1.a. Full Title: PCSK9 Loss-of-Function Mutations and Lipoprotein (a) levels: the ARIC study

b. Abbreviated Title (Length 26 characters): PCSK9 LOF and Lp(a) levels

2. Writing Group:

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I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. ___YP___ [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 analyses are available. The analysis and manuscript preparation will take place within 1 year from approval of the proposal.

4. **Rationale:**

Plasma lipoprotein(a) [Lp(a)] is currently considered to be the strongest genetic risk factor for coronary heart disease (CHD), which is independent of other risk factors such as low-density lipoprotein (LDL) cholesterol (LDL-C). However to date, there is no available pharmacological treatment that has been shown to lower cardiovascular events in people with elevated Lp(a). Lp(a) is made up of a LDL molecule which is linked by disulfide bond with apolipoprotein(a) [apo(a)]. Assembly of Lp(a) requires formation of the disulfide bond between the apolipoprotein B (apoB) in LDL particles and apo(a), which probably occurs in the hepatocyte surface or in plasma. It is unclear where and how Lp(a) is removed. Evidence of LDL receptor (LDL-R) mediated Lp(a) clearance include the fact that Lp(a) are high in familial hypercholesterolemia (FH) patients who have mutations in LDL-R, as well as that Lp(a) is decreased in transgenic mice overexpressing LDL-R. In contrast, statins, which work eventually by up-regulation of LDL-R, markedly reduces LDL-C but do not significantly alter Lp(a) concentration.

Proprotein convertase subtilisin/kexin 9 (PCSK9) loss-of-function (LOF) mutations are associated with lifelong lower levels of LDL-C and significantly lower risk for CHD as seen both in ARIC and other study. Conversely, PCSK9 gain-of-function mutations are associated with lifelong higher LDL-C levels and increased CHD risk. These findings later lead to drug discovery and now several monoclonal antibodies to PCSK9 (commonly known as PCSK9 inhibitors) are in phase 3 clinical trials. It is known that circulating PCSK9 bind to LDL-R, and the receptor undergoes lysozomal degradation. Monoclonal antibodies bind to PCSK9 adjacent to the region where LDL-R binds, essentially blocking binding of PCSK9 with LDL-R and preventing lysozomal degradation of LDL-R, which are then available to be recycled back to the cell surface and thus plasma LDL-C level decreases. PCSK9 inhibitors studied so far have shown dramatic reductions in LDL-C levels, but outcome studies assessing reductions in atherosclerotic cardiovascular events are currently being assessed.

All phase 1-3 studies of PCSK9 monoclonal antibodies have shown that they consistently lower Lp(a), suggesting that this is a class effect. However, it is not clear how PCSK9 inhibitors lower Lp(a) levels and several hypotheses have been proposed. One possibility is that Lp(a) is cleared by LDL-R as Lp(a) contains apoB, which is the ligand for LDL-R. This is consistent with the fact that PCSK9 inhibitors work by eventually increasing LDL-R. However, as mentioned earlier statins which also eventually increase LDL-R, do not significantly change Lp(a) levels, and therefore, this hypothesis has been questioned. Another hypothesis relates to the fact that more than one third of circulating PCSK9 is associated directly with plasma LDL particles and is also associated with Lp(a) (personal communication, Sergio Fazio). Therefore, it is possible that reduction of Lp(a) levels is related to removal of anti-PCSK9 monoclonal antibodies bound to PCSK9.
and Lp(a) particles by the reticuloendothelial system scavenger pathway as antigen-antibody complexes are usually removed by this pathway. If reductions in Lp(a) levels are secondary to this mechanism, then other approaches such as siRNA inhibitors to lower production or small molecule inhibitors would not be expected to reduce levels of Lp(a). An alternative hypothesis is that PCSK9 modulates the levels of Lp(a) by a mechanism that is not the same as up-regulation of the LDL receptor by statin therapy. To our knowledge, it is not known if PCSK9 LOF mutations are associated with reduced levels of Lp(a) levels. Such a study will give insight into a mechanism by which Lp(a) inhibition using monoclonal antibodies may lower levels of Lp(a).

The ARIC study is ideal to assess the associations of PCSK9 LOF mutations and Lp(a) levels, as evidenced by a previous paper from the ARIC study that showed PCSK9 LOF mutations were associated with lifelong reductions in LDL-C and CHD. In that study 3 variants were associated with PCSK9 LOF mutations [R46L (N=301) in whites, and Y142X (N=26) and C679X (N=60) in blacks]. These LOF mutations were associated with reductions in both LDL-c levels and CHD.

5. Main Hypothesis/Study Questions:

Hypothesis: PCSK9 LOF mutations are associated with reductions in Lp(a) levels.

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).

Inclusion/exclusion criteria: Whites and blacks in whom fasting lipids, Lp(a) levels and PCSK9 genotype are available will be included.

We will separately use Lp(a) level measurements from V1 and V4 because of different assays used at those visits. The latter is isofrom size-insensitive assay. For the V1 Lp(a) assay, we will correct for isofrom size as was performed in a previously published paper from the ARIC study.

Other measurements may be used at each visit (if using V1 assay these risk factors will be from V1 visit) including age, gender, body mass index, total cholesterol, triglycerides, LDL-C, HDL-C, hypertension, systolic BP, diastolic BP, diabetes, current smoking, estrogen or progesterone use, CHD, ischemic stroke, total and CV deaths.

Sample characteristics: We will construct race-specific baseline characteristics by presence or absence of carrier state of the specific variants described above. Categorical variables will be analyzed using chi-square tests and continuous variables using t-tests. Non-parametric tests will be used for variables with skewed distributions, such as Lp(a).
Analysis section:
To test our hypothesis, we will examine the distribution of the Lp(a) levels according to the presence or absence of PCSK9 LOF mutations similarly to that shown in the initial paper from the ARIC study examining LDL-C levels by PCSK9 genotype.\textsuperscript{17} We will construct bar-graphs displaying Lp(a) levels. Since Lp(a) is known to be higher in blacks as shown in the ARIC study,\textsuperscript{25} we will analyze the distribution of Lp(a) by PCSK9 LOF carrier states in blacks and whites separately.\textsuperscript{17}

Limitations:
Lack of availability of apo(a) isoform size in the ARIC 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 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 ___X__ No
(This file ICTDER 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? ___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.cscce.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
   ___ A. primarily the result of an ancillary study (list number* __________)
   __X__ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* 2010.12)

*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.


