Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring 1 (2015) 464-471

Alzheimer's & Dementia

Blood-Based Biomarkers

Lipoprotein-associated phospholipase A2, homocysteine, and Alzheimer's disease

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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

http://dx.doi.org/10.1016/j.dadm.2015.08.001

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 $\geq$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 neurofibrillary 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

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R.S.D. served as an ad hoc consultant to AbbVie, Accera, Avanir, Banner Health, Baxter, BioTE, Cardeus, Chiesi, Genzyme, Hoffman-La Roche, Merck, Novartis, NeuroCog, Nutricia, Pfizer, QR Pharma, Shire, Sonexa, Suven, Targacept, Takeda, and Toyoma. She is on the Scientific Advisory Boards of AC Immune, AZ Therapies, CereSpir, and QR Pharma and on a Data Safety Monitoring committee for Hoffman-La Roche. She is the principal investigator on clinical trials funded by Accera, Avanir, Genentech, Merck, Pfizer, and Takeda and holds stock options in AZ Therapies, QR Pharma, Sonexa, and Transition. She receives support from the NIH funded Alzheimer's Disease Cooperative study for chairing a committee, and for clinical trials, and receives support from the NIH funded Alzheimer's Disease Neuroimaging Initiative. She is coinvestigator on NIH 1UF1AGO46143. C.M.B. served on scientific advisory boards for Pfizer; Merck; the Council on Atherosclerosis, Thrombosis, and Vascular Biology; and the Council on Nutrition, Physical Activity, and Metabolism of the American Heart Association. He is on the Data Safety Monitoring Board for the NHLBI Cardiovascular Inflammation Reduction Trial. He holds a patent on "Non-Human Animal Having Predefined Allele of Cellular Adhesion Gene" and a provisional patient "Biomarkers to Improve Prediction of Heart Failure Risk." He receives royalties from the book *Clinical Lipidology: a Companion to Braunwald's Heart Disease*. He has received honoraria for speaking for Abbott and is on the speakers' bureaus for Abbott, GlaxoSmithKline, and Merck. He has consulted for Abbott, AdNexus, Aegerion, Amarin, Amgen, Amylin, Arena, AstraZeneca, Bristol-Myers-Squibb, Cerenis, Esperior, Genentech, Genzyme, GlaxoSmithKline, Idera, Kowa, Merck, Novartis, Omthera, Pfizer, Regeneron, Resverlogix, Roche, Sanofi-Synthelabo, and Takeda. R.B. has received travel expenses and honoraria for lectures and educational activities not funded by industry and has a patent pending on "Methods and devices for diagnosing AD" patent #13/697,978. S.P. is an editor for Modern Pathology and Archive of Pathology & Laboratory Medicine. She has received honoraria for speaking at academic institutions. V.P. is a subinvestigator on a study supported by Takeda, was principal investigator on an AHRQ funded grant and is coinvestigator on another, and was coinvestigator on an NHLBI grant. J.D. and W.C. report no disclosures.

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198 controls) who were examined in TARCC institutions using a standard protocol and had a laboratory panel and neuropsychological assessments completed as of May 2010. Diagnoses were assigned during a consensus review using National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria [17]. Inclusion criteria for AD cases were men and women $\geq$50 years with a diagnosis of probable AD residing in Texas or surrounding states with available information regarding the duration of symptoms, who were willing to provide blood specimens, had a reliable surrogate, and who agreed to follow-up. Patients with a Hachinski score of $>$4, history of major cortical infarction or lacunar infarct(s) in a critical area, and/or persistent focal neurologic deficit were excluded. Also excluded were individuals with neurologic diseases that could contribute to the dementia (Parkinson disease, dementia with Lewy bodies, and multiple sclerosis); active cancer within the past 5 years (nonmelanoma skin cancer and nonmetastatic prostate cancer were allowed); depression or other psychiatric or systemic disorders that could impact cognition (e.g., vitamin-B12 deficiency, thyroid disorders, normal pressure hydrocephalus); and individuals using systemic steroids, chemotherapy, or anti-inflammatory drugs (except nonsteroid drugs). Controls were cognitively normal men and women aged $\geq$50 years within the same residential areas as AD cases, with clinical dementia rating (CDR) global score of 0 and CDR sum of box score of 0, with normal cognition and function based on the information provided by a reliable informant, and who were willing to provide blood specimens and commit to follow-up visits. Controls were judged to be cognitively normal based on neuropsychological testing and in a consensus review and could not be first-degree biological relatives of TARCC patients. Individuals with a primary neurologic disease or psychiatric illness and who used psychoactive medications in amounts that would be expected to compromise cognition were excluded. Subjects with a mild head injury without evidence of residual cognitive impairment; medically controlled chronic diseases such as diabetes mellitus, hypertension, and thyroid disease; and depression not active at the time of examination were allowed. The recruitment methodology did not call for cases and controls to be formally matched on demographic or other variables.

2.3. Clinical and neuropsychological measurements

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.

2.5. Histopathologic assessments

Formalin-fixed, paraffin-embedded (FFPE) brain sections cut at 7 μ of 10 deceased AD patients from the TARCC cohort and 10 nondemented controls from the Department of Pathology of the Methodist Hospital in Houston, TX, USA were obtained. Sections included cortex (frontal, parietal, occipital, cingulate gyrus, and hippocampus), brainstem (midbrain, pons, and medulla), cerebellum, basal ganglia, amygdala, thalamus, and pituitary gland. The 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 infraction, stent placement, congestive heart failure, diabetes mellitus, or high risk for CHD with any two of hypertension, hyperlipidemia, or current cigarette smoking, as categorical variables) [34]. To control for potential confounding, all models included adjustment for age, sex, and body mass index (BMI). All statistical analyses were performed using the SAS statistical package, version 8 (SAS Institute Inc, Cary, NC, USA).

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 μM. 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
Baseline characteristics* of AD cases and controls

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	19.18 (6.22)	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
Adjusted* odds ratio of Alzheimer's disease in relation to Lp-PLA2 values[†]

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

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.

[†] Values dichotomized at median, odds ratio for values above median.

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.

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

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 $\times$ 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 Hcy with 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.

†Lp-PLA2 above median, +Lp-PLA2 below median.

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; the interaction term between CVDE and homocysteine is insignificant ($P = .38$).

†Homocysteine above median.

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, Wenyaw 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, Douglas 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.

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