Journal of Alzheimer's Disease 79 (2021) 573–584
DOI 10.3233/JAD-200987
IOS Press

Quantitative Measurement of Cerebrospinal Fluid Amyloid-β Species by Mass Spectrometry

Yusuke Seino[a], Takumi Nakamura[b], Tomoo Harada[c], Naoko Nakahata[d], Takeshi Kawarabayashi[e], Tetsuya Ueda[c], Masamitsu Takatama[e] and Mikio Shoji[e,*]
[a]*Department of Neurology, Hirosaki National Hospital, Hirosaki, Aomori, Japan*
[b]*Department of Neurology, Gunma University Graduate School of Medicine, Maebashi, Gunma, Japan*
[c]*Bioanalysis Department, LSI Medience Corporation, Itabashi-ku, Tokyo, Japan*
[d]*Department of Speech-Language-Hearing Therapy, Hirosaki University of Health and Welfare, Hirosaki, Aomori, Japan*
[e]*Dementia Center, Geriatrics Research Institute and Hospital, Maebashi, Gunma, Japan*

Accepted 26 October 2020
Pre-press 15 December 2020

Abstract.
Background: High sensitivity liquid chromatography mass spectrometry (LC-MS/MS) was recently introduced to measure amyloid-β (Aβ) species, allowing for a simultaneous assay that is superior to ELISA, which requires more assay steps with multiple antibodies.
Objective: We validated the $Aβ_{1-38}$, $Aβ_{1-40}$, $Aβ_{1-42}$, and $Aβ_{1-43}$ assay by LC-MS/MS and compared it with ELISA using cerebrospinal fluid (CSF) samples to investigate its feasibility for clinical application.
Methods: CSF samples from 120 subjects [8 Alzheimer's disease (AD) with dementia (ADD), 2 mild cognitive dementia due to Alzheimer's disease (ADMCI), 14 cognitively unimpaired (CU), and 96 neurological disease subjects] were analyzed. Aβ species were separated using the Shimadzu Nexera X2 system and quantitated using a Qtrap 5500 LC-MS/MS system. $Aβ_{1-40}$ and $Aβ_{1-42}$ levels were validated using ELISA.
Results: CSF levels in CU were 666 ± 249 pmol/L in $Aβ_{1-38}$, 2199 ± 725 pmol/L in $Aβ_{1-40}$, 153.7 ± 79.7 pmol/L in $Aβ_{1-42}$, and 9.78 ± 4.58 pmol/L in $Aβ_{1-43}$. The ratio of the amounts of $Aβ_{1-38}$, $Aβ_{1-40}$, $Aβ_{1-42}$, and $Aβ_{1-43}$ was approximately 68:225:16:1. Linear regression analyses showed correlations among the respective Aβ species. Both $Aβ_{1-40}$ and $Aβ_{1-42}$ values were strongly correlated with ELISA measurements. No significant differences were observed in $Aβ_{1-38}$ or $Aβ_{1-40}$ levels between AD and CU. $Aβ_{1-42}$ and $Aβ_{1-43}$ levels were significantly lower, whereas the $Aβ_{1-38/1-42}$, $Aβ_{1-38/1-43}$, and $Aβ_{1-40/}Aβ_{1-43}$ ratios were significantly higher in AD than in CU. The basic assay profiles of the respective Aβ species were adequate for clinical usage.
Conclusion: A quantitative LC-MS/MS assay of CSF Aβ species is as reliable as specific ELISA for clinical evaluation of CSF biomarkers for AD.

Keywords: $Aβ_{1-38}$, $Aβ_{1-40}$, $Aβ_{1-42}$ $Aβ_{1-43}$, cerebrospinal fluid, ELISA, liquid chromatography mass spectrometry

INTRODUCTION

Cerebrospinal fluid (CSF) amyloid-β $(Aβ)_{1-42}$ is a definite biomarker of Alzheimer's disease (AD). A decrease in the $Aβ_{1-42}$ level, which begins 25 years earlier, allows the prediction of the onset and

*Correspondence to: Mikio Shoji, MD, PhD, Director, Dementia Center, Geriatrics Research Institute and Hospital, 3-26-8 Ootomo-machi, Maebashi, Gunma, 371-0847 Japan. Tel.: +81 27 253 3311; Fax: +81 27 252 7575; E-mail: m-shoji@ronenbyo.or.jp.

progression of Aβ amyloidosis in the AD brain. CSF $A\beta_{1-42}$ is the earliest and most reliable biomarker for the prediction and diagnosis of AD [1–3]. Low CSF $A\beta_{1-42}$ and cortical amyloid PET ligand binding have been adopted as biomarkers of Aβ plaques (labeled "A") in the ATN classification system of the NIA-AA research framework and have been proposed as diagnostic criteria based on the biological definition of AD for observational and interventional research [4].

γ-Secretase cleaves the β-carboxyl terminal fragment (β-CTF) of the amyloid-β protein precursor (AβPP) at the ε-site into long Aβs ($A\beta_{1-49}$ and $A\beta_{1-48}$), which are ultimately converted from $A\beta_{1-43}$ to $A\beta_{1-40}$ and from $A\beta_{1-42}$ to $A\beta_{1-38}$, respectively, by the successive cleavage of tripeptides and tetrapeptides [5, 6]. The half-life of Aβ in the brain is approximately 9 hours. However, the faster turnover of $A\beta_{1-42}$ than $A\beta_{1-38}$ and $A\beta_{1-40}$ in CSF has been reported with aging and AD [7, 8]. γ-Secretase activity in AD is enhanced by the conversion of $A\beta_{1-43}$ to $A\beta_{1-40}$ and $A\beta_{1-42}$ to $A\beta_{1-38}$, leading to low CSF levels of $A\beta_{1-42}$ and $A\beta_{1-43}$ [9]. $A\beta_{1-38}$ and $A\beta_{1-40}$ have been shown to attenuate $A\beta_{1-42}$ toxicity [10]. $A\beta_{1-43}$ is known to be enriched in the AD brain [11–13] and promotes the rates and extent of protofibril aggregation [14]. CSF $A\beta_{1-43}$ is of similar diagnostic accuracy to $A\beta_{1-42}$ [15, 16]. The quantification of $A\beta_{1-38}$, $A\beta_{1-40}$, and $A\beta_{1-42}$ in CSF using mass spectrometry and ELISA has already revealed that the $A\beta_{1-42/1-40}$ and $A\beta_{1-42/1-38}$ ratios are superior to CSF $A\beta_{1-42}$ alone for the detection of amyloid deposition in AD and the differentiation of AD dementia (ADD) from non-AD dementias [17–19]. However, $A\beta_{1-40}$ and $A\beta_{1-38}$ have lower discriminative powers [20–23]. Collectively, these findings imply that the precise quantitation of these Aβ species, including $A\beta_{1-38}$, $A\beta_{1-40}$, $A\beta_{1-42}$, and $A\beta_{1-43}$, is relevant to biomarkers for observational and interventional research.

However, antibody-based immunoassays, such as ELISA, have several disadvantages [24, 25]. Currently available ELISA systems using antibodies against different epitopes or the conformation of Aβ species measure the levels of various forms of Aβ species. The specific characteristics of Aβ with self-aggregation, binding to tubes, post-translational modifications, and cross-reactions with endogenous components of CSF decrease assay sensitivity and lead to variable results depending on the ELISA systems. Therefore, discrepant findings have been reported across each assay procedure and laboratory. This variability represents a major issue for global clinical observational and interventional studies. Commercial ELISA kits and systems are expensive, time-consuming (often include overnight incubations), and indicate the variation of measured values due to the different lots of the ELISA kits.

An approach based on liquid chromatography mass spectrometry (LC-MS/MS) has been developed to overcome these methodological issues [26–29]. The direct confirmation of $A\beta_{1-38}$, $A\beta_{1-40}$, $A\beta_{1-42}$, and $A\beta_{1-43}$ is possible, and detailed comparisons can be made with advanced LC-MS/MS equipment [8, 17–19]. However, the lower sensitivity and intra-assay variability of this system compared to immunological methods have yet to be reliably improved [26–29]. Therefore, we herein developed and validated a novel LC-MS/MS method to assay CSF $A\beta_{1-38}$, $A\beta_{1-40}$, $A\beta_{1-42}$, and $A\beta_{1-43}$, and further confirm their contribution as biomarkers for AD pathological changes by comparing the results obtained by the conventional ELISA system for $A\beta_{1-40}$ and $A\beta_{1-42}$.

MATERIALS AND METHODS

Subjects

One hundred and twenty CSF samples from 106 patients with neurological diseases and 14 cognitively unimpaired subjects (CU) were examined. The AD group consisted of 4 cases of autosomal inherited ADD with PSEN-1 mutations, 4 of ADD, and 2 of AD due to mild cognitive impairment (ADMCI) based on the criteria of NIA-AA [4, 30, 31]. They were all defined by positive *A*+T+N+biomarker changes. Clinically diagnosed neurological diseases by the respective recent criteria included 17 cases of amyotrophic lateral sclerosis [32], 9 of corticobasal degeneration [33], 6 of encephalopathy, 3 of frontotemporal dementia [34, 35], 14 of multiple sclerosis and related disorders, 6 of multiple system atrophy [36], 11 of Parkinson's disease [37], 4 of peripheral neuropathy, 11 of progressive supranuclear palsy [38], and 15 of spinocerebellar degeneration. Basic profiles are summarized in Table 1 and no significant differences were observed in age among the disease groups. CSF samples were taken in polypropylene vials by lumber puncture in the morning. Cell numbers and protein levels were examined in the first 1 mL of CSF. Each 1 mL of remaining CSF sample was transferred into polypropylene vials after centrifugation at 1,500 × g for 10 min and stored at –80°C until analysis.

Table 1
Subject profiles. Subjects consisted of 10 cases of Alzheimer's disease with dementia (ADD) and AD due to MCI (ADMCI), 17 of amyotrophic lateral sclerosis (ALS), and 9 of corticobasal degeneration (CBD). Six cases of encephalopathy (ENC) consisted of meningoencephalitis, Creutzfeldt-Jacob disease, leukoencephalopathy, superficial siderosis, or cerebral angiitis. Three cases of frontotemporal dementia (FTD), 14 cognitively unimpaired healthy controls (CU), 14 cases of multiple sclerosis (MS) and related disorders including neuromyelitis optica in the multiple sclerosis group, 6 of multiple system atrophy (MSA), 11 of Parkinson's disease (PD), 4 of peripheral neuropathy (PN), 11 of progressive supranuclear palsy (PSP), and 15 of spinocerebellar degeneration (SCD) were analyzed. No significant differences were observed in age among the CU and other groups

Disease	Number			Mean age	Age range
	Total	Males	Females		
ADD/ADMCI	10	5	5	59	41-80
ALS	17	13	4	67	53-83
CBD	9	4	5	72	67-78
ENC	6	1	5	60	41-78
FTD	3	2	1	73	70-75
CU	14	5	9	61	22-80
MS	14	3	11	40	19-75
MSA	6	2	4	66	58-81
PD	11	6	5	65	49-79
PN	4	2	2	51	30-62
PSP	11	6	5	74	67-82
SCD	15	7	8	64	39-80
Total	120	56	64	62	19–83

LC-MS/MS chemicals and reagents

Synthetic human $A\beta_{1\text{-}38}$, $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$, and $A\beta_{1\text{-}43}$ were purchased from the Peptide Institute (Osaka, Japan). Uniformly stable isotope-labeled peptides manufactured by rPeptide (Watkinsville, GA, USA), corresponding to each peptide ($A\beta_{1\text{-}38}$, $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$, and $A\beta_{1\text{-}43}$), were used as internal standards (ISs). Artificial CSF was purchased from Tocris Bioscience (Bristol, UK), and rat plasma was purchased from Charles River Laboratories Japan (Yokohama, Japan). Other materials and reagents were purchased from Kanto Chemical (Tokyo, Japan), Kokusan Chemical (Tokyo, Japan), and Sigma-Aldrich (St. Louis, MO, USA). Ultrapure water was prepared using Milli-Q Integral 10 (Merck, Darmstadt, Germany).

LC-MS/MS sample preparation by solid-phase extraction

Human CSF samples (300 μL) and IS solution were mixed in 1500 μL of water/propionic acid (PA)/trifluoroacetic acid (TFA) (950:50:1) and loaded onto an Oasis HLB 96-well plate (Waters Corporation, MA, USA). After loading, the plate was washed with 1000 μL of water/Methanol (MeOH)/PA/TFA (900:50:50:1) and then analytes and ISs were eluted with 100 μL of EtOH/2,2,2-trifluoroethanol (TF-EtOH)/PA/Ethylene glycol (1:1:1:0.1) twice. Eluates from individual samples were evaporated to dryness at approximately 40°C under nitrogen. The resulting residue was re-dissolved with 100 μL of water/MeOH/TF-EtOH/PA (85:5:5:5). Calibration standards were also prepared by spiking standard solutions to 300 μL of artificial CSF containing 0.25% rat plasma, with concentration ranges of 50–5000 pmol/L for $A\beta_{1\text{-}38}$, 100–10000 pmol/L for $A\beta_{1\text{-}40}$, 10–1000 pmol/L for $A\beta_{1\text{-}42}$, and 2–200 pmol/L for $A\beta_{1\text{-}43}$.

LC-MS/MS analysis

Chromatographic separation was performed using the Shimadzu Nexera X2 system (Shimadzu Corporation, Kyoto, Japan) with a column of Acquity UPLC BEH C8 (1.7 μm, 2.1 × 100 mm, Waters Corporation, MA, USA) set at 50°C. The mobile phases of (A) water/acetic acid (AA) (8:2) and (B) MeOH/AA (8:2) were used with a linear gradient program as follows (Time – B conc.): 0 min-25%, 3.5 min-55%, 4.0 min-100%, 6.0 min-100%, 6.1 min-25%, and 8.0 min-25%. The flow rate was set at 0.4 mL/min. Mass spectra were obtained by a Qtrap 5500 system (AB Sciex, CA, USA) with the positive electrospray ionization and multiple reaction monitoring (MRM) mode. MRM transitions are shown in Table 2. MS/MS conditions were as follows: ion spray voltage at 5500 V, temperature at 600°C, curtain gas at 10 psi, ion source gas 1 at 90 psi, ion source gas 2 at 90 psi, and collision gas at level 11. Quantification was performed using Analyst 1.6.2 (AB Sciex, Redwood City, CA, USA). Calibration curves were constructed by analyzing calibration standards. The peak area ratios of analytes to the corresponding ISs

Table 2
MRM transitions. Mass spectra were obtained by the Qtrap 5500 system with the positive electrospray ionization (ESI) and multiple reaction monitoring (MRM) mode. MRM transitions are shown, respectively

Analyte and IS	Precursor ion $[M+5H]^{5+}$ (m/z)	Product ion (m/z)
$A\beta_{1\text{-}38}$	827.3	801.1
$[^{15}N_{51}]$-$A\beta_{1\text{-}38}$	837.2	810.6
$A\beta_{1\text{-}40}$	867.0	843.8
$[^{15}N_{53}]$-$A\beta_{1\text{-}40}$	877.2	853.6
$A\beta_{1\text{-}42}$	903.7	863.2
$[^{15}N_{55}]$-$A\beta_{1\text{-}42}$	914.7	873.7
$A\beta_{1\text{-}43}$	924.1	1000.9
$[^{15}N_{56}]$-$A\beta_{1\text{-}43}$	935.1	1012.8

were plotted against analyte concentrations, and the curves were fit by a linear least-squares regression method (a weighting factor of 1/X2).

ELISA

Sandwich ELISA was used to quantify $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ levels using Human β Amyloid (1-40) ELISA Kit Wako II and a Human/Rat β Amyloid (1-42) ELISA Kit Wako, High-Sensitive according to the manufacturer's instructions (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Microplates were precoated with monoclonal BAN-50 (IgG, anti-Aβ1-16) and sequentially incubated with 25 μL of samples followed by horseradish-peroxidase-conjugated BA 27 (anti-$A\beta_{1\text{-}40}$ (Fab')2, specific for $A\beta_{40}$) or BC05 (anti-$_{A\beta35}$-43 (Fab')2, specific for $A\beta_{42/43}$) [39]. Sensitivities were 0.019 pmol/L (assay range 1.0–100 pmol/L) in the $A\beta_{1\text{-}40}$ assay and 0.06 pmol/L (assay range 0.01–20.0 pmol/L) in the $A\beta_{1\text{-}42}$ assay. Intra- and inter-assay coefficients of variation were less than 10% in both assay systems.

Data analysis

Simple linear regression models were used in statistical analyses. The linear regression line was drawn by the method of least squares. Comparisons among the groups of assay values were performed using the Kruskal-Wallis test with multiple comparison tests for non-parametric data. Two-tailed *p*-values less than 0.05 were considered to be significant. These analyses were performed with IBM SPSS Statistics, version 24 (IBM Japan, Tokyo) and GraphPad Prism, version 8 (GraphPad Software, San Diego, CA, USA).

RESULTS

The minimal to maximal LC-MS/MS assay ranges of $A\beta_{1\text{-}38}$, $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$, and $A\beta_{1\text{-}43}$ were 193.231∼1,224.771, 391.624∼3,560.087, 29.326∼328.450, and 0.714∼20.624 pmol/L, respectively (Table 3). CSF levels as mean ± standard deviation values of CU were 666 ± 249 pmol/L in $A\beta_{1\text{-}38}$, 2199 ± 725 pmol/L in $A\beta_{1\text{-}40}$, 153.7 ± 79.66 ± 79.7 pmol/L in $A\beta_{1\text{-}42}$, and 9.78 ± 4.58 pmol/L in $A\beta_{1\text{-}43}$. The ratios of $A\beta_{1\text{-}38/1\text{-}42}$, $A\beta_{1\text{-}38/1\text{-}43}$, $A\beta_{1\text{-}40/1\text{-}42}$, $A\beta_{1}$-40/1-$_{43}$, and $A\beta_{(1\text{-}40/1\text{-}43)}/A\beta_{(1\text{-}38/1\text{-}42)}$ were 4.66 ± 0.94, 71.7 ± 15.8, 15.7 ± 3.7, 241 ± 58, and 53 ± 13, respectively. The ratio of the amounts of $A\beta_{1\text{-}38}$, $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$, and $A\beta_{1\text{-}43}$ was approximately 68:225:16:1 (Table 4).

First order regression analysis showed a correlation between $A\beta_{1\text{-}38}$ and $A\beta_{1\text{-}40}$ ($Y = 0.3035*X + 8.409$; $r^2 = 0.8395$; $p < 0.0001$, Fig. 1a), between $A\beta_{1\text{-}38}$ and $A\beta_{1\text{-}42}$ ($Y = 2.686*X + 247.8$; $r^2 = 0.547$; $p < 0.0001$, Fig. 1b), between $A\beta_{1\text{-}38}$ and $A\beta_{1\text{-}43}$ ($Y = 36.4*X + 317.9$; $r^2 = 0.424$; $p < 0.0001$, Fig. 1c), between $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ ($Y = 8.204*X + 860.3$; $r^2 = 0.5601$; $p < 0.0001$, Fig. 1d), between $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}43}$ ($Y = 112.8*X + 1064$; $r^2 = 0.4469$; $p < 0.0001$, Fig. 1e), and between $A\beta_{1\text{-}42}$ and $A\beta_{1\text{-}43}$ ($Y = 13.4*X + 27.09$; $r^2 = 0.7576$; $p < 0.0001$, Fig. 1f).

$A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ values measured by LC-MS/MS were strongly correlated with those obtained by ELISA. The first order regression analysis was $Y = 0.9726*X + 165.9$ ($r^2 = 0.8127$, $p < 0.0001$) for $A\beta_{1\text{-}40}$ values measured by LC-MS/MS and ELISA (Fig. 2a). Regarding $A\beta_{1\text{-}42}$ values, the regression curve was $Y = 0.8584*X + 2.545$ ($r^2 = 0.6207$, $p < 0.0001$) between LC-MS/MS and ELISA (Fig. 2b). Thus, these quantitative assays of $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ by LC-MS/MS were equivalent to those by specific ELISA. Comparisons between $A\beta_{1\text{-}42} + A\beta_{1\text{-}43}$ levels by LC-MS/MS and $A\beta_{1\text{-}42}$ values by ELISA showed the same relationship as those between LC-MS/MS $A\beta_{1\text{-}42}$ levels and $A\beta_{1\text{-}42}$ by ELISA ($Y = 0.9100*X + 2.389$, $r^2 = 0.6245$, $p < 0.0001$; Fig. 2c). These results show that conventional ELISA using BCO5 for Aβ42/43 measures both $A\beta_{1\text{-}42}$ and $A\beta_{1\text{-}43}$ in CSF.

The regression curve between the $A\beta_{1\text{-}38/1\text{-}42}$ ratio and $A\beta_{1\text{-}40/}A\beta_{1\text{-}43}$ ratio measured by LC-MS/MS

Table 3
Basic profile of the LC-MS/MS analysis. The minimal to maximal assay ranges, median, mean, and standard deviations of $A\beta_{1\text{-}38}$, $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$, and $A\beta_{1\text{-}43}$ values measured by LC-MS/MS assay and ELISA

Item	$A\beta_{1\text{-}38}$ LC-MS/MS	$A\beta_{1\text{-}40}$ LC-MS/MS	ELISA	$A\beta_{1\text{-}42}$ LC-MS/MS	ELISA	$A\beta_{1\text{-}43}$ LC-MS/MS
Max	1224.771	3560.087	3401.273	328.450	306.233	20.624
Min	193.231	391.624	634.258	29.326	41.120	0.714
Median	506.495	1739.419	1555.430	100.789	122.838	5.798
Mean	546.038	1771.370	1619.538	111.056	126.683	6.267
SD	198.920	600.511	561.217	54.778	50.425	3.559

Table 4

Assay data obtained by LC-MS/MS. Mean ± standard values (pmol/L) of $A\beta_{1\text{-}38}$, $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$, and $A\beta_{1\text{-}43}$, and the ratios of $A\beta_{1\text{-}38/1\text{-}42}$, $A\beta_{1\text{-}38/1\text{-}43}$, $A\beta_{1\text{-}40/1\text{-}42}$, $A\beta_{1\text{-}40/1\text{-}43}$, and $A\beta_{(1\text{-}40/1\text{-}43)}/A\beta_{(1\text{-}38/1\text{-}42)}$ in respective disease groups including with ADD and ADMCI, ALS, CBD, 6 ENC, 3 FTD, MS, MSA, PD, PN, PSP, and SCD

Disease	mean level ± standard deviation pmol/L				ratio±standard deviation						
	$A\beta_{1\text{-}38}$	$A\beta_{1\text{-}40}$	$A\beta_{1\text{-}42}$	$A\beta_{1\text{-}43}$	$A\beta_{1\text{-}38/1\text{-}42}$	$A\beta_{1\text{-}38/1\text{-}43}$	$A\beta_{1\text{-}40/1\text{-}42}$	$A\beta_{1\text{-}40}/A\beta_{1\text{-}42+}A\beta_{1\text{-}43}$	$A\beta_{1\text{-}40}/_{1\text{-}43}$	$A\beta_{1\text{-}42/1\text{-}43}$	$A\beta_{(1\text{-}40/1\text{-}43)}/_{(1\text{-}38/1\text{-}42)}$
ADD/ ADMCI	498 ± 166	1552 ± 527	71.93 ± 27.7**a	3.15 ± 2.66****b	7.38 ± 2.28*e	250.2 ± 184.2***f	22.5 ± 6.8	21.6 ± 6.6	759 ± 520**g	32.5 ± 19.8	101 ± 61
ALS	647 ± 260	1986 ± 615	115.8 ± 44.6	6.68 ± 2.75	6.06 ± 2.55	107.4 ± 48.3	19.1 ± 8.3	18.0 ± 7.8	333 ± 138	18.0 ± 4.8	57 ± 13
CBD	474 ± 160	1634 ± 526	90.4 ± 50.1	4.82 ± 2.65	6.24 ± 2.54	125.1 ± 71.2	22.0 ± 10.5	20.6 ± 9.1	452 ± 324	21.7 ± 11.4	78 ± 50
ENC	503 ± 156	1571 ± 405	107.8 ± 54.4	4.39 ± 2.49*c	5.05 ± 1.19	133.7 ± 53.2	15.9 ± 3.7	15.3 ± 3.6	429 ± 199	26.2 ± 2.1*h	85 ± 30
FTD	671 ± 62	2132 ± 438	146.4 ± 63.1	9.07 ± 4.46	5.26 ± 2.36	91.4 ± 53.0	16.2 ± 5.9	15.3 ± 5.6	279 ± 134	16.8 ± 2.1	53 ± 3
CU	666 ± 249	2199 ± 725	153.7 ± 79.7	9.78 ± 4.58	4.66 ± 0.94	71.7 ± 15.8	15.7 ± 3.7	114.7 ± 3.3	241 ± 58	15.7 ± 3.4	53 ± 13
MS	468 ± 138	1604 ± 554	97.2 ± 37.6	5.58 ± 3.07	5.27 ± 2.14	120.2 ± 121.7	17.8 ± 6.6	16.9 ± 6.4	393 ± 352	20.6 ± 8.5	69 ± 26
MSA	498 ± 191	1531 ± 702	83.2 ± 33.9	5.41 ± 1.61	6.42 ± 2.72	93.6 ± 33.2	19.5 ± 7.9	18.2 ± 7.1	282 ± 82	15.3 ± 4.0	46 ± 13
PD	489 ± 127	1702 ± 518	97.6 ± 33.9	4.98 ± 2.17*d	5.29 ± 1.23	110.7 ± 49.1	18.2 ± 4.2	17.3 ± 3.9	385 ± 181	21.2 ± 8.7	74 ± 34
PN	464 ± 165	1546 ± 491	114.4 ± 48.7	7.54 ± 1.94	4.30 ± 1.04	60.7 ± 10.3	14.3 ± 2.9	13.3 ± 2.5	202 ± 17	14.7 ± 4.2	50 ± 14
PSP	537 ± 260	1729 ± 830	129.6 ± 87.4	7.07 ± 5.31	4.61 ± 1.42	106.5 ± 81.3	14.8 ± 4.0	14.0 ± 3.8	342 ± 262	22.6 ± 15.1	73 ± 49
SCD	550 ± 124	1759 ± 352	118.1 ± 27.2	6.67 ± 2.15	4.71 ± 0.60	86.4 ± 17.8	15.2 ± 2.3	14.3 ± 2.2	284 ± 96	18.6 ± 4.7	62 ± 29
total	546 ± 199	1771 ± 601	111.1 ± 54.8	6.27 ± 3.56	5.44 ± 1.96	111.2 ± 89.1	17.7 ± 6.3	16.7 ± 5.9	370 ± 272	20.45 ± 10.2	67 ± 35

**a $p = 0.0066$; ****b $p < 0.0001$; *c $p = 0.0297$; *d $p = 0.0281$; *e $p = 0.021$; ***f $p = 0.0004$; **g $p = 0.0033$; *h $p = 0.0123$. ADD, Alzheimer's disease with dementia; ADMCI, AD due to MCI; ALS, amyotrophic lateral sclerosis; CBD, corticobasal degeneration; ENC, encephalopathy; FTD, frontotemporal dementia;), CU, cognitively unimpaired; MS, multiple sclerosis; MSA, multiple system atrophy; PD, Parkinson's disease; PN, peripheral neuropathy; PSP, progressive supranuclear palsy; SCD, spinocerebellar degeneration.

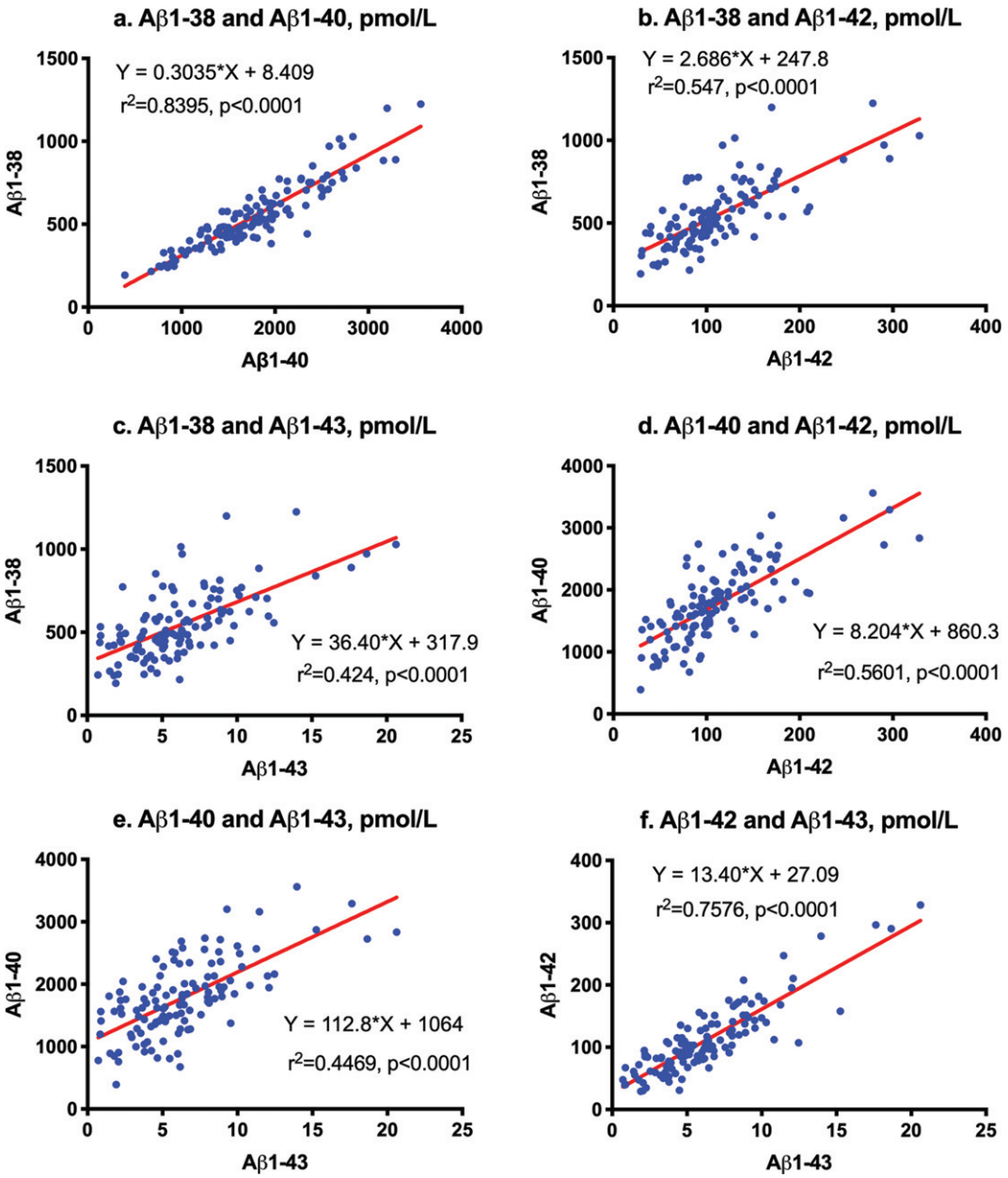

Fig. 1. Correlation among Aβ species measured by LC-MS/MS. First order regression analysis between $A\beta_{1\text{-}38}$ and $A\beta_{1\text{-}40}$ (a), between $A\beta_{1\text{-}38}$ and $A\beta_{1\text{-}42}$ (b), between $A\beta_{1\text{-}38}$ and $A\beta_{1\text{-}43}$ (c), between $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ (d), between $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}43}$ (e), and between $A\beta_{1\text{-}42}$ and $A\beta_{1\text{-}43}$ (f).

was $Y = 0.003858*X+4.017$ ($r^2 = 0.2857$, $p < 0.0001$; Fig. 2d), suggesting that this ratio is almost constant. The ratios of 8 out of 10 AD patients were outside the linear regression curve (Fig. 2d: Red circles indicate ADD and triangles indicate ADMCI). Although correlation analysis between measured levels by LC-MS/MS and ELISA in $A\beta_{1\text{-}40}/A\beta_{1\text{-}42}$ and $A\beta_{1\text{-}40}/A\beta_{1\text{-}42} + A\beta_{1\text{-}43}$ showed close relationships, most values were present in the leftmost regression curve (Fig. 1e, f).

We then examined the efficacy of LC-MS/MS assay values as biomarkers for AD by comparisons with assay values among ADD/ADMCI, CU, and other neurogenerative diseases. No significant differences were observed in $A\beta_{1\text{-}38}$ and $A\beta_{1\text{-}40}$ levels among ADD/ADMCI and other neurological

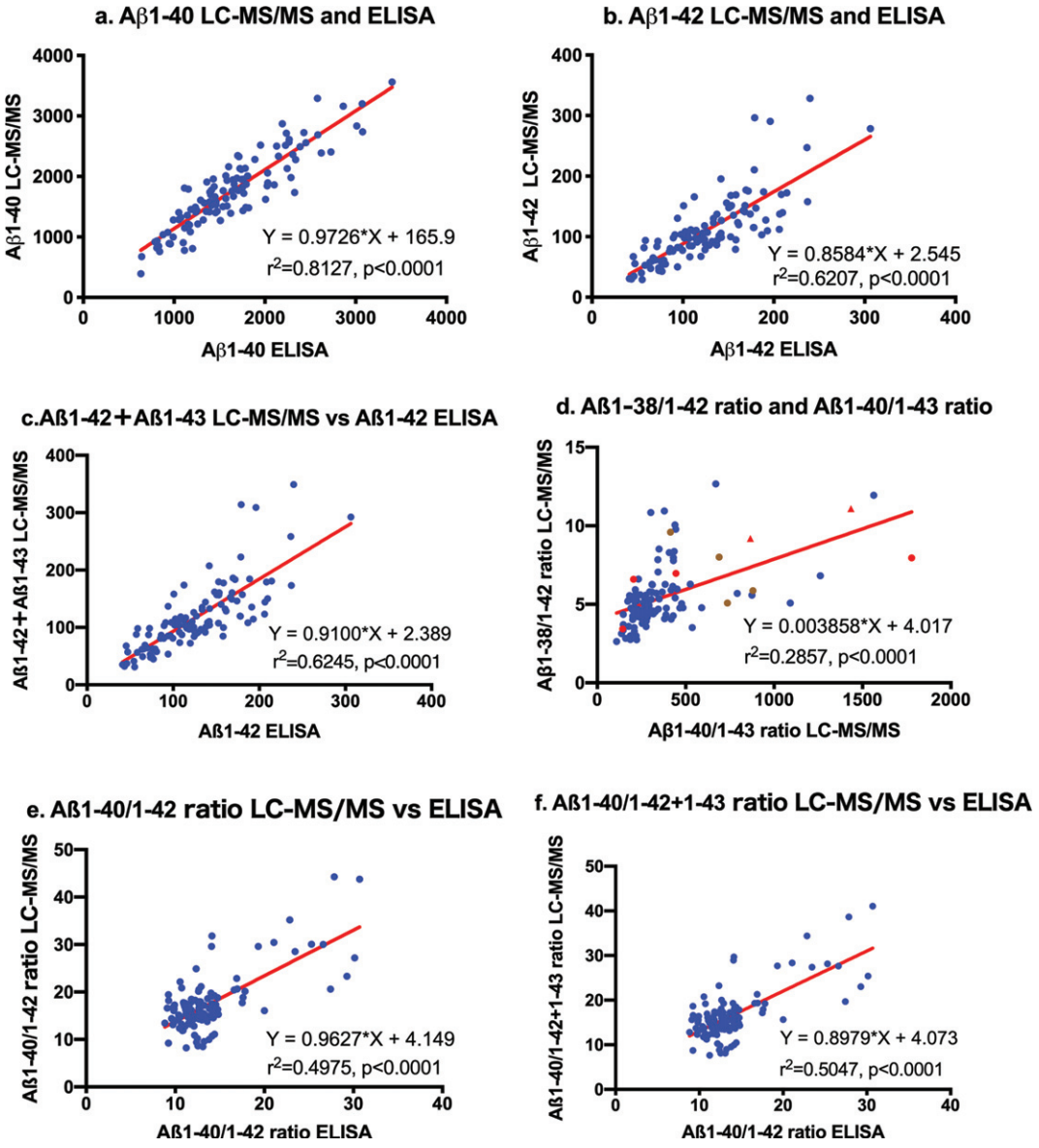

Fig. 2. Correlation between LC-MS/MS and ELISA. $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$, and $A\beta_{1\text{-}42} + A\beta_{1\text{-}43}$ values measured by LC-MS/MS were strongly correlated with those obtained by ELISA (a: $Y = 0.9726*X + 165.9$, $r^2 = 0.8127$, $p < 0.0001$ for $A\beta_{1\text{-}40}$ values; b: $Y = 0.8584*X + 2.545$, $r^2 = 0.6207$, $p < 0.0001$ for $A\beta_{1\text{-}42}$; and c: comparisons between $A\beta_{1\text{-}42} + A\beta_{1\text{-}43}$ levels by LC-MS/MS and $A\beta_{1\text{-}42}$ values by ELISA: $Y = 0.9100*X + 2.389$, $r^2 = 0.6245$, $p < 0.0001$). The regression curve between the $A\beta_{1\text{-}38}/_{1\text{-}42}$ ratio and $A\beta_{1\text{-}40}/A\beta_{1\text{-}43}$ ratio measured by LC-MS/MS was almost constant. The ratios of 8 out of 10 AD patients were outside the linear regression curve (d: Red circles ● represent sporadic ADD, red triangles ▲ represent sporadic ADMCI, brown circles ● represent familial AD). Correlation analysis between measured levels by LC-MS/MS and ELISA in $A\beta_{1\text{-}40}/A\beta_{1\text{-}42}$ and $A\beta_{1\text{-}40}/A\beta_{1\text{-}42} + A\beta_{1\text{-}43}$ showed close relationships, but most values were present in the leftmost regression curve (e and f).

diseases and CU (Fig. 3a, b). $A\beta_{1\text{-}42}$ levels were significantly lower in ADD/ADMCI than in CU ($p = 0.0066$; Fig. 3c). CSF $A\beta_{1\text{-}43}$ values were significantly lower in ADD/ADMCI (Fig. 3d; $p < 0.0001$), encephalopathy ($p = 0.0297$), and Parkinson's disease ($p = 0.0281$) than in CU. The $A\beta_{1\text{-}38}/A\beta_{1\text{-}42}$ and $A\beta_{1\text{-}38}/A\beta_{1\text{-}43}$ ratios were significantly higher in ADD/ADMCI than in CU ($p = 0.021$ in $A\beta_{1\text{-}38/1\text{-}42}$, Fig. 3e; $p = 0.0004$ in $A\beta_{1\text{-}38/1\text{-}43}$, Fig. 3f). Increased ratios of $A\beta_{1\text{-}40}/A\beta_{1\text{-}42}$ and $A\beta_{1\text{-}40}/A\beta_{1\text{-}42} + A\beta_{1\text{-}43}$ were not significant ($p = 0.1226$ in $A\beta_{1\text{-}40/}A\beta_{1\text{-}42}$ ratio, Fig. 3g; $p = 0.0895$ in $A\beta_{1\text{-}40}/A\beta_{1\text{-}42} + A\beta_{1\text{-}43}$ ratio). The $A\beta_{1\text{-}40}/A\beta_{1\text{-}43}$ ratio was significantly increased ($p = 0.0033$, Fig. 3h). The

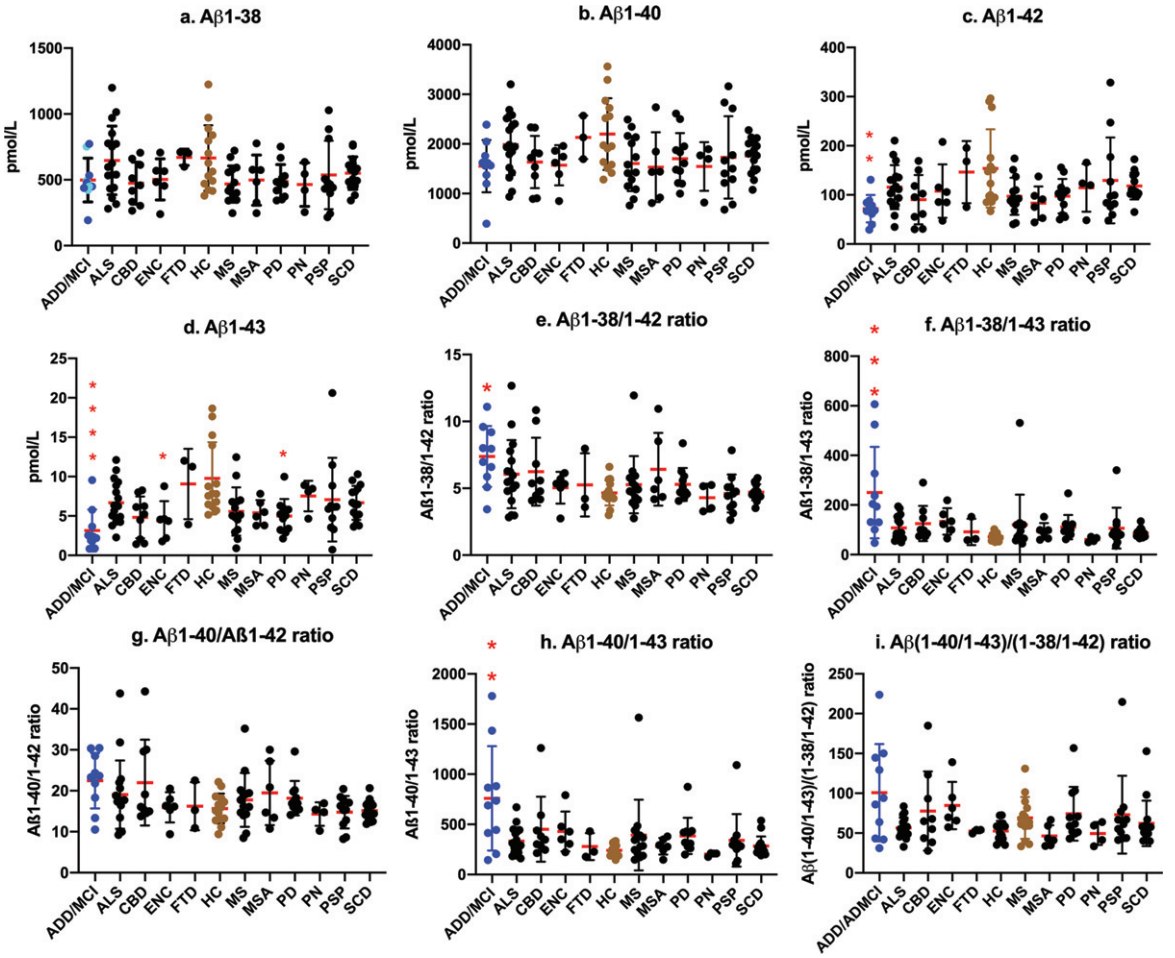

Fig. 3. Values of CSF Aβ species and ratios among neurological diseases and controls. No significant differences were observed in $A\beta_{1\text{-}38}$ and $A\beta_{1\text{-}40}$ levels among ADD/ADMCI and other neurological diseases and CU (a, b). $A\beta_{1\text{-}42}$ levels were significantly lower in ADD/ADMCI than in CU ($p = 0.0066$; c). CSF $A\beta_{1\text{-}43}$ values were significantly lower in ADD/ADMCI (d; $p < 0.0001$), ENC ($p = 0.0297$), and PD ($p = 0.0281$) than in CU. The $A\beta_{1\text{-}38}/A\beta_{1\text{-}42}$ and $A\beta_{1\text{-}38}/A\beta_{1\text{-}43}$ ratios were significantly higher in ADD/ADMCI than in CU (e: $p = 0.021$; f: $p = 0.0004$). Ratios of $A\beta_{1\text{-}40}/A\beta_{1\text{-}42}$ and $A\beta_{1\text{-}40}/A\beta_{1\text{-}42} + A\beta_{1\text{-}43}$ were not significant (g: $p = 0.1226$ in $A\beta_{1\text{-}40}/A\beta_{1\text{-}42}$ ratio; $p = 0.0895$ in $A\beta_{1\text{-}40}/A\beta_{1\text{-}42} + A\beta_{1\text{-}43}$ ratio). The $A\beta_{1\text{-}40}/A\beta_{1\text{-}43}$ ratio was significantly increased (h: $p = 0.0033$). The $A\beta\ (_{1\text{-}40}/_{1\text{-}43})/A\beta\ (_{1\text{-}38}/_{1\text{-}42})$ ratio was not significantly changed in ADD/ADMCI and other diseases (i). (a: Blue circles ● represent sporadic ADD and ADMCI, cyan circles ● represent familial AD).

$A\beta(_{1\text{-}40}/_{1\text{-}43})/A\beta(_{1\text{-}38}/_{1\text{-}42})$ ratio was not significantly changed in ADD/ADMCI and other diseases. These results imply that successive cleavage from $A\beta_{1\text{-}43}$ to $A\beta_{1\text{-}40}$ and from $A\beta_{1\text{-}42}$ to $A\beta_{1\text{-}38}$ was enhanced in parallel in AD.

DISCUSSION

Mass spectrometry is a device that is capable of detecting the masses of various substances, as well as accurate peptide sequence lengths. Due to advances in the operability and analytical devices for extremely small amounts, this method has been clinically applied to the measurement of various substances in recent years. MS/MS is the combination of two mass analyzers in one mass spectrometric instrument. The combination with a highly selective separation method by LC allows for more accurate and reliable quantification for clinical application. Therefore, the LC-MS/MS technique has been used to measure Aβ peptides [7–9, 17–19, 26–29]. This method eliminates time-consuming immune assays with a large number of steps and multiple antibodies or immunoprecipitation steps for pre-clinical studies. This approach also allows for performance of a single assay to measure several different Aβ species simultaneously in one sample with selectivity, specificity, and in a high-throughput format, while still achieving

the high sensitivity required for low level endogenous Aβ peptides (such as CSF $A\beta_{1\text{-}43}$), plasma Aβ species, and modified tau products.

Comparative assays using the same samples between the LC-MS/MS assay and commonly used ELISA systems for $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$, and $A\beta_{1\text{-}42} + A\beta_{1\text{-}43}$ confirmed a strong correlation ($Y = 0.9726*X + 165.9$ for $A\beta_{1\text{-}40}$; $Y = 0.8584*X + 2.545$ for $A\beta_{1\text{-}42}$; $Y = 0.9100*X + 2.389$ for $A\beta_{1\text{-}42} + A\beta_{43}$; Fig. 2a–c). These results suggest that this LC-MS/MS assay is as reliable and reproducible a system as conventional ELISA systems. We did not validate $A\beta_{1\text{-}38}$ or $A\beta_{1\text{-}43}$ assay values by comparisons with ELISA systems. This LC-MS/MS assay requires further improvements in sensitivity for lower $A\beta_{1\text{-}43}$ values. However, studies on relationships with the respective Aβ species showed strong linear correlations among $A\beta_{1\text{-}38}$, $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$, and $A\beta_{1\text{-}43}$ (Fig. 1). Reliable ELISA for $A\beta_{1\text{-}38}$ and $A\beta_{1\text{-}43}$ are not commonly available. The present results support the application of the LC-MS/MS assay system for clinical usage, similar to conventional ELISA systems.

We previously proposed that $A\beta_{1\text{-}38/1\text{-}42}$ and $A\beta_{1\text{-}40/1\text{-}43}$ were distinct product/precursor pairs and reflected γ-secretase activity [9]. Significantly decreased CSF levels of $A\beta_{1\text{-}42}$ and $A\beta_{1\text{-}43}$ accompanied by increased $A\beta_{1\text{-}38/1\text{-}42}$ and $A\beta_{1\text{-}40/1\text{-}43}$ ratios and an enhanced proportional ratio of $A\beta_{1\text{-}40/1\text{-}43}$ versus $A\beta_{1\text{-}38/1\text{-}42}$ suggested altered successive cleavage by γ-secretase in AD. In the present study, strong correlations between $A\beta_{1\text{-}38}$ and $A\beta_{1\text{-}40}$ ($r^2 = 0.8395$) and between $A\beta_{1\text{-}42}$ and $A\beta_{1\text{-}43}$ ($r^2 = 0.7576$) also support this cleavage being processed in parallel. The processing ratio from $A\beta_{1\text{-}42}$ to $A\beta_{1\text{-}38}$ was 2.686 (Fig. 1b), and that from $A\beta_{1\text{-}43}$ to $A\beta_{1\text{-}40}$ was 112.8 (Fig. 1f). The accurate ratios of $A\beta_{1\text{-}38/1\text{-}42}$ were 4.66 in CU and 7.38 in AD whereas those of $A\beta_{1\text{-}40/1\text{-}43}$ were 241 in CU and 795 in AD. Both cleavage processes appear to have been enhanced in parallel twice in AD (Fig. 2d). Recent studies revealed that CNS-derived Aβ is cleared into the CSF [7] and peripheral blood [40], and that the clearance rate decreases in late onset AD [8] and is differently regulated by age, *APOE* isoforms, and the presence of Aβ amyloidosis [41–44]. Therefore, the measurement of precise amounts of $A\beta_{1\text{-}38}$, $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$, and $A\beta_{1\text{-}43}$ is relevant for clinical application of biomarkers and understanding of the processing of Aβ species in AD.

Comparisons between 66 cases of early-onset AD and 25 of late-onset AD by ELISA showed that CSF $A\beta_{1\text{-}43}$ significantly varied with age in AD. However, $A\beta_{1\text{-}43}$ levels were not superior to $A\beta_{1\text{-}42}$ for diagnostic accuracy [15]. CSF $A\beta_{1\text{-}43}$, but not $A\beta_{1\text{-}42}$, differentiated between patients with amnestic MCI who progressed to AD during a 2-year follow-up [16]. Goda and Kobayashi evaluated dog CSF $A\beta_{1\text{-}38}$, $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$, and $A\beta_{1\text{-}43}$ using peptide adsorption-controlled LC-MS/MS and showed that the Aβ assay was reproducible [17]. Aβ quantitation by LC-MS/MS showed satisfactory intra- and inter-day reproducibility as well as freeze-thaw and room temperature stability [17, 18]. The findings of a recent large study by Janelidze et al. using three different immunoassays revealed that the CSF $A\beta_{42}/A\beta_{40}$ and $A\beta_{42/38}$ ratios were significantly better than CSF $A\beta_{42}$ alone for the detection of brain amyloid deposition in prodromal AD and differentiating AD dementia from non-AD dementias [20]. Widely used ELISA for the quantitation of $A\beta_{1\text{-}42}$/total tau, $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}/A\beta_{1\text{-}38}$, and $A\beta_{1\text{-}42}$ showed the greatest accuracy for identifying amyloid-positive subjects. $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}38}$ showed significantly lower discriminative powers [21]. The first report of ELISA assays for $A\beta_{1\text{-}38}$, $A\beta_{1\text{-}40}$, and $A\beta_{1\text{-}42}$ showed only decreased levels of $A\beta_{1\text{-}42}$ compared with $A\beta_{1\text{-}38}$ and $A\beta_{1\text{-}40}$ in CSF from AD [22]. CSF $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}/A\beta_{1\text{-}43}$ were positively correlated with in gait variability and ADL in mild ADD [23]. A UPLC-MS-MS assay of CSF $A\beta_{1\text{-}42}$ showed equivalent diagnostic utility to those of commonly used AlzBio3 ELISA systems [26]. Oe et al. reported that the validated methods had limits of quantitation of 44 fmol/mL for $A\beta_{1\text{-}42}$ and 92 fmol/mL for $A\beta_{1\text{-}40}$ in CSF. A strong correlation was observed between LC/MS/MS and ELISA for $A\beta_{1\text{-}42}$ ($r^2 = 0.915$), and those correlations were weak for $A\beta_{1\text{-}40}$ ($r^2 = 0.644$) [27]. The accuracy and precision of LC-MS/MS will contribute to the clinical diagnosis and monitoring of therapeutic interventions [27].

The present results suggest that the quantitative LC-MS/MS assay for CSF Aβ species is as reliable as specific ELISA for the clinical evaluation of CSF biomarkers for AD. Since the present study was not a large scale or prospective control study, further large-scale studies are necessary. However, the reliable and convenient LC-MS/MS multiple assay of Aβ species is equivalent to specific multiple ELISA assays. Therefore, the LC-MS/MS multiple assay of Aβ species may be applicable and contribute to kinetic [7, 8] and drug monitoring [44] studies on Aβ species. The establishment of a multiple Aβ and tau species assay by LC-MS/MS is warranted.

ACKNOWLEDGMENTS

We thank Yasuhito Wakasaya for his research assistance. This study was supported by the Amyloidosis Research Committee Surveys and Research on Special Diseases, the Longevity Science Committee of the Ministry of Health and Welfare of Japan; Scientific Research (C) (18K07385 MS, 19K07989 T.K.) from the Ministry of Education, Science, and Culture of Japan, the Hirosaki University Institutional Research Grant, and the Center of Innovation Science and Technology-based Radical Innovation and Entrepreneurship Program from the Japan Science and Technology Agency. This study was approved by the Ethics Committee of Hirosaki University (2017-112) and Geriatrics Research Institute and Hospital (2019-78). All participants provided written informed consent.

Authors' disclosures available online (https://www.j-alz.com/manuscript-disclosures/20-0987r1).

REFERENCES

[1] Kanai M, Matsubara E, Isoe K, Urakami K, Nakashima K, Arai H, Sasaki H, Abe K, Iwatsubo T, Kosaka T, Watanabe M, Tomidokoro Y, Shizuka M, Mizushima K, Nakamura T, Igeta Y, Ikeda Y, Amari M, Kawarabayashi T, Ishiguro K, Harigaya Y, Wakabayashi K, Okamoto K, Hirai S, Shoji M (1998) Longitudinal study of cerebrospinal fluid levels of tau, A beta1-40, and A beta1-42(43) in Alzheimer's disease: A study in Japan. *Ann Neurol* **44**, 17-26.

[2] Shaw LM, Vanderstichele H, Knapik-Czajka M, Clark CM, Aisen PS, Petersen RC, Blennow K, Soares H, Simon A, Lewczuk P, Dean R, Siemers E, Potter W, Lee VM, Trojanowski JQ, Alzheimer's Disease Neuroimaging Initiative (2009) Cerebrospinal fluid biomarker signature in Alzheimer's Disease Neuroimaging Initiative subjects. *Ann Neurol* **65**, 403-413.

[3] Bateman RJ, Xiong C, Benzinger TL, Fagan AM, Goate A, Fox NC, Marcus DS, Cairns NJ, Xie X, Blazey TM, Holtzman DM, Santacruz A, Buckles V, Oliver A, Moulder K, Aisen PS, Ghetti B, Klunk WE, McDade E, Martins RN, Masters CL, Mayeux R, Ringman JM, Rossor MN, Schofield PR, Sperling RA, Salloway S, Morris JC, Dominantly Inherited Alzheimer Network (2012) Clinical and biomarker changes in dominantly inherited Alzheimer's disease. *N Engl J Med* **367**, 795-804.

[4] Jack CR Jr, Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, Holtzman DM, Jagust W, Jessen F, Karlawish J, Liu E, Molinuevo JL, Montine T, Phelps C, Rankin KP, Rowe CC, Scheltens P, Siemers E, Snyder HM, Sperling R; Contributors (2018) NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimers Dement* **14**, 535-562.

[5] Qi-Takahara Y, Morishima-Kawashima M, Tanimura Y, Dolios G, Hirotani N, Horikoshi Y, Kametani F, Maeda M, Saido TC, Wang R, Ihara Y (2005) Longer forms of amyloid beta protein: Implications for the mechanism of intramembrane cleavage by gamma-secretase. *J Neurosci* **25**, 436-445.

[6] Takami M, Nagashima Y, Sano Y, Ishihara S, Morishima-Kawashima M, Funamoto S, Ihara Y (2009) γ-Secretase: Successive tripeptide and tetrapeptide release from the transmembrane domain of beta-carboxyl terminal fragment. *J Neurosci* **29**, 13042-13052.

[7] Bateman RJ, Munsell LY, Morris JC, Swarm R, Yarasheski KE, Holtzman DM (2006) Human amyloid-beta synthesis and clearance rates as measured in cerebrospinal fluid *in vivo*. *Nat Med* **12**, 856-861.

[8] Patterson BW, Elbert DL, Mawuenyega KG, Kasten T, Ovod V, Ma S, Xiong C, Chott R, Yarasheski K, Sigurdson W, Zhang L, Goate A, Benzinger T, Morris JC, Holtzman D, Bateman RJ (2015) Age and amyloid effects on human central nervous system amyloid-beta kinetics. *Ann Neurol* **78**, 439-453.

[9] Kakuda N, Shoji M, Arai H, Furukawa K, Ikeuchi T, Akazawa K, Takami M, Hatsuta H, Murayama S, Hashimoto Y, Miyajima M, Arai H, Nagashima Y, Yamaguchi H, Kuwano R, Nagaike K, Ihara Y; Japanese Alzheimer's Disease Neuroimaging Initiative (2012) Altered γ-secretase activity in mild cognitive impairment and Alzheimer's disease. *EMBO Mol Med* **4**, 344-352.

[10] Moore BD, Martin J, de Mena L, Sanchez J, Cruz PE, Ceballos-Diaz C, Ladd TB, Ran Y, Levites Y, Kukar TL, Kurian JJ, McKenna R, Koo EH, Borchelt DR, Janus C, Rincon-Limas D, Fernandez-Funez P, Golde TE (2018) Short Aβ peptides attenuate Aβ42 toxicity *in vivo*. *J Exp Med* **215**, 283-301.

[11] Iizuka T, Shoji M, Harigaya Y, Kawarabayashi T, Watanabe M, Kanai M, Hirai S (1995) Amyloid beta-protein ending at Thr43 is a minor component of some diffuse plaques in the Alzheimer's disease brain, but is not found in cerebrovascular amyloid. *Brain Res* **702**, 275-278.

[12] Welander H, Frånberg J, Graff C, Sundström E, Winblad B, Tjernberg LO (2009) Abeta43 is more frequent than Abeta40 in amyloid plaque cores from Alzheimer disease brains. *J Neurochem* **110**, 697-706.

[13] Sandebring A, Welander H, Winblad B, Graff C, Tjernberg LO (2013) The pathogenic aβ43 is enriched in familial and sporadic Alzheimer disease. *PLoS One* **8**, e55847.

[14] Conicella AE, Fawzi NL (2014) The C-terminal threonine of Aβ43 nucleates toxic aggregation via structural and dynamical changes in monomers and protofibrils. *Biochemistry* **53**, 3095-3105.

[15] Lauridsen C, Sando SB, Møller I, Berge G, Pomary PK, Grøntvedt GR, Salvesen Ø, Bråthen G, White LR (2017) Cerebrospinal fluid Aβ43 is reduced in early-onset compared to late-onset Alzheimer's disease, but has similar diagnostic accuracy to Aβ42. *Front Aging Neurosci* **9**, 210.

[16] Lauridsen C, Sando SB, Shabnam A, Møller I, Berge G, Grøntvedt GR, Bakken IJ, Salvesen Ø, Bråthen G, White LR (2016) Cerebrospinal fluid levels of amyloid beta 1-43 in patients with amnestic mild cognitive impairment or early Alzheimer's disease: A 2-year follow-up study. *Front Aging Neurosci* **8**, 30.

[17] Goda R, Kobayashi N (2012) Evaluation of peptide adsorption-controlled liquid chromatography-tandem mass spectrometric (PAC-LC-MS/MS) method for simple and simultaneous quantitation of amyloid β 1-38, 1-40, 1-42 and 1-43 peptides in dog cerebrospinal fluid. *J Chromatogr B Analyt Technol Biomed Life Sci* **895-896**, 137-145.

[18] Lame ME, Chambers EE, Blatnik M (2011) Quantitation of amyloid beta peptides Aβ(1-38), Aβ(1-40), and Aβ(1-42)

in human cerebrospinal fluid by ultra-performance liquid chromatography-tandem mass spectrometry. *Anal Biochem* **419**, 133-139.

[19] Pottiez G, Yang L, Stewart T, Song N, Aro P, Galasko DR, Quinn JF, Peskind ER, Shi M, Zhang J (2017) Mass-spectrometry-based method to quantify in parallel tau and amyloid β 1-42 in CSF for the diagnosis of Alzheimer's disease. *J Proteome Res* **16**, 1228-1238.

[20] Janelidze S, Zetterberg H, Mattsson N, Palmqvist S, Vanderstichele H, Lindberg O, van Westen D, Stomrud E, Minthon L, Blennow K; Swedish BioFINDER study group, Hansson O (2016) CSF Aβ42/Aβ40 and Aβ42/Aβ38 ratios: Better diagnostic markers of Alzheimer disease. *Ann Clin Transl Neurol* **3**, 154-165.

[21] Adamczuk K, Schaeverbeke J, Vanderstichele HM, Lilja J, Nelissen N, Van Laere K, Dupont P, Hilven K, Poesen K, Vandenberghe R (2015) Diagnostic value of cerebrospinal fluid Aβ ratios in preclinical Alzheimer's disease. *Alzheimers Res Ther* **7**, 75.

[22] Schoonenboom NS, Mulder C, Van Kamp GJ, Mehta SP, Scheltens P, Blankenstein MA, Mehta PD (2005) Amyloid beta 38, 40, and 42 species in cerebrospinal fluid: More of the same? *Ann Neurol* **58**, 139-142.

[23] Koychev I, Galna B, Zetterberg H, Lawson J, Zamboni G, Ridha BH, Rowe JB, Thomas A, Howard R, Malhotra P, Ritchie C, Lovestone S, Rochester L (2018) Aβ42/Aβ40 and Aβ42/Aβ38 ratios are associated with measures of gait variability and activities of daily living in mild Alzheimer's disease: A pilot study. *J Alzheimers Dis* **65**, 1377-1383.

[24] Shaw LM, Vanderstichele H, Knapik-Czajka M, Figurski M, Coart E, Blennow K, Soares H, Simon AJ, Lewczuk P, Dean RA, Siemers E, Potter W, Lee VM, Trojanowski JQ; Alzheimer's Disease Neuroimaging Initiative (2011) Qualification of the analytical and clinical performance of CSF biomarker analyses in ADNI. *Acta Neuropathol* **121**, 597-609.

[25] Mattsson N, Andreasson U, Persson S, Arai H, Batish SD, Bernardini S, Bocchio-Chiavetto L, Blankenstein MA, Carrillo MC, Chalbot S, Coart E, Chiasserini D, Cutler N, Dahlfors G, Duller S, Fagan AM, Forlenza O, Frisoni GB, Galasko D, Galimberti D, Hampel H, Handberg A, Heneka MT, Herskovits AZ, Herukka SK, Holtzman DM, Humpel C, Hyman BT, Iqbal K, Jucker M, Kaeser SA, Kaiser E, Kapaki E, Kidd D, Klivenyi P, Knudsen CS, Kummer MP, Lui J, Lladó A, Lewczuk P, Li QX, Martins R, Masters C, McAuliffe J, Mercken M, Moghekar A, Molinuevo JL, Montine TJ, Nowatzke W, O'Brien R, Otto M, Paraskevas GP, Parnetti L, Petersen RC, Prvulovic D, de Reus HP, Rissman RA, Scarpini E, Stefani A, Soininen H, Schröder J, Shaw LM, Skinningsrud A, Skrogstad B, Spreer A, Talib L, Teunissen C, Trojanowski JQ, Tumani H, Umek RM, Van Broeck B, Vanderstichele H, Vecsei L, Verbeek MM, Windisch M, Zhang J, Zetterberg H, Blennow K (2011) The Alzheimer's Association external quality control program for cerebrospinal fluid biomarkers. *Alzheimers Dement* **7**, 386-395.

[26] Korecka M, Waligorska T, Figurski M, Toledo JB, Arnold SE, Grossman M, Trojanowski JQ, Shaw LM (2014) Qualification of a surrogate matrix-based absolute quantification method for amyloid-β in human cerebrospinal fluid using 2D UPLC-tandem mass spectrometry. *J Alzheimers Dis* **41**, 441-451.

[27] Oe T, Ackermann BL, Inoue K, Berna MJ, Garner CO, Gelfanova V, Dean RA, Siemers ER, Holtzman DM, Farlow MR, Blair IA (2006) Quantitative analysis of amyloid beta peptides in cerebrospinal fluid of Alzheimer's disease patients by immunoaffinity purification and stable isotope dilution liquid chromatography/negative electrospray ionization tandem mass spectrometry. *Rapid Commun Mass Spectrom* **20**, 3723-3735.

[28] Nakamura A, Kaneko N, Villemagne VL, Kato T, Doecke J, Doré V, Fowler C, Li QX, Martins R, Rowe C, Tomita T, Matsuzaki K, Ishii K, Ishii K, Arahata Y, Iwamoto S, Ito K, Tanaka K, Masters CL, Yanagisawa K (2018) High performance plasma amyloid-β biomarkers for Alzheimer's disease. *Nature* **554**, 249-254.

[29] Lin PP, Chen WL, Yuan F, Sheng L, Wu YJ, Zhang WW, Li GQ, Xu HR, Li XN (2017) An UHPLC-MS/MS method for simultaneous quantification of human amyloid beta peptides Aβ1-38, Aβ1-40 and Aβ1-42 in cerebrospinal fluid using micro-elution solid phase extraction. *J Chromatogr B Analyt Technol Biomed Life Sci* **1070**, 82-91.

[30] McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR Jr, Kawas CH, Klunk WE, Koroshetz WJ, Manly JJ, Mayeux R, Mohs RC, Morris JC, Rossor MN, Scheltens P, Carrillo MC, Thies B, Weintraub S, Phelps CH (2011) The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* **7**, 263-269.

[31] Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, Gamst A, Holtzman DM, Jagust WJ, Petersen RC, Snyder PJ, Carrillo MC, Thies B, Phelps CH (2011) The diagnosis of mild cognitive impairment due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* **7**, 270-279.

[32] de Carvalho M, Dengler R, Eisen A, England JD, Kaji R, Kimura J, Mills K, Mitsumoto H, Nodera H, Shefner J, Swash M (2008) Electrodiagnostic criteria for diagnosis of ALS. *Clin Neurophysiol* **119**, 497-503.

[33] Armstrong MJ, Litvan I, Lang AE, Bak TH, Bhatia KP, Borroni B, Boxer AL, Dickson DW, Grossman M, Hallett M, Josephs KA, Kertesz A, Lee SE, Miller BL, Reich SG, Riley DE, Tolosa E, Tröster AI, Vidailhet M, Weiner WJ (2013) Criteria for the diagnosis of corticobasal degeneration. *Neurology* **80**, 496-503.

[34] Rascovsky K, Hodges JR, Knopman D, Mendez MF, Kramer JH, Neuhaus J, van Swieten JC, Seelaar H, Dopper EG, Onyike CU, Hillis AE, Josephs KA, Boeve BF, Kertesz A, Seeley WW, Rankin KP, Johnson JK, Gorno-Tempini ML, Rosen H, Prioleau-Latham CE, Lee A, Kipps CM, Lillo P, Piguet O, Rohrer JD, Rossor MN, Warren JD, Fox NC, Galasko D, Salmon DP, Black SE, Mesulam M, Weintraub S, Dickerson BC, Diehl-Schmid J, Pasquier F, Deramecourt V, Lebert F, Pijnenburg Y, Chow TW, Manes F, Grafman J, Cappa SF, Freedman M, Grossman M, Miller BL (2011) Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. *Brain* **134**, 2456-2477.

[35] Chare L, Hodges JR, Leyton CE, McGinley C, Tan RH, Kril JJ, Halliday GM (2014) New criteria for frontotemporal dementia syndromes: Clinical and pathological diagnostic implications. *J Neurol Neurosurg Psychiatry* **85**, 865-70.

[36] Gilman S, Wenning GK, Low PA, Brooks DJ, Mathias CJ, Trojanowski JQ, Wood NW, Colosimo C, Dürr A, Fowler CJ, Kaufmann H, Klockgether T, Lees A, Poewe W, Quinn N, Revesz T, Robertson D, Sandroni P, Seppi K, Vidailhet

M (2008) Second consensus statement on the diagnosis of multiple system atrophy. *Neurology* **71**, 670-676.

[37] Postuma RB, Berg D, Stern M, Poewe W, Olanow CW, Oertel W, Obeso J, Marek K, Litvan I, Lang AE, Halliday G, Goetz CG, Gasser T, Dubois B, Chan P, Bloem BR, Adler CH, Deuschl G (2015) MDS clinical diagnostic criteria for Parkinson's disease. *Mov Disord* **30**, 1591-1601.

[38] Höglinger GU, Respondek G, Stamelou M, Kurz C, Josephs KA, Lang AE, Mollenhauer B, Müller U, Nilsson C, Whitwell JL, Arzberger T, Englund E, Gelpi E, Giese A, Irwin DJ, Meissner WG, Pantelyat A, Rajput A, van Swieten JC, Troakes C, Antonini A, Bhatia KP, Bordelon Y, Compta Y, Corvol JC, Colosimo C, Dickson DW, Dodel R, Ferguson L, Grossman M, Kassubek J, Krismer F, Levin J, Lorenzl S, Morris HR, Nestor P, Oertel WH, Poewe W, Rabinovici G, Rowe JB, Schellenberg GD, Seppi K, van Eimeren T, Wenning GK, Boxer AL, Golbe LI, Litvan I; Movement Disorder Society-endorsed PSP Study Group (2017) Clinical diagnosis of progressive supranuclear palsy: The movement disorder society criteria. *Mov Disord* **32**, 853-864.

[39] Seino Y, Nakamura T, Kawarabayashi T, Hirohata M, Narita S, Wakasaya Y, Kaito K, Ueda T, Harigaya Y, Shoji M (2019) Cerebrospinal fluid and plasma biomarkers in neurodegenerative diseases. *J Alzheimers Dis* **68**, 395-404.

[40] Roberts KF, Elbert DL, Kasten TP, Patterson BW, Sigurdson WC, Connors RE, Ovod V, Munsell LY, Mawuenyega KG, Miller-Thomas MM, Moran CJ, Cross DT 3rd, Derdeyn CP, Bateman RJ (2014) Amyloid-β efflux from the central nervous system into the plasma. *Ann Neurol* **76**, 837-844.

[41] Mawuenyega KG, Sigurdson W, Ovod V, Munsell L, Kasten T, Morris JC, Yarasheski KE, Bateman RJ (2010) Decreased clearance of CNS beta-amyloid in Alzheimer's disease. *Science* **330**, 1774.

[42] Nakamura T, Kawarabayashi T, Seino Y, Hirohata M, Nakahata N, Narita S, Itoh K, Nakaji S, Shoji M (2018) Aging and APOE-ε4 are determinative factors of plasma Aβ42 levels. *Ann Clin Transl Neurol* **5**, 1184-1191.

[43] Swaminathan S, Risacher SL, Yoder KK, West JD, Shen L, Kim S, Inlow M, Foroud T, Jagust WJ, Koeppe RA, Mathis CA, Shaw LM, Trojanowski JQ, Soares H, Aisen PS, Petersen RC, Weiner MW, Saykin AJ; Alzheimer's Disease Neuroimaging Initiative (2014) Association of plasma and cortical amyloid beta is modulated by APOE ε4 status. *Alzheimers Dement* **10**, e9-e18.

[44] Bateman RJ, Siemers ER, Mawuenyega KG, Wen G, Browning KR, Sigurdson WC, Yarasheski KE, Friedrich SW, Demattos RB, May PC, Paul SM, Holtzman DM (2009) A gamma-secretase inhibitor decreases amyloid-beta production in the central nervous system. *Ann Neurol* **66**, 48-54.