

ARIC Manuscript Proposal # 1642

PC Reviewed: 4/13/10
SC Reviewed: _____

Status: A
Status: _____

Priority: 2
Priority: _____

1.a. Full Title: The Association of Lipoprotein-Associated Phospholipase A₂ (Lp-PLA₂) Activity and Mass with Incident CHD and Stroke: the Atherosclerosis Risk in Communities (ARIC) Study

b. Abbreviated Title (Length 26 characters): Lp-PLA₂ 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
E-mail: ronh@bcm.tmc.edu

Fax: (713) 798-7400

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
E-mail: cmb@bcm.tmc.edu

Fax: (713) 798-3057

- 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 CHD¹. The oxidative modification of low-density lipoproteins (LDL) within the arterial wall is a key early event in the development of atherosclerosis². 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-PtdCho³. The increased lyso-PtdCho content of oxLDL is a chemoattractant for human monocytes and induces endothelial dysfunction^{4, 5}.

Lp-PLA₂, 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-PtdCho⁶. Lp-PLA₂ co-purifies with LDL and is responsible for >95% of the phospholipase activity associated with LDL⁷. Its expression is regulated by mediators of inflammation and inhibition of Lp-PLA₂ 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-PLA₂ (Lp-PLA₂ mass) are associated with increased risk for incident CHD and ischemic stroke⁹⁻¹⁴. However, currently available data on the relationship between Lp-PLA₂ 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-PLA₂, which are associated with altered Lp-PLA₂ activity and risk for incident CHD and stroke. In 1988, Miwa et al. first described the complete absence of serum Lp-PLA₂ activity in 4% of the Japanese population¹⁵. Stafforini et al. showed that this deficiency is caused by a missense mutation (V279F) in exon 9 of the Lp-PLA₂ gene¹⁶, which has also been discovered in subjects from Turkey, Azerbaijan, and Kyrgyzstan¹⁷. This loss-of-function mutation has been shown to be associated with an increased risk for CHD^{18, 19} and stroke²⁰ 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-PLA₂ gene have been described in Caucasians²¹⁻²⁴. Interestingly, one of these three missense mutations, A379V, results in a 2-fold decrease in the affinity of Lp-PLA₂ 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-PLA₂ mass and Lp-PLA₂ 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-PLA₂ 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-PLA₂ activity for future cardiovascular events and stroke in comparison to that of Lp-PLA₂ mass.

5. Main Hypothesis/Study Questions:

- 1) Are high plasma levels of Lp-PLA₂ 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-PLA₂ activity and incident CHD and stroke influenced by gender, race, baseline LDL-cholesterol levels, or hypertension or diabetes status?
- 3) Is Lp-PLA₂ activity or the “Lp-PLA₂ mass/activity index” a stronger predictor of risk for incident CHD and stroke in Caucasians and African Americans than Lp-PLA₂ 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 intraclass 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-PLA₂ 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-PLA₂ 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-PLA₂ 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 LpPLA₂ 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
 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
 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.csc.unc.edu/ARIC/search.php>

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? Yes No

11.b. If yes, is the proposal

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.csc.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.

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