January 19, 2006

ARIC Publications Committee:

Upon completion and publication[1] of MS# 1002, eight additional SNPs in the P-selectin gene and three SNPs in the P-selectin Glycoprotein Ligand-1 gene have been genotyped on the entire ARIC cohort. Therefore, we would like to extend our previous manuscript proposal to include all SNPs in new analyses. Since the gene and study questions remain largely the same as MS# 1002, we suggest considering this extension as MS# 1002B. A brief summary of the timeline and study questions is presented below, and no change in writing group members is expected.

Timeline: Genotyping of 9 PSEL and 3 PSGL-1 polymorphisms is complete for the entire ARIC cohort. Statistical analyses will begin immediately, with a first draft manuscript prepared by April 2006.

Main Hypothesis/Study Questions:

1. To estimate the frequency distribution of PSEL and PSGL-1 gene variation in a population-based sample of whites and African-Americans.
2. In a race-specific manner, to evaluate the independent effects of PSEL and PSGL-1 gene variation on P-selectin levels. P-selectin levels are available for only a subset (stratified random sample) of the ARIC cohort, therefore these analyses will be evaluated utilizing the SUDAAN software package to adjust for the sampling strategy. Age, gender, field center and case status (CHD and stroke) will be included as covariates.
3. In a race-specific manner, to evaluate the ability of PSEL and PSGL-1 gene variation to independently predict incident CHD and ischemic stroke.
   Analyses for CHD will be carried out taking into account age, gender, field center, BMI, HDL and total cholesterol, smoking, diabetes and hypertension status.
   Analyses for stroke will be carried out taking into account age, gender, field center, smoking, diabetes and hypertension status.

Please let us know whether the above proposal can serve as an extension of MS# 1002, or if a new manuscript proposal is warranted.

Thank you for your time and consideration.

Best regards,

Kelly Volcik

[1]Volcik KA, Ballantyne CM, Coresh J, Folsom AR, Wu KK, Boerwinkle E. P-selectin Thr715Pro polymorphism predicts P-selectin levels but not risk of incident coronary heart disease or ischemic stroke in a cohort of 14595 participants: the Atherosclerosis Risk in Communities Study. Atherosclerosis 2005 *In Press*.

ARIC Manuscript Proposal # 1002

PC Reviewed: 04/08/04	**Status: A**	**Priority: 2**
SC Reviewed: 04/13/04	**Status: A**	**Priority: 2**

1.a. Full Title: The P-Selectin Thr715Pro Polymorphism and Risk of Stroke and Coronary Heart Disease

b. Abbreviated Title (Length 26 characters): PSEL T715P, Stroke and CHD

2. Writing Group (list individual with lead responsibility first):

Lead: Eric Boerwinkle

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Writing group members: Kelly Volcik, Christie Ballantyne, Josef Coresh, Aaron Folsom, Kenneth Wu

3. Timeline: Genotyping of the P-selectin (PSEL) Thr715Pro polymorphism is complete for the stroke and CHD case-cohort samples. Analyses will be completed subsequently.

4. Rationale:

The pathogenesis of atherosclerosis is known to contain an important inflammatory component, involving the adhesion of circulating leukocytes, particularly monocytes, to the vascular endothelium at sites of injury (Ross 1999). The recruitment and binding of circulating leukocytes to the vascular endothelium is mediated through a diverse family of adhesion molecules, including the selectins, which mediate the initial rolling of leukocytes along the endothelium (Albelda et al. 1994). Adhesion of leukocytes to the endothelium and subsequent transendothelial migration is an important step in the initiation and aggravation of atherosclerotic lesions (Price and Loscalzo 1999). P-selectin, a member of the selectin family of adhesion receptors, is expressed on platelets and activated endothelium where it mediates both the binding of activated platelets to monocytes and of monocytes to injured endothelium (Johnson et al. 1997).

Evidence of a key role for P-selectin in the development of atherosclerotic lesions is provided by multiple knockout mouse models showing reduced fatty streaks and attenuated lesion formation resulting from disruption of the P-selectin gene (Molenaar et al. 2003, Manka et al. 2001, Collins et al. 2000, Dong et al. 1999). Studies have shown p-selectin expression to be positively correlated with the extent of atherosclerotic lesion development as well as with arterial wall thickness and arterial stiffness (Koyama et al. 2003, Molenaar et al. 2003). Furthermore, increased levels of P-selectin have been observed in various cardiovascular disorders, including hypercholesterolemia, myocardial infarction and unstable angina, as well as being associated with progressive vascular damage in persons with essential and malignant hypertension (Davi et al. 1998, Xu et al. 1998, Ikeda et al. 1995, Verhaar et al. 1998).

The P-selectin Pro715 allele was previously reported to be potentially protective for myocardial infarction (Herrmann et al. 1998). Another analysis found a strong association between P-selectin gene polymorphisms (including Thr715Pro) and serum P-selectin levels, but was unable to replicate the previously reported association between myocardial infarction and the Thr715Pro polymorphism (Barbaux et al. 2001). Zee et al. recently reported that another P-selectin polymorphism, Val640Leu, is an independent predictor of incident stroke (2004). To our knowledge, there are no published reports of the association between the P-selectin Thr715Pro polymorphism and stroke. Therefore, due to the evident role of P-selectin in the progression of atherosclerosis and vascular damage, and due to the conflicting reports regarding the role of the Thr715Pro polymorphism in heart disease, we propose to determine the association of the P-selectin Thr715Pro polymorphism with stroke and coronary heart disease.

References:

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Barbaux SC, Blankenberg S, Rupprecht HJ, et al. 2001. Association Between P-Selectin Gene Polymorphisms and Soluble P-Selectin Levels and Their Relation to Coronary Artery Disease. Arterioscler Thromb Vasc Biol 21:1668-1673.

Collins RG, Velji R, Guevara NV, et al. 2000. P-Selectin or Intercellular Adhesion Molecule (ICAM-1) Deficiency Substantially Protects Against Atherosclerosis in Apolipoprotein E-Deficient Mice. J Exp Med 191:189-194.

Davi G, Romano M, Mezzetti A, et al. 1998. Increased Levels of Soluble P-Selectin in Hypercholesterolemic Patients. Circulation 97:953-957.

Dong ZM, Brown AA, Wagner DD. 2000. Prominent Role of P-Selectin in the Development of Advanced Atherosclerosis in ApoE-Deficient Mice. Circulation 101:2290-2295.

Herrmann SM, Ricard S, Nicaud V, et al. 1998. The P-Selectin Gene is Highly Polymorphic: Reduced Frequency of the Pro715 Allele Carriers in Patients with Myocardial Infarction. Hum Mol Genet 7(8):1277-1284.

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Manka D, Collins RG, Ley K, et al. 2001. Absence of P-Selectin, but not Intercellular Adhesion Molecule-1, Attenuates Neointimal Growth After Arterial Injury in Apolipoprotein E-Deficient Mice. Circulation 103:1000-1005.

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Verhaar MC, Beutler JJ, Gaillard CA, et al. 1998. Progressive Vascular Damage in Hypertension is Associated with Increased Levels of Circulating P-Selectin. J Hypert 16:45-50.

Xu D, Zhao S, Peng W. 1998. Elevated Plasma Levels of Soluble P-Selectin in Patients with Acute Myocardial Infarction and Unstable Angina: An Inverse Link to Lipoprotein(a). Int J Cardiol 64:253-258.

Zee RYL, Cook NR, Cheng S, et al. 2004. Polymorphism in the P-Selectin and Interleukin-4 Genes as Determinants of Stroke: A Population-Based, Prospective Genetic Analysis. Hum Mol Genet 13(4):389-396.

5. **Main Hypothesis/Study Questions**: PSEL T715P polymorphism predicts stroke case and/or CHD status after controlling for traditional risk factors

6. **Data (variables, time window, source, inclusions/exclusions)**: ARIC's stroke and CHD case-cohort groups will be utilized for the analyses. The dependent variable is stroke or CHD case status. Independent variables include, but are not limited to, PSEL T715P genotype status and traditional risk factors such as age, gender, race, smoking status, plasma lipid levels, body mass index, hypertension status, 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
(This file ICTDER02 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 ICTDER02 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)? The most closely related manuscript proposals are #871 and #677, both of which Eric Boerwinkle is a part of the writing group.

11. 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.

__X__ Yes _____ No