ARIC Manuscript Proposal # 1221

PC Reviewed: _2_/_13/07	**Status: ___A_**	**Priority: __2_**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: P-selectin Gene Variation Influences (does not influence) Cell Surface Levels of P-selectin and P-selectin Ligand: the ARIC Carotid MRI Study

b. Abbreviated Title (Length 26 characters): PSEL Gene Variation, Cell Surface Levels & CVD

2. Writing Group: Kelly Volcik, Eric Boerwinkle, Nena Matijevic, Diane Catellier, Aaron Folsom, and others

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

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3. Timeline: All data (genotype and cell surface measures) are available, so analyses can begin immediately. For this MS proposal, we will focus on those PSEL and PSGL-1 SNPs already genotyped on the entire ARIC cohort (PSEL: rs6131, rs6127, rs6133, rs6136; PSGL-1: rs8179131, rs2228315).

4. Rationale: P-selectin (PSEL) initiates the rolling of platelets and leukocytes on activated endothelial cells, and mediates the interactions of leukocytes with the endothelium, platelets with the endothelium, and leukocytes with platelets. These leukocyte-endothelium and leukocyte-platelet interactions require the presence of a counter-ligand on the leukocyte surface, P-selectin glycoprotein ligand-1 (PSGL-1). Previous studies, including ARIC, have shown genetic variation in the PSEL gene (i.e. T715P, Volcik MS#1002) to be associated with plasma PSEL levels but not disease. However, the major biological function of most cell

adhesion molecules is at the cell surface, and it has not been determined whether plasma levels of these molecules correlate with cell surface levels. Most clinical and population studies to date have focused on PSEL plasma levels. Recently, we demonstrated specific PSEL and PSGL-1 genotypes/haplotypes to be associated with incident CHD and ischemic stroke (Volcik MS#1002b). This manuscript will examine if PSEL and PSGL-1 genotypes/haplotypes are associated with cell surface measures.

Through our measurements of cell surface PSEL and PSGL-1, we will study 1) the relationship between PSEL and PSGL-1 cell surface measures, 2) the association of PSEL and PSGL-1 genetic variation with cell surface levels, and 3) the association of PSEL and PSGL-1 cell surface levels with disease.

5. Main Hypothesis/Study Questions:

1. Evaluate the relationship (i.e. correlation) between PSEL and PSGL-1 cell surface measures.

2. Evaluate whether PSEL gene variation is associated with PSEL cell surface measures, and whether PSGL-1 gene variation is associated with PSGL-1 cell surface measures. For PSGL-1, genotype-level associations will be evaluated for each of the three cell types studied (lymphocytes, monocytes, granulocytes).

3. Evaluate whether PSEL and PSGL-1 cell surface measures are associated with disease status (CHD and stroke).

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).

The usual DNA restriction, ethnic group, and missing data exclusion criteria will be used. From our previous analyses involving the PSEL and PSGL-1 SNPs that will be evaluated in this proposed study, we know there is a marked allele frequency difference between whites and African Americans for each of the SNPs of interest; therefore, all analyses will be conducted race-specific. Routine descriptive statistics will be used for the genotype and allele counts. Analysis variables include but are not limited to PSEL and PSGL-1 genotype status, PSEL and PSGL-1 cell surface measures, incident CHD and stroke case status, and traditional risk factors such as age, gender, smoking, diabetes and hypertension status.

To account for our sampling design, we performed all weighted descriptive and regression analyses with SUDAAN software using sampling weights inversely proportional to the stratum-specific sampling fractions. Using these weights allows us to make inferences back to the original ARIC cohort. The percentages and odds ratios (e.g., for risk of CHD or stroke) therefore represent weighted values. SUDAAN also adjusts the standard errors to account for the weighting and stratification of the sampling design, to ensure that *P* values are valid.

Hypothesis 1: Linear regression models will be used to examine the correlation between PSEL and PSGL-1 cell surface measures.

Hypothesis 2: Linear regression models will be used to examine the association between PSEL gene variation and PSEL cell surface marker values and the corresponding gene-surface

marker association for PSGL-1. A basic model will include age, race, sex and case status (CHD and stroke) as covariates. Additional models including additional covariates will be considered.

Hypothesis 3: Logistic regression models will be used to examine the univariate relationships between PSEL and PSGL-1 cell surface measures, singly or in combination, and having a history of CHD or stroke. The odds ratio of having disease will be calculated for a difference in the cell surface value equal to the inter-quartile range (IQR). To explore further the relationships between cell markers and medical histories, multivariate analyses will be performed with the inclusion of control variables: age, race, sex, and CHD risk factors (e.g., obesity, smoking, diabetes history, hypertension history, etc).

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 ICTDER02 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

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 ICTDER02 must be used to exclude those with value RES_DNA = "No use/storage DNA"? _X_ Yes ____ No

9. The lead author of this MS proposal has reviewed the list of existing ARIC Study MS proposals and found no overlap between this proposal and previously approved proposals either published or still in active status. _X_ Yes ___ No

10. What are the most related MS proposals in ARIC? (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)

1002: (K Volcik et al.) P-selectin Thr715Pro polymorphism predicts PSEL levels but not risk of incident CHD or ischemic stroke in a cohort of 14595 participants: the ARIC study
1002b: (K Volcik et al.) Specific P-selectin and P-selectin glycoprotein ligand-1 genotypes/ haplotypes are associated with risk of incident CHD and ischemic stroke: the ARIC study
1205: (N Matijevic et al.) Association of platelet markers with PAD
1207: (N Matijevic et al.) Association of monocyte markers with PAD

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

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 MS proposal will expire.