ARIC Manuscript Proposal #1497

PC Reviewed: 4/14/09	**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: an ARIC study

b. Abbreviated Title (Length 26 characters): How VWF SNPs affect the known impact of ABO blood group on VWF levels.

2. Writing Group: Marco Campos, Christie Ballantyne, Eric Boerwinkle, Aaron Folsom, Weihong Tang, Woody Chambless, Fuli Yu, Wei Sun, Jing-fei Dong

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. MC **[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 initiating hemostasis at the site of vessel injury. VWF is also prothrombotic. It mediates platelet adhesion to inflamed endothelial cells and platelet aggregation under pathological states of high shear stress found at sites of stenosis. VWF-induced platelet aggregation not only occludes arteries, but also activates platelets to release a large amount of proinflammatory cytokines and chemokines which 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 of 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 showing VWF-dependent occlusive coronary thrombosis.[3] Given these findings, it is important to understand and eventually control the variables that impact variations in VWF availability.

It is known that both environmental and genetic factors impact VWF levels with genetic factors having a surprising influence. Studies have demonstrated that 66% of the variations in plasma VWF levels are genetic and 30% is determined by ABO blood type.[4] The VWF gene is extremely polymorphic. Some VWF coding SNPs have been individually studied and found to have various impacts on VWF synthesis, stability, adhesion activity, and clearance. However, VWF haplotype structure and its impact on VWF antigen have not been studied. As a multimeric glycoprotein that can reach 20 million Dalton in size, VWF structure and conformation (and therefore adhesion activity) is greatly influenced by these genetic variations.

As mentioned above, another genetic modifier of VWF antigen is ABO blood group. The ABO blood group gene consists of a single gene located on chromosome 9 which has three different alleles. The A and B alleles encode different glycosyltransferases and the O allele does not encode a functional enzyme. The A and B alleles add N-acetylgalactosamine and D-galactose, respectively, to a common precursor side chain on erythrocytes, the H determinant. This modification converts the H antigen into the A or B antigens, whereas the H antigen remains unmodified because the O allele does not encode a functional glycosyltransferase. The different glycosyltransferase activity dictates the major antigenicity in blood type, but also impacts VWF glycosylation, a posttranslational modification that is known to affect VWF survival, which drives the differences in absolute levels of plasma VWF. The VWF glycosylation includes addition of A, B and H oligosaccharide structures on the N-linked oligosaccharide chains of VWF, catalyzed by glycosyltransferase activity based on the blood type of the individual.[5] It has been proposed that these carbohydrate modifications (or lack thereof) mediate the impact that ABO blood group has on VWF levels.[6]

In a comparison of subjects with type A versus type O blood, O'Donnell et al showed that type A subjects had higher levels of A glycosyltransferase activity which correlated with higher levels of A antigen determinants on VWF and higher total plasma levels of

VWF. They also found that the A2 allele which encodes a less functional enzyme than the A1 allele[7], has lower levels of glycosyltransferase activity and VWF Ag levels than the A1 allele. Expanding on these findings, Gallinaro *et al* posited that ABO determinants affect VWF levels by modifying survival and clearance.[8] In that study, VWF Ag levels were monitored post-DDAVP infusion (DDAVP induces the release of VWF from endothelial cells). The half life of VWF was significantly shorter in O than non-O subjects. There was also a direct correlation between baseline VWF Ag levels and half-life; subjects with lower VWF levels having a shorter half-life. These findings suggest that genetic variations in posttranslational glycosylation mediated by ABO phenotype could alter the rate of VWF clearance.

5. Main Hypothesis/Study Questions:

We hypothesize that structural and conformational differences in VWF substrate determined by VWF gene haplotypes will impact how VWF is glycosylated by various glycosyltransferease, including those defined by ABO blood group. We propose in ARIC to first determine VWF haplotypes and then measure how major haplotypes will affect VWF antigen levels within each of the four different blood groups (A, B, O and AB). Furthermore, we would like to explore whether an association exists between identified VWF haplotypes and incidence of myocardial infarction, stroke and peripheral arterial disease in ARIC.

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. Establish a haplotype map of the VWF 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 vWF locus. fastPHASE implements a clustering algorithm allowing efficient inference on the local haplotypes from unrelated individuals, which suits our proposed specific aim. In addition, "GOLD" maps illustrating the degree of linkage-disequilibrium (i.e. genetic correlations) measured by D' and r^2 among VWF SNPs will be produced for direct visualization of the "local haplotypes map".
2. Infer ABO blood group from proxy SNPs. ABO serologic blood-typing was not performed in the ARIC cohort. As a result, we will have to infer ABO phenotype from proxy SNPs. There are at least 7 genotypic blood groups (O1, O1v, 02, A1-1, A1-2, A2 and B).[9] In the ARIC GWAS database, we were able to identify 4 SNPs that can serve as tagging SNPs for 4 out of the 7 ABO genotypic blood groups as follows:

   A1-1 versus O1: rs14659 (r^2=0.71)

   A1-1 versus B: rs8176749 (one of the functional variants that distinguish between the 2 blood groups).

A1-1/A1-2 versus A2: rs8176704 (r^2=1)
O1 versus O1v/O2: rs512770 (the functional variant itself)

We were not able to find SNPs to separate A1-1 versus A1-2/A2 or O1v versus O2. However, since our analysis will focus primarily on blood phenotype this should not impact the manuscript.

3. Determine the association of VWF haplotypes with baseline ARIC VWF Ag level. This analysis will be performed separately for the four different blood groups (A, B, O and AB). Essentially there will be four ABO subgroups and the analysis will be performed in each subgroup. We anticipate that we will need to establish a normal range of VWF Ag level for each subgroup. Each subgroup will then be evaluated to see if certain VWF haplotypes can be significantly associated with the variation of VWF Ag levels. VWF Ag level is treated as a quantitative trait. We plan to study the haplotype association using a linear mixture model approach[10], which is three different methods. One method 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 [11] and a linear mixture model approach[12]. 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 using analysis of covariance whether there are interactions between any VWF haplotype and blood type (cohort).
4. Perform a cohort analysis to determine if any VWF haplotypes (overall or within a specific blood type) associate with the incidence of (1) definite plus probable myocardial infarction plus definite fatal CHD and (2) ischemic stroke and perform a cross sectional analysis to determine association with prevalence of peripheral arterial disease. The analysis will involve proportional hazards regression for the incidence endpoints and logistic regression for prevalence endpoints.
5. Confounding variables for analysis: gender, diabetes, total cholesterol, HDL, age, smoking status, obesity, hypertension and race.

Inclusion/exclusion:
The entire ARIC cohort, including subjects who have data on plasma VWF antigen (Visit 1) and SNPs in VWF gene. ABO status will be inferred by ABO allele.

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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[12] 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.