ARIC Manuscript Proposal #3585

PC Reviewed: 3/10/20	**Status: _____**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Plasma proteomic signatures of ARIC participants at genetic risk for abdominal aortic aneurysm

b. Abbreviated Title (Length 26 characters): Proteomics and genetic risk of AAA

2. Writing Group: Brian Steffen, Weihong Tang, Jim Pankow, Faye Norby, Weihua Guan, Pam Lutsey, Nathan Pankratz, Kunihiro Matsushita, Ryan Demmer, and Adrienne Tin. We welcome any further nominations.

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

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3. Timeline: Begin analysis immediately
Draft of paper by midsummer, 2020

4. Rationale:

Abdominal aortic aneurysm (AAA) is a potentially life threatening condition typified by a bulging aorta in the abdominal cavity with 80-90% occurring in the infrarenal section of the vessel (1). So far, the root causes of AAA have proven elusive, but the nascent stages of AAA and its progression have been characterized by both degradation and remodeling of extracellular matrix in the aortic wall (2-4), which appears to involve the activation and perpetuation of inflammation, an abnormal response of the innate or adaptive immune systems, up-regulation of matrix metalloproteinases (MMPs) as well as other proteinases, and an impaired compensatory repair of the extracellular matrix (4-11). Reflecting these processes, elevated plasma levels of white blood cells, fibrinogen, D-dimer, troponin T, N-terminal pro-brain natriuretic peptide, high-sensitivity C-reactive protein, interleukin-6 and MMP-9 have been shown to be related to incident AAA in prospective analyses (12, 13). Despite our understanding of these phenotypic manifestations of AAA, its etiology is not well-established, although genetic factors are known contributors.

Apart from smoking, genetic susceptibility is one of the stronger risk factors for AAA development. Indeed, it is estimated that having a first degree relative with AAA translates to a two to eight-fold higher risk for the disease (14, 15), and twin studies have reported a 70-77% rate of heritability (16,17). Given this genetic component, family linkage and genome-wide association studies have been conducted to identify gene variants that are associated with AAA (18-21). In the most recent and comprehensive GWAS meta-analysis followed by a large validation study, Jones et al. (2017) confirmed five previously identified AAA-associated gene variants and reported four additional loci that were unique to AAA (22). These genes and their corresponding coding regions suggest that inflammation, vessel wall remodeling, cell survival, and lipid handling may be involved in AAA development. And yet, no comprehensive large-scale study has taken a broader approach to evaluate downstream proteomic signatures associated with these loci, which may provide evidence for specific protein and pathway targets for disease treatment or prevention. To address this, we propose to examine the associations of those variants identified by Jones et al. (2017) with protein data measured by the SOMAscan v 4.0 platform. We will also develop a composite polygenic risk score (PRS) that will identify individuals with, as far as these variants are concerned, the greatest genetic risk of developing AAA. We posit that characterization of their protein signatures will provide additional insights for risk stratification and shed further light on potential mechanisms of genetically increased risk of AAA.

SOMAscan v 4.0 quantifies 4,931 human proteins through an aptamer-based platform that avoids the inherent issues of mass-spectrometry methodologies (23), and it has so far identified novel proteomic signatures of cardiovascular disease, inflammatory bowel disease, early chronic obstructive pulmonary disease, and muscular dystrophy (24-27). Among the analytes included in SOMAscan are inflammatory cytokines and their receptors, proteases, protease inhibitors, and vessel wall degradation enzymes as well as other protein families likely involved in, or otherwise markers of, AAA development and/or progression (28). The substantial

protein coverage of SOMAscan v 4.0 and the over eleven thousand ARIC participants with available data provide a unique opportunity to characterize proteomic signatures associated with the genetic risk of AAA development and/or its progression.

5. Main Hypothesis/Study Questions:

Hypotheses: 1) Specific proteomic profiles will be associated with high risk AAA alleles, which might point to specific pathways/mechanisms related to AAA development or progression; 2) ARIC participants with a high genetic risk of AAA will show unique proteomic signatures that may be related to known and unknown mechanisms of AAA.

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).

Design: cross-sectional. Proteomic data were measured in Visit 3 samples. Data from Visit 5 will serve as an internal replication sample. Visit 3 was selected for the primary analysis due to the larger sample size, fewer comorbidities, and less extensive medication use.

Inclusion/Exclusion: We will exclude participants who are not black or white, do not have SOMAscan data at visit 3 or the corresponding genetic variants, or failed QC for SNPs or proteins.

Exposure:

1) AAA-associated gene variants:
   PSRC1-CELSR2-SORT1 (rs602633)
   IL6 (rs4129267)
   CDKN2BAS1/ANRIL (rs10757274)
   DAB2IP (rs10985349)
   LDLR (rs6511720)
   SMYD2 (rs1795061)
   LINC00540 (rs9316871)
   PCIF1/MMP9/ZNF335 (rs3827066)
   ERG (rs2836411)

2) PRS comprised of genotypes from the above AAA-associated SNPs (detailed below).

Outcome: Natural log2-transformed SomaScan protein levels will serve as the outcome variable, and ARIC analytic recommendations for the SomaScan data will be followed. The proteins were

assessed using a Slow Off-rate Modified Aptamer (SOMAmer)-based capture array (SomaLogic, Inc, Boulder, Colorado). Non-human proteins and proteins with unacceptable QC will be removed (eg have large CV (e.g. > 20%), poor reproducibility between the blind duplicate pairs, or non-specific binding).

Data analysis:
Multiple linear regression analysis in which the genotype of each SNP or PRS will serve as the independent variable and SOMAscan protein levels will serve as dependent variables. For each SNP, the number copy of AAA risk allele as reported in the AAA GWAS will be modeled. The PRS will be calculated as the weighted sum of the number of risk alleles. Weighted sums will be derived from odds ratios reported by Jones et al. (19). Environmental factors are not expected to affect observations; however, two models will be generated: model 1) covariates will include: age at visit 3, sex, principal components for ancestry, estimated glomerular filtration rate, field center; model 2) model 1 + diabetes diagnosis, systolic blood pressure, uncontrolled hypertension, BMI, smoking status, LDL-cholesterol concentration, and use of lipid-lowering therapy. We will analyze the PRS as both a continuous variable and categorical (i.e., top quintile vs remaining). The categorical PRS may capture additional proteins that are associated with high genetic risk. Statistical significance will be determined after Bonferroni correction of the number of proteins and SNPs tested. We will compare associations between Black and white individuals. For significant proteins, we will also evaluate whether the strength of associations differs in smokers vs nonsmokers and males vs females. As a replication step, we will repeat the analyses using data collected at visit 5. We will also seek replication of significant findings in external cohorts that have published GWAS for SOMAscan proteins.

To aid in the interpretation of the results, we will use Ingenuity Pathway Analysis to identify the most relevant signaling and metabolic pathways, molecular networks, and biological functions for associated proteins.

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/mantrack/maintain/search/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)?

No proposals have been generated that examine proteomics and SNPs of AAA.

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* _AS2017.27___)

___ 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 https://www2.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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