ARIC Manuscript Proposal # 1249

PC Reviewed: _5_/_8_/07	**Status: _A___**	**Priority: _2__**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Prospective Study of Low Protein C levels with Risk of Ischemic Stroke in the ARIC cohort

b. Abbreviated Title (Length 26 characters): Protein C levels & Ischemic Stroke

2. Writing Group:

Writing group members: Sarah Solarz, Tetsuya Ohira, Mary Cushman, Aaron Folsom

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

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3. Timeline: Masters project/paper by July 2007

4. Rationale: Several markers of hemostatic function and inflammation have been associated with an increased risk of coronary heart disease and ischemic stroke. Plasma protein C, measured by ARIC, is a zymogen, and when activated by thrombomodulin-bound thrombin, APC (activated protein C) joins with protein S to degrade factors Va

and VIIIa, thus it is a physiological anticoagulant. Protein C deficiency is an established risk factor for venous thromboembolism and excessive clotting.

An ARIC paper by Conlan et al. (1993) showed that protein C had a normal distribution with a mean value of 3.17 micrograms/ml. Additionally, women had higher values than men, whites were higher than blacks, values were higher in postmenopausal women and further increased with hormonal supplements. Protein C levels were also positively correlated with body mass index, LDL-C, HDL-C, and triglycerides, while being negatively associated with cigarette smoking.

Deficiencies of protein C are classified into two types. Type I deficiency is defined by the reduction of the functional activity and plasma antigen levels of the anticoagulant. Type II deficiency is defined by reduced activity but normal antigen levels. The prevalence of protein C deficiency in the general population has been estimated to be between 0.2% and 0.4% (Tait et al. and Miletich et al.).

Numerous clinical studies have demonstrated genetically determined protein C deficiency increases risk of ischemic. For example, Strater et al. found that protein C deficiency was an important risk factor for ischemic stroke in children with underlying cardiac disorders. Sakata et al. evaluated families with protein C deficiency and showed that when stroke occurred, it was at a younger age. These findings were duplicated in a 2000 study by Sakata et al. There are no high quality studies of protein C deficiency in relation to stroke in adults.

The role of inflammation in the pathogenesis of ischemic stroke is well known, but its association with the clinical picture is unclear (Akyol et al). It remains uncertain whether protein C deficiency is a risk factor of ischemic stroke. Griffin et al. summarized clinical observations, animal model experimentation, and in vitro studies that advance knowledge of the protein C system, including activated protein C (APC), in the setting of ischemic stroke. They found that low levels of plasma APC and a poor response to APC in clotting assays may be markers or risk factors for ischemic stroke. This may indicate that there is compelling evidence that ischemic stroke is an attractive target for therapy with APC.

An ARIC paper by Folsom et al. (1999) showed that protein C was weakly inversely but not statistically significantly associated with incident ischemic stroke, but only in a multivariable model. With further follow-up for strokes in ARIC, we want to study more specifically the association of low protein C with ischemic stroke and subtypes.

References:

Conlan, MG et al. Correlation of plasma protein C levels with cardiovascular risk factors in middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. *Thromb Haemost* 1993;70(5):762-767.

Tait RC, et al. Prevalence of protein C deficiency in the healthy population. *Thromb Haemost.* 1995;73:87-93.

Miletich J et al. Absence of thrombosis in subjects with heterozygous protein C deficiency. *N Engl J Med.* 1987;317:991-996.

Strater R et al. Genetic risk factors of thrombophilia in ischaemic childhood stroke of cardiac origin. A prospective ESPED survey. *Eur J Pediatr* 1999;158 Suppl3:S122-5.

Sakata T et al. Analysis of 45 episodes of arterial occlusive disease in Japanese patients with congenital protein C deficiency. *Thromb Res* 1999;94(2):69-78.

Sakata T et al. Studies on congenital protein C deficiency in Japanese: prevalence, genetic analysis, and relevance to the onset of arterial occlusive disease. *Semin Thromb Hemost* 2000;26(1):11-6.

Akyol, Ali et al. The relationship between protein C, protein S and cytokines in acute ischemic stroke. *Neuroimmunomodulation.* 2006;13;187-193.

Griffin JH et al. Activated protein C and ischemic stroke. *Crit Care Med.* 2004 May;32(5 Suppl):S247-53.

Folsom, AR et al. Prospective study of markers of hemostatic function with risk of ischemic stroke. *Circulation* 1999;100:736-742.

5. Main Hypothesis/Study Questions: Low levels of protein C are associated with increased incidence of ischemic stroke in ARIC.

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).

Dependent variable: Ischemic stroke incidence. This will be defined as a hospitalization due to definite or probable ischemic stroke between baseline and 2003. In addition, we will examine subtypes: lacunar, non-lacunar, and cardioembolic, as defined in a previous ARIC paper by Ohira et al. (Risk factors for ischemic stroke subtypes the atherosclerosis risk in communities study. *Stroke* 2006;37:2493-2498.)

Independent variable: Levels of Protein C. Levels of Protein C, in μg/mL, will be placed into percentiles (quintiles) for analysis.

Follow-up: Follow-up times will be time to event for those with incident ischemic stroke, time from enrollment to December 31, 2003 for those without an ischemic stroke event, and time censored for those who died during the course of the study or who were lost to follow-up.

Covariates: Adjustment and consideration for baseline levels of the main stroke risk factors: age, race, sex, smoking, hypertension (SBP and BP meds), diabetes, education, fibrinogen, von Willebrand factor, BMI, ECG LVH, previous CHD, LDL-C and HDL-C.

Analysis: Main analysis will use proportional hazard regression models to compute hazard ratios and corresponding 95% confidence intervals with Protein C levels as the predictor of incident ischemic stroke. Protein C levels will be placed into percentiles, and person-years will be calculated for the analysis. Adjustment for sex, age, race, and other covariates will be made, as well as a multivariate adjustment model. Spline techniques will be used to look at the shape of the relation and dose-response effects.

Exclusion: Previous stroke at baseline, transient ischemic attack (TIA) at baseline, unknown baseline stroke, missing data on protein C levels, and use of warfarin at baseline.

Limitation: The low measurement reliability of protein C. Reliability coefficient for protein C in ARIC has been reported as r = 0.56 (Folsom, AR et al. Protein C, Antithrombin, and Venous Thromboembolism Incidence: A Prospective Population-Based Study. *Arterioscler. Thromb. Vasc. Biol.* 2002;22;1018-1022). Some attempt will be made to estimate the degree of regression dilution bias. There may also be some misclassification due to unreliability of stroke subtype.

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? ____ Yes ___x_ 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"?
____ 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)?

Folsom, AR et al. Prospective study of markers of hemostatic function with risk of ischemic stroke. *Circulation* 1999;100:736-742.

11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? ____ Yes __x__ No

11.b. If yes, is the proposal

___ A. primarily the result of an ancillary study (list number* _____)

___ B. primiarly 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.