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Pramlintide: the effects of a single drug injection on blood phosphatidylcholine profile for Alzheimer's disease

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Abstract

Studies suggest that a single injection of pramlintide, an amylin analog, induces changes in Alzheimer's disease (AD) biomarkers in the blood of AD mouse models and AD patients. The aim of this study was to examine whether a pramlintide challenge combined with a phosphatidylcholine (PC) profile diagnoses of AD and mild cognitive impairment (MCI) better than PC alone. Non-diabetic subjects with cognitive status were administered a single subcutaneous injection of 60 mcg of pramlintide under fasting condition. A total of 71 PCs, amyloid- β peptide (A β) and total tau (t-tau) in plasma at different time points were measured and treated as individual variables. A single injection of pramlintide altered the levels of 7 PCs in the blood, while a pramlintide injection plus food modulated the levels of 10 PCs in the blood ($p < 0.05$). The levels of 2 PCs in MCI and 12 PCs in AD in the pramlintide challenge were significantly lower than the ones in controls. We found that while some PCs were associated with only A β levels, other PCs were associated with both A β and t-tau levels. A receiver operating characteristic (ROC) analysis of the PCs was combined with the A β and t-tau data to produce an area under the curve predictive value of 0.9799 between MCI subjects and controls, 0.9794 between AD subjects and controls and 0.9490 between AD and MCI subjects. A combination of

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AD biomarkers and a group of PCs post a pramlintide challenge may provide a valuable diagnostic and prognostic test for AD and MCI.

Keywords

Alzheimer's disease (AD); Mild cognitive impairment (MCI); Amylin; Pramlintide; phosphatidylcholine (PC)

Background

As the number of Alzheimer's disease (AD) patients rapidly increases [1], there is an urgent need for simple and specific blood tests that can be easily performed in a doctor's office to diagnose AD. Because there is no effective treatment for AD, there is also a need for new drug targets that are based on the disease-relevant pathways that have been identified in humans [2]. Several studies in recent years have demonstrated that there is a link between low blood phospholipid levels and AD in humans [3–6]. Data suggest that these factors could be useful biomarkers in blood diagnostic tests for AD and potential disease-modifying pathway targets for therapeutics aimed at treating AD.

Amylin is a gut-brain axis hormone that consists of 37 amino acids and is produced and secreted by the pancreas and intestine. Amylin easily crosses the blood brain barrier (BBB) [7, 8] and mediates important brain functions. Using AD mouse models, two independent studies demonstrated that treatment with amylin or its clinical analog, pramlintide, reduced AD pathology in the brain and improved learning and memory [9, 10]. A single peripheral injection of amylin or pramlintide result in the transfer of amyloid- β peptide (A β) from the brain into the blood in AD mouse models [10] and AD patients [11]. Because amylin regulates glucose and lipid metabolism [12], pramlintide treatment is likely to influence the phospholipid profile in AD patients, and a test combining a pramlintide challenge and an analysis of phospholipid levels may therefore be useful for diagnosing AD.

Pramlintide has 3 amino acid differences from human amylin and is an FDA-approved drug for treating diabetes [13]. The aim of the present study was to determine whether pramlintide modulates the phospholipid profile in a way that can be used to diagnose AD. Here, we report the results of a pilot study of phosphatidylcholine (PC) levels in which we administered a single subcutaneous injection of pramlintide to human subjects. Because diabetes is associated with phospholipid levels in the blood and because pramlintide is a diabetes drug, we recruited only subjects who did not have diabetes so that we could study the specific effects of pramlintide on those with AD risk.

Methods and Materials

Participants

We recruited a group of 30 subjects from The Healthy Outreach Program for the Elderly (HOPE) study [14] at Boston University Alzheimer's Disease Center (BU ADC). The subjects were aged 50–90 years old and did not have diabetes, a stroke or a history of brain

injury. The protocol and consent forms were approved by the Institutional Review Board of Boston University School of Medicine. All enrolled participants provided informed consent.

The participants were being followed annually by the BU ADC, where they underwent cognitive evaluations according to the National Alzheimer's Disease Coordinating Center (NACC) protocol [15, 16]. All subjects carried a consensus diagnosis of normal cognition ($n = 14$), probable amnesic MCI ($n = 8$) or probable AD ($n = 8$). A diagnosis of dementia was based on the DSM-IV criteria. The NINCDS-ADRDA guidelines [17] were used to determine whether the diagnostic criteria for potential or probable AD were met. The diagnostic criteria for MCI were based on the guidelines of Petersen et al. [18] and the updated criteria by Albert et al. [19]. Subjects that had neither dementia nor MCI were considered cognitively normal (CN) and were enrolled as controls.

Study design

The study design is illustrated in Figure 1. Participants arrived at the General Clinical Research Unit, Boston University Medical Center in the morning after fasting for > 9 hours overnight. After written consent was obtained, baseline blood draws, vital sign checks and IV line placements were performed, and blood glucose concentrations were determined. If the blood glucose concentration was higher than 80 mg/dl, a subcutaneous injection of pramlintide (60 mcg) was administered, blood draws were conducted at 5, 30, 60 and 180 minutes after the injection, and the blood samples were immediately centrifuged. Plasma was isolated and stored at -80°C until used. One hour after pramlintide challenge, the subjects were provided breakfast. At each time point, glucose concentrations and vital signs were monitored.

Measurements

A Biocrates Absolute IDQ p180 kit assay (Innsbruck, Austria) was used to quantify the levels of different metabolites, including PCs [20]. Sandwich A β ELISA assays were used to measure A β 1-42 and A β 1-40 levels in plasma samples with some modifications [21] [22]. Briefly, plates were coated with 2G3 (anti- $\alpha\beta$ 40) and 21F12 (anti- $\alpha\beta$ 42) antibodies overnight at 4°C . Samples were then loaded and incubated overnight at 4°C followed by incubating with a biotinylated monoclonal anti-N terminus A β antibody (3D6B) for 2 hrs. Finally, streptavidin-conjugated alkaline phosphatase (Promega, USA) was added and incubated, and the signal was amplified by adding alkaline phosphatase fluorescent substrate (Promega, USA), which was then measured [23]. Single molecule array (Simoa) testing was used to measure the total tau protein (t-tau) in the plasma samples (RayBiotech, GA, USA).

We also obtained and used the PC data from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD). For up-to-date information, see www.adni-info.org.

Statistical Analyses

All statistical analyses were performed using R (version 3.3.2). The A β , t-tau and PC variables were log-normal distribution and the data were standardized by using the scale function (the individual values were subtracted from the mean and then were divided by the standard deviation, which is similar to computing z-scores of a normal distribution). Therefore, the mean of each PC variable was treated as zero, and each SD was treated as $\pm$ scales for the distribution. Heatmaps were used to illustrate the mean differences in PC measurements at different treatment time points (Figure 2) and among different diagnostic groups and different time points (Figure 3). After adjusting for confounding factors, including age, gender, ethnicity and treatment time points, we then used the PC level at each time point as repeated measurements in a mixed model of analysis to study the differences of average (mean) PC levels (as outcomes) among controls, MCI and AD groups (as the determining factors). The PCs were then ranked based on the p values obtained in each mixed model, and the top candidate PC biomarkers were selected based on a cut-off p value of $p < 0.05$. Pairwise Pearson's correlation was used to study the relationships among selected AD biomarkers, including Tau, A β 1-42, A β 1-40 and PCs, in the plasma. We used logistic regression models to predict the risks of AD or MCI versus controls, respectively, given a set of those selected AD biomarkers (including Tau, A β 1-42, A β 1-40 and PCs) and the Area under a receiver operating characteristic (ROC) curve (AUC) was used to provide an overall measure of fit the model. An ROC analysis was used to assess the performance of the classifier models used for diagnostic group classification. The parameters of the predictive model were selected according to the least MSE of leave-one-out and 5-fold cross-validation to compare the final model to alternative models. In addition, the similar predictive logistic regression models were used to validate the effects of those candidate PC biomarkers for AD in the ADNI data.

Results

Study sample

Thirty non-diabetic individuals, including control (n = 14), MCI (n = 8) and AD (n = 8) subjects, participated in the present study. While there were no differences in gender, ethnicity, ApoE4 alleles, or body mass index (BMI) among the three diagnostic groups, both the MCI and AD groups were older than the control group ($p < 0.05$) (Table 1). The AD group but not the MCI group had a lower average score on the Mini-Mental State Examination (MMSE) than was found in the control group (means $\pm$ SE: 29.2 ± 1.5 vs. 21.1 ± 1.3 , $p < 0.001$). Under fasting conditions, a single injection of pramlintide did not cause hypoglycemia or other known side effects of the drug in the included non-diabetic subjects (data not shown).

Phosphatidylcholines in the pramlintide challenge test

Seventy-one plasma PC levels were measured at different time points (Figure 1 and Table S2) and then compared. Using the whole study sample, we found that the levels of 17 PCs, including 7 in the group treated with a single injection of pramlintide and 10 in the group treated with pramlintide plus a meal, were modestly altered from baseline levels, and the results are shown using a heatmap ($p < 0.05$) (Figure 2A). A single injection of pramlintide

lowered the levels of 6 PCs (PC ae C38:1, PC ae C40:5, PC ae C40:3, PC ae C42:5, PC ae C38:3, and PC ae C40:4) after 5 minutes and 1 PC (PC ae C38:2) after 30 minutes (Figure 2B). In addition, the levels of 10 PCs were altered at 180 minutes after pramlintide injection plus breakfast, including 7 that were higher (PC aa C38:6, PC aa C38:5, PC aa C36:4, PC aa C38:4, PC aa C40:6, PC aa C40:5, PC aa C36:5) and 3 that were lower (PC aa C32:2, PC aa C30:0, PC ae C34:3) (Figure 2B). After adjusting for multiple comparisons, the changes in the level of 8 of these PCs (PC aa C30:0, PC aa C38:4, PC aa C38:5, PC aa C38:6, PC aa C40:6, PC ae C34:3, PC ae C38:1, and PC ae C40:3) remained significant ($p < 0.05$).

Phosphatidylcholines in the pramlintide challenge test for MCI and AD

We next compared the levels of PCs following the pramlintide challenge test in the three diagnostic groups. A heatmap is used to illustrate the data in response to the drug alone (Figure 3A and Supplement Table-S2). After adjusting for age, gender and ethnicity, the average levels of 2 PCs (PC aa C38:5 and PC aa C40:5) were associated with MCI ($p < 0.05$), and the levels of 12 PCs (PC aa C32:0, PC aa C40:2, PC aa C42:4, PC ae C36:5, PC ae C38:0, PC ae C38:1, PC ae C38:2, PC ae C38:3, PC ae C38:6, PC ae C40:1, PC ae C40:3, and PC ae C40:5) were associated with AD ($p < 0.05$) (Figure 3B). The levels of PC ae C38:1 and PC ae C38:2 were lower and the level of PC aa C42:6 was higher in AD than in MCI ($p < 0.05$) (Figure 3C). The average levels of PCs at different time points are also illustrated using heatmaps (Figure 3A and 3C) after the data were adjusted for age, gender, ethnicity and time points. Table 2 shows the data for the evaluated 3 PCs. The data for some of the PCs are shown in Figure 4 to illustrate the differences among the three diagnostic groups. The levels of 3 PCs (PC aa C38:0, PC aa C38:5, PC aa C40:5) were lower in both MCI and AD (Figure 4A) than in controls, while the levels of 3 other PCs (PC ae C38:1, PC ae C40:3, PC aa C42:4) were lower only in AD (Figure 4B) than in controls.

The pramlintide challenge test for AD diagnosis

We next examined typical AD biomarkers and their relationships with PCs following pramlintide challenge. In the univariate correlation analysis, PC aa C38:5, PC aa C40:5, PC aa C40:2, PC aa C42:4, and PC ae C36:5 were associated with both A β 1-40 and A β 1-42 ($p < 0.05$) but not with t-tau. In contrast, PC ae C38:0, PC ae C38:1, PC ae C40:5, PC ae C38:3, PC ae C40:3, and PC ae C40:1 were associated with A β 1-40, A β 1-42 and t-tau ($p < 0.05$) (Table 3).

We used the PC data and the A β 1-40, A β 1-42 and t-tau data that were obtained during an untargeted LASSO analysis to generate separate linear classifier models to distinguish the MCI and AD groups from the control group. For the group classification, ROC analyses showed that the initial LASSO-identified metabolites yielded a robust AUC of 0.9799 for MCI vs. the CN controls (Figure 5A), 0.9794 for AD vs. the CN controls (Figure 5B), and 0.9490 for AD vs. MCI (Figure 5C).

To further confirm and validate these findings in AD, we used the ADNI study in combination with our data for AD diagnoses, AD biomarkers and PCs. Similar to the outcomes observed following pramlintide challenge, the ADNI study showed that AD subjects had lower levels of a group of PCs, including two identical PCs (PC aa C40:5 and

PC aa C38.5), than were observed in CN controls (Figure 6A), even after adjusting for confounders (Figure 6B). Unlike the outcomes we observed following pramlintide challenge, in the ADNI study, there were no differences in plasma A β 1-42, A β 1-40 and t-Tau levels between the AD and CN control groups.

Discussion

Currently, a blood test for diagnosing AD is not available, and there are clear challenges to developing blood diagnostic tests for AD. The data shown in the present study suggest that combining typical AD biomarkers with a PC profile following pramlintide challenge could be used as a valuable blood test for obtaining a differential diagnoses between cognitively normal controls and patients with MCI or AD. The results of the present study also suggest that pramlintide modulates phospholipid levels and may therefore represent a potential therapeutic target for AD.

Previous studies show that a combination of 10 blood PCs showed promise as a prognostic test for AD [5], but the PCs could not satisfactorily distinguish between control, AD and MCI subjects [4]. Our recent study shows that pramlintide challenge induces increases in A β and a decrease in t-tau levels in AD patients [11]. This study further demonstrated that a combination of mobilized direct AD biomarkers from the brain into blood [10] and an analysis of signature PC levels in the pramlintide challenge test may be a valuable diagnostic test for AD (Figure 5). The existence of BBB and blood-CSF barrier (BCSFB) prevent brain-originating AD biomarkers in the blood from accurately reflecting brain pathology [24, 25] and cause challenges to develop blood diagnostic tests for AD. For example, although plasma A β levels are significantly different between AD and controls when a large sample size is used [26, 27], the sensitivity and specificity of these differences are too low to be useful as AD diagnostics [28] [29, 30]. Amylin binds to the amylin receptor to relax cerebral arteries and increase local cerebral blood flow [31, 32]. This could be the mechanism by which pramlintide, an amylin analog, dilates cerebral blood vessels to remove AD specific peptides like A β from the brain into the blood. Thus using a pramlintide challenge test to examine changes in a combination of typical AD biomarkers and a group of PCs may provide a valuable diagnostic and prognostic test for AD and MCI.

This study showed that AD and MCI patients already had low levels of PCs at baseline (Figure 2), which are consistent with the findings of the ADNI (Figure 6) and other studies. Both our study and the ADNI study show that AD patients had low levels of PCs [33]. It is shown PC levels are lower in AD brains than in controls and in patients with other types of dementia [34, 35]. In addition, low PC levels are associated with brain atrophy in AD [36]. Since PCs are the major components of cellular membranes, these data suggest that AD brains have experienced membrane breakdown. PCs are obtained from diet and are composed of choline as the headgroup and with a variety of fatty acids, which determines different types of PCs. Probably due to different long-term diet patterns, the PCs that are lower in AD patients vary across different studies, although the patterns of low PC levels in AD are consistent.

Although a single injection of pramlintide only modestly altered the levels of some PCs (Figure 2), a long-term treatment with pramlintide may reach therapeutic purposes for AD. We observed that pramlintide plus food resulted in significantly higher levels of PCs than the ones observed when only the drug was administered. Many AD patients have poor appetites and inadequate food intake, which could potentially lead to lower levels of PCs [37]. In addition, although PCs have been shown to increase acetylcholine levels in the brain and improve memory in an animal model [38], clinical trials in which dementia patients were supplemented with PCs failed to show cognitive improvement in humans [39]. On the other hand, a clinical trial did show that a nutritional intervention increased the levels of a few species of PCs and improved cognitive function in mild AD patients [40]. It is likely that molecules which regulate PC metabolism, not PCs alone, should be supplemented in AD patients. Our study suggests that amylin and pramlintide could regulate lipid metabolism including PCs.

Two studies have shown that the plasma concentration of amylin is lower in AD patients than in controls [9, 10, 41]. Chronic, peripheral amylin treatment once on daily basis reduced AD pathology in the brain, and improved learning and memory, in AD mouse models [9, 10] [42]. Thus the neuroprotective effects of pramlintide are long-acting, which is different from the short-acting effects of the drug to inhibit appetite for diabetes. In addition, if pramlintide is given post the meal, it should not affect AD patients' food intakes. Ultimately, whether pramlintide can be an effective therapeutic for AD should only be concluded through a double blind, placebo controlled clinical trial in humans.

One limitation of the present pilot study was the relatively small size of each diagnostic group. In addition, we did not obtain data that could be used to compare the results of pramlintide challenge between subjects with positive vs. negative brain imaging for AD. In the present study, we also did not evaluate the influence of pramlintide on changes in PC levels in AD patients with diabetes. Nevertheless, we provide evidence supporting the use of a potential challenge test for AD that is analogous to the oral glucose tolerance test used to diagnose diabetes in which affected patients are found to exhibit abnormal glucose metabolism after glucose challenge. Once established, this test would be simple and specific and could easily be performed in a doctor's office to diagnose AD. The results of the present study provide additional evidence indicating that pramlintide could be repurposed as an alternative treatment for AD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Study design of the pramlintide challenge test

A diagram of the study design is shown. Human subjects in the BU ADC registry who did not have diabetes participated in the study. The subjects were administered a single subcutaneous (S.C.) injection of pramlintide, and blood draws (BD) were performed at different time points. Sixty minutes after pramlintide challenge, the subjects were provided breakfast.

Figure 2. The effects of pramlintide on phosphatidylcholines in the blood

The mean level of each PC was treated as zero, and each SD was treated as a $\pm$ scale for the distribution of the study sample. Changes were observed in 17 PCs, including 7 PCs that were altered by administration of the drug alone and 10 PCs that were altered by the drug plus a meal, as shown ($p < 0.05$) (A). A heatmap is shown to illustrate the mean differences in PC measurements at different time points during the test (B).

C.

*Difference between AD and MCI groups

Figure 3. Characterization and comparisons of plasma PC changes in the pramlintide challenge test in different diagnostic groups

In the heatmap, the mean is shown as zero, and SDs are shown as a +/- scale for each PC at each time point prior to the time point at which food was provided in the following three diagnostic groups: controls, MCI and AD (A). A mixed model analysis was used to compare the control group to the MCI or AD group at each time point after adjusting for confounders, and all PCs were ranked based on p values and a cut-off p value of $p < 0.05$, as shown (B). A group of PCs were significantly different among the control, MCI and AD groups ($p < 0.05$), and these results are illustrated using a mixed model analysis and a heatmap (C). Significant p values (* $p < 0.05$, ** $p < 0.01$) are indicated for comparisons between the MCI and AD groups.

A.

B.

Figure 4. Comparisons of plasma PC levels in the pramlintide challenge test for different diagnostic groups

Six PCs are illustrated to compare different diagnostic groups with statistical significance ($p < 0.05$). The mean of all samples as zero and SE as $\pm$ scale for each PC at each time point before taking food was used to compare controls and MCI or AD. A mixed model analysis was used to compare the control group to the MCI or AD group at each time point after adjusting for confounders. The levels of 3 PCs (PC aa C38:0, PC aa C38:5, PC aa C40:5) were useful to separate both MCI and AD from controls (A). The levels of 3 other PCs (PC ae C38:1, PC ae C40:3, PC aa C42:4) separated AD from MCI and controls (B).

Figure 5. ROC curve analysis of changes in biomarker levels in the pramlintide challenge test

Etiological analysis and diagnostic (predictive) models were used to evaluate the fold-changes in PC levels after adjusting for the typical AD biomarkers ($A\beta$ and t -tau), times of blood draws during challenge test, age and gender. These data were used to predict AD and MCI. The final model (with collinear effects removed) were based on a stepwise logistic regression analysis to selecting a subset biomarkers for the classification of AD versus Control (A), MCI versus Control (B), and AD versus MCI (C), respectively. The area under the curve (AUC) of the receiver operating characteristic (ROC) curves in the final predictive models for AD vs. controls including $A\beta$ 1-42 + t -tau + times of blood draws + PC ae C38:1 + PC ae C40:5 + PC aa C32:0 (Model A); for MCI vs. controls including $A\beta$ 1-40 + t -tau + times of blood draws + gender + PC ae C38:0 + PC aa C32:0 + PC ae C38:1 + PC ae C40:1 + PC aa C36:1 (Model B); and for AD vs. MCI including $A\beta$ 1-40 + $A\beta$ 1-42 + times of blood draws + gender + PC ae C38:1 + PC aa C32:0 (Model C) are shown.

A.

B.

Figure 6. Characterization and comparisons of plasma PC for AD in the ADNI study

The data of plasma PCs and A β from the ADNI study were downloaded and used to compare the AD and control groups. A mixed model analysis was used to compare the control group to the AD group before (A) and after adjusting for confounders (B). All biomarkers were ranked based on p values and a cut-off p value of $p < 0.05$ as shown.

Table 1
Comparisons of the demographics and cognitive ability in the control, MCI and AD participants

Characteristics	Controls n = 14		MCI n = 8		AD n = 8	
	n (%) or Mean $\pm$ SE		n (%) or Mean $\pm$ SE	p value [†]	n (%) or Mean $\pm$ SE	p value [†]
Age (years)	66.7 $\pm$ 1.9		77.2 $\pm$ 2.5	0.003	76.2 $\pm$ 2.5	0.006
Gender						
Female	8 (57.1)		5 (62.5)		1 (12.5)	0.19
Male	6 (42.9)		3 (37.5)		7 (87.5)	
Race				0.12		1.00
Black	1 (7.1)		3 (12.5)		0 (0)	
White	13 (92.9)		5 (87.5)		8 (100)	
ApoE4 Carrier				0.19		1.00
Yes	2 (40.0)		0 (0.0)		3 (42.9)	
No	3 (60.0)		5 (100.0)		4 (57.1)	
BMI (kg/m ²)	27.4 $\pm$ 1.7		29.3 $\pm$ 2.3	0.51	27.5 $\pm$ 1.9	0.95
MMSE (score)	29.2 $\pm$ 1.5		27.8 $\pm$ 1.6	0.55	21.1 $\pm$ 1.3	< 0.001

[†]The control group is the reference group in the comparisons. The comparisons of counts variables or the numbers at each time point are based on Fisher's exact test for small samples. The comparisons of continue variables are based on t-test to analyze the differences the control and MCI or AD groups. SE: Standard Error; BMI: Body Mass Index; MMSE: Mini-Mental State Exam. P values are shown.

Table 2
Mixed model analyses showing the relationship between PC biomarkers following pramlintide challenge for MCI and AD

Groups	PC aa C38:5			PC aa C40:5			PC ae C38:1		
	Value	SE	p value	Value	SE	p value	Value	SE	p value
Control (Reference)									
MCI	-1.15	0.33	<0.001	-0.30	0.38	0.44	+0.04	0.36	0.90
AD	-0.32	0.34	0.36	-0.85	0.39	0.04	-0.99	0.37	0.06
Time 0 ' (Reference)									
Time 5 '	+0.04	0.06	0.47	-0.30	0.12	0.01	-0.24	0.10	0.02
Time 30 '	+0.09	0.06	0.17	-0.28	0.12	0.02	-0.19	0.10	0.06
Time 60 '	+0.10	0.06	0.08	-0.13	0.12	0.26	-0.07	0.10	0.46

All participants with diagnoses and multivariate analyses were used with each PC biomarker at all time points as an outcome and with MCI and AD as determining factors in each model. The confounders of age, gender and race were adjusted for each model.

Table 3

Pearson correlation coefficients between AD biomarker and PC levels in the pramlintide challenge test

Phospholipids	A β 1-40		A β 1-42		t-tau	
	r	p values	r	p values	r	p values
Associate with Aβ only						
PC aa C38:5	-0.19	0.02	-0.31	<0.01	-0.07	0.37
PC aa C40:5	-0.19	0.02	-0.19	0.02	-0.02	0.92
PC aa C40:2	-0.20	0.01	-0.28	<0.01	-0.02	0.77
PC aa C42:4	-0.19	0.02	-0.29	<0.01	-0.11	0.18
PC ae C36:5	-0.32	<0.01	-0.21	0.01	-0.03	0.72
PC ae C38:6	-0.24	<0.01	-0.13	0.11	-0.06	0.49
Associate with Aβ and t-tau						
PC ae C38:0	-0.34	<0.01	-0.32	<0.01	-0.31	<0.01
PC ae C38:1	-0.25	<0.01	-0.26	<0.01	-0.26	<0.01
PC ae C40:5	-0.17	0.03	-0.25	<0.01	-0.23	<0.01
PC ae C38:3	-0.24	<0.01	-0.29	<0.01	-0.21	0.01
PC ae C38:2	-0.24	<0.01	-0.12	0.13	-0.22	0.01
PC aa C32:0	-0.20	0.02	0.06	0.45	-0.19	0.02
PC ae C40:3	-0.21	0.01	-0.39	<0.01	-0.17	0.04
PC ae C40:1	-0.30	<0.01	-0.28	<0.01	-0.17	0.04

Pearson univariate analyses were used to study the relationships between the typical AD biomarkers including A β 1-40, A β 1-42 and t-tau and PC biomarkers in plasma. R and p values are shown.