ARIC Manuscript Proposal #2818

PC Reviewed: 8/9/16	**Status: _____**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title:

The association of remnant-like particle cholesterol (RLP-c) and LDL-TG with cardiovascular events: ARIC study

b. Abbreviated Title (Length 26 characters):
RLP-C and LDL-TG in CVD events

2. Writing Group:

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I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. __AS____

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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).

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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.

4. Rationale:

A well-known association exists between elevated Triglycerides (TGs) and atherosclerotic cardiovascular disease (ASCVD) [1, 2]. However, it is generally believed that high TGs per se are not the cause of atherosclerosis but are merely a marker of high levels of cholesterol in triglyceride-rich lipoproteins (TGRLs). Elevated TGs are linked to deregulated lipoprotein metabolism, leading to a delayed clearance of TGRLs[3]. Recent scientific data is suggestive of a role of TGRLs and their cholesterol content as a strong predictor of ASCVD. Much of these signals have been based on genetic evidence from genome-wide association studies and mutational analysis which have provided evidence that TGRLs are in the causal pathway for atherosclerotic CV disease thus, highlighting their pathogenic role[4,5].

TGRL hydrolysis (of both intestinal and liver generated TGRLs) results in atherogenic remnants. Since the cholesterol to triglyceride content in TGRLs varies, it may be clinically more appropriate to measure TGRLs and their cholesterol content which is the remnant cholesterol. The remnant cholesterol can be predicted as all the cholesterol that is not found in the LDL and HDL (Total cholesterol – LDL cholesterol minus HDL cholesterol) as done previously[6]. Ultracentrifugation and nuclear magnetic resonance spectroscopy are other ways of measuring remnant cholesterol however both are rather expensive for routine use in the clinical settings. Recently, an assay has been developed that allows immunoseparation of remnant-like particles (RLP) as well as the measurement of cholesterol (RLP-c) and triglyceride (RLP-TG).

Previous publications have focused on the role of RLP-c and RLP-TG as markers of CHD. McNamara et al. showed the RLP-c measured via an immunochemistry mechanism, as an independent risk factor for CVD in women. RLP-c increases the

susceptibility of the coronary endothelium to oxidative stress thereby, inhibiting the NO-mediated arterial dilation[3]. It has also been determined that RLP-c upregulates endothelial expression of intracellular adhesion molecule-1 and vascular cell adhesion molecule-1, which are responsible for monocyte recruitment into the arterial wall, and tissue factor, which is essential for thrombotic events. Thus, these pro-inflammatory and pro-atherothrombogenic effects of RLP-c are likely to explain the association with high RLP-C levels with an increased cardiovascular event incidence.

There has been limited evidence in the role of LDL-TG as an independent risk marker for ASCVD. In a prior published cross sectional study by Marz et. Al, LDL-TG (measured by B-quantification) was linked to CHD independent of LDL-C or CRP levels[7]. Interestingly, in a secondary analysis of the AIM-HIGH trial population, LDL-TG was not found to have predictive value in cardiovascular outcomes of that cohort[8].

We aim to study these two different lipoprotein measurements linked to elevated TGs namely, LDL-TG and RLP-c and their association to ASCVD. We hypothesize that elevated levels of LDL-TG and RLP-c will be associated with increased risk of adverse cardiovascular outcomes (CVD).

5. Main Hypothesis/Study Questions:

In this study, we will look at the comparison between calculated remnant cholesterol and measured RLP-c and LDL-TG levels and incidence of cardiovascular events.

Primary: We hypothesize that individuals with elevated LDL-TG and RLP-c will be associated with higher risk of adverse cardiovascular outcomes as compared to individuals with lower LDL-TG and RLP-c levels.

Secondary: We will use genetic array analysis to investigate associations of genetic polymorphisms with levels of LDL-TG and RLP-c.

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 from ARIC visit 4 will be used. These values 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), lipids, current smoking, diabetes, and hypertension. Follow up period included will be from ARIC study visit 4 (1996-1998) up to December 31st 2013.

Inclusion/ exclusion criteria:

All eligible ARIC participants will be included in the study.

Standard ARIC exclusions (race exclusions for different communities) will apply. The major exclusion criteria include a preexisting diagnosis of CHD or stroke (prior to visit 4), participants without data on exposure, outcome, or covariates.

Analysis:

The lipid and lipoprotein values will be divided into appropriate quartiles and the association with incident CHD, stroke, and CVD will be assessed by quartiles with

quartile one being the referent quartile. The distributions of continuous variables will be evaluated to assess normality. The basic model 1 will be adjusted for age, gender, and race. Model 2 will adjust for covariates in model 1 as well as total and high-density lipoprotein cholesterol levels, systolic blood pressure, use of antihypertensive medications, smoking status, diabetes mellitus status (the variables for the pooled cohort risk equation. In an expanded model analysis, the data will also be adjusted for BMI.

We will perform further sensitivity analysis by excluding those participants who are on lipid lowering therapy. Associations between LDL-TG and RLP-c with anticipated outcomes will be determined using Cox proportional-hazards modeling.

Furthermore, a genetic analysis will be performed for mapping any possible genetic polymorphisms associated with the LDL-TG and RLP-c. We propose to analyze the relationship of the LDL-TG and RLP-c with coding variants captured on the Illumina Human Exome Beadchip. The LDL-TG and RLP-c will be analyzed using a single variant analysis and a gene-based analysis. Polymorphic variants with MAF ≥ 1% will be analyzed individually in the single variant analysis. The effects of functional variants (MAF < 1%) within a gene will be aggregated by summing up the score statistics using the collapsing method in Li and Leal[9]. A variant will be considered functional if it is (1) a stop gain/loss, (2) splice altering or (3) missense. The analysis will be carried out using the R seqMeta package. Both analyses will adjust for age, gender and population stratification. The significance threshold will be adjusted for the number of single variants/genes examined.

Methodological limitations/ challenges**:**

A potential limitation of our study is that the measurements of genetic analysis will be performed at only one time point using frozen plasma samples.

7.a. Will the data be used for non-CVD analysis in this manuscript? _x___ Yes ___ 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? _X__ Yes ____ 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.cscc.unc.edu/ARIC/search.php

_____ 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)?

Hoogeveen RC[1], Gaubatz JW, Sun W, Dodge RC, Crosby JR, Jiang J, Couper D, Virani SS, Kathiresan S, Boerwinkle E, Ballantyne CM. Small dense low-density lipoprotein-cholesterol concentrations predict risk for coronary heart disease: the Atherosclerosis Risk In Communities (ARIC) study. Arterioscler Thromb Vasc Biol. 2014 May;34(5):1069-77.

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:

- AS#2014.39 PI: Ron Hoogeveen

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:

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2. Sarwar N, Danesh J, Eiriksdottir G, Sigurdsson G, Wareham N, Bingham S, Boekholdt SM, Khaw KT, Gudnason V. Triglycerides and the risk of coronary heart disease: 10,158 incident cases among 262,525 participants in 29 Western prospective studies. *Circulation*. 2007;115: 450 – 458.
3. K. Kugiyama, H. Doi, T. Motoyama, H. Soejima, K. Misumi, H. Kawano, O. Nakagawa, M. Yoshimura, H. Ogawa, T. Matsumura, S. Sugiyama, T. Nakano, K. Nakajima, H. Yasue.

Association of remnant lipoprotein levels with impairment of endothelium-dependent vasomotor function in human coronary arteries. Circulation, 97 (1998), pp. 2519–2526
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5. Irvin MR, Zhi D, Joehanes R, Mendelson M, Aslibekyan S, Claas SA, Thibeault KS, Patel N, Day K, Jones LW, Liang L, Chen BH, Yao C, Tiwari HK, Ordovas JM, Levy D, Absher D, Arnett DK. Epigenome- wide association study of fasting blood lipids in the Genetics of Lipid- lowering Drugs and Diet Network study. Circulation 2014;130: 565e572.
6. Nordestgaard BG. Triglyceride-Rich Lipoproteins and Atherosclerotic Cardiovascular Disease: New Insights From Epidemiology, Genetics, and Biology. Circ Res. 2016 Feb 19;118(4):547-63.
7. Marz W, Scharnagl H, Winkler K, Tiran A, Nauck M, Boehm O. B, Winkelman R. B. Low-Density lipoprotein triglycerides associated with low-grade systemic inflammation, adhesion molecules, and angiographic coronary artery disease: the Ludwigshafen risk and cardiovascular health study. Circulation. 2004; 110:3068-2074.
8. Albers JJ, Slee A, Fleg JL, O'Brien KD, Marcovina SM. Relationship of baseline HDL subclasses, small dense LDL and LDL triglyceride to cardiovascular events in the AIM-HIGH clinical trial. Atherosclerosis. 2016 Jun 11. pii: S0021-9150(16)30264-7.
9. Li, B. & Leal, S.M. Methods for detecting associations with rare variants for common diseases: application to analysis of sequence data. Am J Hum Genet 83, 311-21 (2008).