

ARIC Manuscript Proposal #2520

PC Reviewed: 4/14/15
SC Reviewed: _____

Status: A
Status: _____

Priority: 2
Priority: _____

1.a. Full Title: Gamma prime (γ') fibrinogen and incident cardiovascular outcomes: the ARIC study

b. Abbreviated Title (Length 26 characters): γ' fibrinogen and CVD

2. Writing Group:

Writing group members: Duke Appiah, Pamela J. Schreiner, Aaron R. Folsom

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

First author: Duke Appiah

Address: Division of Epidemiology and Community Health, University of Minnesota, 1300 S 2nd St. Suite 300 Minneapolis MN 55454

Phone: 612 626 5458

Fax: 612-624-0315

E-mail: dappiah@umn.edu

ARIC author to be contacted if there are questions about the manuscript and the first author does not respond or cannot be located (this must be an ARIC investigator).

Name: Aaron R. Folsom, MD, MPH

Address: Division of Epidemiology and Community health, University of Minnesota, 1300 S 2nd St. Suite 300 Minneapolis MN 55454

Phone: 612-624-8862

Fax: 612-624-0315

E-mail: folso001@umn.edu

3. Timeline: A draft will be sent to the coauthors by the end of June 2015 and a final draft will be submitted to the P&P Committee by August 2015

4. Rationale:

Several epidemiologic studies have shown a positive association between elevated levels of fibrinogen and cardiovascular disease (CVD); however, the underlying mechanisms are not well understood (1-3). Fibrinogen is associated with several traditional CVD risk factors, suggesting that elevated fibrinogen levels may be one pathway by which these CVD risk factors exert their influence on the cardiovascular system (4, 5).

Fibrinogen, the main coagulation protein in plasma, has been proposed to influence atherothrombotic events through several mechanisms. Elevated concentrations of fibrinogen promote production of fibrin under the action of thrombin, which also activates factor XIII (5). Coagulation factor XIII then catalyzes bonds between other proteins, such as fibronectin and collagen, which increase the adherence of clots that are formed to vessel wall (6). Other mechanisms by which fibrinogen may lead to CVD outcomes include altered clot structure (7), and increased plasma viscosity and platelet aggregation (5). Although elevated concentrations of fibrinogen may reflect a procoagulation state (8), it remains an independent predictor of cardiovascular outcomes independent of other procoagulation and inflammatory factors (2). Recent findings that elevated plasma fibrinogen increases clot lysis time provide novel mechanistic insight and rationalizations to explain the relationship between fibrinogen and CVD (9).

Fibrinogen is a six-chain molecule containing 2 copies each of the A α , B β , and γ chains with the latter having two isoforms γ A and γ' arising from alternative mRNA processing (8, 10). γ' fibrinogen constitutes approximately 7% of plasma fibrinogen, but this percentage is higher among individuals with pathological conditions such as coronary heart disease (CHD) and stroke (8). There are some biochemical and biophysical properties of γ' fibrinogen which are different from other isoforms of fibrinogen that highlight its unique and important mechanistic roles in thrombus formation. It is known that γ' fibrinogen has a binding site for thrombin, which in the presence of factor XIII results in formation of clots that have altered clot architecture and are very resistant to lysis (8, 10). Accordingly, γ' fibrinogen has been proposed as an emerging independent risk factor for atherothrombotic events independent of total plasma fibrinogen levels (11-15). However, all prior studies have only assessed this relationship retrospectively or cross-sectionally among individuals already diagnosed with CVD events which limit the determination of temporality.

Therefore the aim of this study is to prospectively investigate the association of γ' fibrinogen and incident cardiovascular outcomes independent of other known risk factors among participants in the Atherosclerosis Risk in Communities (ARIC) study, a biracial cohort of white and black men and women. Although prior studies have assumed the relationship between γ' fibrinogen and CVD events to be linear, we would further investigate whether a dose-response relationship exists. Finally, we would evaluate the added predictive ability of γ' fibrinogen in CVD risk prediction models.

5. Main Hypothesis/Study Questions:

γ' fibrinogen is independently associated positively with incident CHD, ischemic stroke, peripheral artery disease, heart failure and total CVD events.

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).

Design: cohort beginning at ARIC visit 3

Exposure: γ' fibrinogen measured at visit 3

Exclusions:

Participants with prevalent Coronary heart disease, stroke (ischemic), heart failure, or peripheral artery disease at baseline (visit 3) will be excluded in analysis assessing incident total CVD as outcome. In CVD-specific outcome models, the corresponding prevalent CVD cases will be excluded. Additionally, participants with missing γ' fibrinogen values and current users of anticoagulant medications will be excluded.

Outcomes:

1. Coronary heart disease (CHD) as defined by a definite or probable diagnosis of myocardial infarction, definite fatal CHD and/or coronary revascularization (CABG or PTCA).
2. Ischemic stroke, definite or probable.
3. Peripheral artery disease (PAD) (hospitalized event or revascularization by ICD)
4. Heart failure (hospitalized events by ICD)
5. Total CVD as defined by the above plus other cardiovascular death.

Covariates (visit 3)

Demographic variables: age, sex, race, educational level (years of education), and medical insurance status.

Anthropometric measures: weight, height and waist circumference.

Health behavioral/lifestyle factors: smoking status (never, current, former) and pack years, physical activity (Baecke PA scores) and alcohol use.

Reproductive (women): age at menopause, type of menopause and hormone therapy use

Health history and conditions: systolic blood pressure, anti-hypertensive medication use, diabetes and lipid-lowering medication use.

Labs: total and HDL cholesterol, total fibrinogen (visit 1 only), C-reactive protein, d-dimer, and interleukin 6.

Statistical analysis

Sex-specific descriptive statistics will be calculated to describe the study participants in the cohort component of the ARIC study according to quartiles of γ' fibrinogen levels. Categorical variables will be compared between groups using chi-square tests while comparisons of continuous measures will be tested using analysis of variance (ANOVA). In instances in which continuous measures are skewed, results will be normalized by Log transformation. When normality is still not achieved by this procedure we would employ Kruskal-Wallis test, a non-parametric test or we may categorize such variables. In the analysis of time to event, incidence rates for CHD, ischemic stroke, heart failure, PAD and total CVD by quartiles of γ' fibrinogen will be reported with Kaplan-Meier curves produced. Log-Rank test will be used to test for differences in survival curves. Cox regression models will be used to assess the association of γ' fibrinogen (modelled as a continuous variable and categorized into quartiles) with incident CHD, ischemic stroke, heart failure, PAD and total CVD, in crude and adjusted models. Formal interaction tests of γ' fibrinogen and sex will be conducted and if found to be significant, sex-specific analyses will be performed. Adjustments will be made for the following confounders (age, race, BMI, smoking status and amount, systolic blood pressure, antihypertensive and lipid-lowering medication use, diabetes, total fibrinogen, total and HDL cholesterol). Further adjustment for other available procoagulation and inflammatory factors will be made in a supplementary analysis. The proportional hazards assumption will be tested using cumulative sums of martingale residuals with a Kolmogorov-type supremum test and also by visually inspecting plots of Schoenfeld residuals versus time. To explore the possibility of nonlinear and dose-response relationships between γ' fibrinogen and total CVD, restricted cubic and natural splines will be used with knots set at the quartiles of γ' fibrinogen levels. Finally, we would explore the added predictive ability of γ' fibrinogen on CHD risk prediction using the ARIC prediction equation and CVD risk using ACC/AHA ASCVD pooled cohort equation in net reclassification models. A two-tailed probability value less than 0.05 will be considered statistically significant in all analyses.

We may consider a supplemental analysis examining whether ARIC measured SNPs in *FGG* contribute to any association seen. Also, if we find γ' fibrinogen associated with some CVD outcomes, the possibility exists for replication in CHS.

REFERENCE

1. Danesh J, Collins R, Appleby P, et al. Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies. *JAMA : the journal of the American Medical Association* 1998;279(18):1477-82.

2. Fibrinogen Studies C, Danesh J, Lewington S, et al. Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an individual participant meta-analysis. *JAMA : the journal of the American Medical Association* 2005;294(14):1799-809.
3. Snieder H, MacGregor AJ, Spector TD. Genes control the cessation of a woman's reproductive life: a twin study of hysterectomy and age at menopause. *The Journal of clinical endocrinology and metabolism* 1998;83(6):1875-80.
4. Stec JJ, Silbershatz H, Tofler GH, et al. Association of fibrinogen with cardiovascular risk factors and cardiovascular disease in the Framingham Offspring Population. *Circulation* 2000;102(14):1634-8.
5. Folsom AR. Epidemiology of fibrinogen. *European heart journal* 1995;16 Suppl A:21-3; discussion 3-4.
6. Warner D, Mansfield MW, Grant PJ. Coagulation factor XIII and cardiovascular disease in UK Asian patients undergoing coronary angiography. *Thrombosis and haemostasis* 2001;85(3):408-11.
7. Ariens RA. Fibrin(ogen) and thrombotic disease. *Journal of thrombosis and haemostasis : JTH* 2013;11 Suppl 1:294-305.
8. Farrell DH. gamma' Fibrinogen as a novel marker of thrombotic disease. *Clinical chemistry and laboratory medicine : CCLM / FESCC* 2012;50(11):1903-9.
9. Kim PY, Stewart RJ, Lipson SM, et al. The relative kinetics of clotting and lysis provide a biochemical rationale for the correlation between elevated fibrinogen and cardiovascular disease. *Journal of thrombosis and haemostasis : JTH* 2007;5(6):1250-6.
10. Lovely RS, Kazmierczak SC, Massaro JM, et al. Gamma' fibrinogen: evaluation of a new assay for study of associations with cardiovascular disease. *Clinical chemistry* 2010;56(5):781-8.
11. Drouet L, Paolucci F, Pasqualini N, et al. Plasma gamma'/gamma fibrinogen ratio, a marker of arterial thrombotic activity: a new potential cardiovascular risk factor? *Blood coagulation & fibrinolysis : an international journal in haemostasis and thrombosis* 1999;10 Suppl 1:S35-9.
12. Lovely RS, Falls LA, Al-Mondhiry HA, et al. Association of gammaA/gamma' fibrinogen levels and coronary artery disease. *Thrombosis and haemostasis* 2002;88(1):26-31.
13. Mannila MN, Lovely RS, Kazmierczak SC, et al. Elevated plasma fibrinogen gamma' concentration is associated with myocardial infarction: effects of variation in fibrinogen genes and environmental factors. *Journal of thrombosis and haemostasis : JTH* 2007;5(4):766-73.
14. Lovely RS, Yang Q, Massaro JM, et al. Assessment of genetic determinants of the association of gamma' fibrinogen in relation to cardiovascular disease. *Arteriosclerosis, thrombosis, and vascular biology* 2011;31(10):2345-52.
15. Cheung EY, Uitte de Willige S, Vos HL, et al. Fibrinogen gamma' in ischemic stroke: a case-control study. *Stroke; a journal of cerebral circulation* 2008;39(3):1033-5.

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

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"?

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.csc.unc.edu/ARIC/search.php>

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)?

There is known overlap with Dr. Folsom's omnibus #2456: Associations of gamma prime fibrinogen, factor XI and D-dimer with venous thromboembolism and atherothrombotic CVD. However, Dr. Folsom agrees that this will be an offshoot of 2456, and so no concern about overlap.

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? Yes 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)* 1998.03)

*ancillary studies are listed by number at <http://www.csc.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.csc.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.