ARIC Manuscript Proposal #3640

PC Reviewed: 6/9/20	**Status: _____**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Association between circulating protein C and incident dementia

b. Abbreviated Title (Length 26 characters): Protein C and dementia

2. Writing Group:

Writing group members: Adrienne Tin, Keenan Walker, Kevin Sullivan, Aozhou Wu, B. Gwen Windham, Michael Griswold, Jan Bressler, A. Richey Sharrett, Rebecca Gottesman, Myriam Fornage, Aaron Folsom, Joe Coresh, Tom Mosley

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: Analysis will start immediately after aricpub approval with a draft manuscript for circulation in 3 months.

4. Rationale:

Vascular pathology is a likely contributor to dementia.[1,2] Protein C is an anticoagulant in the hemostasis mechanism, which protects the vasculature by maintaining the flow of blood and

stopping bleeding.[3,4] Lower circulating protein C levels have been associated with incident stroke, which is an established risk factor for dementia.[5-7] Lower protein C levels could contribute to subclinical vascular damage leading to dementia without having stroke events preceding dementia. Whether lower circulating protein C levels are associated with incident dementia independent of dementia risk factors has not been assessed.

A major function of protein C is to prevent the excessive formation of blood clots.[8] In reaction to vascular injury, protein C is activated by the thrombomodulin-thrombin complex to become activated protein C (APC), which can deactivate two coagulants: active form of factor V (FVa) and active form of factor VIII (FVIIIa). Most protein C in circulation is inactive and serves as a reservoir.

Our knowledge on hemostasis regulation in the brain is much more limited compared with our knowledge of hemostasis in other organs.[3] Circulating blood biomarkers, including C-reactive protein, von Willebrand factor (vWF), activated factor VIII (FVIIIa), fibrinogen, IL-10, IL-1beta, IL-4 and IL-2, have been associated with dementia, cognitive decline, or brain imaging measures of neurodegeneration in prospective studies and generated insight on the development of dementia.[9-13] In the peripheral vasculature, the impact of hemostatic factors varies by vascular bed.[3] For example, protein C deficiency tends to affect venous thrombosis rather than arterial thrombosis.[14] Little is known on the effects of hemostatic factors by vascular bed in the brain. In addition, the venous contribution to subclinical features of dementia pathologies, such as white matter hyperintensities and microinfarcts is still unclear.[15,16] Studying the association between circulating protein C and incident dementia may provide insight on potential contribution of anticoagulant on dementia pathologies.

In the ARIC study, circulating protein C levels have been measured at visit 1 (1987-89) as part of the Hemostasis Study[17-20] and are also available as part of the SomaScan assay at visits 3 and 5. The SomaScan assay also has measures of the active form of protein C (APC). Incident dementia events have been ascertained in all participants after visit 1. An association study on circulating protein C levels and incident dementia may provide novel insight on hemostasis and dementia pathologies.

5. Main Hypothesis/Study Questions:

Given that measures of protein C at visit 1 were from a validated assay with absolute quantification, we will focus our main hypothesis on using visit 1 as the baseline. The analysis on using the measures of protein C from the SomaScan assay at visits 3 and 5 will serve to investigate whether the association of protein C with incident dementia may be consistent across assay platforms, baseline age, and follow-up time.

Main hypothesis:
H1. Lower protein C levels at visit 1 (1987-89) will be associated with higher risk of dementia independent of risk factors of dementia, including prevalent and incident stroke. The dementia events will be censored at the end of visit 6 (2016-17) with approximately 29 years of follow-up.

Secondary hypotheses:
H2.1. Lower protein C levels at visit 3 (1993-95, SomaScan assay) will be associated with higher risk of dementia independent of risk factors of dementia, including prevalent and incident stroke. The dementia events will be censored at the end of visit 6 (2016-17) with approximately 24 years of follow-up.

H2.2. Lower protein C levels at visit 5 (2011-13, SomaScan assay) will be associated with higher risk of dementia independent of risk factors of dementia, including prevalent and incident stroke. The dementia events will be censored at the end of visit 6 (2016-17) with approximately 6 years of follow-up

Exploratory hypotheses:
These exploratory hypotheses are not intended for publication. The goal for testing them is to explore the coherency of the relations of the main and secondary results with related data. The exploratory results are not expected to be necessary for supporting the main and secondary results for publication.

Exploratory hypotheses related to APC:
H3.1a. Higher SomaScan APC levels at visit 3 will be associated with higher risk of dementia ascertained up to visit 6. APC is the active form that confers the anticoagulant effect. However, the levels of APC may be reactions to injuries and could have risk association with incident dementia, rather than protective association. In addition, the effect size of APC will likely be weak because APC has more variability then protein C due to activation.
H3.1b. Higher SomaScan APC levels at visit 5 will be associated with higher risk of dementia ascertained up to visit 6.

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: longitudinal cohort study

Inclusion criteria: Participants with self-reported race as white (from Washington county, MD; Forsyth, NC; and Minneapolis suburb, MN) and black (from Jackson, MS and Forsyth, NC) and with data in the exposure, outcome, and covariates

Exclusion criteria: Due to small numbers, we will exclude participants from Washington county and Minneapolis suburb who were not self-reported white, participants from Jackson who were not self-reported black, and participants from Forsyth who were not self-reported white and black.

Exposure:
Primary exposure: Circulating protein C levels measured at visit 1.
Secondary exposures: Circulating protein C levels from SomaScan assay measured at visits 3 and 5.

Exploratory exposure: Circulating APC from SomaScan assay at visits 3 and 5.

Outcome:

Primary outcome: Incident dementia with baseline at visit 1 (1987-89) up to the end of visit 6 (2016-17) using the level 3 definition published previously.[21] This definition incorporated results from longitudinal cognitive evaluation from visits 2, and 4 (3 cognitive measures), comprehensive neuropsychological battery at visits 5 and 6 (10 cognitive measures), dementia classified based on Telephone Interview for Cognitive Status–Modified (TICSm), dementia based on informant telephone interviews using a modified version of the Clinical Dementia Rating and the Functional Activities Questionnaire among a subset identified as having suspect dementia, or dementia cases identified solely by surveillance based on a prior discharge hospitalization ICD-9 or death certificate code for dementia.[21]

Secondary outcomes:

1) Incident dementia with baseline at visit 3 (1993-95) up to visit 6 (2016-17) using the same definition as the primary outcome.
2) Incident dementia with baseline at visit 5 (2011-13) up to visit 6 (2016-17) using the same definition as the primary outcome.

Other variables for primary analysis

Visit 1 variables

Demographics and genetics: age, sex, race, center, education levels, APOE genotype
Vascular risk factors: BMI, current smoking, hypertension and diabetes status, total cholesterol, prevalent coronary heart disease and stroke, incident stroke up to visit 6 based on the ARIC adjudicated definition and incident stroke follow-up time.[21,22]
Hemostasis-related: platelet count, fibrinogen, FVIIIc, and vWF. These coagulants are included because they have been associated with dementia-related outcomes in ARIC.[13]

Other variables for secondary analysis

The secondary analyses will include the same covariates at visits 3 and 5 corresponding to the respected baseline as the primary analysis if they are available for the full cohort. SomaScan proteins are considered available if they are in the assay and have flag2=0 in the SomaScan data annotation. Platelet count is not available at visit 3 and available at visit 5. vWF is not available from the SomaScan assay (flag2 = 1). Factor VIII and fibrinogen from SomaScan assay are available.

Visit 3 variables

Demographics: age, sex, race, center
Vascular risk factors: BMI, current smoking, hypertension and diabetes status, total cholesterol, prevalent coronary heart disease and stroke, incident stroke up to visit 6 based on the ARIC adjudicated definition and incident stroke follow-up time.[21,22]
Hemostasis-related: Factor VIII and fibrinogen from SomaScan assay

Visit 5 variables
Demographics: age, sex, race, center (use v5center, instead of center)
Vascular risk factors: BMI, current smoking, hypertension and diabetes status, total cholesterol, prevalent coronary heart disease and stroke, incident stroke up to visit 6 based on the ARIC adjudicated definition and incident stroke follow-up time.[21,22]
Hemostasis-related: platelet count, Factor VIII and fibrinogen from SomaScan assay

Data analysis:

Modeling of protein C levels. First, protein C levels will be analyzed by quintile for inspection of potential non-linear association. Next, protein C levels will be modeled as a cubic spline to evaluate the association across the full range of values. Appropriate transformation will be determined based on the inspection of the distribution. The reference point and knots of the cubic spline will be informed by the quintile analysis. Analysis using protein C measures from visit 1 will additionally consider reference range used in clinical practice.

Survival analysis methods
The survival analysis methods and models for the primary and secondary analyses will be the same, except for the hemostasis-related covariates due to availability as stated in the Other Variable section.

Given the long follow-up time and the age of the cohort, death can preclude the development of dementia. Therefore, the primary analysis will use deaths unrelated to dementia as competing risk events. We will use the Fine and Gray method, which is a proportional hazards model that estimates subhazards for dementia.[25] The proportional subhazards assumption will be checked using Schoenfeld residuals.

Survival analysis models. A series of models will be used to assess the influence of potential confounders on the strength of association between protein C and incident dementia.

Model 1 will be an unadjusted analysis for estimating the crude association.
Model 2 will include demographic and genetic variables (age, sex, race-center, education levels, and APOE genotype)
Model 3 will add the vascular risk factors (BMI, current smoking, hypertension and diabetes status, total cholesterol, prevalent coronary heart disease and stroke)
Model 4 will add incident stroke, which will be modeled as a time varying covariate.
Model 5 will add hemostasis-related variables.

Sensitivity analyses:

1) Repeat the competing risk models excluding participants with incident stroke prior to incident dementia to evaluate potential residual confounding from incident stroke
2) Repeat the models using Cox regression to evaluate the potential bias due to the competing risk of death. If the results are similar, for publication, the Cox regression may be easier to understand. The competing risk results can be included as supplementary analysis.

Additional Exploratory analyses

The analysis of SomaScan APC at visits 3 and 5 will be the same as the analysis for SomaScan protein C at the same visit.

Limitations:

1) Other hemostatic factors that are available in ARC, mainly coagulants, are not included in this proposal. Only Protein C, an anticoagulant, is included. Given that hemostasis is complex and little is known about hemostasis in the brain, it is helpful to take a detailed look at protein C, especially the protein C measures in SomaScan at visits 3 and 5 have acceptable quality (flag2=0).
2) External validation plan has not been established. A potential validation cohort is the Age, Gene/Environment Susceptibility (AGES) – Reykjavik Study.[26] Given that we focus on one protein and the protein C measures at visit 1 have been published previously, external validation is not critical for publication.

7.a. Will the data be used for non-CVD analysis in this manuscript? _X_ Yes ____ 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? _X_ 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)?

#3096 Systemic inflammation, cognitive decline and dementia

#2698 Hemostasis and cognitive decline

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? __X_ Yes ____ No

ARIC NCS

11.b. If yes, is the proposal

_X_ A. primarily the result of an ancillary study (list number* _ARIC Hemostatic factor and NCS ancillary studies**____)**

___ 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.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.

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.

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

References

1. Cortes-Canteli M, Iadecola C. Alzheimer's Disease and Vascular Aging. Journal of the American College of Cardiology 2020;75:942.
2. Sweeney MD, Montagne A, Sagare AP, et al. Vascular dysfunction-The disregarded partner of Alzheimer's disease. Alzheimer's & dementia : the journal of the Alzheimer's Association 2019;15:158-67.
3. Fisher MJ. Brain regulation of thrombosis and hemostasis: from theory to practice. Stroke 2013;44:3275-85.
4. De Luca C, Colangelo AM, Alberghina L, Papa M. Neuro-Immune Hemostasis: Homeostasis and Diseases in the Central Nervous System. Front Cell Neurosci 2018;12:459.
5. Folsom AR, Ohira T, Yamagishi K, Cushman M. Low protein C and incidence of ischemic stroke and coronary heart disease: the Atherosclerosis Risk in Communities (ARIC) Study. J Thromb Haemost 2009;7:1774-8.
6. Kuzma E, Lourida I, Moore SF, Levine DA, Ukoumunne OC, Llewellyn DJ. Stroke and dementia risk: A systematic review and meta-analysis. Alzheimer's & dementia : the journal of the Alzheimer's Association 2018;14:1416-26.

7. Pendlebury ST, Rothwell PM. Prevalence, incidence, and factors associated with pre-stroke and post-stroke dementia: a systematic review and meta-analysis. Lancet Neurol 2009;8:1006-18.
8. Clouse LH, Comp PC. The Regulation of Hemostasis: The Protein C System. New England Journal of Medicine 1986;314:1298-304.
9. Tao Q, Ang TFA, DeCarli C, et al. Association of Chronic Low-grade Inflammation With Risk of Alzheimer Disease in ApoE4 Carriers. JAMA Netw Open 2018;1:e183597.
10. King E, O'Brien JT, Donaghy P, et al. Peripheral inflammation in prodromal Alzheimer's and Lewy body dementias. J Neurol Neurosurg Psychiatry 2018;89:339-45.
11. Engelhart MJ, Geerlings MI, Meijer J, et al. Inflammatory proteins in plasma and the risk of dementia: the rotterdam study. Arch Neurol 2004;61:668-72.
12. Walker KA, Power MC, Hoogeveen RC, et al. Midlife Systemic Inflammation, Late-Life White Matter Integrity, and Cerebral Small Vessel Disease: The Atherosclerosis Risk in Communities Study. Stroke 2017;48:3196-202.
13. Walker KA, Gottesman RF, Wu A, et al. Systemic inflammation during midlife and cognitive change over 20 years: The ARIC Study. Neurology 2019;92:e1256-67.
14. Rosenberg RD, Aird WC. Vascular-bed--specific hemostasis and hypercoagulable states. N Engl J Med 1999;340:1555-64.
15. Alber J, Alladi S, Bae HJ, et al. White matter hyperintensities in vascular contributions to cognitive impairment and dementia (VCID): Knowledge gaps and opportunities. Alzheimers Dement (N Y) 2019;5:107-17.
16. Hartmann DA, Hyacinth HI, Liao F-F, Shih AY. Does pathology of small venules contribute to cerebral microinfarcts and dementia? Journal of Neurochemistry 2018;144:517-26.
17. Papp AC, Hatzakis H, Bracey A, Wu KK. ARIC hemostasis study--I. Development of a blood collection and processing system suitable for multicenter hemostatic studies. Thromb Haemost 1989;61:15-9.
18. Wu KK, Papp AC, Patsch W, Rock R, Eckfeldt J, Sharrett R. ARIC hemostasis study--II. Organizational plan and feasibility study. Thromb Haemost 1990;64:521-5.
19. Chambless LE, McMahon R, Finch A, et al. ARIC hemostasis study--III. Quality control. Atherosclerosis Risk in Communities. Thromb Haemost 1993;70:588-94.
20. Nguyen ND, Ghaddar H, Stinson V, Chambless LE, Wu KK. ARIC hemostasis study--IV. Intraindividual variability and reliability of hemostatic factors. The Atherosclerosis Risk in Communities (ARIC). Thromb Haemost 1995;73:256-60.
21. Gottesman RF, Albert MS, Alonso A, et al. Associations Between Midlife Vascular Risk Factors and 25-Year Incident Dementia in the Atherosclerosis Risk in Communities (ARIC) Cohort. JAMA Neurol 2017;74:1246-54.
22. Koton S, Sang Y, Schneider ALC, Rosamond WD, Gottesman RF, Coresh J. Trends in Stroke Incidence Rates in Older US Adults: An Update From the Atherosclerosis Risk in Communities (ARIC) Cohort Study. JAMA Neurol 2019.
23. Folsom AR, Aleksic N, Wang L, Cushman M, Wu KK, White RH. Protein C, antithrombin, and venous thromboembolism incidence: a prospective population-based study. Arterioscler Thromb Vasc Biol 2002;22:1018-22.
24. Fashanu OE, Heckbert SR, Aguilar D, et al. Galectin-3 and venous thromboembolism incidence: the Atherosclerosis Risk in Communities (ARIC) Study. Res Pract Thromb Haemost 2017;1:223-30.
25. Fine JP, Gray RJ. A Proportional Hazards Model for the Subdistribution of a Competing Risk. Journal of the American Statistical Association 1999;94:496-509.
26. Emilsson V, Ilkov M, Lamb JR, et al. Co-regulatory networks of human serum proteins link genetics to disease. Science 2018:eaaq1327.