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

b. **Abbreviated Title (Length 26 characters):** CP and AF in ARIC.

2. **Writing Group:** Antonio Arenas, Faye Norby, Lin Y Chen, Elsayed Z. Soliman, Ron Hoogeveen, Dan Arking, Alvaro Alonso; others welcome

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. __AA__ [please confirm with your initials electronically or in writing]

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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:**

Atrial fibrillation (AF) is the most common clinically-significant arrhythmia in the world. It is estimated that, in the United States alone, the number of people who suffer AF is approximately 2.5 million, occurring 1.5 times more often in men than in women.\(^1\) Despite the decline in morbidity and mortality from cardiovascular disease due to advances in prevention and treatment, AF has not followed a similar trend. Over the coming years, the incidence of AF is expected to increase.\(^2\)

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.\(^3,4\)

CP appears to promote structural changes in the atrium making it more arrhythmogenic. If this relationship between AF and CP is confirmed, new prevention approaches could be researched and we could identify individuals at increased risk of AF.

A recently published study showed that higher concentrations of blood CP were associated with increased AF risk. In this same study, rs11708215, a single nucleotide polymorphism (SNP) located in the CP gene promoter and associated with higher CP concentrations in blood, was strongly associated with AF.\(^5\) These results, however, have not been replicated in other studies. Another SNP, rs13072552, also in the CP gene has been associated with CP plasma concentration.\(^4\)

We propose to address the association between rs11708215 and rs13072552, circulating CP and AF 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 AF in the ARIC study.

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

We hypothesize that individuals with higher CP concentrations will have an increased risk for AF and that genetic variants associated with higher CP levels will also be associated with increased AF.
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 (1) with prevalent AF or atrial flutter at baseline based on electrocardiogram (ECG) or prior AF diagnosis, (2) missing baseline CP data, (3) missing other covariates, (4) with a race other than white or African-American, and (5) non-whites from the Minnesota and Washington County sites.

Variables of interest:

Main outcome of interest: Atrial fibrillation incidence
The time to incident AF cases from baseline through December 31, 2012, will be the main outcome variable. Incident AF cases were ascertained from three sources: ECGs completed during the study exams, ICD-9 codes of 427.31 or 427.32 from hospital discharges, and death certificates that include AF as a cause of death (ICD-9 code 427.3 or ICD-10 code I48). AF incidence date will be defined as the date of the first ECG showing AF, the first hospital discharge date for an AF or atrial flutter diagnosis, or date when death occurred due to AF, whichever occurred first.6

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) and N-terminal pro–B-type natriuretic peptide (NT-proBNP), hs-CRP levels and cardiac troponin T was measured as previously described.4

rs11708215 was genotyped using the Sequenom iPLEX assay. rs13072552 was genotyped with the Affymetrix Genome-Wide Human SNP Array 6.0.

Covariates
From visit 4, other measured covariates to be included in the analysis are age, gender, race, study site, body mass index (BMI), height (because this variable is a strong predictor of AF independently of BMI), drinking status, diabetes mellitus, educational level (collected at visit 1), smoking status, systolic and diastolic blood pressure, use of medications (antihypertensive and corticosteroids), circulating liver enzymes, history of HF, myocardial infarction (MI), and stroke. We will also adjust for other AF-related biomarkers including C-reactive protein, troponin T, and NT-proBNP.

Statistical analysis:
Cox proportional hazards models will be used to determine the association between CP concentrations and incident AF. Initially, we will explore the shape of the association of CP with AF 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-AF association:

- Model 1: adjustment for age, gender, race, and ARIC study site
- Model 2: Model 1 + adjustment for BMI, height, alcohol drinking, diabetes mellitus, educational level, smoking status, systolic and diastolic blood pressure, total cholesterol and its fractions, liver enzymes and use of medications (antihypertensive and corticosteroids).
- Model 3: Model 2 + history of heart failure, MI, and stroke.
- Model 4: Model 3 + biomarkers CRP, BNP and troponin.

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 AF risk will be performed with adjustment for age, sex, race, and ARIC study site.

Additional models will adjust for covariates listed above in Models 2-4, 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 >1000 incident events of AF, which will provide sufficient power to study the association of circulating CP with AF risk in the entire sample. However, limited power might exist to study race-specific associations, particularly in African Americans, and the CP-AF association by the three different AF ascertainment sources.

Strengths and limitations:

Strengths of the study include the large sample size and power to measure associations between CP and AF. However, there are a couple of limitations. Although hospital discharge codes being used for identifying incident AF cases have shown to be valid, there is some likelihood of AF cases being missed in outpatient settings. 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  ____ 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
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 AF.

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

11.b. If yes, is the proposal

___ A. primarily the result of an ancillary study (list number* __________)

___ 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.csc.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.csc.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:


