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PROTEOME-BASED PLASMA MARKERS OF BRAIN AMYLOID BETA DEPOSITION IN NON-DEMENTED OLDER INDIVIDUALS

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Abstract

Blood-based markers reflecting core pathological features of Alzheimer's disease (AD) in pre-symptomatic individuals are likely to accelerate the development of disease-modifying treatments. Our aim was to discover plasma proteins associated with brain amyloid-beta (A β) burden in non-demented older individuals. We performed discovery-phase experiments using two dimensional gel electrophoresis (2DGE) and mass spectrometry-based proteomic analysis of plasma in combination with ¹¹C-PiB PET imaging of the brain in samples collected 10 years prior to the PET scans. Confirmatory studies used ELISA assays in a separate set of blood samples obtained within a year of the PET scans. We observed that a panel of 18 2DGE plasma protein spots effectively discriminated between individuals with high and low brain A β . Mass spectrometry identified these proteins, many of which have established roles in A β clearance, including a strong signal from apolipoprotein-E (apoE). In validation-phase studies, we observed a strong association between plasma apoE concentration and A β burden in the medial temporal lobe. Targeted voxel-based analysis localized this association to the hippocampus and entorhinal cortex. APOE ϵ 4 carriers also showed greater A β levels in several brain regions relative to ϵ 4 non-carriers. These results suggest that both peripheral concentration of apoE protein and APOE genotype are related to early neuropathological changes in brain regions vulnerable to AD pathology even in the non-demented elderly. Our strategy combining proteomics with *in vivo* brain amyloid imaging holds promise for the discovery of biologically relevant peripheral markers in those at risk for AD.

Keywords

Alzheimer's disease; proteomics; biomarker; amyloid beta; brain; plasma

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Introduction

Amyloid beta (A β) deposition within the brain is a key event in Alzheimer's disease (AD) pathogenesis, preceding the onset of symptoms [1–2]. Several emerging treatments for AD target brain A β deposition [3]. Even in non-demented elderly, A β deposition is linked to longitudinal cognitive decline [4–5]. The development of radioligands to visualize brain amyloid has helped delineate the pattern and temporal profile of its deposition in at-risk individuals [6].

However, imaging modalities such as ^{11}C -PiB PET are not widely available and are unlikely to be employed in the routine screening of individuals at risk of AD. Although molecular imaging may be useful in early stage clinical trials, it may be too costly for use as an outcome measure in all research participants in phase III trials or in guiding prescribing practice. Other markers of pathology include CSF concentrations of A β and tau, which have a high sensitivity for detection of AD [7–8]. However, lumbar puncture causes patient discomfort and CSF markers may be impractical for repeated sampling. Minimally invasive peripheral markers associated with brain amyloid deposition would therefore represent a significant advance, accelerating the development of newer therapies.

Some studies suggest that blood-based signatures associated with the core neuropathological features of AD are detectable and may be sensitive to both disease severity and progression [9] [10] [11–13]. These approaches include unbiased proteomic methods, array-based and single-analyte analyses of candidate proteins as well as peripheral gene expression signatures in AD. We have previously combined unbiased mass spectrometry-based proteomic analyses of plasma with ^1H -magnetic resonance spectroscopy to identify plasma biomarkers associated with hippocampal metabolite abnormalities in AD [14]. More recently, we have combined plasma proteomics with structural magnetic resonance imaging (MRI) and with ^{11}C -PiB PET imaging to show that plasma clusterin (also known as apolipoprotein-J/apoJ) concentration is associated with brain atrophy in AD and amyloid deposition in non-demented older individuals [15]. The aim of the present study was to identify a blood-based signature of *in vivo* brain amyloid deposition through proteomic analysis of plasma in non-demented older adults.

Methods

Subjects and samples

Blood samples were obtained from participants in the neuroimaging substudy of the Baltimore Longitudinal Study of Aging (BLSA) [16]. We excluded individuals with clinical strokes and those meeting consensus criteria (NINCDS-ADRDA) for AD.

In the initial two dimensional gel electrophoresis (2DGE) experiments, the primary aim was to identify proteins associated with brain amyloid burden. We used plasma samples collected 10 years prior to the ^{11}C -PiB PET scans (N=57). In subsequent experiments, where the principal aim was to validate target proteins using quantitative methods in an independent set of samples, ELISA assays were performed in plasma samples obtained within ± 1 year of the ^{11}C -PiB PET scans. These samples were available in 42 of the 57 participants (figure-1). This study was approved by the local institutional review board. All participants provided written informed consent.

2DGE experiments to discover plasma proteins associated with brain amyloid burden

We first performed a discovery-phase proteomics experiment using 2DGE and liquid chromatography tandem mass spectrometry (LC/MS/MS), as previously described [17], with mean cortical distribution volume ratio (DVR) of PiB deposition as the independent

variable. Mean cortical DVR data were available in 57 participants (table-1). We divided the mean cortical DVR data into tertiles and categorized values into 'high' (> 1.245), 'intermediate' (≤ 1.245 and ≥ 1.039) and 'low' (< 1.039) groups.

Gels were analyzed using SameSpots software (Non-linear Dynamics). The optical density of each spot was normalized to the total optical density of all spots on a gel. Approximately 1700 silver stained protein spots were matched between the 'high' (N=18) and 'low' (N=19) DVR groups. 2DGE data from one subject was excluded due to poor quality of silver-staining.

To identify the protein content within 2D gel spots, peptides were analyzed by LC/MS/MS as previously reported [17].

APOE genotype analysis

Of the 57 individuals who underwent ^{11}C -PiB PET imaging, APOE genotype data were available in 54 participants (37 APOE $\epsilon 4$ non-carriers and 17 APOE $\epsilon 4$ carriers). Of the 42 individuals whose plasma samples were used for the apoE ELISA assays, APOE genotype data were available in 39 (10 $\epsilon 4$ carriers and 29 $\epsilon 4$ non-carriers).

APOE genotyping was performed on DNA extracted from fresh blood by restriction enzyme isoform genotyping [18]. The two groups were APOE $\epsilon 4$ carriers (N=17) (both heterozygous; N=13 and homozygous; N=4 i.e. $\epsilon 4/\epsilon 4$) and non-carriers (N=37) (table-3).

Plasma samples and apoE ELISA assays—Plasma samples were collected after overnight fasting and stored at -80°C prior to use. Concentration of apoE protein was assayed by a commercially available sandwich ELISA method that recognized all three isoforms of apoE (Medical and Biological Laboratories Ltd, Japan). Plasma samples were diluted 1:500 in assay diluent according to the manufacturer's instructions. Samples were run in duplicate (coefficient of variation; $< 3\%$) and the mean absorbance value calculated. Sigmaplot v.11 systat software was used to calculate the standard curve for apoE concentration ($\mu\text{g/ml}$).

Statistical analyses of proteomic data

SIMCA-P (v11.5 Umetrics, Umeå, Sweden) was used for multivariate partial least squares (PLS) regression analysis of 2DGE data. PLS regression is a data reduction method especially suited to datasets where the predictor variables greatly outnumber the observations, or are themselves correlated with each other [19]. Spot data were scaled to unit-variance and $\log_{10}$ transformed where appropriate. PLS discriminant analysis (PLS-DA) was used to build models wherein integrated optical densities of silver-stained spots on 2DGE gels were used to discriminate samples in the 'high' and 'low' DVR groups. The PLS model took the scaled spot volumes as predictor variables and mean cortical DVR (high versus low) as the response variables.

^{11}C -PiB studies—Dynamic ^{11}C -PiB PET studies were performed as described previously [20]. PET scanning started immediately after an intravenous bolus injection of 540.2 ± 33.3 MBq (14.6 ± 0.9 mCi) of ^{11}C -PiB with a specific activity of 208.68 ± 111 GBq/ μmol (range, 36.26–540.94 GBq/ μmol).

MRI-based Region-of-Interest (ROI) definition—Spoiled gradient-recalled MRI scans were co-registered to the mean of the first 20-min dynamic PET images using the mutual information method in the Statistical Parametric Mapping software (SPM 2; Wellcome Department of Imaging Neuroscience, London, U.K.). Besides the cerebellum, which was

used as a reference region, 15 ROIs (caudate, putamen, thalamus, lateral temporal, medial temporal, orbital frontal, prefrontal, occipital, superior frontal, parietal, anterior cingulate, posterior cingulate, pons, midbrain, and white matter) were manually drawn on the co-registered MR images [21].

Quantification of distribution volume ratios (DVRs)—The DVR values for ROIs were estimated by simultaneous fitting of a reference-tissue model to the 15 measured ROI time–activity curves using linear regression and spatial constraint [22]. The mean cortical DVR was calculated by averaging values from orbitofrontal, prefrontal, superior frontal, parietal, lateral temporal, occipital, and anterior and posterior cingulate regions.

Voxel-based correlation analysis of ^{11}C -PiB images—SPM5 (Statistical Parametric Mapping 5; Wellcome Department of Imaging Neuroscience, London, U.K.) was used to investigate the association between plasma apoE protein concentration and ^{11}C -PiB retention. Based on results from the analysis of associations between plasma apoE concentration and the PiB DVR values for selected ROIs, voxel-based analysis was performed using a targeted search of regions showing significant associations. Regional definitions in these analyses used the WFU Pick-Atlas [23].

Results

Identification of 2DGE spots discriminating ‘high’ and ‘low’ PiB DVR groups

A PLS-DA model with three components, consisting of the integrated optical densities of all matched spots was first fitted to the ‘high’ and ‘low’ mean cortical DVR groups. The spots were then ranked according to their regression coefficients in the PLS-DA model and the top 100 spots i.e. those with the highest regression coefficients and thereby contributing the most to the discriminant model were selected for further analysis. Visual inspection of these 100 spots was carried out in every gel and 18 spots were judged to be well-defined, discrete and present in a majority of the gels. A single-component PLS-DA model with the integrated optical densities of these 18 spots was then fitted to the DVR data. This PLS-DA model yielded a robust discrimination between the ‘high’ and ‘low’ DVR groups, accounting for nearly 60% of variance in mean cortical DVR ($R^2Y=0.593$ and represents explained variance in the outcome variable) (figure-2A) ($R^2X=0.189$ and represents explained variance in the predictor variables contributing to the model; $Q^2=0.431$ and represents the cross-validated explained variance in the outcome variable).

In order to address the issue of overfitting which is commonly encountered when high-dimensional proteomic/transcriptomic data are used to predict a relatively small number of outcome events [24], we tested the validity of the PLS-DA model by adopting a permutation response testing procedure. In this procedure, the positions of the Y data in relation to their corresponding rows in the X matrix are randomized (100 separate row permutations were performed) and the effect of this randomization on the R^2Y and Q^2 values is evaluated. Randomization of the Y data considerably reduced R^2Y and Q^2 (figure-2B) in every model in comparison to the original model. Furthermore, the results suggest that the likelihood of deriving a model with comparable predictive ability by chance is less than 1%, providing further evidence for the validity of the PLS-DA model for predicting mean cortical DVR.

We then identified the proteins constituting the 18 silver-stained 2DGE spots by LC/MS/MS. We successfully identified 17 of the 18 spots and determined that these spots represented six distinct proteins i.e. apolipoprotein-E (apoE; six spots), complement-C3 (four spots), albumin (three spots), plasminogen (two spots), haptoglobin and IgG-C chain region (figure-2C) (table-2).

Selection of apolipoprotein-E (apoE) as a target for validation-phase studies

The discovery-phase proteomic experiments, designed to identify a peripheral signature of brain amyloid burden without *a priori* assumptions on the nature of such proteins, uncovered a prominent signal originating from plasma apoE. Given the large body of evidence implicating both the APOE gene and apoE protein in AD risk and A β metabolism [25–26], we selected this protein as a target for subsequent validation studies. In these experiments we measured apoE concentration in a separate set of plasma samples, obtained on average 10 years later, to test its association with brain amyloid burden. We also examined the association of APOE genotype with brain amyloid burden (figure-1).

Sample characteristics of APOE ϵ 4 carriers and non-carriers

APOE ϵ 4 carriers and non-carriers did not differ significantly in age, sex, years of education, family history of dementia or mean score on the Mini-Mental State Examination (MMSE) (table-3). We assessed memory by the California Verbal Learning Test (CVLT) and the Benton Visual Retention Test (BVRT). Visuospatial performance was tested by the card rotation test. Performance in these cognitive domains did not differ significantly between APOE ϵ 4 carriers and non-carriers.

APOE genotype and PiB DVR—We observed widespread and statistically significant increases in amyloid burden in APOE ϵ 4 carriers relative to non-carriers (one-tailed non-parametric Wilcoxon two-sample test). These differences were observed in mean cortical DVR ($p=0.005$) as well as in the orbitofrontal, prefrontal ($p=0.01$), superior frontal cortex ($p=0.007$), anterior ($p=0.01$) and posterior cingulate cortex ($p=0.003$) and in the parietal ($p=0.005$), medial ($p=0.05$) and lateral temporal ($p=0.005$) as well as occipital cortices ($p=0.04$) (figure-3).

APOE genotype and plasma concentration of apoE protein—Mean plasma apoE protein concentration was significantly higher in APOE ϵ 4 carriers ($N = 10$; $175.4 \pm 35.7 \mu\text{g/ml}$) relative to non-carriers ($N=29$; $146.8 \pm 26.9 \mu\text{g/ml}$) ($p=0.004$).

Plasma concentration of apoE protein and brain amyloid burden—To investigate associations between plasma apoE protein levels and PiB retention, we selected, *a priori*, specific regions for testing in addition to mean cortical DVR in order to reduce multiple comparisons and the risk of type-I error. The selection of these ROIs was based on prior evidence suggesting:

- a. Predilection for amyloid deposition as measured by ^{11}C -PiB-PET (prefrontal, parietal and posterior cingulate cortex) [27].
- b. Hypometabolism in cognitively normal older individuals at risk for AD (lateral temporal, prefrontal and posterior cingulate cortex) [28–29].
- c. Vulnerability to early atrophic changes in MCI and AD (medial temporal cortex) [30].

Partial correlation analysis, adjusting for age, sex and years of education, was performed between plasma apoE concentration and amyloid burden in the ROIs specified above with Bonferroni correction for multiple comparisons ($p<0.006$). We found a significant correlation between amyloid burden in the medial temporal cortex and plasma apoE concentration ($N=42$, $r=0.46$; $p=0.003$). This association appeared to be driven primarily by APOE ϵ 4 non-carriers ($r=0.52$; $p=0.007$).

Based on the observation of a significant association between plasma apoE protein concentration and PiB retention in the medial temporal cortex ROI, we performed a targeted

voxel-based analysis using the region defined by the WFU Pick-Atlas [23]. Local associations within the medial temporal lobe were investigated using the SPM2 multiple regression module. These results indicated that higher plasma apoE protein concentrations were associated with greater PiB retention in the hippocampus bilaterally (right hippocampus; $p=0.007$, left hippocampus; $p=0.01$). A similar association was also observed within the right parahippocampal gyrus ($p=0.008$) and entorhinal cortex (ERC) ($p=0.008$) (figure-4 and table-4).

Discussion

Our principal objective in this study was firstly, to identify and validate a peripheral signature of brain amyloid burden in non-demented older individuals. We first combined proteomic analysis of plasma with ^{11}C -PiB PET imaging of the brain in an exploratory study and observed a prominent plasma signal from apoE protein associated with brain amyloid burden in samples collected 10 years preceding the ^{11}C -PiB PET scans. To confirm this finding, we performed quantitative ELISA assays of plasma apoE concentration in a separate set of blood samples obtained within a year of the corresponding ^{11}C -PiB PET scans. Confirmation of discovery-phase findings in an independent test set is a key requirement of a robust biomarker study [31]. Moreover, by using samples obtained recently and concurrent to the ^{11}C -PiB PET scans, we sought to control for the possibility that protein instability/deterioration in archived samples used in the 2DGE experiments may have confounded the results. A related objective was also to confirm recently reported findings on the relationship between APOE genotype and brain amyloid burden during normal aging [32–33].

Our previous studies combining proteomics with imaging endophenotypes of AD pathology in a distinct dataset have shown that plasma concentration of complement factor-H (CFH) and alpha2 macroglobulin (A2M) are associated with hippocampal metabolite abnormalities in patients with AD [14]. More recently, we showed that plasma concentration of clusterin, also known as apolipoprotein-J (apoJ), is associated with brain amyloid burden in non-demented older individuals [15]. This approach, employing imaging endophenotypes in biomarker studies overcomes certain inherent limitations of the standard case-control design, wherein the primary endpoint is binary discrimination between patients and healthy controls. This strategy ignores the clinical heterogeneity in patients with established AD as well as significant neuropathology in subjects assessed to be healthy controls [34–35] and may not yield peripheral markers associated with the core pathological features of AD.

By applying multivariate PLS regression in a discriminant analysis in our 2DGE experiments, we examined whether the integrated optical densities of silver-stained protein spots on 2DGE gels could discriminate between individuals with the highest and lowest tertiles of mean cortical DVR, chosen as a metric of global brain A β deposition. We derived a PLS-DA model consisting of 18 2DGE protein spots that could robustly discriminate between these two groups of subjects and confirmed the validity of the model by permutation response testing.

Of the 17 protein spots identified using LC/MS/MS, six were identified as apoE with the other proteins being haptoglobin, plasminogen, complement-C3, albumin and IgG. It is likely that both alternative splicing and post-translational modifications are responsible for altered electrophoretic mobilities of protein spots in 2DGE gels, accounting for multiple spots containing the same protein. Given that our initial discovery-phase study was undertaken without any *a priori* hypotheses on the nature and identity of candidate amyloid biomarkers, it was striking that we detected a strong signal from proteins with well established roles in amyloid clearance. The role of apoE as an amyloid chaperone protein

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closely associated with AD neuropathology is well-known [26, 36]. In addition, haptoglobin has been shown to both suppress amyloid fibril formation as well as protect neuroblastoma cells from A β -mediated toxicity. [37] [38], Plasminogen is a member of the tissue plasminogen activator (tPA)-plasmin system with an established role in A β degradation [39]. Some recent studies also suggest that the tPA-plasminogen proteolytic system may be a viable target of disease-modifying treatments for AD [40]. Our decision to select apoE as the target for subsequent validation-phase experiments was based both on the relatively large contribution from this protein to the PLS-DA model for brain amyloid, and the substantial body of evidence implicating both the APOE gene and apoE protein in several aspects of AD pathogenesis (18, 19, 21, 22, 29, [41], 37). In this context, it is important to note that while the relationship between APOE genotype and AD risk is well established, the association between variation in plasma apoE protein levels and AD pathogenesis is less clear. A recent study by van Vliet and colleagues suggests that lower plasma apoE levels in middle age is associated with greater risk for AD even after controlling for differences in APOE genotype [41].

Consistent with recent reports [32–33], we first confirmed the association between APOE genotype and brain A β deposition, observing widespread increases in fibrillar amyloid burden in APOE ϵ 4 carriers compared to non-carriers.

We then confirmed findings from our proteomic analysis suggesting that the plasma concentration of apoE protein is a marker of *in vivo* brain amyloid burden by showing that plasma apoE protein concentration is associated with extent of amyloid deposition in the medial temporal lobe, the site of early neuropathological changes in AD [42]. We also examined differences in plasma apoE protein concentration between APOE ϵ 4 carriers and non-carriers. We observed a higher concentration of plasma apoE protein in APOE ϵ 4 carriers. While previous studies have established that apoE protein concentration in plasma is highest in ϵ 2, intermediate in ϵ 3 and lowest in ϵ 4 carriers [43], a few studies have found higher plasma concentration of apoE protein in ϵ 4 carriers relative to non-carriers [44]. APOE genotype is only one of several factors, including APOE gene promoter polymorphisms, diet, age and sex that are likely to influence plasma apoE protein concentration [45–46].

In analyzing associations between plasma apoE protein concentration and brain amyloid burden, we adopted several measures to reduce the risk of type-I error due to multiple comparisons. First, we restricted our ROI analyses to *a priori* selected regions known to be vulnerable to pathological processes relevant to AD, including hypometabolism, amyloid deposition and atrophy. We then applied Bonferroni correction to adjust the threshold for statistical significance due to multiple comparisons. Finally we restricted our voxel level analysis, to the ROI showing a significant association between amyloid burden and plasma apoE concentration. We found a significant correlation between plasma apoE concentration and A β deposition in the medial temporal lobe in the combined cohort of APOE ϵ 4 carriers and non-carriers. Voxel-based analysis confirmed this result and localized the association to the hippocampus, parahippocampal gyrus and entorhinal cortex. Together with previous evidence for a role of the apoE protein in A β deposition and clearance [36, 47], these results indicate that plasma apoE concentration may be a biologically relevant peripheral marker of *in vivo* amyloid deposition in brain regions vulnerable to AD pathology.

Although the medial temporal lobe (MTL) is the site of the earliest pathological changes in AD [42], these predominantly involve neuronal loss and neurofibrillary degeneration [48]. The relatively low amyloid burden in this region raises questions about the role of amyloid in the MTL in AD pathogenesis. However, recent studies indicate that the MTL is especially vulnerable to the neurotoxic effects of A β and that amyloid deposition in this region, even in

non-demented elderly persons, may mediate cognitive impairment by disrupting default mode network connectivity [49–50].

Our findings suggest that plasma apoE protein concentration is indicative of brain amyloid burden, and together with the widespread increases in amyloid deposition in APOE $\epsilon 4$ carriers, provide evidence for association of both APOE gene and apoE plasma protein concentration with the brain amyloid cascade in normal aging. One limitation of the present study that must be considered while interpreting these results is the relatively small number of individuals which precluded a robust analysis of the dose effect of the number of $\epsilon 4$ and non- $\epsilon 4$ alleles on brain amyloid burden. Nevertheless, our strategy combining brain amyloid imaging with proteomic analyses of plasma holds promise for the discovery of peripheral markers of AD neuropathology and may help accelerate the development of several emerging disease-modifying treatments that target this mechanism in AD pathogenesis [51].

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Figure 1. Study design

Schematic diagram illustrating the experimental design and work flow of the present study for discovery and validation of plasma proteins associated with brain amyloid burden in non-demented older individuals.

Figure-2A Scores plot of a PLS-DA model with integrated optical densities of 18 silver-stained 2DGE protein spots discriminating individuals in the ‘high’ (black squares) from ‘low’ (red squares) mean cortical DVR groups.

Figure-2B Internal validation of the final PLS-DA model predicting brain amyloid burden. Clear decreases in model performance are observed as the DVR data are permuted relative to the predictor variables. R2Y describes how well the derived model fits the data and is the proportion of the sum of squares explained by the model. Q2 describes the predictive ability of the derived model and is the cross validated R2Y.

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Figure 3. Aβ deposition in APOE ε4 carriers and non-carriers

Mean [^{11}C] PiB parametric images in 17 non-demented APOE ε 4 carriers and 37 APOE ε4 non-carriers. Parametric images were generated by the SRTM-LRSC algorithm and overlaid on a template MRI. Color scale from blue = low (1.0 DVR) to red = high (2.0 DVR) levels of PiB retention. Selected sagittal (top) and axial slices are shown.

Figure 4. Plasma concentration of apoE is associated with amyloid deposition in the medial temporal lobe in non-demented older individuals

SPM analysis shows correlation between plasma apoE concentration and [^{11}C]PiB uptake, $p < 0.05$; uncorrected. Highlighted areas denote regions in the hippocampus of both hemispheres that show significant association with plasma apoE concentration.

Table-1

Demographic characteristics (mean (SD)) of participants included in the 2DGE experiments to identify plasma proteins associated with brain amyloid burden.

	Whole Sample	Female	Male
N	57	24	33
Education (yrs)	16.7 (2.4)	17.0 (1.9)	16.5 (2.8)
Baseline Age (yrs)	68.2 (5.8)	67.6 (6.4)	68.5 (5.5)
Age at PET (yrs)	78.6 (6.1)	78.2 (6.9)	78.8 (5.5)
Interval (yrs)	10.4 (1.0)	10.5 (1.0)	10.4 (1.0)

Table-2

Plasma proteins identified by LC/MS/MS and constituting the PLS-DA model for brain amyloid burden.

Spot ID	Protein ID	Accession number
370	Apolipoprotein-E precursor	P02649
1679	Apolipoprotein-E precursor	P02649
1676	Apolipoprotein-E precursor	P02649
1659	Apolipoprotein-E precursor	P02649
1669	Apolipoprotein-E precursor	P02649
1644	Apolipoprotein-E precursor	P02649
534	Plasminogen precursor	P00747
533	Plasminogen precursor	P00747
713	No id obtained	
784	Complement C3 precursor	P01024
748	Complement C3 precursor	P01024
91	Complement C3 precursor	P01024
660	Complement C3 precursor	P01024
385	Haptoglobin precursor	P00738
1340	Serum albumin precursor	P02768
925	Serum albumin precursor	P02768
1544	Serum albumin precursor	P02768
1595	Ig gamma-1 chain C region	P01857

Table-3

Demographic characteristics of APOE ε4 carriers and non-carriers.

	N	Sex M/F	Mean age	MMSE score	Education (years)	Family history of dementia
APOE ε4–	37	25/12	79.0 (6.5)	29.2 (1.0)	17.1 (2.3)	7
APOE ε4+	17	8/9	77.5 (3.5)	28.1 (2.2)	15.9 (2.8)	4
Difference (<i>p</i>)		0.1511	0.2597	0.0895	0.1125	0.7133

Local maxima within areas of significant associations between plasma apoE protein concentration and ¹¹C-PiB retention using a restricted search of the medial temporal region. Coordinates are in stereotactic space and Brodmann areas are in parentheses.

Table-4

Region	Side	Coordinate			Tissue	
		x	y	z	T-value	Volume (mm ³)
Hippocampus	R	26	-32	-10	2.58	362
Parahippocampal gyrus (35/36)	R	22	-10	-24	2.55	1332 ¹
Entorhinal cortex (28)	R	22	6	-38	2.51	1332 ²
Hippocampus	L	-26	-20	-18	2.31	196

¹⁻²Denotes local maxima contained within the same cluster