ARIC Manuscript Proposal #2366

PC Reviewed: 5/13/14	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Genetic Associations and Mendelian Randomization Analyses of Plasma Phospholipid n-6/n-3 Fatty Acid Related Genetic Variants with Coagulation Factors in the Atherosclerosis Risk in Communities Study (ARIC) Study

b. Abbreviated Title (Length 26 characters): SNPs for PUFA and Coagulation

2. Writing Group:

Writing group members: Lu-Chen Weng, Weihong Tang, Lyn M. Steffen, Weihua Guan, James S. Pankow, and Aaron R. Folsom (other coauthors from are welcome)

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

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3. Timeline:

6 months for analysis, and 6 months for manuscript preparation

Manuscript draft is anticipated by the end of 2014.

4. Rationale:

Venous thromboembolism (VTE) is a disease that results from a blood clot formed in the veins, including deep vein thrombosis (DVT) and pulmonary embolism (PE). It is a major health problem in the United States (U.S.). Reducing the incidence of VTE does not only decrease the total mortality but also decrease economic cost to the U.S health care system.

Age, obesity, trauma, cancer, and history of VTE were associated with VTE risk. [1,2] Several coagulation factors, such as coagulation factor VII, factor VIII, protein C, fibrinogen, and von Willebrand factor (vWF) were related to VTE risk as well. [2-5]

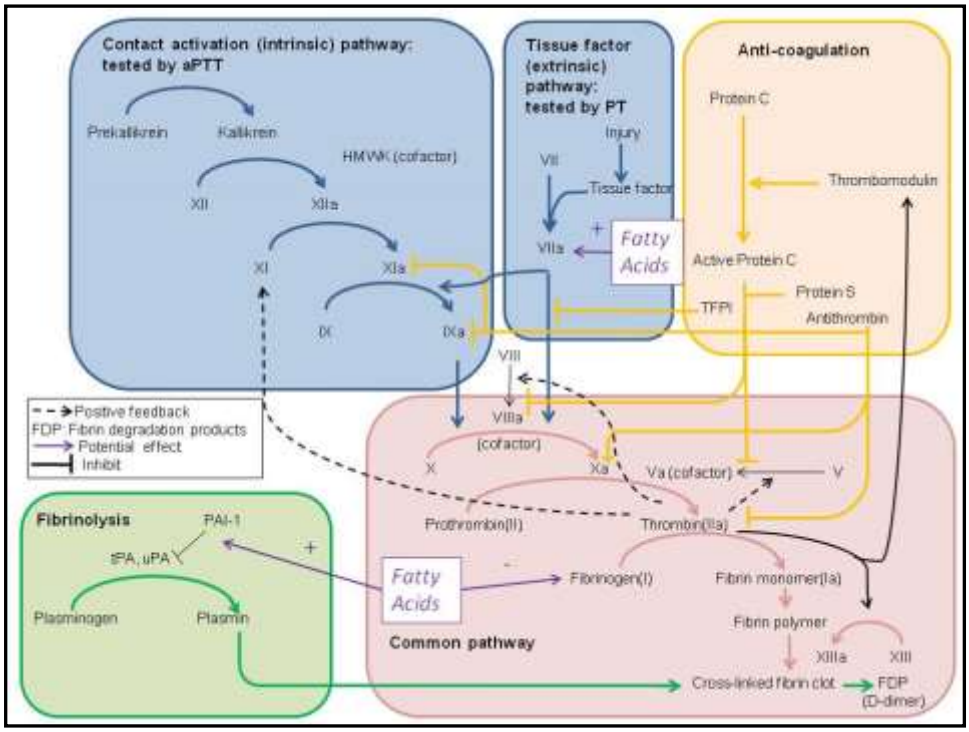

Figure 1. Coagulation system where the possible roles of fatty acids are shown (Adapted from http://en.wikipedia.org/wiki/File:Coagulation_full.svg)

Genetic variants for several coagulation factors or measures, including factor VII, VIII, fibrinogen, prothrombin time (PT), activated partial prothrombin time (aPTT), protein C, and vWF, have been identified in ARIC and other populations. [6-10] However, total variances of these traits explained by the identified genetic variants were limited (~7.7% for FVII, ~10% for FVIII, ~13% for vWF, ~12% for protein C, <2% for fibrinogen, and ~29% of aPTT). We hypothesize that additional genetic variants can be identified for the

coagulation factors by analyzing known genetic variants for biomarkers that are related to coagulation factors, e.g., plasma polyunsaturated fatty acid (PUFA).

Potential Biological Mechanisms between Dietary PUFA and Coagulation Factors

There are two possible mechanisms that fatty acids involve in the hemostatic system. First, n-3 PUFA may participate in the regulation of platelet function [11]. Consumption of n-3 fatty acid may lead to a replacement of platelet phospholipid n-6 PUFA by n-3 PUFA [12]. Thus, arachidonic acid (AA) is replaced by eicosapentaenoic and docosahexaenoic acids (EPA and DHA), and the metabolites converted in platelet activation would be prostaglandin H_3 and thromboxane A_3 but not prostaglandin H_2 and thromboxane A_2 [12]. Because prostaglandin H_3 and thromboxane A_3 are less effective in platelet activation, this replacement suppresses platelet activation. In addition, platelet formation is regulated promoted by thrombin; therefore, this replacement of fatty acids may also indirectly influence the level of coagulation factors by modulating thrombin level.

Furthermore, PUFA may influence coagulation factor level by affecting gene transcription. n-3 PUFA may inhibit sterol regulatory element binding protein 1 (SREBP-1) release from endoplasmic reticulum or down-regulate the hepatic mRNA level of SREBP-1 through accelerating the rate of SREBP-1 mRNA decay [13,14]. It suggests the effect of PUFA on hepatic lipoprotein gene transcription [15]. Lipoproteins are shown to be related coagulation factors [16,17]. Therefore, polyunsaturated acid may indirectly alter the expression and secretion of coagulation factors.

Observational Studies of Blood PUFA level with Coagulation Factors

Because the traditional methods of fatty acid measurements are time-consuming, associations of circulating fatty acid levels with coagulation factors have not been well investigated. A study focusing on middle-aged adults in the FINRISK Hemostasis Study identified that the serum linoleic acid (LA; 18:2n-6) level was negatively associated with fibrinogen and plasminogen in both men and women. In addition, dihomo-γ-linolenic acid (DGLA; 20:3n-6) was shown to be positively associated with FVII antigen in men, while DGLA was positively associated with both FVII antigen and coagulation activity in women [18]. Interestingly, the associations of coagulation factors with n-6 PUFA are stronger than with n-3 PUFA in this study, and the only association for n-3 PUFA was observed between serum DHA (22:6n-3) level and lower FVII coagulation activity in women. On the other hand, the Prospective Epidemiology Study of Myocardial Infarction (PRIME) study observed an inverse association of n-3 fatty acids and DHA with tissue plasminogen activator antigen, but no association of n-6 or n-3 fatty acids with fibrinogen, PAI-1 activity and antigen, FVII coagulation activity and antigen, or vWF was identified. [19] Notably, a large amount of circulating n-3 fatty acid showed a stronger effect with the levels of coagulation factors. In a study investigating the associations of n-3 fatty acid with coagulation factors in different ethnic groups, serum n-3 fatty acid was negatively associated with plasma fibrinogen level in Japanese but not in whites or Japanese Americans when the n-3 level in Japanese was almost twice as high as the level in the other two populations. [20]

Common Genetic Variants Identified for Fatty Acids in GWAS

Recently, several genetic loci had been identified to be associated with levels of plasma phospholipid n-3 and n-6 PUFA in GWAS. [21,22] However, it is unclear whether these genetic variants are also related to coagulation factors. Last year, one newly published study suggested the potential intra-system pleiotropy for triglycerides and coagulation factors. [23] This study identified statistically significant associations between several triglyceride related single nucleotide polymorphisms (SNPs) and FVII level, which demonstrated the pleiotropy for hemostasis and lipids. We hypothesize that genetic variants influencing plasma phospholipid PUFA also play a role in the inter-individual variation of coagulation factors. There are two aims in this proposed study. First, our study proposes to investigate the association of the known SNPs for plasma phospholipid PUFA levels with coagulation factors, in order to identify new genetic determinants for coagulation factors. To the best of our knowledge, there have not been any published studies to evaluate the association of these SNPs with coagulation factors. The second aim of this proposed study is to evaluate the causal relationship between n-3 or n-6 fatty acids and coagulation factors using a Mendelian randomization approach. Mendelian randomization analysis is a technique that allows one to estimate causal relationships in observational studies. Once the associations of the known SNPs for PUFA with coagulation factors have been determined, the causal relationship between PUFA levels and coagulation factors will be evaluated as well.

5. Main Hypothesis/Study Questions:

We hypothesize that fatty acid-associated genetic variants play a role in the inter-individual variation of coagulation factors. In addition, plasma phospholipid n-3 or n-6 PUFA levels may be causally related to coagulation factors.

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: Based on the known plasma phospholipid related genetic variants, a cross-sectional study design will be used to evaluate the association of these variants with coagulation factors.

Population: European American participants with genetic data and phenotype measurements will be included in our primary analysis. In the secondary analysis, identified associations in European Americans will be tested in African American participants as well.

Exclusions: Participants who were missing for phenotype measurements or genotype data, or were anti-coagulant users or extreme outliers will be removed.

Analysis Plan:

A. Linear regression models
   Two models will be used to evaluate the association between each SNP and each plasma coagulation factor level. The models are as follows.

Model1: Coagulation factor=$ß_0$+$ß_1$age+$ß_2$sex+$ß_3$field center+$ß_4$ principal components +$ß_5$SNP
Model2: Coagulation factor=$ß_0$+$ß_1$age+$ß_2$sex+$ß_3$field center+$ß_4$ principal components +$ß_5$fatty acid+ $ß_6$SNP

Independent variable: A total of 15 genetic variants identified for plasma phospholipid n-3 and n-6 fatty acid levels in GWAS [21,22]

Dependent variable: levels of plasma coagulation factors measured at baseline, including coagulation factor VII, VIII, vWF, fibrinogen, protein C, or aPTT.

Covariates of interest: Age in years, sex, field center (F, J, M, W), and baseline plasma phospholipid n-3 and n-6 fatty acid levels, including α- -linolenic acid (ALA), EPA, docosapentaenoic acid (DPA), DHA, LA, γ-linolenic acid (GLA), DGLA, and AA, and principal components to control for potential confounding by genetic admixture.

Modeling of genetic effect:
Additive genetic model will be used (degree of freedom=1). Beta coefficient for SNPs will be explained as the change of coagulation factors per one minor allele increment. Because the functional SNPs may not be identified in the published GWAS for fatty acids, we will expand our analysis by ±50k base pairs if any significant associations are identified between the coagulation factors and SNPs. Because some of the 15 identified SNPs were in moderate to high LD with each other, only 9 of them are judged independent based on r^2<0.3. The significant p-value threshold will thus be set at $5.6x10^{-3}$(=0.05/9), accounting for multiple testing.

B. Mendelian randomization approach
   Using genetic variants as instrument variables, the causal relationship between plasma phospholipid PUFA levels and coagulation factors will be evaluated.
   a. Instrument variable
      1. single genetic variant
      2. multiple genetic variants
      3. a genetic score, a combination of information from multiple genetic variants
   b. Dependent variable: coagulation factors
   c. Independent variable: plasma PUFA levels
   d. Covariates of interest: age in years, sex, field center (F, J, M, W), and the first 10 principal components

C. Limitation and challenges
   There are some limitations in this proposed study. First, plasma phospholipid levels were only available for ARIC white participants in the Minnesota field center. Thus, the sample size in our model 2 will be substantially reduced, and the association identified in model 1 may be undetectable in this small sample. Secondly, the PUFA associated SNPs were identified in populations of European

descent. Therefore, our findings may not be generalizable to other race groups. However, this study is the first to test the association of plasma PUFA related SNPs with coagulation factors. Findings from our study may still be useful to clarify the unknown mechanisms for the associations between plasma phospholipid fatty acids and coagulation factors.

D. Replication: we will seek collaboration with the other cohorts in CHARGE to replicate findings from ARIC if interesting signals are obtained.

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

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 ICTDER 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

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)?

#1710 Genome-Wide Association Study (GWAS) of Plasma Fatty Acid Biomarkers in the De Novo Lipogenesis Pathway: CHARGE Fatty Acid Consortium Lead author: Jason Wu
#2013 Genome-wide association study of monounsaturated fatty acids in Chinese and Caucasian cohorts: CHARGE Consortium Fatty Acid Working Group Lead author: Jingwen Zhu

#1788 Genome-wide Association Study of Plasma Phospholipid N6 Fatty Acids within the CHARGE Consortium Lead authors: Michael Tsai & Weihua Guan
#1600 Genome-wide Association Study of Plasma Phospholipid Fatty Acids within the CHARGE Consortium Lead author: Rozenn Lemaitre
#1736 Does fatty acid intake modify the relation of hemostatic and inflammatory biomarkers with incident ischemic stroke and CHD? The Atherosclerosis Risk in Communities (ARIC) Study Lead author: Huifen Wang
#2172 Long-chain omega3 fatty acids and incident CHD Lead author: Liana Del Gobbo
#416 Plasma Fatty Acid Composition and 6-Year Incidence of Hypertension in Middle-Aged Adults Lead author: Zhi-Jie Zheng
#890 Plasma Fatty Acid Composition and Incidence of Coronary Heart Disease in Middle Aged Adults: The Atherosclerosis Risk in Communities (ARIC) Study Lead author: Lu Wang
#1735 Inflammation mediates the impacts of fatty acids on CHD and ischemic stroke incidence: the Atherosclerosis Risk in Communities (ARIC) Study Lead author: Huifen Wang

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* _1998.03_)
___ 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/

12a. 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.

12b. The NIH instituted a Public Access Policy in April, 2008 which ensures that the public has access to the published results of NIH funded research. It is **your responsibility to upload manuscripts to PUBMED Central** whenever the journal does not and be in compliance with this policy. Four files about the public access policy from http://publicaccess.nih.gov/ are posted in http://www.cscc.unc.edu/aric/index.php, under Publications, Policies & Forms. http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals automatically upload articles to Pubmed central.

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