ARIC Manuscript Proposal #3614 (revised)

PC Reviewed: 6/9/20	**Status: _____**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Remnant cholesterol discordance with low-density lipoprotein cholesterol and Apolipoprotein B and incident atherosclerotic cardiovascular disease

b. Abbreviated Title (Length 26 characters): Remnant cholesterol and ASCVD

2. Writing Group:

Renato Quispe	Johns Hopkins University	1st author, Analyst
Isha Lamba	Weill Cornell Medical College-Qatar	
Erin D. Michos	Johns Hopkins University	
Seth S. Martin	Johns Hopkins University	
Roger S. Blumenthal	Johns Hopkins University	
Anum Saeed	University of Pittsburgh	
Rishi Puri	Cleveland Clinic	
Steven R. Jones	Johns Hopkins University	
Joao A. C. Lima	Johns Hopkins University	
Sarah Nomura	University of Minnesota	
Michael Tsai	University of Minnesota	
Stephen J Nicholls	Monash University – Australia	
Christie Ballantyne	Baylor College of Medicine	
Mohamed B. Elshazly	Weill Cornell Medical College-Qatar	Senior Author

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

First author: Renato Quispe, MD
Address: Clinical Fellow
Department of Cardiology
Johns Hopkins University School of Medicine
600 North Wolfe Street
Carnegie 591
Baltimore MD 21287

Phone: 410-955-7376 Fax: 410-614-9190
E-mail: jquispe1@jhmi.edu

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).

Name: Erin D. Michos, MD, MHS
Address: Blalock 524-B, Division of Cardiology, 600 N. Wolfe Street, Baltimore, MD 21287 Phone: 410-502-6813 Fax: 410-502-0231
E-mail: edonnell@jhmi.edu

Timeline:
We aim to submit the manuscript within the next 3 months.

Background:

Atherosclerotic cardiovascular disease (ASCVD) has prevailed as the leading cause of mortality globally for the past fifteen years. Despite leaps in pharmacotherapy targeting ASCVD – from fibrates to statins – ischemic heart disease and stroke continue to be the world's biggest killers, accounting for 15.2 million deaths in 2016 alone[1].

Statins, which are well-established to lower low-density lipoprotein cholesterol (LDL-C), have tremendously reduced ASCVD globally[2]. Nevertheless, studies have found that there still remains a considerable residual risk of ASCVD in statin-treated individuals[3]. One of the theories is that some lipid parameters, other than LDL-C or non-HDL-C, could contribute to residual risk. A burgeoning target of interest is remnant cholesterol (RC)[4,5] – the atherogenic cholesterol content of triglyceride-rich lipoproteins, commonly estimated as [Non-HDL-C minus LDL-C][6].

Previous studies have established RC to be causally associated with atheroma progression and ischemic heart disease (IHD). For instance, a 2016 study of 5414 Danish patients diagnosed with IHD showed that increased levels of remnant cholesterol were significantly correlated with increased all-cause mortality[7], with a multivariable-adjusted hazard ratio (HR) of 1.5 for RC level ≥1.5mmol/L compared to patients with RC level <0.5mmol/L (P<0.001). A 2013 Mendelian randomization study of 60,608 participants found that nonfasting remnant cholesterol level was causally associated with higher rates of ischemic heart disease[8], with a 1mmol/L increase in RC corresponding to a causal risk ratio for IHD of 3.3 (95% confidence interval, 2.1-5.2). In the same study, a 1mmol/L increase in RC was also causally associated with a 28% higher level of C-reactive protein (95% confidence interval, 10-48), suggesting that residual cholesterol may play a role in driving the low-grade inflammation associated with atherosclerosis. Our group recently showed that higher on-treatment levels of RC are significantly associated with greater percentage atheroma volume progression rates and higher 2-year major adverse cardiovascular events (MACE) in patients on statin therapy[9].

Applying analytical approaches used by our group in previous ARIC studies[10,11], studying the association of RC with incidence of cardiovascular diseases when discordant with LDL-C levels (i.e. when levels of LDL-C are low but levels of RC are high) would provide further insight into the additional prognostic ability of RC beyond LDL-C. Furthermore, assessing discordance

with plasma levels of apolipoprotein B (apoB) would help to elucidate the risk driven by RC independent of total concentration of atherogenic particles in serum[12]. Complementary analyses in several subgroups, such as individuals with diabetes, presence of subclinical inflammation (determined by hsCRP), or females would provide additional insights related to the potential role of RC in the mechanism of disease in these high-risk groups.

Aims:

Our aim is to investigate whether discordance between RC and LDL-C levels is associated with incidence of cardiovascular events in an asymptomatic primary prevention cohort with long-term follow-up, independent of traditional cardiovascular risk factors and, most importantly, levels of apoB (as a comprehensive measure of total burden of apoB-containing particles).

Study Design: We will examine the independent association between RC levels and incident cardiovascular events. The baseline for this analysis will be ARIC visit 4. This is the visit that apoB was measured at. We will use data from two other primary prevention cohorts: Coronary Artery Risk Development in Young Adults (CARDIA) and Multi-ethnic Study of Atherosclerosis (MESA), pending approval.

Inclusions: We will include all participants free of an ASCVD event who attended ARIC visit 4.

Exclusions: We will exclude participants who had prevalent coronary heart disease (CHD) at baseline or who had an incident ASCVD event before visit 4, our baseline. We will exclude individuals with missing data for the standard lipid profile and apoB. We will exclude participants who are neither white nor black race, as well as blacks from MN and MD sites due to small numbers.

Exposures: The main exposure of interest will be RC, which will be estimated as TC minus HDL-C minus LDL-C estimated by the Martin equation[13]. Although there is no definite estimation for RC, our definition has been used in several studies[13,14,15].

Levels of RC will be assessed as medians (< and ≥ median) in the primary analysis across categories of LDL-C (< and ≥ median) estimated by the Martin equation[13]. Of note, LDL-C will be estimated (using the Martin equation), and no directly measured LDL-C will be used in order to make our analysis more reproducible and to make our findings more clinically applicable. In a secondary analysis, we will perform a similar analysis comparing RC with apoB (< and ≥ median).

Outcomes: The primary outcome will be ASCVD events, defined as incident CHD, fatal CHD, and stroke occurring after the Baseline (Visit 4) through December 31, 2016 (or most recent follow-up available). Incident ASCVD will be defined as definite or probable nonfatal myocardial infarction or fatal CHD, definite or probable stroke (defined as sudden or rapid onset of neurological symptoms that lasted for 24 hours or led to death in the absence of another cause). As secondary outcomes, we will include total mortality and incident heart failure occurring after baseline visit through December 31, 2016 (or most recent follow-up available).

Covariates: Other covariates that will be further included in models are: age, sex, race/center, BMI (in kg/m^2), systolic blood pressure (in mmHg), use of antihypertensive medications, log-triglycerides, apoB levels, hsCRP, diabetes mellitus (defined as fasting plasma glucose ≥126 mg/dl, or self-reported physician diagnosis of diabetes or use of diabetes medications), smoking status (in pack-years), physical activity, use of lipid-lowering medication.

Main Analyses:

1) We will exclude participants based on the above exclusion criteria.
2) We will estimate "Martin" RC and divide our population using medians: below (< median) and at or greater than the median (≥median).
3) We will identify the number of individuals with concordance/discordance between LDL-C and RC following the same categories:
   - Individuals with LDL-C < median and RC < median (*REFERENCE group*)
   versus
   - Individuals with LDL-C < median and RC ≥ median
   - Individuals with LDL-C ≥ median and RC < median
   - Individuals with LDL-C ≥ median and RC ≥ median
4) Baseline characteristics of the study participants will be analyzed in each of these groups will be described.
5) Kaplan-Meier estimates of cumulative event-free survival will be used to describe the incidence of our primary (cardiovascular events) and secondary outcomes (total mortality and incident heart failure).
6) We will construct Cox proportional hazard models to estimate hazard ratios (95% confidence intervals) for each outcome (primary and secondary) using the following models:
   -Model 1: adjusted by age, sex and race
   -Model 2: Model 1 + smoking status + BMI+ systolic blood pressure + treatment for hypertension + diabetes + statin use
   -Model 3: Model 2 + apoB
   -Model 4: Model 3 + log-hsCRP
   -Model 5: Model 4 + log-HDL-C
7) We will reproduce analyses shown in (6) by non-HDL-C (< and ≥ median) and apoB (< and ≥ median).
8) As secondary analysis, we will perform similar analyses in steps 3-6 in the following groups:
   - Individuals with apoB < median and RC < median (*REFERENCE group*)
   versus
   - Individuals with apoB < median and RC ≥ median
   - Individuals with apoB ≥ median and RC < median
   - Individuals with apoB ≥ median and RC ≥ median
9) We will reproduce all analyses stratifying by: sex (male vs. female), diabetes (diabetics vs. nondiabetics), elevated hsCRP (<2 vs. ≥2 mg/L), statins use, risk categories based

on the Pooled Cohort Risk Equations (low <7.5% vs. high risk ≥7.5%), and triglyceride categories (<100, 100-199, 200-399 mg/dL).

Limitations:

- There is the likelihood for some residual confounding by other risk factors not included in these models.
- Interim initiation of lipid lowering medication likely will modify the association between lipid discordance and ASCVD events.
- We will not have serial apoB measurements over the follow-up time.

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)?

Similar ARIC Manuscripts:
2753 (Quispe) The clinical impact of TC/HDL-C discordance with LDL-C, non-HDL-C and apoB: The Atherosclerosis Risk in Communities (ARIC) Study

Notes: this proposal looked at discordance between TC/HDL-C vs. LDL-C and non-HDL-C using median cutpoints in the overall population. ApoB discordance was not assessed. The baseline population for this proposal was ARIC 1st visit, whereas for the current proposal we will use ARIC 4th (where apoB data is available).

3095 (Quispe): The prognostic utility of high-sensitivity C-reactive protein when discordant with atherogenic lipoproteins in a primary prevention bi-racial population: The Atherosclerosis Risk in Communities (ARIC) study
Notes: this proposal did not include RC as one of the covariates.

2818 (Saeed): The association of remnant-like particle cholesterol (RLP-c) and LDL-TG with cardiovascular events: ARIC study

Notes: This proposal used RLP-c - measured by immunochemistry from an ancillary study by Ron Hoogeveen, PhD (AS#2014.39) – categorized by quartiles (1st quartile was reference group) and did not include apoB as a covariate in the models. They did not use discordance analysis. Drs. Saeed and Ballantyne were coauthors on that study and are now coauthors on our current proposal. Finally, in our proposal we are estimating RC similar to what previous studies have done, using information readily available from the standard lipid panel.

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.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.

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