ARIC Manuscript Proposal #4185

PC Reviewed: 10/8/24 **Status: _____** **Priority: 2**

1.a. Full Title:

Associations of proteins in fractionated plasma non-high-density lipoproteins (LDL, IDL and VLDL) with brain amyloid deposition, brain volumes, white matter hyperintensity volume and central arterial stiffness in older adults

b. Abbreviated Title (Length 26 characters):

Associations of proteins in fractionated LDL, IDL, VLDL with neuroimaging outcomes and central arterial stiffness

2. Writing Group:

Writing group members:
Danni Li, Alvaro Alonso, Pamela Lutsey, Michelle Mielke, Lin Zhang, Fang Yu, Ron Hoogeveen, Keenan Walker, Rebecca Gottesman, Michelle Meyer, Binchong An, other interested Investigators welcome

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

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3. Timeline:
This manuscript proposal is a companion proposal to the already approved manuscript proposal (#4014) which focuses on fractionated HDL. This manuscript proposal will focus on fractionated plasma non-HDL (VLDL, IDL, and LDL). We plan to submit a manuscript for publication within one year from the date the proposal is approved.

4. Rationale:

Vascular health is critical for brain and cognitive health in older adults. Recent studies from ARIC suggest that higher levels of plasma non-HDL lipoproteins (e.g., LDL-cholesterol, triglycerides [mainly existed in VLDL], apoB [main protein in LDL], small density LDL-cholesterol) in mid-life were associated with greater later-life cognitive decline.[1,2] Although the estimated magnitudes of effect are small, these studies suggest roles of non-HDL plasma lipoproteins, in particular LDL-cholesterol, with cognitive decline in older adults. Furthermore, a Mendelian randomization study suggested that low LDL-C levels may reduce the risk of Alzheimer's disease (AD),[3] the most common cause of dementia in old adults. AD is characterized by brain amyloid deposition, a hallmark pathology, and progressive brain atrophy known as neurodegeneration. Causes for AD pathophysiology are multifactorial and heterogeneous. Vascular pathology substantially increases the risk of AD and AD-related dementias (ADRDs) and coexists with AD pathology (brain amyloid deposition) in most AD/ADRD cases. In addition, plasma non-HDL plasma lipoproteins in the form of LDL-C, triglycerides, apoB have been extensively examined and associated with central arterial stiffness,[4-8] cerebral small vessel diseases (cSVDs) and WMH volume,[9,10] and stroke,[11] well-established risk factors for ADRDs. However, plasma non-HDL lipoproteins LDL, IDL, VLDL each contain up to a hundred of protein cargos, such as anti-oxidative enzymes and protease inhibitors, that regulate oxidative and inflammatory responses and coagulation, beyond the major apolipoprotein apoB.[12]

In the last few years, we have developed an anion exchange fast protein liquid chromatography (AEX-FPLC) method to physically separate or fractionate LDL, IDL, and VLDL from plasma. Furthermore, we developed a label free quantitative mass spectrometry method to identify proteins in fractionated LDL, IDL, and VLDL and their relative abundance. As part of the ARIC Ancillary 2015.19, we applied these methods for biochemical analysis of plasma samples of 66 ARIC participants without dementia collected at the ARIC-NCS baseline and identified 89, 82, 56 proteins in fractionated LDL, IDL, and VLDL from plasma. Many of these proteins are involved in inflammation, innate immunity (complement activation), and coagulation cascade.

The objective of this study is to identify proteins in fractionated LDL, IDL and VLDL and their cross-sectional associations with four ADRD-related outcomes in older adults in ARIC: a) AD pathology (brain amyloid deposition); b) brain volume in the temporoparietal meta region of interest [ROI]), known to be affected by AD; c) WMH volume, a neuroimaging outcome related to cSVDs; d) central arterial stiffness.

5. Main Hypothesis/Study Questions:

Study Question 1a. Which proteins identified in fractionated LDL are significantly associated with the four ADRD-related outcomes [brain amyloid deposition measured by PET, temporoparietal meta-ROI volume, WMH volume, central arterial stiffness] in older adults?

Study Question 1b. Which proteins identified in fractionated IDL are significantly associated with the four ADRD-related outcomes in older adults?

Study Question 1c. Which proteins identified in fractionated VLDL are significantly associated with the four ADRD-related outcomes in older adults?

Study Question 2a. Do proteins in fractionated LDL have stronger associations with the four ADRD-related outcomes than the same proteins in unfractionated plasma? We will compare the associations between the 89 proteins we identified in fractionated LDL versus the same 89 proteins whose concentrations were already measured in plasma based on SomaLogic.

Study Question 2b. Do proteins in fractionated IDL have stronger associations with the four ADRD-related outcomes than the same proteins in unfractionated plasma? We will compare the associations between the 82 proteins we identified in fractionated IDL versus the same 82 proteins whose concentrations were already measured in plasma based on SomaLogic.

Study Question 2c. Do proteins in fractionated VLDL have stronger associations with the four ADRD-related outcomes than the same proteins in unfractionated plasma? We will compare the associations between the 56 proteins we identified in fractionated VLDL versus the same 56 proteins whose concentrations were already measured in plasma based on SomaLogic.

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).

Study design

We will use a cross-sectional study design to determine the associations between proteins in fractionated LDL, IDL, VLDL, and four ADRD-related outcomes [brain amyloid deposition measured by PET, temporoparietal meta-ROI volume, WMH volume, central arterial stiffness] in older adults. We randomly chose 66 ARIC participants from the ARIC-PET study (please see the eligibility criteria) whose plasma samples were collected at the ARIC Visit 5/ARIC-NCS baseline.

Eligibility criteria

ARIC-NCS baseline participant inclusion criteria for the study were: 1) have “frozen never-thawed” fasting plasma samples available at the ARIC-NCS baseline; 2) were adjudicated without dementia at the time of the ARIC-NCS baseline blood collection; 3) had undergone brain MRI scans at the ARIC-NCS baseline; and 4) had undergone PET scans with 18F-AV-45 (florbetapir) in the ARIC-PET study in 2012–15 (baseline).

Biochemical Analysis

Label Free Quantitative Proteomics analysis of proteins in fractionated LDL, IDL, VLDL

We established an anion exchange fast protein liquid chromatography (AEX-FPLC) to fractionate LDL, IDL and VLDL. We applied this AEX-FPLC to fractionated plasma lipoproteins (VLDL, IDL, LDL) using frozen-never thawed plasma samples from the 66 participants. We then applied a label free quantitative MS-based proteomics method, based on data independent acquisition (DIA), for untargeted analysis of proteins in LDL, IDL, and VLDL. We used Spectronaut™ (Biognosis, Switzerland), a proprietary software for DIA proteomics analysis, to process the MS data and establish protein identification and relative abundance for subsequent data processing and statistical analysis. We will use the data on the 89, 82, 56 proteins identified in fractionated LDL, IDL and VLDL in this manuscript proposal.

Plasma concentrations of 89, 82, 56 proteins we found in fractionated LDL, IDL, VLDL based on SomaLogic

ARIC study has SomaLogic proteomics data for over 4000 proteins measured in plasma samples collected at the ARIC-NCS baseline for these 66 participants. We will extract from the SomaLogic data plasma concentrations of these proteins that we identified in fractionated LDL, IDL, VLDL to help answer Hypothesis 2a, b, c. Based on our preliminary data analysis, there are 67, 57, 44 proteins we found in fractionated LDL, IDL, VLDL with Somalogic data. We will also check measurement coefficient variants (CV%) of these proteins.

Outcomes

Central Arterial stiffness. The ARIC-NCS study already have central arterial stiffness (carotid-femoral PWV [cfPWV] and hfPWV)[13] measured for 5,638 ARIC-NCS study participants and had follow-up measures at visit 6 or 7. We will use cfPWV and hfPWV collected at the ARIC-NCS baseline/ARIC Visit 5.

Neuroimaging outcomes. The ARIC-NCS study already have neuroimaging measures analyzed for 326 ARIC-NCS study participants collected as part of the ARIC-PET study at the ARIC Visit 5/ARIC-NCS baseline, including ***(i) amyloid deposition.*** We will use global SUVR from the 18F-AV-45 (florbetapir) PET scans in the ARIC-PET study for amyloid deposition in the brain; ***(ii) WMH volume***. We will use WMH volume obtained via brain MRI scans; ***(iii)*** **temporoparietal meta-ROI volume.** We will use temporoparietal meta-ROI volume, which is total combined volume of the following brain regions: the parahippocampal, entorhinal, and inferior parietal lobules, hippocampus, precuneus, and cuneus.[14]

Other variables of interest with specific references to the time of their collection

All the variables of interests are measured at the ARIC Visit 5/ARIC-NCS baseline, except age, sex, race, and APOE genotypes. We will include age, sex, race/center, APOE genotypes, BMI, educational level, center/site, diabetes, prevalence of heart disease, heart failure, or stroke at baseline, use of antihypertensive medication, use of statin medication, smoking, as covariates.

Summary of data analysis

Study Questions 1a, b, and c. We will first evaluate single proteins' associations with each of the four ADRD-related outcomes, with adjustment for covariates mentioned above. We will process

the mass spectrometry proteomics data. If batch effects remain after data normalization, we will inspect post-processed data and conduct sensitivity analysis (e.g., inclusion vs exclusion of certain batches) when appropriate. We will log-transform data when appropriate so that the distributions of transformed data are at least approximately normal. We will use linear-regression models for continuous outcomes to identify single proteins that are significantly associated with the outcomes, with adjustment for covariates. We will use Benjamini-Hochberg (BH)'s false discovery rate method[15] to control overall type 1 errors for adjustment of multiple proteins and/or multiple phenotypes when appropriate.

Study Questions 2a, b and c. We will perform a simple correlation between the 89, 82, and 56 proteins measured with SomaLogic vs. with MS-based proteomics. We will compare the associations of a single protein in fractionated LDL, IDL or VLDL versus the same protein unfractionated plasma with each ADRD-related outcome.

Anticipated methodological limitations or challenges

Since we have only 66 participants, a challenge is the limited sample size. Preliminary data analysis suggests that a sample size of 66 gave >99% and >80% power to detect statistical significance associations between IgM in LDL and temporoparietal meta-ROI without and with covariate adjustments, respectively.

Other limitations include: 1) for the proteins we identified in fractionated LDL/IDL/VLDL, we may not find all proteins have equivalent plasma protein data in SomaLogic data set; 2) we acknowledge that proteins in fractionated LDL/IDL/VLDL and proteins in unfractionated plasma were measured by different methodologies; 3) if some proteins still have skewed or bimodal distributions after log-2 transformed, we have to figure out an alternative statistical model for these proteins.

7.a. Will the data be used for non-ARIC analysis or by a for-profit organization in this manuscript? ____ Yes __X__ No

b. If Yes, is the author aware that the current derived consent file ICTDER05 must be used to exclude persons with a value RES_OTH and/or RES_DNA = "ARIC only" and/or "Not for Profit" ? ____ Yes ____ No
(The 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 current derived consent file ICTDER05 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/aricproposals/dtSearch.html

__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)?

None.

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* 2015.19___)

___ 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 https://sites.cscc.unc.edu/aric/approved-ancillary-studies

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.

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