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Circulating ceruloplasmin, ceruloplasmin-associated genes, and the incidence of atrial fibrillation in the Atherosclerosis Risk in Communities Study

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Abstract

Background—Ceruloplasmin (CP) may promote structural changes in the atrium making it more arrhythmogenic. We assessed the associations between CP, CP-associated genetic variants, and incident atrial fibrillation (AF) in the Atherosclerosis Risk in Communities (ARIC) study.

Methods and results—We studied 10,059 men and women without prevalent AF aged 53 to 75 years in 1996–1998 and followed through 2012. Circulating CP was measured in stored blood samples obtained in 1996–1998. Polymorphisms rs11708215 and rs13072552, previously associated with CP concentrations, were measured in 10,059 and 8,829 participants respectively. AF was ascertained from study electrocardiograms, hospital discharge codes, and death

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certificates. Multivariable Cox models were run to study the association between circulating CP, CP-associated polymorphisms, and the incidence of AF. Over 10.5 years of mean follow-up, 1357 cases of AF were identified. After adjusting for traditional risk factors and biomarkers, higher levels of circulating CP were associated with incident AF (hazard ratio [HR] 1.33, 95% confidence interval [CI] 1.11, 1.61 comparing top to bottom quartiles). Both rs11708215 and rs13072552 were significantly associated with CP levels. Presence of the CP-increasing alleles in rs11708215 and rs13072552, however, were significantly associated with lower risk of AF in whites (HR 0.84, 95%CI 0.76, 0.94, $p = 0.002$ and HR 0.83; 95%CI 0.69, 0.99, $p = 0.043$ respectively per CP-increasing allele in the final adjusted model) but not in African Americans.

Conclusions—Even though higher CP concentrations were associated with increased AF risk, genetic variants associated with higher CP decreased the risk of AF in whites. Our results suggest that circulating CP levels may not be causally related to risk of incident AF.

Keywords

atrial fibrillation; ceruloplasmin; single nucleotide polymorphism; oxidative stress

INTRODUCTION

Atrial fibrillation (AF) is the most common clinically-significant arrhythmia worldwide. It is estimated that, in the United States alone, the number of people who suffer AF is approximately 2.5 million, with men 1.5 times as likely to be affected compared to 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, and 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 seem to underlie several illnesses such as myocardial infarction, arteriosclerosis, unstable angina, abdominal aortic aneurysm, vasculitis and peripheral arterial disease, and even dementia. (3,4,5,6)

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. (7)

A recently published study showed that higher concentrations of CP in blood were associated with increased AF risk. In this same study, a variant of rs11708215, a single nucleotide polymorphism (SNP) located in the CP gene promoter, was associated with both higher CP concentrations in blood and increased AF risk. (7) 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. This SNP was selected based on a GWAS in ARIC. (4)

We addressed the association between rs11708215 and rs13072552, circulating CP and AF incidence in the Atherosclerosis Risk in Communities (ARIC) Study. We hypothesized that higher concentrations of circulating CP would be associated with AF incidence and, following a Mendelian randomization framework, that if the association between circulating CP and AF incidence is causal then genetic variants associated with higher circulating CP would also increase the risk of AF.

METHODS

Study population

The ARIC study is a community-based population study designed to investigate the causes of atherosclerosis and its clinical outcomes, as well as variation in cardiovascular risk factors, medical care, and disease by race and sex. (8) From 1987 to 1989 (ARIC study baseline), 15,792 adults (55.2% women; age, 45–64 years) from 4 US communities (Washington County, MD; suburbs of Minneapolis, MN; Jackson, MS; and Forsyth County, NC) were enrolled and underwent a home interview and clinic visit. Additional examinations were conducted in 1990 to 1992, 1993 to 1995, 1996 to 1998, and 2011 to 2013. Participants were mostly white in the Washington County and Minneapolis sites, exclusively black in Jackson, and a mix of both races in Forsyth County. Of the 11,656 participants in visit 4 (1996–1998), 11,484 had CP data available. Individuals with prevalent AF (N=524) at visit 4 and those with missing data for CP (N=166), missing information on rs11708215 (N=367) or any other variable used in the statistical models (N=473) were excluded from the study. We additionally excluded individuals who were not white or African American and any African American participants at the Minnesota and Washington County field centers because of small enrollment numbers (N=67). After all exclusions, 10,059 participants remained and were included in this analysis. Medical history, demographic data, anthropometric data, blood pressure measurements, and fasting lipid assessments were obtained during visit 4 at the same time as the blood draw for CP measurement. The ARIC study has been approved by the Institutional Review Board at the University of Minnesota, Johns Hopkins University, Wake Forest University, University of North Carolina, Baylor College of Medicine, University of Texas Health Sciences Center at Houston, and University of Mississippi Medical Center. Participants provided written informed consent.

Ascertainment of AF

AF cases were identified from study visit ECGs, death certificates and by review of hospital discharge records.(9,10) At each study examination, a standard supine 12-lead resting ECG was recorded with a MAC PC Personal Cardiograph (Marquette Electronics, Milwaukee, WI) and transmitted to the ARIC ECG Reading Center (Epidemiological Cardiology Research Center, Wake Forest School of Medicine, Winston Salem, NC) for automatic coding. A cardiologist visually confirmed all AF cases automatically detected from the study ECGs. Information on hospitalizations during follow-up was obtained from annual follow-up calls and surveillance of local hospitals, with hospital discharge diagnosis codes collected by trained abstractors. AF during follow-up was defined as *International Classification of Disease, 9th Revision (ICD-9), Clinical Modification* diagnostic codes 427.31 or 427.32. AF

cases detected in the same hospitalization with open cardiac surgery were not counted as cases. AF cases were also identified if code ICD-9 427.3 or *International Classification of Diseases, 10th Revision* (ICD-10) code I48 was listed as a cause of death. A participant was considered to have prevalent AF at visit 4 (baseline for this analysis) if he or she had a prior AF hospitalization or had AF diagnosed through any of the study ECGs. In this analysis, the AF incident date was defined as the date of the first ECG showing AF (4% of our AF cases), the first hospital discharge with AF coded (96% of AF cases), or when AF was listed as a cause of death (0.1% of cases), whichever occurred earlier.

Covariates

At each study visit, participants underwent a physical exam, provided blood samples, and answered questionnaires. For the present analysis, information on all covariates was obtained at visit 4, with the exception of education, which was only assessed at baseline. Sex, race, date of birth, education, smoking, and alcohol use were self-reported by the study participant. Self-reported educational achievement, a surrogate measure of socioeconomic status, was categorized into 3 levels: less than high school, high school graduate, and greater than high school. Smoking status was categorized as never, former or current based on self-report. Alcohol consumption was ascertained by means of an interviewer-administered dietary questionnaire, and classified into three alcohol-use groups: never, former, or current. (11) Weight and height were measured with the participant wearing light clothing. Body mass index (BMI) was calculated as weight (in kilograms) divided by height squared (in meters). Blood pressure was measured twice and were averaged to define systolic and diastolic blood pressure. Hypertension was defined as a systolic blood pressure of ≥ 140 mm Hg or a diastolic pressure of ≥ 90 mm Hg or use of antihypertensive medication. Diabetes was defined as a fasting blood glucose ≥ 126 mg/dL, a non-fasting blood glucose >200 mg/dL, a self-reported physician diagnosis of diabetes, or use of antidiabetic medication. (12)

Prevalent heart failure (HF) was identified by the Gothenburg criteria (13) or self-report of HF medication use in the past 2 weeks at the baseline visit. During follow-up, prevalent HF at each visit was identified as having a hospitalization with an ICD-9 428.0 code during follow-up prior to that exam. (14) Myocardial infarction was based on self-report at visit 1 and adjudicated events between visit 1 and visit 4. (15) History of stroke was defined as an adjudicated definite or probable hospitalized stroke occurring in a participant prior to visit 4 or a history of physician-diagnosed stroke at the baseline interview. (16,17)

Biomarker assays and genotyping

Plasma CP concentrations were measured in 2010–2011 from Visit 4 plasma samples (stored at -70°C since collection in 1996–1998) by immunoturbidimetric assay using an automated chemistry analyzer (Olympus AU400e, manufacturer Olympus Life Science Research Europa GmbH). The Cp turbidimetric procedure was calibrated every 14 days by using Olympus Serum Protein Multi-calibrator 2 (Cat #ODR3023), which was traceable to IFCC International Reference Preparation CRM470 (RPPHS). The inter-assay coefficient of variation for CP was 6.8%. Alanine transaminase (ALT), aspartate aminotransferase (AST), and gamma-glutamyltransferase (GGT) were simultaneously measured in Visit 4 plasma

samples using an Olympus AU400e automated chemistry analyzer (manufacturer Olympus Life Science Research Europa GmbH) according to the manufacturer's protocol. Inter-assay coefficients of variation were 11.1% for ALT, 8.5% for AST and 9.3% for GGT. (18)

N-terminal pro-B-type natriuretic peptide (NT-proBNP) was measured by using an electrochemiluminescent immunoassay on an automated Cobas e411 analyzer (Roche Diagnostics, Indianapolis, IN) with lower limit of detection 5 pg/mL and coefficient of variation 3.5 to 4.7%. (16) High sensitivity C-reactive protein (hs-CRP) levels were measured by using an immunonephelometric assay on a BNII autoanalyzer (Siemens Healthcare Diagnostics, Deerfield, IL) with a reliability coefficient of 0.9. Cardiac troponin T (cTnT) levels were measured by using a novel precommercial highly sensitive assay, Elecsys Troponin T (Roche Diagnostics), on an automated Cobas e411 analyzer with a lower limit of detection of 0.003 µg/L.

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

Statistical analysis

Baseline characteristics of the overall population were tabulated by CP quartiles. We report means for continuous variables and counts with percentages for categorical variables.

Cox proportional hazards models were used to determine the association between CP concentrations and incident AF. Follow-up time was calculated from the date of visit 4 to the incidence of AF, death, lost to follow-up, or December 31, 2012, whichever occurred first. Separate analyses were performed with circulating CP categorized into quartiles and as a continuous variable (in standard deviation units). The following models with incremental adjustments were used to analyze the CP-AF association: model 1: adjustment for age, sex, race, and ARIC study site; model 2: model 1 plus adjustment for BMI, height, alcohol drinking, smoking status, diabetes mellitus, educational level, systolic and diastolic blood pressure, total cholesterol and its fractions, liver enzymes and use of medications (antihypertensive and corticosteroids); model 3: model 2 plus history of heart failure, MI, and stroke; and model 4: model 3 plus biomarkers CRP, NT-proBNP and troponin. We conducted a sensitivity analysis excluding cases identified during the first 2 years of follow-up to avoid reverse causation (undiagnosed AF increasing circulating CP).

Secondly, race-specific linear regression models were used to test the association between *CP* gene SNPs (rs11708215, rs13072552) and CP concentrations. Analyses in African Americans were adjusted for the first 10 genetic principal components to correct for population stratification.

Thirdly, a race-specific Cox model was used to test associations between *CP* gene SNPs rs11708215 and rs13072552 separately, and AF risk, adjusting for age, sex, and ARIC study site and for covariates listed above in Models 2–4 as well as for CP concentrations to test whether any association with rs11708215 or rs13072552 was mediated by concentrations of circulating CP. We explored the associations using additive genetic models and estimating the associations by specific genotypes, using homozygous for the allele associated with

lower CP as reference. We also used a 2-stage least square model to estimate the effect of circulating ceruloplasmin on AF risk using the two SNPs as instrumental variables.

Finally, we created a CP-related genetic risk score (GRS) adding up the number of CP-increasing alleles in the two SNPs (rs11708215 and rs13072552) (range, 0–4) and also categorized the population by haplotypes formed by combinations of the 2 SNPs, studying the association of GRS and *CP* gene haplotypes with CP concentrations and the incidence of AF in multivariable race-specific models. We used an unweighted genetic score since there are no large studies that could provide validated weights.

RESULTS

After the exclusion of the participants listed above, this analysis included 10,059 (mean age 62.7 ± 5.6 years, 21.9% African American and 56.7% female). Mean CP levels were higher in African Americans than whites (311.6 ± 71.1 mg/L versus 296.8 ± 78.3 mg/L). The baseline demographic characteristics stratified by CP quartiles for the overall population are shown in Table 1. Overall, those with higher concentrations of circulating CP were more likely to be women, African American, had higher concentrations of hsCRP and NT-proBNP, but lower concentrations of cTnT.

Associations of CP concentration with incident AF

Table 2 presents the associations between circulating CP, stratified by quartiles and as a continuous variable, and AF risk. During a mean follow-up of 10.5 years, a total of 1357 individuals developed AF (212 AF events in African American and 1145 in whites). Higher levels of circulating CP were associated with incident AF in all the adjusted models. Individuals with CP in the highest quartile had significantly higher risk for AF than those in the lowest quartile (HR 1.38, 95%CI 1.08, 1.55) after adjusting for traditional risk factors. The association was comparable in the fully adjusted model including other biomarkers (HR 1.33, 95% CI 1.11, 1.61). A similar direct association was observed when we modeled circulating CP as a continuous variable (HR 1.06, 95%CI 0.99, 1.13, per 1-standard deviation difference in CP). Results were essentially unchanged after excluding 33 events occurring in the first 2 years of follow-up.

Association between rs11708215, rs13072552 and CP concentration

We performed a race-stratified analysis between CP concentration and the SNPs rs11708215 and rs13072552 located in or near the *CP* gene in chromosome 3 in 10,059 and 8829 subjects respectively (Table 3). The CP-increasing alleles frequency was different in whites and African Americans. A higher number of CP-increasing alleles in both SNPs was associated with higher concentrations of CP: 14.4 (95%CI 8.8, 20.1; $p < 0.001$) and 13.3 (95%CI 10.9, 15.8, $p < 0.001$) mg/L in African Americans and whites respectively for rs11708215, with the corresponding results being 5.9 (95%CI 1.7, 10.1; $p = 0.006$) and 21.6 (95%CI 17.6, 25.6; $p < 0.001$) mg/L for rs13072552. The R-squared for the association of each SNP with circulating CP ranged between 0.006 and 0.012.

Association between rs11708215, rs13072552 and AF risk

We next investigated the relation between rs11708215, rs13072552 and incidence of AF separately in whites and African Americans (Table 4). Contrary to our initial hypothesis, presence of the CP-increasing alleles in rs11708215 and rs13072552 were significantly associated with lower risk of AF in whites (HR 0.84, 95%CI 0.76, 0.94, $p = 0.002$ and HR 0.83, 95%CI 0.69, 0.99, $p = 0.04$ respectively for each CP-increasing allele in the final adjusted model) but not in African Americans (corresponding HRs 0.92, 95% CI 0.67, 1.25, $p = 0.58$ and 1.01, 95% CI 0.81, 1.26, $p = 0.92$). Additional adjustment for polymorphisms rs11708215 and rs13072552 did not affect the association between circulating ceruloplasmin and AF incidence (data not shown). Results from a two-stage least squares regression analysis with the two polymorphisms as instrumental variables were consistent with these results, showing an inverse association of genetically-determined CP with AF risk in whites but not in African Americans (Table 5).

Difference in CP concentration and AF risk by number of risk alleles and haplotypes in rs11708215 and rs13072552

The CP-related GRS showed a linear direct association with circulating CP. Participants with 3 or 4 CP-increasing alleles had the highest blood CP concentration. This difference was significant in both African Americans (Beta 34.5, 95%CI 18, 51 mg/L) and whites (Beta 36.3, 95%CI, 28.6, 49.9 mg/L). In contrast, we did not find any significant association between GRS and increased AF risk in African Americans and a potentially lower risk of AF with higher GRS in whites. Similar results were obtained when participants were categorized according to haplotypes in both SNPs.

DISCUSSION

Our study is the largest prospective study to date showing that higher concentrations of circulating CP, an inflammatory plasma protein, are associated with AF. Consistent with previous observations, we found that variants in SNPs rs11708215 and rs13072552, which are in or near the *CP* gene in chromosome 3, were associated with circulating concentrations of CP in a biracial cohort. In contrast, effect alleles associated with higher CP concentrations in these 2 alleles were not associated with higher incidence of AF. In fact, and contrary to our initial hypothesis, variants associated with *higher* CP concentrations were associated with *lower* risk of AF in whites. These findings do not support a direct causal role of CP on AF risk, though statistical power is possibly limited.

A previous publication from the Malmö Preventive Project in southern Sweden, including 3900 participants, described an association between CP concentrations and AF incidence.⁽⁷⁾ Our study confirms and extends these findings to the large, middle-aged, biracial cohort of men and women in the ARIC study. After adjusting for traditional risk factors and different biomarkers, higher levels of circulating CP were associated with incident AF. In contrast to our results, the Malmö Preventive Project analysis described a higher risk of AF associated with the CP-increasing allele in SNP rs11708215. This discrepancy may be due to differences in sociodemographic and clinical characteristics of the study populations, diverse sample sizes, and heterogeneity in AF ascertainment method.

CP has been reported to possess both oxidative and anti-oxidative functions. It has been seen that the overproduction of reactive oxygen species (ROS) is associated with both AF and CP pathogenicity (19). When this occurs, the body's antioxidant systems such as catalase, superoxide dismutase and glutathione are saturated and unable to counteract oxidative stress, leading to tissue damage. CP is capable of promoting the activation of the NO oxidase and consume NO catalytically reducing its bioavailability in plasma. Animal studies have shown that NO and NO synthases play a key role in the normal cardiac physiology. (20,21) NO is a cardioprotective agent due to, among other mechanisms, inhibition of oxidative stress. Therefore, high levels of CP in the body can stimulate the activity of NO oxidase causing a decrease of NO and tilting the balance towards an oxidative role. This oxidative stress may produce cardiac electrical activity alterations, damaging ion channels and finally leading to the AF development. (22,23)

Our study has several potential clinical implications. First, since patients with high levels of CP are more likely to develop AF, information on CP concentrations may facilitate efforts to identify high-risk individuals. And, second, future studies could determine whether CP measurements can be used to monitor the efficacy of interventions aimed to prevent AF.

Limitations

Although hospital discharge codes being used for identifying incident AF cases have shown to be valid, (9) AF cases managed exclusively in outpatient settings and those who are asymptomatic are certainly 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. Finally, our Mendelian randomization analysis is limited due to the weak association between the CP-related variants and circulating CP and to the potential association of CP-related variants with other cardiovascular risk factors (pleiotropy).

CONCLUSION

High levels of circulating CP are associated with increased incidence of AF. The two SNPs studied were associated with increased CP levels in both whites and African Americans. Opposite to our initial hypothesis, the presence of the effect allele in rs11708215 SNP was associated with significantly lower risk of AF in whites, but not in African Americans. Our results suggest that CP can be one of many inflammatory intermediaries involved in the development of AF. Therefore, additional studies are needed to understand the causal mechanism behind this association to advance our ability to prevent this common arrhythmia.

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Figure 1.
Difference in CP concentration by number of CP-increasing alleles in rs11708215 and rs13072552, ARIC study, 1996–1998

Figure 2.
Atrial fibrillation risk by number of CP-increasing alleles in rs11708215 and rs13072552,
ARIC study, 1996–2012

Table 1

Baseline Characteristics of the Overall Population by Ceruloplasmin (CP) Quartiles, ARIC study, 1996–1998

CP mg/L	Quartile 1 < 248.6	Quartile 2 248.6 to <285.3	Quartile 3 285.3 to < 336.8	Quartile 4 336.8
N	2517	2515	2513	2514
Age	63.1 ± 5.6	63.2 ± 5.6	62.8 ± 5.6	61.8 ± 5.5
Gender (% Women)	21	45	68	92
African American (%)	13	20	28	26
BMI (kg/m ²)	28.8 ± 5.0	28.7 ± 5.3	29.1 ± 5.9	28.3 ± 5.9
Height (cm)	172.6 ± 8.8	169.1 ± 9.4	165.8 ± 8.9	162.7 ± 6.9
Diabetes mellitus (%)	17	17	17	12
HTN medication (%)	42	41	43	44
SBP (mmHg)	126 ± 18	127 ± 18.9	129 ± 19.4	127 ± 19.3
DBP (mmHg)	71 ± 9.8	71 ± 10.2	71 ± 10.6	70 ± 10.3
Smoker, current (%)	22	25	26	27
Alcohol, current (%)	27	26	23	24
Total cholesterol (mg/dL)	191 ± 34.2	200.2 ± 34.6	205.3 ± 37.3	206 ± 36.8
LDL-c (mg/dL)	119.4 ± 31.2	125.4 ± 31.8	127.7 ± 34.6	118.4 ± 35.1
HDL-c (mg/dL)	43.8 ± 13.4	47.7 ± 14.7	50.7 ± 15.7	59.3 ± 17.6
Triglycerides (mg/dL)	138.5 ± 69.1	135.2 ± 66.6	134 ± 65.8	140.8 ± 66.8
hs-cTnT (μg/L)	0.9 ± 2.9	0.7 ± 0.8	0.7 ± 1.4	0.5 ± 0.8
hsCRP (mg/L)	3.5 ± 7.2	3.3 ± 4.7	4.2 ± 5.2	6.6 ± 7.9
NT-proBNP (pg/mL)	127.2 ± 525	131.1 ± 470.6	132.1 ± 524.4	141.7 ± 244.6
GGT (U/L)	29 ± 26.3	30.7 ± 39.9	31 ± 35.8	29.5 ± 44.9
AST (U/L)	24 ± 14.7	24.1 ± 13.8	24.1 ± 11.2	23.1 ± 10.5
ALT (U/L)	20.2 ± 13.7	19.5 ± 13	19 ± 11.3	16.7 ± 9.1

Values correspond to means or percent. Plus-minus values are means ± SD

BMI, body mass index; HTN, hypertension; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL, low-density lipoprotein; HDL, high-density lipoprotein; hs-cTnT, high-sensitivity cardiac troponin T; hs-CRP, high-sensitivity C-reactive protein; NT-proBNP, N-terminal pro-B-type natriuretic peptide. GGT, γ glutamyl transpeptidase; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Table 2

Association between CP concentration and AF risk, ARIC study, 1996–2012

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	Continuous*	P-value [^]
CP (mg/L)	248.6	248.6 to <285.3	285.3 to <336.8	336.8	CP/ σ	
# AF cases	359	362	335	301	1357	
N	2514	2503	2492	2550	10059	
Hazard ratios (95% confidence intervals)						
Model 1	1 (ref.)	1.11 (0.96, 1.29)	1.18 (1.01, 1.39)	1.30 (1.08, 1.55)	1.04 (0.98, 1.11)	0.18
Model 2	1 (ref.)	1.13 (0.98, 1.32)	1.21 (1.03, 1.42)	1.38 (1.15, 1.66)	1.07 (1.00, 1.14)	0.05
Model 3	1 (ref.)	1.12 (0.96, 1.30)	1.19 (1.02, 1.40)	1.37 (1.14, 1.65)	1.06 (0.99, 1.14)	0.06
Model 4	1 (ref.)	1.12 (0.97, 1.30)	1.19 (1.01, 1.40)	1.33 (1.11, 1.61)	1.06 (0.99, 1.13)	0.1

* per 1-standard deviation increase in CP. σ = Standard deviation = 77.07 mg/L

[^] P-value for the continuous analysis

Model 1: adjustment for age, sex, 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.

Table 3

Difference in ceruloplasmin concentration by rs11708215 and rs13072552 genotype by race, ARIC study, 1996–1998

rs11708215					
	AA	AG	GG	Additive model	P-value [^]
African Americans (N=2205)	1621	527	57	2205	
CP mean values (mg/L)	307.4	322.4	330.7	311.6	
Model 1	ref.	14.6 (7.7, 21.6)	25.5 (6.9, 44.1)	14.0 (8.2, 19.8)	<0.001
Model 2	ref.	15.2 (8.4, 22.0)	26.0 (8.0, 44.1)	14.4 (8.8, 20.1)	<0.001
Whites (N=7854)	5008	2540	306	7854	
CP mean values (mg/L)	291.4	303.8	327.6	296.8	
Model 1	ref.	12.9 (9.8, 16.1)	32.0 (24.4, 36.9)	14.2 (11.6, 16.7)	<0.001
Model 2	ref.	11.9 (8.9, 14.9)	31.0 (23.8, 38.2)	13.3 (10.9, 15.8)	<0.001
rs13072552					
	GG	GT	TT	Additive model	P-value [^]
African Americans (N=1925)	628	958	339	1925	
CP mean values (mg/L)	308.8	309.6	323.6	311.8	
Model 1	ref.	1.3 (–5.3, 7.9)	11.8 (2.9, 20.6)	5.2 (0.8, 9.5)	0.02
Model 2	ref.	1.1 (–5.4, 7.6)	13.6 (4.9, 22.2)	5.9 (1.7, 10.1)	0.006
Whites (N=6904)	5941	921	42	6904	
CP mean values (mg/L)	294.1	314.4	341.5	297.1	
Model 1	ref.	20.9 (16.4, 25.6)	52.9 (32.9, 72.9)	21.9 (17.7, 26.1)	<0.001
Model 2	ref.	20.7 (16.4, 25.1)	51.8 (32.8, 70.7)	21.6 (17.6, 25.6)	<0.001

Measure of association: Beta coefficient (95% confidence intervals)

[^] P-value for the additive model.

Model 1: adjustment for age, sex, ARIC study site and GWAS PCs.

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, use of medications (antihypertensive and corticosteroids), history of heart failure, MI, stroke and biomarkers like CRP, BNP and troponin.

Table 4

Association of rs11708215 and rs13072552 genotype with incidence of atrial fibrillation by race, ARIC study, 1996–2012

rs11708215					
	AA	AG	GG	Additive model	P-value [^]
African Americans (N=2205)	1621	527	57	2205	
AF cases	159	46	7	212	
Model 1	1 (ref.)	0.87 (0.62, 1.20)	1.23 (0.58, 2.63)	0.90 (0.66, 1.21)	0.47
Model 2	1 (ref.)	0.89 (0.64, 1.24)	1.39 (0.65, 2.99)	0.92 (0.68, 1.26)	0.61
Model 3	1 (ref.)	0.88 (0.63, 1.23)	1.38 (0.64, 2.96)	0.92 (0.67, 1.25)	0.58
Whites (N=7854)	5008	2540	306	7854	
AF cases	766	339	40	1145	
Model 1	1 (ref.)	0.84 (0.74, 0.96)	0.82 (0.60, 1.13)	0.87 (0.77, 0.96)	0.008
Model 2	1 (ref.)	0.84 (0.73, 0.95)	0.77 (0.56, 1.05)	0.85 (0.77, 0.95)	0.003
Model 3	1 (ref.)	0.83 (0.73, 0.94)	0.75 (0.54, 1.03)	0.84 (0.76, 0.94)	0.002
rs13072552					
	GG	GT	TT	Additive model	P-value [^]
African Americans (N=1925)	628	958	339	1925	
AF cases	54	92	33	179	
Model 1	1 (ref.)	0.98 (0.73, 1.31)	0.99 (0.66, 1.47)	1.08 (0.87, 1.34)	0.47
Model 2	1 (ref.)	0.89 (0.66, 1.20)	0.92 (0.61, 1.38)	1.01 (0.81, 1.27)	0.91
Model 3	1 (ref.)	0.89 (0.66, 1.20)	0.91 (0.61, 1.37)	1.01 (0.81, 1.26)	0.92
Whites (N=6904)	5941	921	42	6904	
AF cases	883	123	4	1010	
Model 1	1 (ref.)	0.91 (0.75, 1.10)	0.62 (0.23, 1.66)	0.89 (0.75, 1.06)	0.19
Model 2	1 (ref.)	0.86 (0.71, 1.03)	0.68 (0.25, 1.82)	0.85 (0.71, 1.02)	0.08
Model 3	1 (ref.)	0.84 (0.69, 1.02)	0.65 (0.24, 1.75)	0.83 (0.69, 0.99)	0.04

Measure of association: Hazard ratio (95% confidence intervals)

[^] P-value for the additive model

Model 1: adjustment for age, sex and ARIC study site and GWAS PCs (in African Americans).

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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, use of medications (antihypertensive and corticosteroids), history or heart failure, MI, stroke and biomarkers like CRP, BNP and troponin.

Model 3: Model 2 + CP concentration.

Table 5

Results from two-stage least squares regression analysis with rs11708215 and rs13072552 as instruments, circulating ceruloplasmin as the main independent variable, and AF risk as the outcome, ARIC study, 1996–2012

	rs11708215		rs13072552	
	Whites	African Americans	Whites	African Americans
B coefficients	– 0.001	0.001	– 0.001	0.001
p-value	0.039	0.726	0.138	0.731