ARIC Manuscript Proposal # 1642

PC Reviewed: 4/13/10	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: The Association of Lipoprotein-Associated Phospholipase A_2 (Lp-PLA_2) Activity and Mass with Incident CHD and Stroke: the Atherosclerosis Risk in Communities (ARIC) Study

b. Abbreviated Title (Length 26 characters): **Lp-PLA_2 activity/mass and Incident CHD and Stroke**

2. Writing Group:

Writing group members: Ron C. Hoogeveen (lead), Christie M. Ballantyne, LE Chambless, Aaron Folsom, Gerardo Heiss, AR Sharrett, others are welcome.

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. _RH__ **[please confirm with your initials electronically or in writing]**

First author: Ron C. Hoogeveen, Ph.D.
Address: Baylor College of Medicine
Department of Medicine
The Methodist Hospital, M.S. F-701
6565 Fannin Street, Suite F-756
Houston, TX 77030

Phone: (713) 798-3407 Fax: (713) 798-7400
E-mail: ronh@bcm.tmc.edu

ARIC author to be contacted if there are questions about the manuscript and the first author does not respond or cannot be located (this must be an ARIC investigator).
Name: Christie M. Ballantyne
Address: Baylor College of Medicine
Department of Medicine
The Methodist Hospital, M.S. A-601
6565 Fannin Street, Suite A-656A
Houston, TX 77030

Phone: (713) 798-7844 Fax: (713) 798-3057
E-mail: cmb@bcm.tmc.edu

3. Timeline: Laboratory analyses will be completed in March of 2010 and data analyses by the ARIC Coordinating Center will start immediately following data submission. We expect to have a first draft of the manuscript circulated by June of 2010 and submit a manuscript for publication by September of 2010.

4. Rationale:

The hypothesis that atherosclerosis is an inflammatory disease is supported by both the discovery of inflammatory cells in the cap of atherosclerotic plaques and recent reports that elevated levels of plasma markers of inflammation are associated with incidence of CHD[1]. The oxidative modification of low-density lipoproteins (LDL) within the arterial wall is a key early event in the development of atherosclerosis[2]. Therefore, numerous studies have focused on enzymes that are involved in the oxidation of LDL and, as a result, alter the pro-inflammatory activities of oxidized LDL (oxLDL). The LDL oxidation process involves the oxidation of the polyunsaturated fatty acid component of phospholipids and ultimately leads to the conversion of phosphatidylcholine (PtdCho) to lyso-PtdCho[3]. The increased lyso-PtdCho content of oxLDL is a chemoattractant for human monocytes and induces endothelial dysfunction[4, 5].

Lp-PLA_2, also known as platelet-activating factor (PAF) acetylhydrolase, is a serine-dependent lipase that has been shown to hydrolyze oxidatively modified PtdCho to release oxidized fatty acids and lyso-PtdCho[6]. Lp-PLA_2 co-purifies with LDL and is responsible for >95% of the phospholipase activity associated with LDL[7]. Its expression is regulated by mediators of inflammation and inhibition of Lp-PLA_2 activity results in a significant decrease in both lyso-PtdCho content and monocyte chemoattractant ability of oxLDL[7, 8].

A number of large population-based studies, including the ARIC study, have previously shown that circulating levels of Lp-PLA_2 (Lp-PLA_2 mass) are associated with increased risk for incident CHD and ischemic stroke[9-14]. However, currently available data on the relationship between Lp-PLA_2 activity and risk of incident CHD and stroke is limited and inconsistent. Studies in Japanese and European populations have identified several polymorphisms in the gene encoding Lp-PLA_2, which are associated with altered Lp-PLA_2 activity and risk for incident CHD and stroke. In 1988, Miwa et al. first described the complete absence of serum Lp-PLA_2 activity in 4% of the Japanese population[15]. Stafforini et al. showed that this deficiency is caused by a missense mutation (V279F) in exon 9 of the Lp-PLA_2 gene[16], which has also been discovered in subjects from Turkey, Azerbaijan, and Kyrgyzstan[17]. This loss-of-function mutation has been shown to be associated with an increased risk for CHD[18, 19] and stroke[20] in Japanese subjects. In contrast to the genetics data in Japanese populations, the V279F loss-of-function mutation has not been found in Caucasian populations. Instead, three different missense mutations in the Lp-PLA_2 gene have been described in Caucasians[21-24]. Interestingly, one of these three missense mutations, A379V, results in a 2-fold decrease in the affinity of Lp-PLA_2 for its substrate PAF *in vitro*[22] and has been found to be associated with a lower risk for CHD in European populations[23, 24]. Although there appears to be a relatively strong positive correlation between Lp-PLA_2 mass and Lp-PLA_2 activity in

general Caucasian populations, this correlation has not been investigated in African Americans.

The purpose of this study is to investigate the association of Lp-PLA_2 activity with risk of incident CHD and ischemic stroke in the biracial cohort of the ARIC study. We will measure both Lp-PLA2 activity and mass in the entire ARIC visit 4 cohort to determine the predictive power of Lp-PLA_2 activity for future cardiovascular events and stroke in comparison to that of Lp-PLA_2 mass.

5. Main Hypothesis/Study Questions:

1) Are high plasma levels of Lp-PLA_2 activity associated with increased risk for developing CHD events and ischemic stroke after adjustment for traditional risk factors and high-sensitivity C-reactive protein (hs-CRP) in Caucasians and African Americans?

2) Is the association between Lp-PLA_2 activity and incident CHD and stroke influenced by gender, race, baseline LDL-cholesterol levels, or hypertension or diabetes status?

3) Is Lp-PLA_2 activity or the "Lp-PLA_2 mass/activity index" a stronger predictor of risk for incident CHD and stroke in Caucasians and African Americans than Lp-PLA_2 mass?

6. Design and analysis (study design, inclusion/exclusion, outcome and other variables of interest with specific reference to the time of their collection, summary of data analysis, and any anticipated methodologic limitations or challenges if present).

We are interested in a number of variables in the ARIC database including:

Sociodemographics	Smoking status	Anthropometry
Family medical history	Alcohol consumption	Blood pressure
Medical history	Physical activity	Medication use
Lipid profile	Inflammatory markers	Diabetes status

Incident cases of cardiovascular events and stroke occurring after ARIC V4

After the lab measurements are transferred to the ARIC Coordinating Center (CC) the CC will look for potential problems, such as missing data, outliers, or replicate pairs with extreme differences, and will discuss these issues with the lab. If remeasurements or corrections are needed, they will be transferred to the CC. We will then assess lab repeatability, using both an intraclasss correlation coefficient and a coefficient of laboratory variation. After applying any additional exclusions related to the particular analyte being studied, we will first implement a descriptive analysis, generally comparing cases with respect to non-cases with respect to several variables of interest, in particular the Lp-PLA_2 being studied. For analysis of the association between an analyte

measured from Visit 4 frozen samples and incident CHD and stroke after visit 4, the main analysis tool will be the Cox proportional hazards survival model, modeling log(hazard) as a linear function of Lp-PLA_2 and potential confounders and effect modifiers. Separate analyses will be performed for CHD and stroke, as well as a combined endpoint. A number of specific analyses will be included, such as analysis stratified by 1) gender, 2) race, 3) LDL cholesterol levels (above vs. below median level), 4) hypertension status,5) diabetes status, and 6) metabolic syndrome status. For these analyses, Lp-PLA_2 mass and activity will be evaluated both as categorical variables (tertiles, quartiles or quintiles) and continuous variables. We will assess improvement in predictivity by using traditional risk factors, with and without $LpPLA_2$ in specific analyses such as area under the ROC curve (AUC), net reclassification index (NRI), and IDI.

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 ICTDER03 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 ICTDER03 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 ICTDER03 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://www.cscc.unc.edu/ARIC/search.php

___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)?

11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? __X__ Yes ____ No

11.b. If yes, is the proposal
__X_ A. primarily the result of an ancillary study (list number* _2009.06_____)

___ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* _____ _____ _____)

*ancillary studies are listed by number at http://www.cscc.unc.edu/aric/forms/

12. 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.

Literature References

1. Ross R. Atherosclerosis—an inflammatory disease. N Engl J Med 1999;340:115-126.
2. Witztum JL, Steinberg D. Role of oxidized low density lipoprotein in atherogenesis. J Clin Invest 1991;88:1785-1792.
3. Parthasarathy S, Steinbrecher UP, Barnett J, Witztum JL, Steinberg D. Essential role of phospholipase A2 activity in endothelial cell-induced modification of low density lipoprotein. Proc Natl Acad Sci U S A 1985;82:3000-3004.
4. Quinn MT, Parthasarathy S, Steinberg D. Lysophosphatidylcholine: a chemotactic factor for human monocytes and its potential role in atherogenesis. Proc Natl Acad Sci U S A 1988;85:2805-2809.
5. Kume N, Cybulsky MI, Gimbrone MA Jr. Lysophosphatidylcholine, a component of atherogenic lipoproteins, induces mononuclear leukocyte adhesion molecules in cultured human and rabbit arterial endothelial cells. J Clin Invest 1992;90:1138-1144.
6. Stremler KE, Stafforini DM, Prescott SM, McIntyre TM. Human plasma platelet-activating factor acetylhydrolase. Oxidatively fragmented phospholipids as substrates. J Biol Chem 1991;266:11095-11103.
7. Tew DG, Southan C, Rice SQ, Lawrence MP, Li H, Boyd HF, Moores K, Gloger IS, Macphee CH. Purification, properties, sequencing, and cloning of a lipoprotein-associated, serine-dependent phospholipase involved in the oxidative modification of low-density lipoproteins. Arterioscler Thromb Vasc Biol 1996;16:591-599.
8. Cao Y, Stafforini DM, Zimmerman GA, McIntyre TM, Prescott SM. Expression of plasma platelet-activating factor acetylhydrolase is transcriptionally regulated by mediators of inflammation. J Biol Chem 1998;273:4012-4020.
9. Packard CJ, O'Reilly DSJ, Caslake MJ, et al. Lipoprotein-associated phospholipase A_2 as an independent predictor of coronary heart disease. *N Engl J Med* 2000;343:1148-1155.
10. Ballantyne C, Hoogeveen R, Bang H, et al. Lipoprotein-Associated Phospholipase A2, High-Sensitivity C-Reactive Protein, and Risk for Incident Coronary Heart Disease in Middle-Aged Men and Women in the Atherosclerosis Risk in Communities (ARIC) Study *Circulation* 2004;109:837-842.
11. Ballantyne C, Hoogeveen R, Bang H, et al. Lipoprotein-Associated Phospholipase A2, High-Sensitivity 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;165:2479-2484

12. Blake GJ, et al. A Prospective Evaluation of Lipoprotein-Associated Phospholipase A2 Levels and the Risk of Future Cardiovascular Events in Women. *J Am Coll Cardiol.* 2001;38(5):1302-1306.
13. Oei H-HS , et al. Lp-PLA2 is Associated with Risk of CAD and Stroke. The Rotterdam Study. Circulation 2005;111:570-575
14. Blankenberg S, et al. Plasma PAF-acetylhydrolase in patients with coronary artery disease: results of a cross-sectional analysis. J Lipid Res 2003; 44: 1381–1386.
15. Miwa M, Miyake T, Yamanaka T *et al.* Characterization of serum platelet-activating factor (PAF) acetylhydrolase. Correlation between deficiency of serum PAF acetylhydrolase and respiratory symptoms in asthmatic children. *J. Clin. Invest*. 82, 1983–1991 (1988).
16. Stafforini DM, Satoh K, Atkinson DL *et al.* Platelet-activating factor acetylhydrolase deficiency. A missense mutation near the active site of an anti-inflammatory phospholipase. *J. Clin. Invest*. 97, 2784–2791 (1996).
17. Balta G, Gurgey A, Kudayarov DK, Tunc B, Altay C. Evidence for the existence of the PAF acetylhydrolase mutation (Val279Phe) in non-Japanese populations: a preliminary study in Turkey, Azerbaijan, and Kyrgyzstan. *Thromb. Res*. 101, 231–234 (2001).
18. Yamada Y, Yoshida H, Ichihara S, Imaizumi T, Satoh K, Yokota M. Correlations between plasma platelet-activating factor acetylhydrolase (PAF-AH) activity and PAF-AH genotype, age, and atherosclerosis in a Japanese population. *Atherosclerosis*. 150, 209–216 (2000).20.
19. Shimokata K, Yamada Y, Kondo T *et al.* Association of gene polymorphisms with coronary artery disease in individuals with or without nonfamilial hypercholesterolemia. *Atherosclerosis.* 172, 167–173 (2004).
20. Hiramoto M, Yoshida H, Imaizumi T, Yoshimizu N, Satoh K. A mutation in plasma platelet-activating factor acetylhydrolase (Val279-->Phe) is a genetic risk factor for stroke. *Stroke*. 28, 2417–2420 (1997).
21. Bell R, Collier DA, Rice SQ *et al.* Systematic screening of the LDL-PLA2 gene for polymorphic variants and case-control analysis in schizophrenia. *Biochem. Biophys. Res. Commun*. 1997; 241:630-5.
22. Kruse S, Mao XQ, Heinzmann A *et al.* The Ile198Thr and Ala379Val variants of plasmatic PAF-acetylhydrolase impair catalytical activities and are associated with atopy and asthma. *Am. J. Hum. Genet*. 66, 1522–1530 (2000).
23. Abuzeid AM, Hawe E, Humphries SE, Talmud PJ. Association between the Ala379Val variant of the lipoprotein associated phospholipase A2 and risk of myocardial infarction in the north and south of Europe. *Atherosclerosis*. 168, 283–238 (2003).
24. Ninio E, Tregouet D, Carrier J-L *et al.* Platelet-activating factor-acetylhydrolase and PAF-receptor gene haplotypes in relation to future cardiovascular event in patients with coronary artery disease. *Hum. Mol. Genet.* 13, 1341–1351 (2004).