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Blood acetylcholinesterase level is a potential biomarker for the early detection of cerebral amyloid deposition in cognitively normal individuals

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ABSTRACT

Cerebral β-amyloid (cAβ) deposition and cholinergic dysfunction have been considered as major pathological and functional hallmarks of Alzheimer's disease (AD). Acetylcholinesterase (AChE) is one of the major cholinergic enzymes, and there is no report to show the relationship between cAβ accumulation and peripheral AChE alteration in early stage of AD pathogenesis. Recent studies demonstrate that cAβ starts to deposit 15–20 years ahead of symptomatic appearance and this preclinical AD is important for early diagnosis of disease. In this study, we investigated the link between cAβ deposition and the peripheral AChE in cognitively normal (CN) individuals. A total of 407 individuals who underwent Pittsburgh compound B (PiB)-positron emission tomography participated in our study. Lower levels of plasma AChE and its enzymatic activity were detected in CN individuals with cAβ deposition than in those without cAβ. Plasma AChE levels and enzymatic activity were negatively correlated with the degree of cAβ deposition. Our results suggest that blood AChE can be used as a potential blood biomarker for the prediction of cAβ deposition in CN individuals.

1. Introduction

Alzheimer disease (AD) is the leading cause of dementia and the fastest growing neurodegenerative disease in the elderly population. Well known as the amyloid hypothesis, cerebral accumulation of the amyloid-β (Aβ) peptide is the main pathological hallmark of AD and is related to the neuronal toxicity and synaptic disruption that occurs during pathogenesis (Hardy and Higgins, 1992; Hardy and Selkoe, 2002). Abnormal accumulation of excess Aβ peptides in the brain leads to Aβ oligomerization, aggregation, fibrillation, and, finally, the formation of senile plaques on interaction with diverse proteins and metal ions in the brain (Han et al., 2016; Wisniewski et al., 1997), which lead to cognitive dysfunction and memory impairment through neuronal death and synaptic dysfunction (Lesne et al., 2006).

AD is a neurodegenerative disease and progresses for many years; brain Aβ accumulation starts to appear 10–15 years before the onset of clinical symptoms (Bateman et al., 2012). Recently, preclinical AD, characterized by the presence of cerebral amyloidosis observed from either amyloid positron emission tomography (PET) or Aβ analysis of the cerebrospinal fluid (CSF) in cognitively normal (CN) individuals, emerged as a promising topic in AD research (Dubois et al., 2016; Epelbaum et al., 2017). However, amyloid PET imaging is fairly expensive and not easily available in many clinical settings, and CSF Aβ analysis has limitations due to its invasive procedure and lack of interinstitution measurement uniformity. Thus, it is hard to use these procedures routinely to screen or diagnose preclinical AD in nontertiary hospital settings (Nordberg et al., 2010). Recently, many researchers have tried to

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find out blood biomarkers representing cerebral Aβ deposition in cognitively impaired subjects (Kang et al., 2016; Park et al., 2017a,b). However, desperate need is unmet for such biomarkers to represent cerebral Aβ deposition in fairly early stage without cognitive dysfunction.

Among many neuronal cell types, cholinergic neurons have been suggested as one of the main victims during AD pathogenesis according to the cholinergic hypothesis of AD (Francis et al., 1999). The neocortex and the hippocampus with abundant cholinergic innervation from basal forebrain nuclei are the major target areas of the AD pathology (Francis et al., 1999), and enzymes responsible for acetylcholine (ACh) synthesis and degradation are vulnerable during the course of AD pathogenesis (Francis et al., 1999; Perry et al., 1977a). Acute and chronic Aβ exposure induces cholinergic toxicity; furthermore, direct interaction of Aβ with nicotinic ACh receptors has also been reported (Kar et al., 2004; Preda et al., 2008). The deterioration of cholinergic neurons and the dysfunction of the cholinergic system are closely related to cognitive impairment in AD (Bartus et al., 1982). Application of cholinomimetic drugs is the main strategy to improve cognitive impairments in AD patients.

Acetylcholinesterase (AChE), one of the main cholinergic enzymes, is a carboxylic ester hydrolase and belongs to the cholinesterase family (Lionetto et al., 2013). It exists in cholinergic synapses and neuromuscular junctions and breaks down the ester of choline and hydrolyzes acetylcholine at the postsynaptic membrane. AChE modulates synaptic transmission and plays a key role in the functioning of the central and peripheral nervous systems. AChE is also found in the membrane of red blood cells as a Yt blood group antigen, although its physiological function in this setting is not yet clear (Daniels, 2007). AChE is widely known to undergo a dynamic alteration during AD pathogenesis. Reduction of brain AChE activity has been demonstrated in patients with mild cognitive impairment (MCI) and AD dementia (Perry et al., 1977a,b; Rinne et al., 2003). Decreased AChE activity has also been reported in the cerebrospinal fluid (CSF) of patients with severe dementia (Sirvio et al., 1989). Furthermore, alteration of the brain AChE system is associated with reduced AChE in lymphocytes and increased plasma AChE activity in AD dementia brains (Atack et al., 1985; Inestrosa et al., 1994). However, to our knowledge, no report so far has shown a direct relationship between brain Aβ accumulation and peripheral AChE alteration.

The purpose of this study is to investigate whether peripheral AChE levels are correlated with cerebral Aβ accumulation. A total of 407 individuals, including CN individuals, patients with MCI, and patients with AD dementia, underwent Pittsburgh compound B positron (PiB)-PET to quantify cerebral Aβ deposition; their plasma AChE levels were quantified as well. We had 3 specific aims: first, to test the association between peripheral AChE levels and cerebral Aβ deposition in CN individuals; second, to investigate whether plasma AChE increases the discrimination power between PiB− CN (CN−) and PiB+ CN (CN+); and third, to explore the influences of AChE inhibitor (AChEI) treatment on the plasma AChE level.

2. Methods

2.1. Participants

This study was a part of the Korean Brain Aging Study for Early Diagnosis and Prediction of Alzheimer's Disease (KBASE) (Byun et al., 2017). The aim of KBASE is to search for possible biomarkers of AD and observe and record AD-related brain changes. Our work was approved by the Institutional Review Board (IRB) of Seoul National University Hospital and SMG-SNU Boramae Medical Center, South Korea. The participants or their legal representatives provided written informed consent to participate in this study, in accordance with the Declaration of Helsinki.

Total 407 individuals, with ≥55 years, including 241 CN individuals, 103 patients with MCI, and 63 patients with AD dementia participated in the study. All of them underwent comprehensive clinical and neuropsychological assessments, blood tests, and neuroimaging exams. Individuals meeting the following criteria were not included in this study: (1) having communication disorders that would create difficulty in conducting assessments, (2) having any psychiatric or neurological disorder that could affect the normal psychological process, (3) with contraindications in magnetic resonance imaging (MRI) scans, (4) lacking a reliable informant, and (5) illiterate. All CN individuals had a clinical dementia rating (CDR) of 0 and performed within the normal range, relative to the age-, sex-, and education-adjusted normative mean on comprehensive neuropsychological assessments (Lee et al., 2002; Morris, 1993).

Patients with MCI met the following criteria: (1) subjective memory complaint proved by the patients themselves, by an informant, or by a clinician, (2) objective memory impairment considering their physical condition, (3) normal functional activities, and (4) no dementia. All MCI patients had a global CDR score of 0.5. In terms of criterion (2), they scored at least 1.0 standard deviations (SDs) below the respective age-, education-, and sex-specific means for at least one of the 4 memory tests that come under the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) neuropsychological battery (Lee et al., 2004). Patients with AD dementia met the following inclusion criteria: (1) criteria for dementia in accordance with the Diagnostic and Statistical Manual, 4th Edition (DSM-IV) (American Psychiatric Association., 2000), (2) the criteria for probable AD, set in accordance with the National Institute of Aging and Alzheimer's Association (NIA-AA) guidelines (McKhann et al., 2011), and (3) CDR score of 0.5 or 1.

2.2. Clinical and neuropsychological assessment

All participants underwent comprehensive clinical and neuropsychological assessments, including the Mini-Mental State Examination (MMSE) based on the KBASE protocol, which is an incorporation and extended version of the Korean version of the Consortium to Establish a Registry for Alzheimer's Disease Assessment Packet (Lee et al., 2002, 2004). To control for the effects of age, sex, and education levels, the MMSE *z*-score was used (Piccinin et al., 2013).

2.3. Pittsburgh compound B positron-positron emission tomography

All participants were subjected to three-dimensional PiB-PET imaging and T1-weighted MR imaging using a simultaneous 3.0 T PET-MR scanner (Biograph mMR scanner [Siemens, Washington DC, USA]). A 30-minute emission scan was acquired after 40 minutes of intravenous injection of 555 MBq of ^{11}C-PiB. The images were resized to a 256 × 256 image matrix using iterative methods (6 iterations with 21 subsets) and corrected for uniformity, UTE-based attenuation, and decay reduction. Sagittal T1-weighted MR images were acquired using the following parameters: repetition time = 1670 ms; echo time = 1.89 ms; field of view = 250 mm; 256 × 256 matrix with a 1.0 mm slice thickness. Image preprocessing was performed using Statistical Parametric Mapping 8. Transformation parameters for a standard Montreal Neurological Institute (MNI) template were obtained after coregistration of PiB-PET images to the individual T1 images. For the inverse transformation parameters that transform the coordinates from the automatic anatomic labeling (AAL) 116 atlas (Weiss, 1989) to an

individual space for each participant (resampling voxel size = 1 × 0.98 × 0.98 mm), Individual Brain Atlases using Statistical Parametric Mapping software were used, and the white matter and CSF space were excluded by applying a gray matter (GM) mask to each individual.

The mean ^{11}C-PiB uptake values in the cerebral regional were computed from the T1-coregistered PiB-PET images using the AAL116 atlas, with the cerebellar GM ^{11}C-PiB uptake value utilized to demarcate the reference region for normalization (Choe et al., 2014; Jack et al., 2008; Park et al., 2017b; Reiman et al., 2009). Brain regions, including the frontal, lateral parietal, posterior cingulate-precuneus (PC-PRC), and lateral temporal areas, where prominent ^{11}C-PiB retention has been reported (Klunk et al., 2004), and the regions of interest (ROIs) were determined by the AAL algorithm with a region-combining method (Yaffe et al., 2011). Then, a standardized uptake value ratio (SUVR) of each ROI was obtained by dividing the mean value for all voxels within each ROI by the mean cerebellar GM uptake value in the same image. Participants were classified as PiB-positive (PiB+) if the SUVR value was over 1.4 in at least one of the 4 ROIs (i.e., frontal, lateral temporal, lateral parietal, and PC-PRC) and PiB-negative (PiB−) if the SUVR values of all 4 ROIs were equal to or less than 1.4 (Choe et al., 2014; Reiman et al., 2009).

2.4. Blood sampling

Blood samples were obtained by venipuncture in the morning (around 9 AM) following an overnight fast and collected into K2 ethylenediaminetetraacetic acid (EDTA) tubes (BD Vacutainer Systems, Plymouth, UK). The tubes were stabilized at room temperature (RT) for 30 minutes and centrifuged at 700*g* for 5 minutes to obtain the plasma supernatants (SPL Life Sciences Co., Gyeonggi-do, Korea). The collected pure plasma supernatants were aliquoted and immediately stored at −80°C.

2.5. Enzyme-linked immunosorbent assay (ELISA)

To determine the concentrations of human plasma AChE, the human acetylcholinesterase (AChE) Quantikine ELISA Kit (R&D system, Minneapolis, MN, USA) was used. In brief, plasma samples and standards were added to each well and incubated for 2 hours at RT. Each well was aspired and washed, the human AChE conjugate was added, and the samples were incubated for 2 hours at RT. After repetition of the aspiration and washing steps, the 3,3′,5,5′-tetramethylbenzidine substrate was used to detect horseradish peroxidase enzyme activity, following which the stop solution was added. The plate was read at 450 nm, within 5 minutes.

2.6. AChE enzymatic activity (colorimetric assay) test

Plasma AChE enzymatic activity was quantified by acetylcholinesterase colorimetric assay kit (abcam, Cambridge, UK) according to manufacturer's instruction. Briefly, we prepared acetylthiocholine reaction mixture containing 20X 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB) stock solution and mixed with AChE standards and plasma samples. DTNB was used to quantify the thiocholine produced from the enzymatic hydrolysis of acetylthiocholine by AChE in plasma. Signal intensity was read by an absorbance at ∼410 nm.

2.7. Statistics

To compare the level of plasma AChE between the CN− and CN+ groups, with statistical control for the effects of covariates (age and sex), analysis of covariance (ANCOVA) was performed; a significant *p* value was obtained. To analyze the relationships between plasma AChE levels and global cerebral Aβ deposition in CN individuals, partial correlation analysis was used, with corrections for age and sex. For the independent association between plasma AChE levels and global cerebral Aβ deposition, multiple regression analyses were also performed, with covariates such as age, sex, apolipoprotein E ε4 (APOE4) positivity, and MMSE score. In addition, logistic regression analysis was performed, followed by receiver operating characteristic (ROC) curve analysis, to show the discrimination power of APOE4 positivity and plasma AChE on PiB positivity in CN individuals. Comparison of ROC curves was conducted as per the process described in a previous report, DeLong et al. (DeLong et al., 1988). Furthermore, the unpaired *t*-test was used to compare the concentration of plasma AChE between non-AChEI takers and AChEI takers, in the case of patients with MCI/AD dementia. Moreover, to show the relationship between plasma AChE and the risk of cerebral Aβ deposition, a relative risk (95% confidence interval; RR) test was conducted. Especially for RR test on cognitively impaired subjects (MCI or AD dementia), z-scores were used because AChEI takers have significantly higher AChE levels (5.52 ± 0.31 ng/mL; mean ± SEM) than non-AChEI takers (1.53 ± 0.06 ng/mL; mean ± SEM) ($^{****}p < 0.0001$). The levels of plasma AChE of AChEI taker group and nontaker group were standardized (z-scored) and used together for RR analysis.

For the demographic data, multifactorial analyses of variance (ANOVAs), followed by Tukey's multiple comparisons tests, were performed to compare values (age, education, MMSE *z*-score, and global amyloid deposition) between 3 groups or more. The χ^2 test was performed to compare the intergroup differences in categorical variables (sex, CDR score, and ApoE ε4 carrier status). All statistical analyses were performed using Medcalc 17.2 (Medcalc Software, Ostend, Belgium) and GraphPad Prism 7 (GraphPad software, San Diego, CA, USA).

3. Results

3.1. Demographic data of the participants

The participants were classified by PiB positivity (PiB+ and PiB−) and/or clinical diagnosis (CN, MCI, and AD dementia). Overall, the 407 individuals were classified as follows: 209 PiB− CN (CN−), 32 PiB+ CN (CN+), 53 PiB− MCI (MCI−), 50 PiB+ MCI (MCI+), 15 PiB− AD dementia (AD dementia−), and 48 PiB+ AD dementia (AD dementia+). See Table 1 for more details.

3.2. Relationship between plasma AChE or its activity and global cerebral amyloid deposition in the CN individuals

The CN+ group had lower plasma AChE protein levels than the CN− group ($^{***}p < 0.001$; Fig. 1A, left), and the partial correlation plot showed a significant association between plasma AChE protein levels and cerebral Aβ deposition in the CN individuals ($r = -0.20$, $^{**}p < 0.01$; Fig. 1A, middle). No correlation was observed between the plasma AChE level and MMSE scores (Fig. 1A, right) or other cognitive memory scores (Table S3). Also, the CN+ group had lower plasma AChE activity levels than the CN− group ($^{***}p < 0.001$; Fig. 1B, left), and the partial correlation plot showed a significant association between plasma AChE activity levels and cerebral Aβ deposition in the CN individuals ($r = -0.19$, $^{**}p < 0.01$; Fig. 1B, middle). No correlation was observed between the plasma AChE activity and MMSE scores (Fig. 1B, right) or other cognitive memory scores (Table S3). To identify the relationship between plasma AChE or its activity and the risk of cerebral Aβ deposition, we conducted relative risk (RR) analysis (Fig. 2). First, the participants were categorized into AChE quartiles (quartile 1, >1.5485 ng/mL; quartile 2, >1.2528 and ≤1.5485 ng/mL; quartile 3, >1.0088 and ≤1.2528 ng/

Table 1
Demographic data of the participants

Characteristics (n)	CN− (209)	CN+ (32)	MCI− (53)	MCI+ (50)	AD Dementia− (15)	AD Dementia+ (48)	*p*-value
Gender, M/F	96/113	16/16	17/36	17/33	3/12	15/33	0.0616[b]
Age, y, mean ± SEM	68.26 ± 0.6	73.59 ± 1.2	73.77 ± 1.0	74.14 ± 0.9	76.73 ± 1.6	71.77 ± 1.2	<0.0001[a]
Education, mean ± SEM	11.78 ± 0.3	12.28 ± 0.8	8.77 ± 0.6	10.34 ± 0.6	6.13 ± 1.4	9.92 ± 0.8	<0.0001[a]
MMSE raw score, mean ± SEM	26.93 ± 0.2	27.00 ± 0.4	22.89 ± 0.4	21.66 ± 0.4	15.40 ± 1.2	16.98 ± 0.6	<0.0001[a]
MMSE z score, mean ± SEM	0.27 ± 0.1	0.32 ± 0.2	−0.79 ± 0.2	−1.58 ± 0.2	−2.65 ± 0.3	−3.08 ± 0.2	<0.0001[a]
CDR (n)	0	0	0.5 (53)	0.5 (50)	0.5 (4), 1 (11)	0.5 (17), 1 (31)	<0.0001[b]
ApoE4 positivity, ε4+/N (%)	35/209 (17%)	12/32 (37%)	5/53 (9%)	27/50 (54%)	2/15 (13%)	32/48 (67%)	<0.0001[b]
Global amyloid deposition (SUVR), mean ± SEM	1.10 ± 0.01	1.69 ± 0.06	1.13 ± 0.01	1.91 ± 0.05	1.13 ± 0.08	2.12 ± 0.05	<0.0001[a]
AChEI positivity, taker/N (%)	0/209 (0%)	0/32 (0%)	10/53 (19%)	26/50 (52%)	13/15 (87%)	46/48 (96%)	<0.0001[b]

Key: − or +, PiB positivity; AChEI, acetylcholinesterase inhibitor; AD Dementia, Alzheimer's disease dementia; ApoE, apolipoprotein E; CDR, clinical dementia rating; CN, cognitively normal; MCI, mild cognitive impairment; MMSE, mini-mental state examination; MMSE z score, a revised value of the MMSE score with consideration for age, gender, and education level; N, total number of participants; PiB, Pittsburgh compound B; SEM, standard error of mean; n, number of participants; SUVR, standardized uptake value ratio.

[a] Significance by one-way analysis of variance (ANOVA) test.

[b] Significance by χ^2 test.

mL; quartile 4, ≤1.0088 ng/mL) in the same manner as that in previous reports (Duarte et al., 2016; White et al., 2005). The RR of incidence of PiB+ in the CN individuals in quartile 4 (≤1.0088 ng/mL) was significantly enhanced (RR = 8.00, $^{**}p < 0.01$; 95% CI, 1.92 to 33.30; Fig. 2A table) compared to that in quartile 1 (>1.5485 ng/mL). A chi-squared test for trend also demonstrated that the ratio of PiB+:PiB− gradually and significantly increased as the quartile number increased (first quartile to the fourth quartile) ($^{***}p < 0.001$, $\chi^2 = 13.915$, Fig. 2A table). Second, similarly to AChE protein quartiles, the participants were categorized into AChE activity quartiles (quartile 1, >197.83 mU/mL; quartile 2, >178.95 and ≤197.83 mU/mL; quartile 3, >157.12 and ≤178.95 mU/mL; quartile 4, ≤157.12 ng/mL). The RR of incidence of PiB+ in the CN individuals in quartile 4 (≤157.12 mU/mL) was significantly enhanced (RR = 3.00, $^{*}p < 0.05$; 95% CI, 1.16 to 7.73; Fig. 2B table) compared to that in quartile 1 (>197.83 mU/mL). A χ^2 test for trend also

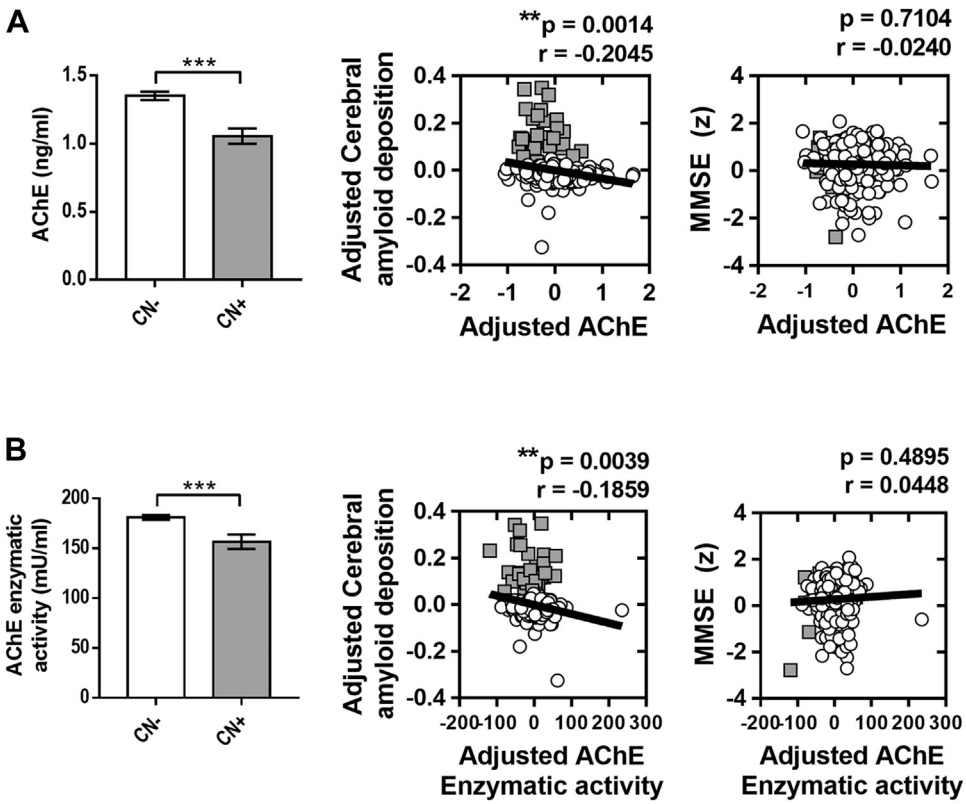
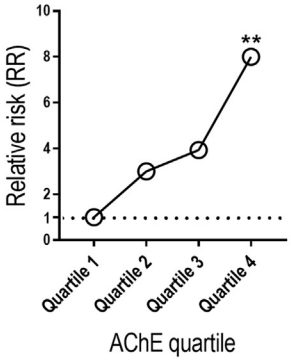


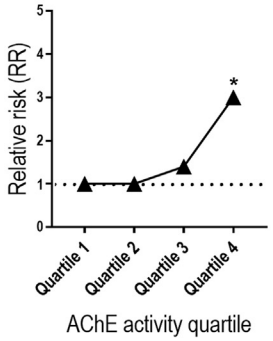
Fig. 1. Association of cerebral amyloid deposition with plasma AChE levels and AChE enzymatic activities in CN individuals. (A) Plasma AChE levels (ng/mL) and cerebral amyloid deposition. CN+ group had lower AChE levels compared to CN− group ($^{***}p < 0.001$, *p* value by ANCOVA comparing adjusted mean after controlling for the effect of age and sex). Partial correlation plot showing the relationship between global cerebral amyloid deposition (SUVR) and plasma AChE (r = −0.2045, $^{**}p = 0.0014$). MMSE z-score (age, sex, and education levels were corrected) has no correlation with plasma AChE (r = −0.0240, $p = 0.7104$). White circles, CN− group; gray squares, CN+ group. (B) Plasma AChE activity (mU/mL) and cerebral amyloid deposition. CN+ group had lower AChE activity compared to CN− group ($^{***}p < 0.001$, *p* value by ANCOVA comparing adjusted mean after controlling for the effect of age and sex). Partial correlation plot showing the relationship between global cerebral amyloid deposition (SUVR) and plasma AChE activity (r = −0.1859, $^{**}p = 0.0039$). MMSE z-score (age, sex, and education levels were corrected) has no correlation with plasma AChE activity (r = 0.0448, $p = 0.4895$). Global cerebral amyloid burden was a natural log transformed to normalize variance. Adjusted values (x-axis) were revised after controlling for the effect of age and sex. White circles, CN− group; gray squares, CN+ group. Abbreviations: AChE, acetylcholinesterase; PiB, Pittsburgh compound B; CN, cognitively normal; + or −, PiB positivity; SUVR, standardized uptake value ratio; MMSE, mini-mental state examination; 209 CN− and 32 CN+ for AChE levels; 208 CN− and 32 CN+ for AChE activity (One CN− sample excluded as an outlier).

A



Plasma AChE level (ng/mL)	PiB status		RR (95% CI)	P‡	P
	PiB-, n (%)	PiB+, n (%)			
Quartile 1 (> 1.5485)	58 (96.7)	2 (3.3)	1	-	
Quartile 2 (> 1.2528 and ≤ 1.5485)	54 (90.0)	6 (10.0)	3.00 (0.63 ~14.27)	0.1674	[a]**0.0018**
Quartile 3 (> 1.0088 and ≤ 1.2528)	53 (86.9)	8 (13.1)	3.93 (0.87 ~17.77)	0.0750	[b]**0.0002**
Quartile 4 (≤ 1.0088)	44 (73.3)	16 (26.7)	8.00 (1.92 ~33.30)	****0.0043**	

B



Plasma AChE activity (mU/mL)	PiB status		RR (95% CI)	P‡	P
	PiB-, n (%)	PiB+, n (%)			
Quartile 1 (> 197.83)	55 (91.7)	5 (8.3)	1	-	
Quartile 2 (> 178.95 and ≤ 197.83)	55 (91.7)	5 (8.3)	1.00 (0.31 ~3.28)	1.0000	[a]**0.0203**
Quartile 3 (> 157.12 and ≤ 178.95)	53 (88.3)	7 (11.7)	1.40 (0.47 ~4.17)	0.5453	[b]**0.0066**
Quartile 4 (≤ 157.12)	45 (75.0)	15 (25.0)	3.00 (1.16 ~7.73)	***0.0229**	

Fig. 2. The relationship between plasma AChE and their enzymatic activities (in quartiles) and the risk of cerebral amyloid accumulation. (A) Quartiles for plasma AChE levels. CN individuals were categorized into quartiles (quartile 1, >1.5485 ng/mL; quartile 2, >1.2528 and ≤1.5485 ng/mL; quartile 3, >1.0088 and ≤1.2528 ng/mL; quartile 4, ≤1.0088 ng/mL). Quartile 4 had the highest proportion of PiB+ (26.7%; $^{**}p = 0.0043$, RR = 8.00), and a χ^2 test for trend showed that the ratio of PiB+: PiB− gradually increased as the quartile number increased (first quartile to fourth quartile) ($^{b}p = 0.0002$). Abbreviations: PiB, Pittsburgh compound B; RR, relative risk; CI, confidence interval; P‡, P of RR; [a]P by χ^2 test = 15.055; [b]P by χ^2 test for trend = 13.915. (B) Quartiles for plasma AChE enzymatic activities. CN individuals were categorized into quartiles (quartile 1, >197.83 mU/mL; quartile 2, >178.95 and ≤197.83 mU/mL; quartile 3, >157.12 and ≤178.95 mU/mL; quartile 4, ≤157.12 mU/mL). Quartile 4 had the highest proportion of PiB+ (25.0%; $^{*}p = 0.0229$, RR = 3.00), and a χ^2 test for trend showed that the ratio of PiB+:PiB− gradually increased as the quartile number increased (first quartile to fourth quartile) ($^{b}p = 0.0066$). Abbreviations: PiB, Pittsburgh compound B; RR, relative risk; CI, confidence interval; P‡, P of RR; [a]P by χ^2 test = 9.808; [b]P by χ^2 test for trend = 7.385; 209 CN− and 32 CN+ for AChE levels; 208 CN− and 32 CN+ for AChE activity (One CN− sample excluded as an outlier).

demonstrated that the ratio of PiB+:PiB− gradually and significantly increased as the quartile number increased (first quartile to the fourth quartile) ($^{**}p < 0.01$, $\chi^2 = 9.808$, Fig. 2B table). Furthermore, multiple regression analysis also showed that the plasma AChE level and enzymatic activity were significantly correlated with cerebral Aβ deposition even after controlling for APOE4 positivity, MMSE score, age, and sex (age, sex, APOE4, and MMSE as covariates, $\beta = -0.032$ and $^{**}p = 0.002$ for AChE levels; $\beta = -0.001$ and $^{**}p = 0.007$ for AChE enzymatic activity; Table 2).

3.3. Plasma AChE and its activity increases discrimination power between CN− versus CN+

Logistic regression analysis and receiver operating characteristic (ROC) curve analysis using independent variables (APOE4 positivity and plasma AChE/AChE activity) were performed. Age and sex were used as covariates, and each ROC curve had a significant p value ($^{***}p < 0.0001$). Area under the curve (AUC) value increased when the AChE or AChE activity variable was added to ApoE ε4 carrier status (for AChE, AUC, 0.728 to 0.798, Fig. 3A, gray and black line, $^{*}p < 0.05$, comparison of ROC curves analysis; for AChE activity, AUC, 0.727 to 0.768, Fig. 3B, gray and black line, nonsignificant p value, comparison of ROC curve analysis).

3.4. Influence of acetylcholinesterase inhibitor (AChEI) treatment on plasma AChE and its activity

The groups of patients with MCI/AD dementia were categorized into the acetylcholinesterase inhibitor (AChEI) taker group and the nontaker group (Table 1). All AChEI takers took donepezil as the AChEI. To identify the effect of AChEI on plasma AChE level, we compared plasma AChE levels of AChEI takers (+AChEI) and those of non-AChEI takers (-AChEI). Interestingly, AChEI takers showed significantly higher levels of plasma AChE in all groups of patients with MCI/AD dementia ($^{*}p < 0.05$, $^{***}p < 0.001$, and $^{\#}p < 0.10$; unpaired t-test; Fig. 4A, left). Furthermore, plasma AChE levels were significantly increased in an AChEI dose-dependent manner ($^{***}p < 0.001$ and $^{****}p < 0.0001$; one-way ANOVA with Tukey's post hoc test; Fig. 4A, right). However, when we checked the enzymatic activity of AChE, there were no significant differences between +AChEI and−AChEI (Fig. 4B). This indicates that AChEI treatment affects the plasma AChE protein level but not AChE enzymatic activity. Therefore, it is hard to interpret the relationship between cerebral Aβ deposition and plasma AChE levels in the case of patients with MCI and AD dementia, as many of them took AChEIs, although their enzymatic activities were not changed.

Table 2
Multiple regression analyses of AChE and global cerebral Aβ deposition in CN individuals (n = 241)

Covariates	β	SE	t	*p*-value	F (df)	R^2-adj
Dependent variable: global cerebral Aβ deposition[a]				<0.001	4.49 (5, 235)	0.068
Age	0.002	0.001	2.575	0.011		
Sex	0.001	0.010	0.101	0.919		
Plasma AChE	−0.032	0.011	−3.065	**0.002		
ApoE ε4 type	0.024	0.012	2.020	0.045		
MMSE score	0.003	0.002	1.250	0.213		
Dependent variable: global cerebral Aβ deposition[a]				<0.001	5.45 (5, 234)	0.085
Age	0.001	0.001	1.810	0.072		
Sex	0.007	0.009	0.731	0.466		
Plasma AChE enzymatic activity	−0.001	0.001	−2.718	**0.007		
ApoE ε4 type	0.021	0.006	3.228	0.001		
MMSE score	0.003	0.002	1.304	0.193		

For AChE activity, 1 sample was excluded by outlier test.
Significant *p*-values for plasma AChE are asterisked (**$p < 0.01$) and highlighted in bold.
Multiple regression analyses with the stratification for CN− and CN+ (separately performed) resulted in no significant *p*-values between plasma AChE (CN−, $p = 0.9976$; CN+, $p = 0.6702$) or enzymatic activity (CN−, $p = 0.1530$; CN+, $p = 0.6696$) and global cerebral Aβ deposition.
Key: β, regression coefficient; Aβ, beta-amyloid; AChE, acetylcholinesterase; ApoE, apolipoprotein E; CN, cognitively normal; MMSE, mini-mental state examination; SE, standard error.
[a] Natural log-transformed to normalize variance.

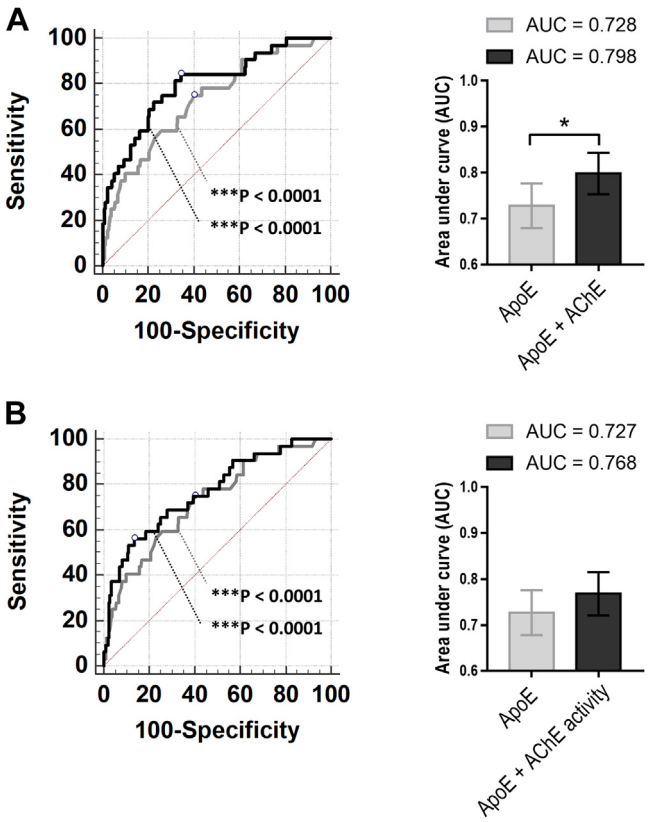


Fig. 3. Logistic regression analyses followed by comparison of receiver operating characteristic (ROC) curves (A) ROC curve models using independent variables (ApoE ε4 and/or plasma AChE). Gray line (AUC: 0.728, ***$p < 0.0001$), ApoE alone; black line (AUC: 0.798, ***$p < 0.0001$), ApoE with plasma AChE. Comparison of ROC curves analysis shows significant difference between the 2 models (*$p < 0.05$, ApoE alone vs ApoE with plasma AChE). (B) ROC curve models using independent variables (ApoE ε4 and/or plasma AChE activity). Gray line (AUC: 0.727, ***$p < 0.0001$), ApoE alone; black line (AUC: 0.768, ***$p < 0.0001$), ApoE with plasma AChE activity. Comparison of ROC curves analysis shows difference between the 2 models (nonsignificant *p* value, ApoE alone vs ApoE with plasma AChE activity). 209 CN− and 32 CN+ for AChE levels; 208 CN− and 32 CN+ for AChE activity (One CN− sample excluded as an outlier).

4. Discussion

Cholinergic dysfunction has been the epicenter of AD pathophysiology. Many studies have aimed to utilize the alterations in the cholinergic system in the central and peripheral nervous systems for the diagnosis of AD and development of therapeutic strategies for patients with AD. However, controversial results on the AChE levels and AChE activity in the brain, CSF, and periphery have been reported in association with AD pathogenesis. These include decreased brain AChE activity in patients with MCI/early AD dementia (Perry et al., 1977a,b; Rinne et al., 2003), decreased AChE activity in CSF in patients with severe dementia (Sirvio et al., 1989), no change in CSF-AChE activity in patients with AD dementia (Lal et al., 1984), increased AChE isoform levels in the AD mouse model expressing the C-terminal fragment of Aβ precursor protein (Sberna et al., 1998), and increased AChE staining around Aβ plaques in the human brain (Ulrich et al., 1990). In the periphery, no changes in AChE activity have been detected in the erythrocytes, lymphocytes, and platelets of patients with mild-to-moderate AD dementia. In addition, it has been proposed that blood AChE levels do not reflect a brain AD pathology (Marquis et al., 1985; Rakonczay et al., 2005). Meanwhile, in the other studies, conflicting results have been presented, including reduced AChE activity in the lymphocytes of patients with sporadic AD dementia (Inestrosa et al., 1994) and significantly elevated plasma AChE activity in patients with AD dementia (Atack et al., 1985). There are some possible explanations for the abovementioned conflicting results. Given that many of these works were carried out during the early period of AD research, these discrepancies may possibly be due to the differences in the clinical severity of AD dementia or cognitive impairment. Also, poor accuracy of clinical diagnoses with respect to the pathological status of the brain could be a major obstacle to selection of reliable AD patient and control group. Especially, conformation of cerebral Aβ positivity is critical in diagnosing AD even among individuals without dementia to assess preclinical AD (Jansen et al., 2015; Ossenkoppele et al., 2015). However, confirmation of cerebral Aβ positivity was rarely performed in previous studies and it is likely that cerebral Aβ positive and negative patients are mixed in each group. To account for the abovementioned limitation, all participants of this study underwent amyloid PET imaging to validate cerebral Aβ positivity and we investigated the direct correlation of plasma AChE levels and the degree of cerebral Aβ deposition.

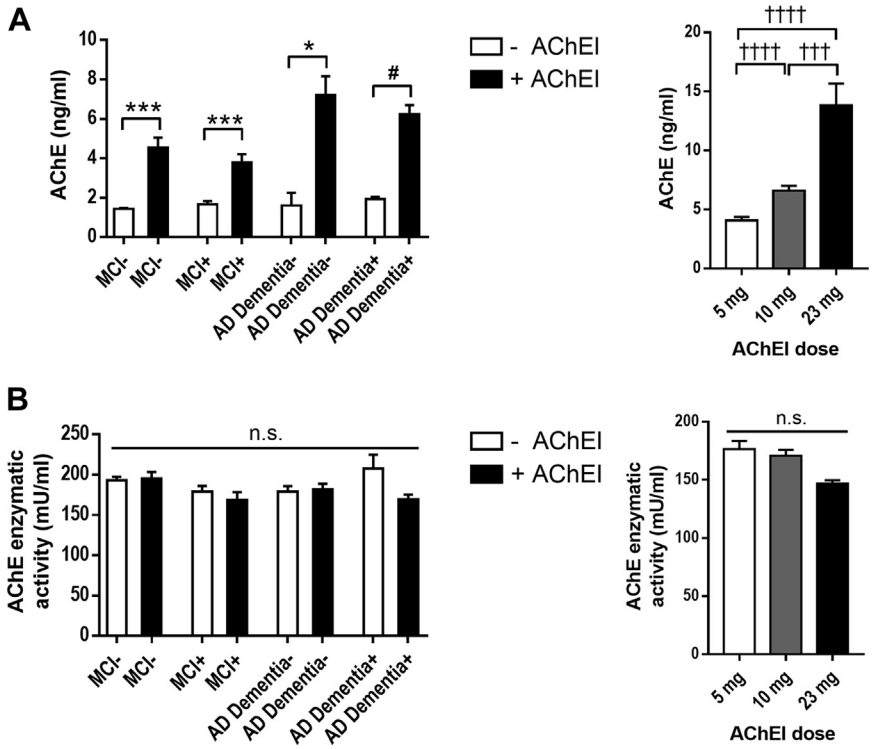


Fig. 4. AChE inhibitor (AChEI) and plasma AChE or its activity. (A) The effect of AChEI treatment on plasma AChE level in cognitively impaired individuals (103 MCI and 63 AD dementia). Right graph shows increasing plasma AChE levels by AChEI treatment in a dose-dependent manner. $^{*}p < 0.05$, $^{***}p < 0.001$, and $^{\#}p < 0.10$ unpaired *t*-test; $^{\dagger\dagger\dagger}p < 0.001$ and $^{\dagger\dagger\dagger\dagger}p < 0.0001$ one-way ANOVA with Tukey's post hoc test. (B) No AChE activity (mU/mL) changes in plasma by the AChEI treatment (103 MCI and 63 AD dementia). Abbreviations: AChEI, acetylcholinesterase inhibitor; -AChEI, non-AChEI taker; +AChEI, AChEI taker; MCI, mild cognitive impairment; AD dementia, Alzheimer's disease dementia; − or +, PiB positivity.

Moreover, our study reveals that the plasma AChE levels drastically changed on AChEI treatment—a therapeutic remedy for AD. Donepezil, the most widely used AChEI for AD, increases the plasma AChE levels significantly in a dose-dependent manner (Fig. 4A). Even though this seems to suggest that a significant increase in the plasma AChE level occurs because of cerebral Aβ deposition in patients with MCI and AD dementia when the MCI and AD dementia are not divided by AChEI treatment, subcategorization by AChEI treatment shows definite influences of AChEI treatment on the peripheral AChEI levels. Given that many patients with AD dementia and MCI are taking AChEIs in clinical settings, similar to the individuals in this study cohort, plasma AChE is not likely to be a good candidate for a cerebral Aβ biomarker for MCI and AD dementia.

Interestingly, increased AChE protein level was not necessarily associated with increased AChE activity in our study. Increased AChE protein level in MCI and AD dementia patients with AChEI treatment, compared to non-AChEI takers, showed no difference in total AChE activity. This result can be explained by either increased AChE protein with lower AChE activity per each protein molecule or enzymatic inhibition from remaining AChEI medication in blood. We are uncertain about the change of central AChE protein level at this point; however, further investigation is needed to clarify the underlying mechanism.

Besides neuron, diverse non-neuronal cell types express AChE, including macrophage, lymphocyte, dendritic cells, endothelial cells, epithelial cells, keratinocytes, and adipocytes (Grando et al., 2003; Kawashima and Fujii, 2003; Wessler and Kirkpatrick, 2008). In periphery, plasma membrane of red blood cells (RBCs) is known to as the richest source of AChE (Freitas Leal et al., 2017; Igisu et al., 1994). We investigated whether the alteration of AChE is associated to the age-dependent change of RBC. We had RBC data for subjects and the number of RBC was highly correlated with age ($^{****}p < 0.0001$, $r = -0.2265$; Fig. S1a). However, AChE levels and its enzymatic activities were not correlated with RBC in all subject groups (Table S2). Also, when we performed partial correlation analyses with the correction for RBC number, there was still correlation between AChE or AChE enzymatic activity and brain Aβ deposition in the CN group (AChE, $n = 241$, $^{**}p = 0.0015$, $r = -0.2035$; enzymatic activity, $n = 240$, $^{***}p = 0.0008$, $r = -0.2151$; Fig. S1b). Taken together, these findings suggest that the relationship between AChE levels and brain Aβ deposition in the brain is independent from age-dependent alteration of RBC number in circulation.

Focusing on the CN groups, in which no one took an AChEI, our study reveals a significant difference in plasma AChE levels and its enzymatic activities on the basis of cerebral Aβ deposition positivity; our results show a decreased blood AChE level and its enzymatic activity in the CN+ group. Decreased plasma AChE levels and activities are also significantly correlated with the degree of global cerebral Aβ deposition. Furthermore, in terms of PiB positivity discrimination, plasma AChE significantly improved the discrimination accuracy of APOE4, indicating the potential of plasma AChE as a screening marker for cerebral Aβ deposition in CN individuals (Fig. 3). It is probable that this decreased tendency of plasma AChE level by cerebral Aβ deposition might occur only in early stage of preclinical AD, and major neurodegeneration by continuous increase of cerebral Aβ deposition might result in varied alterations during further disease progression in MCI and AD dementia. This can be supported by our additional data showing that AChE levels and its enzymatic activities in cognitively impaired subjects (MCI or AD dementia) have weaker correlation with PiB positivity, even after controlling for the effects of AChEI on plasma AChE levels using z-score calculation (See details in the method section; Fig. S2).

The exact mechanism for this peripheral alteration of AChE level by cerebral Aβ deposition remains unclear. Early brain cholinergic disturbances caused by cerebral Aβ deposition may disturb the AChE levels in the brain, possibly through neuronal cell death, which influences the balance of AChE levels between the central and peripheral nervous systems in preclinical AD. Other mechanisms are possible as well. A study suggests that the cholinergic benefit of AChEI treatment in AD is attributable to the acetylcholine-mediated improvement of neuronal transmission and the anti-inflammatory action of the AChEI, including protection from free radicals and Aβ injury and the inhibition of cytokine release from microglia and monocytes (Tabet, 2006). This result shows the possibility of a relationship between AChE and the inflammatory response. Cerebral Aβ deposition is strongly associated with inflammatory responses in the brain. Inflammation is not a bystander phenomenon; rather, it plays a major role in AD pathogenesis (Mandrekar-Colucci and Landreth, 2010; Salminen et al., 2009). Therefore, preclinical cerebral Aβ deposition may influence the AChE levels in the brain and further in the periphery via the inflammatory response. This speculation is supported by previous study suggesting the possibility that cerebral Aβ deposition might increase secretion of proinflammatory cytokines, which activate cholinergic anti-inflammatory pathway and provide the connecting relationship between cerebral Aβ deposition and plasma AChE activity (Alkalay et al., 2013). This group proposed positive correlation between plasma AChE activity and PIB index, whereas our study demonstrated negative correlation. This discrepancy may be caused by different characteristics of the participant cohorts because they studied heterogeneous dementia sample including AD and non-AD who were either under AChEI treatment or AChEI-free, whereas our study focused on AChEI-free CN individuals. In addition, AChE has been proposed to play a significant role in the amyloid pathophysiology of AD, including Aβ fibrilization and plaque formation (Rees et al., 2003). Furthermore, there is a report that Aβ influences the AChE expression in a vicious cycle (Hu et al., 2003). Therefore, Aβ and AChE affect each other during AD pathogenesis, and early cerebral Aβ accumulation has a potential to diversely modulate the AChE levels in the central nervous system through reciprocal pathways. This interactive mechanism could be amplified, causing alterations in the peripheral AChE levels via cerebral Aβ deposition. In future studies, the mechanism underlying the association between cerebral Aβ deposition and peripheral AChE levels needs to be further clarified. Future investigations into alteration of specific isoforms or variants of AChE by cerebral Aβ deposition are needed. Also, examination of the relationship between CSF Aβ level and peripheral AChE level would be informative during AD pathogenesis.

Taken together, our results suggest that blood AChE levels could be a possible indicator of cerebral Aβ deposition in cognitively intact middle-aged and elderly individuals. Considering the limitations of amyloid PET and CSF assessment in detecting cerebral amyloidosis, including the high cost, limited availability of amyloid PET, and the invasiveness and poor interinstitutional reliability of CSF measurements, early detection of cerebral Aβ using the plasma AChE level may be useful for the primary screening of preclinical AD in clinical research and therapeutic trial participants.

5. Conclusions

Blood AChE level is associated with cerebral Aβ deposition in CN individuals and has great potential for a biomarker to predict cerebral Aβ deposition in CN individuals. Further study is needed to validate the possibility of blood AChE level as biomarker for preclinical AD.

Disclosure statement

The authors declare that they have no competing interests, financial or otherwise, that are related to this present work.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.neurobiolaging.2018.09.001.

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