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Apolipoprotein E Highly Correlates with A β PP- and Tau-Related Markers in Human Cerebrospinal Fluid

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Abstract

We assessed cerebrospinal fluid (CSF) levels of apolipoprotein E (apoE), phospholipid transfer protein (PLTP) activity, cholesterol, secreted amyloid- β protein precursor α and β (sA β PP α , sA β PP β), amyloid- β peptides 1-40 (A β ₄₀) and 1-42 (A β ₄₂), total tau and tau phosphorylated at threonine 181 (pTau) in neurologically healthy, cognitively intact adults. ApoE significantly correlated with sA β PP α ($r = 0.679$), sA β PP β ($r = 0.634$), A β ₄₀ ($r = 0.609$), total and pTau ($r = 0.589$ and $r = 0.673$, respectively, all $p < 0.001$), PLTP activity ($r = 0.242$, $p = 0.002$) and cholesterol ($r = 0.194$, $p < 0.01$). PLTP activity significantly correlated with sA β PP α ($r = 0.292$), sA β PP β ($r = 0.281$), total and pTau ($r = 0.265$ and 0.258 , respectively; all $p \leq 0.001$). Using partial correlations of CSF biomarkers with apoE, PLTP activity, age and gender, apoE remained significantly correlated with sA β PP α , sA β PP β , A β ₄₀, total and pTau ($p < 0.001$). The presence of apoE $\epsilon 2$ was associated with lower levels of apoE, PLTP activity and A β ₄₂, while *APOE* $\epsilon 4$ had no significant impact on any of the measured variables. Our data suggest that there is a significant physiological link between apoE and A β PP, as well as between apoE and tau in neurologically healthy, cognitively intact individuals.

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Keywords

Amyloid- β ; amyloid- β protein precursor protein; apolipoprotein E; cerebrospinal fluid; phospholipid transfer protein; tau

INTRODUCTION

Late onset Alzheimer's disease (AD) is the most common neurological disorder of old age. Formation of amyloid plaques and development of neurofibrillary tangles (NFTs) represent the hallmarks of histologically verified changes in the brain tissue of patients with AD, originally discovered by Alois Alzheimer in 1907 [17]. Pathophysiological contribution of amyloid- β protein precursor (A β PP) and its catalytic fragments, secreted A β PP α and β (sA β PP α , sA β PP β), amyloid- β peptide 1-40 (A β ₄₀) and 1-42 (A β ₄₂), as well as the role of phosphorylated tau in development of AD are well documented.

Apolipoprotein E (apoE) genotype is currently the only known and well-established genetic risk factor for development of AD, with *APOE* $\epsilon 4$ isoform conferring an increased risk and accelerated development of AD, and *APOE* $\epsilon 2$ isoform apparently being protective against AD [5,8,23,27]. Although numerous published studies have established the association of apoE genotype to AD development, the relevance of apoE levels and functions in relationship with A β PP and tau are not fully understood, particularly in terms of their physiological role in the healthy human central nervous system (CNS).

ApoE is the main apolipoprotein in the brain [24,34] secreted by glial cells in a form of nascent, lipid-poor lipoprotein particles [33]. ApoE plays a critical role in binding of the lipoprotein particles to the receptors on the cell membrane, leading to the uptake of the lipoprotein particles [16,47]. Apart from its lipid transport properties, apoE has numerous effects on cellular metabolism that are not related to its lipid transfer functions (reviewed in [40]). For example, binding of apoE, which is secreted by glial cells, to apoE receptors on neuronal membrane modulates phosphorylation of tau in neurons, which has been shown to be one of the key factors for maintenance of the structural and functional stability of the intracellular tubular system [15,29]. Similarly, apoE binding to its receptors modulates A β PP processing [18]. A β peptides released by the brain cells bind to lipoproteins, most of which carry apoE, thus preventing A β peptide aggregation and fibrillization [30]. This process is putatively very important for prevention of the peptide oligomerization and subsequent fibrillization, which leads to cell death *in vitro* [30]. These findings suggest that levels of apoE may be relevant for modulation of normal physiological functions of tau and A β PP in neurons, and therefore potentially affect the pathophysiological processes associated with development of AD. However, despite the relative wealth of information regarding effects of apoE on tau phosphorylation and A β PP processing, the relationship between levels of apoE and levels of the markers of neurodegeneration in cognitively intact, generally healthy individuals of different age is not known.

ApoE secretion by astrocytes is modulated by phospholipid transfer protein (PLTP), and cerebrospinal fluid (CSF) apoE levels correlate with CSF PLTP activity, suggesting a strong functional link between apoE and PLTP in the brain [45]. PLTP is a multifaceted lipid transfer protein found on lipoprotein particles, expressed and secreted by a wide variety of cells and tissues, including the brain [1,45]. PLTP is involved in transfer of phospholipids between lipoproteins and cells, efflux of cholesterol and phospholipids through the ABCA1 pathway, lipoprotein particle fusion and remodeling, and is critical for transfer of α -tocopherol, and possibly other lipid-soluble vitamins, to the cells and tissues, including the brain [9,20,28,31,35,42,49]. Patients with AD have significantly lower CSF PLTP activity, and increased levels

of PLTP in the brain tissue, suggestive of PLTP sequestration in the brain of AD patients [44,45]. Potential functional importance of PLTP in pathophysiology of AD is currently unknown, although PLTP functions are relevant for maintenance of the brain antioxidative potential, lipid and lipoprotein metabolism, all of which have been implicated in AD development [14,43,50]. Additionally, due to its effect on apoE secretion from glial cells [45], PLTP may indirectly affect numerous apoE-dependent processes through modulation of apoE levels in the brain.

In this article, we are reporting the relationship between apoE and PLTP, and the biomarkers of neuronal degeneration relevant for AD - sA β PP α , sA β PP β , A β ₄₀, A β ₄₂, tau phosphorylated at threonine 181 and cholesterol - in CSF samples obtained from healthy adults without measurable cognitive impairment.

STUDY PARTICIPANTS, MATERIALS AND METHODS

Study participants and samples

Participants for the study were recruited through six Alzheimer's Disease Research Centers (ADRC). The study was approved by the Human Subjects Committees of the ADRC's respective organizations, and written informed consent was obtained from all subjects. Subjects ($n = 168$, age range 21-88 years old) were medically healthy, had Mini-Mental State Examination scores ≥ 26 , Clinical Dementia Rating scores of zero, and no evidence or history of cognitive or functional decline. For subjects older than 50 years, scores on delayed recall were above a cutoff set at 1.5 SD below the age-adjusted means for both the Wechsler Logical Memory and New York University paragraph recall tests, to rule-out Mild Cognitive Impairment. All individuals with history of stroke, head trauma or chronic depression were excluded from the study. Plasma lipid analyses were performed in all study participants, and 10 out of 168 (5.9%) were hypercholesterolemic based on National Cholesterol Education Program guidelines (NCEP AtP III; total cholesterol ≥ 240 mg/dl and LDL cholesterol ≥ 160 mg/dl). Blood counts were performed in all study participants, and no obvious abnormalities were observed.

CSF samples were drawn by modified lumbar puncture (LP) [32] between 9:00 AM and 11:00 AM after fasting since midnight. Before each LP, subjects were placed at bed rest for 60 minutes. LPs were performed with a 24g Sprotte atraumatic spinal needle while the subject was maintained in the lateral decubitus position. CSF was collected in sequential 5 ml syringes and then frozen on dry ice in sequential 0.5 ml aliquots at the bedside. CSF samples were then stored at -70°C until assayed.

Biochemical analyses

CSF samples were assessed for blood contamination by analyses of total protein, albumin, glucose, and cell count. Only samples that were assessed as free of blood contamination were used for analyses. Albumin index was used to evaluate the integrity of the blood-brain barrier, which was shown to be intact in all study participants.

Measurement of AD biomarkers

Concentrations of A β ₄₀ and A β ₄₂, total tau, and tau phosphorylated at threonine 181 (pTau) were measured in the 9th ml of collected CSF. A β peptides were measured by sensitive and well-validated enzyme-linked immunosorbent assays (ELISAs) using monoclonal antibodies developed by Takeda Pharmaceutical and methods described previously [22,39]. Total tau and pTau were measured by ELISA (Innogenetics Inc., Belgium). Concentrations of sA β PP α and sA β PP β in the 10th ml of CSF were measured by ELISA using 8E5 as capturing antibody, and

2H3 (for sA β PP α) and 192 (for sA β PP β) as detecting antibodies, as previously described in detail [19].

Apolipoprotein E

CSF apoE concentration was measured by an immunonephelometric assay (BNII, Dade-Behring), with intra- and inter-assay variability below 5%. The assay was calibrated using Dade-Behring apoE standards, and it was linear between 0.170 mg/dl and 4.0 mg/dl. CSF samples were assayed undiluted, and over 99% of the assayed samples were within the linear range of the assay. Previous analyses have shown that apoE is not affected by the CSF gradient, allowing us to use various CSF aliquots for analyses (unpublished observations). ApoE genotyping was performed using previously described methodology [10].

PLTP activity

PLTP-mediated phospholipid transfer activity was assessed using PLTP phospholipid transfer activity assay [7]. Previous analyses showed that PLTP activity measurements are not affected by the CSF gradient (unpublished observations). Ten microliters of CSF were assayed for the ability to transfer C14-labeled phospholipid from donor liposome particles to the acceptor particles (plasma HDL without measurable PLTP activity). Phospholipid transfer activity was expressed in μ M/ml/h. Previous studies have shown that over 98% of phospholipid transfer in CSF is due to PLTP [44], and other studies have shown that PLTP activity is not affected by storage of CSF samples at -80°C (unpublished observations).

Statistical analyses

Parametric statistical analyses were performed using Statistica for Windows, StatSoft, Inc., 2000 (Tulsa, OK). Difference between groups was established using the unpaired t-test, and correlation analyses were performed using Pearson's test. Assessment of the independent associations of apoE and PLTP activity with the markers of neurodegeneration was conducted using multiple linear regression analyses of each individual biomarker with covariates apoE, PLTP activity, age and gender. From these models we estimated partial correlation coefficients for the relationship between CSF biomarker and apoE or PLTP activity. P-values < 0.05 were considered statistically significant.

RESULTS

CSF apoE and PLTA and markers of neurodegeneration

We measured levels of apoE, PLTP-mediated phospholipid transfer activity (PLTP activity), cholesterol, sA β PP α and sA β PP β , A β ₄₀ and A β ₄₂, total tau, pTau and albumin in CSF samples obtained from cognitively intact, neurologically healthy human volunteers with intact blood-brain barrier, age range 21-88 years old (Table 1). ApoE and sA β PP α levels were increased in middle-aged adults compared to young adults, and all markers except CSF cholesterol and A β ₄₂ were increased in older adults compared to young adults (Table 2). There was a statistically significant difference between middle-aged and older adults in levels of apoE, PLTP activity, albumin, sA β PP β , A β ₄₂, total and pTau. Levels of total and pTau were particularly increased in older adults compared to both young and middle-aged adults.

CSF apoE and PLTP activity significantly correlated with A β PP-related markers and tau in CSF (Table 3). The correlations between apoE and sA β PP α and sA β PP β , A β ₄₀, total tau, pTau and albumin were similar in men and women except correlation between apoE and cholesterol, which was significant in women but not in men. PLTP phospholipid transfer activity significantly correlated with sA β PP α , sA β PP β , A β ₄₀, total tau, pTau and albumin in men, but not in women (Table 3).

Multiple regression analyses

Because CSF apoE and PLTP activity were positively correlated ($r = 0.242$; $p = 0.002$), we evaluated the independent contribution of CSF apoE and PLTP activity to each CSF biomarker levels also taking into account age and gender by using multiple linear regression of each biomarker on covariates apoE, PLTP activity age and gender. Partial correlation coefficients obtained from these models for apoE and PLTP activity are presented in Table 4. In our study, CSF apoE concentration was significantly correlated with A β PP-derived CSF markers, total and pTau. After adjustments for age, gender and PLTP activity, an increase in apoE levels was associated with an increase in sA β PP α , sA β PP β , A β ₄₀, A β ₄₂, total and pTau levels. There was no significant association between the CSF biomarkers and PLTP activity with adjustment for age, gender, and apoE (Table 4). To exclude the possibility that the observed associations between apoE and PLTP with other biomarkers in CSF is due to amount of protein in CSF, analyses were repeated with adjustment for CSF albumin. Adjustment for CSF albumin did not notably alter the observed associations (data not shown).

Effect of APOE genotype on markers of neurodegeneration

To evaluate the effect of *APOE* genotype, we compared the levels of the CSF variables in participants with present or absent *APOE* $\epsilon 4$ or *APOE* $\epsilon 2$ allele. Interestingly, study participants with at least one *APOE* $\epsilon 2$ allele had significantly lower CSF apoE, A β ₄₂ and PLTP activity compared to participants with no apoE2, while *APOE* $\epsilon 4$ positive participants had no significant alterations in any of the measured variables in our study group (Table 5). The *APOE* $\epsilon 2$ positive participants also tended to have lower sA β PP β and A β ₄₀, but the differences did not reach statistical significance ($p = 0.069$ and 0.052 , respectively; Table 5). Comparisons between study participants homozygous for *APOE* $\epsilon 2$ and *APOE* $\epsilon 4$ were not possible due to small numbers.

DISCUSSION

Data from our study suggest that CSF apoE concentration, regardless of apoE genotype, is significantly associated with A β PP-derived markers, and total and phosphorylated tau in CSF of neurologically healthy, cognitively intact individuals.

Although effects of age were not the primary focus of our study, it is known that some of these markers in CSF change with increasing age. For example, a recent study by Glodzik-Sobanska and colleagues has shown an effect of aging on levels of total tau and tau phosphorylated at threonine 231 in CSF [13]. We have shown that age had significant impact on levels of apoE, albumin, sA β PP α and β , A β ₄₀, total and pTau₁₈₁, as well as on PLTP activity in CSF of healthy adults. This effect, however, was most pronounced in older adults compared both to the young and middle-age group, with exception of apoE and sA β PP α , which were also significantly increased in middle-age. These findings suggest that age is a significant determinant of the levels of nearly all measured markers, with exception of CSF cholesterol, which did not change significantly with aging. The fact that most markers increased with increasing age also suggests compensatory changes in the brain synthesis of these molecules as the brain ages, and loss of these compensatory effects may be detrimental in brain diseases, including neurodegeneration. It would be, therefore, important to understand the mechanisms involved in regulation of levels of these molecules in the aging brain, thus bringing new insight into mechanism of healthy aging and pathophysiology of brain disorders.

Furthermore, we have shown that gender affects some of the reported correlations, although the effect was more pronounced in correlations of PLTP with different markers. All significant correlations between PLTP and other markers observed in our study population were significant only in men, and none were significant in women, suggesting that gender is a major

determinant of PLTP activity in CSF. On the other hand, apoE correlations seem to be less affected by gender. One notable exception is the correlation between apoE and cholesterol in CSF, which was significant only in women, and not in men. Given the fact that apoE is the most important lipid carrier in the brain, this finding that apoE is not a significant determinant for CSF cholesterol levels in men is somewhat surprising. Therefore, it would be important to evaluate the relationship of other apolipoproteins and cholesterol in CSF, as well as potential differences in CSF lipoprotein size and composition in women and men.

Published studies suggest that cholesterol is the critical factor in synaptogenesis [26]. ApoE and PLTP are involved in cholesterol transfer between cells, most probably having synergistic effect on the cellular cholesterol levels. The complementary functions of apoE and PLTP are potentially of particular significance in the brain, given the fact that apoE is the main apolipoprotein in the brain, and PLTP is the key lipid transfer protein that facilitates not only phospholipid transfer, but also transfer of unesterified cholesterol and α -tocopherol [9,20,35]. Given high concentration of cholesterol in the brain and its significance in structural and functional stability of the brain cells, these putative functions of apoE and PLTP and their synergistic relevance in the brain lipid metabolism need to be further elucidated.

In our study, the presence of *APOE* $\epsilon 4$ was not associated with significant alterations in levels of any of the measured markers, which differs from previously published studies by Sunderland and colleagues and Glodzik-Sobanska and colleagues [13,38]. It should be noted, however, that majority of the participants in these studies were middle-aged or older, or were first-degree relatives of patients with AD, which could significantly alter the relationship between apoE genotype and levels tau or $A\beta_{42}$. In contrast, we found that presence of at least one *APOE* $\epsilon 2$ was associated with significantly lower apoE and $A\beta_{42}$ levels and lower PLTP-mediated phospholipid transfer activity in CSF. These findings might be considered counter-intuitive, as *APOE* $\epsilon 2$ has been suggested to have a protective effect on development of AD [23], and AD patients have significantly lower levels of CSF $A\beta_{42}$ and PLTP activity [12,45]. However, reduced CSF $A\beta_{42}$ and PLTP activity in patients with AD are most probably a consequence of sequestration of these molecules in the brain tissue. The lower CSF $A\beta_{42}$ and PLTP activity associated with the presence of *APOE* $\epsilon 2$ in our study population of cognitively intact healthy adults is not likely due to retention in the brain, but more likely a result either of decreased production, increased clearance, or combination of these two mechanisms. A recent study by Berlau and colleagues suggested that patients with AD who are *APOE* $\epsilon 2$ positive may have preserved cognition despite overwhelming neurohistopathological findings concurrent with AD [4]. A study by Reddy and colleagues showed a selective loss of synaptic protein in patients with AD [36], and study by Tannenberg and colleagues suggested that the increased severity of the neuronal loss is associated with the presence of *APOE* $\epsilon 4$ [41]. These studies raise an interesting possibility that apoE isoforms play a pivotal role in modulation of cognitive functions through not yet fully defined mechanisms.

In our study, apoE significantly and positively correlates with $A\beta$ PP-derived fragments present in CSF, suggesting that under physiological conditions there is a positive relationship between apoE and $A\beta$ PP levels. *In vitro* studies have shown that apoE, which is secreted predominantly by astrocytes, modulates γ -secretase cleavage of $A\beta$ PP in neurons, a process that leads to formation of $A\beta_{40}$ and $A\beta_{42}$ [18]. We found that apoE levels were highly correlated with $A\beta_{40}$, but to a lesser extent with $A\beta_{42}$, suggesting that factors other than apoE are important for regulation of $A\beta_{42}$ levels *in vivo*. Alternatively, the observed *in vitro* effect of apoE on $A\beta_{42}$ may be affected by other processes, such as retention, re-uptake or degradation, before this peptide reaches CSF.

Previously published studies have shown that apoE either accelerates [48] or inhibits fibril formation *in vitro* [11], while *in vivo* studies have shown that apoE also plays a significant role

in deposition of A β in neuritic plaques in the brain tissue [2,3]. ApoE knock-out transgenic mice expressing mutated human A β PP have no ability to develop amyloid plaques, while presence of at least one allele of *APOE* leads to development of amyloid plaques in this mouse model [2]. These data demonstrate that apoE is necessary for formation of amyloid plaques *in vivo*. Lauderback and colleagues have shown that apoE knock-out mice are more vulnerable to toxic effects of A β [21]. Another study reported that pharmacological blocking of apoE binding to A β reduces formation of amyloid plaques in mice [37]. In contrast, some published studies have shown that binding of lipoprotein particles to A β peptides significantly reduces their ability to form fibrils, suggesting that apoE-containing lipoproteins, which are predominant in the brain, may act as a stabilizing agent for A β peptides in the brain extracellular spaces, preventing A β oligomerization and aggregation [30]. These findings suggest that physiological interactions between apoE and A β peptides may be of critical importance for maintenance of a healthy steady-state in the brain.

Other *in vitro* studies have shown that apoE binding to its receptors on neuronal membrane reduces tau phosphorylation, suggesting that apoE functions as an important modulator of tau phosphorylation in neurons [15,29]. Furthermore, animal and clinical studies have shown that raising of apoE concentration in the CSF, by infusion or by using medications such as fenofibrates, may have a beneficial effect on formation of amyloid plaques and tangles [6,25, 46]. These data indicate the necessity for better understanding of physiological roles of apoE in the human brain, and its interactions with molecules involved in the pathophysiology of AD.

We have previously shown that CSF apoE and PLTP-mediated phospholipid transfer activity strongly correlate [45], a finding that was confirmed in this study, suggesting that PLTP and apoE in the brain are physiologically related. We previously reported that apoE secretion by astrocytes is modulated by PLTP [45], suggesting that PLTP may also have an indirect effect on measured markers through its modulation of apoE secretion in the brain. It is, therefore, plausible that the major impact of PLTP on the markers of neurodegeneration occurs through its relationship with apoE, as our data indeed suggest. Furthermore, we have shown that PLTP activity has a minor, yet measurable, independent effect on the levels of some of the neurodegenerative markers in CSF.

In summary, our data suggest that there is a significant physiological link between apoE and A β PP, as well as between apoE and tau in neurologically healthy, cognitively intact individuals. Furthermore, our data suggest a physiological relationship between apoE and PLTP in the brain. These findings underscore the importance of further elucidation of the mechanisms underlying these relationships, and their effects on processes of neurodegeneration.

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Table 1
Characteristics and CSF biomarkers in cognitively normal adults (*n* = 168).

Variable (unit)	Mean ± SD [range]
Age (years)	49 ± 20 [21-88]
Gender (M/F)	84/84
Albumin (mg/dl)	19.9 ± 9.3 [6.9-64.9]
ApoE (mg/dl)	0.39 ± 0.11 [< 0.17 -0.88]
PLTP activity (μM/ml/h)	2.02 ± 0.59 [0.57-4.84]
Cholesterol (μg/ml)	2.62 ± 0.69 [0.06-4.06]
sAβPPα (nM)	24.14 ± 8.79 [10.2-54.5]
sAβPPβ (nM)	16.26 ± 5.41 [5.4-32.3]
Aβ ₄₀ (nM/ml)	1.934 ± 0.31 [1.256-3.000]
Aβ ₄₂ (nM/ml)	0.121 ± 0.13 [0.034-0.193]
Total tau (pg/ml)	250.5 ± 125 [76.5-973.2]
pTau _{Thr181} (pg/ml)	49.01 ± 17.6 [24.2-126.5]

Table 2
Average values of CSF variables in young ($n = 64$), middle age ($n = 58$) and older adults ($n = 46$). All data are presented as mean $\pm$ SD [range].

Variable	Young	Middle-aged	Old
Age (years)	27.6 $\pm$ 5.2 [21-39]	50.5 $\pm$ 5.5 [40-59]	73.1 $\pm$ 7.0 [60-88]
ApoE (mg/dl)	0.35 $\pm$ 0.1 [0.16-0.5]	0.39 $\pm$ 0.1 ^d [0.16-0.9]	0.44 $\pm$ 0.1 ^{c,e} [0.16-0.9]
PLTA (μ M/ml/h)	1.82 $\pm$ 0.6 [0.94-4.00]	1.87 $\pm$ 0.6 [0.57-4.38]	2.18 $\pm$ 0.6 ^{c,e} [1.00-4.84]
Albumin (mg/dl)	16.4 $\pm$ 6.7 [4.6-42.1]	18.1 $\pm$ 6.8 [7.0-36.2]	25.1 $\pm$ 9.6 ^{c,e} [9.8-64.9]
Cholesterol (mg/ml)	2.52 $\pm$ 0.7 [0.1-3.7]	2.51 $\pm$ 0.7 [0.1-3.8]	2.71 $\pm$ 0.6 [1.9-4.1]
sA β PP α (nM)	20.3 $\pm$ 6.1 [10.2-39.8]	24.8 $\pm$ 8.5 ^b [11.3-54.5]	28.6 $\pm$ 9.9 ^c [14.9-53.7]
sA β PP β (nM)	14.6 $\pm$ 4.5 [5.7-24.6]	16.0 $\pm$ 5.0 [5.4-29.6]	18.6 $\pm$ 5.7 ^{c,d} [6.8-32.3]
A β ₄₀ (nM/ml)	1.84 $\pm$ 0.3 [1.26-2.34]	1.94 $\pm$ 0.3 [1.29-2.44]	2.04 $\pm$ 0.3 ^c [1.26-3.00]
A β ₄₂ (nM/ml)	0.12 $\pm$ 0.06 [0.08-0.19]	0.13 $\pm$ 0.03 [0.05-0.18]	0.11 $\pm$ 0.04 ^d [0.03-0.19]
Tau, total (pg/ml)	194.9 $\pm$ 63 [101-441]	216.7 $\pm$ 90 [77-535]	346.5 $\pm$ 151 ^{c,e} [122-973]
pTau _{T181} (pg/ml)	41.3 $\pm$ 11 [24-73]	45.6 $\pm$ 15 [25-103]	61.2 $\pm$ 43.6 ^{c,e} [24-127]

^a $p < 0.05$ difference between young and middle-aged.
^b $p < 0.01$ difference between young and middle-aged.
^c $p < 0.01$ difference between young and old.
^d $p < 0.05$ difference between middle-aged and old.
^e $p < 0.01$ difference between middle-aged and old.

Table 3
Pearson's correlation coefficients (r) for CSF apoE or PLTP activity and CSF markers in all study participants (n = 168) and by gender (men = 84, and women = 84).

	ApoE			PLTA		
	All	Men	Women	All	Men	Women
Albumin	0.336 ^c	0.272 ^b	0.424 ^c	0.276 ^c	0.306 ^c	0.150
Cholesterol	0.194 ^b	0.060	0.315 ^b	-0.138	-0.088	-0.194
sAβPPα	0.679 ^c	0.625 ^c	0.725 ^c	0.292 ^c	0.382 ^c	0.170
sAβPPβ	0.634 ^c	0.636 ^c	0.621 ^c	0.281 ^c	0.356 ^c	0.206
Aβ ₄₀	0.609 ^c	0.611 ^c	0.611 ^c	0.128	0.239 ^d	0.037
Aβ ₄₂	0.153	0.134	0.183	-0.121	-0.194	-0.009
Total Tau	0.589 ^c	0.525 ^c	0.744 ^c	0.265 ^c	0.368 ^c	0.201
pTau ₁₈₁	0.673 ^c	0.573 ^c	0.744 ^c	0.258 ^c	0.355 ^c	0.196

^a $p < 0.05$,
^b $p < 0.01$.
^c $p \leq 0.001$.

Table 4

Partial correlations of CSF apoE and PLTP activity with the AD biomarkers, adjusted for age and gender.

	ApoE	PLTP	activity
Cholesterol	0.206 ^a	-0.210 ^a	
AβPP			
sAβPPα	0.530 ^b	0.140	
sAβPPβ	0.557 ^b	0.144	
Aβ ₁₋₄₀	0.545 ^b	-0.019	
Aβ ₁₋₄₂	0.279 ^b	-0.083	
Tau			
Total tau	0.476 ^b	0.117	
pTau	0.500 ^b	0.070	

Note: For apoE partial correlations, model contained PLTP activity, age and gender as independent variables. For PLTP activity, regression model contained apoE, age and gender as independent variables. Adjustment for CSF albumin did not change these partial correlations, and therefore it is not reported.

^a
 $p < 0.01$

^b
 $p \leq 0.001$

Table 5
Concentration (Mean ± SD) of CSF apoE, PLTP, and AD biomarkers by presence of APOE2 or APOE4.

Variable	APOE2+	APOE2-	APOE4+	APOE4-
<i>n</i>	28	140	61	107
ApoE (mg/dl)	0.35 ± 0.1	0.40 ± 0.1 ^b	0.38 ± 0.1	0.40 ± 0.1
PLTP activity (μM/ml/h)	1.87 ± 0.6	2.05 ± 0.6 ^d	1.97 ± 0.5	2.05 ± 0.7
Cholesterol (μg/ml)	2.47 ± 0.7	2.66 ± 0.7	2.58 ± 0.7	2.71 ± 0.6
sAβPPα (nM)	22.79 ± 8.2	24.48 ± 9.0	23.80 ± 8.6	24.92 ± 9.3
sAβPPβ (nM)	14.68 ± 4.7	16.60 ± 5.5	16.14 ± 5.8	16.51 ± 4.8
Aβ ₄₀ (nM/ml)	1.836 ± 0.284	1.956 ± 0.311	1.926 ± 0.322	1.952 ± 0.288
Aβ ₄₂ (nM/ml)	0.109 ± 0.028	0.124 ± 0.033	0.124 ± 0.031	0.117 ± 0.024
Total tau (pg/ml)	235.3 ± 117	254.4 ± 127	246.3 ± 121	259.7 ± 134
pTau _{Thr181} (pg/ml)	44.6 ± 14.9	50.1 ± 18.1	48.4 ± 16.8	50.5 ± 19.1

^a Difference between APOE2 + and APOE2 -, *p* < 0.05
^b Difference between APOE2 + and APOE2 -, *p* < 0.01