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Serum Phosphatidylethanolamine and Lysophosphatidylethanolamine Levels Differentiate Alzheimer's Disease from Controls and Predict Progression from Mild Cognitive Impairment

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Abstract.
Background: There is intense interest in the development of blood-based biomarkers, not only that can differentiate Alzheimer's disease (AD) from controls, but that can also predict conversion from mild cognitive impairment (MCI) to AD. Serum biomarkers carry the potential advantage over imaging or spinal fluid markers both in terms of cost and invasiveness.
Objective: Our objective was to measure the potential for serum lipid markers to differentiate AD from age-matched healthy controls as well as to predict conversion from MCI to AD.
Methods: Using a publicly-available dataset, we examined the relationship between baseline serum levels of 349 known lipids from 16 classes of lipids to differentiate disease state as well as to predict the conversion from MCI to AD.
Results: We observed that several classes of lipids (cholesteroyl ester, phosphatidylethanolamine, lysophosphatidylethanolamine, and acylcarnitine) differentiated AD from normal controls. Among these, only two classes, phosphatidylethanolamine (PE) and lysophosphatidylethanolamine (lyso-PE), predicted time to conversion from MCI to AD. Low levels of PE and high levels of lyso-PE result in two-fold faster median time to progression from MCI to AD, with hazard ratios 0.62 and 1.34, respectively.
Conclusion: These data suggest that serum PE and lyso-PE may be useful biomarkers for predicting MCI to AD conversion. In addition, since PE is converted to lyso-PE by phospholipase A2, an important inflammatory mediator that is dysregulated in AD, these data suggest that the disrupted serum lipid profile here may be related to an abnormal inflammatory response early in the AD pathologic cascade.

Keywords: Alzheimer's disease, biomarker, lipids, lysophosphatidylethanolamine, mild cognitive impairment, phosphatidylethanolamine

[1]Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (http://adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of AD NI 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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INTRODUCTION

Alzheimer's disease (AD) is an aging-related illness that manifests as widespread synaptic loss and cognitive decline. Pathological hallmarks of the illness are the deposition of extracellular beta-amyloid peptide, known as amyloid plaques, and intracellular hyperphosphorylated tau, known as tangles. There are currently no disease-modifying treatments for AD, though many are in development and are likely to be most effective in the earliest stages of the illness. Therefore, it is imperative that early indicators of AD be widely available to predict which at-risk individuals are most likely to decline and therefore benefit from disease-modifying therapy.

The most well-characterized pre-AD clinical state is mild cognitive impairment (MCI). MCI patients have cognitive, but not functional, decline, and bear many of the pathological hallmarks of AD [1–3]. Therefore, it is likely that biological signatures of AD found in MCI patients would enhance prediction of which individuals are likely to develop AD and to predict the rapidity of progression. In addition, identifying individuals with a greater speed of cognitive decline and selecting them for entry into clinical trials may shorten trial duration. Currently, many biomarkers exist that enhance the prediction of the likelihood and rate of MCI to AD conversion [4–6], though most of these involve either expensive (MRI or PET imaging) or invasive (cerebrospinal fluid [CSF]) modalities that limit their widespread use.

Therefore, there has been substantial effort over the years to develop blood-based biomarkers, which carry the advantages of being less expensive and less invasive, and thus more widely available, to both diagnose AD and predict MCI to AD conversion. For example, efforts have established particular species of plasma phosphorylated tau that may be useful for diagnostic and predictive purposes [7, 8]. However, additional recent work has expanded outside of the traditional amyloid-β/tau axis to examine the potential for other biomarkers to predict MCI to AD conversion. For example, serum lipid classes co-segregate with AD diagnostic category [9]. These findings are consistent with a long history of studies showing metabolic changes in the AD brain. For example, the lipid composition of the brain, particularly that of phosphatidylethanolamine (PE) has been found to be markedly altered in AD. PE is a glycerophospholipid and major component of the membrane lipid bilayer (approximately 20% of the lipid content in biological membranes is PE) as well as the inner mitochondrial membrane [10]. PEs play many important roles in the cell, including modulation of membrane fusion, autophagy, and mitochondrial function. Mice engineered to not produce PE are not viable [11], supporting the vital role of PE in development. A number of autopsy studies have found that PE levels are diminished in the AD brain [12–17]. PE levels are also depressed in the MCI brain [18]. Treatment with PE has been shown to reverse amyloid-β related cognitive deficits in a rat model of AD [19]. Recent data has also shown declines in blood-levels of PE in AD subjects [20, 21].

Therefore, in the current study, we examine the ability for serum levels of PE as well as other lipid classes to predict the progression of MCI to AD. We examined measured levels of 349 lipids in 16 classes in a publicly-available database in normal, MCI, and AD subjects. We found that four classes of lipids significantly differentiated AD and control subjects, including PE and lyso-PE, which is a PE metabolite. We then examined the ability of serum levels of those four classes of lipids to predict time to conversion from MCI to AD in MCI subjects that were followed for at least 36 months. We found that only two: PE (low levels) and lyso-PE (high levels), significantly predicted a more rapid rate of conversion. These data suggest that serum PE and lyso-PE levels may be additional markers for the prediction of MCI to AD conversion. In addition, given the role of inflammatory mediators in the conversion of PE to lyso-PE, these data suggest that serum markers of inflammation may be elevated very early in the AD pathologic process.

METHODS

Database

Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (http://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 MCI and early AD. For up-to-date information, see www.adni-info.org. This study was registered under clinicaltrials.gov under Clin-

icalTrials.gov Identifier: NCT00106899. The study was conducted across multiple clinical sites and was approved by the Institutional Review Boards of all of the participating institutions. Informed written consent was obtained from all participants at each site. The following individual ethics boards approved the study: Albany Medical College Institutional Review Board, Boston University Medical Campus Institutional Review Board (BU IRB), Butler Hospital Institutional Review Board, Cleveland Clinic Institutional Review Board, Columbia University Institutional Review Board, Dartmouth-Hitchcock Medical Center Committee for the Protection of Human Subjects, Duke University Health System Institutional Review Board, Emory University Institutional Review Board Georgetown University Institutional Review Board, Human Investigation Committee Yale University School of Medicine, Human Subjects Committee, University of Kansas Medical Center, Indiana University Institutional Review Board, Research Compliance Administration, Institutional Review Board of Baylor College of Medicine, Institutional Review Board of the Mount Sinai School of Medicine, Johns Hopkins University School of Medicine Institutional Review Boards, Lifespan—Rhode Island Hospital Institutional Review Board, Mayo Clinic Institutional Review Board, Nathan Kline Institute Rockland Psychiatric Center Institutional Review Board (NKI RPC IRB), New York University Langone Medical Center School of Medicine, Institutional Review Board Human Research Program, Northwestern University Institutional Review Board Office, Office of the Washington University School of Medicine IRB (OWU MC IRB), Oregon Health and Science University Institutional Review Board, Partners Human Research Committee, Research Ethics Board Jewish General Hospital, Research Ethics Board Sunnybrook Health Sciences Centre, Roper St. Francis Institutional Review Board, Rush University Medical Center Institutional Review Board, Stanford University, Administrative Panel on Human Subjects in Medical Research, The Ohio State University Institutional Review Board, The University of Texas Southwestern Medical Center Institutional Review Board, UCLA Office of the Human Research Protection Program Institutional Review Board, UCSD Human Research Protections Program, University Hospitals Case Medical Center Institutional Review Board, University of Alabama at Birmingham Institutional Review Board, University of British Columbia, Clinical Research Ethics Board (CREB), University of California Davis Office of Research IRB Administration, University of California Irvine Office Of Research Institutional Review Board (IRB), University of California San Francisco Committee on Human Research (CHR), University of Iowa Institutional Review Board, University of Kentucky Office of Research Integrity, University of Michigan Medical School Institutional Review Board (IRBMED), University of Pennsylvania Institutional Review Board, University of Pittsburgh Institutional Review Board, University of Rochester Research Subjects Review Board (RSRB), University of South Florida Division of Research Integrity & Compliance, University of Southern California Health Science Campus Institutional Review Board, University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB), University of Wisconsin Health Sciences Institutional Review Board, Wake Forest University Institutional Review Board, Weill Cornell Medical College Institutional Review Board, Western Institutional Review Board and Western University Health Sciences Research Ethics Board. Data used for the analyses presented here were accessed on June 25, 2020.

Clinical diagnosis

AD was diagnosed using NINCDS/ADRDA criteria for probable AD [22]. MCI patients had a memory complaint, an abnormal score on the Logical Memory II subscale from the Wechsler Memory Scale, an MMSE score between 24–30 and a Clinical Dementia Rating scale score of 0.5. Normal subjects (NL) did not have a memory complaint, had a normal score on the Logical Memory II subscale and had a Clinical Dementia Rating scale score of zero.

Lipid analysis

Details of lipid extraction and measurement as well as quality control measures have been previously described [23] and summarized [24]. In brief, fasting serum samples were obtained from subjects during the baseline visit. Lipids were extracted using organic solvents. Serum extracts were then analyzed using liquid chromatography with mass spectrometry. After quality control measures, data were available from a total of 349 known lipids from 16 classes (see Table 1 for a listing of lipid classes). The lipid subclasses in the ADNI serum lipidomics data set used in this study include acylcarnitine, fatty acid, cholesteryl ester, lysophosphatidylcholine, lysophosphatidylethanolamine, phosphatidylcholine, phosphatidylethanolamine,

Table 1
Listing of lipid classes in the current study

Lipid Classes	Lipid Count
Acylcarnitine	9
Ceramide	19
Cholesterol	1
Cholesteroyl ester	8
Diacylglycerol	13
Fatty acid	29
Glactoylceramide	1
Glucosylceramide	6
Lactosylceramide	1
Lysophosphatidylcholine	22
Lysophosphatidylethanolamine	4
Phosphatidylcholine	82
Phosphatidylethanolamine	25
Phosphatidylinositol	11
Sphingomyelin	34
Triacylglycerol	84

Table 2
Demographic variables

		NL	AD
Number of subjects		226	185
Gender (n)	F	108	90
	M	118	95
Age in years (Mean ± SD)		75.8 (5.0)	75.3 (7.4)
BMI in kg/m^2 (Mean ± SD)*		26.7 (4.3)	25.5 (3.9)
ADAS13 (Mean ± SD)*		9.5 (4.2)	29.0 (7.7)
		Stable MCI	MCI to AD converters
Number of subjects		102	196
Gender (n)	F	32	73
	M	70	123
Age in years (Mean ± SD)		74.7 (7.8)	74.6 (7.2)
BMI in kg/m^2 (Mean ± SD)		26.5 (3.5)	25.8 (4.1)
ADAS13 (Mean ± SD)*		14.8 (5.3)	20.8 (5.8)

*$p < 0.05$.

phosphatidylinositol, plasmalogen phosphatidylcholine, plasmalogen phosphatidylethanolamine, ceramide, glucosylceramide, sphingomyelin, diacylglycerol, and triacylglycerol.

Statistical methods

The effect of each of the 16 known lipid classes for differentiating AD and age-matched healthy control subjects was assessed via "lipid set analysis" (LSA). This LSA analysis of the lipid classes was based on the maxmean statistic of the gene-set-analysis algorithm [25], which was applied on the residuals from the analysis of covariance (ANCOVA) model on the 349 individual lipid species to adjust for the effects of age and gender. Individual subject-level standardized composite scores were determined for each lipid class from this algorithm. These scores were then used to assess the significance of each of the lipid classes for differentiating AD and control subjects. The results were summarized in terms of the area under the receiver operating characteristic curve (ROC AUC), covariates-adjusted significance (p-value), and false discovery rate (q-value). Lipid classes with q-value<0.05 were considered as statistically significant.

To focus the analysis on lipid classes that track with AD pathology, the lipid classes that were significantly differentiated between AD and control subjects from the above analyses (false discovery rate $q < 0.05$) were then analyzed in subjects with MCI to assess their ability to predict future conversion to AD. This was first carried out within the framework of the LSA algorithm described above on the stable MCI subjects and MCI to AD converters using the residuals from the ANCOVA model to adjust for age and gender effect. Individual subject-level standardized composite scores were then derived for each lipid class from this algorithm. An optimal cutoff on these standardized scores of each lipid class was then derived via the BATTing algorithm [26] to optimize their association with the time to conversion of MCI subjects to AD. Results of the association of these lipid classes with time for MCI to AD progression were summarized in terms of hazard ratio (HR) and median time to progression (MTP) from MCI to AD, with the covariates adjusted significance (p-value) derived from log-rank test.

RESULTS

Demographics

Data were obtained from 185 subjects with AD, 102 subjects with stable MCI, 196 subjects with MCI that converted to AD, and 226 NL subjects. AD and NL subjects were similar in age and differed in body mass index and ADAD13 scores. AD subjects had slightly lower body mass index (NL = 26.7 ± 4.3 [SD], AD = 25.5 ± 3.9 [SD], $p < 0.05$), as has been previously described [27, 28], and as expected, had a higher ADAS13 score (NL = 9.5 ± 4.2 [SD], AD = 29.0 ± 7.7 [SD], $p < 0.05$). Among the MCI subjects, when comparing those with stable MCI versus those that converted, there were no significant differences in age, sex or BMI. Those that converted to AD had a slightly higher baseline ADAS13 score (stable MCI = 14.8 ± 5.3 [SD], MCI-AD converters = 20.8 ± 5.8 [SD], $p < 0.05$).

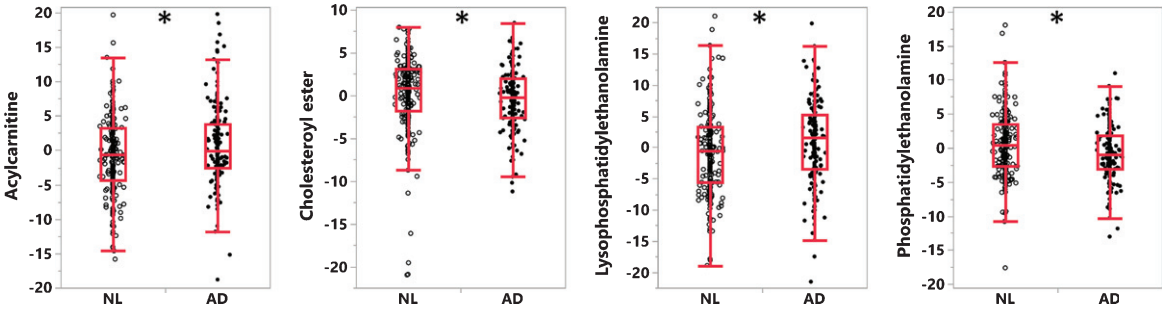

Fig. 1. Box plots showing the median, first and third quartiles of the distributions of the four classes of lipids that significantly differentiated normal controls (NL) and AD subjects. Shown are standardized values (centered by mean and divided by standard deviation), after adjusting for age and gender as covariates. *q-value < 0.05 (Benjamini-Hochberg false discovery rate).

Lipids and lipid classes that differentiate AD from NL

Serum levels of 349 lipids across 16 classes were compared between AD and NL subjects. Because of the varying numbers of different lipids in each class, and to gain intuition about potential mechanism, lipids were grouped into chemical classes. Area under the receiver operating characteristic curve (ROC-AUC), p- and q-values were computed for each of 13 classes (3 of the 16 classes had only one entry) after adjusting for age and sex. Four classes of lipids were found to significantly differ between AD and NL subjects: cholesteroyl ester, PE, lyso-PE and acylcarnitine with cholesteroyl ester and PE showing decreases and lyso-PE and acylcarnitine showing increases (Fig. 1).

Lipid classes that predict MCI to AD conversion

To focus our analyses on those markers that track with AD pathology, we examined the capacity of serum levels of the four lipid classes above that differentiate AD and NL subjects (cholesteroyl ester, PE, lyso-PE, and acylcarnitine) to predict time to conversion from MCI to AD. Only two of these classes, PE and lyso-PE were significantly associated with time for MCI to AD conversion. The levels of these lipid classes showed opposite directions of modulation with low levels of PE (HR 0.62, $p = 0.0034$) and high levels of lyso-PE (HR 1.34, $p = 0.042$; Fig. 2 and Table 4), resulting in greater speed of decline, though both groups had similar asymptotic likelihood of conversion. In both cases, the median time for MCI subjects to convert to AD was two-fold faster,

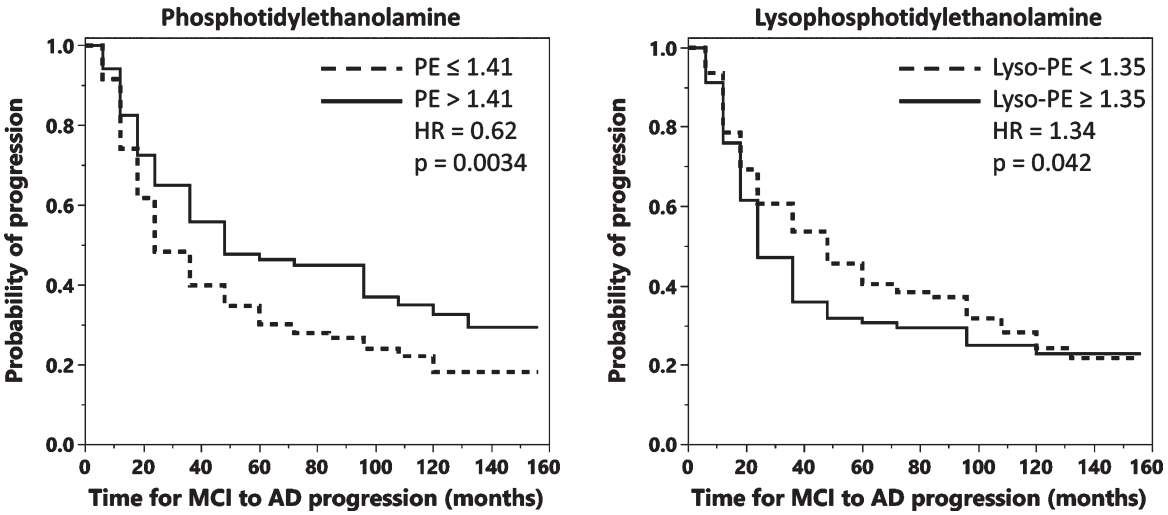

Fig. 2. Kaplan-Meier curves for the two lipid classes (PE and lyso-PE) whose baseline serum levels both differentiated NL and AD subjects and significantly predicted MCI to AD conversion.

Table 3
Table of lipid classes and their performance in distinguishing NL from AD subjects. ROC AUC, receiver-operator characteristic area under the curve

Lipid Classes (known)	Median (NL)	Median (AD)	ROC AUC	*p* (unadjusted)	q (FDR)
Cholesteroyl.ester	0.93	–0.24	0.59	0.0023	0.0247
Phosphatidylethanolamine	0.39	–0.96	0.57	0.0054	0.0247
Lysophosphatidylethanolamine	–0.59	1.67	0.58	0.0057	0.0247
Acylcarnitine	–0.66	–0.13	0.56	0.0117	0.0380
Phosphatidylinositol	–0.6	0.4	0.57	0.0282	0.0663
Phosphatidylcholine	0.3	–0.5	0.55	0.0357	0.0663
Triacylglycerol	–0.21	–1.18	0.55	0.0357	0.0663
Fatty.acid	0.58	–0.08	0.55	0.0747	0.1214
Ceramide	–0.57	0.98	0.55	0.1235	0.1508
Lysophosphatidylcholine	0.12	–0.17	0.53	0.1246	0.1508
Sphingomyelin	0	0.1	0.47	0.1276	0.1508
Diacylglycerol	0.11	–0.56	0.54	0.1655	0.1793
Glucosylceramide	–0.73	0.62	0.54	0.2653	0.2653

Table 4
Table of lipid classes that separate NL from AD with their optimal cutoff and their performance in predicting conversion from MCI to AD

Lipid Class	Optimal Cutoff	Hazard Ratio	*p*
Phosphatidylethanolamine	1.41	0.62	0.0034
Lysophosphatidy lethanolamine	1.35	1.34	0.042
Cholesteroyl.ester	–4.07	1.57	0.063
Acylcarnitine	–3.55	1.63	0.0778

from 48 months to 24 months if the patient had a positive biomarker (serum PE $\leq$ 1.41 or serum lyso-PE $\geq$ 1.35).

DISCUSSION

In the current study, 349 serum biomarkers across 16 lipid classes were measured in a total of 709 subjects with no cognitive deficits, MCI, or AD. In exploratory analyses, several classes of lipids differentiated AD from NL subjects. Among these were PE, which showed a significant decrease in AD subjects and its hydrolytic metabolite, lyso-PE, which was significantly increased, suggesting an increase in PE turnover in AD subjects. These lipids were then used to predict conversion from MCI to AD, and low serum PE, as well as high serum levels of lyso-PE, significantly enhanced the prediction of the rate of MCI to AD conversion. These data suggest that serum PE and lyso-PE levels may be useful adjuncts to more traditional AD biomarkers for the prediction of MCI to AD conversion.

Limitations in the study

The utility of PE or lyso-PE as biomarkers remain unknown for several reasons. The data in this study were obtained from an observational study and from a single time point. It is not yet known if these lipids will track the disease over time or whether incorporating PE or lyso-PE into clinical trial design can be used to enrich MCI subject pools for those with more rapid decline. Therefore, future studies would benefit from serial sampling of PE and lyso-PE levels. It is also not known if blood levels of PE and lyso-PE reflect general neuronal dysfunction and would also be elevated in other neurodegenerative diseases. However a recent study did not find increases in PE levels in patients with frontotemporal dementia compared to controls [29]. The magnitudes of the effects were relatively small, such that all ROC AUCs were less than 0.6, as evident from the extensive overlap in lipid levels between NL and AD subjects, as shown in Fig. 1. However, because the metabolic pathways producing PE and lyso-PE are likely independent from those involving phosphorylation of tau, it is likely that combining PE and lyso-PE with other serum markers, such as p-tau 181 or 217 may provide more powerful biomarkers to differentiate disease state and predict MCI to AD conversion [7, 8].

Phosphatidylethanolamine, phospholipase A2, and Alzheimer's disease

Lipids make up a substantial percentage (up to 40%) of grey matter dry weight [30]. Given the degeneration seen in brain gray matter in AD, it is not surprising that the lipid content of the AD and MCI

brain is altered compared to normal subjects. For example, it has consistently been found that PE levels are lower in the AD brain and several studies also showed an increase in PE metabolites [12–17, 31], suggesting that AD is associated with an increase in PE turnover. Our data similarly point to an increase in PE turnover, with a decrease in serum PE and increase in lyso-PE. Phospholipase A2 (PLA2) converts PE to lyso-PE. This enzyme has been found to be dysregulated in AD and its activity may be tied to AD pathology. For example, PLA2 levels have been found to be elevated [32] or decreased [33] in the AD brain and PLA2 may be activated by amyloid beta peptide [34], and products of PLA2, such as arachidonic acid, may exacerbate AD pathology [35]. In addition, reduction in PLA2 may mitigate the effects of AD pathology in a mouse model [36]. It is not yet known if the latter finding is related to changes in PE levels. In addition, given the increasing evidence that inflammation plays a key role in the pathogenesis of AD [37–39], it is possible that the changes in PE metabolism in the study may, in part, be due to changes in the overall inflammatory state in AD patients and in MCI patients that will convert to AD.

CONCLUSION

In the current study, we confirm previous findings that brain and serum PE levels are depressed in AD patients and report the new finding that low serum PE and high serum lyso-PE levels predict time to conversion from MCI to AD. These findings indicate that the decrease in PE levels may be due to an increase in PE turnover, given the increase in levels of its metabolite, lyso-PE, in the serum. We speculate that increased levels of PLA2, which has previously been implicated in the inflammatory cascade that potentiates AD pathology, may be responsible for these changes in PE metabolism. Thus, we propose that not only do PE and lyso-PE serve as potential new biomarkers for the prediction of conversion from MCI to AD, but also may provide novel insights about the etiopathogenesis of AD.

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Authors' disclosures available online (https://www.j-alz.com/manuscript-disclosures/20-1420r1).

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