

Aging and APOE- ϵ 4 are determinative factors of plasma A β 42 levels

(加齢と APOE- ϵ 4 は血漿 A β 42 の決定因子である)

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Abstract

Objective: The aim of this study was to confirm determinative factors for plasma A β and its association with cognitive function.

Methods: Fasting plasma A β 40 and A β 42 levels were measured by ELISA in 1,019 participants in the Iwaki Health Promotion Project. The relationships between plasma A β and health-related items, including physical characteristics, cognitive function tests, blood chemistry, and *APOE- ϵ 4* genotype were analyzed.

Results: The plasma levels of A β 40 and A β 42, and A β 40/42 ratio were found to significantly increase with aging. The age-dependent increase in A β 42 level was significantly suppressed by *APOE- ϵ 4*. Renal function was an associated factor for the plasma A β 40 level. The plasma A β 42 level and A β 40/42 ratio correlated with cognitive function.

Interpretation: Age and *APOE- ϵ 4* are major determinative factors of plasma levels of A β 42 and the A β 40/42 ratio. These factors are critical adjustment factors for the usage of plasma A β as a biomarker of central nervous system amyloidosis.

Introduction

Alzheimer's disease (AD) is observed at a critical rate due to the aging population. The latest research suggests that it is possible to prevent pathological processes in AD by developing disease-modifying therapies, such as anti-A β antibodies and BACE-1 inhibitors, against A β amyloidosis, which act on pathological cascades, including tauopathy. Prospective cohort studies have reported that the ratio of A β 40/42 is significantly associated with late-life cognitive decline ¹, and risk of developing MCI and AD ²⁻⁶. Systematic reviews and meta-analyses have also suggested that the plasma A β 40/42 ratio can predict the development of AD and dementia ⁷. However, these findings indicated significant heterogeneity ⁷, and plasma levels of A β 40 and A β 42 alone were not significantly associated ^{8,9}.

The Alzheimer's Disease Neuroimaging Initiative (ADNI) and the Dominantly Inherited Alzheimer Network (DIAN) have confirmed the efficacy of neuropsychiatric tests and neuroimaging using cerebrospinal fluid (CSF) biomarkers, including amyloid PET, demonstrating that signatures of brain A β amyloidosis can be found approximately 30 years before the onset of dementia ^{10,11}. Recent studies have clarified that the plasma A β 42/40 ratio is inversely correlated with cortical amyloid burden in AD, which can be converted to MCI ^{12,13}, and that the plasma A β 42/40 ratio is a useful screening marker for brain A β amyloidosis in normal individuals ^{14,15}. Approximately 30~50% of A β in the plasma originates from the brain ¹⁵. Age, *APOE- ϵ 4* and

AD pathology are specific determinants of A β turnover kinetics from the brain to CSF, and finally to plasma ^{15,16}.

We therefore focused on determinant factors of plasma A β levels. As A β amyloidosis initiates mid-life, it is necessary to analyze these factors in large community-based studies on young adolescent to elderly subjects. Age and *APOE- ϵ 4* are two major factors accelerating CNS amyloidosis leading to the onset of AD dementia ¹⁷. The gene dose of *APOE- ϵ 4* may decrease plasma A β 42 levels with natural aging, or long-term preclinical stage of AD dementia ^{10,17}. For this reason, basic information on how plasma A β levels are regulated over time by blood biochemical factors, cognitive function, and lifestyle remains to be clarified in order to adjust plasma A β levels for CNS amyloidosis-specific markers ^{18,19}. Here, we analyzed definite factors of plasma A β of participants in The Iwaki Health Promotion Project (IHPP) in 2014, a community-based annual health checkup study designed to prevent and improve lifestyle-related diseases and quality of life.

Materials and Methods

Subjects

A total of 1,109 participants with complete data sets out of 1,167 enrolled participants were analyzed. The age of 619 participants ranged from 19 to 59 years (mean age of 54 years;

365 females) and 490 participants were older than 60 years of age (mean age of 68 years; 323 females). The baseline characteristics of participants are presented in Table 1. Clinical diagnoses of dementia, Alzheimer dementia (AD), and mild cognitive impairment (MCI) were based on the NIA-AA clinical criteria^{20,21}. A total of 200 medical and paramedical staff examined participants between 6:30 to 13:00 over 10 days at Iwaki culture center. After written informed consent, a mini-mental state examination (MMSE) for all participants, the logical memory II tests (delayed recall: LM-II) from the Wechsler Memory Scale-Revised (WMS-R), and a detailed questionnaire for memory disturbances and ADL conditions were performed for participants older than 60 years of age. During and after these items, medical and neurological examinations, motor performance, blood pressure, height, body weight, BMI, and body fatty ratio (BFR) were evaluated, and common laboratory tests were performed for complete blood cell count, nutrition, liver and renal function, diabetes mellitus, cholesterol and lipid metabolism, endocrine system, immunology, cardiovascular biomarkers, and urine analysis (details in Supplementary table 1 and 2).

AB40 and AB42 Quantitation

Ten milliliters of morning fasting blood was taken into an EDTA-2Na tube and immediately centrifuged at 3,000 rpm for 10 minutes, separated to plasma in a polypropylene tube, and stored frozen at -80°C until use. Sandwich ELISA was used to quantify plasma A β x-

40 and A β x-42 levels using a Human/Rat β Amyloid (40) ELISA Kit Wako II and a Human/Rat β Amyloid (42) ELISA Kit Wako High-Sensitive (Wako Pure Chemical Industries, Ltd, Osaka, Japan)^{22,23}. Microplates were pre-coated with monoclonal BNT77 (IgA, anti-A β 11-28, specific for A β 11-16) and sequentially incubated with 25 μ l of samples, followed by application of horseradish-peroxidase-conjugated BA27 (anti-A β 1-40, specific for A β 40) or BC05 (anti-A β 35-43, specific for A β 42/43). The sensitivity was 0.049 pmol/L (assay range 1.0-100 pmol/L) in the A β 40 assay and 0.024 pmol/L (assay range 0.01-20.0 pmol/L) in the A β 42 assay. Intra- and inter-assay coefficients of variation were less than 10% for both A β 40 and A β 42. After excluding samples with mean values over +3 standard deviation by Grubbs' method^{24,25}, 1,091 assay values were analyzed.

***APOE* genotyping**

DNA of 1,151 Iwaki residents was purified from peripheral whole blood using the QIAamp® 96 DNA Blood Kit (QIAGEN, Hilden, Germany), and *APOE* genotype was determined by Toshiba corporation using the Japonica Array consisting of population-specific SNP markers designed from the 1,070 whole genome reference panel^{26,27}. Fifty-three samples that were not determined by the microarray analysis were genotyped by direct sequencing by the Greiner corporation using the following primer set: Forward primer; 5' TGG ACG AGA CCA TGA AGG AGTT and reverse primer; CAC CTG CTC CTT CAC CTC GTCCA, except for 11

samples that we analyzed using the following primer set: Forward primer; 5' TGG ACG AGA CCA TGA AGG AGT and reverse primer; CAC CTG CTC CTT CAC CTC GTCCA.

Statistical Analysis

Plasma A β 40, A β 42, A β 40/42 ratios did not deviate significantly from normal distribution according to the histograms. To clarify the relationships between plasma A β levels and other factors, including blood examination data, life style, and motor functions, correlation analysis was used. For comparison of normal distribution factors, Pearson's correlation coefficient analysis was applied. If normalization was not possible, Spearman's rank correlation coefficient analysis was used. To examine the effects on plasma A β by aging, linear regression models were used. To plot the age-dependent changes in plasma A β , the simple linear regression model was applied, and the linear regression line was drawn by the method of least squares. To compare the significance between the slopes of the linear regression models and to adjust for confounding factors, multiple regression analysis was applied. To examine whether A β and cognitive function are related, we compared the plasma A β levels between the high MMSE scores group (29 or 30) and low MMSE scores (less than 29) in subjects aged 60 years and over. In this group comparison, multiple logistic regression was used to adjust for age. Two-tailed *p*-values less than 0.05 were considered significant. These analyses were performed with IBM SPSS Statistics, version 24 (IBM Japan, Tokyo) and GraphPad Prism, version 7 (GraphPad Software, San Diego,

CA). In this study, statistical analyses were conducted with all 1,019 participants, including 991 normal, 26 MCI and 2 AD dementia individuals.

Results

Plasma A β Levels and relationship with *APOE* genotype

The mean \pm SD of the A β 40 plasma level was 106.2 \pm 15.5 pmol/L, that of the A β 42 level was 11.36 \pm 1.7, and that of the A β 40/42 ratio was 9.42 \pm 1.1 in all participants. A significant linear increase with age was observed for A β 40 levels ($Y=0.4724X+79.65$, $r^2=0.2208$, $p<0.0001$), A β 42 levels ($Y=0.02466X+10.04$, $r^2=0.04898$, $p<0.0001$), and the A β 40/42 ratio ($Y=0.02234X+8.113$, $r^2=0.09725$, $p<0.0001$) (Fig. 1A-C).

To evaluate whether the *APOE*- ϵ 4 alleles affect plasma A β levels, age-dependent changes in plasma A β levels between *APOE*- ϵ 4 carriers and non-carriers were analyzed. Age-dependent increases in A β 40 levels were observed in both non-*APOE*- ϵ 4 allele carriers ($Y=0.4619X+80.29$, $r^2=0.2163$, $p<0.0001$) and *APOE*- ϵ 4 carriers ($Y=0.5153X+77.08$, $r^2=0.2389$, $p<0.0001$). A β 42 levels were increased in non-carriers ($Y=0.02984X+9.842$, $r^2=0.07497$, $p<0.0001$) but not in *APOE*- ϵ 4 carriers ($Y=0.0001912X+10.92$, $r^2=0.00002616$, $p=0.8068$) with aging. The A β 40/42 ratios were increased both in non-carriers ($Y=0.01701X+8.327$, $r^2=0.066$, $p<0.0001$) and carriers ($Y=0.04561X+7.159$, $r^2=0.2658$). Plasma A β 40 and A β 42 levels, and the A β 40/42 ratio

increased with aging, except for A β 42 levels in *APOE- ϵ 4* carriers by simple linear regression (Fig. 2A-F).

After adjusting for total protein, platelet count and creatinine levels, which were previously reported as confounding factors for plasma A β levels^{18,19}, the multiple linear regression model was used to clarify whether the age-dependent increases in A β levels were affected by *APOE- ϵ 4*. There were significant differences between carriers and non-carriers in regression lines of A β 42 ($p < 0.0001$) and A β 40/42 ($p < 0.0001$) but not A β 40 ($p = 0.76$) (Fig. 3A-B, details in Supplementary table 3). To further validate these results, multiple linear regression model analyses were performed after adjustments for hemoglobin, platelet count, albumin, creatinine, blood urea nitrogen, fasting plasma glucose (FPG), free fatty acid, hemoglobin A1c and cystatin C, which were all found to be correlated with both plasma A β 40 and A β 42 levels in our study. There were also significant differences between carriers and non-carriers in regression lines of A β 42 ($p = 0.001$) and A β 40/42 ($p < 0.0001$) but not A β 40 ($p = 0.923$) (details in Supplementary table 4). Thus, the age-dependent increases in A β 42 levels were suppressed by *APOE- ϵ 4*, whereas age-dependent increases in the A β 40/42 ratio were enhanced by *APOE- ϵ 4*.

Association between MMSE scores and plasma A β levels

Subjects aged 60 years old and over were separated into high MMSE score (30, 29 points; $n = 340$) or low MMSE score (less than 28 points; $n = 150$) groups. Plasma A β 40, A β 42, and

A β 40/42 ratio levels were plotted, and an asterisk was plotted when there were significant differences between the two groups on multiple logistic regression analyses after adjusting for age (Fig.4A-C). There was no significant difference in variables for A β 40 levels ($p=0.25$). However, significant differences in variables for both age and A β 42 were observed for A β 42 ($p<0.0001$ and $p=0.04$), and also by the model chi-squared test ($p<0.0001$). The Hosmer-Lemeshow test demonstrated good predictability ($p=0.502$), with a discrimination predictive value of 69.0%. On analysis of the plasma A β 40/42 ratio, there were significant differences in both age and A β ratio (<0.0001 and $p=0.046$), and by the model chi-squared test ($p<0.0001$). Predictability was good ($p=0.502$), with a discrimination predictive value of 70.2% (details in Supplementary table 5). There were no significant differences in A β concentrations between “AD and MCI group” and “randomly selected age and APOE genotype-matched high MMSE score group (28 participants)”. Each p value was 0.8838 in A β 40 level, 0.4647 in A β 42 level, and 0.2158 in A β 40/42 ratio.

Factors affecting plasma levels of A β

Although the other blood chemistry test items were found to have significant linear correlations with A β levels, the correlation coefficients were very low. A strong correlation was only noted between cystatin C levels and A β 40 levels ($r = 0.5276$). These results are shown in supplementary table 1 and 2. We additionally analyzed the correlation between plasma A β levels

and habits or physical conditions. Weak correlations between both A β 40 and A β 42 levels, and alcohol intake, smoking amount, body fat ratio, and muscle mass were observed. Measurements of 4 major complex motor reaction tests, including the ruler drop test, timed up and go test, 10 meter walk test, and whole-body reaction time test, were more associated with plasma A β 40 and A β 42 levels than simple muscle strength, but the correlation coefficients were low.

Discussion

Our results demonstrated the following: 1) Fasting plasma levels of A β 40 and A β 42, and the A β 40/42 ratio age-dependently increased from 20 years old. 2) The presence of *APOE- ϵ 4* suppressed these age-dependent increases in plasma A β 42 levels. 3) Age and *APOE- ϵ 4* were most significant factors for plasma A β 42 levels and A β 40/42 ratios after adjusting for previously indicated and newly examined factors, including blood chemistry, life style, and activity. 4) Only renal function was a definitive factor for plasma A β 40 levels. 5) Plasma A β 42 levels and A β 40/42 ratios were correlated with lower MMSE scores in subjects aged over 60 years.

With a longer follow-up, repeated measurement of plasma A β may be useful as a simple and minimally invasive screening procedure to detect brain A β amyloidosis¹⁴⁻¹⁶. A β in plasma does not only originate in the brain because it is also involved in amyloid precursor protein (APP) metabolism in peripheral organs, it binds to several proteins and lipoproteins, is partially released

from activated platelets, and is metabolized in the liver and cleared through the kidneys ¹⁹. However, a recent study suggested that 30~50% of plasma A β originates from the CNS ¹⁵. *APOE- ϵ 4* is the strongest genetic risk factor for sporadic late onset AD, and markedly accelerates A β amyloid deposition in the brain and the onset age of dementia by approximately 10 years ^{10,17}. Recent studies have revealed that CNS-derived A β is cleared into the CSF ²⁸ and peripheral blood ²⁹, and that the clearance rate is decreased in late onset AD ³⁰, and is differently regulated by age and presence of A β amyloidosis ^{15, 31}. Association of plasma A β levels and cortical amyloid burden is also modulated by *APOE* isoforms ³². Together with these data, our findings that aging and *APOE- ϵ 4* are critical factors for plasma A β 42 levels from 20 years of age are consistent with A β 42 clearance from the brain to peripheral plasma. For this reason, adjustments of the plasma A β 42 level and A β 40/42 ratio for age, and *APOE- ϵ 4* allele at any age are essential for evaluating plasma A β levels as biomarkers of the progress of brain A β amyloidosis or clinical trials of disease modifying drugs.

Technical problems, including storage tubes, temperature, periods, buffers, and pipetting, during the assay procedure affect plasma A β levels ²⁷. Sleep-wake cycles of A β production and clearance also affect CNS A β levels ³³. We carefully managed fasting morning blood sampling, storage, and assay procedures, and obtained intra- and inter-assay coefficients with a variation of less than 10% in both A β 40 and A β 42 assays. We then analyzed the correlations among plasma

A β and other blood factors. In the ADNI cohort, platelet count, creatinine, and total protein affected plasma A β levels^{18,19}. However, the IHPP cohort comprising a wide range of age did not report similar findings. Creatinine levels were correlated with plasma A β 40 and A β 42 as well as previous study^{18,34}. The present study demonstrated a strong correlation between plasma A β 40 and cystatin C levels. Cystatin C may respond to plasma A β and renal function more sensitively than creatinine. Higher LDL-C and Lower HDL-C levels were both associated with cerebral amyloidosis³⁵ but not with late life cholesterol or AD neuropathology³⁶. Our results suggested that serum cholesterol levels are not directly correlated with plasma A β levels. Type 2 diabetes mellitus is a well-known risk factor for AD. Type 2 diabetes is positively associated with CSF A β 42, but negatively associated with cerebral cortical A β burden³⁷. Although a few large scale-studies have reported an association between glucose metabolism and plasma A β by strict sampling of morning fasting blood, we found no correlation among plasma A β levels, FPG, hemoglobin A1c and glycoalbumin, indicating no direct relationship between plasma A β and blood glucose levels. In conclusion, there were no strong determinant factors directly related with plasma A β levels, except Cystatin C for A β 40 level, in the IHPP cohort.

Regarding the relationship between plasma A β and lifestyle, no direct association was found with systolic or diastolic blood pressure^{38,39}, nor with alcohol intake, hours of sleep or smoking amount by questionnaire survey. Physical and motor activity, including 10MWT, RDT,

TUG, and WBRT as candidates for integrated cognitive processes that require attention, planning, visuospatial, and motor processes, demonstrated linear associations with the plasma A β 40/42 ratio. However, these correlation coefficients were weak, suggesting that plasma A β 40/42 is not a predictor for complex motor activity related with cognitive function ⁴⁰.

Prior major cohort studies have reported that plasma A β is a risk factor or predictive marker for AD onset in healthy older community members aged at least 55 years ¹⁻¹². In contrast, after analyzing fasting blood samples from healthy individuals of a wide age range, we observed the natural course of and factors affecting plasma A β 40 and A β 42. The period from mid-life to elderly is critical for preclinical progression of A β amyloidosis. Consistent with other reports, we found that decreased plasma A β 42 levels and increased A β 40/42 ratio were associated with low cognitive ability in participants aged over 60 years. Furthermore, plasma A β 42 levels were stably regulated mainly by age and *APOE- ϵ 4*. As this study was cross-sectional, we were unable to validate plasma A β 42 and A β 40/42 ratio as a predictive biomarker for the onset of AD. This is one limitation of our study. Furthermore, we were also unable to analyze the association between A β and vascular factors by MRI. To resolve these limitations, longitudinal confirmation is necessary. To confirm this basic data from the 2014 study, we are repeating the same annual surveys from 2015~2017, to clarify the factors of plasma A β and evaluate plasma A β 40 and A β 42 as biomarkers of onset of A β amyloidosis in the brain.

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Author Contributions

T.N., S.N., and M.S. conceptualized and designed the study. T.N., N.N., S.N., and K.I. acquired and analyzed the data. T.N., T.K., Y.S., M.H., K.I., S.N., and M.S. drafted the text and prepared the figures.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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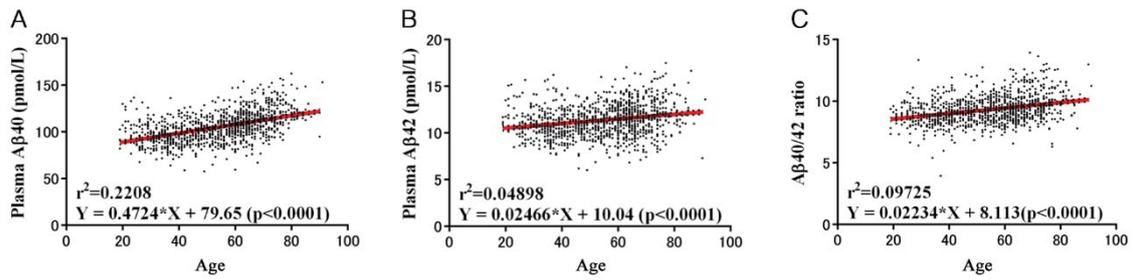
Table 1. Baseline characteristics of participants in the IHPP.

Characteristics (average and SD)	Total population	19~59 y	60~92 y
Number of Participants	1,109	619	490
Age (y)	54.2 (15.3)	43.1 (10.4)	68.2 (6.4)
Gender (female / male)	688/421	365/254	323/167
Height (cm)	160.1 (9.3)	163.6 (8.6)	155.6 (8.1)
Weight (kg)	58.4 (11.3)	60.2 (12.4)	56.1 (9.2)
Education (years)	11.8 (1.8)	12.5 (1.5)	11.0 (1.8)
MMSE score	29.3 (1.3)	29.7 (0.7)	28.7 (1.7)
A β 40 (pmol/L)	106.2 (15.5)	100.3 (12.9)	113.5 (15.3)
A β 42 (pmol/L)	11.36 (1.70)	11.0 (1.55)	11.8 (1.80)
A β 40/A β 42 ratio	9.42 (1.10)	9.16 (0.98)	9.74 (1.16)
Number of <i>APOE-ϵ4</i> alleles			
0 (ϵ 2/ ϵ 3, ϵ 3/ ϵ 3)	878	478	400
1 (ϵ 2/ ϵ 4, ϵ 3/ ϵ 4)	225	135	90
2 (ϵ 4/ ϵ 4)	6	6	0
Alzheimer's dementia	2	N.D.	2
Mild cognitive impairment	26	N.D.	26
Normal	1,081	619	462

SD: standard deviation; MMSE: mini-mental state examination;

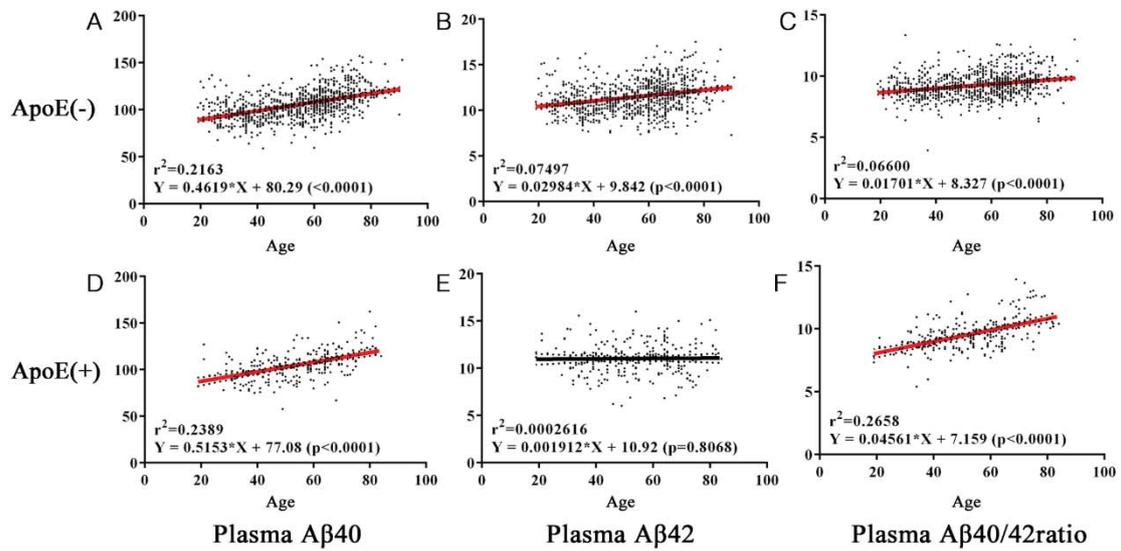
y: years of age; N.D.: not determined.

Figure 1.



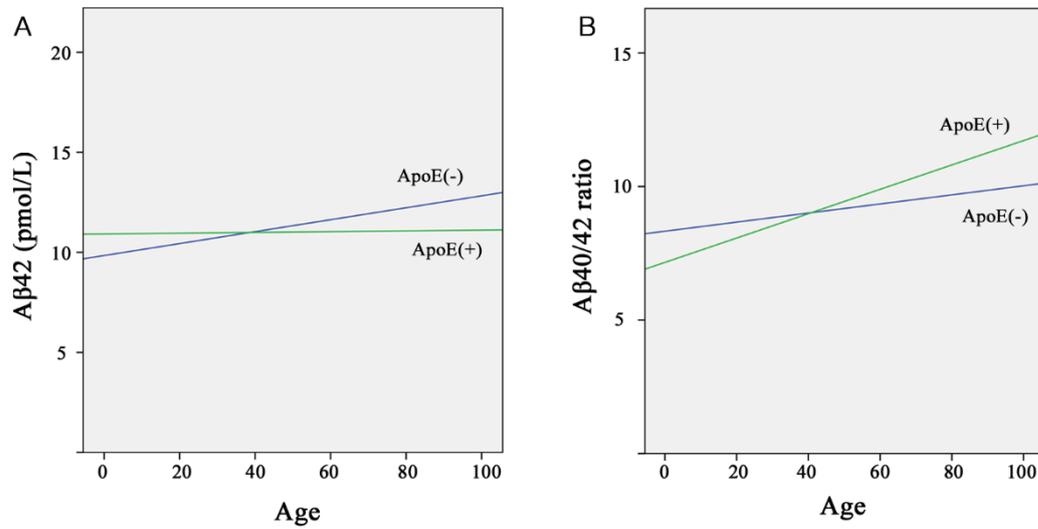
Age-related plasma Aβ changes. The relationship between age and plasma levels of Aβ or the Aβ40/42 ratio analyzed by linear regression. Determination coefficients (r^2) and regression equations are shown (N = 1109). Significant linear increases with age were observed for plasma Aβ40 and Aβ42 levels, and Aβ40/42 ratio (A-C).

Figure 2.



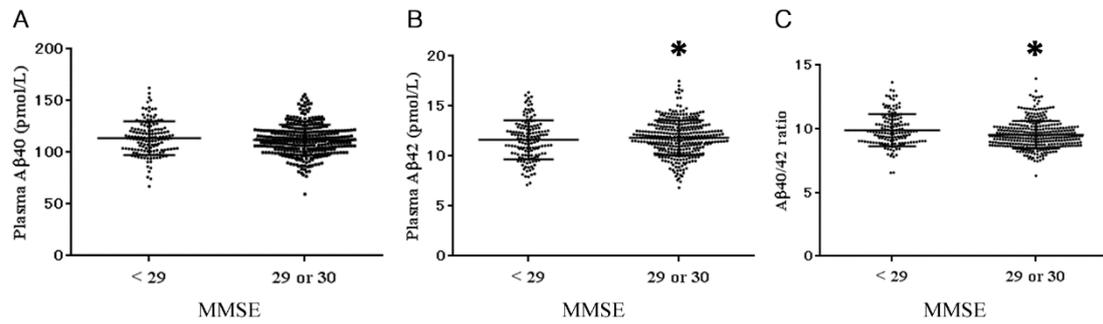
APOE-ε4 suppresses age-dependent plasma Aβ increases. Analyses of the age-related plasma Aβ changes were performed for APOE-ε4 carriers and noncarriers separately. Age-dependent increases in Aβ40 levels and the Aβ40/42 levels were observed in both noncarriers (A, C) and APOE-ε4 carriers (D, F). Levels of Aβ42 were increased in noncarriers but not in APOE-ε4 carriers with aging (B, E).

Figure 3.



APOE-ε4 alters age-dependent Aβ42 levels and Aβ40/42 ratio. The regression lines for age-related plasma Aβ42 and the Aβ40/42 ratio in APOE-ε4 carriers and noncarriers were merged. There were significant differences between carriers and noncarriers in regression lines for Aβ42 (A) and Aβ40/42 (B) after adjusting for total protein, platelet count, and creatinine levels.

Figure 4.



Correlation between MMSE scores and plasma Aβ levels. Comparison of plasma Aβ levels between high MMSE score and low MMSE score groups of subjects aged 60 years and over.

There were significant differences (*) between the two groups in Aβ42 levels and the Aβ40/42 ratio on multiple logistic regression analyses after adjusting for age (A-C).

Supplementary table 1. Correlation between plasma levels of A β and other blood tests 1.

	A β 40		A β 42		A β 40/A β 42 ratio	
	p	r	p	r	p	r
White Blood Cell	0.6166	0.01527	0.0866	-0.05148	0.0166	0.07193
Hemoglobin	0.0003	-0.1092	<0.0001	-0.2028	<0.0001	0.1214
Platelets	0.0314	-0.06468	0.0176	-0.07134	0.7577	0.009284
Total protein	0.9856	-0.0005	0.1341	-0.04501	0.1818	0.04013
Albumin	<0.0001	-0.1453	<0.0001	-0.1326	0.4957	-0.02048
Total bilirubin	0.4357	-0.02343	0.0577	-0.05702	0.1283	0.0457
AST	0.0067	0.0814	0.2364	-0.0356	<0.0001	0.1615
ALT	0.0597	-0.05659	<0.0001	-0.1205	<0.0001	0.1202
γ -GTP	0.1693	-0.04132	<0.0001	-0.1272	<0.0001	0.1443
Creatinine	<0.0001	0.1815	0.0026	0.009037	0.0015	0.09536
Blood Urea Nitrogen	<0.0001	0.311	<0.0001	0.1852	<0.0001	0.1486
Cystatin C	<0.0001	0.5276	<0.0001	0.3327	<0.0001	0.2476
Uric acid	0.4007	0.02526	0.1835	-0.03997	0.0086	0.07883
Total cholesterol	0.5331	0.01873	0.8033	-0.00749	0.2708	0.03309
Triglyceride	0.0003	0.1083	0.7971	-0.007727	<0.0001	0.1608
HDL-Cholesterol	0.007	-0.08089	0.8505	0.005665	0.0003	-0.1092
LDL-Cholesterol	0.1806	0.04023	0.8422	-0.005986	0.0419	0.06112
Free fatty acid	<0.0001	0.2309	<0.0001	0.1302	0.0001	0.1166

Supplementary table 2. Correlation between plasma levels of A β and other blood tests 2.

	A β 40		A β 42		A β 40/A β 42 ratio	
	p	r	p	r	p	r
FPG	<0.0001	0.2131	0.0019	0.09328	<0.0001	0.1671
Hemoglobin A1c	<0.0001	0.1866	0.0001	0.1141	0.0005	0.1041
Glycoalbumin	<0.0001	0.2105	<0.0001	0.1773	0.3076	0.03068
BNP	<0.0001	0.2464	<0.0001	0.1635	0.0093	0.07809
Adiponectin	<0.0001	0.1467	<0.0001	0.1337	0.6344	0.0143
Alcohol intake	<0.0001	-0.1934	<0.0001	-0.197	0.281	0.0324
Hours of sleep	<0.0001	0.119	0.1134	0.04757	0.0024	0.09103
Smoking amount	0.014	-0.07374	<0.0001	-0.1207	0.0037	0.0872
Lung capacity	0.0015	0.09499	0.4681	0.02181	0.0017	0.09401
Body fat ratio	0.0001	0.1153	<0.0001	0.1314	0.4863	-0.02099
Muscle mass	<0.0001	-0.2493	<0.0001	-0.2768	0.006	0.0827
Bone density (Z-score)	0.9476	-0.001975	0.1972	-0.03877	0.0774	0.05307
Systolic blood pressure (BP)	<0.0001	0.1294	0.8067	0.007361	<0.0001	0.1495
Diastolic BP	0.9134	-0.00327	0.0195	-0.07015	0.0058	0.08285
Ruler drop test	<0.0001	0.2211	<0.0001	0.1334	0.001	0.1101
Timed up and go	<0.0001	0.2067	0.0072	0.09169	0.0001	0.1321
10 meter walk	<0.0001	0.2731	0.0002	0.1519	<0.0001	0.136
Whole body reaction	<0.0001	0.1676	<0.0001	0.1541	0.6789	-0.01682

Supplementary table 3. Result of multiple linear regression model analysis about whether age-dependent increases in A β levels are affected by presence of *APOE- ϵ 4* adjusting for total protein, platelet count and creatinine levels.

	Partial regression coefficient	Standard regression coefficient	partial P-value	95% confidence interval
Fixed number (A β 40)	64.46		0.00	49.69~79.23
Age	0.49	0.49	0.00	0.44~0.54
<i>APOE</i>	-0.30	-0.01	0.76	-2.23~1.62
Fixed number (A β 42)	10.79		0.00	8.95~12.62
Age	0.024	0.22	0.00	0.018~0.031
<i>APOE</i>	-0.42	-0.10	0.00	-0.66~-0.18
Fixed number (A β 40/42)	6.54		0.00	5.40~7.68
Age	0.024	0.00	0.00	0.020~0.030
<i>APOE</i>	0.37	0.08	0.00	0.22~0.52

Supplementary table 4. Result of multiple linear regression model analysis about whether age-dependent increases in A β levels are affected by presence of *APOE- ϵ 4* after adjustments for hemoglobin, platelet count, albumin, creatinine, blood urea nitrogen, fasting plasma glucose, free fatty acid, hemoglobin A1c and cystatin C.

	Partial regression coefficient	Standard regression coefficient	partial P-value	95% confidence interval
Fixed number (A β 40)	64.14		0.00	48.10~80.18
Age	0.18	-0.002	0.00	0.11~0.24
<i>APOE</i>	-0.084	0.17	0.92	-1.80~1.63
Fixed number (A β 42)	11.60		0.00	9.54~13.66
Age	-0.009	-0.082	0.03	-0.017~-0.001
<i>APOE</i>	-0.357	-0.085	0.00	-0.58~-0.14
Fixed number (A β 40/42)	5.68		0.00	4.29~7.06
Age	0.024	0.034	0.00	0.018~0.03
<i>APOE</i>	0.33	0.12	0.00	0.18~0.48

Supplementary table 5. Result of multiple logistic regression analyses between plasma A β and MMSE scores after adjusting for age.

	Partial regression coefficient	P-value	95% confidence interval
Age	-0.081	0.00	0.89~0.95
Plasma A β 40	-	0.398	-
Fixed number	6.40	0.00	
Age	-0.087	0.00	0.89~0.95
Plasma A β 42	0.117	0.04	1.01~1.26
Fixed number	5.415	0.00	
Age	-0.074	0.00	0.90~0.96
Plasma A β 40/42	-0.175	0.046	0.71~1.00
Fixed number	7.626	<0.0001	