ARIC Manuscript Proposal #1718

PC Reviewed: 11/9/10 **Status: A** **Priority: 2**
SC Reviewed: _____ **Status: _____** **Priority: ____**

1.a. Full Title: (tentative)
Genetic determinants of plasma von Willebrand factor antigen levels and correlation with atherothrombosis: A comparison between subjects of African and European descent

b. Abbreviated Title (Length 26 characters):
Association of VWF haplotypes and VWF plasma antigen level in subjects of African and European descent

2. Writing Group: Marco Campos, Fuli Yu, Maja Barbalic, Christie Ballantyne, Aaron Folsom, Woody Chambless, Eric Boerwinkle, Jing-fei Dong

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

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3. Timeline:
Data analysis to be started upon approval of the proposal and manuscript to be completed within 6 month after data analysis is completed.

4. Rationale:

von Willebrand factor (VWF) is a multimeric glycoprotein ligand that is essential in the initiation of hemostasis at the site of vessel injury. VWF can also be prothrombotic by mediating platelet adhesion to inflamed endothelial cells and platelet aggregation under pathological high shear stress found at sites of arterial stenosis. VWF-induced platelet aggregation not only occludes arteries, but also activates platelets to release proinflammatory cytokines and chemokines that propagate endothelial injury. VWF multimers are secreted by endothelial cells and platelets under conditions such as systemic inflammation and hyperlipidemia. As such, plasma VWF antigen serves as one of the most widely used markers for endothelial injury. Multiple correlation studies have shown that elevated plasma levels of VWF are associated with smoking, elevated cholesterol levels, diabetes mellitus, hypertension, and atherothrombosis.[1] Some studies have shown that VWF levels are an independent predictor for ischemic heart disease and stroke.[2] There is also substantial evidence that implicates VWF in the terminal thrombotic complications of acute coronary syndromes, including animal models of VWF-dependent occlusive coronary thrombosis.[3] Given these findings, it is important to understand and eventually regulate VWF expression.

It is known that both environmental and genetic factors impact plasma levels of VWF multimers. Studies have demonstrated that 66% of the variations in plasma VWF levels are genetic and 30% of which is determined by ABO blood type.[4] We have recently constructed VWF haplotypes for European (EA) and African Americans (AA) and, as expected, found them to be significantly different (Figure 1). We have also analyzed 78 VWF SNPs for ARIC subjects of European descent and found 18 of them (and associated haplotypes) to significantly associate with VWF antigen levels (the manuscript [#1497] has been approved and submitted recently). However, we were unable to compare SNP and haplotype distributions between subjects of European and African descent because ABO for African American has only recently been genotyped for the entire ARIC cohort. In addition, the recent International 1000 Genomes Project promises to survey more than 25 world wide ethnicities using the next generation sequencing platforms. The data is quickly becoming available to the community. With this information now available, we propose to conduct a follow up study to specifically 1) identify SNPs and haplotypes of the VWF gene that are associated with VWF antigen levels in African Americans, 2) identify differential SNP and haplotypes that may be unique for either ethnic group, and 3) determine whether any SNPs and haplotypes found in African American subjects correlate with cardiovascular or cerebrovascular disease. 4) characterize the SNP and haplotype distributions in control individuals in the ethnicities studies by the 1000 Genomes Project.

5. Main Hypothesis/Study Questions:

We hypothesize that 1) African Americans have a different set of SNPs (and haplotypes) that associate with VWF antigen levels in plasma; 2) ABO genotypes have different impacts on VWF antigen levels in subjects of African and European descents, and 3) specific SNPs and haplotypes in the VWF gene of African Americans associate with incidence of CHD and stroke.

ALTERNATIVE HYPOTHESIS:

We hypothesize that:

1) In spite of having different haplotype maps; African American haplotypes with an association with VWF Ag level will localize to a similar location on the VWF gene as the haplotypes found to have an association in subjects of European descent. If this is true, it will lend support to the hypothesis that this particular place on the VWF gene plays an important role in VWF regulation/synthesis and is more than an association.

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:

We propose to:

1. In collaboration with Dr. Fuli Yu of the Human Genome Sequencing Center at Baylor College of Medicine, we have constructed separate VWF gene haplotype maps for subjects of African and European descent using fastPHASE program (Figure 1).
2. Determine the association of VWF SNPs and haplotypes with baseline ARIC VWF Ag levels in African Americans. VWF Ag level is treated as a quantitative trait. We plan to study the haplotype association using a linear mixture model approach[5], which has three different methods. First is a linear regression with the haplotype estimates from fastPHASE. The other two methods do not require prior estimation of haplotypes: a score test method[6] and a linear mixture model approach[7]. The latter might be more powerful than the traditional approach, the score test method, due to it inherent dimension reduction treatment. After the stratified analyses are performed, we will then evaluate (analysis of covariance) whether there are interactions between any VWF haplotype and ABO blood type (cohort).
3. Identify specific SNPs and haplotypes that are common or unique in their association with VWF antigen levels in subjects of African and European descent.
4. Carry out similar SNP and haplotype analyses in the 1000 Genomes data set.
5. We did not detect any correlation between VVWF SNPs/Haplotypes and atherothrombotic disease in subjects of European descent. Here, we propose to perform analyses to determine if any VWF SNPs and haplotypes (overall or within a specific blood type) associate with the incidence of (1) definite or probable myocardial infarction plus definite fatal CHD, (2) ischemic stroke, (3) diabetes in African Americans. The analysis will involve proportional hazards regression for the incidence endpoints and logistic regression for prevalence endpoints.
6. Confounding variables for analysis: gender, total cholesterol, HDL, age, smoking status, and hypertension.

Inclusion/exclusion:

The entire ARIC cohort, including subjects who have data on plasma VWF antigen (Visit 1) and SNPs in VWF gene. ABO status has been genotyped for the ARIC cohort.

7.a. Will the data be used for non-CVD analysis in this manuscript? __Y__ 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? __Y__ Yes ____ No
(This file ICTDER03 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

8.c. If yes, is the author aware that the participants with RES_DNA = 'not for profit' restriction must be excluded if the data are used by a for profit group?
__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)?

ARIC 1457 CHARGE GWAS for factors VII, VIII, and von Willebrand factor. We have verified with the lead author that this proposal does not overlap.

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.cscc.unc.edu/aric/forms/

12. 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.

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[1] Spiel AO, Gilbert JC, Jilma B. Von Willebrand factor in cardiovascular disease: Focus on acute coronary syndromes. Circulation 2008;117:1449-1459.

[2] Whincup PH, Danesh J, Walker M, Lennon L, Thomson A, Appleby P, Rumley A, Lowe GD. Von Willebrand factor and coronary heart disease: prospective study and meta-analysis. Eur Heart J. 2002;23:1764-1770.

[3] Brinkhous KM, Reddick RL, Read MS, Nichols TC, Bellinger DA, Griggs TR. von Willebrand factor and animal models: Contributions to gene therapy, thrombotic thrombocytopenic purpura, and coronary artery thrombosis. Mayo Clin Proc 1991;66:733.

[4] Orstavik KH, Magnus P, Reisner H, Berg K, Graham JB, Nance W. Factor VIII and factor IX in a twin population: evidence for a major effect of ABO locus on factor VIII level. Am J Hum Genet. 1985;37:89-101.

[6] Schaid DJ, Rowland CM, Tines DE, Jacobson RM, Poland GA. Score tests for association between traits and haplotypes when linkage phase is ambiguous. Am J Hum Genet. 2002 Feb;70(2):425-34.

[7] Kwee LC, Liu D, Lin X, Ghosh D, Epstein MP. A powerful and flexible multilocus association test for quantitative traits. Am J Hum Genet. 2008 Feb;82(2):386-97.

Figure 1. Haplotypes of VWF SNPs in ARIC cohort (top panel: EA and bottom panel: AA).