ARIC Manuscript Proposal # 1827

PC Reviewed: 8/9/11	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Relation of Lipoprotein (a) and small dense LDL (sdLDL) to incident CVD: the ARIC study

b. Abbreviated Title (Length 26 characters): **Lp(a) and sdLDL and incident CVD in ARIC**

2. Writing Group:

Writing group members: John Gaubatz, David Couper, Salim Virani, Jennifer Jiang, Eric Boerwinkle, Christie Ballantyne, Ron Hoogeveen, others are welcome.

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

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3. Timeline: All laboratory measurements of plasma Lp(a) and sdLDL levels on the entire ARIC Visit 4 cohort have been completed and data was submitted to the ARIC CC. Manuscript preparation will start as soon as the manuscript proposal has been approved. We anticipate journal submission of the completed manuscript within 4 months after manuscript proposal approval.

4. Rationale:

1. A number of large population-based studies, including the ARIC study, have previously shown that circulating levels of Lp(a) are associated with increased risk for incident CHD. Furthermore, Lp(a) was also associated with ischemic stroke in the ARIC study among women but not men. In the ARIC study, Lp (a) measurements were made at the baseline examination using an immunological method which is sensitive to the apo(a) isoform size. Recently, a Lp(a) assay has been developed which is not affected by apo(a) size. This assay has been shown to reclassify 5–15% of individuals who are at risk for cardiovascular disease. The purpose of this study is to investigate the association of Lp(a) with risk of incident CHD and ischemic stroke in the biracial cohort of the ARIC study using the newly developed Lp(a) assay. We will measure Lp(a) in the entire ARIC visit 4 cohort to determine the predictive power of Lp(a) concentration, independent of apo (a) size, for future cardiovascular events and stroke in comparison to previous ARIC data obtained with the "older generation" Lp(a) assay.

2. Small dense LDL (sdLDL) has been found to be associated with increased risk for vascular disease in cross-sectional studies as well as prospective observational studies. Furthermore, sdLDL concentration is highly correlated with triglyceride concentration and is increased in individuals with an atherogenic lipoprotein profile, e.g., patients with diabetes and patients with the metabolic syndrome. The purpose of this study is to investigate the association of sdLDL with risk of incident CHD, ischemic stroke and metabolic syndrome in the biracial cohort of the ARIC study.

Background:

Lp(a) contains a lipoprotein moiety that is similar to LDL in lipid composition and the presence of apo B but contains a unique glycoprotein, apo(a), which is covalently attached to apo B by a single disulfide bond.[1] Apo(a) contains a variable number of identically repeated copies of kringle IV type 2 domains, leading to differences in Lp(a) size and molecular weight. The sequence of apo(a) is similar to that of plasminogen[2]. The presence of the plasminogen-like moieties in Lp(a) have led investigators to hypothesize that Lp(a) constitutes a unique link between atherosclerosis and thrombosis. Lp(a) is present in atherosclerotic lesions, with plaque accumulation related to levels of Lp(a) in plasma.[3]

Levels of Lp(a) have much greater variability in humans than levels of LDL or HDL, and this is believed to be primarily due to production rather than catabolism.[4] In Caucasians, 90% of variability in plasma Lp(a) levels is thought to be determined at the level of the gene, and the number of kringle IV type 2 domain repeats contributes a large amount to the variation in levels.[5] In general, there is an inverse relation between apo(a) isoform size and plasma levels of Lp(a).[6] However, isoform size does not explain plasma levels for many individuals, as African Americans have higher Lp(a) levels than Caucasians despite similar apo(a) isoform size.[7]

The role of Lp(a) in CHD risk has been examined in many studies. A meta-analysis of 27 prospective studies with more than 5,000 incident CHD cases and mean follow-up of 10 years showed that Lp(a) levels in the top third were associated with a 70% increase in risk for CHD compared with those in the bottom third.[8] In a case–control analysis from the ARIC study, which included 725 incident CHD cases, Lp(a) was found to be an independent predictor of incident CHD, with a relative risk of 1.17 per standard deviation increase in a model that also included LDL-C, HDL-C, and triglycerides.[9] Lp(a) was also associated with stroke in the ARIC study among women but not men.[10] Several studies have suggested that

the risk associated with elevated Lp(a) levels may be augmented in individuals with high levels of LDL-C. In the Prospective Epidemiological Study of Myocardial Infarction (PRIME), Lp(a) levels were associated with increased risk for MI and angina, with the greatest effect in individuals with high LDL-C.[11] In addition, the Women's Health Study found that high levels of Lp(a) (>90th percentile) were associated with increased cardiovascular risk especially in women with high LDL-C levels.[12]

Lack of standardization of Lp(a) measurement has been a major barrier to both clinical research and defining the role of Lp(a) in clinical practice. Measurement of Lp(a) usually relies on immunological methods, and antibodies that react with the repeating kringle IV type 2 domain are sensitive to the apo(a) isoform size. Marcovina has developed an assay for Lp(a) using a monoclonal antibody to a unique epitope located in apo(a) kringle IV type 9 which does not repeat.[13] This assay was shown to reclassify 5–15% of individuals.[14] The Lp (a) assay manufactured by Denka Seiken, which we intend to use in the current study, has been validated against the Marcovina assay using WHO approved reference materials. In the Physicians' Health Study, baseline Lp(a) assessed by this method was associated with the subsequent development of angina whereas Lp(a) assessed by a commercial assay was not associated with subsequent angina.[15] Because this method is not affected by apo(a) size, Lp(a) values are less likely to be over- or underestimated than with other immunological methods. Although some studies have shown apo(a) isoforms to be associated with cardiovascular disease independent of Lp(a) concentration,[15, 16] this association has not been consistently found in other studies.[17, 18]

LDL particles are heterogeneous in size and composition. Considerable *in vitro* evidence indicates that small dense LDL is more atherogenic than large buoyant LDL. Small dense LDL particles can enter the arterial wall more easily than large buoyant LDL.[19] Studies have shown that small dense LDL particles are more susceptible to oxidation, exhibit increased toxicity to vascular endothelial cells,[20, 21] have greater affinity for glycoproteins of the arterial wall, and bind more readily to scavenger receptors than to the classic LDL receptor.[22, 23] The distribution of LDL subfractions is determined by both genetic and environmental factors.[24, 25] However, the single most important determinant of the LDL particle size is the size of the pool of triglyceride-rich lipoproteins (i.e. VLDL). Therefore, it is not surprising that small dense LDL concentration is highly correlated with triglyceride concentration and is increased in individuals with an atherogenic lipoprotein profile, e.g., patients with diabetes and patients with the metabolic syndrome.[26] Although LDL can be separated on the basis of size into as many as 7 subclasses by electrophoresis,[27] LDL is most commonly separated into two phenotypes. These phenotypes are commonly known as "pattern A" (characterized by a preponderance of large buoyant LDL particles) and "pattern B" (characterized by a preponderance of small dense LDL particles).[28]

Small dense LDL has been found to be associated with increased risk for vascular disease in cross-sectional studies[29-31] as well as prospective observational studies.[32-34] Furthermore, several clinical trials have shown that lipid-lowering therapy slowed the rate of progression of CHD, which was associated with a decrease in small dense LDL concentration.[35-38] However, in most of these studies, small dense LDL did not remain an independent risk predictor when adjusted for other cardiovascular risk factors such as triglyceride and total cholesterol/HDL-C ratio. The Québec Cardiovascular Study showed that the presence of small dense LDL did not substantially increase the risk of CHD in subjects that did not have an increased number of LDL particles.[34] However, in subjects who had an increased number of LDL particles as well as small dense LDL present (pattern B), the risk for CHD was increased 6-fold.[39] Furthermore, in contrast to LDL size, LDL particle number as measured by nuclear magnetic resonance (NMR) spectroscopy has more consistently been shown to be an independent predictor of CHD.[40] Taken together, these data indicate that it is the combination of increased numbers of LDL particles and the presence of small dense LDL that is highly atherogenic.[41]

These findings have sparked debate regarding the importance of LDL particle size versus LDL particle number. Some investigators have argued that measurement of LDL particle size does not add independently (statistically) to the risk prediction of CHD when LDL particle number is assessed either by measuring apo B or by NMR spectroscopy.[42] However, a number of imaging studies [36, 43] have demonstrated that the therapeutic modification of LDL size or the number of small dense LDL particles is associated with reduced progression of atherosclerosis. It is currently not known whether the increased cardiovascular risk associated with small dense LDL is a consequence of its increased atherogenicity or is instead caused by a broader underlying dyslipidemic pathophysiology. Although some have argued that changes in LDL particle size may be a target of therapy, additional research is necessary, as some therapies that increase LDL particle size (CETP inhibition, rosiglitazone) have not reduced CHD risk.

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5. Main Hypothesis/Study Questions:

Primary Hypotheses:

1) Lp(a) and sdLDL-C are associated with increased risk for developing CHD events after adjustment for traditional risk factors and high-sensitivity C-reactive protein (hs-CRP) in Caucasians and African Americans.

2) Lp(a) and sdLDL-C are associated with increased risk for developing ischemic stroke after adjustment for traditional risk factors and high-sensitivity C-reactive protein (hs-CRP) in Caucasians and African Americans.

Secondary Hypotheses:

3) Elevated plasma levels of sdLDL-C are associated with the presence of individual Components of the metabolic syndrome and risk for developing diabetes in Caucasians and African Americans.

4) Elevated plasma levels of Lp(a) and sdLDL-C are associated with carotid atherosclerosis as determined by MRI after adjustment for traditional risk factors and hs-CRP.

5) The recent completion of Lp-PLA_2 mass and activity measurements on the entire visit 4 cohort will allow us to explore possible relationships between Lp-PLA_2 (mass and

activity) and sdLDL-C and Lp(a). To our knowledge, the ARIC study is the largest study with data available on all these lipid 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).

Overview: To test our hypotheses, we will utilize plasma samples from 11,490 participants from the Atherosclerosis Risk in Communities Study Visit 4.

Plasma levels of Lp(a) and sdLDL-C have recently been measured in the entire ARIC Visit 4 cohort. We request access to the extant ARIC data analysis files, and their periodic updates, for cohort data collected by ARIC and the ancillary study on risk factors and incident CHD and stroke.

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
Inflammatory markers	Diabetes status	Lipid risk factors (including Lp-PLA_2 activity and mass, apo A-I, and apo-B)

AFU: incident cases of cardiovascular events and stroke occurring after ARIC V4
ARIC MRI evidence of carotid atherosclerosis

For analysis of the association between Lp(a) and sdLDL 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(a) and sdLDL and potential confounders and effect modifiers. To test our secondary hypotheses, we will investigate the association of sdLDL with individual components of the metabolic syndrome as defined by the NCEP ATPIII criteria using stratified analysis based on prevalent diabetes status. To investigate the association of sdLDL with risk of developing diabetes we will use self-reported diabetes status from annual follow-up after the visit 4 examination. Furthermore, we will investigate the association of Lp(a) and sdLDL with a number of MRI variables in those ARIC cohort members who have participated in the ARIC carotid MRI examination. The following MRI variables are of particular interest: max wall thickness, presence of lipid core, lipid core measurements (max core volume and max core area), max calcium area, and fibrous cap thickness (e.g.mean min cap thickness).

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* 2010.12)
___ 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.