ARIC Manuscript Proposal # 1401

PC Reviewed: 08/12/08	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Associations of platelet surface marker genotype polymorphisms with platelet activation and plaque characteristics.

b. Abbreviated Title (Length 26 characters): Platelet polymorphisms

2. Writing Group: Anna Kucharska-Newton, Kelly Volcick, Nena Aleksic, Lloyd Chambless, Keri Monda, Lynne Wagenknecht, Richey Sharrett, Gerardo Heiss; Others welcome

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

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3. Timeline: : Data analysis to be started immediately following approval of the proposal and completed by April 2009. Manuscript preparation to be completed by July 2009.

4. Rationale:
Platelets play an essential role in the development of atherosclerosis and subsequently coronary heart disease. Platelet activation occurs as a result of exposure to inflamed endothelial cells or following injury to the blood vessel wall and resulting from it exposure to the sub-endothelial

matrix (1). Platelet activation manifests through increased expression of receptors which allow platelets to bind to the extracellular matrix and to each other (2). Glycoprotein Ibα, glycoprotein VI, glycoprotein Ia/IIa (integrin $\alpha_2\beta_1$), and glycoprotein IIb/IIIa have been identified as the major surface receptors involved in those processes.

Polymorphisms of the GVI glycoprotein, a platelet-specific collagen receptor, have been suggested to impart a decreased risk of fatal (3)and non-fatal (4, 5)events. Polymorphisms of the GPIbα, GPIIIa, integrin $\alpha_2\beta_1$,on the other hand have been found to be associated with increased coronary heart disease risk (6-8)), although the data is far from conclusive and age and gender, as well as severity of the event may modify the final estimate. Some studies have examined the association of platelet surface markers with density of their respective surface antigens (9), however, an analysis of the association of those markers with plaque characteristics and indicators of platelet activation, such as platelet-monocyte aggregates, is lacking.

The CarMRI study, through flow cytometry study of platelet and monocyte markers and through information on plaque characteristics, is uniquely suited to examine associations of platelet polymorphisms with intermediate prothrombotic phenotypes.

We propose to examine the associations of gene variation in the following platelet surface markers (Table) with expression of their respective antigens, as well as with levels of platelet-monocyte aggregates, and with plaque characteristics. Within the constraints of study size, effect modification by age, race, gender, BMI, and smoking will be considered in all analyses. Possible synergistic effects of the selected gene polymorphisms on expression of platelet surface antigens will be explored.
Markers selected for this study were identified, on the basis of current literature, as the major collagen, fibrinogen, and von Willebrand factor receptors.

Information concerning platelet surface marker levels, platelet-monocyte aggregates, and genotype variation will be obtained from the CarMRI flow cytometry study.
In our analysis we will examine all SNPs not in linkage disequilibrium, selected based on HapMap data of the CEU population.
Of the identified platelet surface markers, only CD41 and CD61 was shown to have good between-visit and within-visit repeatability in the CarMRI flow cytometery study, therefore densities of only those two markers will be examined in association with their respective gene polymorphisms.

Platelet surface marker (ref)	Surface antigen	Function	Gene
GVI (4, 5)		Collagen receptor	GP6
GP Ibα (11)	CD42b	vWF receptor	GP1BA
GP IIb (12)	CD41	Fibrinogen and vWF receptor	ITGA2B
Integrin $\alpha_2\beta_1$ (13)	CD49	Fibrinogen and vWF receptor	ITGA2
GP IIIa (12)	CD61	Fibrinogen and vWF receptor	ITG3B

References:

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5. Main Hypothesis/Study Questions:

1. Selected platelet glycoprotein polymorphisms are associated with MRI-detectable carotid wall and plaque characteristics
2. Selected platelet polymorphisms are associated with changes in levels of platelet-monocyte aggregates
3. Polymorphisms in the ITGA2B and ITG3B genes are associated with increased densities of the respective platelet surface glycoproteins: CD41 and CD61.

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 population: This study will consist of two components, a cross-sectional analysis based on the CarMRI ARIC study (ARIC Visit 5) and a longitudinal analysis of change in retinal microvascular characteristics from ARIC Visit 3 to Visit 5 and its association with platelet

activation . The CarMRI study, an ancillary ARIC study, was conducted in 2004-2005. Approximately 2000 ARIC cohort participants were selected for the study; 60% of individuals were selected based on their carotid artery wall thickness greater than 85th percentile and 40% of individuals constituted a weighted sample selected from the remaining carotid intima media thickness distribution. Whole blood flow cytometry analysis of platelet and monocyte surface markers was performed as part of the CarMRI study.

Data analysis: Hardy-Weinberg equilibrium will be checked for each SNP. Analyses will be performed using multivariate linear regression with weighted analysis to account for sampling design.

Exclusions: missing covariates, participants who did not consent to genotyping.

Dependent variables:

- Flow cytometry determined median fluorescence intensity (MFI) of the selected platelet markers (CD41, CD61) and of the platelet monocyte aggregates
- Plaque characteristics: wall volume, maximum wall thickness, presence of lipid core, size of lipid core, mean fibrous cap thickness

Covariates: Within the constraints of study size, effect modification by age, race, gender, BMI, and smoking will be considered in all analyses. Possible synergistic effects of the selected gene polymorphisms on expression of platelet surface antigens will be explored.

SNP selection: All single nucleotide polymorphisms (SNPs) not in linkage disequilibrium, selected based on HapMap data of the CEU population. Genetic polymorphisms selected for this study have been genotyped as part of the CARE 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?
__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)?

No overlapping manuscripts were identified

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* _2004,11)

___ 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.