Lipoprotein Phospholipase A2 and Cerebral Microbleeds in the Framingham Heart Study

José Rafael Romero, MD; Sarah R. Preis, PhD; Alexa S. Beiser, PhD; Charles DeCarli, MD; Dong Young Lee, MD, PhD; Anand Viswanathan, MD, PhD; Emelia J. Benjamin, MD, ScM; Joao Fontes, MD; Rhoda Au, PhD; Aleksandra Pikula, MD; Jimmy Wang, MD; Carlos S. Kase, MD; Philip A. Wolf, MD; Michael C. Irrizary, MD; Sudha Seshadri, MD

Background and Purpose—Cerebral microbleeds (CMB) attributable to cerebral amyloid angiopathy generally occur in lobar regions, whereas those attributable to hypertensive vasculopathy are deep. Inflammation may be an underlying mechanism for CMB, with varying associations according to CMB location. Lipoprotein phospholipase-A2 (Lp-PLA2) is a circulating enzyme marker of vascular inflammation associated with risk of ischemic stroke and dementia. We hypothesized that higher Lp-PLA2 levels would be related to higher prevalence of CMB, with possible regional specificity.

Methods—Framingham Offspring participants aged 65 years or older with available Lp-PLA2 measures and brain magnetic resonance imaging were included. Logistic regression models were used to relate Lp-PLA2 activity and mass to presence of CMB, adjusted for age, sex, medication use (aspirin, anticoagulants, and statins), systolic blood pressure, APOE, current smoking, and diabetes.

Results—Eight-hundred nineteen participants (mean age, 73 years; 53% women) were included; 106 (13%) had CMB, 82 (10%) were lobar, and 27 (3%) were deep. We did not observe significant associations of CMB and LpPLA2 measures in multivariable adjusted analyses. However, there was a significant interaction between APOE genotype and Lp-PLA2 activity in their relation to presence of deep CMB (P interaction=0.01). Among persons with APOE ε3/ε3, the odds ratio for deep CMB was 0.95 (confidence interval, 0.59-1.53; P=0.83), whereas among those with at least 1 ε2 or ε4 allele, odds ratio was 3.46 (confidence interval, 1.43-8.36; P=0.006).

Conclusions—In our community-based sample of older adults, there was no significant association of Lp-PLA2 with total or lobar CMB. The association of higher levels of Lp-PLA2 activity with deep CMB among those with at least 1 APOE ε2 or ε4 allele merits replication. (Stroke. 2012;43:3091-3094.)

Key Words: cerebrovascular microbleed □ intracranial hemorrhage □ inflammation □ lipids and lipoprotein □ magnetic resonance □ microcirculation

Cerebral microhemorrhages (CMB) detected on brain magnetic resonance imaging (MRI) are implicated as a risk factor for clinical outcomes such as stroke, dementia, and cognitive impairment. Although CMB in different locations have the same MRI appearance, CMB in lobar regions are generally attributed to cerebral amyloid angiopathy and those in deep regions are attributed to hypertensive vasculopathy. Recent studies suggest an association of inflammation with both cerebral amyloid angiopathy and hypertension affecting the cerebral vessels. Inflammation has been related to CMB and diseases with high prevalence of CMB, such as stroke and dementia. We selected a specific marker of vascular inflammation, lipoprotein phospholipase A2 (Lp-PLA2), and studied its relation to CMB, testing the hypothesis that higher Lp-PLA2 levels are associated with a greater prevalence of CMB and that the association would be stronger for lobar CMB.

Materials and Methods

Framingham Offspring participants (baseline characteristics; Table 1) who attended the 7th Offspring examination (1999–2001) had blood drawn at this examination for Lp-PLA2 mass and activity...
We observed a significant interaction between APOE genotype and Lp-PLA2 activity in their relation to presence of deep CMB (P interaction=0.01). Among persons with APOE ε3/ε3, the multivariable-adjusted odds ratio for deep CMB was 0.95 (95% confidence interval, 0.59-1.53; P=0.83), whereas among those with at least 1 ε2 or ε4 allele, the multivariable adjusted odds ratio was 3.46 (95% confidence interval, 1.43-8.36; P=0.006; Table 2). No significant interaction was observed with antihypertensive medication use.

**Discussion**

The present study explores for the first time to our knowledge the relation of LpPLA2 with cerebral microhemorrhages detected on brain MRI. No significant association of LpPLA2 measures with CMB presence was seen in multivariable adjusted models. There was a significant interaction with APOE status such that in participants who were carriers of at least 1 APOE ε2 or ε4 allele, higher LpPA2 activity was related to the presence of deep CMB. However, because this was an interaction of only Lp-PLA2 activity (not mass) in the small subgroup of deep CMB, further study is required.

LpPLA2 is a recognized marker of vascular inflammation and cardiovascular risk, which has been related to ischemic small vessel disease detected on brain MRI. Our results did not support a role for LpPLA2 measures in hemorrhage-prone

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**Table 1. Characteristics of Study Sample (N=819)**

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>No CMB (n=713)</th>
<th>All CMB (n=106)</th>
<th>Lobar CMB (n=82)</th>
<th>Deep CMB (n=27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure, mm Hg, mean (SD)</td>
<td>129 (18)</td>
<td>134 (19)</td>
<td>132 (16)</td>
<td>138 (18)</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>54 (17)</td>
<td>50 (17)</td>
<td>49 (17)</td>
<td>52 (17)</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>120 (31)</td>
<td>120 (36)</td>
<td>120 (37)</td>
<td>126 (37)</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>351 (49.2)</td>
<td>66 (62.3)</td>
<td>48 (58.5)</td>
<td>22 (81.5)</td>
</tr>
<tr>
<td>Hypertension treatment, n (%)</td>
<td>255 (35.8)</td>
<td>50 (47.2)</td>
<td>48 (58.5)</td>
<td>22 (81.5)</td>
</tr>
<tr>
<td>Aspirin use, n (%)</td>
<td>10 (1.4)</td>
<td>3 (2.8)</td>
<td>3 (3.7)</td>
<td>2 (7.4)</td>
</tr>
<tr>
<td>Statin use, n (%)</td>
<td>142 (19.9)</td>
<td>34 (32.1)</td>
<td>28 (34.2)</td>
<td>9 (33.3)</td>
</tr>
<tr>
<td>APOE status, n (%)</td>
<td>31</td>
<td>36</td>
<td>21</td>
<td>15</td>
</tr>
<tr>
<td>ε3/ε3</td>
<td>440 (62.7)</td>
<td>67 (64.4)</td>
<td>51 (63.8)</td>
<td>21 (77.8)</td>
</tr>
<tr>
<td>ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε4, ε4/ε4</td>
<td>262 (37.3)</td>
<td>37 (35.6)</td>
<td>29 (36.3)</td>
<td>6 (22.2)</td>
</tr>
<tr>
<td>LpPLA2 activity nmol/mL/min, mean (SD)</td>
<td>143 (34)</td>
<td>145 (36)</td>
<td>147 (35)</td>
<td>152 (48)</td>
</tr>
<tr>
<td>LpPLA2 mass ng/mL, mean (SD)</td>
<td>293 (91)</td>
<td>292 (86)</td>
<td>295 (83)</td>
<td>315 (113)</td>
</tr>
</tbody>
</table>

CMB indicates cerebral microbleed; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LpPLA2, lipoprotein phospholipase-A2; MRI, magnetic resonance imaging; SD, standard deviation.

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**Results**

CMB prevalence was 13% (106/819), with most participants having single CMB and with CMB located more frequently in lobar regions.

We did not observe a significant association of LpPLA2 measures and CMB (Supplementary Table II) and the relation did not change according to CMB location or additional adjustment for low-density lipoprotein and high-density lipoprotein cholesterol levels.

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**Discussion**

The present study explores for the first time to our knowledge the relation of LpPLA2 with cerebral microhemorrhages detected on brain MRI. No significant association of LpPLA2 measures with CMB presence was seen in multivariable adjusted models. There was a significant interaction with APOE status such that in participants who were carriers of at least 1 APOE ε2 or ε4 allele, higher LpPA2 activity was related to the presence of deep CMB. However, because this was an interaction of only Lp-PLA2 activity (not mass) in the small subgroup of deep CMB, further study is required.

LpPLA2 is a recognized marker of vascular inflammation and cardiovascular risk, which has been related to ischemic small vessel disease detected on brain MRI. Our results did not support a role for LpPLA2 measures in hemorrhage-prone
In conclusion, we did not observe a significant association of Lp-PLA2 activity or mass with CMB. The present study is limited by its cross-sectional nature, assessing MRI and Lp-PLA2 measures at only a single time point. The predominant white, European descent of the Framingham Heart Study participants limits generalization to other groups, and the small sample size of participants with deep CMB does not allow excluding that chance accounts for the findings.

In conclusion, we did not observe a significant association of Lp-PLA2 with total or lobar CMB. The interaction of APOE status and the relation of Lp-PLA2 activity to CMB, with higher Lp-PLA2 activity in participants with CMB in deep cerebral regions, needs to be interpreted with caution and further studies are required. If it is confirmed by others, then Lp-PLA2 measures may potentially contribute to development of risk models for hypertensive hemorrhage and support mechanistic effects of Lp-PLA2 and hypertension on cerebrovascular integrity. Both elevated Lp-PLA2 and hypertension are potentially modifiable conditions.

**Sources of Funding**

This work (design and conduct of the study, collection, and management of the data) was supported by the Framingham Heart Study’s National Heart, Lung, and Blood Institute contract (N01-HC-25195) and by grants from the National Institute of Neurological Disorders and Stroke (R01 NS17950), the National Institute on Aging (R01 AG16495; AG08122; AG033193; AG031287; K23AG038444), and National Institutes of Health grants (R01 HL64753; R01 HL076784; 1 R01 AG028321).

**Disclosures**

Statistical analyses were performed by Sarah R. Preis and Alexa S. Beiser (academic). Lp-PLA2 activity measurements were provided by GlaxoSmithKline and mass measurements were provided by diaDexus at no cost to the FHS. M.C.I. is a stock and options holding employee of GlaxoSmithKline. J.R.R. received funding grant award from GlaxoSmithKline protocol WEUSRTP3833. The current analysis was partially supported by GlaxoSmithKline.

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### Table 2. Interaction Between APOE Genotype and Lipoprotein Phospholipase-A2 Measures in Relation to Presence of Cerebral Microbleeds

<table>
<thead>
<tr>
<th>APOE Group</th>
<th>Any CMB</th>
<th>LpPLA2 Activity</th>
<th>LpPLA2 Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P</td>
<td>P Interaction†</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any CMB</td>
<td>33</td>
<td>21</td>
<td>0.89 (0.57–1.40)</td>
</tr>
<tr>
<td></td>
<td>33</td>
<td>37</td>
<td>2.76 (1.33–5.73)</td>
</tr>
<tr>
<td>Lobar</td>
<td>33</td>
<td>51</td>
<td>1.14 (0.82–1.59)</td>
</tr>
<tr>
<td></td>
<td>33</td>
<td>29</td>
<td>1.06 (0.73–1.53)</td>
</tr>
<tr>
<td>Deep</td>
<td>22,23,34,44</td>
<td>6</td>
<td>3.46 (1.43–8.36)</td>
</tr>
</tbody>
</table>

CI indicates confidence interval; CMB, cerebral microbleed; LpPLA2, lipoprotein phospholipase-A2; MRI, magnetic resonance imaging; OR, odds ratio; SD, standard deviation. N represents number of CMB. OR of CMB per SD change in LpPLA2 activity (1 SD=31 for women, 33 for men) and mass (1 SD=84 for women, 96 for men).

†Interaction between LpPLA2 and APOE group.
‡Model 2 additionally adjusts for aspirin use, anticoagulant use, statin use, diabetes status, smoking status, systolic blood pressure.
§Model 2a adjusts for age at MRI, sex, time interval between examination 7 and MRI.
References

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Methods supplement

Study Sample

The Framingham Offspring Cohort recruited in 1971 includes 5,124 offspring of the Original Cohort and their spouses, and is examined approximately every 4 years.\(^1\) The seventh examination (1999-2001) of the Offspring Cohort was completed on 3,539 participants (82% of surviving cohort). The present study includes all Framingham Offspring participants attending the 7\(^{th}\) Offspring examination, older than 65 years at this examination and with available measures studied, including Lp-PLA2 mass and activity assay, and brain MRI with gradient-echo sequences.

Participants were excluded if they refused MRI, had a contraindication to MRI (pacemaker or other implantable devices, metallic foreign body, claustrophobia), had clinical stroke, dementia or another neurologic illness that could affect MRI measurements (multiple sclerosis, brain tumor, head injury with loss of consciousness for > 30 minutes). Of the participants attending examination 7 who underwent brain MRI, 838 subjects had GRE MRI scans through 2007. All of them also had available LpPLA2 measurements; 15 participants were excluded due to the presence of clinical stroke (n=5), prevalent dementia (n=7) or other neurological problem (n=3) as noted above; 1 due to poor quality of MRI GRE sequence; and 3 due to presence of large intracerebral hemorrhage (ICH), yielding a study sample of n=819 participants.

Brain MRI Measurements

Offspring Cohort participants attending the 7\(^{th}\) examination were invited to undergo MRI brain imaging beginning in March 1999. MRI protocols sensitive to CMBs (gradient echo imaging sequences, GRE) were added in December 2000. A 1.5-tesla MR machine (Siemens Magnetom) was used to obtain the following sequences: coronal T2-weighted 2470/20 to 80 (TR/TE), echo train length 8, field of view 22 cm, acquisition matrix 192x256 interpolated to 256x256 with 1 excitation, 4-mm slice thickness from nasion to occiput, sagittal T1-weighted 11.4/4.4, 3D FLASH, 192 mm slab, 128 slices of 1.5-mm thickness, 12-degree flip angle and axial T2*gradient echo 656/26 (TR/TE), field of view 22cm, acquisition matrix 144x256, 30-degree flip angle, 19 slices of 5-mm thickness, and 2 mm gap.

After MRI acquisition, data were analyzed using a custom-designed image analysis package, QUANTA 2 written for the Linux operating system. All analyses were done blind to the subject’s demographic and clinical characteristics including Lp-PLA2 levels.

CMB definition and characteristics

CMBs were defined using standard criteria recently published\(^2\) as rounded or ovoid hypointense lesions on T2*-GRE weighted sequence measuring 10mm or less in diameter, surrounded by brain parenchyma over at least half the circumference of the lesion. Hypointense areas not considered to represent CMB included those that were symmetrical and located in the basal ganglia (considered to represent calcifications), contiguous in sequential slices, in or near sulci (considered to represent flow void from vessels), low-signal lesions adjacent to bone (considered to be signal averaging from adjacent bone), hypointense lesions with heterogeneous signal hyperintensity on T1 or T2-weighted sequences (considered to represent cavernous malformations) or lesions consistent with arteriovenous vascular malformations. The presence, size, number and
location of CMB were determined. CMBs were classified by location into lobar (cerebral cortical gray and subcortical white matter), deep (deep gray matter [basal ganglia and thalamus] and white matter of the internal and external capsules and corpus callosum), and infratentorial (brainstem and cerebellum).

CMB detection and reliability measures

For the present study using recent guidelines for CMB detection, one of the readers (JRR) underwent further training in CMB detection with the collaboration of colleagues at the Massachusetts General Hospital. Inter-rater reliability comparing the FHS study readings (JRR) of the training dataset and MGH reader (AV) was excellent (Kappa 0.81). All the scans were read by this investigator (JRR) blinded to the subjects’ demographic and clinical information. The intra-rater reliability based on blinded reading of 50 scans on two separate occasions was excellent (kappa statistic 0.81). Scans with single CMB were reviewed by two readers including a vascular neurologist (JRR) and a neurologist with extensive experience in MRI reading (CD), and lesions identified as possible CMB with conflicting interpretation were confirmed by consensus.

Lipoprotein phospholipase A2 measurements

Lp-PLA2 activity and mass were measured from overnight fasting plasma specimens that were stored at −80 °C. Lp-PLA2 activity was measured using a colorimetric activity method (diaDexus CAM Kit, Inc., San Francisco, CA). Mean coefficients of variation for lower and upper quality control specimens were 7.0% and 5.9%, respectively. Samples with duplicate coefficients of variation greater than 12.4% were repeated. Lp-PLA2 mass was measured using a commercially available sandwich enzyme immunoassay (diaDexus PLAC® test, Inc., San Francisco, CA). For Lp-PLA2 mass, twenty-four percent of samples were run in duplicate; all produced coefficients of variation <15%. Coefficients of variation for lower and upper quality range specimens were 6.0% and 8.0%, respectively. Although previous studies have suggested that both LpPLA2 measures, mass and activity, are related to cardiovascular risk and correlate with each other, other studies have shown the relation of either measure with cardiovascular risk factors and outcomes varies depending on the measure used. Both measures were available for the present study and were included in the analyses.

Covariate definition and measures

Extensive risk factor information was gathered at Examination 7 (1998 – 2001) of the Framingham Offspring Study. The following covariates were selected: Systolic and diastolic blood pressures were each taken as the average of the Framingham clinic physician’s two measurements. Hypertension was defined using JNC-7 criteria (SBP >=140 mm Hg or DBP>=90 mm Hg) or use of antihypertensive medications. Diabetes was defined as fasting glucose ≥126 mg/dl (≥7 mmol/L) or use of insulin or oral hypoglycemic medications. Medications, including lipid, antihypertensive, antiplatelet and anticoagulant therapies were assessed by self-report. Current cigarette smoking was defined as self-reported use in the year prior to the examination. Total and high-density lipoprotein (HDL) cholesterol, and triglycerides were measured on fasting specimens. LDL-cholesterol concentrations were calculated according to the formula $\text{LDL} = (\text{total cholesterol-HDL-cholesterol-triglycerides})/5$ in participants with triglyceride levels
below 400mg/dL, and missing in those with higher levels. APOE genotype status was determined using standard techniques of DNA amplification and restriction isotyping. Since both the ε2 and ε4 alleles have been associated with a higher risk of intracerebral hemorrhage (ICH),\(^8\) we decided to dichotomize participants as having only ε3 alleles or having either one or more of the ε2 or ε4 alleles (ε3/ε3 versus ε2/ε2- ε2/ε3- ε2/ε4- ε3/ε4- ε4/ε4).

**Statistical Analysis**

Descriptive statistics of CMB characteristics and regional distribution of CMBs are presented. Multiple logistic regression analyses were used to relate Lp-PLA2 activity and mass to presence of CMBs, overall and stratified by CMB location, given the different pathophysiology suggested for CMB according to their location. All models were adjusted for age, sex and time interval between exam 7 and brain MRI acquisition. Secondary models were additionally adjusted for systolic blood pressure, diabetes, current smoking, statin use, aspirin use, anticoagulants, and APOE status (ε3/ε3 versus ε2/ε2- ε2/ε3- ε2/ε4- ε3/ε4- ε4/ε4); we chose this dichotomization since both the ε2 and the ε4 alleles have been associated with an increased risk of lobar and deep ICH.\(^9\)

We tested for interaction with the APOE genotype and antihypertensive medication use in the association between LpPLA2 measures and presence or absence of CMB. All models were adjusted for age at MRI, sex, and time interval between exam 7 and MRI. Additional models were also adjusted for aspirin use, anticoagulant use, statin use, diabetes status, smoking status, systolic blood pressure. In the subgroup analyses of deep CMB, the secondary model (Model 2a, Table 2 in manuscript) did not include diabetes or smoking as covariates as there were no participants with those risk factors in one of the strata.

All analyses were determined a priori and performed using Statistical Analyses System (SAS) software version 9.2 (SAS Institute, Cary, NC). A two-sided p-value <0.05 was considered statistically significant.

Power calculations prior to the initiation of the study start indicated that an unadjusted logistic regression relating the presence of CMBs to continuous, normally distributed Lp-PLA2 activity or mass with a sample size of 500 individuals would achieve an 80% power (α=0.05) to detect an OR per SD increase of Lp-PLA2 of at least 1.42, if the predicted prevalence was 15%. In a multivariable adjusted logistic regression where the covariates explain 50% of the variance in Lp-PLA2, the study would achieve an 80% power to detect an OR of at least 1.64 (NCSS, PASS, and GESS [computer program]. Kaysville, Utah: NCSS; J Hintze 2006).

**Results supplement**

Participants included in our MRI analyses had a lower prevalence of traditional cardiovascular risk factors and were generally considered healthier (data not shown) than 7\(^{th}\) examination participants over 65 years old who did not have an MRI. The mean age of the sample was 73 years and 53% were women. Forty participants had multiple CMB, and 66 had only one. In those with multiple CMB, most CMB were located in lobar regions. Twenty-one participants had strictly lobar CMB, 4 had strictly deep, 2 had strictly cerebellar and 13 mixed location. CMB distribution included 21 participants with strictly lobar CMB, 4 strictly deep, 2 strictly cerebellar and 13 mixed location. The 13
cases with mixed location were assigned to the location where CMB were detected (i.e. any lobar to lobar location, any deep to deep location) for the analysis. CMB were found more commonly in men, and participants with CMB were older, had higher systolic blood pressure, and were more likely to be using aspirin, anticoagulants and statins compared to those without detected CMB.

**Relation of Lp-PLA2 measures and cerebral microbleeds (supplement Tables 1 and 2)**

We studied both Lp-PLA2 measures, mass and activity, as their relation to cardiovascular risk factors and outcomes could differ. Differences were observed for cholesterol levels, smoking and statin use across LpPLA2 quartiles, but we did not observe a significant association of Lp-PLA2 measures and CMB, in the age and sex adjusted or multivariable adjusted analysis. The relation did not change according to CMB location; point estimates were higher for Lp-PLA2 and risk of deep CMB (OR 1.20-1.29 per SD) than for lobar CMB (OR 0.97-1.03 per SD), but power was limited by the low number of deep CMBs. Additional adjustment for LDL and HDL cholesterol levels did not change the results (data not shown).

Test for interaction of antihypertensive medication use in the association between LpPLA2 measures and CMBs revealed no significant interaction.

**Discussion supplement**

The prevalence of CMB in the present study was in line with previous reports including persons of similar age groups. For instance, in the AGES Reykjavik study CMB prevalence was 11.1% (mean age 77 years). We also observed a regional distribution of CMB with predominance in lobar regions as reported in previous studies. Our results expand on a prior report from our group by including a larger sample and using a 1.5 T MRI scanner. Similar to our prior findings, we observed that men were more likely to have CMB. We observed a higher CMB prevalence, likely due to the older mean age in our current sample (73 years versus 63 years) and use of a more sensitive MRI scanner for the present study. The observation of greater use of antiplatelet and anticoagulant medications in participants with CMB is in accord with prior studies that reported an association of use of these medications with a greater prevalence of CMB.

Lp-PLA2 is a recognized marker of vascular inflammation and cardiovascular risk. Inflammation is proposed as a mechanism underlying subclinical cerebrovascular disease. Lp-PLA2 has been related to ischemic small vessel disease detected on brain MRI, but its role in the pathophysiology of CMB, a form of hemorrhage-prone microvascular disease, is less clear. Our results did not show a significant association of Lp-PLA2 measures with all CMB taken together or with lobar CMB, but we observed a significant relation of Lp-PLA2 activity and deep CMB in persons with an APOE ε2 or ε4 allele. The relation was only present for one of the Lp-PLA2 measures, in line with previous studies that have shown that both measures are not always congruous in their relation to outcomes of interest. Possible explanations for this difference have been proposed, including varying distributions of Lp-PLA2 across lipoprotein classes not accounted for in the current analysis, and/or differences in measurement precision. At present Lp-PLA2 mass is the only measure approved for use in clinical practice.
APOE plays a role in several brain functions, modifies the risk of cerebrovascular disease and dementia, as well as the response to traditional cardiovascular risk factors, and has a role in both ischemic and hemorrhagic cerebral microvascular disease. For instance, APOE status has been related to white matter hyperintensities (marker of ischemic small vessel disease), where persons with at least one APOE ε2 or ε3 allele were noted to have a greater volume of white matter hyperintensities, but APOE ε4 has also been related to white matter hyperintensities in deep regions. APOE also has been related to hemorrhagic cerebrovascular disease. Individuals with APOE ε2 or ε4 alleles have a higher risk of lobar ICH. A recent study including a large sample of patients with ICH supports the role of APOE modifying risk for lobar and deep ICH, and suggests that both APOE genotypes ε2 and ε4 alleles increased the risk, with the ε4 alleles showing a relation to both deep and lobar ICH, and the ε2 allele relating to deep ICH. Similarly, a relation has been found in previous studies between CMB and APOE ε2 and ε4 alleles. Overall previous results are not conclusive to show definite differences in the risk conferred by the ε2 versus ε4 alleles in terms of CMB location, but it is suggested that the ε4 allele may have a stronger effect on lobar CMB.

The effect of APOE allele status on the relation of Lp-PLA2 and CMB may be mediated by different mechanisms. One possibility is that APOE modulates inflammation in the brain as suggested by animal studies, which may play a role in the pathophysiology of hypertensive vasculopathy represented by deep CMB. Higher circulating levels of other inflammatory markers were associated with CMB in both deep and lobar regions in a prior study. APOE may also modify CMB via an alternate mechanism such as endothelial dysfunction, implicated in hypertensive vasculopathy and Lp-PLA2 has been related to endothelial dysfunction. Last, APOE may affect Lp-PLA2 measurements since APOE status can affect circulating lipoproteins levels, and Lp-PLA2 is highly bound to lipoproteins in serum. The APOE ε4 allele has been postulated to increase LDL, total cholesterol, and ApoB levels, while the ε2 allele decreases them. We adjusted our analysis for cholesterol levels and the results did not change, but we did not adjust for other unmeasured lipoproteins.

If replicated in further studies, our results suggest that APOE status may possibly modify the relation of markers of inflammation with CMB with regional specificity.

Strengths and Limitations

The present study has several strengths including: a large sample size; inclusion of both men and women; the community-based sample, and interpretation of brain MRI by readers blinded to all clinical data. Limitations of our study include its cross-sectional nature, assessing MRI and Lp-PLA2 measures at only a single time-points. As a result, we cannot draw conclusions regarding a causal relation between LpPLA2 and CMB. Although Lp-PLA2 measures were assessed an average of 6 years prior to brain MRI, and may have changed in the interim, a prior study showed stability and good reproducibility of serial Lp-PLA2 measurements over a mean follow up of 7.3 months. We attempted to address the non-contemporaneous assessment by adjusting for the interval between Lp-PLA2 measurement and MRI acquisition. Another limitation is the fact that Framingham Heart Study participants are of predominantly white, European descent; thus, our findings cannot be generalized to other ethnic and racial groups is limited and requires additional study. Finally, the small sample size of participants with
the deep CMB subtype is small, thus chance cannot be excluded to account for the results.

References


### Table 1 supplement. Clinical characteristics across Lp-PLA2 measures

<table>
<thead>
<tr>
<th>Clinical Characteristics</th>
<th>LpPLA2 activity ng/mL</th>
<th>LpPLA2 mass ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Q1</td>
<td>Q2</td>
</tr>
<tr>
<td>Men, n (%)</td>
<td>98 (47.6)</td>
<td>96 (47.1)</td>
</tr>
<tr>
<td>Age at Exam 7, yrs, mean [SD]</td>
<td>67.1 (5.6)</td>
<td>66.8 (5.8)</td>
</tr>
<tr>
<td>Age at MRI, yrs, mean [SD]</td>
<td>73.0 (5.3)</td>
<td>72.9 (5.6)</td>
</tr>
<tr>
<td>Time interval between Exam 7 and MRI, yrs, mean [SD]</td>
<td>5.9 (2.3)</td>
<td>6.0 (2.2)</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg, mean [SD]</td>
<td>132 (18)</td>
<td>128 (18)</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>61 (19)</td>
<td>56 (17)</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>100 (24)</td>
<td>114 (25)</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>27 (13.1)</td>
<td>25 (12.3)</td>
</tr>
<tr>
<td>Current smokers, n (%)</td>
<td>10 (4.9)</td>
<td>16 (7.8)</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>120 (58.3)</td>
<td>91 (44.6)</td>
</tr>
<tr>
<td>Hypertension treatment, n (%)</td>
<td>92 (44.7)</td>
<td>69 (33.8)</td>
</tr>
<tr>
<td>Aspirin use, n (%)</td>
<td>92 (44.9)</td>
<td>89 (43.6)</td>
</tr>
<tr>
<td>Anticoagulant use, n (%)</td>
<td>0 (0.0)</td>
<td>4 (2.0)</td>
</tr>
<tr>
<td>Statin use, n (%)</td>
<td>66 (32.0)</td>
<td>49 (24.0)</td>
</tr>
</tbody>
</table>
Table 2 supplement. Multivariable adjusted OR of Lp-PLA2 as a predictor of presence or absence of CMB

<table>
<thead>
<tr>
<th>Outcome</th>
<th>LpPLA2 Activity</th>
<th>LpPLA2 Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>All CMB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1*</td>
<td>1.00 (0.81-1.23)</td>
<td>0.96</td>
</tr>
<tr>
<td>Model 2**</td>
<td>1.03 (0.83-1.28)</td>
<td>0.79</td>
</tr>
<tr>
<td>Lobar CMB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1*</td>
<td>1.04 (0.82-1.31)</td>
<td>0.77</td>
</tr>
<tr>
<td>Model 2**</td>
<td>1.08 (0.84-1.37)</td>
<td>0.55</td>
</tr>
<tr>
<td>Deep CMB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1*</td>
<td>1.24 (0.85-1.80)</td>
<td>0.27</td>
</tr>
<tr>
<td>Model 2**</td>
<td>1.29 (0.87-1.93)</td>
<td>0.21</td>
</tr>
</tbody>
</table>

Odds Ratio (OR) of CMB per standard deviation change in LpPLA2 activity (1 SD=31 for women, 33 for men) and mass (1 SD=84 for women, 96 for men).

*Model 1 adjusted for age at MRI, sex, and time interval between exam 7 and MRI.

**Model 2 additionally adjusted for aspirin use, anticoagulant use, statin use, diabetes, smoking, APOE status (ε3/ε3 versus ε2/ε2- ε2/ε3- ε2/ε4- ε3/ε4- ε4/ε4), systolic blood pressure.