ARIC Manuscript Proposal # 1678

PC Reviewed: 8/10/10 **Status: A** **Priority: 2**
SC Reviewed: _____ **Status: _____** **Priority: ____**

1.a. Full Title: Genetic Cross-determination of plasma levels of Factor VIII and von Willebrand factor and Correlation with Atherothrombosis: an ARIC study (tentative)

b. Abbreviated Title (Length 26 characters): Genetic Cross-influence between factor VIII and VWF

2. Writing Group: Marco Campos, Fuli Yu, Maja Barbalic, Christie Ballantyne, Woody Chambless, Aaron Folsom, 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 proposal approval and manuscript to be completed within 3 month after data analysis is completed.

4. Rationale:

Human hemostasis is composed of three separate, but interconnected systems of platelets, coagulation and fibrinolysis. When a blood vessel is injured, platelets are initially tethered to the injured site by multiple ligand receptor interactions. Von Willebrand factor is essential in the initial adherence of platelets to subendothelium at the site of injury. Activated platelets are crosslinked (aggregated) by fibrinogen to form an unstable platelet plug. During this process, platelets expose phospholipids, primarily phosphatidylserine, on their surface. Tissue factor binds to these exposed, negatively charge lipids to form a membrane-bound complex with factor VIIa and calcium. This complex activates factor X which activates the serine protease thrombin that cleaves and cross-links fibrin to form a stable clot. In parallel with the propagation of the extrinsic coagulation cascade, thrombin also activates the intrinsic coagulation pathway to increase thrombin generation. A key coagulation factor in the intrinsic pathway is factor VIII (FVIII). Factor VIII is a serine protease that circulates in blood in an inactive form. Factor VIII is very sensitive to proteolysis and has a very short half-life when it circulates alone in plasma. The majority of the bioavailable Factor VIII exists as a complex with VWF, which stabilizes factor VIII and regulates its activity. As a result the activities of these two proteins are intimately intertwined. In fact, Hemophilia A (a genetic defect of Factor VIII) was historically confused with von Willebrand disease (a group of genetic defects of

the VWF gene). Von Willebrand disease always associates with low factor VIII levels.

We have recently detected 18 VWF SNPs and their haplotypes from a total of 78 SNPs investigated that are significantly associated with VWF antigen levels in ARIC subjects of European decent. Strikingly, all 18 positive SNPs are located in a 50 kb region (26% of total sequence), even though the 78 SNPs were dispersed throughout the entire VWF gene. More importantly, these positive SNPs are located within the region that encodes the D2, D' and D3 domains of VWF. These domains are critical for VWF multimerization, but more importantly, they contain the binding site(s) for FVIII. Since FVIII has a extremely shorter half-life without being VWF bound, the SNPs associated with VWF antigen levels may also affect plasma FVIII levels by influencing the VWF-FVIII association. Inversely, SNPs in the factor VIII may also affect VWF antigen levels by altering the rate and kinetics of the association between the two molecules. There has so far been no large cohort study that has investigated the cross-genetic determinants of VWF SNPs on FVIII levels and *vice versa*.

5. Main Hypothesis/Study Questions:

We hypothesize that1) SNPs and associated haplotypes that correlate with plasma VWF antigen levels also affect plasma FVIII levels, 2) Factor VIII SNPs and haplotypes associate with plasma VWF levels, and 3) SNPs that result in high levels of VWF and factor VIII associate with the incidence of CHD and Stroke and prevalence of PAD.

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) Identify specific VWF SNPs that associate with plasma Factor VIII levels (and a factor VIII/VWF ratio in order to control for the absolute levels of both molecules), with emphasis on determining whether VWF SNPs known to associate with VWF levels also associate with factor VIII levels.

2) To detect SNPs in the FVIII gene (the SNP data will be obtained from the ARIC Affimatrix 6.0 SNP data set I thought the VIII gene was on the X chromosome and that these had to be imputed) that associate with plasma

FVIII levels in the entire ARIC cohort and to determine whether factor VIII is also affected by ABO blood group.
3) To establish a haplotype map of the FVIII gene in collaboration with Dr. Fuli Yu of the Human Genome Sequencing Center at Baylor College of Medicine. We will apply fastPHASE program to reconstruct haplotypes from the unphased SNP genotype data from the FVIII locus. In addition, “GOLD” maps illustrating the degree of linkage-disequilibrium (i.e. genetic correlations) measured by D’ and r^2 among FVIII SNPs will be produced for direct visualization of the “local haplotypes map”.
4) To measure the difference of FVIII SNPs and associated haplotypes between European and African Americans.
5) To identify SNPs in the factor VIII gene and haplotypes that associate with VWF antigen levels and to identify SNPs in both genes that associate with high levels of plasma VWF and factor VIII.
6) To perform a analysis to determine whether any SNPs associate with the incidence of (1) definite or probable myocardial infarction plus definite fatal CHD and (2) ischemic stroke, or (3) prevalence of PAD. The analysis will involve proportional hazards regression for the incidence endpoints and logistic regression for prevalence endpoints. A similar analysis will also be used to identify associations between Factor VIII SNPs and incidence of DVT/PE.

Confounding variables for analysis: gender, diabetes, smoking status, obesity, and race.

Finally, not to be included, but could serve as the follow up of this manuscript, Dr. Fuli Yu is leading the 1000 Genomes Project analysis efforts at Baylor, which is expected to have most of the data released by the end of 2010. Fuli will take a lead to investigate the distribution of VWF and FVIII SNPs and haplotypes in ~25 different populations in the world.

Inclusion/exclusion:
The entire ARIC cohort, including subjects who have data on plasma FVIII and VWF antigen levels (Visit 1) and SNPs in the FVIII and VWF genes. ABO status will be ABO genotypes completed recently. For incidence analyses, subjects with prevalent disease will be excluded.

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

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.