ARIC Manuscript Proposal # 3808

PC Reviewed: 3/9/21 **Status: _____** **Priority: 2**
SC Reviewed: _____ **Status: _____** **Priority: ____**

1.a. Full Title: Proteomics of cerebral small vessel disease

b. Abbreviated Title (Length 26 characters):

2. Writing Group:

Writing group members:

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

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3. Timeline:

1-3 months: analysis of data
1-3 months: writing of manuscript

4. Rationale:

Cerebral small vessel disease (SVD) is a major cause of ischemic stroke and a significant contributor to cognitive decline and dementia.[1–3] Consequences of SVD can be characterized on neuroimaging by white matter hyperintensities (WMHs), cerebral microbleeds (CMBs), and lacunar infarcts.[4,5] WMHs,[6–8] CMBs[9] and lacunar infarcts,[10] markers of diffuse cerebrovascular damage, have been associated with poor cognitive outcomes and higher odds of dementia. Both the presence and progression of SVD lesions have been linked to decline in cognitive function.[11] Certain cerebrovascular pathology subtypes (i.e. cerebral arteriolosclerosis severity) have been shown to increase risk for AD type dementia.[12] Notably, WMHs, appearing decades before onset of clinical symptoms, not only confer increased risk for Alzheimer's disease (AD), but may also constitute an integral feature of AD pathogenesis.[13] SVD carries extensive clinical burden and can manifest in cognitive[14], motor[15], and affective[16] dysfunction.

Understanding the complex pathogenesis of SVD and its capacity to engender neurodegenerative and clinical sequelae is critical. It is clear that peripheral processes, including vascular disease/risk factors (e.g., smoking, hypertension) and cardiometabolic dysfunction (e.g., obesity, diabetes) confer increased risk for SVD and SVD progression, which, in turn, tracks with cognitive decline.[11] While these findings make it clear that clinical and subclinical disease processes occurring outside the central nervous system (CNS) can influence SVD risk, much of the explanatory variance in SVD risk is still unaccounted for. This is underscored by the finding that generally healthy individuals have been found to display SVD, even during early and middle adulthood.[17] A deeper understanding of peripheral biological changes that promote SVD development may be achievable using a data-driven analysis of the plasma proteome.

Genome-wide association studies (GWAS's) have highlighted susceptibility loci for SVD, stroke, and stroke subtypes. These findings support the involvement of immune and inflammatory pathways,[18,19] cell signaling and cell survival,[20] and endothelial function.[21] Identification of plasma biomarkers associated with increased risk for SVD neuroimaging characteristics using a proteome-wide analysis may shed additional light on the peripheral molecular processes that promote SVD development. Additionally, these findings may identify plasma biomarkers that can be used to determine which individuals are at greatest risk for SVD. We hypothesize that circulating protein markers of vascular inflammation, endothelial dysfunction, and loss of blood brain barrier integrity may be associated with SVD risk and implicated in SVD pathogenesis.[22] Though the roles of discrete protein pathways have been investigated in the context of SVD, the plasma proteomic signature of SVD burden is largely unexplored.

The goal of the current study is to use SomaScan Multiplexed Proteomic technology[23,24] to examine the relationship between the plasma level of a set of proteins and neuroimaging measures of SVD within the Atherosclerosis Risk in Communities (ARIC) Cohort. In this proteome-wide association study of cerebral small vessel disease, we first aim to identify proteins, measured in late-life, that are associated with WMHs, CMBs, and lacunar infarcts measured in late-life, defined as ARIC Visit 5. Identified SVD-associated proteins will be examined during middle adulthood (ARIC Visit 3) to determine whether they continue to demonstrate an association with MRI measures of vascular injury when measured well before the typical onset of age-related brain changes. SVD-associated proteins will then be validated in external samples, which may include the AGES and MESA cohorts, pending available data. Using available SVD GWAS summary statistics, we will conduct two-sample Mendelian randomization to investigate potential causal relationships between the proteins of interest and

SVD characteristics. Finally, systems level analyses will be applied to identify protein networks represented by the candidate proteins in order to better understand the peripheral biological processes and regulatory mechanisms underlying SVD pathogenesis.

5.Main Hypothesis/Study Questions:

Objective 1. Identification of plasma proteins associated with SVD MRI variables.

Hypothesis. A number of proteins measured during late-life (Visit 5) will be associated with SVD (i.e., WMH volume, and the presence of CMBs and lacunar infarcts), after correction for multiple comparisons.

Hypothesis. Expression of protein networks defined using weighted correlation network analysis (WGCNA) will be associated with SVD.

Objective 2. Midlife replication of SVD-associated proteins

Hypothesis. A subset of identified SVD-associated proteins will also be associated with late-life SVD when examined in blood collected during midlife (Visit 3).

Objective 3. External replication of SVD-associated proteins

Hypothesis. Proteins-SVD associations identified in objective #1 will be validated in external cohorts (including AGES and MESA, if/when data become available).

Objective 4. Two-sample Mendelian randomization for examination of causal effects

Hypothesis. Using protein quantitative trait loci (pQTL) information from ARIC and/or Sun et al. (2018), and available SVD GWAS summary statistics,[18,19,21,25] two-sample Mendelian randomization[26] will support a causal inference of one or more SVD-associated protein identified in objective #1.

Objective 5. Pathway analyses to identify biological mechanisms associated with SVD

Hypothesis. Protein pathway analysis of SVD-associated proteins and WGCNA-defined protein networks (identified in Objective #1) will implicate endothelial function, inflammatory/immune processes, cellular signaling/plasticity, hemostasis/vascular function, and insulin signaling.

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).

Participants

Inclusion criteria:

- Participants who have SOMAscan protein measurements available from blood collected at Visit 3 or Visit 5.
- Underwent brain MRI at visit 5 and WMH, CMB, and lacunar infarcts assessed.

Exclusion Criteria:

- Non-white or non-black race

- Non-white participants in Washington County and Minnesota
- Missing proteomic data
- Missing covariate information
- Missing information needed to classify cognitive status (i.e., normal/MCI/dementia classification) after Visit 3.

Exposure Variables

Proteomic measurement: Using plasma collected at Visit 3 (1993-95) and Visit 5 (2011-13), proteins were measured using a Slow Off-rate Modified Aptamer (SOMAmer)-based capture array (SomaLogic, Inc, Boulder, Colorado). Using chemically modified nucleotides, this process transforms protein signals to a nucleotide signal quantifiable using relative florescence on microarrays. Previous work indicates a median intra- and inter-run coefficient of variation of approximately 5% and intra-class correlation coefficients of ~0.9.[27–30]

Individual protein levels (N=4,877 after QC) will be examined as the primary exposure. Proteins were log2 transformed and outliers >5 SD away from the mean were winsorized.

Proteins will also be grouped into networks/modules using weighted gene coexpression network analysis (WGCNA) R package. WGCNA will be used to identify networks of correlated proteins within the set of 4877 measured proteins using the full set of participants included in the primary analysis. WGCNA converts the protein-protein correlation matrix into an adjacency matrix that filters weak correlations based on a power threshold chosen to meet scale-free topology criteria. These algorithms use hierarchical cluster analyses and dynamic tree cutting implemented to group proteins based on patterns of coexpression. After modules are identified, module expression values (module eigenproteins [MEs]) are calculated from the first principal component of each module for each participant. These values represent measures of protein network expression that can be related to participant SVD outcome measures. Modules will be further characterized using Ingenuity Pathway Analysis to gain an improved understanding of the biological pathways and upstream regulators (e.g., transcription factors) associated with expression of SVD-relevant protein modules.

Primary outcome variables:

<u>MRI Variables</u>. 3T MRIs were conducted in approximately 2,000 participants at Visit 5 as part of the ARIC Neurocognitive Study (NCS). The acquisition sequence for the ARIC Visit 5 MRI has been described previously[14]. At each ARIC site, a common set of sequences were performed for all participants: MP-RAGE, Axial T2*GRE, Axial T2 FLAIR, and Axial DTI.

- *White Matter Hyperintensity (WMH) Volume.* WMH volume (mm^3) was assessed quantitatively from FLAIR images using a computer-aided segmentation program (FLAIR-histoseg) to assess the total volumetric burden.[31] All analyses of WMH volume will be adjusted for total intracranial volume. WMH volume will be log-transformed to adjust for skewness.
- *Cerebral microbleeds (CMBs) and infarction.* CMBs and lacunar infarcts were identified by trained imaging technicians and confirmed by radiologists; this has been previously

described.[32] Lacunar infarcts are defined as a hyperintense subcortical lesion with a dark center (≥3mm and ≤15mm in size) within the white matter, infratentorial, or central grey/capsular regions that is distinguishable from perivascular space. CMBs are defined using a T2* GRE MRI sequence.

- *Cerebral SVD Burden*. To examine the overall burden of SVD, we will also consider using an SVD composite score,[33,34] which has been described previously, and which will be calculated to reflect the cumulative presence of elevated WMH volume, CMBs, and lacunar infarcts.

Other Variables

Visit 1 demographic variables, including race (black/white), sex (male/female), education (less than high school/high school, general education diploma [GED], or vocational school/college, graduate or professional school), *APOE* ε4 status, and center will be extracted. A combined-race-center variable will be created as follows: white-Washington County, white-Forsyth County, black-Forsyth County, white-Minneapolis, or black-Jackson. Additionally, participant age and laboratory and physiologic data, including systolic and diastolic blood pressures, total/high density lipoprotein cholesterol, body mass index (BMI, kg/m^2), and measures of kidney function will be extracted from the visit concurrent with plasma proteomic measurement (i.e., Visit 3 and Visit 5). Cardiovascular risk factors and disease information (i.e., diabetes, hypertension, coronary heart disease, and cigarette use) as well as medication information (i.e. treatment with antihypertensive drugs) will also be extracted from Visit 3 and Visit 5.

Data Analysis

Identification of plasma proteins associated with SVD MRI variables. Multivariable linear regression models will be used to examine the association between the relative level of each protein and WMHs. Multivariable logistic regression will be used to assess the relationship between relative protein levels and both CMBs and lacunar infarcts, separately. Analyses will be first adjusted for age at sample acquisition, sex, education, race-center, and intracranial volume (model 1). Second, analyses will be adjusted for cardiovascular risk factors—i.e., BMI, hypertension, diabetes, and smoking status—and kidney function (model 2). FDR corrected $P<0.05$ will be used to identify candidate proteins. Sensitivity analyses will be conducted excluding participants with (a) prevalent stroke and (b) vasoactive medication use (including ARBs and ACE inhibitors, among others) at the time of plasma collection.

Additionally, analyses will be repeated using weighted correlation network analysis (WGCNA) - defined protein network expression as the exposure variable. WGCNA will be used to identify networks of correlated proteins within the set of 4,877 proteins using the full set of participants with available SomaScan proteins at visits 3 and 5. WGCNA converts the protein-protein correlation matrix into an adjacency matrix that filters weak correlations based on a power threshold chosen to meet scale-free topology criteria. These algorithms use hierarchical cluster analyses and dynamic tree cutting implemented to group proteins based on patterns of coexpression. After modules are identified, module expression values (module eigenproteins [MEs]) are calculated from the first principal component of each module for each participant.

These values represent measures of IPN expression that can be related to participant traits and outcomes.

Midlife Replication Analysis. Using the candidate proteins identified by the primary analyses (Visit 5) that pass FDR corrections, the same models outlined above will be replicated at midlife (Visit 3).

Replication of SVD-associated proteins in the AGES-Reykjavik and MESA studies. To assess the generalizability of the protein associations with SVD metrics found in the ARIC cohort, a selection of proteins associated with MRI variables in the primary analyses will be assessed in analogous models in the AGES-Reykjavik[35] and/or MESA[36] samples, which have been described in detail previously. Participants with available MRI-defined SVD measures and complete covariate information will be included in the replication study. Models will be adjusted for baseline age, sex, education, BMI, diabetes, hypertension, smoking status, and kidney function.

Mendelian Randomization Analysis. We will use a two-sample MR design[26] to determine whether there is evidence for a causal link between SVD-associated proteins and SVD. Genetic instrumental variables (IVs) will be derived from protein GWAS's conducted in ARIC or using an external cohort, e.g., Sun et al. (2018). We will use summary statistics from WMH[18,19], ICH[21] (as the closest available proxy for CMB), and small vessel stroke[25] (as the closest available proxy lacunar infarct) GWAS's to determine the association between IVs and each of the respective outcomes. Inverse variance weighted regression, which examines the SNP-exposure association against the inverse of the variance of SNP-outcome association, will be used determine the causal estimate. We will also conduct a series of sensitivity analyses using multiple tests robust to violations in MR assumptions, including the Mendelian Randomization-Egger and weighted median method.

Ingenuity pathway analysis. In order to understand the regulatory mechanisms underlying the association between the candidate proteins and SVD, we will use Ingenuity Pathway Analysis (IPA), a bioinformatics platform providing interpretations of omics data using the Ingenuity Knowledge Base (IPA, QIAGEN Inc., https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis). We will use candidate proteins associated with MRI variables at a to-be-determined threshold (we will select a threshold that includes >100 of the top proteins, as recommended for IPA analyses). We will perform IPA canonical pathway, upstream regulator, and mechanistic pathway analyses to construct biological interpretations of the pattern of candidate proteins identified to be associated with SVD.

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 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

___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)?

MP# 3327. A proteomic analysis of incident dementia: The ARIC Study

MP# 3903. Multi-omic data integration using systems approaches for mechanistic understanding of disease in the Atherosclerosis Risk in Communities (ARIC) Study

MP#3113. Identification of novel genetic variants associated with Alzheimer's disease in the Alzheimer's Disease Sequencing Project (ADSP)

MP#3728. Proteomics and risk prediction for incident cardiovascular disease, recurrent cardiovascular disease, and chronic kidney disease progression: A validation analysis

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* _____)
"Proteomic longitudinal ARIC study: SOMAscan of multiple visits"

___ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s) * 2013.10)

*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.
Understood

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.
Understood

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 pingping_wu@unc.edu. I will be using CMS data in my manuscript ____ Yes __X__ No.
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