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Association of plasma and cortical amyloid beta is modulated by *APOE* ε4 status

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Abstract

Background: Apolipoprotein E (*APOE*) ε4 allele's role as a modulator of the relationship between soluble plasma amyloid beta (Aβ) and fibrillar brain Aβ measured by Pittsburgh compound B positron emission tomography ([^{11}C]PiB PET) has not been assessed.

Methods: Ninety-six Alzheimer's Disease Neuroimaging Initiative participants with [^{11}C]PiB scans and plasma $A\beta_{1–40}$ and $A\beta_{1–42}$ measurements at the time of PET scanning were included. Regional and voxelwise analyses of [^{11}C]PiB data were used to determine the influence of *APOE* ε4 allele on association of plasma $A\beta_{1–40}$, $A\beta_{1–42}$, and $A\beta_{1–40}/A\beta_{1–42}$ with [^{11}C]PiB uptake.

Results: In *APOE* ε4− but not ε4+ participants, positive relationships between plasma $A\beta_{1–40}/A\beta_{1–42}$ and [^{11}C]PiB uptake were observed. Modeling the interaction of *APOE* and plasma $A\beta_{1–40}/A\beta_{1–42}$ improved the explained variance in [^{11}C]PiB binding compared with using *APOE* and plasma $A\beta_{1–40}/A\beta_{1–42}$ as separate terms.

Conclusions: The results suggest that plasma Aβ is a potential Alzheimer's disease biomarker and highlight the importance of genetic variation in interpretation of plasma Aβ levels.

Keywords: Alzheimer's disease; Mild cognitive impairment; Alzheimer's Disease Neuroimaging Initiative; Amyloid beta; Plasma amyloid beta; Positron emission tomography; Pittsburgh compound B; Apolipoprotein E

[†]Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (http://adni.loni.ucla.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data, but did not participate in analysis or writing the report. A complete listing of ADNI investigators can be found at http://adni.loni.ucla.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

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1. Introduction

Alzheimer's disease (AD) is the most common type of dementia, affecting an estimated 5.4 million Americans [1]. Currently, there are no treatments that can stop its progression. However, worldwide research efforts are being conducted to identify improved methods to prevent, diagnose, and treat this disease [1]. Objective measures of biological or pathogenic processes, called biomarkers, can help in the evaluation of disease risk or prognosis. To date, no reliable biomarkers for AD in peripheral blood have been found [2].

AD is characterized by declining memory and cognition. Amnestic mild cognitive impairment (MCI) is a clinical condition thought to be a prodromal stage of AD, in which individuals have cognitive problems not normal for their age, but are not severe enough to interfere significantly with daily life activities. An estimated 14% to 18% of individuals 70 years and older have MCI, and approximately 10% to 15% of these individuals with MCI will progress to dementia, mostly AD, each year [3,4].

Accumulation of amyloid beta (Aβ) fragments into amyloid plaques in the brain is one of the defining pathologies of AD. Attempts to monitor the presence and/or progression of amyloid deposition have focused primarily on measurements of Aβ in the brain and cerebrospinal fluid (CSF). The level of CSF $A\beta_{1–42}$ has been shown to be a sensitive biomarker for detection and diagnosis of AD [5–7]. Positron emission tomography (PET) imaging techniques with ligands such as Pittsburgh compound B ([^{11}C]PiB) [8] and [^{18}F]florbetapir [9,10], which bind fibrillar Aβ plaques with high affinity, are being studied for their efficacy in predicting and diagnosing AD and have shown some promise [11–13].

Identifying a peripheral biomarker of central Aβ deposition may help in the diagnosis and treatment of the disease at earlier stages. Measuring soluble Aβ levels in plasma would provide an easy method to study Aβ because the procedure is minimally invasive and relatively inexpensive. The utility of plasma Aβ as a potential AD biomarker has been assessed in previous studies, but the results have been inconsistent [14–17]. Possible reasons for the inconsistent results could be the use of different enzyme-linked immunosorbent assays and platforms, and timing of sample collection in relation to the stage of disease progression. Therefore, additional studies are needed to characterize more fully the utility of plasma Aβ measures as sensitive and effective biomarkers of AD.

Genetic factors, such as the apolipoprotein E (*APOE*) gene, may play a role in amyloid accumulation and the development of AD. The *APOE* gene is expressed as three variants: ε2, ε3, and ε4. The *APOE* ε4 allele is the strongest genetic risk factor of late-onset AD and confers a dose-dependent increase in AD risk of approximately fourfold in carriers compared with noncarriers [18–20]. The ε4 allele is also associated with increased fibrillar Aβ [21] and decreased soluble plasma $A\beta_{1–42}$ [22] in a dose-dependent manner. The *APOE* gene codes for the apoE protein, which is essential for maintaining blood–brain barrier (BBB) integrity [23]. The apoE4 form of the apoE protein, coded for by the ε4 allele, has been associated with reduced Aβ clearance from the brain [24] and plasma [25], and with impaired tight junction integrity [26].

To our knowledge, only four studies have investigated the relationship of soluble plasma Aβ and fibrillar brain Aβ as measured by [^{11}C]PiB [22,27–29]. The first study [27] did not identify any relationships between plasma $A\beta_{1–40}$ and $A\beta_{1–42}$ levels and [^{11}C]PiB binding. In the other studies, inverse correlations were observed between plasma $A\beta_{1–42}$ and [^{11}C]PiB uptake [22,28] and between $A\beta_{1–42}/A\beta_{1–40}$ and brain amyloid [28,29]. However, none of these studies examined the potential influence of genetic variation in AD-related genes (e.g., *APOE* ε4) on relationships between peripheral and central markers of Aβ. Furthermore, these studies only included regional measures of [^{11}C]PiB uptake rather than voxel-based mapping across the whole brain, which may have limited the findings because extracting information from spatially large regions may dilute or obscure relevant results that are spatially constrained.

We studied the associations among [^{11}C]PiB brain uptake, soluble plasma Aβ measurements, and *APOE* ε4 genotype status in 96 participants from the Alzheimer's Disease Neuroimaging Initiative (ADNI) to determine whether the relationship of soluble plasma Aβ measures and fibrillar brain amyloid was influenced by *APOE* ε4 status. First, we used the average regional [^{11}C]PiB uptake across four target brain regions known to have amyloid deposition in AD as a quantitative phenotype in regression analyses. We then conducted whole-brain, voxelwise regression analyses to identify spatially specific clusters in which the *APOE* ε4 genotype modulated the association of plasma and brain PET measurements of Aβ.

2. Methods

2.1. Alzheimer's Disease Neuroimaging Initiative

Data used in the preparation of this report were obtained from the ADNI database (http://adni.loni.ucla.edu). The ADNI was initiated in 2003 as a $60 million, 5-year public–private partnership by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, the Food and Drug Administration, private pharmaceutical companies, and nonprofit organizations. The primary goal of the ADNI is to test whether serial magnetic resonance imaging (MRI), PET, other biological markers, genetics, and clinical and neuropsychological assessments can be combined to detect and measure the progression of MCI and early AD. Determining sensitive and specific markers of very early AD progression can aid researchers and clinicians in developing new treatments and monitoring their effectiveness, as well as lessen the time and cost of clinical trials.

Michael W. Weiner, MD, Veterans Affairs Medical Center and University of California at San Francisco, is the principal investigator of this initiative. ADNI is the result of the efforts of many co-investigators from a broad range of academic institutions and private corporations. As part of the initial phase of ADNI, more than 800 participants, age 55 to 90 years, were recruited from more than 50 sites across the Unites States and Canada, including approximately 200 cognitively healthy older individuals (healthy control subjects [HCs]) to be monitored for 3 years, 400 people with MCI to be monitored for 3 years, and 200 people with early AD to be monitored for 2 years. Further information about ADNI can be found in work by Weiner and colleagues [30] and at http://www.adni-info.org.

The study was conducted after institutional review board approval at each site. Written informed consent was obtained from all study participants or their authorized representatives.

2.2. Participants

Data from 96 participants in the ADNI cohort were evaluated. Participant selection was based on the availability of the following data: [^{11}C]PiB PET scans, plasma measurements of $A\beta_{1–40}$ and $A\beta_{1–42}$ at time of PET scan, and *APOE* ε4 genotype data. At the time of PET scan, 22 participants were in the AD group, 52 in the MCI group, and 22 in the HC group. The participants included 89 non-Hispanic whites, two non-Hispanic blacks, two non-Hispanic Asians, two Hispanic whites, and 1 white participant of unknown ethnicity. Additional demographic information about the included sample is presented in Table 1.

2.2.1. [^{11}C]PiB PET image data

For all participants, PET data consisted of each participant's initial [^{11}C]PiB scan in the ADNI longitudinal imaging protocol. Initial scans were acquired at either the participant's baseline visit, 12-month visit, or 24-month visit (Table 1). Methods for the acquisition and processing of [^{11}C]PiB PET scans for the ADNI sample have been described elsewhere [31,32]. The PET data used in the current study were what was available as of October 2010. The ADNI database includes normalized whole-brain [^{11}C]PiB images as well as normalized regional [^{11}C]PiB average uptake values extracted from anatomically defined regions of interest (ROIs). Both preexisting ROI data and whole-brain [^{11}C]PiB images were downloaded and analyzed in the current study. Regional [^{11}C]PiB standardized uptake value ratios from four ROIs (anterior cingulate, frontal cortex, parietal cortex, and precuneus) were averaged and used as a quantitative phenotype, referred to as average regional [^{11}C]PiB uptake. This metric has been used previously to classify participants as positive or negative for amyloid deposition [32].

The whole-brain [^{11}C]PiB PET images evaluated in the current study were preprocessed (PIB Coreg, Avg, Std Img and Vox Size, Uniform Resolution), as described previously described [32]. Briefly, the images were set to a standard orientation and voxel size, intensity normalized using a cerebellar gray matter (GM) ROI, and smoothed to a common resolution of 8 mm full-width at half maximum. These preprocessed scans were downloaded in Neuroimaging Informatics Technology Initiative format from the ADNI scan repository (http://adni.loni.ucla.edu) and processed further using Statistical Parametric Mapping [33] version 5 (SPM5; http://www.fil.ion.ucl.ac.uk/spm/) implemented via MATLAB v7.1.0 (MathWorks, Natick, MA) [34]. Specifically, for all participants, 1.5-T T1-weighted three-dimensional magnetization-prepared rapid acquisition gradient echo magnetic resonance (MR) images [35] acquired at the same time point as the [^{11}C]PiB scans were also downloaded from the ADNI site (http://adni.loni.ucla.edu). The preprocessed [^{11}C]PiB PET image of each participant was coregistered to their corresponding MR image. PET and MR data were then

Table 1
Sample characteristics

Characteristic	AD (n = 22)	MCI (n = 52)	HC (n = 22)	*P* value*
Initial [^{11}C]PiB scans at baseline/12-month/24-month visit	3/13/6	11/36/5	0/20/2	.034
Age at time of scan, years; mean ± SD	74.06 ± 9.09	75.35 ± 7.93	77.14 ± 6.17	.428
Sex, n; male/female	15/7	35/17	14/8	.940
Education, years; mean ± SD	15.73 ± 3.04	16.31 ± 2.65	15.50 ± 3.32	.492
Handedness, n; right/left	20/2	48/4	17/5	.165
APOE ε4 status, n; ε4−/ε4+	8/14	24/28	16/6	.039
Average regional [^{11}C]PiB uptake†, mean ± SD	2.01 ± 0.31	1.81 ± 0.44	1.56 ± 0.34	.001
Plasma $A\beta_{1–40}$, pg/mL; mean ± SD	160.99 ± 47.89	171.56 ± 48.60	168.75 ± 36.57	.666
Plasma $A\beta_{1–42}$, pg/mL; mean ± SD	36.05 ± 9.19	40.81 ± 12.48	41.93 ± 9.06	.160
Plasma $A\beta_{1–40}/A\beta_{1–42}$, mean ± SD	4.53 ± 1.20	4.38 ± 1.19	4.11 ± 0.81	.440

Abbreviations: AD, Alzheimer's disease; MCI, mild cognitive impairment; HC, healthy control participant; PiB, Pittsburgh compound B; SD, standard deviation; *APOE*, apolipoprotein E; Aβ, amyloid beta.

*For categorical variables, Pearson χ^2 was used to compute the *P* value. For continuous variables, one-way analysis of variance was used to compute the *P* value.

†Average regional [^{11}C]PiB uptake is the average of [^{11}C]PiB uptake values from four brain regions—anterior cingulate, frontal cortex, parietal cortex, and precuneus—normalized to cerebellum.

spatially normalized to Montreal Neurological Institute (MNI) space using transformation parameters estimated from the SPM segmentation algorithm [36]. These spatially normalized PET images were used for the whole-brain voxelwise analysis.

2.2.2. *Plasma Aβ data*

Plasma $A\beta_{1–40}$ and $A\beta_{1–42}$ levels for participants with [^{11}C]PiB PET scans from the same time point that the PET data were acquired were obtained from the ADNI database. The methods for the collection, measurement, and quality control of plasma samples have been described previously [22,37].

2.2.3. APOE *ε4 genotyping*

The *APOE* ε4 status of all participants was determined by two single nucleotide polymorphisms (rs429358 and rs7412), as described previously [38]. Participants were classified as *APOE* ε4− (absence of the ε4 allele) or *APOE* ε4+ (presence of the ε4 allele).

2.3. *Statistical analyses*

The influence of *APOE* ε4 status on the association between plasma Aβ and average regional [^{11}C]PiB uptake was assessed in R version 2.10.0 [39] and SAS 9.3 (SAS Institute Inc., Cary, NC) using the following regression models:

1. Average regional [^{11}C]PiB uptake = Plasma $A\beta_{1–40}$ + *APOE* ε4 status + (Plasma $A\beta_{1–40}$ × *APOE* ε4 status)
2. Average regional [^{11}C]PiB uptake = Plasma $A\beta_{1–42}$ + *APOE* ε4 status + (Plasma $A\beta_{1–42}$ × *APOE* ε4 status)
3. Average regional [^{11}C]PiB uptake = Plasma $A\beta_{1–40}$/$A\beta_{1–42}$ + *APOE* ε4 status + (Plasma $A\beta_{1–40}$/$A\beta_{1–42}$ × *APOE* ε4 status)

Models with significant interactions between plasma Aβ and *APOE* ε4 status on average regional [^{11}C]PiB uptake were identified. For these models, the variance in average regional [^{11}C]PiB uptake explained by different terms in the model was determined using the following regression models:

1. Variance in average regional [^{11}C]PiB uptake explained by plasma Aβ term alone:

Average regional [^{11}C]PiB uptake = Plasma Aβ

2. Variance in average regional [^{11}C]PiB uptake explained by *APOE* ε4 status term alone:

Average regional [^{11}C]PiB uptake = *APOE* ε4 status

3. Variance in average regional [^{11}C]PiB uptake explained by plasma Aβ and *APOE* ε4 status terms together:

Average regional [^{11}C]PiB uptake = Plasma Aβ + *APOE* ε4 status

4. Variance in average regional [^{11}C]PiB uptake explained by plasma Aβ, *APOE* ε4 status, and (plasma Aβ × *APOE* ε4 status) terms together:

Average regional [^{11}C]PiB uptake = Plasma Aβ + *APOE* ε4 status + (Plasma Aβ × *APOE* ε4 status)

The influence of *APOE* ε4 status on the association between plasma Aβ and [^{11}C]PiB uptake was assessed further in whole-brain voxelwise analyses in SPM5 using the following regression models:

1. Voxel [^{11}C]PiB uptake = Plasma $A\beta_{1–40}$ + *APOE* ε4 status + (Plasma $A\beta_{1–40}$ × *APOE* ε4 status)
2. Voxel [^{11}C]PiB uptake = Plasma $A\beta_{1–42}$ + *APOE* ε4 status + (Plasma $A\beta_{1–42}$ × *APOE* ε4 status)
3. Voxel [^{11}C]PiB uptake = Plasma $A\beta_{1–40}$/$A\beta_{1–42}$ + *APOE* ε4 status + (Plasma $A\beta_{1–40}$/$A\beta_{1–42}$ × *APOE* ε4 status)

In the voxelwise analyses, an explicit GM mask was used to restrict analyses to GM regions. Significant interactions were determined using a voxel-level threshold of $P < .005$ (uncorrected) and a cluster-level threshold of $k \geq 200$ contiguous voxels to achieve cluster-level uncorrected $P < .05$. Clusters identified in the left or right cerebellum were not considered because the [^{11}C]PiB PET images had been intensity normalized using a cerebellar GM ROI. Voxels at which significant relationships existed were displayed on a three-dimensional-rendered brain. The MNI coordinates of voxels that were peak maxima and local maxima (voxels > 4 mm apart) in each cluster were converted to Talairach coordinates, and queried in Talairach Client v2.4.2 [40,41] software to determine the associated anatomic labels. Random field theory corrected P values (P_{corr}) were used to identify significant clusters. Mean [^{11}C]PiB uptake from each significant cluster was extracted for all participants, and their distribution in *APOE* ε4− and *APOE* ε4+ participants was further examined in R version 2.10.0 and SAS 9.3. We then evaluated further the relationship of mean [^{11}C]PiB uptake from the biggest and most significant cluster and plasma Aβ levels. The variance in mean [^{11}C]PiB uptake extracted from the significant cluster explained by different terms in the model was determined using the following regression models:

1. Variance in mean [^{11}C]PiB uptake from the significant cluster explained by plasma Aβ term alone:

Mean [^{11}C]PiB uptake from the significant cluster = Plasma Aβ

2. Variance in mean [^{11}C]PiB uptake from the significant cluster explained by *APOE* ε4 status term alone:

Mean [^{11}C]PiB uptake from the significant cluster = *APOE* ε4 status

3. Variance in mean [^{11}C]PiB uptake from the significant cluster explained by plasma Aβ and *APOE* ε4 status terms together:

Mean [^{11}C]PiB uptake from the significant cluster = Plasma Aβ + *APOE* ε4 status

4. Variance in mean [^{11}C]PiB uptake from the significant cluster explained by plasma Aβ, *APOE* ε4 status, and (plasma Aβ × *APOE* ε4 status) terms together:

Mean [^{11}C]PiB uptake from the significant cluster = Plasma Aβ + *APOE* ε4 status + (Plasma Aβ × *APOE* ε4 status)

3. Results

We first investigated the influence of *APOE* ε4 status on the association between plasma Aβ and average regional [^{11}C]PiB uptake. No significant interactions between plasma $A\beta_{1–40}$ and *APOE* ε4 status or between plasma $A\beta_{1–42}$ and *APOE* ε4 status were observed on average regional [^{11}C]PiB uptake. However, a significant interaction between plasma $A\beta_{1–40}/A\beta_{1–42}$ and *APOE* ε4 status ($P = .025$) on average regional [^{11}C]PiB uptake was observed. Inclusion of age at time of scan and gender as covariates did not alter this finding. *APOE* ε4 genotype status (ε4− or ε4+) conferred different patterns of association between plasma $A\beta_{1–40}/A\beta_{1–42}$ and average regional [^{11}C]PiB uptake. Specifically, in the *APOE* ε4− participants, there was a positive relationship between plasma $A\beta_{1–40}/A\beta_{1–42}$ and average regional [^{11}C]PiB uptake (slope = 0.162, $P = .008$, $r^2 = 0.141$; Fig. 1A). However, this relationship did not exist in the *APOE* ε4+ participants (slope = −0.005; $P = .901$; $r^2 < 0.001$; Fig. 1B). The *APOE* ε4 status and plasma $A\beta_{1–40}/A\beta_{1–42}$ terms explained 17% and 6% of variation in average regional [^{11}C]PiB uptake, respectively. The two terms together explained 19% of variation in average regional [^{11}C]PiB uptake. Inclusion of the (plasma $A\beta_{1–40}/A\beta_{1–42}$ × *APOE* ε4 status) interaction term in the model increased the explained variance in average regional [^{11}C]PiB uptake to 24%.

To examine further the spatial extent of the potential influence of *APOE* ε4 status on the association of plasma Aβ and [^{11}C]PiB uptake, we performed whole-brain, voxelwise regression analyses. *APOE* genotype did not affect significantly the positive or negative associations between plasma $A\beta_{1–40}$ and [^{11}C]PiB uptake, and between plasma $A\beta_{1–42}$ and [^{11}C]PiB uptake in cerebral regions. However, *APOE* genotype significantly altered the

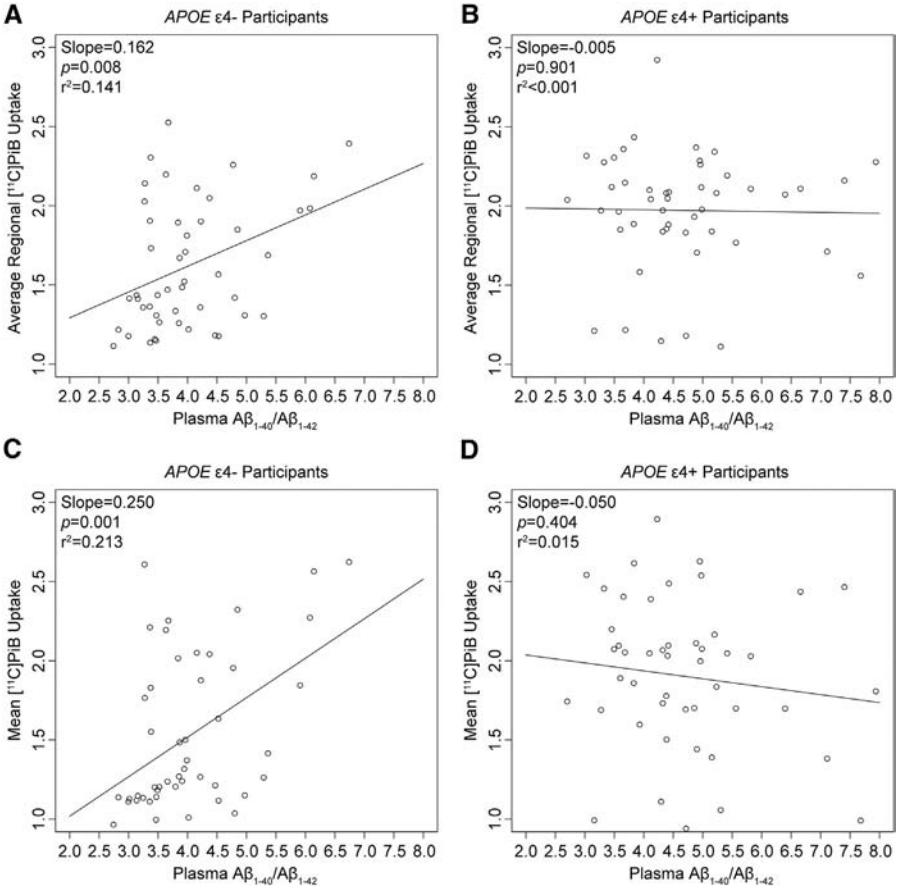

Fig. 1. (A–D) Scatterplots of plasma $A\beta_{1–40}/A\beta_{1–42}$ vs average regional [^{11}C]PiB uptake from the (Average regional [^{11}C]PiB uptake = Plasma $A\beta_{1–40}/A\beta_{1–42}$ + *APOE* ε4 status + [Plasma $A\beta_{1–40}/A\beta_{1–42}$ × *APOE* ε4 status]) model (A and B), and plasma $A\beta_{1–40}/A\beta_{1–42}$ vs mean [^{11}C]PiB uptake from the cluster identified in the (Voxel [^{11}C]PiB uptake = Plasma $A\beta_{1–40}/A\beta_{1–42}$ + *APOE* ε4 status + [Plasma $A\beta_{1–40}/A\beta_{1–42}$ × *APOE* ε4 status]) model (C and D). Aβ, amyloid beta; PiB, Pittsburgh compound B; *APOE*, apolipoprotein E.

negative correlation of plasma $A\beta_{1-40}/A\beta_{1-42}$ and [^{11}C]PiB uptake in a significant cluster in the left inferior frontal gyrus (MNI peak coordinates: $x = -40$, $y = 18$, $z = -6$; $k = 6152$ voxels; cluster-level $P_{corr} < .001$; Fig. 2 and Table 2). Similar to the data from the average regional [^{11}C]PiB uptake analysis, there was a positive relationship between plasma $A\beta_{1-40}/A\beta_{1-42}$ and mean [^{11}C]PiB uptake from the inferior frontal gyral cluster in the *APOE* ε4− participants (slope = 0.250, $P = .001$, $r^2 = 0.213$; Fig. 1C), but not in the *APOE* ε4+ participants (slope = −0.050, $P = .404$, $r^2 = 0.015$; Fig. 1D). Inclusion of age at time of scan and gender as covariates again did not alter this finding. The *APOE* ε4 status and plasma $A\beta_{1-40}/A\beta_{1-42}$ terms by themselves explained 13% and 4% of variation in mean [^{11}C]PiB uptake from the significant cluster, respectively. The two terms together explained 14% of variation in mean [^{11}C]PiB uptake from the significant cluster. Inclusion of the (plasma $A\beta_{1-40}/A\beta_{1-42}$ × *APOE* ε4 status) interaction term along with the two terms in the model increased the variance explained in mean [^{11}C]PiB uptake from the significant cluster to 23%. No clusters were identified when considering the positive correlation of plasma $A\beta_{1-40}/A\beta_{1-42}$ and [^{11}C]PiB uptake.

4. Discussion

The investigation of Aβ species in plasma offers advantages over conventional methods for measuring Aβ levels in the brain and CSF. Obtaining and analyzing plasma samples is relatively inexpensive, minimally invasive, and can be performed easily at multiple time points. Therefore, a plasma-based biomarker for early detection and diagnosis of AD would be ideal. In the ADNI cohort, a strong association has been observed between CSF $A\beta_{1-42}$ and fibrillar brain Aβ (indexed with [^{11}C]PiB) [31], whereas a weak but significant association has been observed between CSF $A\beta_{1-42}$ and soluble plasma $A\beta_{1-42}$ [37]. However, the association between soluble plasma Aβ and fibrillar brain Aβ is unclear.

We investigated the relationship of soluble plasma Aβ ($A\beta_{1-40}$, $A\beta_{1-42}$, and $A\beta_{1-40}/A\beta_{1-42}$), *APOE* ε4 status, and fibrillar brain Aβ (indexed with [^{11}C]PiB) in the ADNI cohort. In two types of analytical approaches, *APOE* ε4 genotype status had a significant effect on the relationship between plasma $A\beta_{1-40}/A\beta_{1-42}$ and [^{11}C]PiB uptake. Specifically, a positive relationship between plasma $A\beta_{1-40}/A\beta_{1-42}$ and [^{11}C]PiB signal was observed in *APOE* ε4− participants, but not in *APOE* ε4+ participants (Fig. 1). This finding may reflect a stronger relationship between plasma Aβ and accumulation of fibrillar amyloid in the brain in individuals at an earlier and/or less severe disease state (e.g., *APOE* ε4−). A recent study suggested that plasma Aβ levels in cognitively stable individuals tend to increase slightly with age [15]. Cognitively normal individuals with higher plasma Aβ levels are thought to be at an increased risk of progression to AD. Plasma Aβ levels in individuals who go on to develop AD tend to be elevated during the predementia stage, reach a peak, and then decrease prior to developing clinical AD symptoms. Increasing brain amyloid deposits in the later stages of disease may perhaps reduce interstitial Aβ in the brain and CSF, confounding the relationship between plasma Aβ and brain amyloid.

The molecular mechanism by which the *APOE* ε4 allele leads to increased risk for AD is unclear. The *APOE* gene codes for the apoE protein, which is essential for maintaining BBB integrity [23]. Furthermore, the various apoE protein isoforms are thought to clear Aβ from the brain differentially into the plasma across the BBB. In a recent study, Castellano and colleagues [24] found that mice expressing the human apoE4 protein had greater Aβ concentrations in the interstitial fluid of the brain and hippocampus, and showed reduced Aβ clearance from the interstitial fluid of the brain when compared with mice expressing the human apoE2 or apoE3 proteins. However, they did not find differences in Aβ synthesis or amyloidogenic processing between mice expressing the different apoE protein isoforms. In another study conducted in *APOE* ε2, ε3, and ε4 knock-in and *APOE* knock-out mice injected with lipidated recombinant apoE2, E3, and E4 proteins, Sharman and colleagues [25]

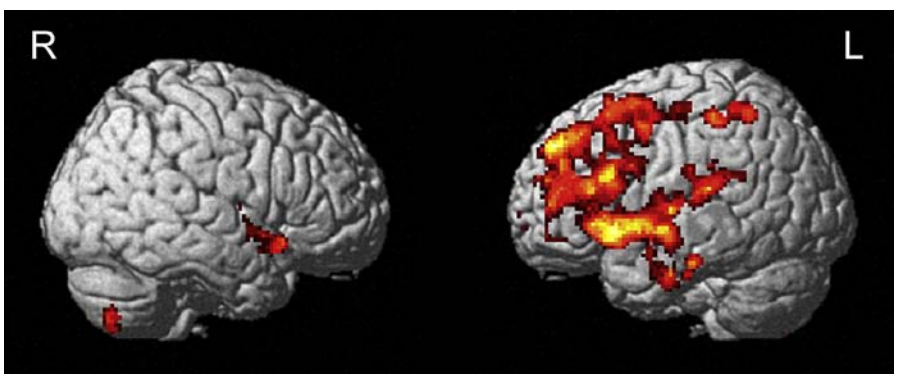

Fig. 2. Brain regions (R, right; L, left) identified in the (Voxel [^{11}C]PiB uptake = Plasma $A\beta_{1-40}/A\beta_{1-42}$ + *APOE* ε4 status + [Plasma $A\beta_{1-40}/A\beta_{1-42}$ × *APOE* ε4 status]) model (voxel-level threshold of $P < .005$ [uncorrected], cluster size ≥ 200 voxels). The red-to-yellow scale indicates increasing statistical significance of association. PiB, Pittsburgh compound B; Aβ, amyloid beta; *APOE*, apolipoprotein E.

Table 2
Brain regions identified in the (Voxel [^{11}C]PiB uptake = Plasma $A\beta_{1-40}/A\beta_{1-42}$ + *APOE* ε4 status + [Plasma $A\beta_{1-40}/A\beta_{1-42}$ × *APOE* ε4 status]) model (voxel-level threshold of $P < .005$ [uncorrected], cluster size ≥ 200 voxels)

Region	Broadmann area	Peak value coordinates, mm			Voxel level		Cluster level		
		x	*y*	*z*	T value	$P_{FWE-corr}$	*k*	P_{uncorr}	P_{corr}
Left inferior frontal gyrus	BA47	−40	18	−6	3.92	.713	6152	<.001	<.001
Left superior temporal gyrus	BA22	−46	6	−4	3.85	.774			
Left middle frontal gyrus	BA6	−30	12	60	3.81	.816			
Left superior frontal gyrus	BA8	−28	20	56	3.76	.855			
Left precentral gyrus	BA4	−34	−20	52	3.68	.910			
Left insula	BA13	−42	−8	−8	3.58	.955			
Left middle frontal gyrus	BA8	−24	24	50	3.43	.990			
Left middle frontal gyrus	BA10	−36	42	14	3.40	.993			
Left middle temporal gyrus	BA21	−58	−10	−8	3.35	.996			
Left superior frontal gyrus	BA9	−18	48	34	3.29	.998			
Left inferior frontal gyrus	BA45	−50	20	20	3.24	.999			
Left anterior cingulate	BA32	0	44	12	3.23	.999			
Left superior frontal gyrus	BA10	−26	42	30	3.19	1.000			
Left medial frontal gyrus	BA10	−2	58	−4	3.17	1.000			
Left inferior parietal lobule	BA40	−54	−22	26	3.16	1.000			
Left transverse temporal gyrus	BA41	−48	−26	10	3.16	1.000			
Left postcentral gyrus	BA40	−48	−34	54	3.73	.878	273	.023	.448
Left inferior parietal lobule	BA40	−36	−52	56	3.54	.968			
Left inferior temporal gyrus	BA20	−64	−8	−26	3.32	.998	231	.035	.586
Left fusiform gyrus	BA20	−62	−4	−28	3.20	1.000			
Left middle temporal gyrus	BA21	−54	0	−18	2.84	1.000			
Right inferior frontal gyrus	BA47	42	14	−14	3.32	.998	262	.026	.482
Right superior temporal gyrus	BA22	46	0	−4	3.16	1.000			
Right subgyral	BA13	44	2	−8	3.08	1.000			
Right insula	BA13	42	−6	4	2.92	1.000			

Abbreviations: PiB, Pittsburgh compound B; *APOE*, apolipoprotein E; Aβ, amyloid beta; $P_{FWE-corr}$, voxel-level *P*-value after familywise error correction; *k*, number of voxels in cluster; P_{uncorr}, cluster-level uncorrected *P*-value; P_{corr}, cluster-level *P*-value after random field theory correction; BA, Broadmann area.

found a difference in peripheral Aβ clearance from the plasma by *APOE* genotype. The results suggested that *APOE* ε4 gene expression results in a protein (apoE4) with lowered efficiency of peripheral Aβ clearance from the plasma. Tight junction integrity in the BBB is also regulated by the apoE protein in an isoform-dependent manner, with impaired tight junction integrity and increased BBB permeability observed in mice with the apoE4 isoform compared with mice with expression of the apoE3 isoform [26]. Thus, reduced Aβ clearance by apoE4 protein may lead to an impaired BBB, which in turn affects Aβ levels in the brain and plasma, and the relationship between these compartments.

Binding of the apoE protein to Aβ may also influence Aβ clearance from the brain into the plasma. The apoE protein binds strongly to Aβ, but the binding characteristics of the apoE4 isoform are different than those of the apoE3 isoform [42,43]. Oxidized apoE4 binds more rapidly to synthetic Aβ than oxidized apoE3. Binding by oxidized apoE4 was also more sensitive to pH changes than oxidized apoE3. In addition, the *APOE* ε4 allele is associated with increased vascular and plaque amyloid deposits [44]. *APOE* ε4 homozygotes have more amyloid deposits in the vasculature and tissue compared with *APOE* ε3 homozygotes. *APOE* ε3/ε4 heterozygotes have intermediate amyloid deposits. In a recent study, free apoE protein was shown to facilitate a greater removal of Aβ from the brain into the periphery across the BBB compared with apoE protein bound to Aβ [45]. Furthermore, apoE isoform-specific differences were observed in Aβ transport. Specifically, Aβ bound to the apoE4 isoform had increased blood-to-brain transport when compared with Aβ bound to the apoE3 isoform. The authors found that the apoE4 isoform had decreased Aβ clearance across the BBB in comparison with the apoE3 isoform. More recently, Bachmeier et al. [46] observed that retinoid X receptor stimulation increased Aβ clearance across the BBB, an effect that was believed to be partially mediated by the apoE protein.

The apoE protein has been shown to be present in greater amounts in the AD brain relative to those of healthy elders. Furthermore, apoE undergoes significantly more cleavage in the AD brain than in HCs, especially in *APOE* ε4 carriers [47]. The N-terminal domain of the apoE protein contains the major receptor-binding region; the C-terminal domain contains the lipid-binding region. The C-terminal domain of both the apoE3 and apoE4 isoforms has been shown to interact closely with Aβ [47]. Isolated C-terminal–truncated apoE4 protein fragments have been shown to be associated with Aβ plaques [47]. Last, inefficient clearance of Aβ peptides produces neuronal and behavioral deficits in mice [48]. Thus, differential clearance of Aβ by the apoE protein

isoform, which is coded for by different *APOE* genotypes, may be a potential explanation for the *APOE* genotype effects observed in the current study. Further investigation of mechanistic explanations is warranted.

It is important to discuss the limitations of the current study. First, the sample size in this study was relatively modest, as only 96 participants in the ADNI cohort had both [^{11}C] PiB PET scans and concomitant plasma measurements of $A\beta_{1-40}$ and $A\beta_{1-42}$. Our results suggest a complex relationship between plasma Aβ, brain Aβ, and *APOE* genotype, warranting further investigation in independent and larger samples. Second, a majority of the participants (n = 52) in the current study had a diagnosis of MCI. Therefore, it is possible that the observed association may have been driven primarily by these participants. Because of the relatively small number of participants in the three diagnostic groups, we were unable to perform analyses within each diagnostic group to determine whether there was an effect of diagnosis. Last, genetic factors other than *APOE* may also play a role in modulating the association of plasma and brain Aβ. Investigation of the impact of other known and novel AD-associated genetic variants on the relationship between plasma and brain Aβ represents a possible future direction of this work.

5. Summary

In summary, we detected an association between soluble plasma Aβ and fibrillar brain Aβ that was modulated by *APOE* ε4 status. Replication and additional study in independent samples is needed to clarify the nature of this interaction as well as to understand the underlying biological mechanisms. Our results suggest that plasma Aβ levels have great potential as an AD biomarker, and emphasize the importance of genetic variation in the interpretation of plasma Aβ levels.

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RESEARCH IN CONTEXT

1. Systematic review: Our scientific issue was to determine the influence of apolipoprotein E (*APOE*) ε4 status on the relationship between soluble plasma amyloid beta (Aβ) and fibrillar brain Aβ measured by Pittsburgh compound B ([^{11}C]PiB) positron emission tomography. We performed a literature search and identified four studies that had investigated the relationship between plasma Aβ and brain Aβ measured by [^{11}C]PiB, but did not examine the role of *APOE* ε4 on modulating the relationship.
2. Interpretation: A positive relationship between plasma Aβ and [^{11}C]PiB uptake was observed in *APOE* ε4− participants, but not *APOE* ε4+ participants. The findings suggests the utility of plasma Aβ as a potential Alzheimer's disease biomarker and highlight the importance of genetic variation in interpretation of plasma Aβ levels.
3. Future directions: Replication and additional study in independent samples is needed to determine the biological mechanisms that can explain the findings. The role of other genetic factors should also be investigated.

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