
b. Abbreviated Title (Length 26 characters): Lp-PLA₂ and Risk of Stroke by RP

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

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Note: Dr. Myerson is a cardiologist who, while a Medical Officer at NHLBI (Epidemiology and Biometry,) served as Project Officer for the ARIC Study. She is currently first author of an ARIC manuscript that is being reviewed by Circulation; the editors have asked for revisions and the revised manuscript is now being considered.
3. Timeline:

This is a secondary data analysis. Statistical analysis will begin as soon as ARIC approval is granted and data will be sent to statisticians Dr. Heejung Bang (a former ARIC statistician at the data coordinating center) and Dr. Stanly Young (a leading expert in recursive partitioning at NISS). Analysis will take place from Sep 2008-Nov 2008 and manuscript preparation will take place from Nov 2008-Jan 2008 with journal submission aiming in the end of Jan 2009.

4. Rationale:

**Lp-PLA2: Background**

Lipoprotein Phospholipase A2 (Lp-PLA2) is a novel cardiovascular marker and is increasingly being recognized for it’s value in predicting risk for stroke and heart attack. Lp-PLA2 is an enzyme that plays a critical role in vascular inflammation, atherogenesis, and formation of vulnerable plaque. Levels are not influenced by systemic inflammation and are independent of age, systolic blood pressure, Triglycerides, LDL-cholesterol, HDL-cholesterol, body mass index, smoking, and other inflammatory markers. Statins, fenofibrates, and niacin lower Lp-PLA2 by 20-40%; Niacin further lowers Lp-PLA2 in patients already on satin therapy (Kuvio Am J Cardiol. 2006;98:743). Based on a consensus panel report, an Lp-PLA2 score greater than 235 ng/mL indicates a patient may be considered at higher risk for stroke and heart attack (Lanman R, et al. Prev Card. 2006;9:138). High risk patients, e.g. coronary risk equivalents, have also been shown to be at risk for recurrent CV events when Lp-PLA2 is elevated. Conversely, patients with an Lp-PLA2 below 200 ng/mL appear to have low risk for recurrent events. In addition, recent research has suggested that Lp-PLA2 may also be a novel therapeutic target (Mohler ER, et al. J Am Coll Cardiol. 2008;51:1632).

As Lp-PLA2 appears to be emerging as an important diagnostic, predictive, and therapeutic tool it is essential that it’s unique and independent contribution is understood. Recursive partitioning-based risk stratification offers another approach with which to evaluate the contribution that Lp-PLA2 may make to prevention and management of cardiovascular disease.

**Lp-PLA2: Epidemiology**

The role of Lp-PLA2 as a marker of risk for CVD has been determined from epidemiological studies where an increased level had prognostic implications in patients with and without CVD.

Lp-PLA2 has been shown to be a useful clinical biomarker independent of traditional and other emerging risk factors. Koenig, et al determined plasma concentrations of Lp-PLA2 in 1051 patients aged 30-70 with coronary artery disease. At four years, Lp-PLA2 elevation was strongly associated with cardiovascular events after controlling for traditional risk factors, severity of CAD, and statin treatment (Koenig W, et al.)
Koenig, et al. also investigated this association in 934 apparently healthy men aged 45-64 sampled from the general population and followed for 14 years. Baseline levels of Lp-PLA₂ were higher in subjects who experienced an event than in event-free subjects (Koenig W, et al. Circulation. 2004;110:1903.).

In the Northern Manhattan Stroke Study, Elkind, et al. looked at 467 patients, mean age 69 years with first ischemic stroke and found that Lp-PLA₂ may be a stronger predictor of recurrent stroke than C-reactive protein (CRP) (Elkind MSV, et al. Arch Intern Med. 2006;166:2073). The West of Scotland Coronary Prevention Study (WOSCOPS) included patients with dyslipidemia but no known coronary artery disease. Lp-PLA₂ levels were shown to be an independent predictor of coronary events. (Packard CJ, et al. N Engl J Med. 2000;343:1148.)

ARIC Studies
Two studies in the ARIC population have already investigated both Lp-PLA₂ and hs-CRP. Both were based on prospective case-cohort studies. The first investigated the relation between Lp-PLA₂, CRP, traditional risk factors, and risk for coronary heart disease events over a period of six years. In case-cohort samples, Lp-PLA₂, and CRP were significantly higher in 608 cases than 740 non-cases. In a model adjusted for traditional risk factors including LDL, Lp-PLA₂ alone did not seem to have high predictive power for coronary heart disease. However, Lp-PLA₂ and hs-CRP interacted each other and the two biomarkers together were significantly associated with coronary heart disease (Ballantyne CM, et al. Circulation. 2004;109:837).

The second study investigated the association of Lp-PLA₂ and hs-CRP with ischemic stroke in the ARIC population. Mean Lp-PLA₂ and CRP levels were elevated in the 194 stroke cases than the 766 non-cases and LDL was not significantly different in the two groups. In a model that adjusted for traditional risk factors, Lp-PLA₂ and hs-CRP levels in the high category in both risk factors (i.e., the highest tertile for Lp-PLA₂ and hs-CRP > 3 mg/L based on AHA/CDC guideline) were associated with an estimated hazard ratio of 11 (95% CI, 3-41 p< 0.001). (Ballantyne CM, et al. Arch Intern Med. 2005;165:2479). In the follow-up paper, it was demonstrated that Lp-PLA₂ and hs-CRP improved the stratification of stroke risk in the same ARIC dataset (Nambi et al. In Press in Stroke, Ballantyne as senior author).

We now propose to reanalyze the ARIC case-cohort data for stroke in order to establish risk stratification strategies for stroke using tree regression.

5. Main Hypothesis/Study Questions:

To develop recursive partitioning-based risk stratification for incident ischemic stroke using traditional risk factors and novel risk factors such as Lp-PLA₂ or hs-CRP. The rationale for this question is straightforward as many previous studies in ARIC have examined whether addition of new risk markers improves risk assessment beyond
established risk assessment equations or algorithms for vascular diseases. Lp-PLA2 has been shown to be an important risk factor for stroke and to improve risk profiling in combination with hs-CRP. Now is a good time to develop a risk stratification algorithm, which may offer informed decision making for prevention and, possibly, treatment of stroke. Recursive partitioning would provide a user-friendly graphical way to stratify patients into groups that have similar risk (e.g., low, mid vs. high risk) and to elucidate the role of Lp-PLA2 in the presence of complex interactive effects among multiple risk factors.

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 will reanalyze a case-cohort data for incident ischemic stroke using tree regression called recursive partitioning (RP) technique focusing on the roles of Lp-PLA2 in risk stratification. For tree analysis that can investigate interactive effects and optimal cutpoints of risk factors, we plan to use Partitionator powered by the patented FIRMplus RP engine. Key advantages that have been noted are that this software can view all multivariate relationships without being confined to fitting empirical correlations that lack predictive power and it can create random forests to find correlations and interactions among mixed types of variables. Statistical significance, reflected in p-values, will be adjusted for multiple comparisons. At each step of the splitting process, complex counting is done. The p-values are adjusted based on massive simulations to build rules. There is an additional ability to use resampling to verify the correctness of a split (Hawkins and Kass 1982; Lambert 2004; Young and Ge 2005; Zaykin and Young 2005). RP will be visualized in a tree shape plot with leaves connected with nodes hierarchically. Model validation will be conducted as well. One disadvantage of tree models is that they produce discontinuous predictions and the main disadvantage of standard regression models such as Cox regression model that we adopted for previous papers is that they enforce strict smoothness everywhere (Ridgeway 2003). We will analyze the data with the outcome as case vs. non-case so we can not account for the design aspects of our case-cohort study.

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://www.cscce.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)? Prediction of ischemic stroke risk in ARIC" and "Lp-PLA₂, hs-CRP, and risk of incident ischemic stroke in ARIC" Ballantyne, et al.

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

11.b. If yes, is the proposal

___ A. primarily the result of an ancillary study (list number* _________)

___ 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.cscce.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.