Neuroimaging

# Effect of apolipoprotein E phenotype on the association of plasma amyloid β and amyloid positron emission tomography imaging in Japan

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**Abstract**

**Introduction:** The plasma concentration of beta-amyloid (Aβ) has been considered another biomarker of Alzheimer's disease and was reportedly associated with cortical Aβ accumulation.

**Methods:** We analyzed 28 subjects with apolipoprotein E4 (ApoE4; E4 group) and 89 subjects without ApoE4 (non-E4 group) to determine the association between cortical Aβ accumulation by standard uptake value ratio with [$^{18}$F]florbetapir positron emission tomography and plasma $A\beta_{1-40}$ and $A\beta_{1-42}$.

**Results:** $A\beta_{1-42}/A\beta_{1-40}$ correlated significantly with mean regional [$^{18}$F]florbetapir standard uptake value ratio in the non-E4 group ($R^2 = 0.06$, $P = .02$) but not in the E4 group, and receiver operating characteristic curve analysis for $A\beta_{1-42}/A\beta_{1-40}$ in the non-E4 group showed sensitivity (92.9%) and specificity (45.9%) with a cutoff value of 0.150 for Aβ positivity.

**Discussion:** We verified that the correlation between $A\beta_{1-42}/A\beta_{1-40}$ and Aβ accumulation differed according to ApoE phenotype. The high sensitivity of plasma $A\beta_{1-42}/A\beta_{1-40}$ for Aβ positivity in non-E4 subjects indicated a possible role of plasma $A\beta_{1-42}/A\beta_{1-40}$ as a screening biomarker before amyloid positron emission tomography in clinical settings.

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*Keywords:* Alzheimer's disease; Plasma beta-amyloid$_{1-40}$; Plasma beta-amyloid$_{1-42}$; Beta-amyloid$_{1-40}$/beta-amyloid$_{1-42}$; Positron emission tomography; Phenotype of apolipoprotein

## 1. Introduction

For early diagnosis of Alzheimer's disease (AD), researchers have been searching for useful biomarkers in terms of accuracy. To the present, both amyloid positron emission tomography (PET) imaging and cerebrospinal fluid (CSF) have been most useful biomarkers, showing good sensitivity and specificity (amyloid PET, 82% and 95% [1]; CSF beta-amyloid (Aβ)$_{1-42}$, 96.4% and 76.9% [2]). However, these methods have some disadvantages that prevent them from coming into wide use, such as high cost, difficulty of ligand synthesis, and high invasiveness. Blood testing has been considered to offer another possible biomarker of AD, and it is less costly and invasive than PET and CSF. Recently, rapid progressing protein analysis has found some proteins as possible plasma biomarker candidates of AD. Proteomic analysis reported that plasma concentrations of apolipoprotein E (ApoE) protein and related proteins (e.g., Apo-A1, Apo-A2M) were identified as preclinical biomarkers of neocortical amyloid burden [3,4], and other studies reported that combinations of plasma proteins and other clinical information could predict neocortical amyloid burden [5,6].

Because Aβ accumulates in cerebral cortex and is detected in both CSF and plasma, plasma Aβ is one of the

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hopeful biomarker candidates of AD. Some studies examined plasma Aβ as a predictor of AD [7–9], but the results were inconsistent. Recently, two large-scale multicenter studies, Australian Imaging, Biomarkers and Lifestyle (AIBL) and Alzheimer's Disease Neuroimaging Initiative (ADNI), successively reported a correlation between amyloid PET and blood test. The AIBL study reported that $A\beta_{1-42}/A\beta_{1-40}$ was negatively correlated with the neocortical Aβ burden evaluated by $[^{11}C]PiB$ uptake [10]. On the other hand, the ADNI study reported a positive correlation between plasma $A\beta_{1-40}/A\beta_{1-42}$ and $[^{11}C]PiB$ uptake, although this correlation was shown only in subjects without ApoE-ε4 [11].

In this study, we examined the correlation between plasma Aβ and the results of amyloid PET imaging with $[^{18}F]$florbetapir in a Japanese population and a single-center study setting. We also tried to verify the effect of ApoE on the relationship between plasma Aβ and Aβ accumulation.

## 2. Methods

### 2.1. Subjects

We analyzed 117 Japanese participants (49 males and 68 females). Participants were excluded if they had other current clinically relevant neurologic illness, were receiving any investigational medications, or had ever received antiamyloid experimental therapy. Participants were also excluded by brain imaging (i.e., magnetic resonance imaging or computed tomography) if it showed evidence of significant brain damage (e.g., stroke, hemorrhage, or traumatic brain injury) that could explain the patients' cognitive decline or dementia.

Their cognitive function was evaluated by Mini–Mental State Examination (MMSE) [12]. The criteria *for* dementia were based on the Diagnostic and Statistical Manual of Mental Disorder (Fourth Edition, text revision). Participants diagnosed with dementia because of other general medical conditions were evaluated if they met the criteria for dementia with Lewy bodies [13] or frontotemporal lobe dementia [14]. The criteria for mild cognitive impairment (MCI) and healthy controls were the same as in our previous report [15].

Our participants included 35 patients with dementia (10 males and 25 females, age 76.2 ± 7.4 [mean ± standard deviation] years, MMSE score 19.7 ± 6.5, 22 AD, 11 dementia with Lewy bodies, one frontotemporal lobe dementia, one dementia unspecified), 58 patients with MCI (27 males and 31 females, age 68.1 ± 14.8 years, MMSE score 25.1 ± 4.1), and 24 healthy controls (12 males and 12 females, age 73.6 ± 5.3 years, MMSE score 28.8 ± 1.4).

### 2.2. PET analysis

A PET scanner system, Eminence SET-3000GCT/X (Shimadzu Corp, Kyoto, Japan), was used to measure regional brain radioactivity. For quantitative analysis of florbetapir-

PET images, we used the same method as described in previous studies [15,16]. Mean cortical (six regions: medial orbital frontal, temporal, anterior and posterior cingulate, parietal lobe, and precuneus) and whole-cerebellar region of interest (ROI) templates were applied to all PET scans to calculate mean regional cerebral-to-cerebellar standard uptake value ratio (SUVR) [16]. A threshold of SUVR greater than 1.099 was used to signify positive Aβ [15].

### 2.3. Blood test

Blood samples were drawn before the injection of $[^{18}F]$ florbetapir. Blood collected in a vacuum collection tube with ethylenediaminetetraacetic acid (EDTA) 2K was centrifuged (5000$g$) at 4°C for 15 minutes to separate plasma. Plasma samples were stored at −80°C until use. The thawed samples were analyzed and discarded after analysis every month. Then ApoE phenotype and plasma concentrations of $A\beta_{1-40}$ and $A\beta_{1-42}$ were examined by the methods given subsequently.

#### 2.3.1. Determination of ApoE phenotype

We pretreated plasma by incubating 10 μL with 100 μL of 5 mmol/L dithiothreitol containing 2.5 mL/L Tween 20 for 15 minutes at 4°C. The ApoE phenotypes were determined by isoelectric focusing using the method of Kataoka et al. [17] with a slight modification. The number of each phenotype of ApoE was as follows: E2/E3, N = 16 (13.7%); E3/E3, N = 71 (60.7%); E3/E4, N = 26 (22.2%); E3/E5, N = 2 (1.7%); and E4/E4, N = 2 (1.7%). We divided the subjects according to ApoE4, with 28 subjects having ApoE4 (E4 group) and 89 subjects being without ApoE4 (non-E4 group).

#### 2.3.2. Measurement of plasma $A\beta_{1-40}$ and $A\beta_{1-42}$

For measurement, the plasma sample was diluted fourfold by standard diluent to avoid the effect of interfering substances in plasma. A sandwich Aβ enzyme-linked immunosorbent assay kit was used (Wako, Osaka, Japan).

The plasma samples were analyzed as follows. We added 100 μL of standard diluent to samples, and they were incubated with a plate seal in a refrigerator overnight. After washing samples five times, 100 μL horseradish peroxidase–conjugated antibody solution was added and incubated in the refrigerator for 1 hour. After washing samples five times, 100 μL 3,3′,5,5′-tetramethylbenzidine solution was added and incubation under a plate seal was done at room temperature in the dark for 30 minutes. Finally, 100 μL of stop solution was added, and absorbance of each well was read at 450 nm with a microplate reader. Human $A\beta_{1-40}$ and $A\beta_{1-42}$ concentrations for the samples and controls were read from the standard curve.

Sensitivity was 0.019 pmol/L (dynamic range, 1.0–100 pmol/L) for $A\beta_{1-40}$ and 0.06 pmol/L (dynamic range, 0.1–20 pmol/L) for $A\beta_{1-42}$. Average intra-assay and interassay coefficients of variation were 2.18% and 6.94%, respectively.

## 2.4. Statistical analysis

Student $t$ test and chi-square test were used for group comparisons (Fisher's exact test if the smallest expected value of cells of the divided figure was less than 5). The influence of ApoE4 status on the relationship between plasma Aβ and SUVR was assessed using the method of Swaminathan et al. [11]: (1) SUVR = $Aβ_{1-40}$ + ApoE status + ($Aβ_{1-40}$ × ApoE status); (2) SUVR = $Aβ_{1-42}$ + ApoE status + ($Aβ_{1-42}$ × ApoE status); and (3) SUVR = $Aβ_{1-42}/Aβ_{1-40}$ + ApoE status + ($Aβ_{1-42}/Aβ_{1-40}$ × ApoE status). Linear correlation coefficients for pairs of variables were calculated for the correlations between SUVR and plasma $Aβ_{1-40}$, $Aβ_{1-42}$, and $Aβ_{1-42}/Aβ_{1-40}$. Receiver operating characteristic (ROC) curve analysis allowed us to examine the diagnostic power of $Aβ_{1-42}/Aβ_{1-40}$ against Aβ positivity, and the area under the curve (AUC) was calculated. We calculated the point that maximized the Youden Index (i.e., sensitivity − (1 − specificity)) as the cutoff value of ROC curve analysis. In all tests, a $P$ value <.05 was considered statistically significant. Statistical analysis of data was carried out using JMP 11.0.0 (SAS Inc, Cary, NC) on Windows 7.

## 3. Results

As shown in Table 1, group comparison revealed no differences between the E4 and non-E4 groups in gender distribution (chi-square = 0.58, $df$ = 1, $P$ = .45), mean age ($t$ = 0.03, $df$ = 115, $P$ = .98), mean MMSE score ($t$ = 1.29, $df$ = 115, $P$ = .26), distribution of diagnosis (chi-square = 4.84, $df$ = 2, $P$ = .09), and $Aβ_{1-42}/Aβ_{1-40}$ ($t$ = −0.26, $df$ = 115, $P$ = .79). However, compared with the non-E4 group, the E4 group had significantly higher SUVR ($t$ = −2.27, $df$ = 115, $P$ = .03), rate of Aβ positivity (chi-square = 7.54, $df$ = 1, $P$ = .006), $Aβ_{1-40}$ ($t$ = −2.30, $df$ = 115, $P$ = .02), and $Aβ_{1-42}$ ($t$ = −2.28, $df$ = 115, $P$ = .02).

Multiple linear regression analysis showed that the multiple regression coefficient was not significant.

No significant interactions between plasma $Aβ_{1-40}$ and ApoE4 status ($t$ = −0.52, $P$ = .61), $Aβ_{1-42}$ and ApoE4 status ($t$ = 1.28, $P$ = .20), and $Aβ_{1-42}/Aβ_{1-40}$ and ApoE4 status ($t$ = 1.90, $P$ = .06) were observed on SUVR.

There was no significant correlation between plasma $Aβ_{1-42}/Aβ_{1-40}$ and SUVR ($R^2$ = 0.02, $t$ = −1.59, $df$ = 116, $P$ = .11) in all 117 subjects. The correlation results between the value of SUVR and plasma $Aβ_{1-42}/Aβ_{1-40}$ by ApoE status are shown in Fig. 1. $Aβ_{1-42}/Aβ_{1-40}$ was significantly correlated with the value of SUVR in the non-E4 group (slope = 0.57, $R^2$ = 0.06, $t$ = −2.37, $df$ = 88, $P$ = .02) but not in the E4 group (slope = −0.56, $R^2$ = 0.04, $t$ = 0.98, $df$ = 27, $P$ = .36) (Fig. 2). Partial correlation analysis including covariate age and gender did not change the results (E4 group: $r$ = 0.17, $df$ = 24, $P$ = .41; non-E4 group: $r$ = −0.25, $df$ = 85, $P$ = .02).

The ApoE4 status and plasma $Aβ_{1-42}/Aβ_{1-40}$ terms by themselves explained 4% and 1% variations in SUVR, respectively. The two terms together explained 5% variation in SUVR, and inclusion of the (plasma $Aβ_{1-42}/Aβ_{1-40}$ × ApoE status) interaction term along with the two terms in the model explained 7% variation in SUVR.

In the E4 group, ROC curve analysis for $Aβ_{1-42}/Aβ_{1-40}$ showed best sensitivity (63.6%) and specificity (52.9%) with a cutoff value of 0.123 for Aβ positivity (AUC, 0.519; 95% confidence interval, 0.285–0.752). There was no significant difference between AUC of E4 group and a random classifier ($P$ = .81). In non-E4 group, ROC curve analysis for the $Aβ_{1-42}/Aβ_{1-40}$ ratio showed best sensitivity (92.9%) and specificity (45.9%) with a cutoff value of 0.150 for Aβ positivity (AUC, 0.648; 95% confidence interval, 0.528–0.767). There was a significant difference between AUC of non-E4 group and a random classifier ($P$ = .01). Positive predictive value (PPV) was 44.1% and negative predictive value (NPV) was 93.3%.

## 4. Discussion

Our results from a Japanese population could not reconfirm the finding that plasma $Aβ_{1-42}/Aβ_{1-40}$ was correlated with the degree of Aβ accumulation as reported by the AIBL study [10], but they did support the hypothesis that this correlation differed according to the ApoE phenotype as indicated by the ADNI study [11]. Compared with multicenter studies, a single-center study has reported larger intervention effects [18], whereas in multicenter studies, standardization of the methods to maintain the quality of the examinations is required, and sometimes this is difficult because of disparities among the various facilities. Both our study (single-center study, Japanese *participants*) and the ADNI study (multicenter study, most participants were Caucasian) demonstrated the same association of plasma Aβ, the degree of Aβ

Table 1
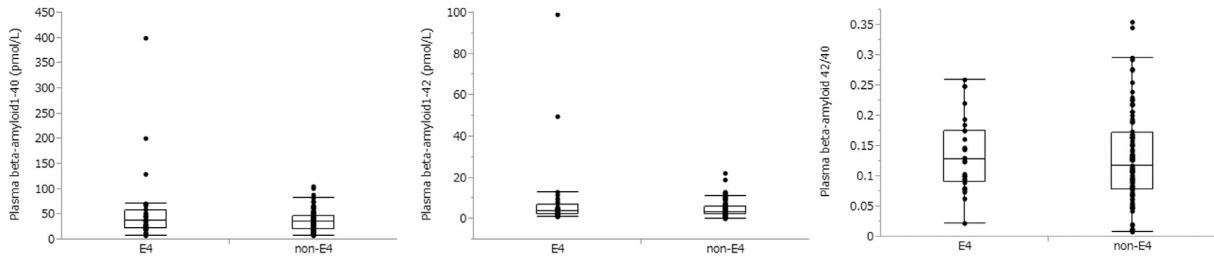Group comparison of subjects with ApoE4 (E4 group) and without ApoE4 (non-E4 group)

|  | E4 group | Non-E4 group | $P$ value |
|---|---|---|---|
| N (male/female) | 28 (10/18) | 89 (39/50) | NS |
| Age, mean ± SD | 71.6 ± 11.2 | 71.7 ± 12.2 | NS |
| MMSE, mean ± SD | 23.2 ± 6.6 | 24.6 ± 5.3 | NS |
| Diagnosis |  |  | NS |
| HC, N (%) | 4 (14.3) | 20 (22.5) |  |
| MCI, N (%) | 11 (39.3) | 47 (52.8) |  |
| Dementia, N (%) | 13 (46.4) | 22 (24.7) |  |
| [$^{18}$F]florbetapir PET |  |  |  |
| SUVR, mean ± SD | 1.18 ± 0.18 | 1.09 ± 0.17 | .03 |
| Beta-amyloid positivity, N (%) | 17 (60.7) | 28 (38.5) | .006 |
| Plasma (pmol/L), mean ± SD |  |  |  |
| $Aβ_{1-40}$ | 57.76 ± 77.67 | 36.99 ± 20.41 | .02 |
| $Aβ_{1-42}$ | 9.56 ± 19.8 | 4.54 ± 3.83 | .02 |
| $Aβ_{1-42}/Aβ_{1-40}$ | 0.14 ± 0.06 | 0.13 ± 0.08 | NS |

Abbreviations: Aβ, beta-amyloid; HC, healthy control; MCI, mild cognitive impairment; MMSE, Mini–Mental State Examination; NS, not significant; PET, positron emission tomography; SD, standard deviation; SUVR, standard uptake value ratio.

Fig. 1. Distribution of plasma beta-amyloid $(A\beta)_{1-40}$, $A\beta_{1-42}$, and plasma $A\beta_{1-42}/A\beta_{1-40}$.

accumulation varied according to ApoE phenotype, and therefore our study was important for allowing us to generalize the results that $A\beta_{1-42}/A\beta_{1-40}$ may be correlated with the accumulation of $A\beta$ among non-E4 subjects.

The extracellular concentration of $A\beta$ might be a result of the balance between synthesis and clearance rates of $A\beta$. A previous study reported that human ApoE allele might differentially regulate the clearance of $A\beta$ from the brain and that the clearance of soluble $A\beta$ from brain interstitial fluid depends on the isoform of the human ApoE expressed (ApoE4 < ApoE3 < ApoE2) [19]. Permeability of blood-brain barrier (BBB) might be another factor to account for the correlation between plasma $A\beta$ and cortical $A\beta$ accumulation. We suppose that $A\beta$ excretion mechanisms through BBB might account for the small contribution of plasma $A\beta$ and ApoE4 toward the variance in SUVR, because the correlation between $A\beta$ and cortical $A\beta$ accumulation in CSF samples [20] was stronger than in plasma samples.

Another interesting finding was that plasma $A\beta_{1-42}/A\beta_{1-40}$ in the non-E4 group showed strong sensitivity (92.9%), NPV (93.3%), and the negative likelihood ratio was 0.16 for $A\beta$ positivity by amyloid PET. A previous study of other minimally invasive biomarkers for MCI converting to AD reported an AUC of combined cytokine, clinical measures, and ApoE genotype of 0.61 [21]. In that study, the combination of cytokine and magnetic resonance imaging showed the best AUC of 0.78, sensitivity of 0.75, specificity of 0.78, PPV of 0.75, and NPV of 0.78 [21]. It has been reported that the ApoE-ε3 allele is present in 50% to 90% of people of all populations, whereas

ApoE-ε4 is present in 5% to 35% and ApoE-ε2 in 1% to 5% [22]. Our results suggested that $A\beta_{1-42}/A\beta_{1-40}$ among non-E4 subjects represented good screening for excluding subjects not needing unnecessary cost and exposure, because the prevalence of cortical $A\beta$ positivity is known not to be so high in non-E4 subjects. However, E4 subjects and non-E4 subjects with lower plasma $A\beta_{1-42}/A\beta_{1-40}$ need to undergo further testing with high specificity for $A\beta$ positivity, as lower PPV of plasma $A\beta_{1-42}/A\beta_{1-40}$ among the non-E4 group and ApoE4 is known to be a high-risk factor of AD.

We need to acknowledge several methodological limitations in our study. First, we studied only 117 subjects, so this result will need to be verified with a larger number of subjects in the future. Second, our subjects comprised three clinically diagnosed *groups*. Therefore, one might suppose that our results may have been driven primarily by the effect of diagnosis. Unfortunately, our study group, especially the E4 group, was not sufficiently large to allow analyses within each clinically diagnosed group, so we could not exclude the possibility of a diagnosis effect. Third, our non-E4 group contained ApoE2, ApoE3, and ApoE5. Although the phenotype of ApoE might influence the clearance of $A\beta$, the number of these subgroups was not large enough to allow analyses within them. Thus, further study will be needed to address whether the relationship of $A\beta_{1-42}/A\beta_{1-40}$ and SUVR differed only with ApoE4 or also with other ApoE phenotypes. Finally, we should conduct further study among subjects with suspected asymptomatic to preclinical stage of AD to clarify the usefulness of $A\beta_{1-42}/A\beta_{1-40}$ as an AD biomarker.
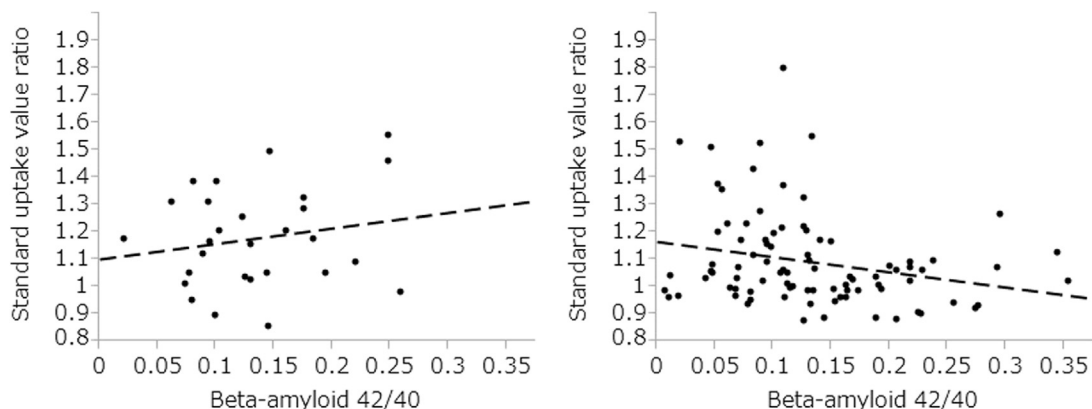


Fig. 2. Correlation results between the value of standard uptake value ratio and plasma beta-amyloid $(A\beta)_{1-42}/A\beta_{1-40}$ by ApoE status. Left side: E4 group, $y = 0.57x + 1.10$, $R^2 = 0.04$, $t = 0.98$, $df = 27$, $P = .34$. Right side: Non-E4 group, $y = -0.56x + 1.17$, $R^2 = 0.06$, $t = -2.37$, $df = 88$, $P = .02$.

In conclusion, the relationship of $A\beta_{1-42}/A\beta_{1-40}$ and SUVR was affected by ApoE phenotype. Although this correlation was very weak for a differential diagnosis, our results suggested that plasma $A\beta_{1-42}/A\beta_{1-40}$, especially among non-E4 subjects, might be a *possible* screening modality for deciding whether subjects should be examined by amyloid PET or not in clinical settings.

## Acknowledgments

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### RESEARCH IN CONTEXT

1. Systematic review: The authors used PubMed and the keywords "Alzheimer's disease", "biomarker", "amyloid positron emission tomography", "plasma beta-amyloid", "apolipoprotein", and "ApoE4".

2. Interpretation: Both the ADNI study, a multi-center study of Caucasians, and our study, a single-center study of Japanese, revealed the same finding that plasma $A\beta_{1-42}/A\beta_{1-40}$ was correlated with the degree of $A\beta$ accumulation and that this correlation differed by ApoE phenotype. The combination of the measurement of plasma $A\beta_{1-42}/A\beta_{1-40}$ and ApoE phenotype might be a useful screening test before amyloid PET in clinical settings.

3. Future directions: Minimally-invasive screening test for AD is required. Thus, in order to confirm our findings, further study with large numbers of subjects and different cohorts, and examining whether the correlation changes by clinical stages, will be needed.

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