1.a. Full Title:

The association of HDL subfractions and incident cardiovascular events: ARIC study

b. Abbreviated Title (Length 26 characters):

2. Writing Group:

Writing group members:

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Additional interested authors are invited to join the writing group.

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

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3. **Timeline:**

Analysis will start as soon as approval is obtained. Manuscript is to be prepared as soon as laboratory analyses are available. The analysis and manuscript preparation will take place within 1 year from approval of the proposal.

4. **Rationale:**

Improving methods to predict incident cardiovascular events has been the goal of a number of clinical studies. HDL-C has long been thought to be protective against cardiovascular disease (CVD)\(^1,2\), however, recent studies have demonstrated that HDL-C may not always be protective against cardiovascular disease\(^3-5\). HDLs are diverse particles that contain a variety of apolipoproteins. Variations in the composition and size of the HDL particles give them different properties that affect their function. We seek to identify if certain HDL subfractions, and lipids/lipoproteins may be better predictors of incident cardiovascular events as compared to HDL-C alone.
Apolipoprotein E (ApoE) is a hepatically synthesized apolipoprotein present on HDL and triglyceride-rich lipoproteins that plays an important role in cholesterol metabolism. Absence of ApoE in mouse models has been shown to be associated with hypercholesterolemia. ApoE is thought to exhibit a cardioprotective effect via two mechanisms. First, it participates in reverse cholesterol transport and plays a pivotal role in the interaction with LDL receptors, specifically scavenger receptor type B class I (SR-BI) and ATP-binding cassette transporter GI (ABCG1). Second, the HDL-C content containing ApoE (ApoE-HDL) has been shown to inhibit arterial stiffening and attenuate atherosclerosis independent of cholesterol levels. We hypothesize that individuals with elevated levels of ApoE-HDL-C would be associated with a lower rate of incident cardiovascular events as compared to lower levels of ApoE-HDL-C.

5. Main Hypothesis/Study Questions:

In this study, we will look at the association between HDL subfractions (ApoE-HDL-C, HDL2, and HDL3) and incident cardiovascular events. We hypothesize that individuals with elevated levels of ApoE-HDL-C would be associated with a lower rate of incident cardiovascular events as compared to lower levels of ApoE-HDL-C and that elevated levels of HDL2 and HDL3 would be associated with lower rate of incident cardiovascular events and that ApoE-HDL-C, HDL2 and HDL3 would be better predictors of incident cardiovascular events as compared to total HDL-C.

If findings regarding the association between HDL subfractions are promising with regard to incident events, we will proceed with GWAS analysis.
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).

In the primary analysis, data on HDL-C and its subfractions from ARIC visit 4 will be used. HDL subfractions will serve as the exposure variable and incident CHD, stroke, and CVD will be the outcomes.

Endpoints to be assessed:

1. Total/All CHD (fatal CHD, definite/probable MI, cardiovascular revascularization)
2. Hard CHD ((fatal CHD, definite probable MI)
3. Stroke (ischemic/ thrombotic stroke)
4. CVD (CHD + stroke)

Covariates will include age, gender, race, body mass index (BMI), total cholesterol, natural log of triglycerides, current smoking, diabetes, and systolic blood pressure, and use of antihypertensive medications.

Inclusion/ exclusion criteria:

All eligible ARIC participants will be included in the study. The major exclusion criteria include a preexisting diagnosis of CHD or stroke (prior to visit 4), participants without data on exposure, outcome, or covariates. We will also exclude race other than African American or white and African American participants from Minnesota and Washington field centers.
Analysis:

We will use multivariate Cox proportional hazard regression models to investigate the association between HDL subfractions and incident CVD. Levels of HDL subfractions will be treated as both continuous variables and categorical variables by dividing them into quartiles. We will further use HDL-C subfractions to total HDL-C ratios and concordance to examine the primary contrast between the subfractions and total HDL-C on CVD prediction. Finally, to check for possible improvement in prediction, we will calculate AUC, NRI, and IDI increments by adding HDL-C or HDL subfractions separately into Cox regression models. Model 1 will adjust for age, gender, and race. Model 2 will adjust for covariates in model 1 as well as systolic blood pressure, use of antihypertensive medications, diabetes, current smoking status, and body mass index. Model 3 will adjust for covariates in models 1 and 2 as well as LDL-C and triglycerides. Model 4 will adjust for the use of lipid lowering medications (statins, niacin, etc.) in addition to the covariates in models 1-3. We will assess for possible interactions of the lipid and lipoprotein parameters by race, gender, obesity, and diabetes. If significant interactions are noted, subgroup analyses will be performed.

Methodological limitations/ challenges:

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
8.a. Will the DNA data be used in this manuscript? __x__ 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”? __x__ 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

_____ Yes _____x____ 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* 2014.39)

___  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/

12a. 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.
12b. The NIH instituted a Public Access Policy in April, 2008 which ensures that the public has access to the published results of NIH funded research. It is **your responsibility to upload manuscripts to PUBMED Central** whenever the journal does not and be in compliance with this policy. Four files about the public access policy from http://publicaccess.nih.gov/ are posted in http://www.cscc.unc.edu/aric/index.php, under Publications, Policies & Forms. http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals automatically upload articles to PubMed central.

13. Per Data Use Agreement Addendum, approved manuscripts using CMS data shall be submitted by the Coordinating Center to CMS for informational purposes prior to publication. Approved manuscripts should be sent to Pingping Wu at CC, at pingping_wu@unc.edu. I will be using CMS data in my manuscript ____ Yes __x__ No.

References: