1.a. **Full Title**: Association of Remnant-Like Protein Cholesterol (RLP-C), Low-Density Lipoprotein Triglycerides (LDL-TG) and Aptamer-Based Proteins with Incidence of Cardiovascular Events in Older Adults: Atherosclerosis Risk in Communities Study

b. **Abbreviated Title**: TGRLs, Somascan proteins and incident CVD

2. **Writing Group**:

Writing Group Members:

[In alphabetical order]

Christie M. Ballantyne, MD
Joe Coresh, MD
Ron Hoogeveen, PhD
Aliza Hussain, MD
Xiaoming Jia, MD
Vijay Nambi, MD, PhD
Elizabeth Selvin, PhD, MPH
Adrienne Tin, PhD

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

**First author:**

Aliza Hussain, MD
Corresponding/senior author (if different from first author correspondence will be sent to both the first author & the corresponding author):

Ron Hoogeveen, PhD
Associate Professor, Section of Cardiovascular Research
Department of Medicine
Baylor College of Medicine
Alkek Tower, Room ALKT-F756
Houston, Texas 77030
Email: ronh@bcm.edu

3. Timeline: Analysis is anticipated to begin as soon as approval is obtained. The manuscript is to be prepared as soon as analyses are available. Both the analysis and manuscript preparation are anticipated to take place within one year of approval of the proposal.

4. Rationale:

Elevated triglyceride (TG) levels have been associated with the development of cardiovascular diseases (CVD)\(^1\). While contemporary trials failed to demonstrate clinical benefit of targeting hypertriglyceridemia, the recently published landmark and REDUCE-IT\(^2\) trial demonstrated that targeting elevated TG levels significantly reduced cardiovascular (CV) events. TG metabolism may therefore play a causal role in atherosclerosis and, and TG pathways may prove potential therapeutic target. Triglycerides are primarily carried by triglyceride-rich lipoproteins (TGRL). While TGRL have been shown to be directly associated with incident CVD, it is less clear whether the TG component itself is involved in disease pathway.
The cholesterol content of TGRL particles, known as remnant like particle cholesterol (RLP-C), is shown to be highly atherogenic by penetrating the arterial wall, leading to accumulation of cholesterol in the intimal space, foam cell formation, and atherosclerosis\textsuperscript{3-4}. Mendelian randomization studies have provided overwhelming evidence for causal role of genes involved in the triglyceride metabolism pathways and incident CVD\textsuperscript{5}.

Saeed et al\textsuperscript{6} were the first to demonstrate a significant association of LDL-TG with incident coronary heart disease (CHD) and ischemic stroke in models adjusted for traditional pooled cohort equation (PCE) risk factors. Recently the LURIC study showed similar positive correlation between elevated LDL-TG and cardiovascular mortality. Furthermore, the study concluded, via two different Mendelian randomization analysis that low hepatic lipase activity may cause high LDL-TG resulting in increased cardiovascular risk.\textsuperscript{7}

Triglyceride metabolism involves a complex interplay between multiple lipoproteins, lipases (e.g. lipoprotein lipase (LPL) and hepatic lipase (HL)), and their activators (e.g. apoAV (A5) and apoC2), and inhibitors (e.g. ANGPTL 4, ANGPTL3 and apoC3). Lipoprotein lipase (LPL), is the rate-limiting enzyme in the TG pathway, and hydrolyzes TGRL to generate smaller triglyceride-depleted remnant lipoproteins and facilitates their removal from circulation via binding of specific ligands (e.g. apoE and apoB) to cellular receptors. Decreased LPL activity from loss of function mutations can lead to increased plasma TGRL and has been shown to increase risk of coronary heart disease as well as ischemic stroke\textsuperscript{8-9}. LPL is a highly regulated enzyme and multiple proteins can affect its function including apoC2, and angiopoietin-like protein (ANGPTL) 3 and 4\textsuperscript{10}. ApoC3 noncompetitively inhibits LPL and promotes atherosclerosis. Loss of function mutations in the gene encoding apolipoprotein C3 (APO3) have been associated with a reduced risk of ischemic cardiovascular events\textsuperscript{11}. ApoE, is involved in clearance of VLDL particles by binding to macrophage scavenger receptor, and is of clinical interest as it has been implicated in progression of atherosclerotic disease\textsuperscript{12}.

Recently, there has been a strong interest in biomarker discoveries and large-scale proteomic profiling via aptamer-based technology. Ngo et al\textsuperscript{13} recently used this aptamer-based platform to evaluate 1129 proteins in the Framingham Offspring cohort and helped identify 156 significant protein associations with myocardial injury. The results demonstrated feasibility of large-scale use aptamer-based approach with high sample throughput. SOMAscan proteomic profiling platform, has been included in the ARIC study, and could be a powerful tool to provide further insight into triglyceride metabolism and identification of potential therapeutic targets.

The ARIC study provides an ideal opportunity for the investigation of lipoproteins and CVD. Saeed et al\textsuperscript{5} examined LDL-TG and RLP-C measured at visit 4 in the ARIC study. LDL-is not a strong predictor of ASCVD in older adults, and it would be interesting to study whether LDL-TG or RLP-C may be better markers in older adults. We aim to study the same TGRLs, LDL-TG and RLP-C, and their association to CVD at
visit 5 and assess whether the association persists in older adults. We hypothesize that elevated levels of LDL-TG and RLP-C will be associated with increased risk of adverse cardiovascular outcomes in older adults. Furthermore, we will apply the now available proteomics data from SOMAscan to identify association of the proteins biomarkers LPL, HL, Apo B, ApoC2, ApoC3, ApoE, ANGPTL 3 and ANGPTL 4 with incident cardiovascular disease.

5. Main Hypothesis/Study Questions:

Hypothesis:
1. LDL-TG and RLP-C are associated with incident cardiovascular disease in older adults.
2. Aptamer-based proteomic measurements of apoproteins, lipases and angiopoietin-like proteins associated with catabolism of TG rich lipoproteins, specifically LPL, HL, Apo B, ApoC2, ApoC3, ApoE, ANGPTL 3 and ANGPTL 4, will show associations with levels of lipids, RLP-C, LDL-TG and incident CVD.
3. Other proteins in a large unbiased proteomic array will show strong associations with the levels of RLP-C and LDL-TG.

Study Aims:
1. Assess whether association of LDL-TG and RLP-C with development of CVD persists in older adults at ARIC visit 5.
2. Examine the association of specific apoproteins known to be important in TG metabolism with lipids, RLP-C and LDL-TG, and with incident CVD, CHD and ischemic stroke.
3. Examine in an unbiased manner the association of 5000 proteins measured by aptamer based proteomics with lipids, LDL-TG and RLP-C. If any strong associations are found, then examine whether these proteins are also associated with incident CVD, CHD and ischemic stroke.

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 methodological limitations or challenges if present).

Study design:
In the primary analysis ARIC visit 5 will be used as the index visit. We will perform prospective analyses on the subset of patients at visit 5 without preexisting diagnosis of CHD or ischemic stroke at baseline with 4-6 years of follow up from visit 5 to assess
whether lipoproteins/proteins involved in TG metabolism are associated with incident CVD.

Baseline data from visit 5 will serve as exposure variables, as described in detail below. Incident CHD, ischemic stroke, and CVD will be the outcomes

**Inclusion/ exclusion criteria:**

All eligible ARIC participants from visit 5 will be included in the study. The major exclusion criteria include a preexisting diagnosis of CHD or ischemic stroke (prior to visit 5), participants without data on exposure, outcome, or covariates.

**Exposures:**

1. LDL-TG and RLP-C will be modeled as both continuous variables as well as categorical variables based on quartile cut points for concentration.

2. For the second and third part of the study we will use proteomic data from SOMAscan v.4 obtained from ARIC visit 5. We will first study a small subset of proteins that include LPL, HL, Apo B, Apo C2, ApoC3, ApoE, ANGPTL 3 and ANGPTL 4. Protein measures using this platform has been shown to be robust with little variation from centrifugation to freezing and has been shown previously to quantify proteins with high precision12-13

3. For the third part we will include all 5000 serum proteins available from SOMAscan v.4 as exposure of interest

**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. Ischemic stroke
4. CVD (CHD + ischemic stroke)

Covariates will include age, sex, race, body mass index (BMI), lipids, current smoking, diabetes, and hypertension.

**Statistical Analysis:**

1. We will use Cox regression to assess the association between lipoproteins, LDL-TG and RLP-C, and incident CVD. The lipoprotein values will be divided into appropriate quartiles and the association with incident CHD, ischemic stroke, and CVD will be assessed by quartiles. In addition, we will also express the
lipoproteins as continuous variables (with transformation, if appropriate) and assess their association with incident CVD. The model 1 will be adjusted for age, sex, and race. Model 2 will adjust for covariates in model 1 as well as total cholesterol, high-density lipoprotein (HDL) cholesterol levels, systolic blood pressure, use of antihypertensive medications, smoking status and diabetes mellitus status (PCE model). We will perform additional adjustment using model 3 which include model 2 and in addition use of lipid lowering medications.

2. For the apatamers, LPL, HL, Apo B, Apo C2, ApoC3, ANGPTL 3, ANGPTL 4 and ApoE, we will first perform adjusted linear regression analysis to evaluate the association of each individual protein with LDL-TG and RLP-C. The model 1 will be adjusted for age, sex, and race. Model 2 will adjust for covariates in model 1 as well as total cholesterol, high-density lipoprotein (HDL) cholesterol levels, systolic blood pressure, use of antihypertensive medications, smoking status and diabetes mellitus status (PCE model). We will perform additional adjustment using model 3 which include model 2 and in addition use of lipid lowering medications. We will then perform, adjusted cox regression analysis to evaluate the association of each individual protein with incident CVD adjusting for similar covariates in models 1 and 2.

3. For the third aim of the study, we will perform adjusted linear regression models for each protein of 4931 proteins measured by aptamer-based proteomics (outcome) versus both LDL-TG and RLP-C. We will run 3 models accounting for baseline covariates: (1) adjusted for age, sex and race (2) adjusted for covariates in model plus total cholesterol level, HDL cholesterol level, systolic blood pressure, use of antihypertensive medications, smoking status and diabetes mellitus (3) adjusted for all covariates in model 2 plus use of lipid lowering medications. If any strong associations are found, we will then perform additional adjusted Cox regression to examine whether the individual protein is also associated with incident CVD adjusted using similar models 1, 2 and 3.

Limitations / Major Challenges:
- Proteomic assay data from visits 2-4 and 6 are not yet available, therefore we are not able to perform any longitudinal analysis of proteomic data.
- We are aware that certain proteins measured with SomaScan technology at ARIC visit 5 (e.g. troponins) show poor correlations with conventional assay methodologies. Currently there are ongoing efforts in a number of clinical/population studies to validate SomaScan protein data against conventional assays. We plan to validate a number of SomaScan proteins, including the specific proteins listed in this study proposal, either through laboratory validation studies using ARIC visit 5 samples or via communications...
with other investigators involved in SomaScan validation efforts in other study cohorts.

7a. 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 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?   _____ Yes   __X_ No

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 * AS#2014.39 and AS#2018.13

____ 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.cscu.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
http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals
automatically upload articles to Pubmed central.

References:

The American Journal of Cardiology. 1998;81(4); 450-458
density, Sf 12-60, and Sf 60-400 lipoproteins between plasma and arterial intima in humans.
Cell Adhesion Molecule (VCAM)-1 Expression via Differential Regulation of Endoplasmic
Reticulum Stress. PLoS ONE 2013;8(10)
Triglycerides, and Incident Cardiovascular Disease. Journal of the American College of
Cardiology. 2018;72(2):156–169
7. Silbernagel G, Scharmagl H, Kleber ME, et al. LDL triglycerides, hepatic lipase activity, and
coronary artery disease: An epidemiologic and Mendelian randomization study. Atherosclerosis
2019;282:37–44.
lipoprotein lipase is associated with increased risk of ischemic heart disease. Journal of Clinical


