ARIC Manuscript Proposal #4014

PC Reviewed: 3/8/22 **Status: _____** **Priority: 2**
SC Reviewed: _____ **Status: _____** **Priority: ____**

1.a. Full Title:

Association of proteins in fractionated high-density lipoprotein (HDL) 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 HDL 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, Rebecca Gottesman, W. Sean Davidson, Keenan Walker, Brian Steffen, Gerardo Heiss and Michelle Meyer, Matthew Glittenberg, Sai Kaushik Shankar Ramesh Kumar, 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]**

First author: **Danni Li**
Address: 420 Delaware St SE, MMC 609, Mayo Building D250-1
Minneapolis, MN 55455

Phone: 6124990751 Fax:
E-mail: dannili@umn.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: **Pam Lutsey**
Address: 1300 South 2nd St; Suite 300
Minneapolis, MN 55454

Phone: 612-624-5812
E-mail: lutsey@umn.edu

3. Timeline:

A manuscript will be submitted for publication within one year from the date the proposal is approved.

4. Rationale:

Plasma HDL (e.g., HDL-Cholesterol or significant HDL proteins such as apoA-1, apoE, apoJ) has potential protective roles in preventing brain amyloid deposition and central arterial stiffness, slowing down cognitive decline, reducing incident dementia, and attenuating brain atrophy in older adults.[1-4] Preclinical studies suggest that these neuroprotective roles of HDL and its associated proteins are likely due to their abilities to clear amyloid in the brain,[5] protect the integrity of brain endothelium, cerebral vasculature, and blood brain barrier (BBB), and reduce chronic neuroinflammation.[6-12] Although apoA-1 is a major HDL protein that makes up its 70% of the HDL protein content, HDL contains over one hundreds of other proteins. Most preclinical studies did not discern individual HDL-associated protein's contributions to the protective effects on neurocognitive outcomes. In addition, there are networks of proteins in HDL (i.e., identified by unweighted protein co-expression analysis) and it is unclear whether a few major networked HDL proteins mediate the effect of apoA-1 through their own subnetworks in HDL. Most of the epidemiological studies evaluated a few significant HDL-associated proteins (apoA-1, apoE, apoJ) in unfractionated plasma[1-3,13-17] HDL is a component of plasma. Because majority of HDL-associated proteins do not exist exclusively on HDL, it is unclear whether the neuroprotective effects of HDL-associated proteins are dependent upon their physically residence on fractionated HDL.

In the last few years, we have developed an anion exchange fast protein liquid chromatography (AEX-FPLC) method to physically separate or fractionate HDL from plasma. Furthermore, we developed a label free quantitative mass spectrometry method to identify proteins in fractionated HDL 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 124 proteins in fractionated HDL from plasma. Many of these proteins in fractionated HDL are involved in inflammation, innate immunity (complement activation), and coagulation cascade.[18] Plasma proteins involved in coagulation and complement pathways (e.g., apoJ) have been induced by exercise interventions, and they reduce neuroinflammation and improve memory in animal models of AD.[19] Interestingly, many of these exercise-induced neuroprotective proteins are also found in HDL, raising the possibility that proteins in fractionated HDL which are involved in innate immunity (complement activation) and coagulation cascade have protective effects on neurocognitive outcomes in older adults.

This manuscript proposal aims to: 1a) identify specific proteins in fractionated HDL that are associated with neuroimaging outcomes [brain amyloid deposition measured by PET, various brain volumes (e.g., AD-signature region volume, frontal and temporal volumes), white matter hyperintensity (WMH) volume] in older adults enrolled in Atherosclerosis Risk in Community (ARIC); 1b) identify the same proteins in fractionated HDL that are associated with central arterial stiffness; 2a) to examine the topology of HDL protein networks and how the major networked proteins mediate the effects of apoA1 on neuroimaging outcomes and central arterial stiffness; 2b) relate HDL protein networks to neuroimaging outcomes and central arterial

stiffness; 3) to examine whether neuroprotective effects of plasma HDL-associated proteins are dependent upon their physical residence on fractionated HDL.

5. Main Hypothesis/Study Questions:

Study Question 1a: Which proteins among the 124 proteins identified in fractionated HDL are significantly associated with neuroimaging outcomes [brain amyloid deposition measured by PET, central arterial stiffness, brain volumes (e.g., AD- signature region volume, frontal and temporal volumes), white matter hyperintensity (WMH) volume in older adults?

Study Question 1b: Which proteins among the 124 proteins identified in fractionated HDL are significantly associated with central arterial stiffness outcomes in older adults?

Study Question 2a: How do the major networked proteins in fractionated HDL mediate the effect of apoA1 on neuroimaging outcomes and central arterial stiffness?

Study Question 2b: How are the protein networks in HDL associated with neuroimaging outcomes and central arterial stiffness?

Study Question 3: Are the associations of 124 proteins identified in fractionated HDL proteins with these neuroimaging outcomes and central arterial stiffness dependent upon their physical residence on fractionated HDL? To answer this question, we will compare the associations between the 124 proteins we identified in fractionated HDL versus the same 124 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 HDL proteins and neuroimaging outcomes [(brain amyloid deposition measured by PET), brain volumes (e.g., AD- signature region volume, frontal and temporal volumes), white matter hyperintensity (WMH) volume] and central arterial stiffness. We will use a cohort study design to determine the associations between proteins in fractionated HDL and incident dementia in an exploratory analysis. We randomly chose 66 ARIC participants who were selected 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 HDL

We established an anion exchange fast protein liquid chromatography (AEX-FPLC) to fractionate HDL, LDL, IDL and VLDL. We applied this AEX-FPLC to fractionated plasma lipoproteins (VLDL, IDL, LDL, and HDL) 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 HDL, 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. Even though we have quantitative data of proteins in fractionated HDL, LDL, IDL and VLDL, we will only use the data on the 124 proteins identified in fractionated HDL in this manuscript proposal.

Plasma concentrations of 124 proteins we found in fractionated HDL 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 124 proteins that we identified in fractionated HDL to help answer Study Question 3. If these 124 proteins are not all available in the SomaLogic data, we will use whatever protein data is available. 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)[20] 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) brain volume data.*** We will use AD signature region volume as AD specific neurodegeneration, which is total combined volume of the following brain regions: the parahippocampal, entorhinal, and inferior parietal lobules, hippocampus, precuneus, and cuneus.[21] We will use other brain region volumes such as frontal and parietal lobe cortical volumes.[22] We will examine the relationship of proteins with lobar brain volumes to determine there is neuroanatomical specificity to the link between HDL proteins and brain volume

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, APOE genotypes, HDL-cholesterol (for fractionated HDL only) as confounders.

Summary of data analysis

Study Questions 1a&b. We will first evaluate single proteins' associations with neuroimaging outcomes and central arterial stiffness, with adjustment of 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 include batch as a covariate 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 or logistic regression for dichotomized outcomes with mixed effects to identify single proteins that are significantly associated with neuroimaging outcomes and central arterial stiffness, with adjustment for key biological variables such as sex or *APOE4* genotype and other potential confounders as appropriate. We will use BH's false discovery rate method[23] to control overall type 1 errors for adjustment of multiple proteins and/or multiple phenotypes when appropriate.

Study Questions 2a&b. We will use the unweighted gene coexpression network analysis to discover how HDL proteins correlate. We will identify "predominant HDL proteins" with most associations and then perform mediation analysis to determine the effects of apoA1 has through each of these predominant proteins. Combination of these results with our univariate analysis for Study Question 1 could demonstrate how adjustment of apoA1 and its mediator can have far reaching neuroprotective effects. In addition, we will explore network topology and associations between network structure and cross-sectional neuroimaging outcomes and central arterial stiffness.

Study Question 3. We will apply the same univariate analysis (described for Study Question 1) for 124 proteins in fractionated HDL and 124 plasma proteins based on SomaLogic proteomics data. We will answer Study Question 3 by comparing the strength of the associations between 124 plasma proteins versus 124 proteins in fractionated HDL with neuroimaging outcomes and central arterial stiffness.

Anticipated methodological limitations or challenges

Since we have only 66 participants, a challenge is the limited sample size. A post hoc power analysis was performed for power estimation, based on effect sizes from the analysis. Power was estimated for the effect of two proteins (CPN2 and F10) on AD signature region volume across two different models (unadjusted model and adjusted for HDL-Cholesterol level, number of apoE4 alleles, sex and age) using simulation. For alpha=0.05 and n=61 (5 samples removed due to outliers in protein measurement), the power for CPN2 in the unadjusted model was found to be 0.934 and the power for the adjusted model was found to be 0.838. For alpha=0.05 and n=61, the power for F10 in the unadjusted model was found to be 0.927 and the power for the adjusted model was found to be 0.804.

Other limitations include: 1) for the 124 proteins we identified in fractionated HDL, we may not find all proteins have equivalent plasma protein data in SomaLogic data set; 2) since we selected the 66 participants randomly, sex and race as potential confounders may not make any difference; 3) we acknowledge that proteins in fractionated HDL and proteins in unfractionated plasma were measured by different methodologies; 4) 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.

References

1. Gupta VB, Wilson AC, Burnham S, et al. Follow-up plasma apolipoprotein E levels in the Australian Imaging, Biomarkers and Lifestyle Flagship Study of Ageing (AIBL) cohort. *Alzheimers Res Ther*. 2015;7(1):16. doi:10.1186/s13195-015-0105-6
2. Thambisetty M, An Y, Kinsey A, et al. Plasma clusterin concentration is associated with longitudinal brain atrophy in mild cognitive impairment. *Neuroimage*. 2012;59(1):212-217.
3. Chernick D, Zhong R, Li L. The role of HDL and HDL mimetic peptides as potential therapeutics for Alzheimer's disease. *Biomolecules*. Sep 4 2020;10(9):1276. doi:10.3390/biom10091276
4. Wang X, Du Y, Fan L, et al. Relationships between HDL-C, hs-CRP, with central arterial stiffness in apparently healthy people undergoing a general health examination. *PLoS One*. 2013;8(12):e81778.
5. Dal Magro R, Simonelli S, Cox A, et al. The extent of human apolipoprotein AI lipidation strongly affects the β-amyloid efflux across the blood-brain barrier in vitro. *Front Neurosci*. 2019;13:419.
6. Lewis TL, Cao D, Lu H, et al. Overexpression of human apolipoprotein A-I preserves cognitive function and attenuates neuroinflammation and cerebral amyloid angiopathy in a mouse model of Alzheimer disease. *J Biol Chem*. Nov 19 2010;285(47):36958-68. doi:10.1074/jbc.M110.127829
7. Lefterov I, Fitz NF, Cronican AA, et al. Apolipoprotein AI deficiency increases cerebral amyloid angiopathy and cognitive deficits in APP/PS1ΔE9 mice. *J Biol Chem*. 2010;285(47):36945-36957.
8. Huynh T-PV, Wang C, Tran AC, et al. Lack of hepatic apoE does not influence early Aβ deposition: observations from a new APOE knock-in model. *Mol Neurodegener*. 2019;14(1):1-23.
9. Lane-Donovan C, Wong WM, Durakoglugil MS, et al. Genetic restoration of plasma apoE improves cognition and partially restores synaptic defects in apoE-deficient mice. *J Neurosci*. Sep 28 2016;36(39):10141-50. doi:10.1523/JNEUROSCI.1054-16.2016
10. De Retana SF, Marazuela P, Solé M, et al. Peripheral administration of human recombinant ApoJ/clusterin modulates brain beta-amyloid levels in APP23 mice. *Alzheimers Res Ther*. 2019;11(1):42.
11. Robert J, Stukas S, Button E, et al. Reconstituted high-density lipoproteins acutely reduce soluble brain Aβ levels in symptomatic APP/PS1 mice. *Biochimica Et Biophysica Acta (BBA)-Molecular Basis of Disease*. 2016;1862(5):1027-1036.
12. Moore EE, Liu D, Li J, et al. Association of Aortic Stiffness With Biomarkers of Neuroinflammation, Synaptic Dysfunction, and Neurodegeneration. *Neurology*. 2021;

13. Schrijvers EM, Koudstaal PJ, Hofman A, Breteler MM. Plasma clusterin and the risk of Alzheimer disease. *JAMA*. Apr 6 2011;305(13):1322-6. doi:10.1001/jama.2011.381
14. Song F, Poljak A, Crawford J, et al. Plasma apolipoprotein levels are associated with cognitive status and decline in a community cohort of older individuals. *PLoS One*. 2012;7(6):e34078. doi:10.1371/journal.pone.0034078
15. Gupta VB, Doecke JD, Hone E, et al. Plasma apolipoprotein J as a potential biomarker for Alzheimer's disease: Australian Imaging, Biomarkers and Lifestyle study of aging. *Alzheimers Dement (Amst)*. 2015;3:18-26. doi:10.1016/j.dadm.2015.12.001
16. Gupta VB, Laws SM, Villemagne VL, et al. Plasma apolipoprotein E and Alzheimer disease risk: the AIBL study of aging. *Neurology*. Mar 22 2011;76(12):1091-8. doi:10.1212/WNL.0b013e318211c352
17. Rasmussen KL, Tybjaerg-Hansen A, Nordestgaard BG, Frikke-Schmidt R. Plasma levels of apolipoprotein E and risk of dementia in the general population. *Ann Neurol*. Feb 2015;77(2):301-11. doi:10.1002/ana.24326
18. Gordon SM, Remaley AT. High density lipoproteins are modulators of protease activity: Implications in inflammation, complement activation, and atherothrombosis. *Atherosclerosis*. Apr 2017;259:104-113. doi:10.1016/j.atherosclerosis.2016.11.015
19. De Miguel Z, Khoury N, Betley MJ, et al. Exercise plasma boosts memory and dampens brain inflammation via clusterin. *Nature*. 2021;600(7889):494-499.
20. Stoner L, Meyer ML, Kucharska-Newton A, et al. Associations between carotid-femoral and heart-femoral pulse wave velocity in older adults: the Atherosclerosis Risk In Communities study. *J Hypertens*. 2020;38(9):1786-1793.
21. Dickerson BC, Stoub TR, Shah RC, et al. Alzheimer-signature MRI biomarker predicts AD dementia in cognitively normal adults. *Neurology*. Apr 19 2011;76(16):1395-402. doi:10.1212/WNL.0b013e3182166e96
22. Kim HJ, Yang JJ, Kwon H, et al. Relative impact of amyloid-beta, lacunes, and downstream imaging markers on cognitive trajectories. *Brain*. Sep 2016;139(Pt 9):2516-27. doi:10.1093/brain/aww148
23. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B (Methodological)*. 1995;57(1):289-300.