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Longitudinal plasma amyloid beta in Alzheimer's disease clinical trials

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Abstract

Introduction: Little is known about the utility of plasma amyloid beta (Aβ) in clinical trials of Alzheimer's disease (AD).

Methods: We analyzed longitudinal plasma samples from two large multicenter clinical trials: (1) donezepil and vitamin E in mild cognitive impairment (n = 405, 24 months) and (2) simvastatin in mild to moderate AD (n = 225, 18 months).

Results: Baseline plasma Aβ was not related to cognitive or clinical progression. We observed a decrease in plasma Aβ40 and 42 among apolipoprotein E epsilon 4 (*APOE* ε4) carriers relative to noncarriers in the mild cognitive impairment trial. Patients treated with simvastatin showed a significant increase in Aβ compared with placebo. We found significant storage time effects and considerable plate-to-plate variation.

Discussion: We found no support for the utility of plasma Aβ as a prognostic factor or correlate of cognitive change. Analysis of stored specimens requires careful standardization and experimental design, but plasma Aβ may prove useful in pharmacodynamic studies of antiamyloid drugs.

Keywords: Alzheimer's disease; Mild cognitive impairment; Plasma amyloid; Biomarkers; Apolipoprotein E; Bioassay; Luminex; Innogenetics; Donepezil; Simvastatin

1. Introduction

Biomarkers of Alzheimer's disease (AD) have profoundly affected the course of AD research, drug development, and clinical practice. Cerebrospinal fluid (CSF) and neuroimaging measures of amyloid, presumably reflecting principal pathology of AD, are among the leading biomarkers. Given the somewhat invasive nature of CSF sampling and the expense of neuroimaging, plasma amyloid beta (Aβ) would be an attractive alternative biomarker. Although it is known that there is communication between the peripheral and central Aβ pools (via receptor mediated and passive mechanisms), the utility of plasma Aβ measurements has remained limited. Some studies have shown correlations between plasma Aβ and dementia risk and/or progression, although many of such findings have been inconsistent. Biological and methodological issues likely contribute to these limitations, thereby underlining the need for a better understanding of the biology and dynamics of plasma Aβ and the need for studies with longer follow-up to determine the clinical utility of measuring plasma Aβ.

As with CSF, changes in plasma Aβ may reflect changes within the brain [1–3], but may also be more affected by peripheral factors. In subjects with familial AD or Down syndrome, plasma Aβ begins to increase before dementia onset, perhaps reflecting increased Aβ production [4–9].

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Investigations of plasma Aβ as a predictor of dementia in sporadic or late-onset forms of AD have had inconsistent results (reviewed in [10]). Relationships have been found with plasma Aβ40 or 42 and dementia, but the direction of these associations varies among studies [11–16]. Some studies have found an association between lower Aβ42:40 ratios and higher risk of AD [17,18]. The sources of variability in findings from existing studies are potentially due to variability in subject age and with disease severity [12,19,20], but may also relate to study size; very few large-scale studies have been attempted. A recently published study in a cohort of N = 997 nondemented elderly patients found that cognitive reserve and plasma Aβ42:40 are associated, and the relationship is accentuated in those with low cognitive [21]. However, the predictive value of the plasma Aβ42:40 ratio was low.

Rodent studies demonstrate that a high cholesterol diet can increase levels of Aβ, which can be reversed by 3-hydroxy-3-methyl-glutaryl-(HMG) CoA reductase inhibitors (statins) drug treatment [22,23]. Simvastatin, an HMG CoA reductase inhibitor penetrates the central nervous system and has been shown to reduce the risk of cardiovascular disease and death. It was selected for use in an Alzheimer's Disease Cooperative Study (ADCS) randomized clinical trial to test the hypothesis that lipid lowering could reduce the clinical progression in subjects with AD who have cholesterol levels not otherwise requiring treatment. The study concluded that cholesterol levels decreased significantly in the statin group, but there was no effect on cognitive decline [24]. The effect of statin treatment on plasma Aβ was not assessed in the primary analysis, although it has been the subject of several investigations [25–28]. No studies of Aβ in plasma or CSF have found an effect of statin treatment [28–31], although several reported changes in amyloid precursor protein and improvements in cognition.

We assessed the relationships among plasma Aβ and clinical progression, treatment, and apolipoprotein E (*APOE*) using banked plasma from two large ADCS clinical trials: (1) donezepil and vitamin E in mild cognitive impairment (MCI; n = 405, 24 months) [32,33] and (2) simvastatin in mild to moderate Alzheimer's (n = 225, 18 months) [24]. Our primary goal was to determine covariates that may be associated with plasma Aβ40, 42, or ratio in the setting of AD clinical trials of 18–24 months duration. We also investigated the value of plasma Aβ as a predictive biomarker of clinical change, or an outcome measure in pharmacodynamic studies.

2. Methods

2.1. ADCS MCI trial

The 36-month, three-arm, placebo-controlled ADCS MCI trial examined the effect of vitamin E or donepezil in MCI patients (clinicaltrials.gov identifier: NCT00000173) [33]. A total of 769 patients with amnestic MCI were randomized to vitamin E, donepezil, or placebo. Complete information on inclusion, exclusion criteria, and the treatment regimen has been reported [32,33]. Serial blood samples were taken and plasma was aliquoted and banked (Appendix A, available in the online Supplementary Materials).

2.2. ADCS simvastatin trial

The potential benefit of 18 months of statin treatment on cognitive decline in AD was examined by the ADCS (clinicaltrials.gov identifier: NCT00053599). Individuals aged 50 years or older with probable AD and Mini-Mental State Examination (MMSE) within the range 12 to 26 were included. Individuals were excluded if they had other neurological or psychiatric diagnoses that could interfere with cognitive function, were taking lipid lowering drugs, or had conditions requiring cholesterol lowering treatment as defined by the Adult Treatment Panel (ATP III) guidelines. They were also excluded if they had low-density lipoprotein cholesterol below 80 mg/dl or triglycerides >500 mg/dl. Complete information on inclusion, exclusion and treatment regimen has been reported [24]. As with the MCI study, blood samples were taken and plasma was banked (Appendix A, available online).

2.3. Plasma analysis and internal standard

Plasma was assayed, quantified, and quality controlled as described in Appendices B and C, available online. Each assay plate also included a plasma sample derived from blood drawn by venipuncture of a 56-year-old cognitively normal volunteer in a single afternoon. This internal standard provided a means for adjusting plate-to-plate variation and assessing freezer storage effects.

2.4. Statistical methods

Storage effects on the internal standard were estimated by ordinary least squares regression of Aβ concentration on the number of years because the sample was obtained from the volunteer. We examined the associations between covariates of interest and plasma Aβ at baseline using linear mixed-effects models adjusting for the internal standard [34]. The covariates of interest include age, education, gender, *APOE* ε4, Alzheimer's Disease Assessment Scale Cognitive Subscale (ADAS-Cog), Activities of Daily Living (ADL), MMSE, urea nitrogen, creatinine, total protein, albumin, total cholesterol, hemoglobin, and platelets. See Appendix D, available online, for details.

To estimate the correlation between *change* in Aβ and *change* specific covariates, we used a multivariate outcome linear mixed-effects model approach [35]. Typically one would estimate the correlation of change by a two-step process: (1) calculate or estimate each individual's change from baseline for each outcome, (2) calculate the usual correlation coefficients for change in each pair of outcomes. Instead we used multivariate outcome mixed-effect models to estimate in a single step the correlation of change in each pair of outcomes. The model directly estimates the correlation

between random slopes for two outcomes in one step. This approach is more efficient and powerful for detecting correlations of change.

To account for the plate effects in our longitudinal models of treatment and *APOE* ε4 group differences in Aβ40, Aβ42, and the log ratio of Aβ42 to Aβ40; we used linear mixed-effects models with subject-specific effects nested within plate-specific effects [34]. The models treat time as categorical and provide estimates of differences between groups at each time point. We also considered adding effect to the model for sample storage time, subject age, creatinine, hemoglobin, total protein, albumin, and platelets. We considered both the baseline level of the labs and change in the labs as potential covariates. Rather than prespecifying which covariates should be included, we used the Akaike Information Criterion (AIC) [36] to objectively select covariates. Briefly, AIC uses the familiar likelihood framework in combination with a penalty for model complexity with the goal of determining which covariates comprise the most predictive model.

3. Results

3.1. Quality control

In the MCI trial, duplicate plasma samples were obtained from n = 480 subjects at baseline, n = 375 at 2 years, and

Table 1
Baseline characteristics

		MCI			
Variable	N	Without Aβ (N = 364)	With Aβ (N = 405)	Combined (N = 769)	*P*-value
Age (yrs)	769	73.70 (7.42)	72.23 (7.10)	72.93 (7.28)	.008
Gender					
Female	769	182 (50%)	170 (42%)	352 (46%)	.030
Education (yrs)	769	14.57 (3.12)	14.70 (3.05)	14.64 (3.08)	.423
APOE ε4 alleles					
0	769	146 (40%)	199 (49%)	345 (45%)	.007
1		186 (51%)	161 (40%)	347 (45%)	
2		32 (9%)	45 (11%)	77 (10%)	
ADAS11	765	11.33 (4.31)	11.23 (4.44)	11.28 (4.38)	.636
ADL	768	46.00 (4.94)	45.91 (4.63)	45.95 (4.77)	.455
MMSE	769	27.13 (1.89)	27.39 (1.81)	27.27 (1.85)	.054
Urea Nitrogen	689	16.90 (4.11)	17.66 (5.16)	17.34 (4.76)	.290
Creatinine	689	0.869 (0.190)	0.915 (0.227)	0.896 (0.213)	.010
Total Protein	689	7.082 (0.431)	7.053 (0.449)	7.065 (0.441)	.240
Albumin	689	4.161 (0.233)	4.175 (0.246)	4.169 (0.240)	.466
Total Cholesterol	688	215.2 (37.2)	213.1 (37.1)	214.0 (37.1)	.480
Hemoglobin	686	13.95 (1.18)	14.11 (1.25)	14.04 (1.22)	.126
Platelets	686	233.1 (54.6)	224.9 (52.8)	228.4 (53.7)	.068
		AD			
Variable	N	Without Aβ (N = 181)	With Aβ (N = 225)	Combined (N = 406)	*P*-value
Age (yrs)	406	74.88 (9.44)	74.35 (9.18)	74.58 (9.29)	.533
Gender					
Female	406	102 (56%)	139 (62%)	241 (59%)	.309
Education (yrs)	406	14.40 (3.38)	14.14 (3.08)	14.25 (3.21)	.290
APOE ε4 alleles					
0	358	64 (39%)	86 (44%)	150 (42%)	.626
1		78 (48%)	84 (43%)	162 (45%)	
2		21 (13%)	25 (13%)	46 (13%)	
ADAS11	403	24.35 (9.82)	24.07 (10.28)	24.19 (10.07)	.669
ADL	406	67.7 (10.0)	68.0 (10.3)	67.9 (10.2)	.491
MMSE	406	20.32 (4.72)	20.37 (4.69)	20.35 (4.70)	.900
Urea nitrogen	405	17.27 (4.87)	17.18 (4.96)	17.22 (4.91)	.779
Creatinine	405	0.904 (0.202)	0.860 (0.208)	0.879 (0.206)	.010
Total protein	405	7.171 (0.437)	7.141 (0.477)	7.154 (0.459)	.267
Albumin	405	4.122 (0.290)	4.174 (0.309)	4.151 (0.301)	.076
Total cholesterol	405	211.8 (30.1)	212.1 (30.8)	212.0 (30.5)	.888
Hemoglobin	401	13.99 (1.23)	14.01 (1.24)	14.00 (1.24)	.975
Platelets	398	246.5 (74.7)	249.1 (57.2)	247.9 (65.4)	.233

Abbreviations: MCI, mild cognitive impairment; ADAS, Alzheimer's Disease Assessment Scale; ADL; activities of daily living; MMSE, Mini-Mental State Examination; AD, Alzheimer's disease.

NOTE. Mean (standard deviation) and counts (percentages) of baseline characteristics among those with plasma Aβ samples that passed quality controls versus not. *P*-values are from Wilcoxon rank-sum or Fisher's exact tests.

n = 338 subjects at 3 years. After excluding samples with coefficient of variance (CV) greater than 20%, we analyzed data from n = 405 subjects at baseline, n = 349 at 2 years, and n = 309 at 3 years. Similarly, for the simvastatin trial we obtained samples from n = 242 subjects at baseline and n = 206 at 1.5 years; and of these n = 225 at baseline and n = 190 at 1.5 years were used in the analysis. The range of storage times of the MCI samples was from 7.81 to 13.4 years across all study visits. The storage time range for samples from the simvastatin trial was 3.95 to 7.82 years.

3.2. Baseline characteristics

Table 1 shows the baseline characteristics of the subjects that had analyzable plasma Aβ samples passing quality control versus those that did not. In the MCI trial, subjects with versus without analyzable plasma Aβ data were younger, less female, more *APOE* ε4 positive, and had higher levels of creatinine. In the simvastatin trial, subjects with analyzable plasma Aβ data had lower levels of creatinine compared with those that did not have analyzable plasma Aβ data.

3.3. Storage effects and plate-to-plate variation of biological standard

Fig. 1 depicts the storage effect that we observed from the biological standard that was aliquoted on each plate. Storage time of the biological standard ranged from 0 to 1.8 years. We found that estimated Aβ40 and Aβ42 concentrations of the biological standard declined significantly over time (−14.42 pg/ml Aβ40 per storage year, standard error of mean (SE) = 1.32, $P < .001$; −1.893 pg/ml Aβ42 per storage year, SE = 0.616, $P = .003$). The standard deviations of the residuals from these models, σ = 6.9 pg/ml Aβ40 and σ = 3.2 pg/ml Aβ42, provide measures of the plate-to-plate variability, controlling for storage. Fig. 1 also demonstrates a wide range of estimated concentrations, even within a short time frame. In the samples assayed within 40 days of venipuncture, for instance, the range is nearly 21.6 pg/ml for Aβ40, and about 7.58 pg/ml for Aβ42. The interplate CV, adjusted for storage effect, was 15.1% for Aβ40 and 24.5% for Aβ42, while the median intraplate CV was 6.0% for Aβ40 and 8.3% for Aβ42.

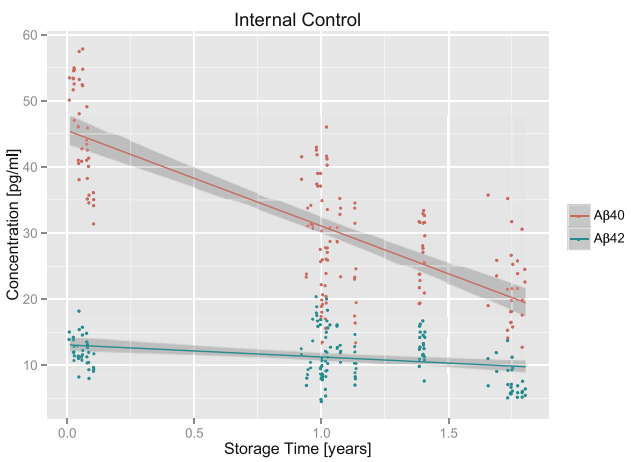

Fig. 1. Storage effects. Each plate included an aliquot from the same healthy control sample. We observed a significant linear effect of storage time on the estimated concentration of this sample. Estimated storage time plots are from an ordinary least squares regression. Shaded regions indicate 95% confidence bounds.

3.4. Baseline associations with plasma Aβ40, Aβ42, and log ratio of Aβ42 to Aβ40

Table 2 summarizes the associations among covariates and Aβ40 and Aβ42. Aβ40 was positively associated with Aβ42 in both trials (2.223 pg/ml Aβ40 per pg/ml Aβ42 SE = 0.118, $P < .001$ in the MCI trial; and 4.606 pg/ml Aβ40 per pg/ml Aβ42 SE = 0.335, $P < .001$ in the simvastatin trial). In the MCI trial, we found Aβ40 and Aβ42 were positively associated with age (1.041 pg/ml Aβ40 per year of age, SE = 0.324, $P = .001$; and 0.163 pg/ml Aβ42 per year of age, SE = 0.083, $P = .047$); urea nitrogen (0.8697 pg/ml Aβ40 per mg/dl urea nitrogen, SE = 0.432, $P = .045$; and 0.3515 pg/ml Aβ42 per mg/dl urea nitrogen, SE = 0.113, $P = .002$); and creatinine (25.712 pg/ml Aβ40 per mg/dl creatinine, SE = 9.656, $P = .008$; and 10.890 pg/ml Aβ42 per mg/dl creatinine, SE = 2.531, $P < .001$). In the simvastatin trial, Aβ40 was positively associated with hemoglobin (3.949 pg/ml Aβ40 per g/dl hemoglobin, SE = 1.954, $P = .044$); and Aβ42 was positively associated with ADAS-Cog (0.0714 pg/ml Aβ42 per ADAS-Cog point, SE = 0.0334, $P = .033$). The log ratio of Aβ42 to Aβ40 was significantly associated with creatinine (0.16 per mg/dl, SE = 0.07, $P = .026$) and platelets (-7.4×10^{-4} per 1000/μl, SE = -3.1×10^{-4}, $P = .016$) in the MCI trial.

3.5. Correlates of change

Table 3 summarizes the correlates of change in Aβ40 and Aβ42. In MCI, change in Aβ40 was positively correlated with change in Aβ42 (≥0.842, 95% CI 0.779 to 0.912) and change in Aβ40 was positively correlated with change in platelets (≥0.170, 95% CI 0.036 to 0.308). Similarly, in the simvastatin trial, change in Aβ40 was correlated with change in Aβ42 (≥0.713, 95% CI 0.606 to 0.804). In the MCI trial, change in log ratio of Aβ42 to Aβ40 was correlated with ADAS-Cog (≥0.145, 95% CI 0.019 to 0.274), ADL (≥−0.178, 95% CI −0.309 to −0.055), and urea nitrogen (≥−0.168, 95% CI −0.305 to −0.039). Note that higher scores on ADAS-Cog indicate *worse* cognition and higher scores on the ADL indicate *better* daily function.

3.6. APOE ε4 group differences in Aβ change

The top of Fig. 2 shows the modeled change in Aβ40 and Aβ42 by the number of *APOE* ε4 alleles. In MCI we see significantly greater change from baseline in Aβ40

Table 2
Baseline associations

	Baseline associations with Aβ40 (pg/ml)					
	MCI			AD		
Variable	Estimate	SE	*P*-value	Estimate	SE	*P*-value
Aβ42 (pg/ml)	2.22	0.12	<.001***	4.61	0.34	<.001***
Age (yrs)	1.04	0.32	.001**	0.00	0.26	.988
Education (yrs)	0.18	0.78	.819	−0.45	0.80	.572
Gender						
Male	160.83	5.75	.268	130.83	5.13	.849
Female	165.99	4.65		130.11	8.44	
APOE ε4						
0	159.15	6.02	.301	128.61	8.40	.625
1	166.71	4.88		123.03	5.76	
2	162.17	7.61		125.63	8.74	
ADAS-Cog	0.44	0.52	.394	0.42	0.24	.077†
ADL	−0.50	0.49	.310	−0.16	0.23	.500
MMSE	−1.49	1.26	.239	−0.46	0.52	.379
Urea nitrogen (mg/dl)	0.87	0.43	.045*	−0.45	0.49	.365
Creatinine (mg/dl)	25.71	9.66	.008**	12.36	11.80	.296
Total protein (g/dl)	−3.60	5.07	.478	−2.08	5.01	.679
Albumin (g/dl)	2.31	9.01	.797	7.06	7.81	.367
Total cholesterol (mg/dl)	0.03	0.06	.580	−0.06	0.08	.473
Hemoglobin (g/dl)	−1.29	1.79	.472	3.95	1.95	.044*
Platelets (1000/μl)	0.08	0.04	.051†	−0.04	0.05	.386
	Baseline Associations with Aβ42 (pg/ml)					
	MCI			AD		
Variable	Estimate	SE	*P*-value	Estimate	SE	*P*-value
Age (yrs)	0.16	0.08	.047*	−0.02	0.04	.663
Education (yrs)	0.33	0.19	.081†	0.07	0.12	.570
Gender						
Male	39.43	1.29	.761	19.09	0.73	.955
Female	39.07	1.18		19.05	0.72	
APOE ε4						
0	40.10	1.37	.419	18.81	0.80	.406
1	38.70	1.24		19.63	0.83	
2	38.25	1.89		18.07	1.26	
ADAS-Cog	−0.13	0.13	.311	0.07	0.03	.033*
ADL	−0.03	0.13	.827	−0.04	0.03	.285
MMSE	0.17	0.32	.591	−0.11	0.07	.135
Urea nitrogen (mg/dl)	0.35	0.11	.002**	0.01	0.07	.883
Creatinine (mg/dl)	10.89	2.53	<.001***	2.75	1.72	.110
Total protein (g/dl)	−1.51	1.30	.244	−0.94	0.74	.201
Albumin (g/dl)	1.37	2.33	.555	0.39	1.12	.731
Total cholesterol (mg/dl)	0.00	0.02	.759	0.00	0.01	.760
Hemoglobin (g/dl)	−0.07	0.47	.884	0.23	0.29	.429
Platelets (1000/μl)	−0.01	0.01	.622	−0.01	0.01	.384
	Baseline associations with log ratio of Aβ42 to Aβ40					
	MCI			AD		
Variable	Estimate	SE	*P*-value	Estimate	SE	*P*-value
Age (yrs)	0.00	0.00	.912	0.00	0.00	.878
Education (years)	0.01	0.01	.115	0.00	0.01	.576
Gender						
Male	−1.42	0.04	.131	−1.89	0.05	.751
Female	−1.47	0.05		−1.90	0.06	
APOE ε4						
0	−1.40	0.05	.056†	−1.90	0.06	.181
1	−1.48	0.05		−1.84	0.06	
2	−1.45	0.06		−1.93	0.08	

(*Continued*)

Table 2
Baseline associations (*Continued*)

	Baseline associations with log ratio of Aβ42 to Aβ40					
	MCI			AD		
Variable	Estimate	SE	*P*-value	Estimate	SE	*P*-value
ADAS-Cog	0.00	0.00	.363	0.00	0.00	.877
ADL	0.00	0.00	.889	0.00	0.00	.591
MMSE	0.01	0.01	.205	0.00	0.00	.638
Urea nitrogen (mg/dl)	0.00	0.00	.226	0.00	0.00	.430
Creatinine (mg/dl)	0.16	0.07	.026*	0.09	0.09	.280
Total protein (g/dl)	−0.07	0.04	.077†	−0.04	0.04	.278
Albumin (g/dl)	−0.01	0.06	.857	−0.02	0.06	.787
Total cholesterol (mg/dl)	0.00	0.00	.912	0.00	0.00	.292
Hemoglobin (g/dl)	0.00	0.01	.765	−0.02	0.01	.254
Platelets (1000/μl)	-7.4×10^{-4}	-3.1×10^{-4}	.016*	0.00	0.00	.944

Abbreviations: MCI, mild cognitive impairment; AD, Alzheimer's disease; ADAS, Alzheimer's Disease Assessment Scale; ADL; activities of daily living; MMSE, Mini-Mental State Examination.

NOTE. Associations between the indicated variables at baseline as estimated by linear mixed effect model with plasma Aβ40 or Aβ42 as the outcome. Each estimate is on a different scale. For example, in MCI, Aβ40 increased an estimated 1.04 pg/ml per year of age.

†p <0.01; *p <0.05; **p <0.01; ***p <0.001.

and Aβ42 at three years among *APOE* ε4 noncarriers compared with carriers. Change in Aβ40 at 3 years was greater in those with no alleles compared with those with one allele (41.7 pg/ml, SE = 6.69, $P < .001$) or two alleles (55.7 pg/ml, SE = 9.54, $P < .001$). Change in the log ratio of Aβ42 to Aβ40 at year 3 in MCI was greater for those with one versus no allele (0.12, SE = 0.04, $P = .019$). The AIC selected model of Aβ40 included age; baseline creatinine; and baseline and change in hemoglobin, albumin, and platelets. The AIC selected model of Aβ42 included age; and change in creatinine, hemoglobin, and platelets. The AIC selected model of log ratio of Aβ42 to Aβ40 included baseline creatinine, total protein, hemoglobin, and platelets; and change in creatinine, total protein, albumin, and platelets. No significant differences between *APOE* ε4 groups were observed in the simvastatin trial.

3.7. Treatment group differences in Aβ change

The bottom of Fig. 2 shows the modeled change in plasma Aβ species by treatment group. In the MCI trial, Aβ40 and Aβ42 increased more at 3 years in the placebo group compared with donepezil (33.9 pg/ml Aβ40, SE = 7.68, $P < .001$; 12.63 pg/ml Aβ42, SE = 2.04, $P < .001$) or vitamin E (39.3 pg/ml Aβ40, SE = 7.53, $P < .001$; 7.81 pg/ml Aβ42, SE = 2.01, $P < .001$). Change in log ratio of Aβ42 to Aβ40 was greater at 3 years with vitamin E compared with placebo (0.14, SE = 0.049, $P = .012$), but no difference was found with donepezil. In the simvastatin trial, both Aβ species increased more at 18 months in the simvastatin group compared with placebo (21.3 pg/ml Aβ40, SE = 6.55, $P = .001$; 4.34 pg/ml Aβ42, SE = 0.923, $P < .001$), but the difference in change of log ratio of Aβ42 to Aβ40 was not significant (−0.10, SE = 0.062, $P = .010$).

3.8. Treatment group differences in Aβ change within APOE ε4 subgroups

Fig. 3 shows the modeled change in plasma Aβ species by treatment group within each *APOE* ε4 group. For *APOE* ε4 carriers in the MCI trial, both Aβ species increase significantly more at 3 years in the placebo group compared with vitamin E (64.8 pg/ml Aβ40, SE = 10.8, $P < .001$; 15.89 pg/ml Aβ42, SE = 2.65, $P < .001$), and Aβ42 increased more at 3 years in the placebo group compared with donepezil (15.96 pg/ml Aβ42, SE = 2.68, $P < .001$). The log ratio of Aβ42 to Aβ40 decreased more with donepezil compared with placebo (−0.21, SE = 0.074, $P = .009$). For *APOE* ε4 carriers in the simvastatin trial, both Aβ species increased significantly at 18 months in the simvastatin group compared with placebo (43.8 pg/ml Aβ40, SE = 8.99, $P = .001$; 8.28 pg/ml Aβ42, SE = 1.37, $P < .001$); and the log ratio decreased more with simvastatin (−0.18, SE = 0.091, $P = .044$). For *APOE* ε4 noncarriers in the MCI trial, both Aβ species increased more at 3 years in the placebo group compared with donepezil (53.4 pg/ml Aβ40, SE = 11.3, $P < .001$; 10.28 pg/ml Aβ42, SE = 3.17, $P = .002$); and there was no difference in change in log ratio of Aβ42 to Aβ40. There were no significant differences in Aβ change between simvastatin and placebo among the *APOE* ε4 noncarriers.

4. Discussion

In comparison to CSF, plasma Aβ has been an inconsistent predictor of dementia in sporadic or late-onset forms of AD. Associations have been found between plasma Aβ40 and 42 and dementia, but the direction of these associations vary among studies [11–15,37]. More consistency has been found in the ratio of plasma Aβ42:40, with non-

Table 3
Correlations of change

Variable	MCI Correlation (95% CI)	AD Correlation (95% CI)
Aβ40		
Aβ42	**0.842 (0.779,0.912)**	**0.713 (0.606,0.804)**
ADAS-Cog	−0.038 (−0.152,0.075)	−0.016 (−0.206,0.179)
ADL	−0.072 (−0.181,0.044)	−0.043 (−0.239,0.144)
MMSE	−0.071 (−0.197,0.051)	−0.015 (−0.204,0.176)
Urea nitrogen	−0.024 (−0.161,0.106)	–
Creatinine	0.034 (−0.094,0.159)	–
Total protein	0.015 (−0.112,0.131)	–
Albumin	0.010 (−0.125,0.128)	–
Total cholesterol	0.039 (−0.090,0.175)	−0.001 (−0.199,0.199)
Hemoglobin	−0.014 (−0.149,0.125)	–
Platelets	**0.170 (0.036,0.308)**	–
Aβ42		
ADAS-Cog	0.044 (−0.062,0.162)	0.033 (−0.147,0.216)
ADL	−0.100 (−0.218,0.006)	−0.040 (−0.219,0.141)
MMSE	−0.115 (−0.251,0.002)	−0.081 (−0.278,0.114)
Urea nitrogen	0.053 (−0.077,0.186)	–
Creatinine	0.032 (−0.108,0.156)	–
Total protein	−0.035 (−0.167,0.085)	–
Albumin	−0.065 (−0.194,0.071)	–
Total cholesterol	−0.021 (−0.148,0.100)	−0.127 (−0.303,0.050)
Hemoglobin	−0.079 (−0.205,0.044)	–
Platelets	0.038 (−0.095,0.161)	–
log ratio of Aβ42 to Aβ40		
ADAS-Cog	**0.145 (0.019, 0.274)**	−0.089 (−0.263, 0.120)
ADL	**−0.178 (−0.309,−0.055)**	0.185 (−0.008, 0.351)
MMSE	0.062 (−0.073, 0.205)	−0.048 (−0.239, 0.143)
Urea nitrogen	**−0.168 (−0.305,−0.039)**	
Creatinine	0.009 (−0.133, 0.152)	
Total protein	−0.060 (−0.193, 0.074)	
Albumin	−0.098 (−0.228, 0.043)	
Total cholesterol	−0.018 (−0.151, 0.118)	0.161 (−0.021, 0.360)
Hemoglobin	−0.087 (−0.222, 0.031)	
Platelets	−0.085 (−0.212, 0.052)	

Abbreviations: MCI, mild cognitive impairment; AD, Alzheimer's disease; ADAS, Alzheimer's Disease Assessment Scale; ADL; activities of daily living; MMSE, Mini-Mental State Examination.

NOTE. Correlations between change in amyloid beta (Aβ40) and change in each other indicated variable as estimated by multivariate outcome mixed-effect models. We estimated the lower and upper bounds of the 95% confidence intervals (CI) by 1000 simulations. Correlations that are significantly different from zero are indicated in bold.

demented patients usually having higher risk of AD with lower Aβ42:40 ratios [17,18]. In terms of predicting whether patients with MCI will convert to AD, no consistent change in plasma Aβ or ratio has been found [12,13,37]. However, studies demonstrate that age-related changes (increases) in plasma Aβ and reduced Aβ40:42 ratio are primarily restricted to MCI patients or individuals with worsening cognitive status [37].

Variability in these findings is potentially due to sample variability in subject age and/or with disease severity [12,20], but may also relate to study size. Very few large-scale studies have been attempted. A recently published study in a large cohort of elderly patients identified an association between low cognitive reserve and plasma Aβ42:40, which accentuated the relationship between low plasma Aβ42:40 and greater cognitive decline in non-demented participants [21]. As mentioned previously, plasma Aβ has been reported to begin increasing before dementia onset in subjects with familial AD or Down syndrome, perhaps reflecting increased Aβ production [5–7]. The same has not been found in sporadic or late-onset forms of AD. Although relationships have been found with plasma Aβ40 or 42 and dementia, the direction of these associations is variable. In particular, a recent Alzheimer's Disease Neuroimaging Initiative (ADNI) study of plasma Aβ42 in normal, mildly impaired and mildly demented cohorts, found that plasma Aβ measurements were not useful in distinguishing among the cohorts, and showed minimal association with disease progression [37]. Although discouraging, this study found a significant association between plasma Aβ42 and brain amyloid, as indicated by CSF Aβ42. The ADNI study also found a correlation of plasma Aβ42 and other biomarkers of Aβ pathology [37]. As opposed to studies examining levels of peptide, reports on ratio of plasma Aβ42:40, have had more consistent results, with lower Aβ42:40 ratios predicting higher risk of AD [17,18]. Furthermore, a large cohort study of elderly patients found that low cognitive reserve and plasma Aβ42:40, which accentuated the relationship between low plasma Aβ42:40 and greater cognitive decline in nondemented participants [21].

We found that *APOE* ε4 carriers demonstrated significant reduction of Aβ compared with noncarriers in our MCI cohort, while this relationship was not observed in the AD statin trial. A possible explanation is that *APOE* ε4 group differences in plasma Aβ are only apparent in milder populations, and populations with more severe impairment are more homogeneous across *APOE* ε4 groups.

Despite the fact that both studies found no effect on their primary outcomes, we did observed significant, although inconsistent, treatment effects on Aβ in both trials. Active groups in the MCI trial demonstrated decreased Aβ and the statin group demonstrated increased Aβ. Although the original statin trial itself was negative, our plasma biomarker data suggests further study of the effect of statins on Aβ is warranted. It is surprising that presumed symptomatic agents, donepezil and vitamin E, appeared to affect plasma Aβ in AD. All our treatment-related findings should be interpreted with caution until confirmed in studies with parallel CSF or amyloid imaging.

We observed greater interassay CVs than some previous reports, but our intra-assay CVs were within the range of many prior reports (e.g. [37]). Collection, preparation and handling of plasma samples can all influence variability. The inter-assay CVs we observed could have been elevated due to preparation, handling, or storage of the samples or the analytic kits. Recent data also suggest that technical precision may also be involved. Using a robotized method for specific steps allowed for a large improvement in consistency over results reported in the

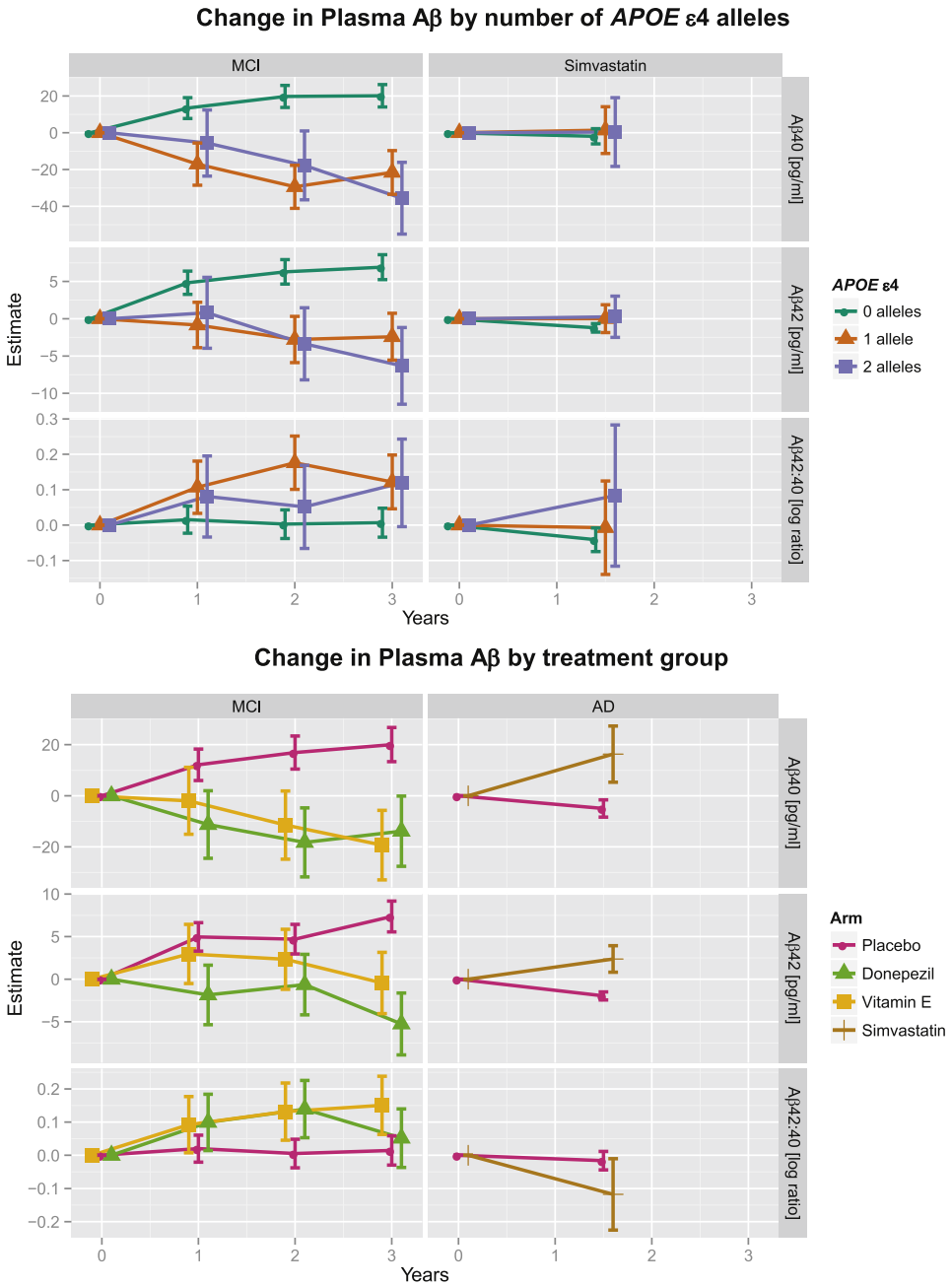

Fig. 2. Linear mixed effects model estimates of change in plasma amyloid beta (Aβ) by treatment and apolipoprotein E ε4 (*APOE* ε4). Change in plasma Aβ was modeled by number of *APOE* ε4 alleles (top) and treatment group (bottom). Covariates in these models were selected by Akaike Information Criterion (AIC). Specifically models of Aβ40 included age; baseline creatinine; and baseline and change in hemoglobin, albumin and platelets. The models of Aβ42 included age; and change in creatinine, hemoglobin, and platelets. Models of Aβ42 to Aβ40 (log) ratios included baseline creatinine, total protein, hemoglobin, and platelets; and change in creatinine, total protein, albumin, and platelets.

literature, and several significant relationships between plasma and CSF biomarkers have been found using this method [38]. Although the authors concluded that these associations are not strong enough to support use of plasma Aβ as a diagnostic screening test, these data and those observed in immunotherapy trials (e.g. [39], for review [40]) suggests that plasma Aβ42 may be useful as a pharmacodynamic marker.

Due to plate-to-plate variability seen with the Innogenetics platform, we find that inclusion of one or more internal standard controls and sound experimental design and analysis are crucial. In particular, we recommend that samples be randomized so that key features (e.g. treatment assignment, *APOE* ε4, gender) are well balanced on each plate. Good experimental design can help ensure that plate effects are not confounded with other effects of interest.

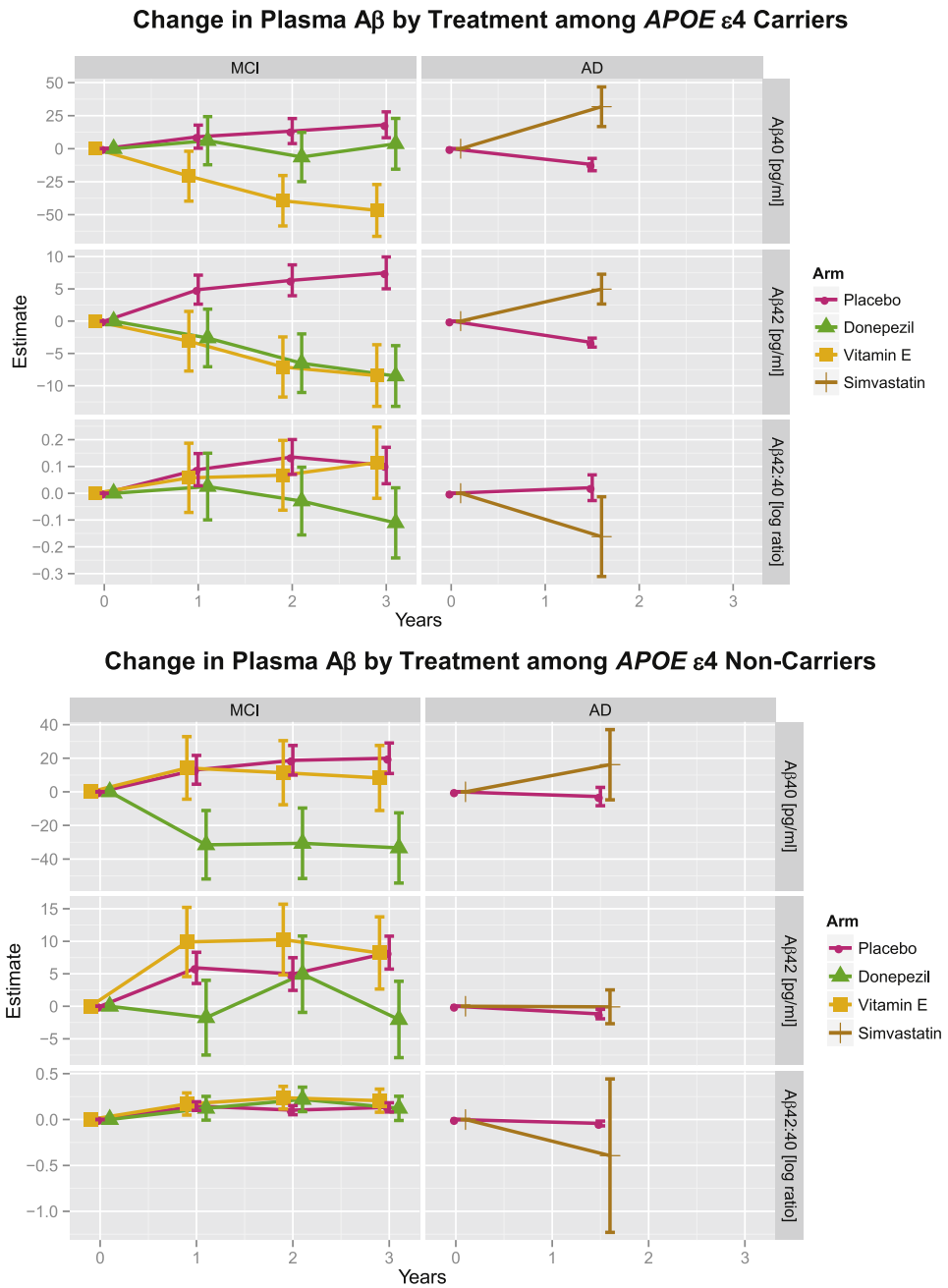

Fig. 3. Linear mixed effects model estimates of change in plasma amyloid beta (Aβ) by treatment within apolipoprotein E ε4 (*APOE* ε4) subgroups. Change in plasma Aβ was modeled by treatment among *APOE* ε4 carriers (top) by treatment among *APOE*-ε4 noncarriers (bottom). Covariates in these models were selected by Akaike Information Criterion (AIC). Specifically models of Aβ40 included age; baseline creatinine; and baseline and change in hemoglobin, albumin and platelets. The models of Aβ42 included age; and change in creatinine, hemoglobin, and platelets. Models of Aβ42 to Aβ40 (log) ratios included age; and baseline and change in creatinine, total protein, hemoglobin, albumin, and platelets.

The statistical models (Appendix D, available online) included fixed-effect covariates for mean-centered biological standard assayed on each plate. This model allows for plate-level covariate adjustment, similar to familiar adjustments for subject-level covariates. A more naïve approach subtracts the biological control from each observation before submitting to the final regression analysis. In a perfectly balanced design, point estimates from the covariate adjustment approach would be identical to the naïve approach, but naïve standard error estimates would be incorrect because they do not account for variability in the biological control. We also include subject- and plate-specific random effects to account for the correlation structure of these repeated measures, plate-clustered data.

We also recommend that samples from an individual be aliquoted to the same plate. This helps ensure that plate effects are not confounded with longitudinal effects. Unfortunately, this means that storage effects are confounded with

longitudinal effects; however we have found that storage effects are small relative to plate-to-plate variation. In this setting, estimates of group differences are valid under the assumption that storage effects are similar in the groups being compared.

When considering our results and those from other groups, an important factor to consider is blood processing time. The ADCS chooses to process blood samples for plasma stores centrally to reduce variations in preanalytic handling. This requires that whole blood samples are shipped in ambient temperature gel packs overnight and processed at approximately 24 h postdraw. Our decision to maintain this strategy is supported by our internal studies (Rissman and Aisen, unpublished observations) and investigations by other groups that have tested stability of Aβ in plasma. Stability experiments assessing the effect of time-to-processing demonstrate that mean (standard deviation) Aβ1–40 decreased from 267 (46) pg/ml at time 0 to 190 (41) pg/ml at 24 h and 143 (33) pg/ml at 48 h; or an average decrease of about 2.6 pg/ml/h [41]. Similarly, Aβ1–42 decreased from 29 (4) pg/ml at time 0 to 2 (4) pg/ml at 24 h and 19 (3) pg/ml at 48 h; or an average decrease of about 0.2 pg/ml/h. Their conclusion was that processing should be done within 24 h and peptide ratios should be created to minimize artificial results. Other groups conducted similar experiments and found plasma concentrations of Aβ (particularly Aβ1–42) appeared stable in whole blood processed as long as 24 h after collection [42]. While comparisons of absolute Aβ across studies is problematic, group comparisons within a study in the present manuscript should be less so. This is because samples from different groups of interest have been handled similarly within a particular study, and samples have been randomized to plates to prevent confounding of plate and group effects.

With improvements of assay conditions (e.g., with increasing sensitivity and reproducibility, and standardization of specimen handling to minimize interactions with other blood constituents and collection materials); and sound experimental design and analysis to control confounding factors such as batch effects, age and renal function; plasma Aβ may become a useful biomarker of brain amyloidosis. This, in turn, could greatly facilitate the development and clinical application of disease-modifying therapies for AD.

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Conflict of Interest Statement: The authors have no conflict of interests relevant to blood biomarker discovery research.

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jalz.2014.07.156.

RESEARCH IN CONTEXT

1. Systematic review: We used banked plasma samples from two Alzheimer's Disease Cooperative Study multicenter studies to determine factors that may influence plasma amyloid beta (Aβ) and whether levels of Aβ in plasma are associated with apolipoprotein E (*APOE*) genotype and/or clinical and cognitive measures of Alzheimer's disease (AD) progression. We assayed levels of Aβ40 and 42 with high throughput multiplex fluorescent bioassays in the context of clinical, cognitive and laboratory data.
2. Interpretation: Our data suggest that plasma Aβ may be a biomarker of interactions between *APOE* genotype and change in Aβ42 in patients with mild cognitive impairment. Our results suggest that detection of plasma Aβ may prove to be a viable biomarker of AD.
3. Future directions: Our data demonstrate the standardization and covariates that should be accounted for when analyzing plasma Aβ as an AD biomarker or for assessing treatment effects. Our future plans are to use determine whether plasma Aβ is altered in treatment trials that specifically impact Aβ.

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