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RESEARCH ARTICLE

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Circulating ethanolamine plasmalogen indices in Alzheimer's disease: Relation to diagnosis, cognition, and CSF tau

Mitchel A. Kling[1,2] | Dayan B. Goodenowe[3] | Vijitha Senanayake[3] | Siamak MahmoudianDehkordi[4] | Matthias Arnold[4,5] | Tyler J. Massaro[6] | Rebecca Baillie[7] | Xianlin Han[8] | Yuk-Yee Leung[9] | Andrew J. Saykin[10] | Kwangsik Nho[10] | Alexandra Kueider-Paisley[4] | Jessica D. Tenenbaum[11] | Li-San Wang[9] | Leslie M. Shaw[9] | John Q. Trojanowski[9] | Rima F. Kaddurah-Daouk[4,12,13] | for the Alzheimer's Disease Metabolomics Consortium (ADMC) and the Alzheimer's Disease Neuroimaging Initiative (ADNI)[1]

[1]Department of Psychiatry, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA

[2]Behavioral Health Service, Crescenz VA Medical Center, Philadelphia, Pennsylvania, USA

[3]Prodrome Sciences Inc., Temecula, California, USA

[4]Department of Psychiatry and Behavioral Sciences, Duke University, Durham, North Carolina, USA

[5]Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany

[6]Duke Clinical Research Institute, Duke University, Durham, North Carolina, USA

[7]Rosa & Co LLC, San Carlos, California, USA

[8]University of Texas Health Science Center at San Antonio, San Antonio, Texas, USA

[9]Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA

[10]Department of Radiology and Imaging Sciences and the Indiana Alzheimer Disease Center, Indiana University School of Medicine, Indianapolis, Indiana, USA

[11]Department of Biostatistics and Bioinformatics, Duke University, Durham, North Carolina, USA

[12]Duke Institute of Brain Sciences, Duke University, Durham, North Carolina, USA

[13]Department of Medicine, Duke University, Durham, North Carolina, USA

Correspondence
Mitchel A. Kling, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA.
E-mail: mitchel.kling@uphs.upenn.edu
Rima Kaddurah-Daouk, Duke University Medical Center, Durham, NC, USA.
E-mail: kaddu001@mc.duke.edu

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Abstract

Introduction: Altered lipid metabolism is implicated in Alzheimer's disease (AD), but the mechanisms remain obscure. Aging-related declines in circulating plasmalogens containing omega-3 fatty acids may increase AD risk by reducing plasmalogen availability.

Methods: We measured four ethanolamine plasmalogens (PlsEtns) and four closely related phosphatidylethanolamines (PtdEtns) from the Alzheimer's Disease Neuroimaging Initiative (ADNI; n = 1547 serum) and University of Pennsylvania (UPenn; n = 112 plasma) cohorts, and derived indices reflecting PlsEtn and PtdEtn metabolism: PL-PX (PlsEtns), PL/PE (PlsEtn/PtdEtn ratios), and PBV (plasmalogen biosynthesis value; a composite index). We tested associations with baseline diagnosis, cognition, and cerebrospinal fluid (CSF) AD biomarkers.

Results: Results revealed statistically significant negative relationships in ADNI between AD versus CN with PL-PX ($P = 0.007$) and PBV ($P = 0.005$), late mild cognitive impairment (LMCI) versus cognitively normal (CN) with PL-PX ($P = 2.89 \times 10^{-5}$) and PBV ($P = 1.99 \times 10^{-4}$), and AD versus LMCI with PL/PE ($P = 1.85 \times 10^{-4}$). In the

UPenn cohort, AD versus CN diagnosis associated negatively with PL/PE ($P = 0.0191$) and PBV ($P = 0.0296$).

In ADNI, cognition was negatively associated with plasmalogen indices, including Alzheimer's Disease Assessment Scale 13-item cognitive subscale (ADAS-Cog13; PL-PX: $P = 3.24 \times 10^{-6}$; PBV: $P = 6.92 \times 10^{-5}$) and Mini-Mental State Examination (MMSE; PL-PX: $P = 1.28 \times 10^{-9}$; PBV: $P = 6.50 \times 10^{-9}$). In the UPenn cohort, there was a trend toward a similar relationship of MMSE with PL/PE ($P = 0.0949$).

In ADNI, CSF total-tau was negatively associated with PL-PX ($P = 5.55 \times 10^{-6}$) and PBV ($P = 7.77 \times 10^{-6}$). Additionally, CSF t-tau/A$\beta_{1\text{-}42}$ ratio was negatively associated with these same indices (PL-PX, $P = 2.73 \times 10^{-6}$; PBV, $P = 4.39 \times 10^{-6}$). In the UPenn cohort, PL/PE was negatively associated with CSF total-tau ($P = 0.031$) and t-tau/A$\beta_{1\text{-}42}$ ($P = 0.021$). CSF A$\beta_{1\text{-}42}$ was not significantly associated with any of these indices in either cohort.

Discussion: These data extend previous studies by showing an association of decreased plasmalogen indices with AD, mild cognitive impairment (MCI), cognition, and CSF tau. Future studies are needed to better define mechanistic relationships, and to test the effects of interventions designed to replete serum plasmalogens.

KEYWORDS

case-control study, dementia, lipidomics, mass spectrometry, metabolomics, mild cognitive impairment, phosphatidylethanolamines, plasmenylethanolamines

1 | INTRODUCTION

Alzheimer's disease (AD) is a major public health problem.[1] While much is known about its natural history, epidemiologically identified risk factors, and characteristic neurodegenerative changes, there are currently no available treatments to modify the underlying disease process or prevent cognitive or functional decline. Moreover, relatively little is known regarding etiologic mechanisms that initiate or promote neurodegenerative processes in sporadic AD. Biomarkers that identify AD in preclinical stages may aid in elucidating these mechanisms. Currently, most accepted biomarkers relate to neuropathologic features of AD, eg, cerebrospinal fluid (CSF) levels of tau and amyloid beta (Aβ) and neuroimaging of brain atrophy and amyloid or tau deposits. Blood-based biomarkers are potentially appealing due to lower participant burden. Serum- and plasma-based biomarkers have been used in other studies to predict cognitive declines, dementia diagnosis, and progression to AD.[2-6]

Altered lipid metabolism is implicated in sporadic AD risk;[7,8] however, the precise mechanisms accounting for findings from observational studies remains to be fully elucidated. Although cholesterol and its content in circulating lipoproteins are perhaps the most well-studied lipids in AD,[8] recent data implicate other classes such as glycerophospholipids.[2,9-14] Plasmalogens represent a subclass of glycerophospholipids carrying a vinyl ether linkage at the sn1 carbon of the glycerol backbone, versus the more familiar fatty acyl ester linkage[15] (see Figure S1 in supporting information). Plasmalogens are integral membrane components that appear to play important roles relevant to AD pathophysiology, including vesicle fusion[16,17] necessary for synaptic neurotransmitter release,[18] modulation of membrane fluidity and microdomain dynamics,[19] membrane antioxidant functions,[20,21] and neuroprotection.[22] Plasmalogens can exert reciprocal effects with cholesterol on membrane fluidity and lipid microdomain composition, promoting the breakdown of amyloid precursor protein (APP) by alpha-secretase to non-amyloidogenic peptide products. This enhancement of alpha-secretase-mediated APP breakdown may reduce the production of beta-secretase-derived peptides and thus to the formation of the amyloid plaques characteristic of AD neuropathology.[23]

The initial steps of endogenous plasmalogen synthesis occur in peroxisomes, notably in the liver.[15,24] Peroxisomes also perform enzymatic functions necessary for synthesis of long-chain fatty acids (LCFA) and very long-chain fatty acids (VLCFA) such as docosahexaenoic acid (DHA, C22:6n-3), particularly beta-oxidation and side chain elongation steps. Plasmalogens are incorporated into circulating lipoproteins,[25] from which they can be transported to other organs including the brain.[26,27] Serum plasmalogen levels decline with increasing age[8,28,29] and previous studies show decreased levels of serum and *post mortem* brain plasmalogens in AD.[9,10,13,29-34]

Ratios of metabolites measured at a given time point are frequently informative regarding the homeostasis of metabolites and

metabolic flux. Thus, these ratios can be used to identify metabolic defects and to elucidate etiopathogenesis. One example from disorders of peroxisome biogenesis is the use of VLCFA ratios in the diagnosis of Zellweger spectrum disorders in which elevation of C24/C22 and C26/C22 ratios are indicative of enzymatic defects in the beta-oxidation of very long chain fatty acids in the peroxisomes.[35] Zellweger syndrome is a peroxisomal biogenesis disorder with multi-enzyme defects in the peroxisomal beta-oxidation pathway.[35]

Another commonly used lipid ratio is that of omega-6/omega-3 fatty acids, which reflects the relative activity of competing metabolic pathways in omega-6 and omega-3 fatty acid synthesis, dietary intake, and rate of use.[36] Plasmalogens act as reservoirs of polyunsaturated fatty acid (PUFA) and they are selectively enriched in certain PUFA side chains.[37] Thus, fatty acid composition in plasmalogens bring valuable insights not only about plasmalogen biosynthesis but also about homeostasis of fatty acids and remodeling of plasmalogens.

Ratios among plasmalogen species (PL/PL) having substrate–product relationships, such as those described below, reflect the relative abundance of DHA, EPA (omega-3) and adrenic acid (omega-6) fatty acid side chains in plasmalogens and represent the results of plasmalogen remodeling, fatty acid biosynthesis/dietary intake, and peroxisomal beta-oxidation. Plasmalogen and phosphatide levels are homeostatically regulated, such that a reduction in the circulating levels of one subclass (eg, plasmalogens) is often accompanied by a compensatory increase in the other (eg, phosphatides [PE]).[38] Therefore, ratios of PL to PE reflect this equilibrium and represent the capacity of the body to synthesize plasmalogens. In addition, the calculation of composite indices from two or more ratios may result in reduced biological variability and thus show greater comparability across various cohorts. Certain indices constructed in this fashion were found to be associated with cognition in previous studies.[10]

We report here data obtained using a focused lipidomics platform to measure four ethanolamine plasmalogens and four closely related phosphatidylethanolamines in baseline serum specimens from the Alzheimer's Disease Neuroimaging Initiative (ADNI)-1, -GO, and -2 cohorts, and in plasma samples from subjects at the Perelman School of Medicine, University of Pennsylvania (UPenn). We selected these species based on previous work in several disparate populations as described in Goodenowe and Senanayake.[10] We then calculated ratios among omega-3 and omega-6-bearing plasmalogens (PL/PL) and of plasmalogens to phosphatides (PL/PE), and constructed composite indices from these ratios based on previous work.[10] Algorithm-based in vitro diagnostic multivariate index assays are used in increasing frequency to identify the risks of complex chronic diseases. Our overarching goal was to create an in vitro diagnostic multivariate index that can be used to assess the risk of AD in pre-clinical stages. The use of these ratios helped create a single index that can be tested in future longitudinal studies for early detection of AD.

We further note that this study was not intended to be an untargeted lipidomic study aimed at generation of hypotheses, but rather was a focused analysis of selected members of certain physiologically important lipid subclasses known to be associated with AD and cognitive dysfunction. The molecules of interest have been well researched and published in peer-reviewed journals.[9,10,13,29-34] Preliminary results on the particular metabolite ratios mentioned here have been presented in Alzheimer's Association conferences from years 2014 to 2016 and published as abstracts in *Alzheimer's and Dementia*,[39,40] as well as in a paper based on some of these data that was recently published.[10] These metabolites and their ratios were selected iteratively from comparisons of data across several large lipidomic datasets in previous studies cited earlier. The objective of this study was to confirm these findings in well characterized cohorts, such as ADNI and UPenn.

We sought to test the following hypotheses:

1. Are indices of plasmalogen and related phosphatide metabolism associated with (a) diagnosis or (b) cognitive function at the time of blood sampling?
2. Are indices of plasmalogen and related phosphatide metabolism associated with CSF biomarkers of AD pathophysiology, ie, amyloid beta (Aβ) and tau?

We present here findings showing a reduction in indices of plasmalogen remodeling that may also reflect altered biosynthesis and peroxisomally derived plasmalogen beta-oxidation associated with AD.

RESEARCH IN CONTEXT

1. **Systematic review**: The authors reviewed the literature using PubMed, Google, Web of Science, and through meeting abstracts and presentations. We included several publications which associate plasmalogens with important aspects of Alzheimer's disease (AD) pathophysiology.
2. **Interpretation**: This is the first large study to show that decreased serum ethanolamine plasmalogens are associated with AD diagnosis, cognitive impairments, and levels of cerebrospinal fluid tau. These data further suggest the hypothesis that interventions that enhanced plasmalogen biosynthesis and availability to the central nervous system may mitigate cognitive decline and/or brain atrophy in those at risk for AD.
3. **Future directions**: Understanding the role of the plasmalogens in aging and related diseases opens potential new hypotheses for AD. Prospective clinical observations and validation in model systems are needed to assess causality and specific mechanisms underlying altered plasmalogens in AD and their potential relationships to genetic variation, neuroimaging measures, and other phenotypic characteristics of AD-type dementia.

2 | METHODS

2.1 | Study cohorts and samples

2.1.1 | Alzheimer's Disease Neuroimaging Initiative (ADNI)

Data were obtained from the ADNI database (www.adni.loni.usc.edu). In this study, we used serum samples obtained at the baseline study visit in the ADNI-1, -GO, and -2 cohorts (see Text S1 in supporting information for a summary of the study design and inclusion criteria for these cohorts). For additional information, see www.adni-info.org. Metabolomics data and results have been made accessible through the Accelerating Medicines Partnership—Alzheimer's Disease (AMP-AD) Knowledge Portal (https://ampadportal.org). The AMP-AD Knowledge Portal is the distribution site for data, analysis results, analytical methodology, and research tools generated by the AMP-AD Target Discovery and Preclinical Validation Consortium and multiple Consortia and research programs supported by the National Institute on Aging. Information on data availability and accessibility is available in the Data Availability section.

2.1.2 | University of Pennsylvania Alzheimer's Disease Clinical Center (ADCC)

We selected subjects from the Integrated Neurodegenerative Diseases Database at the Perelman School of Medicine, University of Pennsylvania for studies of plasma and CSF metabolomics (see Text S1 for selection criteria). All subjects gave informed consent for participation in research protocols approved by the Penn Institutional Review Board for the collection of clinical and genetic data and biofluid samples. A total of 127 samples were selected, representing 112 unique subjects; 12 subjects underwent plasma sampling at two different times, and 1 subject was sampled on three occasions. In all cases, the earliest plasma sample was used for the analyses described here.

2.2 | Procedures (blood draw, sample storage/handling)

Blood samples were collected in the morning after an overnight fast (water was permitted) prior to collection. Serum was obtained from blood drawn into 10-mL plain red top plastic tubes, allowed to clot for 30 minutes at room temperature in a vertical position, and centrifuged at 1,500 relative centrifugal force (rcf) for 15 minutes. Serum was collected into polypropylene tubes, frozen, and shipped on dry ice to the ADNI Biorepository. Serum samples were subsequently thawed and divided into 0.5-mL aliquots at the ADNI Biorepository, and frozen at −80 °C until retrieved for analysis.

2.3 | Apolipoprotein E genotype

Apolipoprotein E (*APOE*) genotyping was performed at the time of participant enrollment. The two single-nucleotide polymorphisms (SNPs; rs429358, rs7412) that define the epsilon 2, 3, and 4 alleles, were genotyped using DNA extracted by Cogenics from a 3-mL aliquot of ethylenediaminetetraacetic acid (EDTA) blood.

2.4 | Cognitive function

Global cognition was measured using the modified Alzheimer Disease Assessment Scale 13-item cognitive subscale (ADAS-Cog13) where higher scores indicate worse cognition and the Mini-Mental State Exam (MMSE) where higher scores indicate better cognition. ADAS-Cog13 and MMSE scores were available in the ADNI cohort, whereas MMSE scores were only available in the UPenn cohort.

2.5 | Biochemical analysis

We measured four plasmenylethanolamine (PlsEtn) and four phosphatidylethanolamine (PtdEtn) glycerophospholipid species with the following sn-1/sn-2 compositions in 20-μL aliquots of serum or EDTA plasma: PlsEtn 16:0/18:2, 18:0/20:5, 16:0/22:4, 16:0/22:6 and PtdEtn 16:0/18:3, 18:0/20:5, 18:0/22:4, 16:0/22:6 by a stable isotope dilution, flow-injection analysis (FIA)-mass spectrographic (MS) method on an Ionics 3Q tandem mass spectrometer operating in the negative ionization atmospheric pressure chemical ionization (APCI) mode. Methods are summarized in Text S2 in supporting information and described elsewhere.[9,13] Presumed structures of phospholipids corresponding to the specific mass/charge (m/z) ratio peaks are shown in Figure S1. Due to the specific hypotheses being tested, the data reported here will concern ratios and indices derived from three of the above plasmalogen species (PlsEtn 18:0/20:5, 16:0/22:4, 16:0/22:6) and one phosphatidylethanolamine species (PtdEtn 16:0/22:6). These ratios and indices have previously been shown to be useful composite measures of plasmalogen physiology in other cohorts.[10]

2.6 | Statistical analysis

2.6.1 | Calculation of phosphoethanolamine ratios and indices and rationale

Each bivariate ratio was scaled to the full dataset for each of the cohorts examined (ie, ADNI and UPenn) by log transformation of the ratio, followed by normalization to the mean and standard deviation of the values for the cohort. ADNI values underwent $\log_2$ transformation before normalization; UPenn data were $\log_{10}$ transformed. Composite scores were then created from these scaled values for each sample to assess:

1. Peroxisomal plasmalogen (PL) biosynthesis and/or remodeling (PL-PX),
2. Relative abundance of PLs versus PEs (PL/PE); and
3. Overall plasmalogen biosynthesis value (PBV).

These indices were calculated as follows and are summarized in Table S1 in supporting information:

(A) PL-PX: Mean of PL226/PL224, PL205/PL224, and PL205/PL226;

provides an index of biosynthesis and/or remodeling of ethanolamine plasmalogens based on ratios of concentration of specific sn2 isoforms measured (ie, those containing adrenic acid or 22:4; eicosapentaenoic acid, EPA or 20:5; and docosahexaenoic acid or 22:6)

(A) PL/PE: Mean of PL226/PE226 and PL205/PE226;

provides an index of the ratio of omega-3 fatty acid-containing plasmalogens to the DHA-containing phosphatide PtdEtn 16:0/22:6.

(A) Overall PBV: Mean of the following five ratios:

PL226/PE226, PL205/PE226 (constituents of index B, above),
PL226/PL224, PL205/PL224, and PL205/PL226 (constituents of index A, above); provides an overall composite index of plasmalogen biosynthesis and/or remodeling.

2.7 | Statistical models

Statistical analyses were conducted using R version 3.2.4 and MATLAB R2016b. Data were analyzed according to previously published methods.[41] The statistical pipeline steps applied to these data are summarized in Figure S2 in supporting information.

Phosphoethanolamines were measured in 829 samples from 809 subjects within the ADNI-1 cohort and in 905 samples from 873 subjects in the ADNI-GO/2 cohort. Biological replicates were averaged for 20 subjects in ADNI-1 and 17 subjects in ADNI-GO/2. From the original set of 1734 samples, we removed 104 who were not fasting, two missing body mass index (BMI), and one missing sex. An additional 26 subjects were removed after determining they were multivariate outliers based on squared Mahalanobis distances significant at the upper one-tailed 0.05 level of confidence adjusted for multiple comparisons by false discovery rate (FDR) correction. This resulted in an analysis dataset containing 1545 subjects, five bivariate ratios, and three composite indices.

The Kruskal-Wallis one-way analysis of variance (ANOVA) on ranks for continuous covariates and the Pearson chi-square test for categorical covariates were used to check for differences among covariate information collected at baseline grouped by baseline diagnosis (cognitively normal [CN] vs late mild cognitive impairment [LMCI] vs AD).

To assess the association of metabolic features and the odds of being in one subgroup relative to being in another subgroup for all pairs of subgroups (ie, MCI vs CN, AD vs CN, and AD vs MCI) binary logistic regression models were used. As early mild cognitive impairment (EMCI) and subjective memory concern (SMC) were not represented in ADNI-1, we only examined comparisons among AD, (L)MCI, and CN subjects in the logistic regression analyses. The MCI subjects in ADNI-1 were combined with the LMCI cohort in ADNI-2, given the comparability of criteria for selecting these groups.[42] In the UPenn cohort, due to the relatively small sample of MCI patients, we only tested the association of AD versus CN with the three composite phosphoethanolamine indices.

To test the association of each metabolic feature with the outcome of interest in both ADNI and UPenn cohorts, a separate linear regression (ADAS-Cog13, MMSE, and CSF t-tau, $A\beta_{1\text{-}42}$, and t-tau/$A\beta_{1\text{-}42}$ ratio) was performed. ADAS-Cog13 and MMSE were square-root transformed and CSF t-tau/$A\beta_{1\text{-}42}$ was $\log_{10}$-transformed prior to analysis. All models included age, sex, and *APOE* $\varepsilon 4$ genotype carrier status as covariates to control for the confounding effects (*APOE* $\varepsilon 4$ genotype carrier status was not included in the analyses of the UPenn cohort due to the number of subjects who had not been genotyped for *APOE*.) Moreover, education in years was controlled for in the analyses modeling cognition.

Because it is known that the genetic variance in the FADS1/FADS2 gene cluster can affect the blood levels of EPA/DHA containing lipids,[43] we conducted a candidate gene association analysis for relationships between plasmalogen ratios and indices with genetic variants in this gene cluster. In the ADNI cohort, to control for the confounding effect of medication and dietary supplements on metabolic levels, we constructed additional models in which each ratio and index was regressed on 42 medication and dietary supplement categories using previously published methods.[41] Figure S3 in supporting information depicts the percentage of ADNI subjects in each diagnostic group who were recorded as taking a member of each of these medication classes at the baseline study visit. Medication classes were backward-selected via Bayesian information criteria to select an optimal combination of medications for minimizing confounding while limiting model complexity. For this analysis, we removed acetylcholinesterase inhibitors and N-methyl-D-aspartic acid (NMDA) receptor antagonist drugs from consideration, to avoid sampling bias due to the extreme degree of heterogeneity among diagnostic groups in the frequency of their use (ie, few if any CN subjects were taking these drugs, while most AD subjects report taking them). The residuals from the regression of metabolic levels on medications and supplements were kept to create a separate analysis dataset. We did not conduct this medication-adjusted analysis on the UPenn cohort due to the size of this sample, which limited our power to conduct a meaningful analysis.

3 | RESULTS

Characteristics of the combined ADNI-1/-GO/-2 participants at the time of baseline serum collection are depicted in Table 1. Characteristics of the University of Pennsylvania cohort are presented in Table 2.

TABLE 1 Baseline demographic characteristics of the ADNI-1, -GO, -2 cohorts

Characteristic	N	Overall group (N = 1545)	CN (n = 364)	SMC (n = 98)	EMCI (n = 282)	LMCI (n = 497)	AD (n = 304)	*P*-value
Age years (SD)	1545	73.67 (7.2)	74.63 (5.7)	72.24 (5.6)	71.14 (7.5)	74.09 (7.5)	74.65 (7.8)	<0.001
Male, % (#)	1545	54 (840)	48 (176)	42 (41)	55 (154)	61 (304)	54 (165)	<0.001
Education years (SD)	1545	15.91 (2.8)	16.30 (2.77)	16.73 (2.5)	15.98 (2.6)	15.86 (2.9)	15.20 (3.0)	<0.001
APOE ε4 +, % (#)	1545	47 (726)	28 (103)	33 (32)	43 (120)	54 (270)	66 (201)	<0.001
ADAS-Cog13, (SD)	1534	16.82 (9.6)	9.18 (4.3)	8.82 (4.0)	12.57 (5.3)	18.70 (6.6)	29.74 (8.1)	<0.001
MMSE (SD)	1545	27.17 (2.6)	29.08 (1.1)	29.00 (1.2)	28.33 (1.5)	27.16 (1.8)	23.23 (2.0)	<0.001
CSF T-tau (SD)	1111	90.10 (53.5)	68.71 (22.3)	64.91 (31.3)	75.82 (47.3)	101.18 (55.8)	125.77 (59.9)	<0.001
CSF $Abeta_{1-42}$ (SD)	1127	174.50 (53.8)	200.72 (51.6)	202.06 (48.5)	184.05 (50.9)	162.8 (51.4)	139.91 (39.5)	<0.001
T-tau/$Abeta_{1-42}$	1111	0.62 (0.50)	0.39 (0.27)	0.35 (0.23)	0.39 (0.47)	0.72 (0.52)	0.97 (0.54)	<0.001
Log T-tau/$Abeta_{1-42}$ (SD)	1111	−0.33 (0.33)	−0.49 (0.25)	−0.52 (0.24)	−0.43 (0.32)	−0.25 (0.32)	−0.08 (0.26)	<0.001

Abbreviations: ADAS-Cog13, Alzheimer's Disease Assessment Scale, 13-item cognitive subscale; *APOE*, apolipoprotein E genotype; ADNI, Alzheimer's Disease Network Initiative; CN, cognitively normal; CSF, cerebrospinal fluid; EMCI, early mild cognitive impairment; LMCI, late mild cognitive impairment; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; SD, standard deviation

TABLE 2 Baseline demographic characteristics of the University of Pennsylvania cohort

Characteristic	N	Overall group (N = 112)	CN (n = 51)	MCI (n = 18)	AD (n = 43)	*P*-value
Age years (SD)	112	69.9 (9.2)	68.1 (9.8)	70.9 (5.9)	71.5 (9.3)	0.186
Male, % (#)	112	47 (53)	41 (21)	78 (14)	42 (18)	0.019
Education years (SD)	112	16.0 (2.9)	16.3 (2.8)	15.7 (3.2)	15.7 (2.9)	>0.05
APOE ε4 +, % (#)	82	37.8 (31)	26.0 (13)	36.4 (4)	66.7 (14)	0.006
MMSE (SD)	109	27.72 (5.3)	29.16 (1.11)	27.35 (1.46)	20.98 (5.85)	<0.001
CSF T-tau (SD)	112	74.1 (51.2)	52.2 (19.0)	66.3 (45.7)	103.3 (64.6)	<0.001
CSF $Abeta_{1-42}$ (SD)	112	232.9 (86.6)	281.3 (77.8)	224.0 (89.8)	179.2 (59.3)	<0.001
T-tau/$Abeta_{1-42}$	112	0.39 (0.37)	0.20 (0.12)	0.39 (0.40)	0.62 (0.43)	<0.001
Log T-tau/$Abeta_{1-42}$ (SD)	112	−0.54 (0.34)	−0.74 (0.22)	−0.57 (0.37)	−0.29 (0.28)	<0.001

*Note for the UPenn Cohort: *APOE* genotype available on 50 CN, 11 MCI, 21 AD. MMSE missing on 1 CN, 1 MCI, and 1 AD subject.
Abbreviations: AD, Alzheimer's disease; ADAS-Cog13, Alzheimer's Disease Assessment Scale, 13-item cognitive subscale; *APOE*, apolipoprotein E genotype; CN, cognitively normal; CSF, cerebrospinal fluid; EMCI, early mild cognitive impairment; LMCI, late mild cognitive impairment; MCI, mild cognitive impairment; MMSE, Mini-Mental State Exam; SMC, subjective memory concerns; T-tau, total tau.

The means and standard deviations of the five ratios and three composite indices in the ADNI diagnostic groups AD, LMCI, and CN are shown in Table S2 in supporting information.

3.1 | Phosphoethanolamine lipid indices by diagnosis

3.1.1 | AD versus CN

The results of phosphoethanolamine lipid ratios and indices in cross-sectional analysis, unadjusted for medications, in the ADNI cohort are shown in Table 3. Of the composite indices examined using pairwise-by-diagnosis logistic regression models, the PL-PX (odds ratio [OR] = 0.784, $P = 0.007$) and overall PBV (OR = 0.775, $P = 0.005$) showed a significant negative relationship with the likelihood of AD versus CN diagnosis that persisted after FDR correction. This result indicates that reduced values of these indices of plasmalogen metabolism were associated with an increased likelihood of having AD versus CN. The other composite index, PL/PE, did not show significant relationships with AD versus CN diagnosis (Table 3).

When all medications except those specific to AD (ie, anticholinesterases and memantine) were included in the models (see Table S3 in supporting information for the medication classes retained in these models due to significant contributions to the analysis of the three composite measures), none of the associations of plasmalogen ratios or indices with the AD versus CN diagnosis was significant (all *P*-values > 0.1). See Table S4 in supporting information for a summary of these results.

In the UPenn ADCC cohort the results of phosphoethanolamine lipid ratios and indices are shown in Table 4. As seen in ADNI, in the UPenn ADCC cohort we observed a statistically significant association of AD versus CN with PBV (OR = 0.695, $P = 0.0296$). We also observed a significant association of AD versus CN with PL/PE (OR = 0.630, $P = 0.0191$). The PL-PX

TABLE 3 Serum plasmalogen/phosphatide indices in ADNI-1/GO/2 Cohort: associations with diagnosis and cognition

Index/ratio	Logistic regression analyses									Linear regression analyses					
	AD versus CN			LMCI versus CN			AD versus LMCI			ADAS-Cog13			MMSE		
	OR (95% CI)	*P*-value	q-value	OR (95% CI)	*P*-value	q-value	OR (95% CI)	*P*-value	q-value	β (95% CI)	*P*-value	q-value	β (95% CI)	*P*-value	q-value
PBV	**0.775 (0.648, 0.925)**	**0.005**	**0.030**	**0.745 (0.638, 0.869)**	**1.99E-04**	**7.97E-04**	0.999 (0.859, 1.162)	0.994	0.994	**−0.130 (−0.187, −0.074)**	**6.92E-06**	**2.77E-05**	**0.038 (0.025, 0.051)**	**6.50E-09**	**2.60E-08**
PL-PX	**0.784 (0.656, 0.935)**	**0.007**	**0.030**	**0.719 (0.615, 0.838)**	**2.89E-05**	**1.73E-04**	1.051 (0.904, 1.222)	0.517	0.564	**−0.135 (−0.192, −0.078)**	**3.24E-06**	**1.94E-05**	**0.040 (0.027, 0.052)**	**1.28E-09**	**1.25E-08**
PL/PE	0.894 (0.745, 1.072)	0.228	0.304	1.141 (0.983, 1.328)	0.085	0.145	**0.757 (0.644, 0.887)**	**6.15E-04**	**2.46E-03**	0.0120 (−0.047, 0.070)	0.696	0.759	−0.003 (−0.016, 0.010)	0.646	0.887
PL205_224	**0.787 (0.658, 0.937)**	**0.008**	**0.030**	**0.843 (0.728, 0.976)**	**0.023**	0.056	0.901 (0.775, 1.047)	0.176	0.235	**−0.077 (−0.133, −0.020)**	**0.008**	**0.023**	**0.032 (0.020, 0.045)**	**5.99E-07**	**1.80E-06**
PL205_226	0.902 (0.754, 1.078)	0.257	0.308	1.163 (1.000, 1.355)	0.051	0.102	**0.718 (0.609, 0.844)**	**6.90E-05**	**4.14E-04**	0.059 (0.002, 0.117)	0.044	0.105	0.003 (−0.010, 0.016)	0.677	0.887
PL226_224	**0.804 (0.673, 0.959)**	**0.015**	**0.046**	**0.684 (0.583, 0.801)**	**2.70E-06**	**3.24E-05**	1.133 (0.972, 1.321)	0.110	0.165	**−0.148 (−0.205, −0.092)**	**2.95E-07**	**3.54E-06**	**0.039 (0.026, 0.052)**	**2.08E-09**	**1.25E-08**
PL226_PE226	0.928 (0.774, 1.111)	0.418	0.456	1.116 (0.961, 1.299)	0.152	0.203	**0.822 (0.702, 0.961)**	**0.014**	**0.024**	−0.002 (−0.060, 0.056)	0.951	0.951	−0.005 (−0.018, 0.008)	0.448	0.767
PL205_PE226	**0.887 (0.740, 1.061)**	**0.190**	**0.285**	**1.191 (1.025, 1.386)**	**0.023**	0.056	**0.692 (0.586, 0.815)**	**1.17E-05**	**1.41E-04**	0.042 (−0.015, 0.099)	0.150	0.225	−0.001 (−0.014, 0.012)	0.891	0.934

Abbreviations: AD, Alzheimer's disease; ADAS-Cog13, Alzheimer's Disease Assessment Scale, 13-item cognitive subscale; *APOE*, apolipoprotein E genotype; CN, cognitively normal; CSF, cerebrospinal fluid; EMCI, early mild cognitive impairment; LMCI, late mild cognitive impairment; MMSE, Mini-Mental State Exam; PBV, plasmalogen biosynthesis value; PE, phosphatidylethanolamines; PL, plasmalogens; PL-PX, plasmalogen remodeling.

TABLE 4 Serum plasmalogen/phosphatide indices in University of Pennsylvania cohort: Associations with diagnosis and cognition

	AD versus CN		MMSE	
Index/Ratio	OR (95% CI)	*P*-value	β (95% CI)	*P*-value
PBV	0.695 (0.039, 1.399)	0.0433	0.948 (−0.808, 2.705)	0.286
PL-PX	0.293 (−0.236, 0.846)	0.285	0.190 (−1.276, 1.655)	0.797
PL/PE	0.630 (0.121, 1.193)	0.02	1.123 (−0.195, 2.442)	0.094

Notes: *P*-values were estimated from logistic (diagnosis) or linear (cognitive function) regression.
Models as described in Methods. All models were adjusted for age, sex, and *APOE* $\varepsilon 4$ status (note: analyses with UPenn cohort did not adjust for *APOE* due to number of subjects not genotyped). ADAS-Cog13 and MMSE models also adjusted for years of education. Above results were not adjusted for concomitant medications.
Abbreviations: ADAS-Cog13, Alzheimer's Disease Assessment Scale, 13-item cognitive subscale; *APOE*, apolipoprotein E genotype; AD, Alzheimer's disease; CI, confidence interval; CN, cognitively normal; MMSE, Mini-Mental State Examination; OR, odds ratio; PBV, plasmalogen biosynthesis value; PE, phosphatidylethanolamines; PL, plasmalogens; PL-PX, plasmalogen remodeling.

index did not show a significant association with AD versus CN.

3.1.2 | LMCI versus CN

The results of cross-sectional logistic regression analysis of phosphoethanolamine lipid ratios and indices for association with baseline diagnoses of LMCI versus CN in ADNI are shown in Table 3 (unadjusted for medications) and Table S4 (adjusted for all but AD-specific medications). In the non-medication adjusted analysis, as in the AD versus CN analysis, both the PL-PX composite index (OR = 0.719, $P = 2.89 \times 10^{-5}$, q = 1.73×10^{-4}) and the PBV composite (OR = 0.745, $P = 1.99 \times 10^{-4}$, q = 7.97×10^{-4}) showed significant associations with the LMCI versus CN comparison (Table 3).

When medications except those specific to AD were included in the model, the associations remained statistically significant for the PL-PX composite (OR = 0.767, $P = 0.0019$) and the PBV composite (OR = 0.802, $P = 0.0096$) with the LMCI versus CN comparison.

3.1.3 | AD versus LMCI

As shown in Table 3, the unadjusted logistic regression analysis in the ADNI cohort for phosphoethanolamine lipid ratios and indices showed a negative association between specific composite indices and AD versus LMCI, indicating that lower values of these ratios and indices were associated with a higher likelihood of AD versus LMCI. In this analysis, the PL/PE (OR = 0.757, $P = 6.15 \times 10^{-4}$) showed a negative association with AD versus LMCI. PL-PX and PBV did not show significant associations with AD versus LMCI.

After adjusting for all non-AD-specific medications in ADNI, as shown in Table S4, the logistic regression analyses on these ratios and indices showed that the association of PL/PE with the likelihood of AD versus LMCI remained significant (OR = 0.73, $P = 1.85 \times 10^{-4}$).

3.2 | Cognitive function

The results of cross-sectional linear regression analysis of baseline ADAS-Cog13 score in the ADNI cohort with phospholipid ratios and indices are shown in Table 3 (unadjusted analyses) and Table S4 (analyses adjusted for all non-AD-specific medications). In the medication-unadjusted analysis (Table 3), statistically significant relationships were seen between baseline ADAS-Cog13 score and PL-PX ($\beta = -0.1349$, $P = 3.24 \times 10^{-6}$) and overall PBV ($\beta = -0.1302$, $P = 6.92 \times 10^{-5}$). Because higher values of ADAS-Cog13 indicate greater degrees of cognitive impairment, the negative sign of the coefficient indicates that lower values of these indices are associated with greater cognitive impairment. The other index, PL/PE, did not show a significant relationship with ADAS-Cog13. When adjusted for all non-AD-specific medications, the above associations remained statistically significant. Thus, the composite indices PL-PX ($\beta = -0.0996$, $P = 0.0018$) and overall PBV ($\beta = -0.0938$, $P = 0.0031$) remained significant after non-AD-specific medications adjustment. The association of the composite measure PL/PE with ADAS-Cog13 remained non-significant.

In ADNI, baseline MMSE showed a significant association with the same two indices as for ADAS-Cog13. Thus, the PL-PX composite ($\beta = 0.0397$, $P = 1.28 \times 10^{-9}$) and the PBV composite ($\beta = 0.0379$, $P = 6.50 \times 10^{-9}$) were significantly and positively associated with MMSE. Because lower values of MMSE indicate greater degrees of cognitive impairment, the positive values of these coefficients indicate that lower values of PL-PX and PBV are associated with lower MMSE scores and thus greater cognitive impairment. After adjustment for all non-AD-specific medications, the above associations remained statistically significant (Table S4). Thus, the PL-PX ($\beta = 0.0343$, $P = 1.71 \times 10^{-6}$) and PBV composite ($\beta = 0.0318$, $P = 8.44 \times 10^{-6}$) showed significant positive associations with baseline MMSE after adjustment for these medications.

In the UPenn ADCC cohort, MMSE showed a trend toward an association with PL/PE ($\beta = 1.123$, $P = 0.0949$; Table 4). There were no other significant findings with MMSE.

3.3 | CSF Aβ_{1-42}, total tau, and total tau/Aβ_{1-42} ratio

In cross-sectional analyses without adjustment for medications in the ADNI, CSF t-tau showed significant negative correlations with the PL-PX ($\beta = -7.193$, $P = 5.55 \times 10^{-6}$) and PBV composite ($\beta = -6.994$, $P = 7.77 \times 10^{-6}$) indices. Similarly, the $\log_{10}$ CSF t-tau/Aβ_{1-42} showed significant negative correlations with these same two indices (PL-PX, $\beta = -0.0422$, $P = 2.73 \times 10^{-6}$; PBV, $\beta = -0.0408$, $P = 4.39 \times 10^{-6}$). CSF Aβ_{1-42} did not show any significant relationships (Table 5).

After adjusting for all non-AD-specific medications in ADNI, the negative correlations of CSF-t-tau with PL-PX ($\beta = -6.654$, $P = 1.13 \times 10^{-4}$) and with PBV ($\beta = -6.332$, $P = 1.89 \times 10^{-4}$), and of the $\log_{10}$ CSF t-tau/Aβ_{1-42} ratio with PL-PX ($\beta = -0.0396$, $P = 5.24 \times 10^{-5}$) and PBV ($\beta = -0.0374$, $P = 1.05 \times 10^{-4}$) remained statistically significant (Table S5 in supporting information).

In the UPenn ADCC cohort, the PL/PE index showed a significant negative relationship with both CSF t-tau ($\beta = -12.231$, $P = 0.0305$) and CSF t-tau/Aβ_{1-42} ($\beta = -0.0973$, $P = 0.0206$), but not with CSF Aβ_{1-42} (Table 6). The PBV and PL-PX indices did not show statistically significant relationships with any CSF measures (Table 6).

3.4 | Association of plasmalogen ratios and indices with variants in the FADS1/FADS2 gene cluster

A gene-based association analysis of the *FADS1/FADS2* gene cluster with plasmalogen ratios and indices reported in this manuscript showed that *FADS2* was associated with the PL226_224 ratio ($P = 0.0348$; Figure S4) in the ADNI-1/-GO/-2 cohort after adjusting for multiple comparisons using permutation. A SNP (rs97384) in *FADS2* was most significantly associated with PL226_224 ($P = 0.0016$; Figure S4 in supporting information—left figure lower left), where subjects with the minor allele (T) of SNP rs97384 had high levels of PL226_224 compared to subjects without the minor allele (central figure at the bottom of Figure S4). An expression quantitative trait locus (eQTL) analysis of SNP rs97384 with *FADS2* gene expression in the temporal cortex from cognitively normal subjects showed that *FADS2* was significantly highly expressed in subjects with the minor allele (T) of SNP rs97384 (lower right figure in Figure S4).

4 | DISCUSSION

Age-related declines in circulating plasmalogens may increase the risk for AD by reducing plasmalogen availability to the central nervous system.[9,10,13,14,29,39,40] Using a focused lipidomics approach we measured four ethanolamine plasmalogens (PlsEtns) and four closely related phosphatidylethanolamines (PtdEtns) and derived indices from a subset of these phosphoethanolamines reflecting PlsEtn and PtdEtn metabolism to explore the association of these indices with baseline diagnosis, cognitive functioning, and CSF biomarkers of AD.

TABLE 5 Serum plasmalogen/phosphatide indices in ADNI-1/GO/2 Cohort: associations with CSF AD biomarkers

	CSF Total tau (Ttau)			CSF Aβ1-42			Log10(CSF Ttau/CSF Aβ1-42)		
Ratio/Index	β (95% CI)	*P* value	Q value	β (95% CI)	*P* value	Q value	β (95% CI)	*P* value	Q value
PBV	**−6.994 (−10.049, −3.94)**	**7.77E-06**	**3.11E-05**	1.970 (−0.868, 4.807)	0.173	0.694	**−0.041 (−0.058, −0.023)**	**4.39E-06**	**1.76E-05**
PL-PX	**−7.193 (−10.285, −4.101)**	**5.55E-06**	**3.11E-05**	2.048 (−0.826, 4.922)	0.162	0.694	**−0.042 (−0.06, −0.025)**	**2.73E-06**	**1.64E-05**
PL/PE	−0.038 (−3.205, 3.13)	0.981	0.981	0.518 (−2.391, 3.428)	0.727	0.988	0.000 (−0.018, 0.018)	0.9664	0.9664
PL205_224	**−5.11 (−8.096, −2.123)**	**8.15E-04**	**2.44E-03**	1.649 (−1.11, 4.407)	0.241	0.724	**−0.029 (−0.046, −0.012)**	**0.0009**	**0.0026**
PL205_226	0.589 (−2.507, 3.685)	0.709	0.945	0.137 (−2.711, 2.984)	0.925	0.988	0.006 (−0.012, 0.023)	0.5157	0.9664
PL226_224	**−7.543 (−10.653, −4.432)**	**2.21E-06**	**2.65E-05**	2.150 (−0.745, 5.045)	0.145	0.694	**−0.045 (−0.062, −0.027)**	**7.38E-07**	**8.86E-06**
PL226_PE226	−0.238 (−3.409, 2.932)	0.883	0.963	0.581 (−2.329, 3.492)	0.695	0.988	−0.002 (−0.02, 0.016)	0.8097	0.9664
PL205_PE226	0.302 (−2.81, 3.414)	0.849	0.963	0.428 (−2.434, 3.289)	0.769	0.988	0.003 (−0.015, 0.021)	0.7322	0.9664

Abbreviations: CI, confidence interval; CSF, cerebrospinal fluid; PBV, plasmalogen biosynthesis value; PE, phosphatidylethanolamines; PL, plasmalogens; PL-PX, plasmalogen remodeling.

TABLE 6 Serum plasmalogen/phosphatide indices in University of Pennsylvania cohort: associations with CSF AD biomarkers

	CSF Total tau (Ttau)		CSF Aβ1-42		log10(CSF Ttau/Aβ1-42)	
Ratio/Index	β (95% CI)	*P* value	β (95% CI)	*P* value	β (95% CI)	*P* value
PBV	−6.444 (−19.997, 7.110)	0.348	−4.118 (−27.553, 19.318)	0.728	−0.011 (−0.103, 0.080)	0.806
PL-PX	0.836 (−10.256, 11.927)	0.882	−9.159 (−28.192, 9.873)	0.342	0.033 (−0.041, 0.107)	0.383
PL/PE	**−12.040 (−22.932, −1.148)**	**0.031**	6.949 (−12.185, 26.083)	0.473	−0.068 (−0.141, 0.005)	0.068

Notes: *P*-values were estimated from linear regression models and adjusted for age and sex. Results from 3A were further adjusted for *APOE* ε4 carrier status. Due to small number of subjects with genotyping in UPenn cohort, results presented are not adjusted for *APOE* e4. Above results were not adjusted for concomitant medications. Three indices were calculated to assess the following: a) **PL-PX**: index of biosynthesis and/or remodeling of ethanolamine plasmalogens using mean of PL226/PL224, PL205/PL224, and PL205/PL226; b) **PL/PE**: index of ratio of omega-3 fatty acid-containing plasmalogens to the DHA-containing phosphatide PtdEtn 16:0/22:6 using mean of PL226/PE226 and PL205/PE226; and c) **PBV**: overall composite index of plasmalogen biosynthesis and/or remodeling using a mean of all five ratios contributing to index (a) and index (b).
Abbreviations: A$\beta_{1\text{-}42}$, amyloid beta peptide, residues 1-42; CSF, cerebrospinal fluid; Ttau, total-tau.

In ADNI, without correcting for concomitant medications, we observed statistically significant associations between AD diagnosis at the baseline visit and several serum indices of ethanolamine plasmalogen biosynthesis, peroxisomal beta-oxidation, and phosphoethanolamine remodeling. Most notably, baseline serum PL-PX and overall PBV were strongly associated with baseline AD versus CN and LMCI versus CN, while baseline serum PL/PE did not show such an association (Table 3). The associations of the PL-PX and PBV indices with AD versus LMCI were not statistically significant; on the other hand, the PL/PE index showed statistically significant relationships with AD versus LMCI diagnosis (Table 3).

In the UPenn ADCC, we observed an association of AD versus CN with PBV, replicating the finding in ADNI, and also observed an association with the PL/PE index. The significant association between AD versus CN and PBV in both cohorts is consistent with findings from the Religious Orders Study[9,10,13,14,29,39,40] Memory and Aging Project cohort,[10] and provides further support for the utility of this measure as an index of plasmalogen biosynthesis and remodeling that is applicable across cohorts, while individual analyte values may show more variability among various subject populations.

In ADNI, using baseline ADAS-Cog13 as a measure of global cognition, we also observed statistically significant negative associations with the same two indices, PL-PX and overall PBV, as seen in comparisons of AD versus CN and LMCI versus CN, indicating an association of reduced values of these plasmalogen metabolic indices with greater cognitive impairment. However, we saw no evidence of an association between the baseline PL/PE index and baseline ADAS-Cog13 (Table 3).

In the UPenn ADCC, we observed trends toward associations of impaired cognition as measured by MMSE with PBV and PL/PE (Table 4), although these did not reach statistical significance. The lack of statistically significant effects in the UPenn cohort may be attributable to its limited sample size (n = 112 versus 1547 for ADNI).

In ADNI, we observed statistically significant negative associations between CSF t-tau and of the CSF t-tau/A$\beta_{1\text{-}42}$ ratio with PL-PX and PBV, ie, the same indices associated with the AD versus CN and LMCI versus CN and with the cognitive scores (Table 5). On the other hand, we did not see an association of CSF A$\beta_{1\text{-}42}$ alone with any of these indices. The negative association of CSF t-tau and its ratio with A$\beta_{1\text{-}42}$ with these indices suggest that altered plasmalogen biosynthesis and/or remodeling may associate more closely with the development of tau than of amyloid pathology. Longitudinal studies including concomitant measurements of plasmalogen indices, biomarkers of amyloid and tau pathology, and cognitive function are needed to address this hypothesis.

After adjustment for concomitant medication classes (other than anticholinesterases and memantine) in ADNI, the association of the plasmalogen indices with the AD versus CN was no longer significant; however, those with LMCI versus CN and AD versus LMCI remained statistically significant (Table S4). In addition, the linear regression models showed persistence of statistical significance of the associations of plasmalogen indices with cognition (Table S4). Likewise, the associations of CSF tau and the CSF t- tau/A$\beta_{1\text{-}42}$ ratio with PL-PX and PBV remained significant (Table S5).

These results are in broad agreement with previous studies of blood-based levels of ethanolamine plasmalogens and related lipids in AD,[9,10,13,29-31] and suggest that reduced indices of plasmalogen biosynthesis and/or remodeling of very long chain-PUFA-containing plasmalogens is associated with an increased risk of AD (see Figure 1 for potential sites in this pathway that may be affected in AD). In addition, significant associations of PL-PX and PBV with LMCI versus CN indicates reduced plasmalogen indices occur at the MCI stage, and thus may be associated with cognitive decline prior to the development of dementia. Finally, the association of the PL/PE index, which is calculated from ratios of omega-3 fatty-acid-bearing plasmalogens to corresponding phosphatides, with AD versus LMCI diagnosis may indicate that the development of dementia is characterized in part by a specific compensatory failure of peroxisomal mechanisms required to sustain plasmalogen biosynthesis in relation to that of corresponding phosphatidylethanolamines which do not require peroxisomes for biosynthesis. Increased remodeling by phospholipases,[44] and degradation through lysoplasmalogenases[45] and oxidative pathways,[46] might contribute to relative reductions in plasmalogen levels (Figure 1). Fatty acids made available by plasmalogen degradation might be directed toward PE synthesis.[38] These three phenomena, namely decreased biosynthesis, decreased remodeling/degradation, and a putative increase in PE synthesis could contribute, individually or collectively, to reduction of the PL/PE ratio. Because our observations were conducted at a single time point, we are

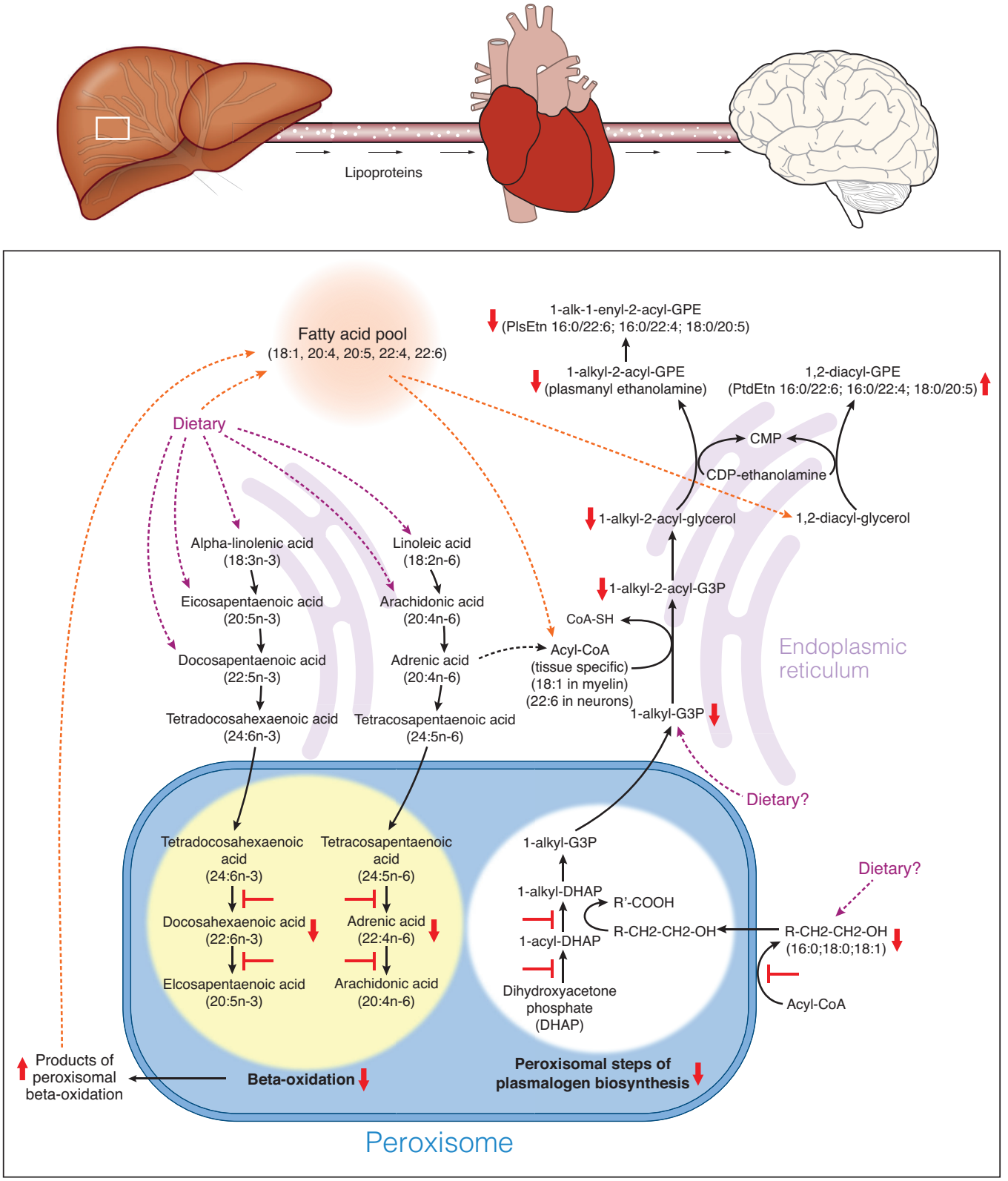

FIGURE 1 Primary lipid pathways for ethanolamine plasmalogen and phosphatidylethanolamine biosynthesis and remodeling. The initial steps in plasmalogen biosynthesis, and the beta-oxidation of very long chain polyunsaturated fatty acids, take place within peroxisomes; later reactions occur in the endoplasmic reticulum. Fundamental lipid species potentially available through dietary sources are metabolized in the endoplasmic reticulum and are incorporated into plasmalogens in the peroxisome. The direction of alteration in constituent lipid species and processes in Alzheimer's disease (AD) based on the data reported here are indicated by red arrows. Red hammerhead symbols indicate putative enzymatic steps proposed to be inhibited in AD

unable to distinguish among these possibilities. Studies examining the kinetics of plasmalogen biosynthesis and metabolism in AD and related conditions are needed to better understand the mechanisms underlying these findings. Attendant changes in signaling pathways, cholesterol metabolism, and neurons might also contribute to pathogenesis of AD.

Because genetic variance in the *FADS1/FADS2* gene cluster can affect the blood levels of EPA/DHA containing lipids (cf. O'Neill and Minihane[43]), we conducted a candidate gene association analysis for relationships between plasmalogen ratios and indices with genetic variants in this gene cluster in the ADNI cohort. We found a significant gene-based association between *FADS2* and the PL226_224 ratio ($P = 0.0348$; Figure S4) in the ADNI-1/-GO/-2 cohort after adjusting for multiple comparisons using permutation. Five SNPs in this region were significantly and independently associated with the PL226_224 ratio, most notably rs97384. Individuals homozygous for the minor allele (T) had higher values of this ratio than those without the minor allele ($P = 0.0016$; see middle figure in Figure S4); conversely, an eQTL analysis showed that individuals homozygous for the major allele (C) had lower levels of *FADS2* gene expression in the temporal cortex of cognitively normal subjects than those with at least one minor allele ($P = 1.8 \times 10^{-5}$, lower right figure in Figure S4). These findings suggest that *FADS2* genotype may influence the relative abundance of omega-3 (DHA)- to omega-6 (adrenic acid)-containing plasmalogens both in circulating blood and in the brain. Further studies are needed to assess the implications of this effect for AD, particularly with regard to the balance of pro- and anti-inflammatory fatty acid-containing plasmalogens in brain.

In contrast to most previous studies of plasmalogens and other phospholipids in AD, we focused on examining indices calculated from specific, preselected ratios of plasmalogens, both to each other and to comparable phosphatidylethanolamine species. The finding of significant alterations of these ratios and indices in relation to cognition and diagnoses appears to be informative in this setting. It will be important to determine whether these ratios and indices are similarly informative in other studies of AD and cognitive impairment conducted in other populations and by different laboratories.

5 | CONCLUSIONS

Further studies are needed to elucidate the specific mechanisms accounting for these findings, including measurement of additional lipids representing upstream and downstream pathways and enzyme activities (eg, phospholipases and oxidative stress). In particular, the steady-state measurements conducted here at a single time point do not allow discrimination between decreased biosynthesis versus increased degradation of plasmalogen species in AD. Studies of the kinetics of plasmalogen biosynthesis may help distinguish between these possibilities. Also, longitudinal measurement of these lipids will likely be informative regarding the relative time courses of changes in serum lipids and of clinical, other biochemical, and imaging outcomes in MCI and AD.

These data further suggest the hypothesis that interventions that enhance plasmalogen biosynthesis and availability to the central nervous system may mitigate cognitive decline and/or brain atrophy in those at risk for AD. Future research will also address the potential relationships of plasmalogen ratios and indices to genetic variation, neuroimaging measures, and other characteristics of AD-type dementias.

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ADDITIONAL CONTRIBUTIONS

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CONFLICTS OF INTEREST

Dayan B. Goodenowe is the CEO and President of Prodrome Sciences Inc., and was the former CEO and President of Phenomenome Discoveries Inc. Vijitha Senanayake is an employee of Prodrome Sciences Inc. and was previously an employee of Phenomenome Discoveries, Inc. Rebecca Baillie is employed as Chief of Staff at Rosa & Co. Leslie M. Shaw received research support from NIH/NIA, ADNI (AG024904), and UPenn ADCC Biomarker Core (AG010124), MJFox Foundation for PD research, Roche, Lilly; provides QC oversight for Roche Elecsys CSF AD biomarker immunoassays for ADNI; and is a consultant for Roche, Lilly, and Novartis. Andrew J. Saykin has been supported by multiple grants from the NIA, NCI, and NCAA/DoD as well as collaborative research support from Eli Lilly unrelated to the work reported here. He also received nonfinancial support from Avid Radiopharmaceuticals and Neurovision, and has served as a consultant to Arkley BioTek and Bayer. He received support from Springer-Nature as Editor in Chief of *Brain Imaging and Behavior*, outside the scope of the work submitted here. John Q. Trojanowski may accrue revenue in the future on patents submitted by the University of Pennsylvania wherein he is a co-inventor and he received revenue from the sale of Avid to Eli Lily as a co-inventor on imaging-related patents submitted by the University of Pennsylvania. Rima F. Kaddurah-Daouk is inventor on key patents in the field of metabolomics including applications for Alzheimer's disease. All other authors report no disclosures.

DATA AVAILABILITY STATEMENT

Metabolomics datasets used in the current analyses for the ADNI-1 and ADNI-GO/-2 cohorts are available via the Accelerating Medicines Partnership-Alzheimer's Disease (AMP-AD) Knowledge Portal and can be accessed at http://doi.org/10.7303/syn5592519 (ADNI-1) and http://doi.org/10.7303/syn9705278 (ADNI-GO/-2). The full complement of clinical and demographic data for the ADNI cohorts are hosted on the LONI data sharing platform and can be requested at http://adni.loni.usc.edu/data-samples/access-data/.

The Alzheimer's Disease Metabolomics Consortium (ADMC): A complete listing of ADMC investigators can be found at: https://sites.duke.edu/adnimetab/who-we-are/

The Alzheimer's Disease Neuroimaging Initiative (ADNI): Data used in the preparation of this article were obtained from the ADNI database (http://adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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