ARIC Manuscript Proposal #2949

1.

a. **Full Title:** The role of lipoprotein-associated phospholipase A2 activity compared to high-sensitivity C-reactive protein in identifying high risk smokers for coronary heart disease and stroke: The Atherosclerosis Risk in Communities (ARIC) study

b. **Abbreviated Title (Length 26 characters):** Lp-PLA2, CRP, smoking and CVD

2. **Writing Group:**
   - Martin Tibuakuu (first author, analyst)  Johns Hopkins
   - Di Zhao (second author, co-analyst)  Johns Hopkins
   - Sina Kianouush (third author)  Johns Hopkins
   - Andrew P. DeFilippis  University of Louisville
   - J. William (Bill) McEvoy  Johns Hopkins
   - Christie M. Ballantyne  Baylor College of Medicine
   - Ron C. Hoogeveen  Baylor College of Medicine
   - Eliseo Guallar  Johns Hopkins
   - Michael J. Blaha  Johns Hopkins
   - Erin D. Michos (senior author)  Johns Hopkins

(*A note about the multiple Hopkins authors: Martin Tibuakuu, an American Heart Association Tobacco Regulation Center (A-TRAC) Fellow and Erin Michos designed the concept. Erin Michos will guide project as senior author. Di Zhao, a senior analyst, will supervise the analysis of this project. Eliseo Guallar is Di’s mentor and will oversee the statistical analyses. Sina Kianouush is also an A-TRAC fellow with experience in tobacco regulatory science. Bill McEvoy has published on cigarette smoking and cardiovascular events in MESA and provided critical scientific input. Michael Blaha is an A-TRAC principal investigator and Martin’s A-TRAC mentor.

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

**First author:** Martin Tibuakuu, MD, MPH

**Address:** The Johns Hopkins School of Public Health
2213 McElderry St, 1st FL
Baltimore, MD 21205
Phone: 443-850-1407
Fax: 410-614-9190
E-mail: mtibuak1@jhu.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).
3. **Timeline:** We aim to submit an abstract for the AHA meeting (submission deadline is June 2017) with full manuscript by end of 2017.

4. **Rationale:**

Cigarette smoking is one of the major preventable causes of death and cardiovascular disease (CVD) globally.[1, 2] However, despite the putative causal role of cigarette smoking in CVD events, the biological mechanisms are not fully understood. Furthermore, while some data report a dose-response relation of smoking intensity and burden with cardiovascular risk factors and disease,[3-5] others suggest that the relationship between smoking and cardiovascular disease has a low “ceiling” making smoking status paramount to smoking burden or intensity.[6-8]

While smoking exposure is assumed to be harmful to everyone, only about 33% of smokers develop a smoking related cardiovascular illness.[2] This relationship is poorly understood and is not exclusively explained by exposure amount or intensity. Identifying current and former smokers who are the most vulnerable to smoking-induced CVD would allow for targeted and more-intensive prevention strategies to be implemented for these high-risk individuals. Before the onset of clinical atherosclerotic cardiovascular disease (ASCVD), subclinical cardiovascular risk markers involving systemic inflammation may help to identify smokers at high risk of clinical ASCVD.[9] Biomarkers of inflammation, including high sensitivity C-reactive protein (hsCRP),[10] interleukin 6 (IL-6),[11] intercellular adhesion molecules (ICAM),[12] and myeloperoxidase (MPO)[13], have all been demonstrated as useful tools in predicting ASCVD risk.

Indeed, prior studies have demonstrated that inflammation is on the causal pathway linking smoking to ASCVD.[14, 15] Among inflammatory biomarkers associated with ASCVD risk, hsCRP has increasingly been identified as a sensitive biomarker for measuring subclinical tobacco-induced atherosclerotic cardiovascular disease.[3, 16] In their MESA study, McEvoy et al. reported that hsCRP could be useful in identifying high-risk smokers needing intensive smoking cessation efforts.[4] However, hsCRP when used alone was not consistent in identifying high-risk smokers.[4] Therefore, more studies are needed to find biomarkers that can be helpful in identifying smokers who are at higher risk for cardiovascular events.

Lipoprotein associated phospholipase A₂ (Lp-PLA₂) is a serine-dependent lipase secreted by inflammatory cells in atherosclerotic plaques.[17] It is a marker of plaque vulnerability and its activity has been found to independently predict the risk for coronary heart disease (CHD), stroke and all-cause mortality.[18, 19]. A consensus panel formed in 2008 to review the use of Lp-PLA₂ in cardiovascular risk prediction recommended that individuals with high Lp-PLA₂ activity level may benefit from intensified preventive measures including use of lipid lowering therapy, blood pressure control and lifestyle changes.[20] In December 2014, the US Food and Drugs Administration approved Lp-PLA₂ activity for clinical use in predicting CHD.[21] Lp-PLA₂ activity may therefore
be independently superior or complementary to hsCRP in identifying high-risk smokers for incident CHD and stroke for prognostication and possibly more aggressive treatment purposes.

The ARIC study’s large sample size and long follow-up time for events, in addition to the presence of detailed smoking data offers an opportunity to reliably investigate markers appropriate for routine clinical use to identify high-risk smokers. This proposal, which focuses on the impact of smoking and inflammation on CVD risk, is part of larger work being conducted by investigators affiliated with the AHA Tobacco Regulation Center (A-TRAC) at Johns Hopkins.

5. Main Hypothesis/Study Questions:

a. Aims:
   1) To evaluate whether there is a linear dose-response relationship between smoking (i.e. status, burden and intensity) and the inflammatory subclinical cardiovascular disease risk markers, measured using Lp-PLA2 activity and hsCRP at baseline.
   2) To evaluate whether Lp-PLA2 activity would be more strongly associated with incident CHD and stroke risk among smokers compared to hsCRP.
   3) To evaluate whether Lp-PLA2 activity improves risk prediction for incident CHD and stroke among active and former smokers beyond what is ascertained with hsCRP alone.

We hypothesize that:
   1) Higher smoking intensity and burden will be associated with higher levels of hsCRP and Lp-PLA2 activity (in a linear dose-response association).
   2) Lp-PLA2 activity will equally or more consistently identify high-risk smokers (those at greater risk of CHD and stroke), as compared to hsCRP.
   3) Lp-PLA2 used together with hsCRP will better predict absolute and relative CHD and stroke risk among current and former smokers than hsCRP or Lp-PLA2 activity alone.

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).

a. Participant inclusion/exclusion criteria
   We will investigate these study questions in ARIC, a large, prospective, predominantly biracial population-based cohort of individuals who were recruited from 4 U.S. field centers and aged 45-64 yrs at enrollment in 1987-1989 (visit 1). Visit 4 (1996-1998), the visit of Lp-PLA2 measurement, will be the baseline for this analysis. All participants with available data on smoking status, Lp-PLA2 activity, and hsCRP levels at visit 4 will be included. We will exclude participants with prevalent CHD and stroke at visit 1 or incident CHD or stroke occurring before or at Visit 4, those with missing key covariates (in our model 1), and participants whose race is neither black nor white (given their small numbers and inability to stratify them by race/center). A prior ARIC study noted that 11,656 individuals attended ARIC visit 4 and 403 were missing Lp-PLA2 values.[22] Thus after additional exclusions for our sample, we estimate our sample size will be approximately ~10,000.

b. Study Design: This will be a two-part analysis:
   2) A longitudinal analysis using proportional hazard model to evaluate the relation of hsCRP and Lp-PLA2 activity with incident CHD and stroke, stratified by categories of smoking
status, burden, and intensity from Visit 4 to December 31, 2013 (or date of last available follow-up).

c. **Exposures:**

Measures of smoking behavior:
- Smoking status: never, former, and current smokers
- Smoking burden: pack-years of smoking (for current and former smokers)
- Smoking intensity: number of cigarettes per day (for current smokers)
- Years since quitting (for former smokers)

Smoking history was ascertained by means of an interviewer-administered questionnaire. Smoking status was established at visit 4 by asking participants whether they currently smoked cigarettes or whether they had done so in the past. Cumulative pack-years and intensity of smoking are also available for participants at visit 4.

**Outcomes:**

The primary outcomes will be:

For cross-sectional analysis:
1) Level of Lp-PLA2 activity in Visit 4 plasma by an automated Colorimetric Activity Method (CAM) assay (diaDexus Inc., South San Francisco, CA) using a Beckman Coulter (Olympus) AU400e autoanalyzer.
2) Level of hsCRP in Visit 4 serum by a CRP-Latex (II) high sensitivity assay from Denka Seiken (Tokyo, Japan).

For longitudinal analysis:
1) Incident CHD defined as definite or probable myocardial infarction, or definite coronary death occurring after Visit 4 through December 31st 2013, or the most recent follow-up available.
2) Incident ischemic stroke defined per the National Survey of Stroke criteria adapted by ARIC[23] and occurring after Visit 4 to most recent follow-up available.

As a secondary outcome, we will consider total ASCVD (CHD plus stroke).

d. **Covariates:** Other covariates will include: age, sex, race*, education, income, family history of CHD, alcohol use, body mass index (BMI), physical activity level, systolic blood pressure, diabetes, total and HDL-cholesterol, use of medications (statins, antihypertensives and NSAIDs/steroids), and estimated glomerular filtration rate (eGFR) using CKD-Epi formula.[24]

*Since race is strongly correlated with field center in ARIC, we will consider race/center groups as previously done in other ARIC papers.

e. **Main analyses:**

Baseline characteristics of the study population will be described using means, medians, and proportions by categories of smoking status.

For cross-sectional analysis, we will naturally log-transform hsCRP and Lp-PLA2 activity if they are not normally distributed. The associations of the smoking variables (the independent variables) with hsCRP and Lp-PLA2 activity (the dependent variables) will be evaluated using multivariable linear regression models. Models will progressively adjust for covariates as follows:
- Model 1 will adjust for age, sex, race-center groups (i.e. Minnesota-whites; Maryland-whites; North Carolina-whites; North Carolina-blacks; Mississippi-blacks), income and education.
Model 2 will be Model 1 plus further adjustment for alcohol use, family history of CHD, physical activity level, BMI, systolic blood pressure, total cholesterol, HDL-cholesterol, diabetes mellitus, eGFR, and medication use (statins, antihypertensives and NSAIDs/steroids)

Additionally, smoking duration will be adjusted for in all Models during intensity analysis to estimate the effect of smoking intensity on the inflammatory subclinical cardiovascular risk markers independent of smoking duration.

To facilitate comparisons with previous studies,[16] we will evaluate the associations of smoking behaviors with hsCRP and Lp-PLA₂ activity per 5-unit increase in cumulative exposure or burden (pack-years) among former and current smokers, per 5-year interval in time since quitting smoking, and per 10-unit increase in number of cigarettes per day among current smokers. Finally, adjusted restricted cubic spline models will be used to test a linear dose-response association of smoking intensity and burden with subclinical cardiovascular risk measured by hsCRP and Lp-PLA₂ activity.

For longitudinal analysis, we will categorize pack-years of smoking as quartiles,[4] intensity as <10, 10-19 and ≥20 cigs/day,[3] hsCRP as <1, 1-3 and >3 mg/L (AHA/CDC risk categories);[25] and Lp-PLA₂ activity as tertiles.[26]

Cox proportional hazard models adjusting for variables in Models 1 and Model 2 will be used to investigate the independent associations of Lp-PLA₂ and hsCRP (analyzed separately) with incident CHD and stroke, stratified by categories of smoking burden and intensity. Interaction testing will be performed between the inflammatory biomarkers with smoking categories for incident CHD and stroke. We will also assess model fitness of Lp-PLA₂ vs. hsCRP for incident CHD and stroke using statistical methods such as AIC and area under the ROC. This will help determine whether Lp-PLA₂ or hsCRP is a better biomarker for identifying which smokers are at highest risk for events. Additionally, we will evaluate Lp-PLA₂ activity and hsCRP in the same adjusted Cox model to evaluate their independent value after adjusting for the other inflammatory marker, for identifying the highest risk smokers for incident CHD and stroke.

All statistical analyses will be performed using Stata 13 (StataCorp LP, College Station, TX) and significance will be considered at P value<0.05.

f. Secondary analyses:
   1) As a secondary outcome, we will consider total ASCVD (CHD plus stroke) as a composite outcome.

g. Sensitivity analyses:
   1) We will repeat the longitudinal analysis using the clinical cut point of ≤225 nmol/min/mL and > 225 nmol/min/mL for Lp-PLA₂ activity.
   2) We will study whether there are interactions between measures of smoking behavior and age, sex, and race in their associations with Lp-PLA₂ and hsCRP in cross-sectional analysis.
   3) Similarly, we will test for various potential interactions of age, sex, and race in longitudinal analysis.

h. Limitations:
Smoking exposure in ARIC is only by self-report and does not include information on urine cotinine levels to corroborate self-reported smoking behaviors to avoid misclassification.
7. Will the data be used for non-CVD analysis in this manuscript? ____ Yes __X__ No

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

ARIC Investigators have access to the publications lists under the Study Members Area of the web site at: http://www.cscu.unc.edu/ARIC/search.php

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)?

There are several manuscripts about Lp-PLA2, hsCRP and CHD and stroke risk, notably ARIC Manuscript Proposal # 1642.

There is a manuscript about smoking and inflammatory biomarkers (ARIC Manuscript Proposal #1862), and a manuscript about smoking and myocardial injury (specifically NT-proBNP and hs-cTnT, ARIC Manuscript Proposal #2463).

But there were no manuscripts proposals on the use of inflammatory biomarkers for CHD/stroke risk stratified by smoking sub-groups with the goal of identifying high-risk smokers who may need intensified cessation therapy or other targeted prevention methods. Therefore there is no overlap. Some of the authors of these above manuscripts have been invited to this writing group to again ensure no overlap.

11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? __X__ Yes ____ No
b. If yes, is the proposal
   1) primarily the result of an ancillary study (list number* __2009.06____)
   2) 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.cscu.unc.edu/aric/forms/ 2009.06 (Hoogeveen)

The Relation of Lipoprotein-Associated Phospholipase A2 (Lp-PLA2) activity and mass to Incident CHD and stroke in Middle-Aged Men and Women

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.
Reference: