ARIC Manuscript Proposal # 1438

PC Reviewed: 10/14/08	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Genome-wide Association Study of Activated Partial Thromboplastin Time (aPTT) and Protein C – the ARIC Study

b. Abbreviated Title (Length 26 characters): **GWAS of aPTT and protein C**

2. Writing Group: Weihong Tang, Saonli Basu, Aaron Folsom, Xiaoxiao Kong, James Pankow, Nena Aleksic, Eric Boerwinkle, others are welcome…

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

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3. Timeline: Data analysis to start immediately (October, 2008). First draft of manuscript expected between November, 2008 and February, 2009 depending on the timeline for obtaining replication data for aPTT in the Caerphilly Study, an UK population-based epidemiological study.

4. Rationale:

Venous thromboembolism (VTE) is a common disease with a high mortality rate. It collectively constitutes the third most common life-threatening cardiovascular disease after coronary heart disease and stroke.[1] Both environmental and genetic risk factors, mostly targeting coagulation system, are important in the etiology of VTE. [2, 3,4,5,6] However, the major genetic variants for VTE have not been identified.

aPTT is a commonly used coagulation test to screen for deficiencies in the coagulation cascade. Protein C is one of the most important anticoagulant regulators of the coagulation pathway. Reduced levels of aPTT and protein C are important risk factors for VTE.[7-10] In the ARIC Study, the risk of VTE was 2-3 times higher for participants with aPTT below the median value[8] and 3.3 times higher for 1.1% of participants with plasma level of protein C values <2.0 mg/L at baseline compared with participants with higher values.[9]

Twin and family studies suggest that aPTT and protein C are heritable, with heritability of 0.36-0.50 for protein C [11, 12] and 0.43-0.83 for aPTT.[11, 12] Genetic linkage analysis identified a region on chromosome 16 that was strongly linked to protein C level in Spanish families.[13] Since aPTT and protein C are important risk factors and intermediate phenotype for VTE, it is possible that genetic factors influencing the levels of aPTT and protein C also influence the risk of VTE. Therefore, identification of genetic factors for aPTT and protein C may shed light on genetic etiology of VTE.

In ARIC, aPTT and protein C were measured at baseline in the entire cohort of 11,422 whites and 4089 African Americans, providing an excellent opportunity for the proposed study to conduct a genome-wide association study for these traits in a large sample of white and African American participants.

5. Main Hypothesis/Study Questions:
We propose to investigate the associations of genome-wide SNPs with protein C and aPTT in both white and African Americans participants. We hypothesize that there are novel genetic variants that are associated with plasma level of aPTT and protein C in white and African American populations.

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).
Population: All white and African American participants with available phenotype (aPTT and protein C) and GWAS data. The analysis will start with Phase 1 and 2 genome-wide SNP data. Phase 3 data is anticipated to arrive soon and will be integrated when it is available. Use of GWAS data in African Americans will follow the CARe procedures. To the best of our knowledge, ARIC is the only CARe cohort that has aPTT data. Protein C was only available in ARIC and a subset of Cardiovascular Health Study (CHS) cohort in the CARE consortium. The sample size for protein C in CHS is limited (~400)[14, 15] and it is unclear how many African Americans were included in this subset.

Exclusion: Participants reporting anticoagulant use will be excluded.
Outcome: Plasma level of protein C and aPTT.
SNP data: Genotyped and imputed SNPs for whites; genotyped SNPs (1 million) for African Americans. Imputation in African Americans will not be pursued at this stage due to a lack of suitable population and SNP database for imputation in African Americans. When adequate information/data is available for imputation in African Americans, we will consider imputation and analyze imputed SNPs in African Americans.
Covariates: Primary analyses will be adjusted for age, sex, and field center; Multivariate adjustment will be conducted for top SNPs and include age, sex, field center, smoking status (current, former, never), BMI, diabetes, CVD, TG, HDL-C, total cholesterol, alcohol consumption, systolic blood pressure, antihypertensive treatment, and hormone replacement treatment; Latent population substructure will be screened, and if substructure is detected, measures of population substructure (eg, principal components derived from Eigenstrat software) will be included as covariates in the primary analyses.
Statistical analysis: Analysis will be stratified by race. The 2 outcome variables will be analyzed as continuous variables. A multiple linear regression will be used with the covariates and each SNP as the predictor and each outcome variable as the response variable. Outliers and trait distribution will be checked and appropriate transformation will be conducted if necessary. An additive genetic model will be assumed. For genotyped SNPs, the analyses will be conducted using the genetic analysis package PLINK.[16] For imputed SNPs, we will use ProbABEL 0.0-5 (http://mga.bionet.nsc.ru/~yurii/ABEL/) that can incorporate dosage information for imputed SNPs into the regression analysis.
Genome-wide significance threshold: We will use Bonferroni adjusted p-value threshold (eg, 5×10^{-8}).
Validation and replication: We have obtained collaboration agreement from the Caerphilly Study, an UK population-based epidemiological study, to provide us aPTT data and DNA samples from this study to replicate the genetic findings for aPTT obtained in the whites of ARIC. In the Caerphilly Study, about 950 subjects had available aPTT measures and DNA samples. We are currently seeking funds to cover cost for the replication study.

7.a. Will the data be used for non-CVD analysis in this manuscript? ____ Yes __X__ 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? ____ 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?

____Yes ____No

(the data will not be used by a for profit group)

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)?

1159 Zakai NA et al. Activated Partial Thromboplastin Time and Risk of Incident Venous Thromboembolism.
783 Folsom AR et al. Protein C, Antithrombin III, and Venous Thromboembolism.

11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? __X__ Yes ____ No

11.b. If yes, is the proposal

_X__ A. primarily the result of an ancillary study (list number* 2006.03—Stampede and Geneva genotype funding in whites, and 2007.02 –- CARe genotype funding in African Americans).

___ 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.

References

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