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Increased Levels of Plasma p3-Alcα35, a Major Fragment of Alcadeinα by γ-Secretase Cleavage, in Alzheimer's Disease

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Abstract. p3-Alcα is a metabolic fragment of Alcadeinα (Alcα). Similar to the generation of the p3 fragment from amyloid-β protein precursor (AβPP) processing, Alcα is cleaved by α- and γ-secretases, leading to the secretion of p3-Alcα peptides into cerebrospinal fluid (CSF). p3-Alcα is also detected in the plasma, similar to amyloid-β (Aβ), which is a metabolic fragment of AβPP cleaved by amyloidogenic β- and γ-secretases. Because p3-Alcα is a non-aggregatable and stable peptide, unlike aggregatable Aβ and metabolically labile p3 of AβPP, the changes of p3-Alcα in quality and/or quantity in CSF and plasma are expected to be a marker for assessing alteration of substrate cleavage by γ-secretase, such as Aβ generation from AβPP. The present study describes a sandwich enzyme-linked immunosorbent assay for quantifying levels of p3-Alcα35, the major form of the p3-Alcα species, and examines levels of p3-Alcα35 in the plasma of three independent Japanese cohorts. In two of the three cohorts, the p3-Alcα35 levels were significantly increased with a concomitant decrease in the Mini-Mental State Examination score, or in clinically diagnosed Alzheimer's disease (AD) patients, when compared with age-matched non-demented subjects. The values were significantly lower in AD subjects who were administered donepezil, when compared to AD subjects without donepezil treatment. The increase in plasma p3-Alcα35 levels may indicate an endophenotype in subjects in whom AD is due to a progressing cognitive impairment in subjects with a γ-secretase malfunction, or a disorder of the clearance of peptides.

Keywords: Alzheimer's disease, alcadein, diagnosis, donepezil, γ-secretase, p3-Alc, plasma biomarker

INTRODUCTION

Alcadeins (Alcα, Alcβ, and Alcγ, also called calsyntenin or XB31) constitute a family of neural type I transmembrane proteins, all of which are encoded by their respective genes, and are highly conserved among mammals [1, 2]. Both Alcα and the amyloid-β protein precursor (AβPP), which is involved in Alzheimer's

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disease (AD), function as cargo receptors for the kinesin-1 motor that transports membrane vesicles anterogradely in neurons [3–6]. Alc is subject to proteolytic processing by a combination of AβPP α- and γ-secretases, but not an amyloidogenic combination of β- and γ-secretases [7]. This processing of Alc secretes a large amino-terminal extracellular domain and small p3-Alc peptide, along with the intracellular release of the cytoplasmic domain fragments, AlcICDs [8]. In neurons, Alc and AβPP form a complex mediated by the cytoplasmic interaction of X11-like (X11L), a neural adaptor protein which stabilizes proteolytic cleavage of both proteins, and facilitate intracellular colocalization of both membrane proteins in the neuron [2, 9, 10]. X11L (also called X11β, Mint2, or APBA2) was recently reported as a possible candidate of ApoE4-related late-onset AD effector [11]. The integrated genomic approach of late-onset/sporadic-type AD suggests that the ApoE4 variant is involved in the intracellular trafficking of AβPP, in which X11L plays an important role [12–14].

In transporting membrane vesicles in the late secretory pathway, plasma membrane, or in the endocytotic recycling pathway, Alc and AβPP are likely to be cleaved by primary secretases. AβPP is cleaved by α-secretase ADAM 10 and ADAM 17, or β-secretase BACE, to leave the membrane associated AβPP carboxy-terminal fragment (AβPP CTFα or CTFβ) [15], while Alc is cleaved by only α-secretase to leave Alc CTF [7]. All CTFs are further cleaved by γ-secretase to secrete a p3 peptide from AβPP CTFα, Aβ peptide from AβPP CTFβ, and p3-Alc from Alc CTF into the exracellular milieu [7, 8]. Therefore, Alc and AβPP perform similar functions [5], show large colocalization in the neuron [2], and are subject to almost the same regulation of proteolytic processing [9]. This suggests that some alteration in the processing systems of the substrates in specific regions of the brain, or the malfunction of the clearance system for secreted short peptides, may appear in the qualitative and/or quantitative alteration of metabolic products derived from AβPP and Alc almost equivalently [16]. In fact, recent reports describe that γ-secretase dysfunction and/or malfunction of the Aβ clearance system are observed in AD patients [16–19], suggesting that some AD pathogenesis is due to an altered membrane substrate cleavage, or a clearance failure of the cleaved products.

The p3 of AβPP is metabolically labile to detect in the cerebrospinal fluid (CSF) and plasma, and Aβ (in particular the more AD-pathogenic $Aβ_{42}$) is progressively aggregatable to detect quantitative or qualitative changes in the plasma. Aβ is a causative metabolic peptide of AD, which detects qualitative and quantitative alterations in the CSF and/or plasma, and is very important in diagnosing AD patients [20]. However, the aggregatable form of this peptide, and various aggregated soluble Aβ oligomers, make it difficult to investigate the alteration of Aβ levels in body fluids precisely. Instead of Aβ, non-aggregatable p3-Alc can be available as a surrogate marker for the detection of changes in the quality and quantity of the γ-cleavage of substrates.

In the adult brain, the expression of Alcα and Alcβ is more prominent than Alcγ, and p3-Alcα and p3-Alcβ are also more prominent in the CSF than p3-Alcγ [7]. In human CSF, p3-Alcα35 is the major peptide among several p3-Alcα species, while p3-Alcβ37 and p3-Alcβ40 are the major products of Alcβ [7, 16]. We previously developed a sandwich ELISA (sELISA) system for quantifying the total amount of p3-Alcα [21]. This sELISA was constructed with pan-p3-Alcα antibodies that can quantify the total amount of all of the p3-Alcα species, including the major p3-Alcα35, and the minor p3-Alcα species in the CSF and plasma. However, the sELISA cannot selectively quantify specific species such as p3-Alcα35 or p3-Alcα38. Despite the restricted conditions of the sELISA, several trials using patients' samples have provided significant information about p3-Alcα: (a) the total p3-Alcα level in the plasma correlates with the level in the CSF of individuals, (b) the total p3-Alcα level correlates with the levels of $Aβ_{40}$, an Aβ species that is less aggregatable than $Aβ_{42}$, in the CSF and plasma samples of several cohorts, and (c) the CSF and total plasma p3-Alcα levels of several cohorts increases in AD patients, when compared to age-matched control subjects [21–23]. These observations suggest that changes in p3-Alcα levels in body fluids may be able to diagnose AD status. The present study develops a novel monoclonal antibody which specifically recognizes p3-Alcα35 and establishes a new sELISA system for quantifying p3-Alcα35 levels. The plasma p3-Alcα35 levels of AD patients, mild cognitive impaired (MCI), and non-demented subjects of three independent Japanese cohorts will be investigated, in addition to the fluctuation of p3-Alcα35 levels in AD subjects treated with donepezil.

METHODS

Antibodies and the ELISA system

p3-Alcα35 is a peptide that includes the sequence from Ala817 to Thr851 of the human Alcα1. The

monoclonal mouse antibody was raised against an antigen peptide containing the sequence between positions Asn839 and Thr851. The antibody reacts with the antigen peptide specifically, but not with another peptide containing the sequence between positions Asn839 and Ile854 (for amino acid sequence of p3-Alcα, see [7]). Clone 63A1 was selected among several clones showing specific reactivity to p3-Alcα35. The affinity-purified antibody 63A1 was used to capture p3-Alcα35. The horseradish peroxidase-conjugated pan-p3-Alcα rabbit polyclonal antibody 817, which was raised against a peptide containing the sequence between positions Ala817 and Val822 [21], and tetramethyl benzidine were used to detect the captured p3-Alcα35.

Total amount of p3-Alcα was quantified with an ELISA kit of pan-p3-Alcα monoclonal antibody to capture all p3-Alcα species, which was supplied from Immuno-Biological Laboratories Co., Ltd. This ELISA kit is different from our previous assay system in which pan-p3-Alcα polyclonal antibody 839 is used to capture all p3-Alcα species [21].

Blood samples were collected from the subjects into tubes containing EDTA and centrifuged. Two hundred microliters of plasma was used per duplicate assay. p3-Alcα was extracted from the plasma, as described for the total extraction of p3-Alcα for ELISA [21], and quantified for p3-Alcα35 using the new sELISA system, in duplicate.

Cohort information

The first cohort (Cohort 1, $n = 135$) is largely composed of MCI and AD patients, many of whom are not hospitalized and living in the countryside together with non-demented control subjects; the second cohort (Cohort 2, $n = 252$) is largely composed of inpatients with normal controls, MCI, AD, and other neurological diseases (OND); and the third cohort (Cohort 3, $n = 91$) is a mixture of inpatients and non-hospitalized subjects living in the city. Detailed descriptions of all subjects are shown in Supplementary Tables 1–3. The cohorts are different from those of previous studies for an analysis of total p3-Alcα [21, 23].

RESULTS

Characterization of the ELISA system with the monoclonal p3-Alcα35 C-terminal end-specific antibody

To develop the new sELISA, the 63A1 antibody was used to capture p3-Alcα35 specifically, instead of the polyclonal pan-p3-Alcα antibody 839 used in previous studies, to examine the total amount of p3-Alcα [21]. The new ELISA recognized p3-Alcα35 specifically, but not p3-Alcα39 (Fig. 1A), and does not react with the p3-Alcβ species (data not shown), indicating the establishment of a new sELISA system specific for p3-Alcα35 analysis. Previously, plasma samples were extracted, and total p3-Alcα was quantified using

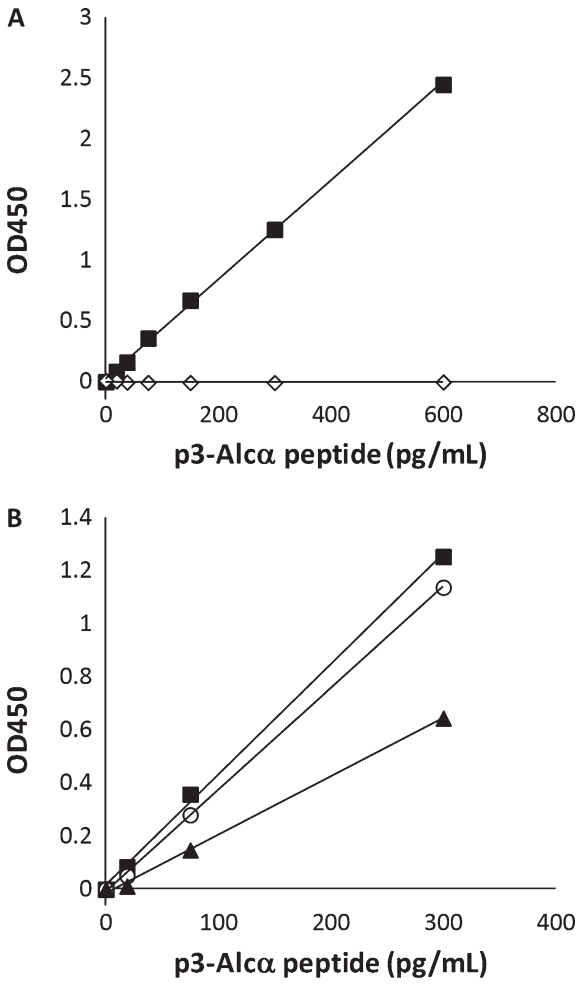

Fig. 1. Specificity of the sELISA. A) Specific reactivity of the sELISA system to p3-Alcα35. The indicated amount of synthetic p3-Alcα35 (closed square) and p3-Alcα39 (open diamond) were dissolved in buffer A (PBS containing 1% (w/v) bovine serum albumin and 0.05% (v/v) Tween-20), and assayed with the ELISA. B) Quantification of the p3-Alcα35 peptide in human plasma with or without an extraction process. The indicated amounts of synthetic p3-Alcα35 were dissolved in human plasma (prepared from a non-AD healthy volunteer; open circle and closed triangle) or buffer A (closed square). The plasma was subject to extraction with (open circle) or without (closed triangle) a standard organic extraction protocol prior to analysis by ELISA, as described [21]. The horseradish peroxidase-conjugated antibody and tetrametyl benzidine were used to detect the captured p3-Alcα35 colormetrically at OD_{450}.

the sELISA, because the treatment of plasma samples with a standard organic extraction protocol removed factor(s) that interfered with the immuno-detection of p3-Alcα [21]. This extraction procedure was also included in the new ELISA to allow significant recovery of p3-Alcα from the plasma, and therefore accurate quantification of p3-Alcα35 levels in the plasma. The ELISA system was performed to quantify the amount of synthetic p3-Alcα35 dissolved in the human plasma (Fig. 1B). After the extraction process, the slope of the standard curve was almost identical to that of the standard curve in which an identical amount of the synthetic p3-Alcα35 was dissolved in assay buffer. This procedure results in a yield of over 90% (compare closed square with open circle in Fig. 1B), which is sufficient to be considered quantitative, while the recovery of synthetic p3-Alcα35 from the plasma was approximately 50% to 60% when plasma samples were assayed without the extraction process (compare closed square with closed triangle in Fig. 1B). Therefore, plasma p3-Alcα35 quantification was carried out by sELISA after extraction of the endogenous p3-Alcα peptides, as described previously [21].

Plasma p3-Alcα35 levels of subjects in three independent Japanese cohorts

The levels of p3-Alcα35 in the plasma of subjects from three cohorts were investigated (Table 1). The correlation between p3-Alcα35 and total amount of p3-Alcα levels was first examined. The p3-Alcα35 levels were significantly correlated with the total amount of p3-Alcα in the three cohorts (Fig. 2 left). Furthermore, in all cohorts, plasma p3-Alcα35 levels significantly increased in an age-dependent manner (Fig. 2 right).

The relationship between p3-Alcα35 levels and MMSE scores was then investigated (Fig. 3). The increase in plasma p3-Alcα35 levels correlates with the decrease of the score significantly in two of the three cohorts (Fig. 3A, B). These analyses suggest that p3-Alcα35 levels increase during the aging process, and that subjects appearing to have cognitive impairment show higher levels of p3-Alcα35 in their plasma.

Table 1
Summary of subjects' data analyzed in Fig. 2

n	A) Cohort 1 135	B) Cohort 2 252	C) Cohort 3 91
Age (years)	74.9 ± 6.50	81.3 ± 10.1	76.6 ± 8.37
p3-Alcα35 (pg/mL)	164.6 ± 46.9	192.1 ± 60.1	140.5 ± 43.8
p3-Alcα total (pg/mL)	241.6 ± 45.0	240.0 ± 74.4	172.5 ± 51.5

Average age and average values of p3-Alcα35 and p3-Alcα total in three cohorts are summarized. Numbers indicate means ± standard deviation. Details of individual subjects are shown in Supplementary Tables 1–3.

The p3-Alcα35 levels of AD and MCI patients were then compared with those of non-demented control subjects or OND patients, in an age-matched population (Fig. 4 and Table 2). In all cohorts, AD and MCI were clinically diagnosed based on two major criteria: the Diagnostic and Statistical Manual of Mental Disorders: 4th Edition (DSM-IV) and the National Institute of Neurological and Communicational Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA), although we realize that there are many different definitions of MCI [24].

In the subjects of Cohort 1 (Fig. 4A and Table 2A), the p3-Alcα35 levels of AD patients were significantly higher than the values of the age-matched non-demented controls. The age-matched MCI subjects showed higher levels of p3-Alcα35 than non-demented controls, although this finding was not statistically significant.

In Cohort 2 (Fig. 4B and Table 2B), the subjects with OND were divided into two subgroups with (MMSE $\leq$22) or without (MMSE $\geq$23) remarkable cognitive impairment [25]. We examined the p3-Alcα35 levels of age-matched controls, MCI, AD, OND (MMSE $\geq$23), and OND (MMSE $\leq$22) subjects. The p3-Alcα35 level in AD subjects was significantly higher when compared to the values of the control subjects. OND patients who show cognitive impairment (MMSE $\leq$22) also presented higher p3-Alcα35 levels than subjects of OND without severe cognitive impairment (MMSE $\geq$23) and control subjects, although these were not significant. The present findings suggest that the increase in the p3-Alcα35 levels may be involved in neurodegeneration and cognitive impairment.

We also analyzed the p3-Alcα35 levels of 67 age-matched subjects between 63 and 83 years of age in Cohort 3 (Fig. 4C and Table 2C). The levels of p3-Alcα35 are statistically identical between non-demented controls, MCI, AD, and OND subjects.

The subjects of Cohorts 2 and 3 were also classified by other criteria for dementia, the Clinical Dementia Rating (CDR) scale [26]. The p3-Alcα35 levels were compared in age-matched populations with a CDR score of CDR 0, CDR 0.5, and CDR 1, 2, 3 (Fig. 5). CDR 0 subjects were selected from clinically diagnosed MCI and OND patients by criteria of non-demented subjects, and CDR 1, 2, 3 subjects were selected from OND and AD subjects. The CDR scores of some subjects did not agree with the clinical diagnosis. Therefore we removed subjects from analysis who

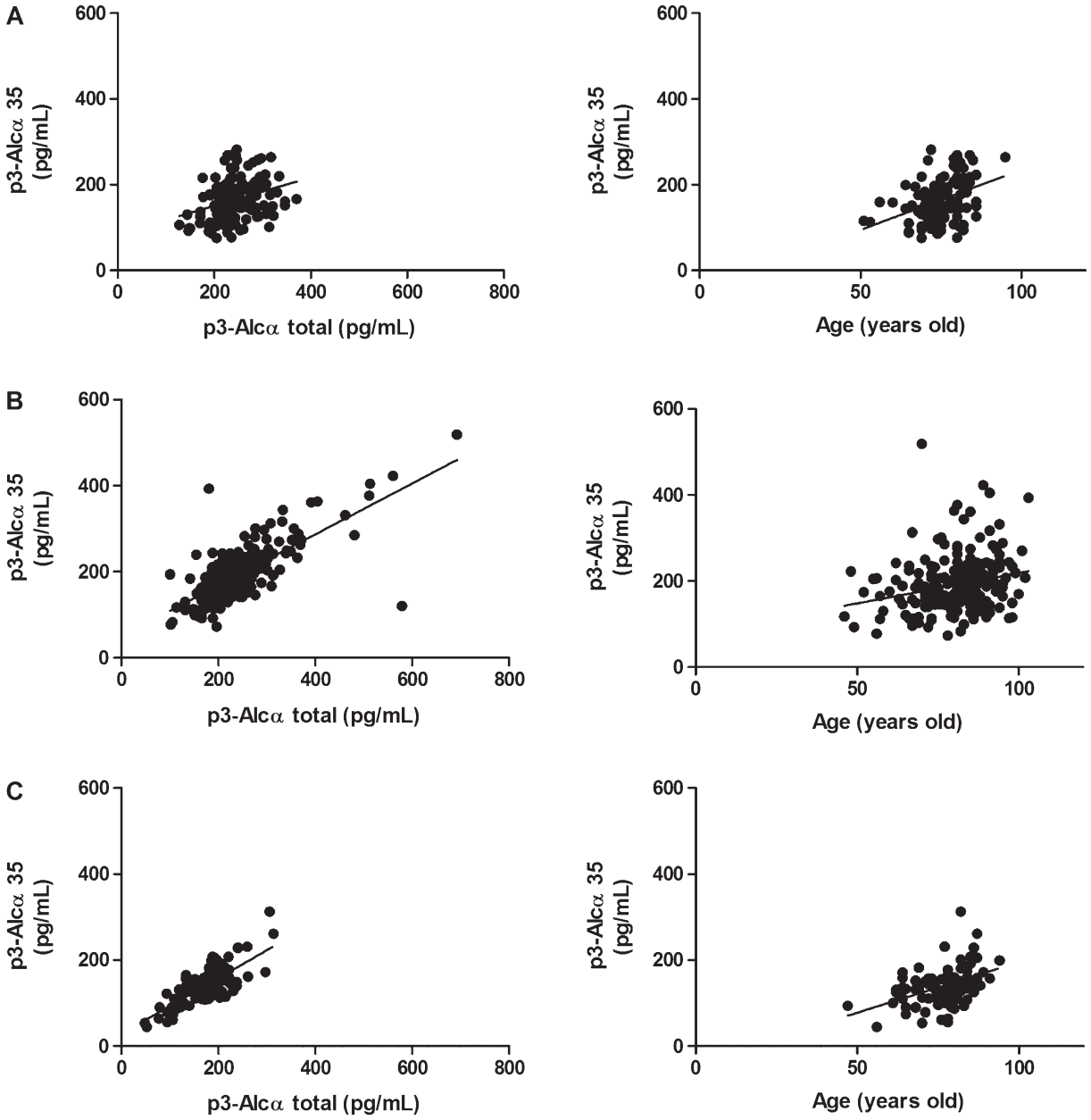

Fig. 2. Correlation of p3-Alcα35 levels with total p3-Alcα levels in plasma and age-dependency of plasma p3-Alcα35 levels. (Left) The correlation between p3-Alcα35 and total p3-Alcα levels are shown. A) Cohort 1 ($r^2 = 0.1009$, $p < 0.001$), B) Cohort 2 ($r^2 = 0.5362$, $p < 0.001$), C) Cohort 3 ($r^2 = 0.5790$, $p < 0.001$). (Right) The correlation between p3-Alcα35 levels and age are shown. A) Cohort 1 ($r^2 = 0.1512$, $p < 0.001$), B) Cohort 2 ($r^2 = 0.055912$, $p < 0.001$), C) Cohort 3 ($r^2 = 0.2005$, $p < 0.001$). Statistical analysis was performed by using the Pearson's correlation coefficient test. Subject numbers (n), average age, and p3-Alcα35 amounts of each cohort are summarized in Table 1.

had remarkably different diagnosis, such as a subject labeled as MCI presenting with CDR 2. In the present analysis of Cohort 2 (Fig. 5A), CDR 1, 2, 3 patients ($n = 197$, 198.0 ± 60.5 pg/mL) presented significantly high p3-Alcα35 levels when compared to those of CDR 0 subjects ($n = 17$, 152.8 ± 44.1 pg/mL) and CDR 0.5 subjects ($n = 6$, 153.1 ± 15.7 pg/mL). In Cohort 3 (Fig. 5B), there are no significant differences between the respective CDR subjects. Based on at least two criteria (Figs. 4 and 5), the p3-Alcα35 levels showed a tendency to be increased in AD and/or demented (CDR 1, 2, 3) subjects.

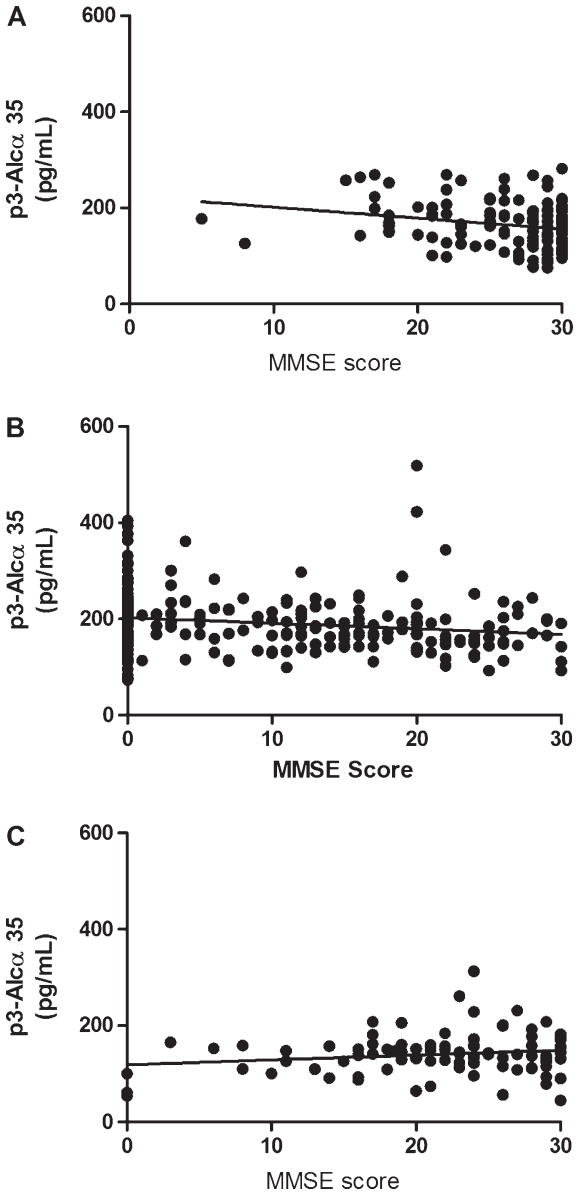

Fig. 3. The relationship between MMSE scores and plasma p3-Alcα35 levels. The correlation between Mini-Mental State Examination (MMSE) scores and p3-Alcα35 levels in the plasma of subjects is shown. In the graphs, statistical analysis was performed by using the Pearson's correlation coefficient test. A) Cohort 1 ($r^2 = 0.05303$, $p < 0.01$), B) Cohort 2 ($r^2 = 0.03525$, $p < 0.01$), C) Cohort 3 ($r^2 = 0.02934$, $p = 0.1045$).

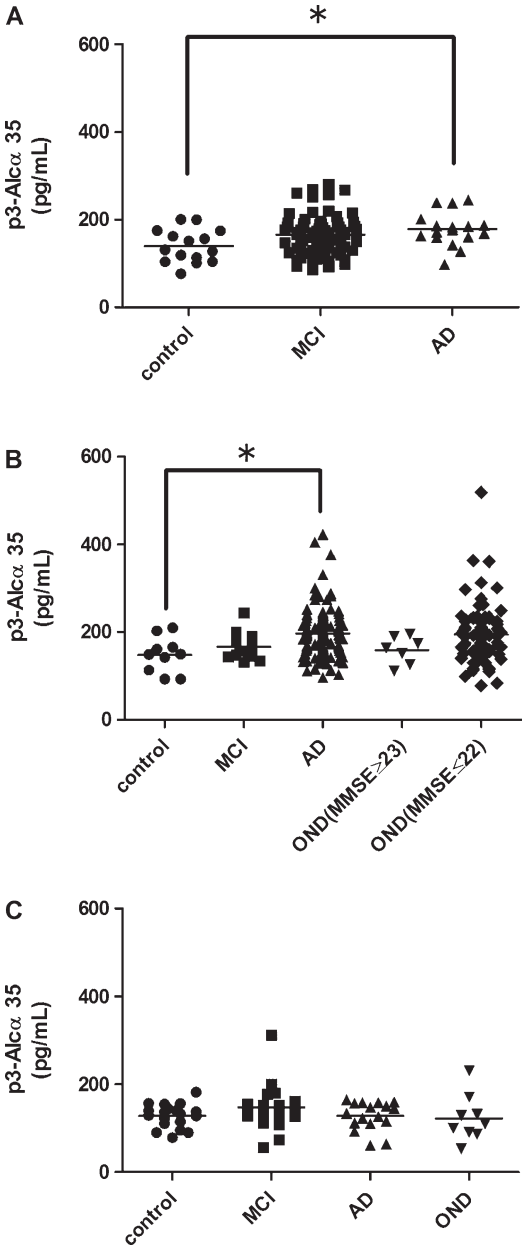

Fig. 4. Levels of plasma p3-Alcα35 following subgrouping into non-demented, MCI, AD, and OND. Subjects from three cohorts were clinically divided into four groups: non-demented subject (control), subjects with mild cognitive impairment (MCI), Alzheimer's disease (AD), and patients with other neurological diseases (OND). In Cohort 2 (B), OND are further distinguished into subjects with (MMSE $\leq$22) or without (MMSE $\geq$23) remarkable cognitive impairment. Plasma p3-Alcα35 levels of age-matched subjects in these subgroups were compared within the respective cohorts. A) Cohort 1 (subjects between 70 and 83 years old), B) Cohort 2 (subjects between 73 and 94 years old), C) Cohort 3 (subjects between 63 and 83 years old). Statistical analysis was performed using the Dunn's multiple comparison test following the Kruskal-Wallis test. *$p < 0.05$. Subject numbers (n), average age, and p3-Alcα35 amounts are summarized in Table 2.

We also investigated the p3-Alcα35 levels of AD patients with or without donepezil hydrochloride (Aricept) administration (Fig. 6). In 31 age-matched AD subjects with CDR 1 and CDR 2 in Cohort 2, non-treated subjects ($n = 18$, average age 80.4 ± 9.2) showed significantly higher levels of plasma p3-Alcα35 (182.7 ± 35.9 pg/mL) when compared to the

Table 2
Summary of subjects' data analyzed in Fig. 4

A) Cohort 1					
n		Control 15	MCI 70	AD 17	
p3-Alcα35 (pg/mL)		140.1 ± 37.8	166.3 ± 45.1	178.7 ± 38.5	
Age (years)		74.3 ± 4.1	75.5 ± 3.7	77.1 ± 4.1	
B) Cohort 2					
n	Control 10	MCI 12	AD 116	OND (MMSE ≥ 23) 7	OND (MMSE ≤22) 85
p3-Alcα35 (pg/mL)	148.0 ± 40.3	166.4 ± 32.6	196.5 ± 55.7	159.2 ± 31.7	194.8 ± 64.0
Age (years)	74.6 ± 10.4	81.5 ± 4.4	82.8 ± 7.7	79.3 ± 11.1	78.9 ± 10.1
C) Cohort 3					
n		Control 19	MCI 22	AD 17	OND 9
p3-Alcα35 (pg/mL)		128.6 ± 26.8	146.0 ± 49.5	130.8 ± 32.7	122.9 ± 52.3
Age (years)		74.5 ± 4.4	76.2 ± 6.4	75.3 ± 6.6	74.4 ± 5.7

Average age and average values of p3-Alcα35 in three cohorts are summarized. Numbers indicate means ± standard deviation. MCI, mild cognitive impairment; AD, Alzheimer's disease; OND, other neurological diseases.

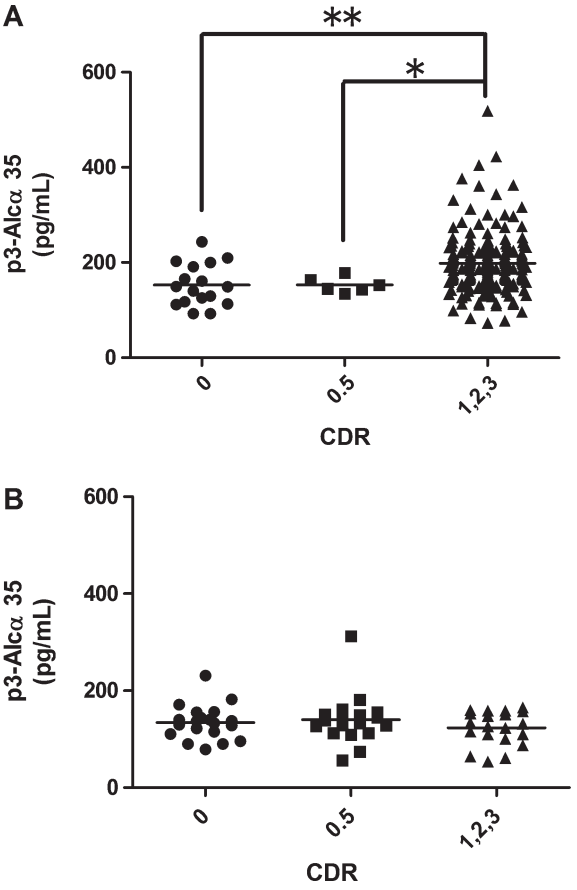

Fig. 5. Levels of plasma p3-Alcα35 of subjects divided into subgroups using the clinical dementia rating (CDR) scale. Age-matched subjects of cohort 2 (A) and cohort 3 (B) were divided into three groups based on CDR scales. Statistical analysis was performed using the Dunn's multiple comparison test following the Kruskal-Wallis test. *$p < 0.05$; **$p < 0.01$.

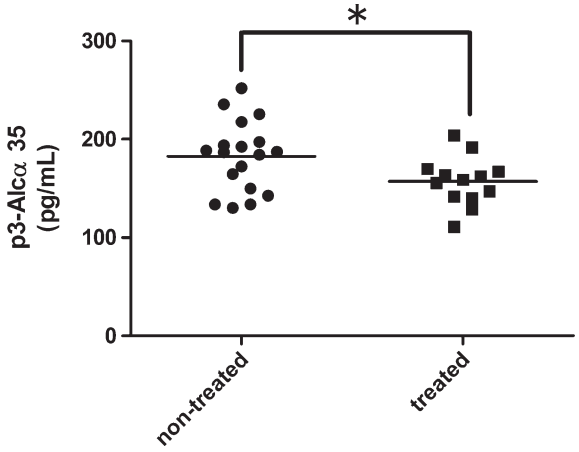

Fig. 6. Influence of donepezil hydrochloride administration on plasma p3-Alcα35 levels. Age-matched AD (CDR 1 and CDR 2) patients treated with Aricept (donepezil hydrochroride) were selected from Cohort 2, and their plasma p3-Alcα35 levels were compared to those of age-matched subjects who were not treated with Aricept (non-treated). Statistical analysis was performed using the Mann-Whitney test. *$p < 0.05$.

levels detected in subjects who were treated with the drug (157.1 ± 24.7 pg/mL; $n = 13$, average age 81.5 ± 5.3), suggesting that the increase in p3-Alcα35 levels may be slowed by the suppression of cognitive impairment by donepezil administration.

DISCUSSION

We previously showed that p3-Alcα35 is the major p3-Alcα species in human CSF by MALDI-TOF/MS spectrometric analysis of p3-Alcα peptides

immuno-isolated with a pan-p3-Alcα antibody. By semi-quantitative estimation with the mass spectrometric analysis, approximately 70% or more of the p3-Alcα species is p3-Alcα35 [7]. Furthermore, using an ELISA system with the pan-p3-Alcα antibody, which can detect all of the p3-Alcα species and quantify total amounts of p3-Alcα, we estimated a total of 5,000–15,000 pg/mL of p3-Alcα in the CSF of human subjects. The levels are comparable to the $A\beta_{40}$ levels, and increase in AD patients with a significant correlation to $A\beta_{40}$ levels in the CSF [22]. p3-Alcα is detectable in the plasma, but the total p3-Alcα content in the plasma is less than that detected in the CSF, and approximately 50–300 pg/mL of total p3-Alcα is quantified by the pan-p3-Alcα sELISA system [21]. Although the total plasma p3-Alcα levels also increase in AD patients, it remains unclear whether the p3-Alcα35 levels increase in AD patients.

In the present study, we analyzed levels of plasma p3-Alcα35, a major species of the p3-Alcα peptides generated from the cleavage of Alcα by γ-secretase, by using a newly established ELISA system with a C-terminal end-specific monoclonal antibody. First, we found that p3-Alcα35 is a major p3-Alcα species in the plasma, and we can estimate approximately 60% or more of total p3-Alcα species is p3-Alcα35 in the plasma, which is a comparable ratio to that of the CSF. We also found that the p3-Alcα35 levels in the plasma increased in subjects with a lower MMSE score in two of three of the cohorts, although the plasma levels appeared to increase in an age-dependent manner. In age-matched subjects, the p3-Alcα35 levels increased in AD patients of two of the cohorts. One cohort showed a significant increase in p3-Alcα35 levels in a CDR-dependent manner among age-matched subjects. Taken together, the plasma p3-Alcα35 levels showed an increase in subjects who appear to have cognitive impairment, as demonstrated by the total p3-Alcα levels [21, 23]. However, the increased magnitude of plasma p3-Alcα35 in AD was small compared to the total amount of p3-Alcα detected in our previous studies using the sELISA system with the polyclonal pan-p3-Alcα antibody [21, 23]. Therefore, we cannot rule out the possibility that the p3-Alcα35 specific monoclonal antibody may lack some component(s) of p3-Alcα that are greatly increased in the blood of AD patients.

Unfortunately, we could not detect a significant increase of p3-Alcα35 levels of AD patients in Cohort 3. This may be due to a cause of heterogeneity of sporadic AD patients. We previously suggested that causes of sporadic AD may be variable [22], and a recent publication for the different Aβ fibril formation in individual AD patients supports this idea [27]. Alternatively, in blood examination, the quantification of p3-Alcα35 alone may be difficult to classify AD patients clearly. Combination assay with another blood marker may be more effective for blood sample.

The age-matched AD subject population of CDR 1 and CDR 2 treated with donepezil showed lower plasma p3-Alcα35 values than the population without treatment. Although it remains unclear how donepezil lowers the level of p3-Alcα35 (and this is a result of limited number of subjects), the improvement of neuronal activity may contribute to the suppression of the increase in p3-Alcα35 levels, or facilitating the removal of p3-Alcα35. A more convincing study, such as a study to compare the levels of p3-Alcα35 in patients with AD before and after donepezil treatment, will be needed to confirm this effect. The plasma p3-Alcα35 levels can be reflected by the levels detected in the CSF of patients with cognitive impairment. If so, the level of plasma p3-Alcα35 may be an indicator of cognitive ability of aged subjects. Another peptide derived from γ-secretase cleavage of the AβPP-like protein 1 (APLP1) is also reported to reflect the amyloidogenic state of AβPP metabolism in the brain [17]. The concentration of this peptide in the CSF (4.5 nM) is comparable to the level of p3-Alcα in the CSF [22]; however, the concentration of the APLP1 peptide in the plasma has not been determined. Aβ and p3-Alcα are the main γ-secretase peptide products of the type I membrane proteins expressed largely in the brain that are detectable in the plasma. AβPP695 shows neuron-specific expression, while other isoforms such as AβPP770/751 are moderately expressed in non-neuronal tissues, including immunocompetent cells in the blood [10, 28, 29]. p3-Alcα is largely derived from the brain because Alcα expression is mostly observed in the brain [2], and increased plasma p3-Alcα levels are detected in Alcα-CTF transgenic mice with a neuron-specific promoter (unpublished observation). Moreover, it should be noted that AβPP and Alc express and colocalize largely in neurons, appear to have the same function as kinesin-1 cargo receptors, and are subject to similar proteolytic metabolism [2, 5, 7, 9]. Therefore, changes in the quality and quantity of the plasma p3-Alcα levels may provide a glimpse into the metabolic state of γ-secretase substrates, such as AβPP, in a specific brain region. Although physiological functions of p3-Alcα in brain and blood remain unclear, it is interesting to know the function for understanding the means of changes of p3-Alcα levels in aging and loss of cognitive functions. Analysis of more

cohorts should be performed to evaluate the changes of plasma p3-Alcα35 in subjects as an indicator of brain cognitive impairment such as that involved in AD.

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SUPPLEMENTARY MATERIAL

Supplementary tables are available in the electronic version of this article: http://dx.doi.org/10.3233/JAD-131610.

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