Published in final edited form as:

J Thromb Haemost. 2019 May ; 17(5): 818–826. doi:10.1111/jth.14420.

Circulating ceruloplasmin, ceruloplasmin-associated genes, and the incidence of venous thromboembolism in the Atherosclerosis Risk in Communities study.

Antonio P. Arenas de Larriva, MD[1,2], **Alvaro Alonso, MD,PhD**[3], **Faye L. Norby, MPH**[4], **Nicholas S. Roetker, MPH**[4], and **Aaron R. Folsom, MD,MPH**[4]

[1]Lipid and Atherosclerosis Unit, IMIBIC/Hospital Universitario Reina Sofía/Universidad de Córdoba, Córdoba, Spain

[2]CIBER Fisiopatología Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain

[3]Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA

[4]Division of Epidemiology and Community Health, School of Public Health, University of Minnesota, Minneapolis, MN

Abstract

Background.—Ceruloplasmin (CP) is an acute-phase reactant and a potential biomarker of atherothrombotic risk. We assessed the associations between CP, CP-associated genetic variants, and incident venous thomboembolism (VTE) in the Atherosclerosis Risk in Communities (ARIC) study.

Methods and results.—In an observational study, 9933 men and women without prevalent VTE aged 53 to 75 years were included in 1996–1998 and followed through 2011. Circulating CP was measured in stored blood samples obtained in 1996–1998. Polymorphisms rs11708215 and rs13072552, previously associated with CP concentrations, were measured in 8439 participants. VTEs were identified from hospital discharge codes and validated by physician review of medical records and imaging reports. Over a mean of 10.5 years follow-up, 376 cases of VTE were identified. The association between circulating CP, CP-associated polymorphisms, and the incidence of VTE was estimated. After adjusting for traditional risk factors and biomarkers, higher levels of circulating CP were associated with greater incident VTE rates (hazard ratio [HR] 1.82,

Corresponding author: Antonio P. Arenas de Larriva, MD. Lipids and Atherosclerosis Unit, IMIBIC/Hospital Universitario Reina Sofía/Universidad de Córdoba, Córdoba, Spain, CIBER Fisiopatología Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain, Phone: + 34 670689539. antonio.arenas@imibic.org.

ADDENDUM

A. Alonso contributed significantly to the design of the study, reviewed the study proposal, participated in drafting the manuscript and to each subsequent revision.

A. Folsom reviewed the study proposal, participated in drafting the manuscript and critically reviewed the manuscript.

N. Roetker designed the database and reviewed the paper.

F. Norby reviewed the paper and provided many recommendations and useful ideas.

A. Arenas analyzed the database and interpreted the results obtained. He wrote and updated the manuscript following the suggested recommendations from other authors.

DISCLOSURE OF CONFLICT OF INTERESTS

The authors state that they have no conflict of interest.

95% confidence interval [CI] 1.12, 2.95 comparing the 87.5–100th percentile to the bottom quartile). Both rs11708215 and rs13072552 were associated with CP levels but not with VTE risk.

Conclusions.—Even though high CP concentrations were associated with increased VTE risk, CP-associated genetic variants were not associated with higher risk of VTE. Our results suggest that circulating CP levels may not be causally related to risk of incident VTE.

Keywords

venous thromboembolism; ceruloplasmin; single nucleotide polymorphism; oxidative stress

INTRODUCTION

Deep vein thrombosis (DVT) and pulmonary embolism (PE) are manifestations of venous thromboembolic disease (VTE), the third most common cardiovascular disease after myocardial infarction and stroke. (1, 2).

It is estimated that at least 900,000 people are affected by VTE (1 to 2 per 1,000 adults) each year in the United States.This results in about 100,000 premature deaths (3, 4), of which over 10% will die within one month of diagnosis. (1, 2). Because of the public health burden and expense of VTE, additional biomarkers are needed to help detect or predict VTE and/or monitor the treatments prescribed.

Ceruloplasmin (CP) is an enzyme synthesized in the liver that is responsible for transport of circulating copper. CP 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 may underlie several illnesses such as myocardial infarction, arteriosclerosis, unstable angina, abdominal aortic aneurysm, vasculitis, peripheral arterial disease, and even dementia (5, 6).

There is strong evidence that inflammation, where CP levels are increased, is associated with increased risk of atherothrombosis (7, 8). Several studies have demonstrated a relationship between various proteins involved in inflammatory processes and VTE. For example, ARIC previously found that elevated hsCRP, but not fibrinogen, was independently associated with increased risk of VTE (9). Inflammation seems to trigger a chain reaction where procoagulant factors are activated and the fibrinolytic pathway is inhibited (10). Yet, Sveinsdottir et al, found no significant relationship between VTE incidence and CP or other inflammatory markers (fibrinogen, orosomucoid, α1-antitrypsin, haptoglobin) (11).

Additionally, two single nucleotide polymorphisms (SNPs) located in the CP gene promoter are associated with CP concentrations in blood (rs11708215 and rs113072552) (12). We addressed the association between these SNPs, circulating CP, and VTE incidence in the Atherosclerosis Risk in Communities (ARIC) study. We hypothesized that higher concentrations of circulating CP would be associated with greater VTE incidence, and following a Mendelian randomization framework, that if the association between circulating CP and VTE incidence is causal then genetic variants associated with higher circulating CP would also increase the risk of VTE.

METHODS

Study population

The ARIC study is a community-based population study designed to investigate the causes of cardiovascular disease. 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, 2011 to 2013, and 2016 to 2017. Participants were mostly white in the Washington County and Minneapolis sites, exclusively African Americans in Jackson, and a mix of both races in Forsyth County (13).

Of the 11,656 participants attending visit 4 (1996–1998), we excluded individuals with prevalent VTE at visit 1 (N=238), incident VTE event before visit 4 (N=49), missing data for CP (N=153), taking anticoagulants at visit 4 (N=235), follow-up ended on date of visit 4 exam (N=2) and with missing information for body mass index (N=38) or any other variable used in the statistical models (N=939). 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=69).

Ascertainment of VTE

Staff contacted ARIC participants annually by phone and asked about all hospitalizations in the previous year. In addition, ARIC conducted surveillance of hospital discharge lists from local hospitals, and obtained all International Classification of Diseases (ICD) discharge codes. For ICD codes harboring possible VTE events, staff obtained copies of the hospital records. To validate VTE events, two physicians reviewed the records using standardized criteria, requiring positive imaging tests for diagnosis of DVT and PE. We restricted DVTs for this analysis to those occurring in the lower extremity or vena cava, because upper extremity DVTs were relatively few and almost always the result of venous catheters (14).

Covariates

At each study visit, participants underwent physical assessments, 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, and hormone replacement therapy (HRT) were self-reported by the study participant. Weight and height were measured with the participant wearing light clothing. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared. Blood pressure was measured twice and averaged to define systolic and diastolic blood pressure. Hypertension was defined as a systolic blood pressure of ≥140 mm Hg, 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 anti-diabetic medication.

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%.

High sensitivity C-reactive protein (hs-CRP) levels were measured by an immunonephelometric assay on a BNII autoanalyzer (Siemens Healthcare Diagnostics, Deerfield, IL) with a reliability coefficient of 0.9.

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

ARIC had exhausted most baseline citrate plasma samples previously. Therefore, we used in this analysis D-dimer and factor XI concentrations measured on fasting citrate plasma collected at ARIC visit 3 (in 1993–95) and stored unthawed at −70°C until analysis in 2014. The Laboratory for Clinical Biochemistry Research at the University of Vermont used an immuno-turbidimetric assay (Liatest D-DI; Diagnostica Stago, Parsippany, NJ) on the Evolution analyzer (Diagnostica Stago, Parsippany, NJ) for D-dimer and sandwich ELISA with affinity-purified polyclonal antibodies from Affinity Biologicals (Ancaster, Ontario, CAN) for factor XI. The analytical coefficient of variation for the D-dimer assay was 4 – 16%. Blind analysis of 73 pairs of ARIC samples split at the time of blood draw and stored until 2014 yielded an intra-class reliability coefficient of 0.92 (15).

For factor XI, the coefficient of variation for control samples during this study averaged 9.6%. Blind analysis of 74 pairs of ARIC samples split at the time of blood draw and stored until 2014 yielded an intra-class reliability coefficient of 0.81 (16).

Other hemostatic factors were measured at ARIC visit 1 (1987–89). Factor VIII activity was measured by determining the ability of the tested sample to correct the clotting time of human factor VIII–deficient plasma obtained from George King Biomedical Inc. Von Willebrand factor (VWF) antigen was determined by ELISA kits from American Bioproducts Co. Activated partial thromboplastin time (aPTT) was measured on an automated coagulometer (Coag-A-Mate X-2, General Diagnostics). The reference material for assays was the Universal Coagulation Reference Plasma (Thromboscreen, Pacific Hemostasis, Curtin Matheson Scientific, Inc). Reliability coefficients (method variance plus intraindividual variance divided by total variance) obtained from repeated testing of individuals over several weeks were 0.86 for factor VIII, 0.68 for VWF and 0.92 for aPTT (17).

Statistical analysis

Cox proportional hazards models were used to estimate the association between CP concentrations and incident VTE. Time to follow-up was defined as the time between visit 4

and VTE occurrence, death, loss to follow-up, or end of 2011, whichever occurred earlier. Initially, we explored the shape of the association of CP with VTE risk using restricted cubic splines, which allowed us to test for linearity. Circulating CP was modeled using quartiles (with the highest quartile split in two) and as a continuous variable (scaled per standard deviation increment).

The following incrementally-adjusted models were used to analyze the CP-VTE association: model 1: adjustment for age, sex, race, BMI and current HRT; model 2: model 1 plus adjustment for diabetes mellitus, systolic blood pressure, activated partial thromboplastin time, VWF, D-dimer, Factor VIII, XI and hsCRP. Other atherosclerotic risk factors such as diastolic blood pressure, smoking, lipid levels and physical activity were not strong VTE risk factors in ARIC and thus not examined. We also ran a sensitivity analysis adjusting for participants cancer status (yes, no), which had no material impact on the results.

In a second step, we ran race-specific linear regression models testing the association between CP gene SNPs (rs11708215, rs13072552) and CP concentration. Analyses in African Americans were adjusted for the first 10 principal components of ancestry (PCAs) to correct for population stratification.

Thirdly, a race-specific Cox model was used to test associations of CP gene SNPs rs11708215 and rs13072552, separately, with VTE risk, adjusting for age, sex, race, BMI, HRT and PCAs (in African Americans), and for covariates listed above in Model 2 as well as for CP concentration to test whether any association between rs11708215 or rs13072552 might be mediated by circulating CP.

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

RESULTS

This ARIC visit 4 sample included 9,933 participants at risk for VTE (mean age 62.7 ± 5.6 years, 20.1% African American and 55.7% female). Mean CP levels were higher in African Americans than whites (311.9 ± 71.7 mg/L versus 296.1 ± 78.1 mg/L). The baseline demographic characteristics stratified by CP quartiles for the overall sample are shown in Table 1. Those with higher concentrations of circulating CP were more likely to be women, African American and have higher concentrations of hsCRP.

Associations of CP concentration with incident VTE

During a mean follow-up of 10.5 years, a total of 376 individuals developed VTE (110 events in African American and 266 in whites). Table 2 presents the associations between circulating CP, stratified by quartiles (last quartile split in two) and as a continuous variable, and VTE risk. Higher levels of circulating CP were associated with greater incidence of

VTE in all the adjusted models. Individuals with CP in the highest category (87.5–100th percentile) had 1.61 times greater risk for VTE than those in the lowest quartile (HR 1.61, 95%CI 1.06, 2.45) after adjusting for traditional risk factors. The association was comparable in the fully adjusted model including other biomarkers (HR 1.50, 95%CI 0.98, 2.29). A similar association was observed when we modeled circulating CP as a continuous variable in model 2 (HR 1.16, 95%CI 1.03, 1.30 per 1-standard deviation increment in CP). Associations between circulating CP and VTE risk were similar in whites (HR 1.16, 95%CI 1.01, 1.34 per 1-standard deviation increment in CP) and African Americans (HR 1.13, 95%CI 0.91, 1.41). We also conducted stratified analysis by sex. Hazard ratios (95% confidence intervals) for the association between CP concentration and VTE stratified by sex are presented as supplementary tables (S1 and S2). Higher levels of ceruloplasmin were associated with increased risk of VTE in both men and women, without evidence of a significant interaction (p for interaction = 0.49). Given the more limited sample size in each analysis, estimates were more imprecise than in the combined analysis. Still, HRs for VTE in top category were 1.5 in women and 1.6 in men.One additional analysis was carried out restricting follow-up to the first eleven years. Associations were stronger than for the entire follow-up indicating that there was some dilution of the effect over time (Table S3).

We also plotted the association between circulating ceruloplasmin and VTE risk modeling ceruloplasmin using a restricted cubic spline. The risk increased up to the 87.5th percentile, plateauing afterwards (Figure 1).

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 8439 subjects (Table 3). The frequencies for CP-increasing alleles differed between whites and African Americans. For both SNPs, a higher number of CP-increasing alleles was associated with higher concentrations of CP: 30.3 (95%CI 11.5, 49.1) and 29.8 (95%CI 22.5, 37.2) mg/L higher in African Americans and whites respectively for rs11708215, with the corresponding results being 13.6 (95%CI 4.6, 22.6) and 53.8 (95%CI 34.9, 72.7) mg/L higher for rs13072552 in the fully adjusted model. The proportion of variability in circulating CP explained by these 2 SNPs was small (r^2 = 0.02). Reported differences in concentration reflect two risk alleles versus no risk alleles of the SNPs.

Association between rs11708215, rs13072552 and VTE risk

We next investigated the relationship of rs11708215 and rs13072552 with incidence of VTE separately in whites and African Americans (Table 4). Presence of the CP-increasing alleles in rs11708215 and rs13072552 were not significantly associated with risk of VTE in whites or African Americans.

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

The CP-related GRS showed a linear association with circulating CP (Table 5). Participants with 3 or 4 CP-increasing alleles had the highest blood CP concentration. This difference was significant in both African Americans (Beta 11.6 mg/L, 95% CI 7.7, 15.5) and whites

(Beta 11.7 mg/L, 95%CI, 9.8, 13.6). In contrast, neither the GRS (Table 5) nor the haplotype categories (Table 6) were associated with VTE risk in whites. In contrast, there was some suggestion that African American with a GRS of 0 (Table 5) or haplotype AA/GG may have lower risk of VTE than those with other genotypes.

DISCUSSION

This is the largest prospective study to date showing that a higher concentration of circulating CP, an inflammatory plasma protein, is associated with modestly increased VTE risk. 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 both whites and African Americans. In contrast, alleles associated with higher CP concentrations in these two SNPs were not associated with greater incidence of VTE. These findings do not support a direct causal role of CP on VTE risk, though statistical power to rule out a small effect was limited.

A previous publication from the Malmö Preventive Project in southern Sweden, including 6068 participants, explored whether raised levels of inflammation-sensitive plasma markers (fibrinogen, haptoglobin, ceruloplasmin, a1-antitrypsin and orosomucoid) were associated with increased VTE risk. They did not find any association between these biomarkers and VTE risk (11).

In contrast with this previous study, we found a positive association of CP with VTE in a large, middle-aged, biracial cohort of men and women. After adjusting for several VTE risk factors and different biomarkers, higher levels of circulating CP remained associated with increased incidence of VTE.

Multiple clinical and molecular lines of evidence suggest a close link between inflammation, thrombosis activation, and VTE (18–22). Inflammation increases the production of procoagulant factors activating blood coagulation and inhibiting the fibrinolytic pathway (23). During the endothelial dysfunction, platelet activating factor and endothelin-1 are released promoting a vasoconstriction, whereas production of Factor V, VWF, plasminogen activator inhibitor-1 and tissue factor augment thrombosis. Furthermore, endothelial cells increase the adhesion molecules in the surface, promoting the activation of leukocytes. This event initiates and amplifies inflammation and thrombosis (24).

It has been reported that inflammation, with the subsequent overproduction of reactive oxygen species (ROS), is associated with VTE risk (18–22). CP has been suggested to have proinflammatory effects on vascular cells, both oxidative and anti-oxidative functions having been reported. So, hypothetically, the overproduction of reactive oxygen species and vascular inflammation could be a cause of VTE in the presence of higher levels of CP.

In relation to CRP, we are uncertain why single measures of CRP were associated previously with incident VTE (25,26) but changes in these biomarkers were not and this appears to be somewhat depending on the follow-up time (27). One possibility is that the original findings were spurious, because of some unrecognized confounding variable. Overall, the lack of longitudinal associations between CRP and VTE incidence suggest that it, or the

fundamental processes that they represent (low-level inflammation and cardiac injury), is not a risk factor for VTE. This is supported by Zacho et al., where genetically elevated CRP levels did not associate with increased VTE risk (28).

During an inflammatory process, multiple factors are involved. Numerous inflammatory markers could be analyzed individually as potential VTE risk factors. Further studies would be needed to determine whether elevated CP is a causal risk factor or merely a risk marker for VTE.

Strengths and limitations

Strengths of the study include the large sample size and power to measure overall associations between CP and VTE. However, there are a few limitations. Although the LITE study validated VTEs, some VTE cases treated in outpatient settings are missed (14). In addition, there may be some misclassification of CP concentrations since there is no follow-up information on circulating CP after visit 4. As a result, if the CP measures changed over time, it would tend to bias our hazard ratios (presumably toward 1). Power is poor for race-specific findings and for the SNPs associations. We considered these SNPs because they have been previously associated with CP concentrations (rs13072552 in an ARIC GWAS and rs11708215 in a candidate SNP analysis) (12); there can be other variants related to CP concentrations that have not been identified yet. Finally, the hemostatic factors were not measured at visit 4 and so there could be residual confounding. Finally, we could not take into account acute risk factors for VTE (immobility, surgery, etc.).

CONCLUSION

High levels of circulating CP are associated with increased incidence of VTE. The two SNPs studied were associated with increased CP levels in both whites and African Americans. Presence of the CP-increasing alleles in rs11708215 and rs13072552 were not significantly associated with risk of VTE in either race group. Our results suggest that CP may be one of many inflammatory intermediaries involved in the development of VTE, or at least reflecting a patient at risk for VTE.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

The Atherosclerosis Risk in Communities Study is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute contracts (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, and HHSN268201100012C), R01HL087641, R01HL59367 and R01HL086694; National Human Genome Research Institute contract U01HG004402; and National Institutes of Health contract HHSN268200625226C. The authors thank the staff and participants of the ARIC study for their important contributions. Infrastructure was partly supported by Grant Number UL1RR02500 5, a component of the National Institutes of Health and NIH Roadmap for Medical Research.

REFERENCES

1. Goldhaber SZ, Bounameaux H. Pulmonary embolism and deep vein thrombosis. Lancet. 2012;379:1835–46. [PubMed: 22494827]
2. Heit JA, Spencer FA, White RH. The epidemiology of venous thromboembolism. J Thromb Thrombolysis. 2016;41:3–14. [PubMed: 26780736]
3. Beckman MG, Hooper WC, Critchley SE, Ortel TL. Venous thromboembolism: a public health concern. Am J Prev Med. 2010;38:S495–501. [PubMed: 20331949]
4. Raskob GE, Silverstein R, Bratzler DW, Heit JA, White RH. Surveillance for deep vein thrombosis and pulmonary embolism: recommendations from a national workshop. Am J Prev Med. 2010;38:S502–9. [PubMed: 20331950]
5. Dadu RT, Dodge R, Nambi V, Virani SS, Hoogeveen RC, Smith NL, Chen F, Pankow JS, Guild C, Tang WH, Boerwinkle E, Hazen SL, Ballantyne CM Ceruloplasmin and heart failure in the Atherosclerosis Risk in Communities study. 2013 Circ Heart Fail 6: 936–943. [PubMed: 23861484]
6. Jeremy JY, Shukla N. Ceruloplasmin dysfunction: a key factor in the pathophysiology of atrial fibrillation? J Intern Med. 2014;275:191–4. [PubMed: 24188106]
7. Casas JP, Shah T, Hingorani AD, Danesh J, Pepys MB. C-reactive protein and coronary heart disease: a critical review. J Intern Med. 2008;264:295–314. [PubMed: 18823504]
8. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med. 1997;336:973–9. [PubMed: 9077376]
9. Folsom AR, Lutsey PL, Astor BC, Cushman M. C-reactive protein and venous thromboembolism. A prospective investigation in the ARIC cohort. Thromb Haemost. 2009;102:615–9. [PubMed: 19806245]
10. Conde I, Lopez JA. Classification of venous thromboembolism (VTE). Role of acute inflammatory stress in venous thromboembolism. J Thromb Haemost. 2005;3:2573–5. [PubMed: 16241956]
11. Sveinsdottir SV, Svensson PJ, Engstrom G. Inflammatory plasma markers and risk for venous thromboembolism. J Thromb Thrombolysis. 2014;38:190–5. [PubMed: 24307292]
12. Adamsson Eryd S, Sjogren M, Smith JG, Nilsson PM, Melander O, Hedblad B, Engström G. Ceruloplasmin and atrial fibrillation: evidence of causality from a population-based Mendelian randomization study. J Intern Med. 2014;275:164–71. [PubMed: 24118451]
13. The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives. The ARIC investigators. Am J Epidemiol. 1989;129:687–702. [PubMed: 2646917]
14. Cushman M, Tsai AW, White RH, Heckbert SR, Rosamond WD, Enright P, Folsom AR. Deep vein thrombosis and pulmonary embolism in two cohorts: the longitudinal investigation of thromboembolism etiology. Am J Med. 2004;117:19–25. [PubMed: 15210384]
15. Folsom AR, Alonso A, George KM, Roetker NS, Tang W, Cushman M. Prospective study of plasma D-dimer and incident venous thromboembolism: The Atherosclerosis Risk in Communities (ARIC) Study. Thromb Res. 2015;136:781–5. [PubMed: 26337932]
16. Folsom AR, Tang W, Roetker NS, Heckbert SR, Cushman M, Pankow JS. Prospective study of circulating factor XI and incident venous thromboembolism: The Longitudinal Investigation of Thromboembolism Etiology (LITE). Am J Hematol. 2015;90:1047–51. [PubMed: 26260105]
17. Folsom AR, Wu KK, Rosamond WD, Sharrett AR, Chambless LE. Prospective study of hemostatic factors and incidence of coronary heart disease: the Atherosclerosis Risk in Communities (ARIC) Study. Circulation. 1997;96:1102–8. [PubMed: 9286936]
18. Goldhaber SZ. Risk factors for venous thromboembolism. J Am Coll Cardiol. 2010;56:1–7. [PubMed: 20620709]
19. Smeeth L, Cook C, Thomas S, Hall AJ, Hubbard R, Vallance P. Risk of deep vein thrombosis and pulmonary embolism after acute infection in a community setting. Lancet. 2006;367:1075–9. [PubMed: 16581406]
20. Libby P, Crea F. Clinical implications of inflammation for cardiovascular primary prevention. European heart journal. 2010;31:777–83. [PubMed: 20185554]

21. Libby P, Ridker PM, Hansson GK, Leducq Transatlantic Network on A. Inflammation in atherosclerosis: from pathophysiology to practice. J Am Coll Cardiol. 2009;54:2129–38. [PubMed: 19942084]

22. Glynn RJ, Danielson E, Fonseca FA, Genest J, Gotto AM Jr., ,Kastelein JJ, Koenig W, Libby P, Lorenzatti AJ, MacFadyen JG, Nordestgaard BG, Shepherd J, Willerson JT, Ridker PM A randomized trial of rosuvastatin in the prevention of venous thromboembolism. N Engl J Med. 2009;360:1851–61. [PubMed: 19329822]

23. Libby P Inflammation in atherosclerosis. Arterioscler Thromb Vasc Biol. 2012;32:2045–51. [PubMed: 22895665]

24. Wakefield TW, Myers DD, Henke PK. Mechanisms of venous thrombosis and resolution. Arterioscler Thromb Vasc Biol. 2008;28:387–91. [PubMed: 18296594]

25. Folsom AR, Lutsey PL, Astor BC, Cushman M C-reactive protein and venous thromboembolism. A prospective investigation in the ARIC cohort. Thromb Haemost 2009; 102: 615–19. [PubMed: 19806245]

26. Folsom AR, Lutsey PL, Nambi V, DeFilippi CR, Heckbert SR, Cushman M, Ballantyne CM Troponin T, NT-pro BNP, and venous thromboembolism: the Longitudinal Investigation of Thromboembolism Etiology (LITE). Vasc Med 2014; 19: 33–41. [PubMed: 24558027]

27. Folsom AR, Lutsey PL, Heckbert SR, Poudel K, Basu S, Hoogeveen RC, Cushman M, Ballantyne. Longitudinal increases in blood biomarkers of inflammation or cardiovascular disease and the incidence of venous thromboembolism. Journal of thrombosis and haemostasis: JTH. 2018;16:1964–72. [PubMed: 30007116]

28. Zacho J, Tybjaerg-Hansen A, Nordestgaard BG. C-reactive protein and risk of venous thromboembolism in the general population. Arterioscler Thromb Vasc Biol. 2010;30:1672–8. [PubMed: 20466978]

Essentials

- Ceruloplasmin (CP) is an acute-phase reactant and a potential biomarker of atherothrombotic risk.
- We assessed associations between CP and venous thromboembolism (VTE) risk in 9933 individuals.
- Higher circulating CP but not CP-related genes were associated with greater incident VTE rates.
- Circulating CP could be considered a non-causal biomarker of VTE risk in the community.

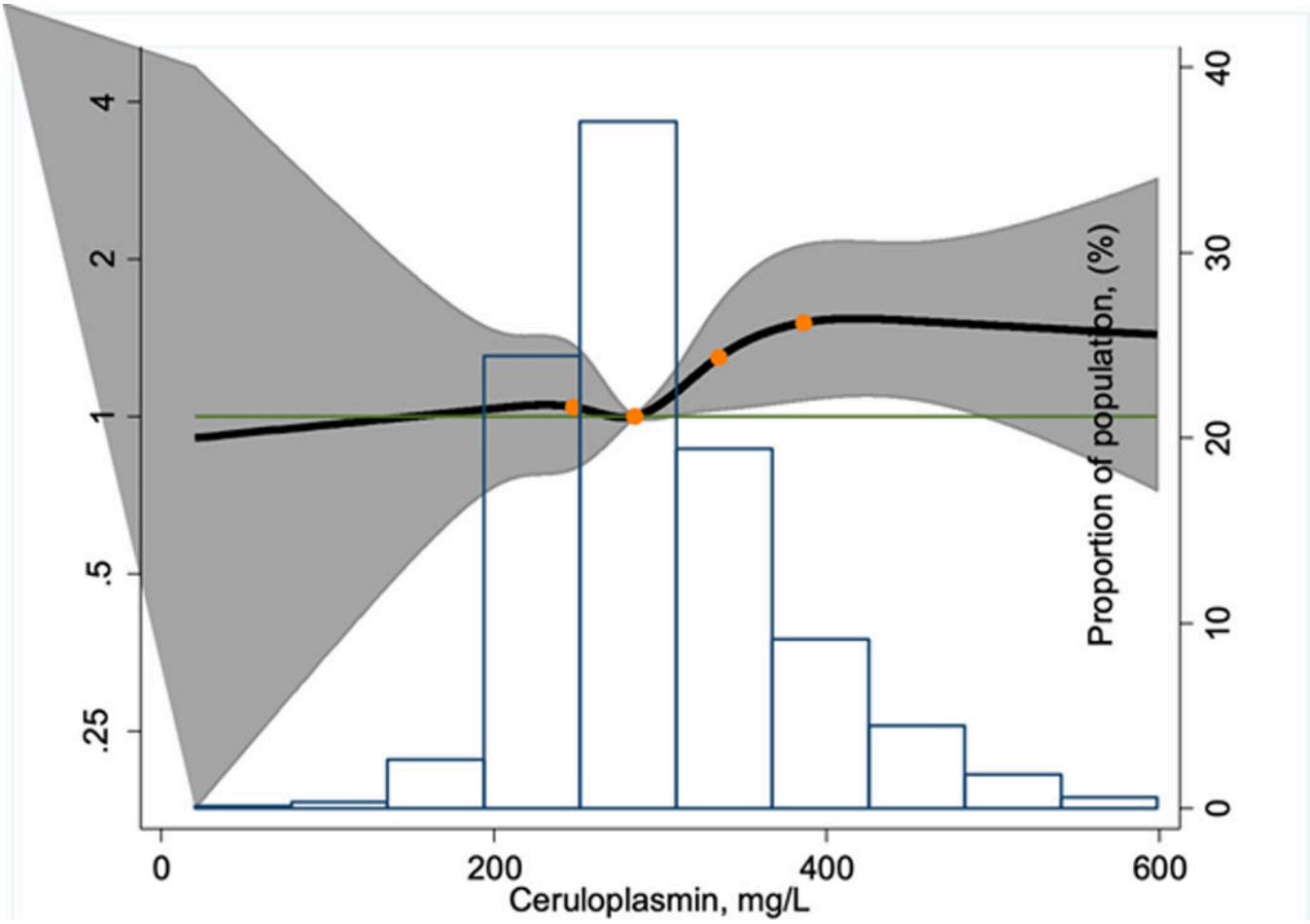

Figure 1.

Association of concentration of circulating ceruloplasmin with incidence of VTE presented as hazard ratio (solid line) and 95% confidence interval (shaded area) adjusted for age, sex, and race. The histogram represents the distribution of circulating ceruloplasmin in the study sample. Orange points corresponds to the values for the 25th, 50th, 75th and 87.5th percentiles of the ceruloplasmin distribution.

Table 1.

Baseline Characteristics (mean or percent) of the Overall Sample by Ceruloplasmin (CP) Quartiles in ARIC, 1996–1998

CP mg/L	Quartile 1 ≤ 248	Quartile 2 248 to <284.6	Quartile 3 284.6 to < 335	Quartile 4 ≥ 335
N	2486	2487	2480	2480
Age (years)	63 ± 5.6	63 ± 5.7	63 ± 5.7	62 ± 5.5
Women (%)	20	44	67	91
African American (%)	12	18	27	25
BMI (kg/m2)	28.9 ± 4.9	28.6 ± 5.2	29.0 ± 5.8	28.2 ± 5.8
HRT use (%) [1]	3	5	11	42
Diabetes mellitus (%)	18	17	17	12
SBP (mmHg)	126 ± 17	127 ± 19	129 ± 19	127 ± 19
DBP (mmHg)	71 ± 9	71 ± 10	71 ± 10	70 ± 10
Smoker, current (%)	21	25	27	26
Total cholesterol (mg/dL)	191 ± 35	200 ± 35	205 ± 38	206 ± 36
LDL-c (mg/dL)	119 ± 30	125 ± 31	127 ± 33	118 ± 34
HDL-c (mg/dL)	44 ± 13	47 ± 14	50 ± 15	59 ± 17
Triglycerides (mg/dL)	147 ± 98	140 ± 83	141 ± 86	146 ± 77
hsCRP (mg/L)	3.6 ± 7.4	3.3 ± 4.7	4.2 ± 5.3	6.5 ± 7.6
aPTT, (seconds) *	29.4 ± 3.0	29.2 ± 2.9	29.1 ± 3.1	28.8 ± 2.8
Factor VIII (%) *	125 ± 37	127 ± 35	129 ± 36	129 ± 35
VWF (%) *	112 ± 43	114 ± 44	116 ± 47	113 ± 43
D-dimer (μg/mL) **	0.39 ± 1.05	0.46 ± 1.33	0.48 ± 1.13	0.62 ± 1.82
Factor XI (%) **	107 ± 24	111 ± 25	115 ± 26	118 ± 28

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

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL, low-density lipoprotein; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; aPPT, activated partial thromboplastin time; VWF, Von Willebrand factor.

[1] Women only.

* Visit 1 value (1987–1989).

** Visit 3 value (1993–1995). The rest of covariates were measured in visit 4.

Table 2.

Hazard ratios (95% confidence intervals) for the association between CP concentration and VTE risk, ARIC, 1996–2011

	Quartile 1	Quartile 2	Quartile 3	75–87.5th percentile	87.5–100th percentile	Continuous*	P-value^
CP (mg/L)	< 248	248 to < 284.6	284.6 to < 335	335 to < 386.4	≥ 386.4		
# VTE cases	87	92	85	60	52	376	
N	2486	2487	2480	1240	1240	9933	
	Hazard ratios (95% confidence intervals)						
Model 1	1 (ref.)	1.09 (0.81, 1.47)	0.99 (0.72, 1.37)	1.53 (1.05, 2.21)	1.61 (1.06, 2.45)	1.20 (1.06, 1.35)	0.003
Model 2	1 (ref.)	1.09 (0.81, 1.46)	0.98 (0.71, 1.36)	1.50 (1.03, 2.18)	1.50 (0.98, 2.29)	1.16 (1.03, 1.30)	0.016

*per 1-standard deviation increase in CP (77.1 mg/L)

^P-value for the continuous analysis

Model 1: adjustment for age, sex, race, BMI and HRT.

Model 2: Model 1 + adjustment for diabetes mellitus, systolic blood pressure, activated partial thromboplastin time, Von Willebrand factor, D-dimer, Factor VIII, Factor XI and hsCRP.

Table 3.

Association between rs11708215, rs13072552 SNPs and difference in CP concentration

rs11708215			
	AA	**AG**	**GG**
African Americans (N=1661)	1225	393	43
CP mean values (mg/L)	307.4	322.4	330.7
Difference (mg/dl)**, Model 1**	ref.	16.6 (9.5, 23.7)	29.6 (10.7, 48.5)
Difference (mg/dl), Model 2	ref.	16.6 (9.6, 23.6)	30.3 (11.5, 49.1)
Whites (N=6778)	4331	2162	285
CP mean values (mg/L)	291.4	303.8	327.6
Difference (mg/dl), Model 1	ref.	11.7 (8.5, 14.9)	30.4 (22.9, 37.9)
Difference (mg/dl), Model 2	ref.	11.7 (8.5, 14.9)	29.8 (22.5, 37.2)
rs13072552			
	GG	**GT**	**TT**
African Americans (N=1661)	550	824	287
CP mean values (mg/L)	308.9	309.2	323.9
Difference (mg/dl), Model 1	ref.	0.9 (−5.9, 7.6)	13.6 (4.5, 22.7)
Difference (mg/dl), Model 2	ref.	1.1 (−5.7, 7.8)	13.6 (4.6, 22.6)
Whites (N=6778)	5830	907	41
CP mean values (mg/L)	293.6	314.3	340.3
Difference (mg/dl), Model 1	ref.	20.9 (16.5, 25.2)	54.9 (35.8, 74.0)
Difference (mg/dl), Model 2	ref.	21.1 (16.8, 25.4)	53.8 (34.9, 72.7)

Model 1: adjustment for age, sex, race, BMI, HRT and PCAs (in African Americans).

Model 2: Model 1 + adjustment for diabetes mellitus, systolic blood pressure, activated partial thromboplastin time, Von Willebrand factor, D-dimer, Factor VIII, Factor XI and hsCRP.

Table 4.

Hazard Ratio (95% confidence interval) for the associations between the rs11708215 and rs13072552 SNPs with VTE risk

rs11708215			
	AA	**AG**	**GG**
African Americans (N=1661)	1225	393	43
VTE cases	61	25	2
Model 1	1 (ref.)	1.31 (0.82, 2.10)	0.90 (0.22, 3.72)
Model 2	1 (ref.)	1.28 (0.80, 2.06)	1.06 (0.25, 4.41)
Whites (N=6778)	4331	2162	285
VTE cases	132	86	8
Model 1	1 (ref.)	1.31 (1.00, 1.72)	0.89 (0.43, 1.81)
Model 2	1 (ref.)	1.31 (1.00, 1.72)	0.89 (0.43, 1.82)
rs13072552			
	GG	**GT**	**TT**
African Americans (N=1661)	550	824	287
VTE cases	21	50	17
Model 1	1 (ref.)	1.52 (0.91, 2.53)	1.40 (0.73, 2.69)
Model 2	1 (ref.)	1.54 (0.91, 2.61)	1.57 (0.81, 3.04)
		GT/TT	
Whites (N=6778)	5830	948	
VTE cases	191	35	
Model 1	1 (ref.)	1.12 (0.78, 1.61)	
Model 2	1 (ref.)	1.11 (0.77, 1.59)	

Model 1: adjustment for age, sex, race, BMI, HRT and PCAs (in African Americans).

Model 2: Model 1 + adjustment for diabetes mellitus, systolic blood pressure, activated partial thromboplastin time, Von Willebrand factor, D-dimer, Factor VIII, Factor XI and hsCRP.

Table 5.

Difference in CP concentration and venous thromboembolism risk by number of CP-increasing alleles in rs11708215 and rs13072552

	0 risk alelles	1 risk alelle	2 risk alelles	3–4 risk alelles
N	4499	2497	1253	190
CP (mg/L)	291.0	303.7	317.7	332.1
VTE cases	133	117	59	5
AFRICAN AMERICANS				
Difference in CP concentration (95% confidence intervals)				
N	353	791	466	51
VTE cases	10	46	29	3
Model 1	Ref.	11.3 (3.5, 19.2)	23.4 (14.7, 32.1)	32.5 (14.2, 50.9)
Model 2	Ref.	11.8 (4.0, 19.7)	23.8 (15.1, 32.4)	32.9 (14.7, 51.2)
Hazard ratios (95% confidence intervals)				
Model 1	1 (ref.)	2.03 (1.02, 4.04)	2.11 (1.02, 4.04)	1.73 (0.47, 6.37)
Model 2	1 (ref.)	2.26 (1.11, 4.60)	2.31 (1.10, 4.87)	2.24 (0.60, 8.38)
WHITES				
Difference in CP concentration (95% confidence intervals)				
N	4146	1706	787	139
VTE cases	123	71	30	2
Model 1	Ref.	9.9 (6.5, 13.5)	23.4 (18.7, 28.11)	39.3 (28.8, 49.8)
Model 2	Ref.	10.1 (6.6, 13.5)	23.2 (18.6, 27.9)	39.2 (28.8, 49.5)
Hazard ratios (95% confidence intervals)				
Model 1	1 (ref.)	1.39 (1.04, 1.86)	1.28 (0.86, 1.91)	0.48 (0.12, 1.94)
Model 2	1 (ref.)	1.38 (1.03, 1.85)	1.29 (0.87, 1.93)	0.45 (0.11, 1.83)

Model 1: adjustment for age, sex, race, BMI, HRT and PCAs (in African Americans).

Model 2: Model 1 + adjustment for diabetes mellitus, systolic blood pressure, activated partial thromboplastin time, Von Willebrand factor, D-dimer, Factor VIII, Factor XI and hsCRP.

Table 6.

Difference in ceruloplasmin concentration, hazard ratios (HR) and 95% confidence intervals (CI) of venous thromboembolism by haplotypes of rs11708215 and rs13072552

African Americans (n = 1661)					
Haplotype (rs11708215 / rs13072552)	N	VTE events	Ceruloplasmin (mg/L)	Difference in CP (adjusted) ***	HR (95%CI) of AF (adjusted) ***
AA / GG	353	10	298.4	Ref.	1 (Ref.)
AA / GT	621	36	305.3	7.6 (−0.5,15.7)	2.19 (1.05,4.55)
AA / TT	251	15	323.4	22.7 (12.6,32.9)	2.35 (1.02, 5.39)
AG / GG	170	10	324.8	26.9 (15.6,38.1)	2.50 (1.02,6.13)
AG / GT	188	13	321.8	22.4 (11.5,33.3)	2.36 (1.01,5.50)
AG / TT	35	2	326.9	30.5 (8.9,51.9)	2.01 (0.43,9.44)
GG / xx *	43	2	339.3	38.9 (19.4,58.4)	1.98 (0.43,9.26)
Whites (n = 6778)					
Haplotype (rs11708215 / rs13072552)	N	VTE events	Ceruloplasmin (mg/L)	Difference in CP (adjusted) ***	HR (95%CI) of AF (adjusted) ***
AA / GG	4146	123	290.4	Ref.	1 (Ref.)
AA / GT	183	9	311.0	23.6 (14.5,32.7)	1.49 (0.76,2.94)
AG / GG	1523	62	299.7	8.5 (4.8,12.1)	1.37 (1.01,1.86)
AG / GT	624	24	312.4	22.6 (17.4,27.7)	1.32 (0.85,2.04)
GG / GG	161	6	318.8	24.7 (15.1,34.4)	1.23 (0.54,2.79)
GG / GT	100	2	332.3	33.5 (21.3,45.6)	0.65 (0.16,2.62)
xx / TT **	41	0	340.3	56.7 (37.9,75.6)	

* GG / xx: 27 GG / GG (1 VTE case), 15 GG / GT (1 VTE case) and 1 GG / TT (0 VTE cases)

** xx / TT: 2 AA / TT (0 VTE cases), 15 AG / TT (0 VTE cases), 24 GG / TT (0 VTE cases)

*** Adjusted for [variables in Model 1+2 + principal components in African Americans]