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Danni Li, *University of Minnesota*
Jeffrey R. Misialek, *University of Minnesota*
Eric Boerwinkle, *University of Texas*
Rebecca F. Gottesman, *Johns Hopkins University*
A. Richey Sharrett, *Johns Hopkins University*
Thomas H. Mosley, *University of Mississippi*
Josef Coresh, *Johns Hopkins University*
Lisa M. Wruck, *University of North Carolina*
David S. Knopman, *Mayo Clinic*
Alvaro Alonso, *Emory University*

Journal Title: Alzheimer's and Dementia
Volume: Volume 6
Publisher: Elsevier | 2017-01-01, Pages 1-10
Type of Work: Article | Final Publisher PDF
Publisher DOI: 10.1016/j.dadm.2016.09.003
Permanent URL: https://pid.emory.edu/ark:/25593/rwh3h

Final published version: http://dx.doi.org/10.1016/j.dadm.2016.09.003

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Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring 6 (2017) 1-10

Blood-Based Biomarkers

Prospective associations of plasma phospholipids and mild cognitive impairment/dementia among African Americans in the ARIC Neurocognitive Study

Danni Li[a,*], Jeffrey R. Misialek[b], Eric Boerwinkle[c], Rebecca F. Gottesman[d], A. Richey Sharrett[e], Thomas H. Mosley[f], Josef Coresh[e], Lisa M. Wruck[g], David S. Knopman[h], Alvaro Alonso[i]

[a]*Department of Lab Medicine and Pathology, School of Public Health, University of Minnesota, Minneapolis, MN, USA*
[b]*Division of Epidemiology and Community Health, School of Public Health, University of Minnesota, Minneapolis, MN, USA*
[c]*School of Public Health, University of Texas Health Science Center, Houston, TX, USA*
[d]*Department of Neurology, Johns Hopkins University, Baltimore, MD, USA*
[e]*School of Public Health, Johns Hopkins University, Baltimore, MD, USA*
[f]*Department of Medicine, University of Mississippi Medical Center, Jackson, MS, USA*
[g]*Collaborative Studies Coordinating Center, University of North Carolina, Chapel Hill, NC, USA*
[h]*Department of Neurology, Mayo Clinic, Rochester, MN, USA*
[i]*Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA, USA*

Abstract

Introduction: The objective of this study was to investigate whether 10 phospholipids/metabolites previously identified as prospectively predictive of mild cognitive impairment (MCI) or dementia in whites would also be predictive in a mostly African-American cohort.

Methods: We repeatedly measured 188 phospholipids/metabolites in plasma samples of 221 participants of the Atherosclerosis Risk in Communities study, 97% African American, who were followed between 2004–2006 and 2011–2013.

Results: After a mean follow-up of 7.3 years, 77 were classified as having MCI and 18 as having dementia. Our study failed to replicate previous findings in this mostly African American cohort, in that the 10 phospholipids/metabolites only achieved a C statistic/AUC of 0.609 in predicting development of MCI or dementia (compared to 0.96) and 0.607 in distinguishing normal from MCI or dementia at the follow-up visit.

Conclusion: A panel of 10 phospholipids/metabolites previously associated with incident dementia was not predictive of MCI or dementia in an independent cohort.

Keywords: Phospholipids; Plasma; Mild cognitive impairment; MCI; Dementia; Metabolomics; Metabolites; ARIC-NCS; ARIC; Alzheimer's disease; AD

1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia in the elderly. In individuals transitioning from normal cognition to mild cognitive impairment (MCI) or dementia, the brain undergoes metabolic changes during the pathophysiological progression of AD and/or other neurodegenerative diseases. Many of these metabolic changes in the brain and/or cerebrospinal fluid, a surrogate of brain environment, have been detected in plasma [1–3]. Therefore, metabolomic characterization of plasma in individuals transitioning from normal cognition to MCI or dementia is important for two general reasons: (a) to

*Corresponding author. Tel.: 612-626-0299; Fax: 612-625-1121.
E-mail address: dannili@umn.edu

http://dx.doi.org/10.1016/j.dadm.2016.09.003

define altered metabolomic pathways and networks that are affected by AD and other neurodegenerative diseases that contribute to cognitive impairment, which may provide novel therapeutic targets for development of disease-modifying drugs and (b) to identify biomarkers to aid in the prediction of subsequent cognitive impairment, especially during the early stages when therapeutic interventions are more likely to be effective [3–15].

The significance of early detection has spurred a growing interest in the discovery of biomarkers for prediction of future development of MCI and dementia. Graham *et al.* [10] reported that polyamine and L-arginine metabolism predicted development of AD dementia up to 2 years later in individuals with MCI. Orešič *et al.* demonstrated that a panel of 3 metabolites, phosphatidylcholine (PC) (16:0/16:0), an unidentified carboxylic acid, and 2, 4-dihydroxybutanoic acid, was predictive of progression to AD dementia up to 3.6 years later in individuals with MCI [5]. Mapstone *et al.* identified a panel of 10 phospholipids that predicted development of amnestic MCI (aMCI) or AD dementia over 2 or 3 years [12]. Mielke *et al.* reported that high levels of sphingomyelins and ceramides predicted development of cognitive impairment in individuals with normal cognitive function up to 9 years later [13]. These plasma metabolomic studies, conducted using diverse methods to characterize plasma metabolites, all support the notion that identifiable metabolomic changes in plasma precede the development of clinical cognitive symptoms such as MCI or dementia. Although there are certain advantages of using various metabolomic methods, namely, comprehensive coverage of metabolites and discovery of novel biomarkers, the homebrew nature of many of these methods has made it challenging for other investigators to reproduce the findings in independent cohorts.

Recently, Casanova *et al.* failed to replicate Mapstone's findings [12] discovered using a commercially available kit (Biocrates Absolute-IDQ P180 [Biocrates, Life Science AG, Innsbruck, Austria]) [16]. Neither study considered race as biological variable. Health disparities between African Americans and white Americans in rates of MCI and dementia, in the patterns of cognitive function decline, and in prevalence of certain risk factors for dementia such as hypertension have been previously described [17]. Therefore, the objective of our study was to investigate whether the biomarkers that were prospectively predictive of MCI and dementia in whites in the previously published Mapstone study would also be predictive in African Americans.

2. Methods

2.1. Study design

The ARIC study is a prospective cohort study investigating the etiology of atherosclerotic diseases in a middle-aged, predominantly biracial population. In 1987–89, 15,792 men and women aged 45–64 years were recruited from four communities in the United States. The entire cohort was invited for follow-up examinations in 1990–92 (visit 2), 1993–95 (visit 3), 1996–98 (visit 4), and 2011–2013 (visit 5). A detailed study design description was published previously [18]. In 2004–2006, 1130 ARIC participants were recruited for the ARIC Brain Magnetic Resonance Imaging (MRI) study, an ancillary study to the ARIC cohort [19,20]. In 2011–2013, participants underwent a detailed cognitive assessment in the context of the ARIC-Neurocognitive Study (NCS; taken place in conjunction with ARIC visit 5) with the general aim of evaluating their cognitive performance and the prevalence of MCI and dementia. For the present study, we selected 221 ARIC participants who participated in the ARIC Brain MRI visit and ARIC-NCS provided blood samples during the Brain MRI examination, had normal cognition at the time of the ARIC Brain MRI visit (see the definition below), were not taking anticonvulsant and antipsychotic medications, which can affect the mass spectroscopy analyses, and did not have severe depressive symptoms (Center for Epidemiological Studies-Depression [CES-D] scale ≤ 9). A large majority of the ARIC Brain MRI participants with stored samples were African Americans. For the ARIC Brain MRI visit, participants underwent a comprehensive neuropsychological battery. Among other tests, participants completed the mini-mental state examination (MMSE), the delayed word recall (DWR) test, digit symbol Substitution (DSS), and word fluency (WF) tests [19]. Scores for the last three tests were standardized to the distribution of tests obtained at the ARIC visit 2. A global cognitive score was calculated as the average of the standardized scores and standardized to a mean of 0 and standard deviation of 1, also following the distribution of scores at ARIC visit 2 [21]. We considered participants to have normal cognition at the ARIC Brain MRI visit if their MMSE score was >23 for African Americans and >24 for whites and if their global cognitive z score was above -1.5. We chose these cutoff points as they have been used in other settings to define cognitive impairment [22,23]. The ARIC Study, the ARIC Brain MRI Study, and ARIC-NCS were approved by the institutional review board of each participating center. Informed consent was obtained from participants at each study visit.

2.2. MCI and dementia

At ARIC visit 5, as part of the ARIC-NCS, participants underwent a detailed neurocognitive battery, which included the MMSE, DWR, DSS, and WF tests, along with a number of other cognitive tests spanning multiple cognitive domains, a neurological examination, and a sample of them also had brain MRI. Using information collected during the ARIC-NCS as well as prior cognitive testing, a committee of experts adjudicated cases of MCI and dementia as previously described [24]. In addition to a syndromic diagnosis (normal, MCI, dementia), the adjudication committee also assigned an etiologic diagnosis when enough information

was available (AD, frontotemporal dementia, vascular dementia, Lewy-body disease, or other mixed etiologies).

2.3. Targeted metabolomic analysis

The targeted metabolomic analysis has been described previously [25]. We used the Biocrates Absolute-IDQ P180 kits (Biocrates, Life Science AG, Innsbruck, Austria), a validated targeted assay that allows for simultaneous detection and quantification of 188 metabolites (40 acylcarnitines, 21 amino acids, 21 biogenic amines, 15 sphingolipids, 90 glycerophospholipids, and 1 hexose) in 10 μL of plasma samples in a high-throughput manner. Please refer to Supplementary Method for the details of the analysis. We used seven p180 kits to analyze all the plasma samples, once for each sample. As part of the quality control, three concentrations of quality controls (1 QC1, 4 QC2, and 1 QC3) were included in each kit to monitor imprecisions (% coefficient variances [CVs]) of measuring these 188 metabolites. We calculated means, standard deviations (SDs), and CVs of 3 levels of QCs for all the 188 metabolites, and imprecisions of >83% of the metabolites were less than 25% for all three concentrations of quality controls (Supplementary Table 1). Initial data analysis was performed using the MetIQ software (Biocrates). The targeted metabolomic profile was measured in blood samples obtained at the ARIC Brain MRI visit in 2004–2006 and repeated in blood samples obtained at the ARIC visit 5/ ARIC-NCS in 2011–2013.

2.4. Other covariates

Education level, sex, and race were self-reported by the participant at the study baseline. Information on smoking and drinking status and medication use was self-reported at all study visits. Body mass index was defined as weight in kilograms divided by the square of height in meters measured with the participant wearing light clothing. A sports index was calculated based on the number of times per month that participants engaged in vigorous, moderate, or light physical activity. Prevalent coronary heart disease, stroke, and heart failure were defined according to published criteria [26,27]. Prevalent diabetes was defined as a self-reported physician diagnosis of diabetes or use of antidiabetic medication. Total cholesterol, HDL cholesterol, and triglycerides were measured in blood samples provided at the ARIC visit 4 (1996–1998).

2.5. Statistical analysis

Metabolite concentrations were log-transformed and modeled in standard deviation units when possible. For metabolites with <50% of missing values or below the limit of detection (LOD), we imputed the lowest nonzero measurement to the missing/below LOD for analysis. Nine metabolites with 50%–80% missing or below LOD values were categorized into three groups: <LOD, ≥LOD to <median, and ≥median. Finally, 35 metabolites that had >80% missing or below LOD or had a failure in model convergence were excluded (Supplementary Table 2). The primary analysis focused on 9 of the 10 phospholipids identified in the previous publication by Mapstone *et al.* as predictors of conversion from normal to cognitive impairment (the 10th metabolite, C16:1-OH, had values below the LOD in all measurements) [12]. We used a multinomial logistic regression to assess the prospective association of the individual metabolites with MCI and dementia (three level dependent variable: normal, MCI, dementia, n = 221). The following variables were used for adjustment in a series of nested models: model 1: age, race, sex, and *APOE* genotype; model 2: model 1 + educational level, diabetes mellitus, body mass index, drinking status, smoking status, sports index, systolic blood pressure, use of antihypertensive medications, prevalent coronary heart disease, prevalent heart failure, prevalent stroke, total cholesterol, HDL cholesterol, and triglycerides. These covariates (except education level, sex, and race assessed at the ARIC visit 1 in 1987–1989 and blood lipids assessed at ARIC visit 4 in 1996–1998) were assessed at the ARIC brain MRI visit, which is the baseline when the plasma samples were taken from the 221 participants. To measure the ability of this panel of nine phospholipids in prediction of MCI and dementia, we calculated the C statistic from binary logistic regression models with MCI/dementia (vs normal) as the outcome with individual phospholipids modeled as a 1-SD difference in the log-transformed

Table 1
Selected characteristics at the ARIC Brain MRI Study, 2004–2006, by cognitive status at the ARIC NCS, 2012–2014

Characteristics	Normal (n = 126)	Mild cognitive impairment (n = 77)	Dementia (n = 18)
Age, y	70.9 (3.6)	71.3 (3.7)	73.1 (5.0)
African American, %	96.8	96.1	100.0
Female, %	66.7	62.3	77.8
Body mass index, kg/m^2	30.0 (4.8)	29.7 (5.4)	29.7 (7.7)
Cholesterol medication, %	35.7	35.1	38.9
Current drinker, %	31.8	29.9	16.7
Current smoker, %	5.6	7.8	11.1
Diabetes, %	31.0	29.9	22.2
High school graduate, %	21.4	32.5	33.3
HDL cholesterol, mg/dL	54.8 (15.9)	55.1 (14.6)	55.5 (18.6)
Hypertension medication, %	64.3	72.7	66.7
Prevalent CHD, %	5.6	6.5	5.6
Prevalent HF, %	5.6	2.6	11.1
Prevalent stroke, %	1.6	2.6	11.1
Systolic blood pressure, mm Hg	130.8 (18.7)	132.3 (19.4)	138.7 (18.9)
Sports index	91.5 (76.7)	78.1 (56.0)	70.1 (52.6)
Total cholesterol, mg/dL	200.8 (35.9)	203.1 (37.9)	203.2 (36.8)
Triglycerides, mg/dL	109.3 (52.5)	105.8 (41.7)	112.2 (41.2)
APOE, %			
44	2.4	5.2	16.7
24 or 34	27.0	33.8	22.2
Other	65.9	59.7	55.6
Missing	4.8	1.3	5.6

Table 2
Odds ratios (OR) and 95% confidence interval (CI) of MCI or dementia by baseline phospholipids (log-transformed per 1-SD change), ARIC Brain MRI Study (2004–2006) and ARIC-NCS (2011–2013)

Phospholipid	N	MCI vs. normal, OR (95% CI)*	Dementia vs. normal, OR (95% CI)*	MCI and dementia vs. normal, OR (95% CI)*
PC aa C36:6				
Model 0	221	1.03 (0.77–1.38)	0.96 (0.61–1.50)	1.01 (0.77–1.36)
Model 1	221	1.04 (0.77–1.40)	0.97 (0.58–1.62)	1.03 (0.78–1.40)
Model 2	221	1.10 (0.79–1.53)	1.36 (0.49–3.77)	1.11 (0.82–1.62)
PC aa C38:0				
Model 0	221	1.04 (0.77–1.41)	0.91 (0.62–1.35)	1.01 (0.76–1.35)
Model 1	221	1.06 (0.77–1.46)	0.86 (0.58–1.28)	1.01 (0.75–1.37)
Model 2	221	1.07 (0.77–1.50)	0.84 (0.51–1.37)	1.02 (0.75–1.44)
PC aa C38:6				
Model 0	221	1.10 (0.78–1.56)	1.09 (0.60–1.98)	1.10 (0.83–1.65)
Model 1	221	1.12 (0.79–1.59)	1.05 (0.56–1.97)	1.11 (0.83–1.68)
Model 2	221	1.16 (0.77–1.74)	1.39 (0.38–5.12)	1.18 (0.85–1.99)
PC aa C40:1				
Model 0	221	0.98 (0.74–1.30)	0.62 (0.33–1.18)	0.92 (0.69–1.20)
Model 1	221	0.97 (0.73–1.30)	0.63 (0.32–1.23)	0.92 (0.69–1.21)
Model 2	221	1.02 (0.74–1.41)	0.56 (0.24–1.29)	0.95 (0.69–1.29)
PC aa C40:2				
Model 0	221	1.11 (0.83–1.49)	0.91 (0.57–1.46)	1.07 (0.82–1.41)
Model 1	221	1.15 (0.85–1.55)	0.91 (0.54–1.52)	1.10 (0.84–1.47)
Model 2	221	1.20 (0.86–1.69)	0.99 (0.50–1.97)	1.16 (0.85–1.62)
PC aa C40:6				
Model 0	221	1.07 (0.79–1.45)	1.15 (0.62–2.15)	1.08 (0.82–1.51)
Model 1	221	1.10 (0.80–1.51)	1.11 (0.57–2.16)	1.10 (0.83–1.57)
Model 2	221	1.16 (0.80–1.70)	1.79 (0.63–5.12)	1.20 (0.87–1.87)
PC ae C40:6				
Model 0	221	1.14 (0.79–1.65)	1.06 (0.62–1.83)	1.12 (0.84–1.69)
Model 1	221	1.18 (0.79–1.77)	1.00 (0.60–1.65)	1.13 (0.84–1.75)
Model 2	221	1.20 (0.76–1.88)	1.05 (0.45–2.42)	1.17 (0.85–1.99)
Lyso PC a C18:2				
Model 0	221	**1.50 (1.01–2.23)**	1.11 (0.64–1.93)	**1.40 (1.00–2.04)**
Model 1	221	**1.49 (1.00–2.23)**	1.11 (0.60–2.06)	**1.40 (1.00–2.07)**
Model 2	221	**1.66 (1.04–2.64)**	1.17 (0.52–2.65)	**1.52 (1.03–2.37)**
Propionyl-L-carnitine (C3)				
Model 0	221	0.97 (0.73–1.29)	1.19 (0.73–1.96)	1.01 (0.77–1.32)
Model 1	221	0.98 (0.73–1.30)	1.24 (0.73–2.11)	1.02 (0.77–1.34)
Model 2	221	0.97 (0.69–1.34)	1.21 (0.62–2.35)	0.98 (0.71–1.35)

NOTE. Model 0: Unadjusted logistic regression; Model 1: Logistic regression adjusted for age, race, sex, & *APOE*. Model 2: Model 1 with additional adjustment for body mass index, cholesterol medications, diabetes mellitus, drinking status, educational level, HDL cholesterol, smoking status, sports index, systolic blood pressure, total cholesterol, triglycerides, use of antihypertensive medications, and prevalent coronary heart disease, heart failure, or stroke at baseline.

The Table 2 results are showing the association of phospholipid measurements at the MRI visit with incident MCI/dementia at visit 5.

Bold indicates statistical significance.

*OR per 1-SD change in the log (phospholipid).

phospholipid unless noted. The C statistic is the proportion of pairs of participants with the outcome and participants without the outcome in which the participant who experienced the outcome had a higher predicted probability of the outcome than the participant without the outcome [28]. This statistic corresponds to the area under the receiver operating characteristic curve (AUC) and is part of the standard output of PROC LOGISTIC in SAS.

Linear regression analysis was performed to estimate the association of concentrations of these nine phospholipids with changes in MMSE, and z scores in DWR, DSS, and WF tests, and a composite z score for global cognition based on DWR, DSS, and WF described previously. Also, logistic regression was performed to determine the association between the difference of log-transformed phospholipids levels (log [ARIC-NCS] − log [ARIC brain MRI]) and MCI/dementia status.

In an exploratory, hypothesis-generating analysis, we repeated the analyses for the rest of 144 metabolites assessed, which excluded the 35 metabolites with >80% values lower than LOD and nine phospholipids from the main analysis. This hypothesis-generating analysis adjusted for the same variables used in model 2 above and was corrected for multiple comparisons using a Bonferroni correction [29]. Therefore, only associations with a P value $< .000347$ (0.05/144 metabolites) were considered statistically significant. We used SAS v 9.3 (SAS Inc., Cary, North Carolina) for the statistical analysis.

Table 3
C-statistic from binary logistic regression models with dementia/MCI (vs normal) at the ARIC-NCS (2012–2014) as the outcome and individual phospholipids (modeled as a 1-SD difference in the log-transformed phospholipid) at the ARIC Brain MRI Study (2004–2006)

	C-statistic	
Phospholipid	Model 0	Model 1
Model (No Phospholipids)		0.698
Model + PC aa C36:6	0.486	0.699
Model + PC aa C38:0	0.486	0.698
Model + PC aa C38:6	0.487	0.699
Model + PC aa C40:1	0.525	0.697
Model + PC aa C40:2	0.504	0.700
Model + PC aa C40:6	0.493	0.701
Model + PC ae C40:6	0.502	0.700
Model + Lyso PC a C18:2	0.566	0.717
Model + Propionyl-L-carnitine (C3)	0.488	0.698
Model + all 9 phospholipids	0.609	0.729

NOTE. Model 0: Unadjusted; Model 1: Adjusted for age, sex, race, *APOE* genotype, body mass index, diabetes mellitus, drinking status, educational level, HDL cholesterol, smoking status, sports index, systolic blood pressure, total cholesterol, triglycerides, use of antihypertensive medications, and prevalent coronary heart disease, heart failure, or stroke at baseline.

3. Results

3.1. Baseline characteristics of individuals with MCI and dementia at the follow-up examination

Of 221 participants (97% African American) cognitively normal at the ARIC Brain MRI examination included in this analysis, 77 were classified as having MCI and 18 as having dementia at the time of the ARIC-NCS examination (mean follow-up, 7.3 years; range, 5.8–9.6 years). Table 1 lists the baseline characteristics of included participants by cognitive status at the follow-up examination. Overall, individuals who developed dementia were older, more likely to be female, less likely to be a drinker, and more likely to be a smoker, had a worse cardiovascular risk profile and a higher prevalence of *APOE ε4/ε4* genotype.

3.2. Prospective associations of baseline plasma phospholipids/metabolites with MCI and/or dementia

Our primary analysis focused on the 10 metabolites previously identified by Mapstone *et al.* Concentrations of C16:1-OH, one of the 10 metabolites, were lower than LOD in all participants. Therefore, we excluded this metabolite from the analysis. Table 2 shows the results from multinomial logistic models estimating the prospective association of the remaining 9 phospholipids with MCI and/or dementia. In the unadjusted model (model 0), higher concentration of lyso PC a C18:2 was significantly associated with higher odds of MCI (OR, 1.50; 95% confidence interval [CI], 1.01–2.23) but not of dementia. After adjusting for covariates in models 1 and 2, the association with MCI remained significant (model 1, OR, 1.49; 95% CI, 1.00–2.23 and model 2, OR, 1.66; 95% CI, 1.04–2.64). There were no significant associations for the other eight phospholipids with MCI/dementia.

Table 3 shows the predictive ability (C statistic, a measure of AUC) of these nine phospholipids in classifying individuals as remaining normal cognition versus developing MCI/dementia. Individual baseline phospholipids had C statistics in the range of 0.486–0.566 in the unadjusted model (model 0). When including all the phospholipids in the unadjusted model, the C statistic slightly improved to 0.609. A model including all the covariates and no phospholipids achieved a C statistic of 0.698. Adding individual phospholipids or all 9 phospholipids simultaneously led to small improvements in the C statistic (a range of 0.697–0.717 for individual phospholipids and a 0.729 when adding simultaneously the nine phospholipids). Furthermore, we also evaluated the predictive ability of these nine phospholipids in distinguishing normal versus MCI/dementia at the ARIC-NCS visit. The C statistic is 0.607 for model 0 (including the nine metabolites) and 0.728 for the final multivariable model (same as model 1 in Table 3). These results were quite similar to the c statistic from our previous study of cross-sectional association of these phospholipids with MCI and dementia (0.602 for model 0 and 0.713 for model 1) [25]. Changes in the nine phospholipids of interest between the brain MRI and ARIC-NCS visits were not prospectively associated with MCI or dementia (Table 4).

We also performed hypothesis-generating analysis of the baseline, prospective association of the remaining 144 metabolites with MCI and dementia (Supplementary Table 3). Higher levels of 17 of these metabolites were associated with dementia at the nominal P value $<.05$; however, none of them were significant after applying a correction for multiple comparisons: arginine, aspartic acid, acetyl-L-carnitine (C2), octadecenoyl-L-carnitine (C18:1), ornithine, PC aa C32:3, PC aa C34:1, PC aa C36:1, PC aa C38:5, PC aa C40:3, PC ae C32:2, PC ae C36:1, SM C18:0, SM C18:1, SM C20:2, SM 26:0, and SM C26:1 were associated with higher incidence of dementia. Higher levels of 9 of them were prospectively associated with MCI (P value $<.05$): lysoPC a C16:0, lysoPC a C16:1, lysoPC a C18:0, lysoPC a C18:1, PC aa C36:3, PC ae C38:1, PC ae C42:2, SM C16:0, and SM C20:2. Higher levels of butenyl-L-carnitine (C4:1) and SM C20:2 were prospectively associated with both MCI and dementia. Among the 28 metabolites that were associated with either MCI or dementia, decreases in concentrations of 5 of them (aspartic acid, acetyl-L-carnitine [C2], butenyl-L-cartinine [C4:1], PC aa C36:1 and PC ae C38:1) between the brain MRI visit and the ARIC-NCS were associated with higher rate of MCI or dementia (P value $< .05$, Supplementary Table 4).

Furthermore, we also analyzed the baseline, prospective association of the nine metabolites with changes in neuropsychological tests including MMSE, DWR, DSS, and WF tests (Table 5). After multivariable adjustment, higher levels of propionyl-L-carnitine (C3) were significantly associated with slower decline in the global cognitive score

Table 4
Odds ratios (OR) and 95% confidence interval (CI) of MCI or dementia associated with 1-SD change in log-transformed phospholipids levels between ARIC-NCS and ARIC Brain MRI examination (log [ARIC-NCS] − log [ARIC brain MRI])

Phospholipid	n	MCI vs. normal, OR (95% CI)*	Dementia vs. normal, OR (95% CI)*	MCI & dementia vs. normal, OR (95% CI)*
PC aa C36:6				
Model 0	221	0.89 (0.65–1.22)	0.82 (0.44–1.55)	0.88 (0.63–1.16)
Model 1	221	0.88 (0.64–1.22)	0.71 (0.35–1.45)	0.85 (0.60–1.14)
Model 2	221	0.86 (0.61–1.23)	0.53 (0.21–1.37)	0.84 (0.57–1.15)
PC aa C38:0				
Model 0	221	0.87 (0.62–1.21)	1.02 (0.65–1.58)	0.90 (0.65–1.19)
Model 1	221	0.86 (0.62–1.20)	1.03 (0.63–1.68)	0.89 (0.64–1.18)
Model 2	221	0.85 (0.60–1.21)	1.08 (0.60–1.94)	0.88 (0.61–1.18)
PC aa C38:6				
Model 0	221	0.74 (0.44–1.25)	0.65 (0.24–1.72)	0.72 (0.42–1.06)
Model 1	221	0.72 (0.42–1.23)	0.52 (0.18–1.51)	0.68 (0.40–1.04)
Model 2	221	0.70 (0.39–1.27)	0.43 (0.12–1.62)	0.66 (0.36–1.04)
PC aa C40:1†				
Model 0	221	1.10 (0.82–1.47)	1.12 (0.67–1.86)	1.10 (0.83–1.45)
Model 1	221	1.07 (0.80–1.45)	1.09 (0.63–1.89)	1.08 (0.81–1.44)
Model 2	221	1.08 (0.78–1.50)	1.09 (0.54–2.19)	1.09 (0.79–1.50)
PC aa C40:2				
Model 0	221	0.96 (0.73–1.28)	0.95 (0.57–1.56)	0.96 (0.73–1.26)
Model 1	221	0.92 (0.69–1.24)	0.87 (0.50–1.52)	0.92 (0.69–1.21)
Model 2	221	0.91 (0.66–1.26)	0.69 (0.33–1.47)	0.89 (0.65–1.21)
PC aa C40:6				
Model 0	221	0.81 (0.56–1.18)	0.65 (0.30–1.38)	0.79 (0.53–1.07)
Model 1	221	0.80 (0.54–1.16)	0.57 (0.25–1.29)	0.76 (0.50–1.05)
Model 2	221	0.77 (0.50–1.17)	0.43 (0.15–1.22)	0.73 (0.46–1.05)
PC ae C40:6				
Model 0	221	0.83 (0.55–1.25)	0.86 (0.42–1.73)	0.83 (0.53–1.13)
Model 1	221	0.80 (0.52–1.24)	0.79 (0.33–1.89)	0.80 (0.50–1.11)
Model 2	221	0.79 (0.50–1.26)	0.69 (0.21–2.26)	0.78 (0.46–1.11)
Lyso PC a C18:2				
Model 0	221	0.82 (0.58–1.15)	1.05 (0.69–1.60)	0.88 (0.63–1.16)
Model 1	221	0.78 (0.55–1.11)	1.09 (0.70–1.70)	0.85 (0.60–1.13)
Model 2	221	0.78 (0.53–1.16)	1.21 (0.73–2.02)	0.86 (0.59–1.17)
Propionyl-L-carnitine (C3)				
Model 0	221	1.12 (0.84–1.50)	0.62 (0.37–1.04)	1.01 (0.77–1.31)
Model 1	221	1.10 (0.82–1.46)	0.60 (0.34–1.05)	0.99 (0.75–1.31)
Model 2	221	1.15 (0.83–1.58)	0.63 (0.32–1.25)	1.07 (0.79–1.45)

NOTE. Model 0: Unadjusted logistic regression; Model 1: Logistic regression adjusted for age, race, sex, and *APOE*; Model 2: Model 1 with additional adjustment for body mass index, cholesterol medications, diabetes mellitus, drinking status, educational level, HDL cholesterol, smoking status, sports index, systolic blood pressure, total cholesterol, triglycerides, use of antihypertensive medications, and prevalent coronary heart disease, heart failure, or stroke at baseline.
*OR per 1-SD change in the log (phospholipid).
†OR for phospholipid as an ordinal variable difference between the MRI visit & visit#5 (below LOD, >LOD−<Median, >Median).

(β = 0.11, 95% CI, 0.01–0.22). For the remaining 144 metabolites, the hypothesis-generating analysis indicated, though none of them were significant after applying a correction for multiple comparisons, that higher levels of three metabolites, alpha-aminoadipic acid (alpha-AAA), DL-carnitine, and valeryl-L-carnitine (C5), were associated with slower decline in the global cognitive score (Supplementary Table 5).

4. Discussion

The objective of this study was to investigate the ability of plasma metabolites to predict individuals who transitioned from normal cognition to cognitive impairment in a mostly African American cohort. We used a commercially available, targeted metabolomic kit (Biocrates AbsoluteIDQ P180 kit) to measure 188 plasma metabolites including amino acids, carnitines, phospholipids, and sphingomyelins. Our analysis was unable to replicate Mapstone's results, which may be due to key differences in study design (Table 6). First, by design, our study focused mostly on African Americans, whereas the Mapstone study only included whites. Second, the Mapstone study and our study were also different in the education and age distribution: the Mapstone study included individuals who were well educated (mean education, >15 years) and older (mean age of normal, baseline converter, and aMCI/AD were 81.6, 80.2, and 82.3, respectively); in contrast, our cohort was less educated (only 26% had a high school degree) and younger (mean age of normal, incident MCI, and

Table 5
Association of phospholipids levels (per 1-SD log-transformed) with the change in cognitive scores from the ARIC Brain MRI visit to the ARIC-NCS, Atherosclerosis Risk in Communities (ARIC) Study, 2004 to 2013

Phospholipid		Delayed word recall test, Z score	Digit symbol substitution test, Z score	Word fluency test, Z-score	Global, Z score	Mini-mental state examination (MMSE)
	n	219	204	218	202	191
PC aa C36:6	β	0.01	−0.002	0.03	0.01	−0.08
	95% CI	−0.17 to 0.19	−0.07 to 0.07	−0.05 to 0.11	−0.09 to 0.11	−0.45 to 0.29
	P value	.92	.96	.44	.78	.68
PC aa C38:0	β	0.05	0.02	0.03	0.03	0.01
	95% CI	−0.14 to 0.23	−0.05 to 0.09	−0.05 to 0.11	−0.07 to 0.13	−0.36 to 0.38
	P value	.60	.58	.47	.54	.96
PC aa C38:6	β	0.04	0.001	0.04	0.03	−0.01
	95% CI	−0.14 to 0.22	−0.07 to 0.07	−0.04 to 0.12	−0.07 to 0.13	−0.37 to 0.35
	P value	.67	.97	.30	.52	.97
PC aa C40:1	β	0.03	0.02	0.03	0.03	0.02
	95% CI	−0.16 to 0.22	−0.06 to 0.09	−0.06 to 0.11	−0.07 to 0.14	−0.39 to 0.43
	P value	.75	.63	.54	.54	.92
PC aa C40:2	β	−0.04	−0.01	−0.03	−0.03	−0.11
	95% CI	−0.23 to 0.15	−0.08 to 0.06	−0.12 to 0.05	−0.14 to 0.07	−0.50 to 0.28
	P value	.69	.75	.46	.53	.56
PC aa C40:6	β	0.01	0.01	0.05	0.03	−0.03
	95% CI	−0.18 to 0.19	−0.06 to 0.08	−0.03 to 0.13	−0.07 to 0.13	−0.40 to 0.34
	P value	.95	.76	.23	.54	.87
PC ae C40:6	β	0.06	0.02	0.03	0.04	−0.07
	95% CI	−0.12 to 0.25	−0.05 to 0.09	−0.05 to 0.11	−0.06 to 0.14	−0.43 to 0.30
	P value	.49	.65	.47	.42	.73
Lyso PC a C18:2	β	0.04	−0.01	−0.003	0.04	−0.24
	95% CI	−0.14 to 0.22	−0.08 to 0.07)	−0.08 to 0.08	−0.06 to 0.14	−0.61 to 0.13
	P value	.67	.88	.95	.47	.20
C3	β	0.13	0.02	0.05	**0.11**	−0.05
	95% CI	−0.06 to 0.32	−0.06 to 0.09	−0.03 to 0.14	**0.01–0.22**	−0.45 to 0.35
	P value	.18	.67	.22	**.03**	.81

NOTE. Model 2: adjusted for age, sex, *APOE*, body mass index, cholesterol medications, diabetes mellitus, drinking status, educational level, HDL cholesterol, smoking status, sports index, systolic blood pressure, total cholesterol, triglycerides, use of antihypertensive medications, and prevalent coronary heart disease, heart failure, or stroke at baseline.

Bold indicates statistical significance.

incident dementia at baseline were 70.9, 71.3, and 73.1, respectively). Third, although both studies were longitudinal in nature, the Mapstone study used a mixed cohort and case-control study design. It included 124 samples in its discovery sample set, 36 of them were longitudinal from the same 18 participants who had converted from cognitive normal condition to cognitive impairment. The rest of 88 samples were cases (35 of them were individuals who were either aMCI or AD) and controls (53 of them were individuals with normal cognitive function). In our study, we had 221 participants, who at the baseline of the Brain MRI visit had normal cognition and developed MCI (n = 77) and dementia (n = 18) at the follow-up of the ARIC-NCS, with plasma metabolites measured at both time points. Fourthly, our study had expert adjudicated MCI and dementia diagnoses at the follow-up, where the Mapstone study–derived composite scores (standardized z scores) for each participant on cognitive tests and categorized the participants into a combined, single aMCI/AD group or a cognitive normal group based on these composite scores. Last but not least, the duration of the follow-up in our study was in the range of 5.8–8.6 years, with a mean of 7.3 years, much longer than the follow-up in the Mapstone study of 1–5 years, with a mean of 2.1 years.

Results from our exploratory analysis showed that higher concentrations of 28 plasma amino acids, carnitines, phospholipids, and sphingomyelins were prospectively associated with clinically significant cognitive symptoms such as MCI or dementia in African Americans. Among the 28 metabolites, decreases in the level of aspartic acid, acetyl-L-carnitine (C2), butenyl-L-carnitine (C4:1), PC aa C36:1, and PC ae C38:1 between the brain MRI visit and the ARIC-NCS were prospective in association with MCI or dementia.

Furthermore, our results showed higher levels of PC and sphingomyelins were predictive of MCI and dementia. Synapses are comprised of PC, and sphingomyelins make up half of the total lipid content of myelins. In addition to the important structural roles, their metabolites function as messengers that modulate signaling for proper neuron functions. Presumptively, increases in neurotransmission, mitochondrial synthesis, and synaptic plasticity are consistent with the emerging evidence that there are abnormal increases in brain activity in individuals that are transitioned from

Table 6
Comparison of participant characteristics, study design, and results between the present study and the Mapstone study [12]

Characteristics	Mapstone (discovery)	Present study
Participants with normal cognition at baseline	53	221
Participants with amnestic MCI (aMCI) or AD at baseline	35	None
Participants with samples collected at both the baseline (pre) and follow-up (post)	18 (Converters)	221
Partcipants who developed aMCI or AD at the follow-up	18 (Converters)	95

Characteristics	Normal (53)	aMCI/AD (35)	Converters (18)	Normal (126)	MCI (77)	Dementia (18)
Age (years), baseline	81.6 (3.6)	82.3 (4.8)	80.2 (3.0)	70.9 (3.6)	71.3 (3.7)	73.1 (5.0)
% Female	66.0%	71.4%	55.6%	66.7%	62.3%	77.8%
% African American	Not reported			96.8%	96.1%	100.0%
Education						
Mean, years	15.68 (2.32)	15.45 (2.19)	15.33 (3.14)	Not reported		
% high school graduate	Not reported			21.40%	32.50%	33.30%
Follow-up interval, mean, years (range)	2.1 (1–5)			7.3 (5.8–8.6)		
Sample for metabolomic analysis	Plasma			Plasma		
Metabolomics method	p180 kits (Biocrates)			p180 kits (Biocrates)		
Performance of the 10-metabolite panel described in the Mapstone study						
Normal versus Converter (pre) AUC	0.96			0.609*		
Normal versus aMCI/AD AUC	0.827			0.607†		

*Including nine metabolites instead of 10 because C16:1-OH levels were lower than LOD in all participants in the present study.
†For the present study, this reflects normal versus MCI/dementia at the follow-up visit.

normal cognition to cognitive impairment, with the inflection point most likely in the presymptomatic and prodromal stages. In particular, Berchtold *et al.* showed that in MCI brains, there were prominent upregulation of genes associated with protein synthesis, mitochondrial energy generation, and synaptic genes for central components of the vesicle function machinery at the synapse, synaptic vesicle trafficking, neurotransmitter receptors, and synaptic structure and stabilization [30]. The precise mechanisms of our findings are not clear: whether the changes in plasma of these metabolites are a consequence of early brain pathology in incipient AD, or a systematic peripheral signature in response to pathophysiological changes in the brain is not known. Peripheral metabolites can indirectly affect brain process. Several studies have shown that phosphatidylcholines and sphingomyelins are implicated in the development of cardiovascular diseases and insulin resistance [31–34]. All these vascular outcomes are known to increase the risk of MCI and dementia.

The limitations of our study are twofold. First, at the time of the Brain MRI visit, participants did not undergo full MCI or dementia assessment; the brain MRI visit did not include informant interviews nor adjudicated evaluation for MCI and dementia, as was included at the ARIC-NCS visit. Therefore, we defined normal cognition at the Brain MRI visit using a combination of a composite cognitive z-score and the MMSE, which might lead to missed MCI or even dementia cases at this earlier visit. Second, we used stored plasma samples. The potential impact of using stored samples for phospholipids analysis using the p180 kits has been discussed previously [35]. Because these samples were collected and processed using standardized protocols, were stored at −80°C, and were never thawed before being used in this study, the specimens were of high quality and considered to be suitable for plasma phospholipids analysis. In conclusion, our study demonstrated that higher levels of certain amino acids, carnitines, phospholipids, and sphingomyelins were prospectively associated with MCI and dementia over a mean follow-up of 7.3 years in a cohort of mostly African Americans with normal cognition at baseline.

Acknowledgments

We thank the staff and participants of the ARIC Study for their important contributions. Drs. Danni Li and Alvaro Alonso had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Dr. Knopman previously served as Deputy Editor for Neurology; serves on a Data Safety Monitoring Board for Lundbeck Pharmaceuticals and for the DIAN study; is an investigator in clinical trials sponsored by TauRX Pharmaceuticals, Lilly Pharmaceuticals, Biogen and the Alzheimer's Disease Cooperative Study; and receives research support from the NIH. Dr. Gottesman serves as Associate Editor for Neurology and receives research support from the NIH. Dr. Boerwinkle receives research support from NIH. Drs. Li, Sharrett, Mosley, Coresh, Wruck, and Alonso report no disclosures; Mr. Misialek reports no disclosures.
Sources of funding: The Atherosclerosis Risk in Community (ARIC) Study is carried out as a collaborative study

supported by the National Heart, Lung, and Blood Institute contracts (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN 268201100009C, HHSN2682011000010C, HHSN268 2011000011C, and HHSN2682011000012C). Neurocognitive data are collected by the support of the National Heart, Lung, and Blood Institute U01 HL096812, HL096814, HL096899, HL096902, and HL096917 with previous brain MRI examinations funded by R01-HL70825.

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.dadm.2016.09.003.

RESEARCH IN CONTEXT

1. Systematic review: Prior studies suggested plasma levels of phospholipids/metabolites might be useful in the prediction of incident mild cognitive impairment (MCI) and dementia. However, limited evidence exists in African Americans. We investigated whether the 10 phospholipids/metabolites that were prospectively predictive of MCI and dementia in whites would also be predictive in a mostly African-American cohort.
2. Interpretation: Our study failed to demonstrate that a panel of 10 phospholipids/metabolites previously associated with incident dementia was not predictive of MCI or dementia in an independent cohort.
3. Future directions: This study does not support the potential of the panel of 10 phospholipids/metabolites for prediction of cognitive impairment.

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