ARIC Manuscript Proposal #2777

PC Reviewed: 7/12/16	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Circulating ceruloplasmin and incidence of venous thromboembolism: the Atherosclerosis Risk in Communities (ARIC) Study.

b. Abbreviated Title (Length 26 characters): Ceruloplasmin and VTE.

2. Writing Group: Antonio Arenas, Alvaro Alonso, Ron Hoogeveen, Nick Roetker, Aaron Folsom.

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

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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).

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3. Timeline: Data analysis: One month from manuscript approval date.

First draft of the manuscript: 2-3 months from the manuscript approval date.

4. Rationale:

Venous thromboembolism (VTE) is the third most common cardiovascular disease after myocardial infarction and stroke. It is estimated that at least 900,000 people could be affected by VTE (1 to 2 per 1,000) each year in the United States. Estimates suggest that 60,000-100,000 Americans die of VTE, of which 10 to 30% of people will die within one month of diagnosis. Sudden death is the first symptom in about one-quarter of people who have a pulmonary embolism (PE) and one-third of people with VTE will have a recurrence within 10 years.[1-3] Because of the public health burden and expense of VTE, we need to find more biomarkers to help us detect or predict VTE and/or monitor the treatment prescribed.

Ceruloplasmin (CP) is an enzyme synthesized in the liver that is responsible for transport of circulating copper and is also involved in iron metabolism. It is an acute-phase reactant that may have antioxidant actions but can also participate in the generation of free radicals that seems to underlie several illnesses such as myocardial infarction, arteriosclerosis, unstable angina, abdominal aortic aneurysm, vasculitis and peripheral arterial disease and even dementia.[4,5]

There is strong evidence that inflammation, where CP levels are increased, is associated with increased risk of atherothrombosis.[6,7] Several studies have demonstrated a relationship between various proteins involved in inflammatory processes and VTE. S. V. Sveinsdottir et al. did not show a significant relationship between inflammatory markers (fibrinogen, orosomucoid, α1-antitrypsin, haptoglobin and ceruloplasmin) and VTE incidence. ARIC previously found that elevated CRP, but not fibrinogen, is independently associated with increased risk of VTE.[8]

Two single nucleotide polymorphisms (SNPs) have been located in the CP gene promoter and associated with higher CP concentrations in blood (rs11708215 and rs113072552).[5,9] We also propose to address the association between these SNPs, circulating CP and VTE incidence in the Atherosclerosis Risk in Communities (ARIC) Study.

5. Main Hypothesis/Study Questions:

Aim #1: To determine the association between circulating CP and the incidence of VTE in the ARIC study.

Aim #2: To determine the association of rs11708215 and rs13072552 with CP concentrations and the incidence of VTE.

We hypothesize that individuals with higher CP concentrations will have an increased risk for VTE and that genetic variants associated with higher CP levels will also be associated with increased VTE.

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:

A follow-up data analysis will be performed utilizing longitudinal data from the ARIC cohort, using visit 4 as baseline.

Inclusion/exclusion criteria:

We will exclude individuals with (1) prevalent VTE at baseline based on prior VTE diagnosis, (2) missing baseline CP data, (3) missing other covariates, (4) race other than white or African-American.

Variables of interest:

Main outcome of interest: Venous thromboembolism incidence

VTE outcomes have been identified through the LITE ancillary study. The time to incident VTE from baseline through December 31, 2011, will be the main outcome variable.

Main independent variables of interest: CP blood concentrations and SNPs

In the ARIC study, CP levels were assessed through laboratory tests at visit 4.

Plasma CP levels were measured by immunoturbidimetric assay using an automated chemistry analyzer (Olympus AU400e, manufacturer Olympus Life Science Research Europa GmbH).

Covariates

From visit 4, measured covariates to be included in the main analysis are age (continous), gender (male/female), race (African American/white), and body mass index (BMI), and hormone replacement therapy, if available. We will also consider some other blood markers measured at visit 1 or visit 3: factor VIII, VWF, aPTT, D-dimer, and factor XI, but do not expect them to be confounders.

Likewise, other risk factors as such as blood pressure, diabetes, smoking, lipid levels and physical activity were not strong VTE risk factors in ARIC, but we will verify that they are not confounders in this analysis.

Statistical analysis:

Cox proportional hazards models will be used to determine the association between CP concentrations and incident VTE. Initially, we will explore the shape of the association of CP with VTE risk using restricted cubic splines. Log transformations will be done if necessary. If appropriate, circulating CP will be divided into quartiles. We will also assess linear associations based on the spline model. The following models will be used to analyze the CP-VTE association:

- Model 1: adjustment for age, gender, race, BMI and HRT, study site
- Model 2: Model 1 + adjustment for other potential confounders
- Model 3: Model 2 + CRP (This model will be run to see whether CP or CRP is the stronger inflammatory biomarker for VTE).

In a second step, we will run linear regression testing association between CP SNP (rs11708215, rs13072552) and CP concentrations. These analyses will be stratified by race.

Finally, a race-stratified Cox models testing associations between CP SNP rs11708215, rs13072552 separately and VTE risk will be performed with adjustment for age, sex, and race.

Additional models will adjust for covariates listed above, as well as for CP concentrations

to test whether any association between rs11708215 or rs13072552, if present, are mediated by concentrations of circulating CP.

We expect to include more than >400 incident events of VTE, which will provide sufficient power to study the association of circulating CP with VTE risk in the entire sample.

Strengths and limitations:

Strengths of the study include the large sample size and power to measure associations between CP and VTE. However, there are a couple of limitations. Although the LITE study validated VTEs, some VTE cases treated in outpatient settings are missed. In addition, there may be some misclassification of the CP concentrations exposure since there is no follow-up information on circulating CP after visit 4. As a result, if the CP measures happened to change over time, there is no additional information to examine such changes from follow-up data.

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)?

No previous manuscript proposals in ARIC have specifically examined the association between CP and VTE.

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* 2006.16)

___ 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 __X__ No.

References:

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4. Jeremy JY, Shukla N. Ceruloplasmin dysfunction: a key factor in the pathophysiology of atrial fibrillation? *Journal of internal medicine*. 2014;275(2):191-4.
5. Dadu RT, Dodge R, Nambi V, Virani SS, Hoogeveen RC, Smith NL, et al. Ceruloplasmin and heart failure in the Atherosclerosis Risk in Communities study. *Circulation Heart failure.* 2013;6(5):936-43.
6. Casas JP, Shah T, Hingorani AD, et al. C-reactive protein and coronary heart disease: a critical review. J Intern Med 2008;264:295–314.
7. Ridker PM, Cushman M, Stampfer MJ, et al. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med 1997;336:973–9.
8. Folsom AR, Lutsey PL, Astor BC, Cushman M. C-reactive protein and venous thromboembolism. A prospective investigation in the ARIC cohort. Thrombosis and haemostasis. 2009;102(4):615-9.
9. Adamsson Eryd S, Sjogren M, Smith JG, Nilsson PM, Melander O, Hedblad B, et al. Ceruloplasmin and atrial fibrillation: evidence of causality from a population-based Mendelian randomization study. Journal of internal medicine. 2014;275(2):164-71.