Blood-Based Biomarkers
Lipoprotein-associated phospholipase A2, homocysteine, and Alzheimer’s disease

Rachelle S. Doody\textsuperscript{a,}\textsuperscript{*}, Jasenka Demirovic\textsuperscript{b}, Christie M. Ballantyne\textsuperscript{c}, Wenyaw Chan\textsuperscript{d}, Robert Barber\textsuperscript{e}, Suzanne Powell\textsuperscript{f}, Valory Pavlik\textsuperscript{g,h}, and the Texas Alzheimer’s Disease Research and Care Consortium

\textsuperscript{a}Alzheimer’s Disease and Memory Disorders Center, Department of Neurology, Baylor College of Medicine, Houston, TX, USA
\textsuperscript{b}Epidemiologic Expertise, Houston, TX, USA
\textsuperscript{c}Section of Atherosclerosis and Lipoprotein Research, Department of Medicine, Baylor College of Medicine, and Methodist DeBakey Heart and Vascular Center, Houston, TX, USA
\textsuperscript{d}Department of Biostatistics, University of Texas Health Science Center, School of Public Health, Houston, TX, USA
\textsuperscript{e}Department of Pharmacology and Neuroscience, University of North Texas Health Science Center, Fort Worth, TX, USA
\textsuperscript{f}Department of Pathology, The Methodist Hospital, Houston, TX, USA
\textsuperscript{g}Department of Family and Community Medicine, Baylor College of Medicine, Houston, TX, USA
\textsuperscript{h}Department of Neurology, Baylor College of Medicine, Houston, TX, USA

Abstract

**Introduction:** Lipoprotein-associated phospholipase A2 (Lp-PLA2) and homocysteine (Hcy) have been linked to inflammation and Alzheimer’s disease (AD). Using a case-control design, we examined their independent effects and interactions with cardiovascular disease equivalent (CVDE), on AD risk.

**Methods:** AD cases and controls were from the Texas Alzheimer’s Research and Care Consortium study. Lp-PLA2 was determined using the PLAC test (diaDexus, Inc), and Hcy by recombinant cycling assay (Roche Hitachi 911). Logistic regression was used to predict AD case status. We assayed for Lp-PLA2 in the brain tissue of cases and controls.

**Results:** AD case status was independently associated with Lp-PLA2 and Hcy above the median (odds ratio [OR] = 1.91; 95% confidence interval [CI] = 1.22–2.97; \( P < .001 \) and OR = 1.81; 95% CI = 1.16–2.82; \( P = .009 \), respectively). Lp-PLA2, but not Hcy, interacted with CVDE to increase risk. Lp-PLA2 was absent from the brain tissue in both groups.

**Discussion:** Higher Lp-PLA2 and Hcy are independently associated with AD. The association of Lp-PLA2 with AD may be mediated through vascular damage.

**Keywords:** Alzheimer’s disease; Lp-PLA2; Homocysteine; Dementia; Biomarkers

1. Introduction

It is widely accepted that inflammation plays an important role in the pathogenesis of Alzheimer’s disease (AD) [1,2] and in cardiovascular disease (CVD) [3,4]. Although there are numerous reports on the relationship between various inflammatory markers and AD [2,5], the association of lipoprotein-associated phospholipase A2 (Lp-PLA2) with AD is far less known [6,7]. Lp-PLA2 is a proinflammatory enzyme that circulates in plasma in active form as a complex with low (LDL) and high (HDL) density lipoproteins and, to a lesser extent, with lipoprotein (a) [8]. It is primarily produced by macrophages and other inflammatory cells, such as activated bone marrow–derived mast cells and activated platelets [9]. Elevated levels of Lp-PLA2 indicate increased oxidative stress and inflammation. Lp-PLA2 is well recognized as both an inflammatory marker and a risk factor for coronary heart disease, stroke, and CVD.
mortality [4,10]. The association of Lp-PLA2 and AD requires far more research, especially as it relates to the mechanisms by which Lp-PLA2 may influence the risk of AD and how Lp-PLA2 relates to prevalent CVD and other risk factors in its association with AD.

Another risk factor implicated in the etiology of both AD and CVD is homocysteine (Hcy), a sulfur-containing amino acid produced in the methionine cycle and regulated by vitamin-B12 and folic acid [11–13]. Epidemiologic studies have shown that the abnormal elevation of circulating Hcy increases the risk of AD [12]. In a study by Seshadri et al. [12], among persons ≥60 years with Hcy levels >14 μM, the risk of AD was almost double the risk among those with lower Hcy. Conflicting results from clinical trials designed to examine whether B-vitamin and folic acid therapy may reduce Hcy and lower the risk of CVD and AD have prompted the question of whether Hcy is a risk factor in the etiology of AD or is just a marker of AD, reflecting the actions of other risk factors [14]. It is also less known whether CVD and other risk factors interact with Hcy in affecting the risk of AD.

Research regarding the precise role of Lp-PLA2 and Hcy in the occurrence of AD is complex and complicated by the fact that AD and CVD often co-exist and share some common risk factors including Lp-PLA2 and Hcy. Lp-PLA2 and Hcy may affect the pathogenesis of AD (1) directly and independently by affecting synaptic and neuronal function, formation of amyloid plaques, and neurofibrillar tangles; (2) interacting with each other in increasing the risk of AD; and (3) indirectly increasing vascular damage and promoting neurodegeneration with loss of cognitive reserve [15].

To further explore the association of Lp-PLA2 and Hcy with AD, we have analyzed data collected from AD patients and cognitively normal controls enrolled in the Texas Alzheimer’s Research and Care Consortium (TARCC) study. We hypothesized that (1) AD cases will have significantly higher levels of Lp-PLA2 and Hcy than controls, (2) the two variables will not interact in their association with AD, and (3) prevalent CVD and/or risk factors, such as hypertension, cigarette smoking, and diabetes mellitus, will interact with Lp-PLA2 and Hcy to modify the association of Lp-PLA2 and Hcy with AD. To examine if the association of Lp-PLA2 with AD was because of a central (brain metabolism of lipids or brain injury) and/or peripheral (systemic lipid metabolism, vascular damage) effect, we examined histopathologically brains of AD cases and nondemented controls from the brain bank of the Alzheimer’s Disease and Memory Disorders Center, Baylor College of Medicine, Houston, TX, USA. Because we found no literature reports on the presence of Lp-PLA2 in the brain, we hypothesized that the association between Lp-PLA2 and AD is mediated through peripheral lipid metabolism and vascular disease.

2. Methods

2.1. Study population

TARCC was established in 1999 and initially included four institutions: Texas Tech University Health Science Center, the University of North Texas Health Science Center, the University of Texas Southwestern Medical Center at Dallas, and Baylor College of Medicine, Houston, TX, USA. The University of Texas Health Science Center at San Antonio and Texas A&M University were added in 2008 and 2013, respectively. The main goal of the Consortium was to develop a longitudinal cohort study of AD patients and cognitively unimpaired controls, examined and followed up annually at the participating institutions [16].

2.2. Study design

In the present report, a case-control study design was used to examine variables of interest and their associations with AD. The analysis includes 398 subjects (197 AD cases and 141 cognitively normal controls).
The protocol was approved by the institutional review boards at each participating institution and informed consent obtained for all participants. All clinical assessments were part of the standard clinical work-up. Demographic characteristics (age, sex, race, ethnicity, marital status, primary language, handedness, years of education, living arrangements, and zip code) and extensive medical history and medication use were taken, also including physician’s estimate of disease duration [18]. Vital signs and assessment of vision and hearing were also documented. Neuropsychological testing included examination of global cognitive functioning status, with mini-mental state examination (MMSE) [19] and CDR [14,20]; attention with digit span [21,22] and Trials A [23]; executive function, with Trials B [23] and CLOX I and II [24]; memory, with Wechsler memory scale (WMS) logical memory I and II [21], the Consortium to Established a Registry for Alzheimer’s disease list learning and recognition [25]; language, with Boston naming test [26], FAS verbal fluency [25] and animal naming [25]; premorbid IQ, with the American version of the Nelson Adult Reading Test (AMNART) [27,28]; visuospatial memory, with WMS-visual reproduction I and II [21], psychiatric status, with geriatric depression scale [29] and neuropsychiatric inventory questionnaire [30]; and functional status, with Lawton-Brody activities of daily living: Physical Self-Maintenance Scale (PSMS), and instrumental activities of daily living scale [31]. Full spelling of all abbreviations may be found in corresponding references.

2.4. Laboratory analyses

Blood samples were taken at the time of entry into the study. For biomarkers analyses, including C-reactive protein (CRP), TARCC uses the technology offered by Rules-Based Medicine, called multi-analyte profiles, a large panel of tests that provide accurate and precise measurements of numerous biological markers of inflammation [32]. This approach reduces interassay variability, which is an important methodological problem facing many comparable studies. Lp-PLA2, Hcy, and lipid profile were measured at the Maria and Alando J. Ballantyne Atherosclerosis Clinical Research Laboratory at Baylor College of Medicine, Houston, TX, USA. Lp-PLA2 was determined using the diaDexus PLAC test (diaDexus, Inc, San Francisco, CA), a dual monoclonal antibody immunoassay for the quantitative (mass) determination of Lp-PLA2. Hcy levels were measured using the recombinant cycling assay (Hitachi 911 analyzer; Roche Diagnostics, Indianapolis, IN, USA). To assess reliability of the Lp-PLA2 and Hcy measurements, we calculated corresponding coefficients of variation (CV). For Lp-PLA2, intraassay (within run) CV was 3.6% and interassay (between runs) CV was 5.7%. The intraassay and interassay CVs for Hcy were 1.4% and 4.4%, respectively.
spinal cord was included when present. A total of 15 blocks per case were prepared, 10 sections cut on each block, to assure adequate material for examination. Frozen tissues were also cut and results compared with those of the FFPE tissue. Standard immunochemical procedures were used using Dako immunohistochemical automated stainers and staining of individual slides by hand in parallel [33]. Catalyzed signal amplification system Dako code K1500 was used to develop/stain slides. Automated and hand staining were performed on the FFPE materials and on the frozen material as well using an antibody to Lp-PLA2 from diaDexus (Lp-PLA2 Monoclonal AB 4b4 PN: 2611 LN: 1012103) using a dilution of 1:2500. Multiple dilutions were attempted in parallel.

2.6. Statistical analyses

Means and standard deviations of continuous variables and proportions of binary variables at baseline were compared in AD cases and controls. We also determined median values and interquartile range for Lp-PLA2 and Hcy. Fisher’s exact test (unadjusted) and multivariable (adjusted) models were used to analyze the association of Lp-PLA2, Hcy, and other baseline characteristics with AD status (case vs. control). In assessing the association of Lp-PLA2 and Hcy with AD status, Lp-PLA2 and Hcy were used as categorical variables, dichotomized at the median value because their values were very skewed. AD status (case vs. control) was used as the outcome variable and Lp-PLA2 and Hcy (above vs. below median) as independent variables. The lower category (Lp-PLA2 and Hcy levels below median) served as referent with which the upper category (above median) was compared. We also examined potential interactions of Lp-PLA2 and Hcy with each other and with prevalent CVD and/or CVD risk factors, using a variable defined as cardiovascular disease equivalent (CVDE). The CVDE was calculated according to the National Cholesterol Education Program—Adult Treatment Panel III guidelines (history of myocardial infarction, stent placement, congestive heart failure, diabetes, or increased risk of CHD with any two of hypertension, hyperlipidemia, or current cigarette smoking).

3. Results

The baseline characteristics of AD cases and controls are listed in Table 1. AD cases were significantly older and, as expected, had lower MMSE scores and lower BMI. In our analyses, we used age as a continuous variable, but it should be noted that 8.1% of cases and 29.9% of controls were aged <65 years. There was no statistically significant difference in the proportion of men among AD cases versus controls or in the proportion of AD cases and controls with prevalent CVDE. Also, there was no statistically significant difference in the mean values of CRP and total cholesterol levels between cases and controls. Unadjusted mean values of both Lp-PLA2 and Hcy were significantly higher among AD cases than among controls ($P < .02$ and $P < .001$, respectively). Median values of Lp-PLA2 and Hcy were also significantly higher among AD cases than among controls. To assess the association of Lp-PLA2 and Hcy with AD, we dichotomized all values (including cases and controls) at median, which was for Lp-PLA2 293.1 and for Hcy 13.1 $\mu$M. About 60% of AD cases had Lp-PLA2 or Hcy levels above the median versus about 40% among controls for both variables. Odds ratio (OR) of AD in relation to Lp-PLA2 levels is listed in Table 2. After adjustment for age, sex, and BMI, subjects with Lp-PLA2 levels above the median were almost twice as likely to have AD than those with levels below the median ($OR = 1.91, P < .0001$). The likelihood of having AD among subjects with higher Hcy levels was also greater compared with the

### Table 1

| Characteristics | AD cases (n = 197) | Controls (n = 198) | P value |
|----------------|-------------------|-------------------|---------|
| Age at visit (y) | 77.41 (8.29) | 70.42 (8.89) | <.001 |
| Sex (% male) | 34.52 | 31.82 | .75 |
| BMI | 25.68 (5.06) | 27.43 (4.81) | <.001 |
| MMSE | 31.82 (4.62) | 29.42 (0.88) | <.001 |
| Lp-PLA2 (ug/L) | 297.0 (71.6) | 281.11 (65.7) | .02 |
| Median (IQR) | 300.08 (83.65) | 276.20 (94.40) | .02 |
| Homocysteine (u/M) | 16.21 (9.01) | 13.3 (5.03) | <.001 |
| Median (IQR) | 14.30 (8.75) | 12.20 (6.30) | <.001 |
| CRP (ug/mL) | 3.23 (4.87) | 3.68 (4.14) | .32 |
| Cholesterol (mg/L) | 210.12 (50.11) | 209.35 (62.04) | .40 |
| CVDE | 48.22 | 46.46 | .73 |

Abbreviations: AD, Alzheimer’s disease; BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters); MMSE, mini-mental state examination; Lp-PLA2, lipoprotein-associated phospholipase A2; IQR, interquartile range; CRP, C-reactive protein; CVDE, cardiovascular disease equivalent; CHD, coronary heart disease.

*Unadjusted mean values (standard deviations) or proportions.

CVDE calculated according to the Adult Treatment Panel III guidelines [34] (history of myocardial infarction, stent placement, congestive heart failure, diabetes, or increased risk of CHD with any two of hypertension, hyperlipidemia, or current cigarette smoking).

### Table 2

| Variables | Odds ratio$^*$ | 95% CI | P value |
|-----------|---------------|--------|---------|
| Lp-PLA2 | 1.91 | 1.22–2.97 | <.001 |
| Age (y) | 1.09 | 1.06–1.24 | <.0001 |
| Sex (M vs. F) | 1.29 | 0.80–2.07 | .29 |
| BMI | 0.97 | 0.93–1.02 | .26 |

*Values dichotomized at median, odds ratio for values above median.

**Abbreviations:** Lp-PLA2, lipoprotein-associated phospholipase A2; CI, confidence interval; M, male; F, female; BMI, body mass index.

$^*$Adjusted for age, sex, and body mass index.
Table 3
Adjusted* odds ratio of Alzheimer’s disease in relation to homocysteine values

| Variables     | Odds ratio* | 95% CI         | P value |
|---------------|-------------|----------------|---------|
| Homocysteine  | 1.81        | 1.16–2.82      | .009    |
| Age (y)       | 1.09        | 1.16–2.82      | <.0001  |
| Sex (M vs. F) | 1.21        | 0.75–1.93      | .44     |
| BMI           | 0.96        | 0.91–1.01      | .08     |

Abbreviations: CI, confidence interval; M, male; F, female; BMI, body mass index.
*Adjusted for age, sex, and body mass index.

subjects with lower levels, with adjusted OR = 1.81, P = .009 (Table 3). To examine if there was an interaction between Lp-PLA2 and Hcy, we included the interaction term (Lp-PLA2 > median × Hcy > median) in the multivariate model and found no significant interaction between the two variables (P = .78). We then examined possible interactions between Lp-PLA2 and CVDE in a multivariable model, we found a significant interaction between Lp-PLA2 and CVDE (P = .02, Table 4). In the model examining the contribution of CVDE, it was of borderline significance regardless of Lp-PLA2 and Lp-PLA2 was not an independent risk variable (P = .79). In a multivariate model with Hcy and CVDE (Table 5), Hcy remained a significant variable (P = .01) and the interaction term between Hcy and CVDE was statistically insignificant (P = .38, not listed in the table).

Histoimmunologic analyses of autopsy brain tissue were performed on 10 AD cases (nine women and one man) and 10 controls (six women and four men).

Mean age of AD cases and controls was 79.2 and 72.5, respectively. The results of Lp-PLA2 staining were negative for both the FFPE and the frozen material in all AD cases and controls.

Table 4
Adjusted* odds ratio of Alzheimer’s disease in relation to CVDE and Lp-PLA2

| Variables and interactions | Odds ratio | 95% CI | P value |
|----------------------------|------------|--------|---------|
| Lp-PLA2 × CVDE             | —          | —      | .02     |
| CVDE                       | —          | —      | .06     |
| CVDE = 1, Lp-PLA2          | 1.72       | 0.89–3.34 | —       |
| CVDE = 0, Lp-PLA2 +        | 0.53       | 0.27–1.02 | —       |
| Lp-PLA2                    | —          | —      | .79     |
| Lp-PLA2 × CVDE = 1         | 3.56       | 1.08–6.99 | —       |
| Lp-PLA2 +, CVDE = 0        | 1.08       | 0.59–2.02 | —       |
| Age (y)                    | 1.10       | 1.07–1.113 | <.001  |
| Sex (M vs. F)              | 1.30       | 0.80–2.09 | .29     |
| BMI                        | 0.98       | 0.93–1.03 | .40     |

Abbreviations: CVDE, cardiovascular disease equivalent [34]; Lp-PLA2, lipoprotein-associated phospholipase 2; CI, confidence interval; Lp-PLA2 × CVDE, interaction term; CVDE = 1, CVDE present; CVDE = 0, CVDE absent; M, male; F, female; BMI, body mass index.
*Adjusted for age, sex, and body mass index.

Table 5
Adjusted* odds ratio of Alzheimer’s disease in relation to CVDE and homocysteine

| Variables     | Odds ratio | 95% CI | P value |
|---------------|------------|--------|---------|
| CVDE          | 0.78       | 0.49–1.24 | .29     |
| Homocysteine  | 1.89       | 1.20–2.97 | .01     |
| Age (y)       | 1.09       | 1.06–1.12 | <.0001  |
| Sex (M vs. F) | 1.23       | 0.77–1.98 | .38     |
| BMI           | 0.97       | 0.92–1.01 | .14     |

Abbreviations: CVDE, cardiovascular disease equivalent [34]; CI, confidence interval; M, male; F, female; BMI, body mass index.
*Adjusted for age, sex, and body mass index.

4. Discussion

The results of our study showed that in the TARCC cohort, after adjustment for age, sex, and BMI, higher levels of both Lp-PLA2 and Hcy were significantly associated with prevalent AD. Subjects with higher levels of Lp-PLA2 were almost twice as likely to have AD compared with subjects with Lp-PLA2 levels below the median. We found a significant interaction between prevalent CVDE and Lp-PLA2. In the model which included the interaction term, prevalent CVDE remained a significant and independent variable, whereas Lp-PLA2 was no longer an independent factor in the prevalence of AD. This would suggest that the association of Lp-PLA2 with AD might be primarily mediated through CVDE.

The relationship between Lp-PLA2 and AD reported in other studies has been inconsistent. The prospective Rotterdam study [6] showed that higher levels of Lp-PLA2 were associated, independent of other CVD and inflammatory factors, with increased risk of dementia in general but not with AD in particular. The prospective Framingham Study showed that, after adjustment for other risk factors, Lp-PLA2 was not a significant risk factor for all-cause dementia or AD [35]. The authors proposed that this lack of association between Lp-PLA2 and AD could be because of a relatively small number of incident AD cases in the sample and the age of the participants at which Lp-PLA2 was measured. The authors also suggested that Lp-PLA2 levels in the blood might not reflect concentrations in the brain tissue or in the cerebrospinal fluid [35]. Similarly to our findings, the most recently published findings from the prospective Cardiovascular Health Study [36] showed that increased levels of Lp-PLA2 were associated with an increased risk of AD. Contrary to our findings, however, this association was independent of the presence of CVD morbidity or CVD risk factors. The authors concluded that Lp-PLA2 may be an important predictor of AD, without or with concurrent vascular dementia. The discrepancy between our findings and the findings from the Cardiovascular Health Study may be explained by different study designs, ascertainment, and potential misclassification of the
subtypes of dementia in the Cardiovascular Health Study where the number of cases of vascular dementia was very small, and the number of variables used in the adjusted models to determine OR or hazard ratio of AD in these two studies. In a small cross-sectional study of 78 AD cases, 59 amnestic mild cognitive impairment cases, and 66 cognitively normal controls, Davidson et al. [7] found no significant association between Lp-PLA2 activity and AD. In that study, the main clinical correlates of Lp-PLA2 activity in AD cases and controls were lipid levels and statin use. One large genetic case-control study conducted in Japan showed that inherited deficiency of Lp-PLA2 activity, due to carriage of the V279F null allele, was not associated with a reduced risk of AD [37].

Extensive basic research into the role of Lp-PLA2 in the pathogenesis of atherosclerosis and in clinical manifestations of CVD [3,4,9] is mainly focused on the role of Lp-PLA2 in oxidative stress, inflammation, cardiometabolic risk, and on modulation of Lp-PLA2 [9]. Lp-PLA2 is a promoter of inflammation, a critical feature of the atherosclerotic process.

A meta-analysis of 32 prospective studies showed that both Lp-PLA2 mass and activity were related to proatherogenic lipids and vascular risk [4]. Because Lp-PLA2 binds with both LDL and HDL, it is possible that Lp-PLA2 exhibits a dual action, depending on its association with proatherogenic LDL or antiatherogenic HDL. This might provide an opportunity for manipulation of Lp-PLA2 modulation, by means of medications and diet [9].

Our results suggest that the association of Lp-PLA2 with AD might be largely mediated through the prevalent CVDE. This would imply the same biological plausibility of the Lp-PLA2-AD and Lp-PLA2-CVD relationship. This was also supported by our histopathologic analysis: we did not find Lp-PLA2 in the brain tissue of AD cases or controls. All this suggests that peripheral lipid metabolism and vascular damage may be a key mediating mechanism by which Lp-PLA2 predisposes to AD. These issues require further research generated from prospective epidemiologic studies and from histopathologic and other laboratory studies. Our findings regarding the association between Hcy and AD are consistent with the results of other epidemiologic and clinical studies [12,38]. After adjusting for age, sex, and BMI, ORs showed that AD cases were more likely to have Hcy above the median than controls. The association between increased Hcy and the occurrence of AD and CVD may be linked to oxidative stress. About 80% of Hcy present in human blood is bound with proteins. There is a causal relationship between the levels of Hcy-bound proteins and oxidative damage. An intramolecular hydrogen atom mechanism provides biochemical rationale for the link between protein oxidation and Hcy [11,39]. Experimental studies have also shown that Hcy promotes oxidative stress via generation of reactive oxygen species (ROS) on disulfide bond formation [39]. An interesting finding is that cysteine is more abundant than Hcy and also undergoes disulfide- and ROS-forming reactions but it is not associated with these diseases. This has prompted further research to find potential alternative mechanisms of Hcy action different from those found in the case of cysteine, glutathione, and other biological thiols [39]. Hcy metabolism is regulated by folic acid and vitamin-B12 [40]. Kruman et al. [40] reported that low folic acid and elevated Hcy may promote accumulation of DNA damage, affect DNA repair in neurons, and sensitize them to amyloid beta protein (A-beta) toxicity. Another explanation offered by Zhang et al. [13] includes the effect of elevated Hcy in enhancing expression of gamma-secretase and its effect on A-beta phosphorylation, leading to overproduction of A-beta. The authors suggested that high Hcy may serve as an “upstream” factor for increased A-beta production as seen in patients with AD.

Further basic research is critical for explaining the mechanism of action of high Hcy, especially in the light of inconsistencies in findings of clinical trials aimed at Hcy lowering by means of B-vitamin and folic acid therapy to reduce AD and CVD risk. We found no interaction between Lp-PLA2 and Hcy in their association with AD. We also did not find a significant interaction between CVDE and Hcy suggesting differences in mechanisms by which these two variables affect the risk of AD. One longitudinal epidemiologic study showed that Hcy is significantly and independently associated with the incidence of AD but not with the incidence of vascular dementia [41] whereas, as shown previously, Lp-PLA2 association with AD seems to be predominantly mediated via vascular damage.

One of the disadvantages of our study is its retrospective case-control design, with all limitations regarding the cause-effect examination. This concern is mitigated by the prospective nature of the systematic data collection for TARCC study which will allow for analyses of the association between Lp-PLA2 and the incidence and the rate of progression of AD. This also applies to other inflammatory biomarkers measured in the TARCC cohort, and it is addressed in another article in preparation by the same group of investigators.

5. Conclusions

There is limited evidence that Lp-PLA2 is a significant and independent risk factor for AD. It is likely that the relationship between Lp-PLA2 and AD is mediated primarily via CVDE. The association of higher levels of Hcy with the prevalence of AD seems to be independent of CVDE. Further research in these areas is of great importance because Lp-PLA2 and Hcy are modifiable risk factors and, if further confirmed, they may be considered as therapeutic targets in preventing AD and in reducing severity of AD.

Acknowledgments

The authors acknowledge that this project was supported by the Texas Alzheimer’s Research and Care Consortium
(TARCC) funded by the State of Texas through the Texas Council on Alzheimer’s Disease and Related Disorders. Investigators from the Texas Alzheimer’s Research and Care Consortium include Baylor College of Medicine: Rachelle Doody, Susan Rountree, Valory Pavlik, Weneyaw Chan, Paul Massman, Eveleen Darby, Tracy Evans, and Aisha Khaleeq; Texas Tech University Health Science Center: Gregory Schrimsher, Andrew Dentino, and Ronnie Orozco; University of North Texas Health Science Center: Thomas Fairchild, Janice Knebl, Sid E. O’Bryant, James R. Hall, Robert C. Barber, Douglass Mains, Lisa Alvarez, Erin Braddock, Rosemary McCallum, and Leigh Johnson; University of Texas Southwestern Medical Center: Perrie Adams, Roger Rosenberg, Myron Weiner, Benjamin Williams, Mary Quiceno, Joan Reisch, Ryan Huebinger, Guanghua Xiao, Doris Svetlik, Amy Werry, and Janet Smith; University of Texas Health Science Center–San Antonio: Donald Royall, Raymond Palmer, and Marsha Polk.

### RESEARCH IN CONTEXT

1. Systematic review: An extensive literature search was conducted using PubMed, covering the areas of basic science, clinical, and epidemiologic aspects of the association between lipoprotein-associated phospholipase 2 (Lp-PLA2), homocysteine, and Alzheimer’s disease (AD). The literature concerning the relationship between Lp-PLA2 and AD, particularly mechanism of action, is relatively scarce compared with that of other biomarkers.

2. Interpretation: Our findings add to the evidence of increased risk of AD associated with higher levels of Lp-PLA2 and homocysteine. These two variables were independently associated with AD. The relationship between Lp-PLA2 and AD may be mediated via vascular damage, which is consistent with our finding that Lp-PLA2 is absent in the brains of both AD cases and controls.

3. Future directions: To establish firmly the role of Lp-PLA2 in the etiology of AD, confirmation of these findings is needed in prospective studies with a larger number of AD cases. This is important because of the potential utility of Lp-PLA2 in the early detection and progression of AD.

### References

[1] Eikelenboom P, van Exel E, Hoozemans JJ, Veehuis R, Rozenmuller AJ, van Gool WA. Neuroinflammation—an early event in both the history and pathogenesis of Alzheimer’s disease. Neurodegener Dis 2010;7:38–41.

[2] Koyama A, O’Brian J, Weuve J, Blacker D, Metti AL, Iaffi K. The role of peripheral inflammatory markers in dementia and Alzheimer’s disease: A meta-analysis. J Gerontol A Biol Sci Med Sci 2013;68:433–40.

[3] Davidson MH, Corson MA, Alberts MJ, Anderson JL, Gorelick PB, Jones PH, et al. Consensus panel recommendation for incorporating lipoprotein-associated phospholipase A2 testing into cardiovascular disease risk assessment guidelines. Am J Cardiol 2008;101:51F–7.

[4] The Lp-PLA2 Studies Collaboration. Lipoprotein-associated phospholipase A2 and risk of coronary disease, stroke, and mortality: collaborative analysis of 32 prospective studies. Lancet 2010; 375:1536–44.

[5] Gorelick PB. Role of inflammation in cognitive impairment: Results of observational epidemiological studies and clinical trials. Ann N Y Acad Sci 2010;1207:155–62.

[6] Van Oijen M, van der Meer JM, Hoffman A, Witteman JC, Koudstaal PJ, Breteler M. Lipoprotein-associated phospholipase A2 is associated with the risk of dementia. Ann Neurol 2006;59:139–44.

[7] Davidson JE, Lackhart A, Amos L, Stirmadet-Farrant HA, Mooser V, Sollberger M, et al. Plasma lipoprotein-associated phospholipase A2 activity in Alzheimer’s disease, amnestic mild cognitive impairment, and cognitively healthy elderly subjects: A cross-sectional study. Alzheimers Res Ther 2012;4:51.

[8] Rosenson RS, Stafforini DM. Modulation of oxidative stress, inflammation, and atherosclerosis by lipoprotein-associated phospholipase A2. J Lipid Res 2012;53:1767–82.

[9] Silva IT, Mello AP, Damasceno NR. Antioxidant and inflammatory aspects of lipoprotein-associated phospholipase A2 (Lp-PLA2): A review. Lipids Health Dis 2011;10:170–80.

[10] Ballantyne C, Hogeveen R, Bang H, Coresh J, Folsom AR, Chambless LE, et al. Lipoprotein-associated phospholipase A2, high sensitive C-reactive protein and risk for incident ischemic stroke in middle-aged men and women in the Atherosclerosis Risk in Communities (ARIC) study. Arch Intern Med 2005;165:2479–84.

[11] Jacobsen DV. Hyperhomocysteinemia and oxidative stress. Arterioscler Thromb Vasc Biol 2000;20:1182–4.

[12] Seshadi S, Beiser A, Sellhub J, Jaques PF, Rosenberg IH, D’Agostino RB, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer’s disease. N Engl J Med 2002;346:476–83.

[13] Zhang CE, Wei W, Liu YH, Peng JH, Liu GP, Zhang Y, et al. Hyperhomocysteinemia increases beta-amyloid by enhancing expression of gamma-secretase and phosphorylation of amyloid precursor protein in rat brain. Am J Pathol 2009;174:1481–91.

[14] Berg L. Clinical dementia rating scale (CDR). Psychopharmacol Bull 1988;24:63.

[15] Stamper MJ. Cardiovascular disease and Alzheimer’s disease: Common links. J Intern Med 2006;260:211–23.

[16] Waring S, O’Bryant SE, Reisch JS, Diaz-Arrastia R, Knebel J, Doody R. Texas Alzheimer’s Research Consortium longitudinal research cohort: Study design and baseline characteristics. Texas Public Health J 2008;63:10–3.

[17] McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical Diagnosis of Alzheimer’s disease. Report of the NINCDS-ADRDA work group under the auspices of the Department of Health and Human Services Task Force for Alzheimer’s Disease. Neurology 1984;34:939–44.

[18] Doody RS, Dunn J, Huang E, Azher S, Katakai M. A method for estimating duration of illness in Alzheimer’s disease. Dement Geriatr Cogn Disord 2004;17:1–4.

[19] Folstein MF, Folstein SE, McHugh PR. “Mini-mental state”: A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12:189–98.

[20] Morris JC. The clinical dementia rating (CDR): Current version and scoring rules. Neurology 1993;43:2412–4.
[21] Wechsler D. Wechsler memory scale-Revised. San Antonio: Psychological Corporation; 1987.
[22] Satz P, Mogel S. An abbreviation of the WAIS for clinical use. J Clin Psychol 1962;18:77–9.
[23] Corrigan JD, Hinkelday NS. Relationship between parts A and B of the Trial Making Test. J Clin Psychol 1987;43:402–9.
[24] Royall DR, Polk M. CLOX: An executive clock drawing task. J Neurol Neurosurg Psychiatr 1998;64:588–94.
[25] Morris JC, Heyman A, Mohs RC, Hughes JP, van Belle G, Fillenbaum G, et al. The Consortium to Established a Registry for Alzheimer’s disease (CERAD). Part I. Clinical and neuropsychological assessment of Alzheimer’s disease. Neurology 1989;39:1159–65.
[26] Kaplan E, Goodglass H, Weintraub S. The Boston naming test. Philadelphia: Lea & Febiger; 1983.
[27] Grober E, Sliwinski M. Development and validation of a model for estimating premorbid verbal intelligence in the elderly. J Clin Exp Neuropsychol 1991;13:933–49.
[28] Nelson HE, O’Connell A. Dementia: The estimation of premorbid intelligence levels using the new adult reading test. Cortex 1978;14:234–44.
[29] Yesevage JA, Brink TL, Rose TL, Lum O, Huand V, Adey M, et al. Development and validation of a geriatric depression screening scale: A preliminary report. J Psychiatr Res 1982;17:37–49.
[30] Kaufer DI, Cummings JL, Ketchel P, Smith V, MacMillan A, Shelley T, et al. Validation of the NPI-Q, a brief clinical form of the neuropsychiatric inventory. J Neuropsychiatry Clin Neurosci 2000;12:233–9.
[31] Lawton MP, Brody EM. Assessment of older people: Self-maintaining and instrumental activities of daily living. Gerontologist 1969;9:179–86.
[32] O’Bryant SE, Xiao G, Barber R, Reisch J, Doody R, Fairchild T, et al. A blood-based algorithm for the detection of Alzheimer’s disease. Dement Geriatr Cogn Disord 2011;32:55–62.
[33] Vickers KC, Maguire CT, Wolfert R, Burns AR, Reardon M, Geis R, et al. Relationship of lipoprotein associated phospholipase A2 and oxidized low density lipoprotein in carotid atherosclerosis. J Lipid Res 2009;50:1735–43.
[34] Grundy SM, Cleeman JL, Merz CN, Brewer HB, Clark LT, Hunninghake DB, et al. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines. Circulation 2004;110:227–39.
[35] van Himberg TM, Beisser AS, Ai M, Seshadri S, Otokozawa MT, Au R, et al. Biomarkers for insulin resistance and inflammation and the risk of all-cause dementia and Alzheimer’s disease: Results from the Framingham Study. Arch Neurol 2012;69:594–600.
[36] Koshy B, Miyashita A, St Jean P, Stirnadel H, Kaise T, Rubio JP, et al. Genetic deficiency of plasma lipoprotein-associated phospholipase A2 (PLA2G7 V297F null mutation) and risk of Alzheimer’s disease in Japan. J Alzheimers Dis 2010;21:775–80.
[37] Fitzpatrick AL, Irizarry MC, Cushman M, Jenny NS, Chi GC, Koro C. Lipoprotein-associated phospholipase A2 and risk of dementia in the Cardiovascular Health Study. Atherosclerosis 2014;235:384–91.
[38] Shen L, Ji HF. Association between homocysteine, folic acid, vitamin B12 and Alzheimer’s disease. J Alzheimers Dis 2015;46:777–90.
[39] Sibrian-Vazquez M, Escobedo JO, Lim S, Samoei GK, Strongin RM. Homocystamides promote free-radical and oxidative damage to proteins. Proc Natl Acad Sci U S A 2010;107:551–4.
[40] Krumar II, Kumaravel TS, Lohani A, Pedersen WA, Cutler RG, Krumar Y, et al. Folic acid deficiency and homocysteine impair DNA repair in hippocampal neurons and sensitize them to amyloid toxicity in experimental models of Alzheimer’s disease. J Neurosci 2002;22:1752–62.
[41] Ravaglia G, Forti P, Maioli F, Chiappelli M, Montesi F, Tumini E, et al. Blood inflammatory markers and risk of dementia: The Conselice Study of Brain Aging. Neurobiol Aging 2007;28:1810–20.