ARIC Manuscript Proposal # 940

PC Reviewed: 06/03/03	Status: A	Priority: 2
SC Reviewed: 06/19/03	Status: A	Priority: 2

1.a. Full Title: The Relation of Lipoprotein-Associated Phospholipase A_2 (Lp-PLA_2) to Incident Stroke in Middle-Aged Men and Women

b. Abbreviated Title (Length 26 characters): **Lp-PLA_2 and Stroke**

2. Writing Group (list individual with lead responsibility first):

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3. Timeline: Measurement of plasma lipoprotein-associated phospholipase A_2 (Lp-PLA_2) has been performed on plasma from visit 2 for stroke cases and cohort random sample (CRS) controls. Statistical analyses will be completed by the end of 2003. Funding will be supported by GlaxoSmithKline.

4. Rationale: Stroke contributes significantly to morbidity and mortality in industrialized countries. In the United States, stroke remains the third leading cause of death and the leading cause of severe neurological disability. The prevalence of stroke in the ARIC study has been estimated to be 6.9% for African-Americans and 5.5% for Caucasian adults between 1988 and 1994 (Rosamond WD et al. *Stroke* 1999;30:736–743). Elevated blood pressure has been identified as the most prominent modifiable risk factor for stroke. However, to increase prevention efforts, new modifiable risk factors for stroke need to be identified.

Lipoprotein-associated phospholipase A_2 (Lp-PLA_2, also known as platelet-activating factor acetylhydrolase) hydrolyzes phospholipids at the sn2 position to generate lysophospholipids and fatty acids. Three recent reports link Lp-PLA2 to atherogenesis and the risk of coronary heart disease (Caslake MJ et al. *Atherosclerosis* 2000;150:413–419; Packard CJ et al. *N Engl J Med* 2000;343:1148–1155; Elinder LS et al. *Arterioscler Thromb Vasc Biol* 1997;17:2257–2263). This enzyme, found in the media of normal and diseased arteries (Elinder LS et al. *Arterioscler Thromb Vasc Biol* 1997;17:2257–2263), may be involved in modifying low-density lipoprotein (LDL) that is present in the artery wall (Leitinger N et al. *Arterioscler Thromb Vasc Biol* 1999;19:1291–1298).

At this time, very little is known about the basic epidemiology of Lp-PLA_2. In the largest study to date (by WOSCOPS researchers), levels of Lp-PLA_2, the expression of which is regulated by mediators of inflammation, had a strong, positive association with risk that was not confounded by other cardiovascular disease risk factors (Packard CJ et al. *N Engl J Med*

2000;343:1148–1155). It was associated with almost a doubling of the risk for coronary events (nonfatal myocardial infarction, death from coronary heart disease [CHD], or a revascularization procedure) in the highest quintile as compared with the lowest quintile. However, the generalizability of these data is limited because they are based on a single sample of middle-aged men. Thus, at this time, there is a need to broaden the epidemiological studies of Lp-PLA_2 to include larger, more diverse populations to test if the association of Lp-PLA_2 with risk factors and cardiovascular risk continues to hold. In addition, the relationship among Lp-PLA_2, LDL, and high-sensitivity C-reactive protein (hs-CRP) will also be investigated. However, in the Women's Health Study, Lp-PLA_2 was no longer significant after multivariate analysis (Blake GJ et al. *J Am Coll Cardiol* 2001;38:1302–1306).

The proposed study will be the first large, epidemiological study of Lp-PLA_2 that includes women and minority populations to assess the levels of Lp-PLA_2 among stroke patients and to assess whether Lp-PLA_2 is an independent risk factor for stroke.

5. Main Hypothesis/Study Questions: (1) To determine whether levels of Lp-PLA_2 at visit 2 are associated with an increased incidence of stroke after adjusting for conventional risk factors. (2) To determine whether levels of Lp-PLA_2 at visit 2 are associated with an increased incidence of stroke after adjusting for conventional risk factors, hs-CRP, and stratification by LDL-C level. (3) To determine the relationship between traditional (blood pressure, cholesterol, HDL-C, LDL-C, triglycerides) and nontraditional (hs-CRP) atherosclerotic risk factors and Lp-PLA_2 among stroke cases.

6. Data (variables, time window, source, inclusions/exclusions): Incident stroke cases will be ascertained and their values of Lp-PLA_2 at visit 2 will be determined. A case–cohort design will be used to ascertain the relationship between Lp-PLA_2 and stroke.

The exposure of interest in this study will be defined by the plasma levels of Lp-PLA_2 in samples of study participants at visit 2. At this point, there is no universally accepted definition for elevated or unhealthy levels of Lp-PLA_2; therefore, the outcomes will be assessed by using Lp-PLA_2 measurements in stroke cases and controls, and by using various cutpoints in the distribution of Lp-PLA_2 values (dichotomization, tertiles, quintiles) as long as sufficient data are available within each category for numerically stable statistical models. Continuous covariate(s) will also be considered together with the test for potential nonlinearity.

The outcome of interest will be defined by the type of stroke, thrombotic brain infarction, cardioembolic stroke, subarachnoid hemorrhage, intracerebral hemorrhage, lacunar infarcts, or deaths associated with stroke. Because some stroke subtypes are infrequent, we will examine all stroke and ischemic versus nonischemic as the 2 categories of stroke. Methods for diagnoses have been described previously (Rosamond WD et al. *Stroke* 1999;30:736–743). Subjects with previous diagnoses of ischemic heart disease, diabetes, or severe hypertension will not be included as cases.

The following covariates will be evaluated during analysis: demographic and lifestyle data (age, gender, ethnicity); traditional risk factors for atherosclerotic vascular disease (smoking [status and cigarette years], hypertension [systolic and diastolic BP, antihypertensive medication], diabetes, lipid abnormalities [hypercholesterolemia, elevated LDL-C, low HDL-C], and aspects of the metabolic syndrome [BMI, triglycerides]); hs-CRP levels; and medications (antidiabetic, hormone replacement therapy, antihypertensive, statins).

Data on Lp-PLA_2 levels will be collected at the ARIC central lipid laboratory and integrated with interview and clinical data already collected at the ARIC coordinating data center to perform statistical analysis. All personal identifiers will be removed from the ARIC data for

statistical analyses. During laboratory analyses, samples will be anonymous to preserve the confidentiality of study participants. Data management and analyses will be conducted by the ARIC study investigators at the coordinating data center. The samples selected for analysis will include replicate observations from about 6%, blinded to the lab, so that we will be able to assess repeatability and potential drift of lab measurement of analytes being studied.

Data Analysis

Univariate analysis of Lp-PLA_2: The distribution for Lp-PLA_2 and lipid levels will be assessed for normality. Skewed distributions will be normalized through logarithmic or other transformations if necessary. The mean, median, and 25th and 75th percentiles will be calculated for Lp-PLA_2 level and all continuous variables. This analysis will be repeated for each subtype of stroke.

Bivariate analysis: Mean levels of Lp-PLA_2 will be calculated for stroke and cohort random sample controls. In addition, correlation of Lp-PLA_2 with all other cardiovascular risk factors will be assessed.

Multivariable analyses: To assess the association between Lp-PLA_2 levels and incident stroke cases, Cox proportional hazard models suitable for case–cohort design will be evaluated after assessment of the proportional hazard assumption for Lp-PLA_2 and each covariate. Demographic-adjusted analysis will be followed by models including age, gender, and race. A final model will add traditional risk factors for stroke (smoking, HDL-C, LDL-C, hypertension by JNC 6, and mean arterial pressure) and other risk factors (alchohol consumption and hs-CRP). Multivariate models will also be stratified by LDL-C levels above and below the mean. We will examine potential confounding, effect modification, and multicollinearity effects of hs-CRP and LDL-C closely. Interaction terms between covariates and the levels of Lp-PLA_2 will be tested in nested models and retained if $p<0.05$. For all the analyses to be conducted, sampling scheme should be properly incorporated.

7.a. Will the data be used for non-CVD analysis in this manuscript? ____ Yes __X_ No

b. If Yes, is the author aware that the file ICTDER02 must be used to exclude persons with a value RES_OTH = "CVD Research" for non-DNA analysis, and for DNA analysis RES_DNA = "CVD Research" would be used? ____ Yes ____ No
(This file ICTDER02 has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

8.a. Will the DNA data be used in this manuscript? ____ Yes __X_ No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER02 must be used to exclude those with value RES_DNA = "No use/storage DNA"? ____ Yes ____ No

9. The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status.
ARIC Investigators have access to the publications lists under the Study Members Area of the web site at: http://bios.unc.edu/units/cscc/ARIC/stdy/studymem.html

___X__ Yes _____ No

10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)? None

11. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire.