-B

Abbreviations: ApoE4 = apolipoprotein E \( \varepsilon 4 \) allele; FDG = [18F]-labelled fluorodeoxyglucose; FWE = family-wise error; MMSE = Mini-Mental State Exam score; PIB = [11C]-labelled Pittsburgh compound-B

**Introduction**

Though generally considered a disease of the elderly, Alzheimer’s disease is also a leading cause of early age-of-onset dementia (Ratnavalli et al., 2002; Knopman et al., 2004; Mercy et al., 2008). Patients who present with early-onset Alzheimer’s disease, defined in most studies as the onset of symptoms before age 65, show more rapid clinical decline and shorter survival than late-onset patients (Seltzer and Sherwin, 1983; Barclay et al., 1985; Heyman et al., 1987; Mortimer et al., 1992; Jacobs et al., 1994; Koss et al., 1996). The majority of studies have also found an effect of age on the pattern of cognitive deficits, with early-onset patients showing greater impairment in attention, language, visuo–spatial and executive functions, and late-onset patients showing comparatively greater deficits in episodic memory (Seltzer and Sherwin, 1983; Loring and Largen, 1985; Filley et al., 1986; Binetti et al., 1993; Jacobs et al., 1994; Koss et al., 1996; Fujimori et al., 1998; Imaamura et al., 1998; Frisoni et al., 2005, 2007; Kim et al., 2005; Snowden et al., 2007). Mirroring these clinical differences, neuroimaging studies show greater cortical atrophy, hypoperfusion and hypometabolism in early-onset Alzheimer’s disease, particularly in parietal and lateral temporal cortices, and relatively greater medial temporal lesions in late-onset Alzheimer’s disease (Grady et al., 1987; Jagust et al., 1990; Yasuno et al., 1998; Salmon et al., 2000; Sakamoto et al., 2002; Kemp et al., 2003; Frisoni et al., 2005, 2007; Ishii et al., 2005; Kim et al., 2005; Shinoh et al., 2006, 2008; Karas et al., 2007). Age-of-onset correlates positively with glucose metabolism in frontal, parietal and temporal cortices, with the youngest patients tending to show the most severe hypometabolism, while age correlates negatively with glucose metabolism in hippocampus (Yasuno et al., 1998). When controlling for dementia severity, patients with early-onset Alzheimer’s disease show more extensive structural and functional neuroimaging changes than their late-onset counterparts (Kim et al., 2005).

The biological mechanisms underlying the clinical differences between early-onset and late-onset Alzheimer’s disease are not well understood. Only a small minority (1–5%) of patients carry disease-causing mutations that lead to autosomal dominant early-onset Alzheimer’s disease via early and rapid aggregation of amyloid-\( \beta \) (Rocchi et al., 2003). Patients with ‘sporadic’ early-onset disease are more likely to be carriers of the apolipoprotein E \( \varepsilon 4 \) allele (Farrer et al., 1997), which is also associated with early deposition of amyloid-\( \beta \) plaques (Kok et al., 2009; Morris et al., 2009). Post-mortem studies comparing the burden of Alzheimer’s disease pathology in early-onset and late-onset patients demonstrate a higher burden of both neuritic plaques and neurofibrillary tangles in younger patients, though this association has been more consistently found for tangles than plaques (Mann et al., 1984; Hansen et al., 1988; Nochlink et al., 1993; Berg et al., 1998; Bigio et al., 2002; Ho et al., 2002; Marshall et al., 2007). Neurochemical differences between early-onset and late-onset patients are also evident, with younger patients showing more diffuse and severe deficits in acetylcholine and norepinephrine, reflecting greater degeneration of the nucleus of Meynert and the locus coeruleus (Bird et al., 1983; Rossor et al., 1984). Taken together, these data suggest a model in which early-onset patients, in part due to genetic factors, develop early and accelerated deposition of pathology, which in turn leads to a precipitous clinical decline. The fact that early-onset patients show a higher burden of pathology and more widespread neurodegeneration in comparison to late-onset patients of similar clinical severity has been interpreted as evidence for greater ‘cognitive reserve’ in younger brains (Kim et al., 2005).

While post-mortem studies comparing early-onset and late-onset Alzheimer’s disease are invaluable, they also have inherent limitations. Early-onset patients typically die in the end-stages of dementia, whereas late-onset patients are more likely to die at an earlier disease stage of an unrelated cause. This has led to a systematic bias towards more severe disease at the time of autopsy in early-onset Alzheimer’s disease (Berg et al., 1998; Ho et al., 2002). A limited number of brain regions are sampled on autopsy, subjecting studies to sampling error while making it difficult to describe the precise anatomic distribution of pathology. Many studies employed semi-quantitative measures of pathology such as CERAD (Consortium to Establish a Registry for Alzheimer’s Disease) scores (Mirra et al., 1991) or Braak staging (Braak and Braak, 1991), limiting statistical inferences. Even studies that more precisely quantified lesion burden did not employ stereological techniques, and thus may have been biased by brain atrophy and tissue shrinkage, which are both greater in younger patients (West, 1993). Finally, in most studies there was a significant delay between the last meaningful clinical evaluation and the time of death, making it difficult to derive clinicopathologic correlations.

A recent advance in neuroimaging allows the measurement of amyloid-\( \beta \) amyloid in vivo using the positron emission tomography
(PET) tracer Pittsburgh compound-B (PIB) (Klunk et al., 2004). PIB is a thioflavin-T analogue that, at PET tracer concentrations, binds fibrillar amyloid-β with high sensitivity and specificity (Klunk et al., 2003). Elevated PIB binding is seen in the vast majority of patients with Alzheimer’s disease in the known distribution of amyloid-β neuritic plaques (Klunk et al., 2004; Kampaipainen et al., 2006). PIB signal during life correlates strongly with the distribution and burden of amyloid-β pathology found at autopsy (Bacskai et al., 2007; Ikonomovic et al., 2008). Thus, PIB allows the quantification of fibrillar amyloid-β during life with high precision and anatomic resolution.

In this study, we applied PIB-PET to investigate in vivo the effects of age-of-onset on the distribution and burden of amyloid-β plaques in Alzheimer’s disease. We selected patients in the mild-to-moderate (rather than severe) stage of disease, and compared the effect of age on amyloid-β to its impact on resting glucose metabolism. Based on previous work, we hypothesized that PIB uptake would be increased in early-onset Alzheimer’s disease compared to patients with late-onset disease, and that this would be accompanied by more severe temporoparietal hypometabolism in younger patients.

**Methods**

**Subject selection and characterization**

All patients were recruited from an Alzheimer’s disease research cohort followed at the University of California San Francisco Memory and Aging Centre. The clinical evaluation includes a history and physical examination by a neurologist, a structured caregiver interview by a nurse, and a battery of neuropsychological tests (Kramer et al., 2003). Clinical diagnosis was assigned by consensus at a multi-disciplinary conference using standard research criteria (McKhann et al., 1984). Patients with significant co-morbid medical or psychiatric illness were not eligible for the study. Patients with significant cerebrovascular disease (as defined by more than one lacunar infarct or severe white matter hyperintensities on MRI [Longstreth scale ≥6 (Longstreth et al., 1996)]) were also excluded.

For this study we included all patients who met research criteria for probable Alzheimer’s disease (McKhann et al., 1984) and underwent PIB-PET imaging at Lawrence Berkeley National Laboratory between April 2005 and January 2009. Enrolment was independent of PIB scan results (i.e. positive or negative for elevated tracer uptake). However, one patient (PIB-negative, age at PET 66.5) was excluded because on longitudinal follow-up the clinical diagnosis was changed from Alzheimer’s disease to frontotemporal dementia. Patients with a clinical diagnosis other than Alzheimer’s disease (e.g. primary progressive aphasia) were not included even if the clinician suspected the clinical syndromes were due to underlying Alzheimer’s disease histopathology. To avoid the confounding effects of co-morbid pathology, patients with a diagnosis of mixed dementia (e.g. mixed Alzheimer’s disease and dementia with Lewy bodies) were also excluded.

Age at onset was determined based on the estimated date of first symptoms as stated by patients or caregivers. Consistent with previous reports, we dichotomized Alzheimer’s disease patients into early-onset (age at onset <65) and late-onset (age at onset ≥65) groups. Our final cohort consisted of 39 Alzheimer’s disease patients (mean age-at-onset 62.0±10.1, median 61.7), 21 with early-onset disease (mean 55.2, range 43.4–64.7) and 18 with late-onset disease (mean 72.0, range 66.7–82.0) (Table 1).

Thirty cognitively normal older volunteers were included as imaging controls. These subjects were recruited from the Berkeley Aging Cohort as previously described (Mormino et al., 2009). All control subjects were deemed to be cognitively normal following a battery of neuropsychological tests (Mormino et al., 2009).

Apolipoprotein E genotyping was performed for 33 Alzheimer’s disease patients and 30 normal controls using previously published methods (Agosta et al., 2009).
Imaging

PET acquisition

\(^{[1]} \text{C}\)-labelled PIB was synthesized at the Lawrence Berkeley National Laboratory's Biomedical Isotope Facility using standard methods (Mathis et al., 2003). \(^{[18]} \text{F}\)-labelled fluorodeoxyglucose (FDG) was purchased from a commercial vendor (Eastern Isotopes, Sterling, VA). PET scans were performed using a Siemens ECAT EXACT HR PET scanner in 3D acquisition mode. Approximately 15 mCi of PIB was injected as a bolus into an antecubital vein and dynamic acquisition frames were obtained for 90 min as previously described (Rabinovici et al., 2007). All 30 controls and 36/39 Alzheimer's disease patients underwent FDG-PET immediately following the PIB scan, while three patients (two early-onset and one late-onset) could not receive an FDG scan for technical reasons. Patients were injected with \(~10\) mCi of FDG and imaged at a minimum of 2 h after PIB injection (six \(^{11} \text{C}\) half-lives). Thirty minutes of emission data were collected at \(t=30-60\) min after tracer injection with patients resting quietly with eyes and ears unoccluded. Ten-minute transmission scans for attenuation correction were obtained either immediately before or after each PET and FDG scan. PET data were reconstructed using an ordered subset expectation maximization algorithm with weighted attenuation. Images were smoothed with a 4 mm Gaussian kernel with scatter correction. All images were evaluated before analysis for patient motion and adequacy of statistical counts. Two FDG-PET scans (one each for early-onset and late-onset Alzheimer’s disease) were excluded from analysis because of technical problems.

MRI acquisition

Thirty Alzheimer’s disease patients (17/21 early-onset patients and 13/18 late-onset patients) underwent research protocol MRI scans at the San Francisco Veterans Affairs Medical Centre within one year of PET imaging (mean interval between MRI and PET 96 ± 99 days). Nineteen patients (10 with early-onset Alzheimer’s disease and nine with late-onset Alzheimer’s disease) were scanned on a 1.5 Tesla Siemens Vision system (Rosen et al., 2002), and 11 patients (seven early-onset and four late-onset) were scanned on a Bruker MedSpec 4 Tesla system controlled by a Siemens Trio\textsuperscript{TM} console (Mueller et al., 2009). All 30 normal control subjects were imaged at Lawrence Berkeley National Laboratory on a 1.5 Tesla Siemens Avanto System (mean interval to PET 95 ± 200 days) (Mormino et al., 2009). Given the heterogeneous MRI acquisition protocols, structural images were used only to assist with PET analysis and a primary MRI analysis was not performed.

PET analysis

Image processing and analysis was performed using Statistical Parametric Mapping 2 software (http://www.fil.ion.ucl.ac.uk/spm). Reference regions in whole cerebellum (for PIB) and pons (for FDG) were created in Montreal Neurological Institute space and then warped to each subject's native space using a 'reverse normalization' procedure (Rabinovici et al., 2007). To allow for inter-subject comparisons, FDG frames for each subject were summed and normalized to mean activity in the pons (Minoshima et al., 1995). For PIB, voxel-wise distribution volume ratios were calculated using Logan graphical analysis, with the cerebellum time-activity curve used as a reference tissue input function \((t=35–90\) min) (Lopresti et al., 2005; Price et al., 2005). The cerebellum is the preferred reference region for PIB because it is relatively free of fibrillar amyloid even in advanced Alzheimer's disease (Joachim et al., 1989).

To ensure that there were no differences in cerebellar PIB binding between early-onset and late-onset Alzheimer’s disease patients, we calculated distribution volume ratios for cerebellum in every Alzheimer’s disease patient using the pons time-activity curve as the reference input function. The pons has been previously shown to be an area of non-specific PIB binding with no difference in tracer uptake between Alzheimer’s disease patients and controls (Klunk et al., 2004). We found no difference in cerebellum distribution volume ratios between early-onset Alzheimer’s disease (mean distribution volume ratio 0.62 ± 0.05) and late-onset Alzheimer’s disease (mean distribution volume ratio 0.61 ± 0.05, \(P=0.55\)), validating the use of cerebellum as a reference region when comparing these two patient groups.

Partial volume correction

Given previous reports of greater cortical atrophy in early-onset versus late-onset Alzheimer’s disease (Frisoni et al., 2005, 2007; Ishii et al., 2005, 2009), we were concerned that brain atrophy may confound our group comparisons by preferentially lowering PET counts in early-onset subjects. We thus corrected PET data for atrophy by applying a two-compartmental partial-volume correction to all subjects with an MRI (Meltzer et al., 1990). The correction procedure involved convolving a brain mask (a sum of the grey and white matter segmented images from the subject’s \(T_1\)-weighted MRI) with the point-spread function specific to the PET tomograph along all axes (previously empirically derived (Klein et al., 1997)). This provided a means for estimating the percentage of brain tissue emitting radioactivity at each voxel. The PET counts for each voxel were then adjusted based on the percentage of estimated brain matter (Meltzer et al., 1999). All primary analyses were performed using uncorrected PET data from all subjects, while confirmatory analyses were conducted with atrophy-corrected data in the subset of patients with an available MRI.

Voxel-wise group comparisons

Individual subject PIB and FDG volumes were spatially normalized to Montreal Neurological Institute space. This was done separately for non-atrophy corrected data (PET-based normalization) and atrophy-corrected data (MRI-based normalization). In the PET-based normalization stream, mean PET and FDG images created during frame realignment were normalized to the Statistical Parametric Mapping PET template. Normalization parameters were then applied to the PIB distribution volume ratio and FDG pons-normalized volumes, respectively. In the MRI-based normalization stream, the subject’s \(T_1\)-weighted MRI was spatially normalized to a template brain derived from older individuals (the minimal deformation template 2) (Sun et al., 2007). The normalization parameters were subsequently applied to the subject’s co-registered, atrophy-corrected PET and FDG volumes. All spatially-normalized images were smoothed with a 12 mm kernel.

Voxel-wise comparisons of PIB distribution volume ratio and pons-normalized FDG images were performed in an analysis of covariance model that included diagnosis (early-onset Alzheimer’s disease, late-onset Alzheimer’s disease or normal control) as the condition and sex, education and Mini-Mental State Exam score (MMSE) as nuisance variables. Pair-wise contrasts were performed between the three groups as follows: (i) early-onset Alzheimer’s disease > normal controls; (ii) late-onset Alzheimer’s disease > normal controls; (iii) early-onset Alzheimer’s disease > late-onset Alzheimer’s disease; (iv) late-onset Alzheimer’s disease > early-onset Alzheimer’s disease; (v) normal controls > early-onset Alzheimer’s disease; and (vi) normal
controls > late-onset Alzheimer’s disease. To allow broad visualization of the data, results were displayed on a template brain as T-maps thresholded at $P < 0.001$ uncorrected for multiple comparisons. Voxels were considered significant at $P < 0.05$ after family-wise error (FWE) correction. All voxel-wise analyses were repeated with atrophy-corrected data using the same analysis of covariance model, post-hoc contrasts and statistical thresholds.

Region of interest definition

PET counts were extracted in normalized space from regions of interest derived from the Automated Anatomic Labelling Atlas (Tzourio-Mazoyer et al., 2002). In order to exclude PET counts from white matter and cerebrospinal fluid, automated regions of interest were masked by the individual subject’s grey matter segmented images (Sun et al., 2007). Pre-defined regions of interest included (separately for left and right hemispheres): frontal cortex (anterior to precentral gyrus), precuneus, posterior cingulate cortex, lateral parietal cortex, lateral temporal cortex, temporal pole, medial temporal cortex (parahippocampal, fusiform and lingual gyrus), occipital cortex (excluding striate cortex), hippocampus and striatum. As a measure of global amyloid burden we calculated a PIb index, representing the subject’s mean distribution volume ratio in frontal, posterior cingulate, precuneus, parietal and lateral temporal cortices (Mormino et al., 2009). Again, primary region of interest analyses were performed with non-atrophy corrected data (available for all subjects), and confirmatory analyses were performed with atrophy-corrected data (available for a subset of subjects).

Statistical analysis

Group differences in continuous variables were examined using one-way analysis of variance (ANOVA) and Tukey post hoc contrasts or Mann-Whitney U-tests when appropriate. Dichotomous variables were compared with the Pearson’s chi-square test or Fisher’s exact test. The effect of age-at-onset as a continuous (rather than dichotomous) variable on regional PIb and FDG binding in Alzheimer’s disease was studied using multi-linear regression, with education and MMSE included as covariates. Sex was not included as a covariate in this analysis since gender distribution did not differ based on age-at-onset.

The study was approved by the University of California Berkeley, University of California San Francisco and Lawrence Berkeley National Laboratory institutional review boards for human research.

Results

Subject characteristics

Patients with early-onset Alzheimer’s disease were significantly younger at onset than late-onset patients, and significantly younger at PET than both late-onset patients and normal controls (Table 1). Age at onset and age at PET were highly correlated ($r = 0.96, P < 0.0001$). The gender distribution differed between normal controls (female preponderance) and late-onset Alzheimer’s disease (male preponderance, $P < 0.005$), and was thus included as a nuisance variable in the three-way voxel-wise analysis. All participants were highly educated. Early-onset and late-onset patients were well matched for disease duration and for functional status, as measured by the Clinical Dementia Rating total and sum-of-boxes (Morris, 1993). Patients performed worse than normal controls on the MMSE ($P < 0.0001$) and there was a trend for worse performance by early-onset versus late-onset patients ($P = 0.06$). MMSE was therefore included as a covariate in group comparisons. There were no differences between early-onset and late-onset Alzheimer’s disease in medication use or in the percentage of patients with a family history of dementia in a first degree relative. Of note, none of the patients had a family history suggestive of autosomal dominant Alzheimer’s disease, though only one patient was screened (and tested negative) for mutations in presenilin-1 and -2. The ApoE4 genotype was more common in patients compared to controls, but there was no difference in ApoeE gene distribution between early-onset and late-onset patients ($P = 0.25$). Only one Alzheimer’s disease patient carried an ApoE2 allele (E2/E3 genotype, age-at-onset 70.4 years).

Neuropsychological profiles

Neuropsychological test batteries obtained within one year of PET were available for 18/21 early-onset patients and 14/18 late-onset patients (Table 2). The mean interval between cognitive testing and PET was 86 ± 82 days. Because of the skewed distribution of most test scores, group medians and ranges are presented in place of means and standard deviations. Early-onset patients performed significantly worse than late-onset patients on digit span backwards, a test of attention and working memory ($P = 0.02$). There were non-significant trends for worse performance by early-onset patients on tests of executive function (Stroop Interference Test), language (syntax comprehension) and visuo-spatial function (copy of modified Rey-Osterrieth figure). Median scores were lower in late-onset Alzheimer’s disease for delayed recall tasks and lower in early-onset Alzheimer’s disease for most tests of language, visuo-spatial and executive function; but these differences were not significant.

Pittsburgh compound-B: voxel-wise comparisons

Compared to normal controls, patients with both early-onset and late-onset Alzheimer’s disease showed diffuse, symmetric PIb binding throughout frontal, parietal and temporal cortices, with sparing of primary sensorimotor and visual cortices [$P_{(FWE-corr)} < 0.05$, Fig. 1 left panels]. On direct contrast of the two patient groups, early-onset patients showed higher PIb binding in left medial temporal cortex and right temporal pole at $P < 0.001$ uncorrected (Fig. 1), but only a small cluster of voxels ($n = 49$) in left fusiform gyrus survived multiple comparisons correction. Late-onset patients showed higher PIb uptake in left superior parietal lobule at $P < 0.001$ uncorrected, but this did not survive FWE correction. There were no voxels in which PIb uptake was greater in normal controls than in early-onset or late-onset patients at $P < 0.001$ uncorrected.

Atrophy correction did not alter the pattern of PIb uptake in patients compared to normal controls, though the statistical significance of the contrasts increased as PIb counts were raised in atrophic regions (Fig. 1, right panels). Following atrophy...
### Table 2 Neuropsychological test scores

<table>
<thead>
<tr>
<th></th>
<th>Early-onset Alzheimer’s disease (n = 18)</th>
<th>Late-onset Alzheimer’s disease (n = 16)</th>
<th>P</th>
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<tbody>
<tr>
<td>MMSE (30)</td>
<td>23.0 (13–29)</td>
<td>25.0 (10–29)</td>
<td>0.20</td>
</tr>
<tr>
<td>Memory</td>
<td></td>
<td></td>
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<tr>
<td>CVLT-SF total learning (36)</td>
<td>16.0 (3–32)</td>
<td>17.5 (6–27)</td>
<td>0.82</td>
</tr>
<tr>
<td>CVLT-SF 10 min recall (9)</td>
<td>0.5 (0–9)</td>
<td>0.0 (0–7)</td>
<td>0.68</td>
</tr>
<tr>
<td>Modified Rey 10 min recall (17)</td>
<td>3.0 (0–14)</td>
<td>0.0 (0–12)</td>
<td>0.34</td>
</tr>
<tr>
<td>Executive function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modified Trails-B time (120’’)</td>
<td>120 (29–120)</td>
<td>99 (32–120)</td>
<td>0.41</td>
</tr>
<tr>
<td>Modified Trails-B no. correct lines (14)</td>
<td>7.0 (1–14)</td>
<td>14.0 (0–14)</td>
<td>0.52</td>
</tr>
<tr>
<td>Digits backward span</td>
<td>3.0 (0–6)</td>
<td>4.0 (3–7)</td>
<td>0.02</td>
</tr>
<tr>
<td>Stroop Interference Test no. correct</td>
<td>5.0 (0–55)</td>
<td>26.0 (9–35)</td>
<td>0.07</td>
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<tr>
<td>Language</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boston Naming Test (15 item)</td>
<td>12.0 (2–15)</td>
<td>11.0 (3–15)</td>
<td>0.84</td>
</tr>
<tr>
<td>Sentence repetition (5)</td>
<td>4.0 (1–5)</td>
<td>3.0 (1–5)</td>
<td>0.54</td>
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<tr>
<td>Syntax comprehension (5)</td>
<td>3.0 (0–5)</td>
<td>4.0 (1–5)</td>
<td>0.09</td>
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<tr>
<td>Letter fluency (D words)</td>
<td>8.0 (2–18)</td>
<td>10.0 (4–15)</td>
<td>0.23</td>
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<tr>
<td>Category fluency (animals)</td>
<td>9.5 (4–14)</td>
<td>10.0 (2–20)</td>
<td>0.51</td>
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<tr>
<td>Visuo–spatial</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Modified Rey copy (17)</td>
<td>11.5 (2–17)</td>
<td>14.0 (3–16)</td>
<td>0.12</td>
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<tr>
<td>VOSP Spatial Location Test (10)</td>
<td>6.0 (2–10)</td>
<td>9.0 (3–10)</td>
<td>0.27</td>
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<tr>
<td>Calculations (5)</td>
<td>3.0 (2–5)</td>
<td>4.0 (0–5)</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Maximum attainable scores are presented in parentheses. Values are presented as medians (range).

Missing data: CVLT learning (late-onset 4), CVLT recall (late-onset 3), Rey recall (early-onset 2, late-onset 1), Modified Trials (early-onset 5, late-onset 6), Stroop (early-onset 7, late-onset 5), Boston Naming Test (late-onset 2), Sentence repetition (late-onset 2), Sentence comprehension (early-onset 2, late-onset 5), Letter fluency (late-onset 3), Rey copy (early-onset 2, late-onset 1), VOSP (early-onset 3, late-onset 3), Calculations (early-onset 2, late-onset 2). CVLT-SF = California Verbal Learning Test – San Francisco (9-item); Modified Trails-B = Trails B test.

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**Figure 1** Patterns of PIB binding in early-onset (EO) and late-onset (LO) Alzheimer’s disease (AD) compared to normal controls (NC) and to each other. Voxel-wise comparisons included sex, education and MMSE as nuisance variables. T-score maps prior to atrophy correction are rendered on the ch2 template brain (left), while T-score maps following atrophy correction are rendered on the minimal deformation template 2 template (right). All results are presented at a threshold of $P < 0.001$, uncorrected for multiple comparisons.
correction, early-onset patients showed greater PIB uptake than late-onset patients in bilateral temporal poles while late-onset patients showed greater uptake in left superior parietal lobule at $P < 0.001$ uncorrected (Fig. 1); however none of these differences survived FWE correction.

**Pittsburgh compound-B: region of interest comparisons**

Early-onset patients showed higher mean PIB distribution volume ratio values than normal controls in all regions of interest except hippocampus, while patients with late-onset Alzheimer’s disease showed higher uptake than normal controls in all regions of interest except hippocampus, right posterior cingulate and right temporal pole (Table 3). Mean PIB index was essentially identical in patients with both early-onset and late-onset Alzheimer’s disease, and no significant differences between patient groups were detected in any regions. Following atrophy correction, early-onset and late-onset patients showed higher PIB than normal controls in all regions except hippocampus. As expected, the atrophy correction procedure raised the PIB index of early-onset patients more than that of late-onset patients (early-onset 29.4% ± 7.8%, late-onset 21.8% ± 9.4%, $P < 0.05$). Nevertheless, even following atrophy correction, there were no differences in PIB index or in regional PIB uptake between patients with early-onset and late-onset disease (Table 3).

### Table 3  PIB distribution volume ratios in regions of interest before and after atrophy correction

<table>
<thead>
<tr>
<th>PIB Region</th>
<th>Normal controls</th>
<th>Early-onset Alzheimer's disease</th>
<th>Late-onset Alzheimer's disease</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 30)</td>
<td>(n = 21)</td>
<td>(n = 18)</td>
<td></td>
</tr>
<tr>
<td>PIB index</td>
<td>1.13 (0.18)</td>
<td>1.58 (0.21)</td>
<td>1.58 (0.33)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>L frontal</td>
<td>1.13 (0.20)</td>
<td>1.60 (0.20)</td>
<td>1.60 (0.35)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>R frontal</td>
<td>1.13 (0.19)</td>
<td>1.59 (0.22)</td>
<td>1.58 (0.35)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>L precuneus</td>
<td>1.08 (0.21)</td>
<td>1.58 (0.18)</td>
<td>1.62 (0.37)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>R precuneus</td>
<td>1.09 (0.21)</td>
<td>1.55 (0.25)</td>
<td>1.64 (0.37)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>L posterior cingulate</td>
<td>1.26 (0.15)</td>
<td>1.62 (0.27)</td>
<td>1.54 (0.32)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>R posterior cingulate</td>
<td>1.28 (0.17)</td>
<td>1.48 (0.30)</td>
<td>1.42 (0.25)</td>
<td>0.01</td>
</tr>
<tr>
<td>L lateral parietal</td>
<td>1.12 (0.20)</td>
<td>1.52 (0.19)</td>
<td>1.60 (0.32)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>R lateral parietal</td>
<td>1.11 (0.20)</td>
<td>1.51 (0.22)</td>
<td>1.58 (0.32)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>L lateral temporal</td>
<td>1.15 (0.18)</td>
<td>1.59 (0.23)</td>
<td>1.53 (0.33)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>R lateral temporal</td>
<td>1.15 (0.16)</td>
<td>1.58 (0.29)</td>
<td>1.55 (0.35)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>L medial temporal</td>
<td>1.08 (0.11)</td>
<td>1.29 (0.15)</td>
<td>1.22 (0.22)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>R medial temporal</td>
<td>1.07 (0.11)</td>
<td>1.30 (0.19)</td>
<td>1.23 (0.23)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>L occipital</td>
<td>1.17 (0.15)</td>
<td>1.45 (0.20)</td>
<td>1.47 (0.30)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>R occipital</td>
<td>1.15 (0.14)</td>
<td>1.44 (0.23)</td>
<td>1.45 (0.30)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>L temporal pole</td>
<td>0.92 (0.12)</td>
<td>1.25 (0.19)</td>
<td>1.15 (0.27)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>R temporal pole</td>
<td>0.92 (0.11)</td>
<td>1.25 (0.22)</td>
<td>1.13 (0.26)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>L hippocampus</td>
<td>1.15 (0.11)</td>
<td>1.21 (0.15)</td>
<td>1.10 (0.21)</td>
<td>0.06</td>
</tr>
<tr>
<td>R hippocampus</td>
<td>1.13 (0.13)</td>
<td>1.20 (0.19)</td>
<td>1.12 (0.21)</td>
<td>0.23</td>
</tr>
<tr>
<td>L striatum</td>
<td>1.22 (0.16)</td>
<td>1.58 (0.25)</td>
<td>1.59 (0.29)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>R striatum</td>
<td>1.22 (0.16)</td>
<td>1.59 (0.24)</td>
<td>1.56 (0.28)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Values are presented as mean (standard deviation).

*Early-onset > normal controls, $P < 0.05$.

Late-onset > normal controls, $P < 0.05$.

Scatter-plots of PIB index by diagnostic group (before and after atrophy correction) are shown in Fig. 2A. As expected, most normal controls showed low PIB uptake, though a minority showed elevated binding in the Alzheimer’s disease range. While overall group means were the same, the distribution of the PIB index appeared different in early-onset and late-onset Alzheimer’s disease. Late-onset patients showed a bimodal distribution, with four subjects falling in the low normal controls range, and the remaining subjects showing high index values. In contrast, patients with early-onset Alzheimer’s disease showed a continuum of PIB uptake beginning at the very top of the normal controls cluster and extending into the high index range (Fig. 2A). Only one of four ‘low PIB’ late-onset patients had an MRI available and this patient continued to show a low PIB index following atrophy correction (bottom subject in the atrophy-corrected late-onset distribution).

**Fluorodeoxyglucose: voxel-wise comparisons**

Compared to normal controls, early-onset patients showed extensive regions of decreased glucose metabolism throughout bilateral temporo–parietal and occipital cortices ($P < 0.001$ uncorrected, Fig. 3). Differences in bilateral angular gyrus and right middle and inferior temporal gyrus remained significant after multiple comparisons correction ($P_{FWE-corr} < 0.05$). There were no regions...
of decreased metabolism in late-onset Alzheimer’s disease compared to normal controls at \( P < 0.001 \) uncorrected, though bilateral temporo-parietal hypometabolism was found if MMSE was removed from the model and the statistical threshold was lowered to \( P < 0.01 \) (data not shown). Compared to late-onset Alzheimer’s disease, early-onset patients showed decreased metabolism in bilateral precuneus, lateral temporo–parietal and occipital cortices \( (P < 0.001 \) uncorrected). After FWE correction, significant differences remained in left precuneus, bilateral angular gyrus, right middle temporal gyrus and left middle occipital gyrus \[ (P_{\text{FWE-corr}}) < 0.05 \]. There were no areas of relative hypometabolism in late-onset versus early-onset Alzheimer’s disease, or in normal controls compared to either patient group \( (P < 0.001 \) uncorrected).

Atrophy correction did not appreciably alter the pattern of hypometabolism in early-onset Alzheimer’s disease compared to normal controls (Fig. 3), with significant differences found in bilateral precuneus, angular gyrus and middle temporal gyrus \[ (P_{\text{FWE-corr}}) < 0.05 \]. Compared to normal controls, late-onset patients now showed decreased metabolism in left angular gyrus, left middle temporal gyrus and right hippocampus at \( P < 0.001 \) uncorrected, though these regions did not survive multiple comparisons correction. In the direct patient contrasts, atrophy correction did restrict the regions of relative hypometabolism in early-onset compared to late-onset Alzheimer’s disease (Fig. 3), though significant differences remained in bilateral precuneus and in right angular/supramarginal gyrus \[ (P_{\text{FWE-corr}}) < 0.05 \]. Late-onset patients showed lower glucose metabolism than early-onset patients in left frontal trigone, right lateral orbital gyrus, bilateral temporal poles and bilateral fusiform gyrri \( (P < 0.001 \) uncorrected), but these differences did not survive FWE correction.

Fluorodeoxyglucose: region of interest comparisons

Compared to normal controls, FDG uptake was decreased in early-onset Alzheimer’s disease throughout frontal,
temporoparietal and occipital regions of interest, while late-onset patients showed lower FDG than normal controls in left posterior cingulate, left lateral temporal cortex and left temporal pole (Table 4). In direct patient contrasts, early-onset Alzheimer’s disease showed lower glucose metabolism in bilateral temporoparietal and occipital regions of interest, while there were no regions of relative decreased metabolism in late-onset Alzheimer’s disease. Following atrophy correction, early-onset Alzheimer’s disease patients continued to show lower metabolism than late-onset patients in left posterior cingulate, while there were again no regions of relative hypometabolism in late-onset Alzheimer’s disease (Table 4). Scatter-plots of FDG uptake in precuneus (pre- and post-atrophy correction) are shown in Fig. 2B.

**Pittsburgh compound-B and fluorodeoxyglucose comparisons, excluding Pittsburgh compound-B-negative subjects**

A number of Alzheimer’s disease patients in our cohort showed low PIB index values, particularly in the late-onset group (Fig. 2A). We wanted to ensure that comparisons between early-onset and late-onset Alzheimer’s disease were not biased by including ‘PIB-negative’ subjects in whom the diagnosis of Alzheimer’s disease is questioned (Engler et al., 2006). We therefore classified all Alzheimer’s disease subjects as ‘PIB-positive’ or ‘PIB-negative’ based on a visual read by an experienced PET investigator (WJJ) blinded to clinical diagnosis (Rabinovici et al., 2007). A ‘PIB-positive’ scan was defined as a distribution volume ratio image in which PIB uptake was greater in cortex or striatum than in white matter. Voxel-wise and region of interest analyses for PIB and FDG were then repeated after excluding all ‘PIB-negative’ Alzheimer’s disease subjects.

Based on visual read, all 21 early-onset Alzheimer’s disease subjects were classified ‘PIB-positive’, while four of 18 late-onset subjects were classified ‘PIB-negative’. The four visually negative scans corresponded to the four subjects with the lowest PIB index values in the late-onset group (Fig. 2A and B, no atrophy correction). Excluding these four, subjects had a minor effect on PIB comparisons between early-onset and late-onset Alzheimer’s disease. Prior to atrophy correction, late-onset patients showed higher PIB binding than early-onset patients in a 36 voxel cluster in the left superior parietal lobule \( P_{(\text{FWE-corr})} < 0.05 \) on voxel-wise analysis, and in bilateral precuneus and lateral parietal cortex on region of interest analysis \( P<0.05 \), Supplementary Table 1). However, these differences were not significant following atrophy correction. There were no regions of greater PIB uptake in early-onset Alzheimer’s disease compared to late-onset Alzheimer’s disease on voxel-wise or region of interest comparisons (Supplementary Table 1).

FDG contrasts also did not change appreciably after excluding PIB-negative Alzheimer’s disease subjects. Without atrophy correction, early-onset patients showed lower glucose metabolism than late-onset patients in bilateral inferior parietal lobule, left precuneus, right superior parietal lobule, right angular gyrus and right middle
occipital gyrus on voxel-wise analysis \(P_{\text{FWE-corr}}<0.05\), and in bilateral posterior cingulate, precuneus, lateral temporoparietal and occipital cortices on region of interest analysis \(P<0.05\), Supplementary Table 2). There were no regions of relatively greater hypometabolism in late-onset Alzheimer’s disease. Following atrophy correction, early-onset patients showed hypometabolism in bilateral precuneus and right angular gyrus \(P_{\text{FWE-corr}}<0.05\) on voxel-wise analysis, while late-onset patients showed lower glucose metabolism in left temporal pole on voxel-wise comparison \(P_{\text{FWE-corr}}<0.05\), and in bilateral temporal poles on region of interest analysis \(P<0.05\), Supplementary Table 2).

### Relationship between age-at-onset, Pittsburgh compound-B and fluorodeoxyglucose

Graphs of age-at-onset as a continuous (rather than dichotomous) variable versus PIB and FDG suggest a positive linear relationship with glucose metabolism (in temporoparietal regions) but no relationship with global or regional amyloid burden (Fig. 4A and B). To assess this further, we performed a series of multi-linear regressions that included age-at-onset, education and MMSE as independent variables, and regional PIB or FDG uptake as the dependent variable. Standardized correlation coefficients relating age-at-onset to regional PIB and FDG are shown in Table 5. There was no correlation between age-at-onset and PIB in any regions. In contrast, age-at-onset was positively correlated with FDG in precuneus, lateral parietal and occipital cortices. MMSE was positively correlated with FDG in precuneus \(\beta=0.36, P=0.02\), posterior cingulate \(\beta=0.45, P=0.007\), lateral parietal \(\beta=0.44, P=0.003\) and lateral temporal cortices \(\beta=0.42, P=0.02\), but did not correlate with PIB in any region. In this highly educated cohort (Table 1), we did not find correlations between education and PIB or FDG in any region. Following atrophy correction, we again did not find a correlation between age-at-onset and PIB in any regions. Positive correlations between age and FDG were weakened [trends in precuneus \(\beta=0.38, P=0.07\) and posterior cingulate \(\beta=0.35, P=0.08\)], while significant negative correlations appeared in hippocampus \(\beta=-0.50, P=0.02\) and temporal pole \(\beta=-0.51, P=0.01\).

### Effect of apolipoprotein E genotype on Pittsburgh compound-B and fluorodeoxyglucose

Apolipoprotein E genotype has been previously reported to affect both PIB binding and glucose metabolism in Alzheimer’s disease, with the E4 genotype associated with higher amyloid load and...
lower glucose metabolism (Drzezga et al., 2005, 2009). ApoE is also a major risk factor for developing Alzheimer’s disease at an early age (Farrer et al., 1997). We thus investigated the relationships between ApoE genotype, PIB and FDG in our Alzheimer’s disease cohort. Patients were dichotomized as E4 carriers (with one or two E4 alleles) and non-carriers for all analyses. Results are presented in Supplementary Table 3.

Prior to atrophy correction we found a surprising trend for lower global and regional amyloid load in ApoE4 carriers compared to non-carriers, though this was only significant in lateral temporal cortex ($P = 0.04$). Following atrophy correction there were no significant differences in PIB binding between patients with and without the E4 allele. As expected, patients with an E4 allele showed lower glucose metabolism than non-carriers in posterior cortical regions (Supplementary Table 3), with significant differences found (following atrophy-correction only) in posterior cingulate ($P = 0.02$) and a trend found in precuneus ($P = 0.06$).

**Figure 4** Relationships between age-at-onset, PIB and FDG. (A) and (B) demonstrate the unadjusted relationships between age-at-onset (as a continuous variable) and (A) PIB or (B) FDG. Regression lines have been added to best fit the data. (C) Plots PIB (x axis) versus FDG (y axis) in precuneus. Reference lines denoting one standard deviation above normal controls mean for PIB (vertical line), and one standard deviation below the normal controls mean for FDG (horizontal line) have been added to divide the data into quadrants. See text for discussion. DVR = distribution volume ratio; EO = early-onset; LO = late-onset; AD = Alzheimer’s disease; CONT = controls.

**Effect of age on the local relationship between Pittsburgh compound-B and fluorodeoxyglucose**

The relationship between amyloid load and glucose metabolism in precuneus is demonstrated in Fig. 4C. Precuneus is chosen for illustrative purposes, with similar data scatter found in posterior cingulate, lateral parietal and occipital cortices. The graph is divided into four quadrants, with lines denoting one standard deviation above the normal control mean for PIB, and one standard deviation below the normal control mean for FDG.

Nearly all normal controls fell into the low PIB/high FDG quadrant, though two fell into the high PIB/high FDG and one into the low PIB/low FDG quadrants. Three of the four PIB-negative patients with late-onset Alzheimer’s disease fell into the low PIB/low FDG quadrant. In the high PIB quadrants there was a clear divide based on age-of-onset, with most early-onset patients
falling into the high PIB/low FDG category, and most late-onset patients found in the high PIB/high FDG quadrant (Fig. 4C). At an individual level, early-onset patients tended to show more severe hypometabolism for a given amyloid burden than late-onset subjects. When all subjects were included we found a trend for a negative correlation between PIB and FDG in precuneus (r = −0.24, P = 0.06). However, when normal controls were excluded, we found no correlation between PIB and FDG in patients with Alzheimer’s disease when tested together (r = 0.11, P = 0.52) or separately as early-onset (r = −0.04, P = 0.87) or late-onset (r = 0.18, P = 0.51) Alzheimer’s disease.

To determine which factors may explain lower glucose metabolism in early-onset patients, we performed step-wise regressions in which regional FDG was the dependent variable. Candidate independent variables included age-at-onset and MMSE (based on our previous regressions, Table 5), and three new variables: regional PIB, ApoE genotype (dichotomized into E4 carriers and non-carriers), and an interaction term between age-at-onset and ApoE (to exclude an age-specific effect of ApoE on glucose metabolism) (Hirono et al., 2002). Regressions were performed in precuneus, lateral parietal and occipital cortices, regions in which we previously found an effect of age-at-onset on FDG (Table 5). The analysis was performed using the Statistical Package for the Social Sciences’ default step-wise regression settings ($P(F_{\text{enter}}) < 0.05$, $P(F_{\text{remove}}) \geq 0.10$).

Age-at-onset (β = 0.51, P = 0.002) and MMSE (β = 0.37, P = 0.02) predicted FDG in precuneus, while PIB (P = 0.52), ApoE (P = 0.34) and the interaction between age and ApoE (P = 0.40) were eliminated from the model. Similarly, age-at-onset (β = 0.44, P = 0.004) and MMSE (β = 0.47, P = 0.003) predicted FDG in lateral parietal cortex, while PIB (P = 0.72), ApoE (P = 0.75) and the interaction term (P = 0.86) were eliminated. Finally, age-at-onset (β = 0.49, P = 0.009) predicted occipital FDG, while MMSE (P = 0.18), PIB (P = 0.34), ApoE (P = 0.43) and the interaction term (P = 0.44) were excluded from the model.

Table 5 Results of multi-linear regressions

<table>
<thead>
<tr>
<th>Region</th>
<th>PIB (n = 39)</th>
<th>FDG (n = 34)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIB index</td>
<td>β = 0.08, P = 0.65</td>
<td>–</td>
</tr>
<tr>
<td>Frontal cortex</td>
<td>β = 0.08, P = 0.62</td>
<td>β = 0.02, P = 0.91</td>
</tr>
<tr>
<td>Precuneus</td>
<td>β = 0.15, P = 0.37</td>
<td>β = 0.48, P = 0.002</td>
</tr>
<tr>
<td>Posterior cingulate cortex</td>
<td>β = −0.10, P = 0.53</td>
<td>β = 0.26, P = 0.11</td>
</tr>
<tr>
<td>Lateral parietal cortex</td>
<td>β = 0.23, P = 0.17</td>
<td>β = 0.45, P = 0.002</td>
</tr>
<tr>
<td>Lateral temporal cortex</td>
<td>β = −0.03, P = 0.86</td>
<td>β = 0.21, P = 0.20</td>
</tr>
<tr>
<td>Medial temporal cortex</td>
<td>β = −0.19, P = 0.27</td>
<td>β = 0.14, P = 0.43</td>
</tr>
<tr>
<td>Occipital cortex</td>
<td>β = 0.01, P = 0.95</td>
<td>β = 0.37, P = 0.03</td>
</tr>
<tr>
<td>Temporal pole</td>
<td>β = −0.19, P = 0.26</td>
<td>β = −0.26, P = 0.14</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>β = −0.21, P = 0.22</td>
<td>β = −0.06, P = 0.74</td>
</tr>
<tr>
<td>Striatum</td>
<td>β = 0.13, P = 0.45</td>
<td>β = 0.12, P = 0.51</td>
</tr>
</tbody>
</table>

β values represent standardized correlation coefficients relating age-at-onset to PIB and FDG after co-varying MMSE and education. Significant correlations are in bold.

Discussion

In this study we applied PIB and FDG-PET to investigate in vivo the relationships between age-at-onset, fibrillar amyloid-β and glucose metabolism in Alzheimer’s disease. We hypothesized that patients with early-onset (age <65) disease would show increased cortical PIB binding compared to late-onset (age >65) patients, and that this would be accompanied by more severe temporalparietal hypometabolism in younger patients. Contrary to our hypothesis, we found no significant effect of age on the burden or distribution of PIB binding, either when directly comparing patients with early-onset and late-onset disease, or when studying age-at-onset as a continuous variable. In contrast, congruent with our a priori hypothesis, we found more severe metabolic deficits in temporoparietal and occipital cortices in patients with early-onset Alzheimer’s disease compared to matched late-onset patients. We further found that in a multi-linear model, glucose metabolism in posterior brain regions was positively correlated with age-at-onset and with degree of cognitive impairment (as measured by the MMSE), but not with amyloid burden or apoE genotype.

Current theories on the pathogenesis of early-onset Alzheimer’s disease have emphasized increased deposition of both amyloid-β and tau pathology as the primary mechanism for the greater functional and structural brain changes and more rapid decline seen in young patients. This hypothesis hinges on observations of greater pathologic burden in early-onset patients at the time of autopsy. In contrast, our finding of similar PIB binding regardless of age-at-onset suggests that increased amyloid-β deposition alone cannot explain the clinical differences between early and late-onset disease. Rather, our data are consistent with a model in which both early amyloid-β accumulation and increased vulnerability to pathology play critical roles in the pathogenesis of Alzheimer’s disease in young patients.

Amyloid-β plaques are relatively uncommon in cognitively normal individuals before the seventh decade of life (Mintun et al., 2006; Kok et al., 2009; Morris et al., 2009), and are strongly correlated with dementia in younger individuals (Savva et al., 2009). Indeed, all patients with early-onset Alzheimer’s disease in our study showed evidence of elevated PIB binding compared to a group of older normal controls, suggesting that early amyloid-β accumulation is an important initial pathogenic step in early-onset Alzheimer’s disease (Figs 1 and 2A). Early-onset patients further showed more severe metabolic deficits in posterior brain regions compared to late-onset patients faced with similar amyloid burden (Figs 3 and 4C). One interpretation of this result is that younger patients have an increased susceptibility to the neurotoxic effects of fibrillar amyloid-β. However, there are a number of alternative explanations. In this cross-sectional study we cannot rule out the possibility that duration of exposure to amyloid-β, rather than absolute amyloid-β burden, leads to more severe hypometabolism in early-onset Alzheimer’s disease. Longitudinal PIB studies suggest that amyloid aggregation reaches a relative plateau early in the disease course (perhaps even in the mild cognitive impairment stage or earlier), while glucose metabolism and brain volume decline in concordance with disease.
that is not imaged by PIB. For example, amyloid-b progression (Engler et al., 2006; Jack et al., 2009). It is thus possible that early-onset patients have a longer biological prodrome than late-onset patients, during which significant plaques are present without clinical symptoms, perhaps as a result of higher cognitive reserve in young brains. By the time symptoms are present, young patients have more widespread atrophy and hypometabolism than older patients, reflecting both the longer duration of exposure to amyloid-b and the more diffuse neuronal dysfunction necessary to cross the threshold into clinical dementia. However, this model would not account for a number of early-onset patients who show severe metabolic disruption in the face of a moderate amyloid burden, well below the typical ‘PIB saturation’ level (Fig. 4C).

It is also possible that differences between early-onset and late-onset Alzheimer’s disease may be explained by pathology that is not imaged by PIB. For example, amyloid-b oligomers, considered by many to be the most neurotoxic of all amyloid-b species (Mucke et al., 2000; Walsh and Selkoe, 2007), are not detected by PIB, which binds to amyloid-b aggregates in direct relation to their fibrillar content (Klunk et al., 2003). Though speculative, one might even consider whether the apparent increased pathogenicity of amyloid-b in early-onset disease may be related to ‘inefficient fibrillation’, with patients developing symptoms at a young age in part because of an inability to sequester highly toxic soluble amyloid-b oligomers effectively into relatively less toxic insoluble plaques. Further support for a potential ‘protective’ effect of fibrillar amyloid-b can be found in our voxel-wise analyses, where trends for higher amyloid were seen for each group in regions of relative metabolic sparing, namely anteromedial temporal cortex for early-onset Alzheimer’s disease and parietal cortex for late-onset Alzheimer’s disease (compare direct patient contrasts in Figs 1 and 3). However, differences in PIB binding in these regions did not meet our conservative significance threshold for voxel-wise comparisons, were not reproduced in region of interest data, and may also have been affected by differential atrophy patterns (greater parietal atrophy in early-onset Alzheimer’s disease and medial temporal atrophy in late-onset Alzheimer’s disease), even after applying partial volume correction. Therefore, we cannot confidently conclude that our data support an inverse association between amyloid burden and glucose metabolism in these regions.

Differences between early-onset and late-onset Alzheimer’s disease may also be explained by a differential burden of neurofibrillary tangles, which do not appreciably bind PIB at PET tracer concentrations (Lockhart et al., 2007; Ikonomovic et al., 2008). Indeed, tangle pathology correlates more strongly with age-at-onset than amyloid-b plaques in most post-mortem studies (Hansen et al., 1988; Nochlín et al., 1993; Berg et al., 1998; Marshall et al., 2007). Whether and how amyloid-b and tau aggregates are related in Alzheimer’s disease remains an area of active debate. Finally, clinical and metabolic differences may in part be explained by greater neurochemical deficits in younger patients that occur independently of cortical amyloid-b deposition (Bird et al., 1983; Rossor et al., 1984).

The apolipoprotein E4 allele is the strongest known genetic risk factor for both early-onset and late-onset Alzheimer’s disease (Farrer et al., 1997), and was present in 74% of early-onset and 50% of late-onset Alzheimer’s disease patients compared to 30% of normal controls in our study (P=0.02). ApoE4 directly promotes amyloid-b fibrillation, but may also contribute to Alzheimer’s disease pathogenesis through a variety of other mechanisms, including promotion of tau phosphorylation, direct mitochondrial toxicity, decreased cognitive reserve and impaired neuronal response to injury (Mahley et al., 2006). The ApoE4 allele decreases age-at-onset in Alzheimer’s disease in a dose-dependant fashion (Farrer et al., 1997; Meyer et al., 1998). Recent autopsy and PIB studies of cognitively normal individuals demonstrate that ApoE4 is strongly associated with amyloid-b deposits between ages 40 and 90 years, and plaque pathology in mid-life is seen almost exclusively in ApoE4 carriers (Kok et al., 2009; Morris et al., 2009; Reiman et al., 2009). Thus, ApoE4 may contribute to the early-onset of Alzheimer’s disease by predisposing carriers to overproduce and deposit amyloid-b at a young age. We did not find evidence for increased PIB binding in Alzheimer’s disease patients with an ApoE4 genotype, and actually found a trend for lower binding in E4 carriers in temporoparietal cortex, though this was ameliorated by atrophy correction (Supplementary Table 3). Our study thus adds to the ambiguity in the literature, where increased amyloid deposition in ApoE4 carriers has been found (on autopsy or with PIB-PET) in some studies of Alzheimer’s disease (Gomez-Isla et al., 1996; Drzegza et al., 2009) but not others (Berg et al., 1998; Rowe et al., 2007). Similar to previous reports (Drzegza et al., 2005), we found lower glucose metabolism in precuneus/posterior cingulate cortex in Alzheimer’s disease patients carrying an ApoE4 allele (Supplementary Table 3).

However, step-wise regression did not identify ApoE genotype as a significant predictor of glucose metabolism after controlling for age-at-onset and MMSE. Thus, our findings suggest that ApoE4 acts as a risk factor for early-onset Alzheimer’s disease primarily by predisposing towards early accumulation of fibrillar amyloid-b, rather than by increasing the overall burden of amyloid-b or by accentuating the metabolic response to pathology.

Our data highlight the precuneus as one of the most metabolically vulnerable regions in early-onset Alzheimer’s disease (Figs 2–4, Tables 4 and 5). The precuneus/posterior cingulate complex is a central cortical ‘hub’ that is structurally and functionally interconnected with other heteromodal association regions in lateral parietal, temporal and prefrontal cortices (Buckner et al., 2009; Greicius et al., 2009). The precuneus/posterior cingulate appears to play a central role in episodic memory processes, with functional MRI experiments demonstrating deactivation during successful stimulus encoding, and activation during episodic memory retrieval (Daseelaar et al., 2004; Wagner et al., 2005; Svoboda et al., 2006). PIB-PET studies of ageing have identified the precuneus/posterior cingulate as one of the earliest sites of amyloid-b deposition (Mintun et al., 2006; Mormino et al., 2009; Reiman et al., 2009), with elevated PIB binding associated with decreased precuneus/posterior cingulate connectivity at rest (Hedden et al., 2009; Sheline et al., 2009), and aberrantly increased precuneus/posterior cingulate activation during encoding (Sterling et al., 2009). The precuneus/posterior cingulate is also a site of early metabolic disruption in Alzheimer’s disease (Minoshima et al., 1997), with hypometabolism apparent even in asymptomatic ApoE4 gene carriers (Reiman et al., 1996).
Buckner and others have observed that amyloid-β pathology and functional and structural changes in Alzheimer’s disease all converge in the precuneus/posterior cingulate, and have hypothesized that this may be related to high neuronal activity in the precuneus/posterior cingulate due to its inter-connectivity and frequent fluctuation between an activated and deactivated state. This elevated level of activity may predispose the precuneus/posterior cingulate to early amyloid-β aggregation (extrapolating from in vitro data that link amyloid-β release to synaptic activity) (Cirrito et al., 2005) and render it more vulnerable to amyloid-β neurotoxicity due to high metabolic stress (Buckner et al., 2005, 2009; Liang et al., 2008). In our study patients with early-onset and late-onset Alzheimer’s disease showed comparably high levels of amyloid-β deposition in the precuneus/posterior cingulate (Fig. 1), but hypometabolism was markedly more severe in early-onset patients, suggesting that metabolic vulnerability in the precuneus/posterior cingulate may be particularly relevant to the pathogenesis of Alzheimer’s disease in young patients (Figs 3 and 4).

Further studies are needed to identify factors that may increase metabolic susceptibility in early-onset Alzheimer’s disease, such as potential genetic variations in key metabolic enzymes and pathways (Li et al., 2004; Liang et al., 2008).

Four clinically diagnosed Alzheimer’s disease patients in our study, all with late-onset disease, did not show evidence of elevated PIB binding (Fig. 2A). Patients with PIB-negative Alzheimer’s disease have been reported in previous series, and are likely to represent a combination of clinical misdiagnosis and true ‘false-negatives’ related to failure of PIB to detect amyloid-β pathology (Klunk et al., 2004; Edison et al., 2007; Leinonen et al., 2008; Rosen et al., 2009). Clinically, all four patients met research criteria for Alzheimer’s disease with a progressive amnestic disorder, though one had very mild symptoms and deficits on cognitive testing, one patient had a prominent aphasias and another developed significant behavioural disturbances, albeit in the moderate stage of dementia. The fourth patient was felt to have a typical Alzheimer’s disease clinical course. Excluding these four patients did not appreciably alter the results of PIB or FDG comparisons between early-onset and late-onset Alzheimer’s disease (Supplementary Tables 1 and 2).

Our finding of similar PIB binding in patients with early-onset and late-onset Alzheimer’s disease deviates from reports of greater amyloid-β plaque burden at autopsy in early-onset disease (Mann et al., 1984; Hansen et al., 1988; Nochlín et al., 1993; Bigio et al., 2002; Ho et al., 2002; Marshall et al., 2007). This incongruity may be accounted for by significant methodological differences between our study and previous post-mortem work. By virtue of applying an in vivo technique, we were able to select subjects in the mild-to-moderate early-onset Alzheimer’s disease, while most autopsies were conducted in patients in the late stages of dementia. We were able to match patients closely for dementia severity, while in autopsy studies older patients are more likely to have died at an earlier disease stage (Berg et al., 1998; Ho et al., 2002). We also excluded patients with evidence of co-morbid pathology, including clinical features of another dementia syndrome or significant vascular lesions on MRI. To our knowledge previous autopsy studies did not apply such strict exclusion criteria, again introducing potential bias since late-onset patients are more likely to suffer from co-pathology (Schneider et al., 2007). To measure amyloid-β burden, post-mortem studies typically applied semi-quantitative measures (Nochlín et al., 1993; Bigio et al., 2002) and rarely differentiated between different plaque morphologies (Ho et al., 2002; Marshall et al., 2007). PIB binding, on the other hand, quantifies amyloid-β pathology in direct relation to its fibrillar content, with stronger binding to more mature plaques. Our results are most comparable to the those of Berg and colleagues, who found that total plaques correlated negatively with age throughout cortex and hippocampus, but found more restricted correlations when the analysis was limited to cored plaques with high fibrillar content (Berg et al., 1998). Finally, the greater degree of cortical atrophy in early-onset patients would tend to confound PIB and autopsy comparisons of early-onset and late-onset Alzheimer’s disease in opposite directions, decreasing PET signal but artificially increasing lesion density in pathologic specimens from younger patients. Notably, our results are consistent with another in vivo biomarker study that found no difference in cerebrospinal fluid levels of amyloid-β₄₀–₄₂, tau and phosphorylated tau in patients with mild-to-moderate early-onset and late-onset Alzheimer’s disease (Bouwman et al., 2009). Thus, both biomarker studies comparing early-onset and late-onset Alzheimer’s disease raise doubts about whether previous post-mortem findings truly reflect the effects of age-at-onset on Alzheimer’s disease pathogenesis in vivo.

Our study has limitations. PIB-PET is a relatively new technique, and although early clinicopathologic studies suggest that in vivo PIB signal correlates strongly with post-mortem measures of amyloid-β (Bacskai et al., 2007; Ikonomovic et al., 2008), the limitations of PIB have not yet been fully defined. For example, two recent case reports suggest that PIB may have reduced binding affinity for fibrillar amyloid in rare individuals (Leinonen et al., 2008; Rosen et al., 2009). We attempted to control for two possible confounders related to the technique. We confirmed that PIB binding did not differ between early-onset and late-onset patients in the cerebellum, which is the reference tissue used to normalize PIB counts across subjects. To account for PET signal loss due to atrophy, we confirmed the results of all PET analyses with atrophy-corrected data, though this analysis was limited to the subset of patients that had a recent research-protocol MRI. However, we cannot exclude the possibility that currently unknown limitations of PIB (e.g. a ceiling effect on tracer binding, age-related effects on binding site availability, etc.) may have influenced our results. Finally, although our study is one of the largest PIB studies in Alzheimer’s disease to date, the size of our early-onset and late-onset groups was modest. However, our sample size was adequate to detect significant effects of age on FDG uptake, and is comparable to many autopsy studies comparing early-onset and late-onset Alzheimer’s disease (Mann et al., 1984; Hansen et al., 1988; Nochlín et al., 1993; Bigio et al., 2002; Marshall et al., 2007).

In summary, using PIB and FDG-PET we found similar amyloid burden but greater posterior cortical metabolic deficits in patients with early-onset Alzheimer’s disease compared to late-onset patients matched for disease severity. Our results suggest that both early amyloid-β accumulation and increased vulnerability to pathology act in complement to cause Alzheimer’s disease in
young patients. Both amyloid-β production and susceptibility to pathology are likely to be governed by a multitude of genetic and environmental factors that interact with each other, modulating an individual’s risk of developing clinical Alzheimer’s disease over the course of the life span.

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Supplementary material

Supplementary material is available at Brain online.

References


