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Brief Report

Plasma amyloid-β precursor protein $_{669–711}$/amyloid-$\beta_{1–42}$ ratio is associated with cognition in Alzheimer's disease

Moeko Noguchi-Shinohara[a], Yasuhiro Sakashita[a], Hiroto Nakano[a], Daiki Muramatsu[a], Sadao Hikishima[a], Junji Komatsu[a], Hidetomo Murakami[b], Yukiko Mori[b], Kenjiro Ono[a,*]

[a] Department of Neurology, Kanazawa University Graduate School of Medical Sciences, Kanazawa, Japan
[b] Department of Neurology, School of Medicine, Showa University, Tokyo, Japan

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ABSTRACT

Plasma amyloid-β (Aβ) markers are significant predictors of Aβ pathology. However, their prognostic value for cognition in patients with Alzheimer's disease (AD) is unknown. We compared plasma amyloid-β precursor protein (APP)$_{669–711}$ and A$\beta_{1–42}$ levels between cognitively unimpaired participants (CU) and those with MCI due to AD and AD dementia. The CU group was divided into CU+ or CU- groups according to presence of Aβ pathology. All patients with AD exhibited Aβ pathology. The plasma APP$_{669–711}$/A$\beta_{1–42}$ ratio was significantly elevated in patients with CU+, MCI+, and AD+ compared with those with CU-. Furthermore, the plasma APP$_{669–711}$/A$\beta_{1–42}$ ratio was significantly correlated with the MMSE score (rs = −0.544, $p < 0.001$). Analysis of the Aβ+ group revealed that the significant relationship between MMSE score and plasma APP$_{669–711}$/A$\beta_{1–42}$ ratio remained unchanged (rs = −0.244, $p = 0.027$). Therefore, we conclude that the plasma APP$_{669–711}$/A$\beta_{1–42}$ ratio is associated with cognition in patients with AD.

1. Introduction

Amyloid-β protein (Aβ) deposition in brain is the earliest pathological signature of Alzheimer's disease (AD) [1]. Aβ is produced by the amyloid-β precursor protein (APP) through consecutive proteolytic cleavage executed by β- and γ-secretases [2]. Amyloid positron emission tomography and cerebrospinal fluid (CSF) A$\beta_{1–42}$/A$\beta_{1–40}$ ratio are well-established and reliable biomarkers of this cerebral Aβ accumulation [3,4]. However, reliable blood biomarkers for AD are desired due to convenience, noninvasiveness, and the low-cost of the measurement methods. We recently demonstrated that plasma Aβ biomarkers are useful indicators of comorbid AD pathology in Lewy body diseases [5], and others have found that plasma biomarkers, such as the APP$_{669–711}$/A$\beta_{1–42}$ and A$\beta_{1–40}$/A$\beta_{1–42}$ ratios, are highly predictive of cerebral Aβ accumulation [6].

Although these blood biomarkers accurately indicate amyloid pathology, their association with cognitive decline is unclear. Many cognitively unimpaired individuals exhibit advanced senile amyloid plaques, implying that cerebral Aβ accumulation does not indicate a one-on-one relationship with cognitive function. This study investigated the prognostic values of multiple plasma Aβ markers for cognitive decline in patients across the AD continuum.

2. Methods

2.1. Participants

The participants consisted of cognitively unimpaired participants (CU) and participants with mild cognitive impairment (MCI) due to AD and AD dementia. Participants classified as CU exhibited subjective cognitive decline but no other involvement of the central nervous system, including functional headache, peripheral neuropathy, myopathy, somatoform disorder, epilepsy, and cervical spondylosis. Patients with MCI due to AD or AD dementia met the criteria established by the National Institute on Aging-Alzheimer's Association Workgroup for MCI [7] as those with a high likelihood of AD or probable AD dementia [8], exhibiting substantial evidence of pathophysiological process. All participants were assessed for the presence of Aβ (+/−), which was determined based on the results of CSF A$\beta_{1–42}$ testing. A CSF A$\beta_{1–42}$ < 490 pg/mL, which was our laboratory's cutoff value [9], was considered positive for Aβ pathology. All patients with MCI due to AD and AD dementia were Aβ+ based on the CSF A$\beta_{1–42}$ marker. The CU group was divided into CU+ or CU- groups according to the presence or absence of Aβ pathology. The CU- group was further divided into young normal control (YNC) and age-matched control (AMC, age ≥ 50 years) groups.

* Corresponding author at: Department of Neurology, Kanazawa University Graduate School of Medical Sciences, 13-1 Takara-machi, Kanazawa 920-8640, Japan.
E-mail address: onoken@med.kanazawa-u.ac.jp (K. Ono).

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The participants included in the final analysis consisted of 28 CU-, including 14 YNC and 14 AMC; 8 CU+; 28 MCI due to AD (MCI+); and 46 AD dementia (AD+). All participants received a comprehensive clinical assessment that included a detailed interview of a collateral source, neurological examination, routine laboratory testing, apolipoprotein E (*APOE*) phenotyping, brain magnetic resonance imaging, lumber puncture, and cognitive testing, including the Mini-Mental State Examination (MMSE).

This study was approved by the Medical Ethics Review Board of Kanazawa University (approval number 775) and Showa University School of Medicine (approval number 1997) and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants or their legal representatives prior to enrollment.

2.2. Blood collection and plasma aβ measurements

Blood samples were collected into ethylenediaminetetraacetic acid disodium salt tubes, centrifuged to isolate the plasma. Individual plasma samples were aliquoted and stored at −80 °C until analysis. Plasma Aβ concentrations were measured by immunoprecipitation–mass spectrometry at Shimadzu Techno-Research, Inc (Shimadzu Corporation, Kyoto, Japan) [6].

2.3. Cerebrospinal fluid biomarker measurements

CSF samples were collected, centrifuged, and frozen at −80 °C. Concentrations of $A\beta_{1-42}$, total tau, and phosphorylated tau (p-tau) 181 were measured by sandwich enzyme-linked immunosorbent assays (Innotest β-amyloid [1–42] and phospho-tau [181 p], respectively; Fujirebio, Tokyo, Japan) as previously described [10].

2.4. Statistical analysis

Clinical characteristics were compared among AMC, CU+, MCI+, and AD+ groups and plasma biomarker values among CU-, CU+, MCI+, and AD+ groups. Categorical variables (sex, *APOE* E4 carrier status, and family history of dementia) were compared by χ^2 test. Numerical variables were first checked for homogeneity of variance using the Levene test, and based on a negative result (inhomogeneity of variance), age, years of education, and MMSE score were compared by the Games–Howell test, while plasma biomarkers with confirmed homogeneity of variance were compared by analysis of variance with post hoc Tukey's honestly significant difference tests for pair-wise comparisons. For sensitivity analysis, we further compared plasma biomarker values among AMC, CU+, MCI+, and AD+ groups. The prognostic performance of biomarkers for Aβ pathology was assessed using receiver operating characteristic (ROC) curve analysis. Associations between plasma biomarkers and CSF biomarkers or MMSE score were tested using Spearman correlation coefficient (rs) analysis. A two-sided p-value < 0.05 was considered statistically significant for all tests. All statistical analyses were conducted using the Statistical Package for the Social Sciences version 28 (SPSS Inc., Chicago, IL, USA).

3. Results

The demographic characteristics of the participants are shown in Table 1. Excluding the YNC group, mean age (± standard deviation) was 71.77 ± 8.14 years, and 46 (47.9 %) were women. The proportions of *APOE* E4 carriers and individuals with a family history of dementia were significantly lower in the AMC group, while mean MMSE scores were significantly lower in MCI+ and AD+ groups compared to the CU- group (both $p < 0.001$). No significant differences were found in age, sex, and years of education among these groups.

There were significant differences in plasma $A\beta_{1-42}$ concentration, $A\beta_{1-40}/A\beta_{1-42}$ ratio, and $APP_{669-711}/A\beta_{1-42}$ ratio among Aβ+ (CU+, MCI+, and AD+) as well as Aβ− (CU−) groups ($A\beta_{1-42}$ concentration: $F = 9.204$, $p < 0.001$; $A\beta_{1-40}/A\beta_{1-42}$ ratio: $F = 18.04$, $p < 0.001$, $APP_{669-711}/A\beta_{1-42}$ ratio: $F = 20.06$, $p < 0.001$), but no significant group-level differences in $A\beta_{1-40}$ concentration ($F = 1.790$, $p = 0.154$) or $APP_{669-711}$ concentration ($F = 0.327$, $p = 0.806$). Tukey's post hoc comparisons showed that CU+, MCI+, and AD+ had significantly lower plasma $A\beta_{1-42}$ levels than CU- ($p = 0.047$, $p < 0.001$, and $p < 0.001$, respectively) (Supplementary Fig. 1a). Post hoc comparisons also showed that MCI+ and AD+ had significantly higher $A\beta_{1-40}/A\beta_{1-42}$ levels than CU- (both $p < 0.001$) (Supplementary Fig. 1b), and that CU+, MCI+, and AD+ had significantly higher $APP_{669-711}/A\beta_{1-42}$ levels than CU- ($p = 0.002$, $p < 0.001$, and $p < 0.001$, respectively) (Supplementary Fig. 1c). Further, plasma $A\beta_{1-42}$ concentration distinguished Aβ+ from Aβ− individuals with 75.0 % sensitivity and 82.9 % specificity at an optimal cutoff value [OCV] of 4.480 (areas under the ROC curve [AUC] = 0.821). Similarly, $A\beta_{1-40}/A\beta_{1-42}$ ratio distinguished Aβ+ from Aβ− individuals with 85.4 % sensitivity and 85.7 % specificity at an OCV of 9.926 (AUC = 0.882), and $APP_{669-711}/A\beta_{1-42}$ ratio distinguished Aβ+ from Aβ− individuals with 75.0 % sensitivity and 95.1 % specificity at an OCV of 1.187 (AUC = 0.896).

There were also significant correlations between CSF $A\beta_{1-42}$ concentration and the plasma $A\beta_{1-42}$ concentration, (rs = 0.542, $p < 0.001$), $A\beta_{1-40}/A\beta_{1-42}$ ratio (rs = −0.398, $p < 0.001$), and $APP_{669-711}/A\beta_{1-42}$ ratio (rs = −0.456, $p < 0.001$) (Fig. 1a–c) as well as between MMSE score and the plasma $A\beta_{1-42}$ concentration (rs = 0.352, $p < 0.001$), $A\beta_{1-40}/A\beta_{1-42}$ ratio (rs = −0.466, $p < 0.001$), and $APP_{669-711}/A\beta_{1-42}$ ratio (rs = −0.544, $p < 0.001$). When we restricted analysis to the Aβ+ group, the significant relationship between MMSE scores and plasma $APP_{669-711}/A\beta_{1-42}$ ratio remained unchanged (rs = −0.244 and $p = 0.027$) (Fig. 1c), whereas the relationships with $A\beta_{1-42}$ concentration

Table 1
Participants characteristics.

	YNC $n = 14$		AMC $n = 14$		CU+ $n = 8$		MCI+ $n = 28$		AD+ $n = 46$	
Demographics										
Age, years	14	35.0 (10.1)	14	67.7 (11.0)	8	74.5 (6.6)	28	73.3 (7.1)	46	71.5 (7.7)
Women, n	14	6 [42.9 %]	14	3 [21.4 %]	8	2 [25.0 %]	28	16 [57.1 %]	46	25 [54.3 %]
APOE -E4 carriers, n	13	2 [15.4 %]	14	4 [28.6 %]*	8	4 [50.0 %]	27	16 [59.3 %]	43	29 [67.4 %]
Years of education, years	14	14.7 (1.6)	14	12.1 (2.8)	8	13.1 (3.7)	28	11.2 (2.5)	46	12.0 (1.9)
FH of dementia, n	14	0 [0 %]	14	0 [0 %]*	8	2 [25.0 %]	28	12 [42.9 %]	46	16 [34.8 %]
MMSE score, points	13	30.0 (0.0)	14	29.2 (0.9)*	8	26.8 (1.9)	28	26.0 (2.2)	46	20.8 (4.1)

Data presented as mean (standard deviation) or number [percent].

* $p < 0.001$, compared to CU+, MCI+, AD+ groups.Abbreviations: AD+, patients with Alzheimer's disease dementia with amyloid β pathology; AMC, age matched control; *APOE*, apolipoprotein E; CU+, cognitively unimpaired participants with amyloid β pathology; FH, family history; MCI+, mild cognitive impairment due to Alzheimer's disease with amyloid β pathology; MMSE, Mini-Mental State Examination; YNC, young normal control.

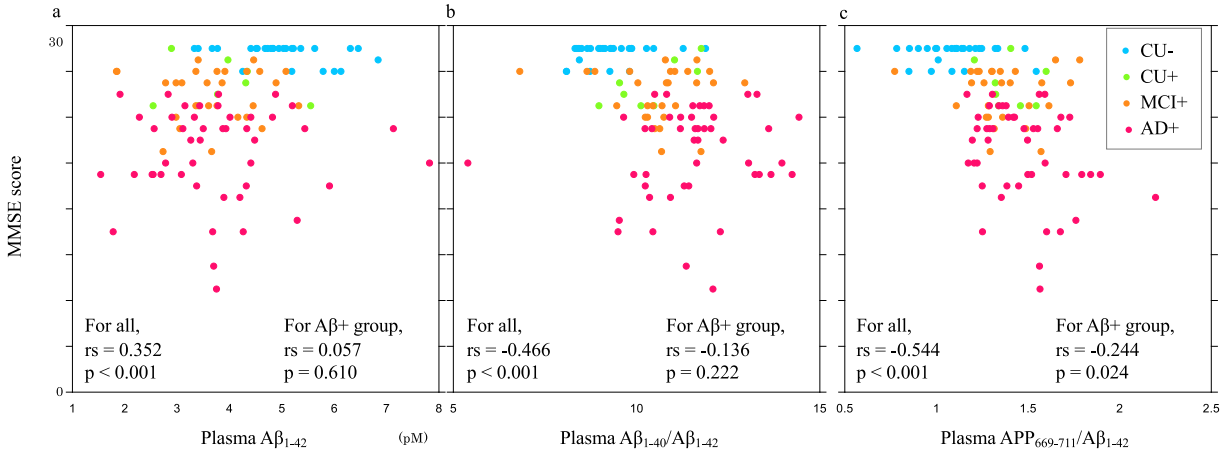

Fig. 1. Association between MMSE score and the levels of plasma amyloid markers. Scatter plot for the MMSE score and plasma $A\beta_{1-42}$ (a), plasma $A\beta_{1-40}/A\beta_{1-42}$ ratio (b), and plasma $APP_{669-711}/A\beta_{1-42}$ ratio (c) ($n = 110$). The colored circles represent clinical categories: AD+ (red), MCI+ (orange), CU+ (green), and CU- (blue). Spearman's correlation coefficients (rs) and their significance (two-sided p-value) are indicated in the plots.
Abbreviations: Aβ, amyloid-β; AD+, patients with Alzheimer's disease dementia with amyloid β pathology; APP, amyloid-β precursor protein; CU+, cognitively unimpaired participants with amyloid β pathology; CU-, cognitively unimpaired participants without amyloid β pathology; MCI+, mild cognitive impairment due to Alzheimer's disease with amyloid β pathology; MMSE, Mini-Mental State Examination.(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and $A\beta_{1-40}/A\beta_{1-42}$ ratio were no longer significant. Sensitivity analysis in which the YNC subgroup was excluded from the CU− group did not produce any differences in significance profile compared to inclusion of both YNC and AMC subgroups in the CU− group.

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4. Discussion

This study demonstrated that plasma $A\beta_{1-42}$ levels were significantly lower in patients with CU+, MCI+, or AD+, whereas plasma $A\beta_{1-40}/A\beta_{1-42}$ ratio was significantly elevated in MCI+ and AD+ groups compared to the CU- group. Furthermore, plasma $APP_{669-711}/A\beta_{1-42}$ ratio was significantly higher in the CU+, MCI+, and AD+ groups than the CU- group. ROC analysis showed that these plasma Aβ markers distinguished Aβ+ (Aβ pathology-positive) from Aβ− individuals with relatively high sensitivity and specificity, in accord with a previous study [6]. Additionally, however, by utilizing a larger case cohort ($n = 110$ vs. $n = 46$ in [6]), we found a strong correlation between CSF $A\beta_{1-42}$ concentration and plasma Aβ marker values. Plasma $APP_{669-711}/A\beta_{1-42}$ ratio and MMSE score were also significantly correlated, even after restricting analysis to the Aβ+ group, suggesting $APP_{669-711}/A\beta_{1-42}$ ratio as a potential biomarker for cognitive decline in AD.

The CSF $APP_{669-711}$ concentration did not differ significantly between Aβ+ and Aβ- groups [11]. In contrast, CSF $APP_{669-711}/A\beta_{1-42}$ ratio was reported to be significantly higher among individuals with Aβ pathology (Aβ+ group in the current study) [11]. The plasma $APP_{669-711}/A\beta_{1-42}$ ratio is a promising surrogate biomarker of cerebral Aβ accumulation [6], although the pathophysiological functions of $APP_{669-711}$ in AD are currently unknown. In the current study, three types of Aβ peptides, $A\beta_{1-40}$, $A\beta_{1-42}$, and $APP_{669-711}$ were measured (Supplementary Figure 2). The $APP_{669-711}$ (or $A\beta_{-3-40}$) is one of the N-terminally elongated Aβ peptides which are generated by the metalloproteinase ADAMTS4 (disintegrin and metalloproteinase with thrombospondin motifs 4) [12] which also generates N-terminally truncated Aβ4-x peptides [13] found at high abundance in human AD brains [14]. Of note, ADAMTS4 is known as a potential AD risk locus [15] as it is associated with Aβ aggregation and early detection of amyloid pathology [16]. It has been reported that $APP_{669-711}$ is capable of aggregation into amyloid fibrils [17], albeit with a lower tendency for self-assembly than $A\beta_{1-42}$ [6]. Our results showed that the Aβ+ group had significantly lower plasma $A\beta_{1-42}$ levels, although there were no significant association between MMSE score and plasma $A\beta_{1-42}$ levels in the Aβ+ group. Considering these results, it is possible that plasma $APP_{669-711}$ levels might increase with cognitive decline in patients with AD, although there have been no reports on the relationship between $APP_{669-711}$ and cognitive function. In addition, in the periphery, ADAMTS4 expression can be upregulated by inflammatory cytokines, and plasma $APP_{669-711}$ levels can be affected by inflammatory conditions [18]. Therefore, further investigations are expected to clarify the mechanisms of the association between $APP_{669-711}/A\beta_{1-42}$ ratio and cognitive function in patients with AD.

Plasma glial fibrillary acidic protein, p-tau 181, p-tau 231, and neurofilament light levels have been significantly correlated with cognition [19], whereas there are no reports on plasma Aβ markers and cognitive function has not been established. This study is the first to report that the plasma $APP_{669-711}/A\beta_{1-42}$ ratio is associated with cognitive decline in patients across the AD continuum.

The major limitation of our study is its cross-sectional design. Future longitudinal studies are needed to clarify the potential of the plasma $APP_{669-711}/A\beta_{1-42}$ ratio as a prognostic biomarker for cognitive decline in AD. Nevertheless, our results indicate that the plasma $APP_{669-711}/A\beta_{1-42}$ ratio is associated with cognition in patients with AD and could be used to distinguish between Aβ+ (CU+, MCI+ and AD+) Aβ- (CU-) individuals.

Data Sharing

The datasets used in the current study are not publicly available as they contain confidential clinical data on the study participants. However, the data are available on reasonable request and after permission from the corresponding author.

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Author Credit Statement

MN-S, acquisition and analysis of data, drafting a significant portion of the manuscript; YS, HN, DM, SH, HM, and YM, acquisition of data; KO, conception and design of the study, interpretation of data, and critical revision of the manuscript and supervision.

Financial disclosures of all authors

Nothing to report.

Declaration of competing interest

Nothing to report.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tjpad.2024.100003.

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