ARIC Manuscript Proposal #2174

1.a. Full Title: Is sickle cell trait a risk factor for stroke and cerebral small vessel disease?

   b. Abbreviated Title (Length 26 characters): **sickle cell trait and stroke**

2.   Writing Group:
     Writing group members: Melissa Caughey, Kari North, Bruce Wasserman, Nigel Key, Laura Loehr, Vimal Derebail, Diane Catellier, Rebecca Gottesman, Abhijit Kshirsagar, Gerardo Heiss

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

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3.   Timeline: Manuscripts to be completed for doctoral dissertation requirement. Projected timeline is one year following genotyping of sickle cell trait polymorphism (rs334) and completion of visit 5 MRI readings.

4.   Rationale:

The prevalence of stroke in African Americans 18 years or older is nearly twice that of non-Hispanic whites (4.0% vs. 2.3%)[1]. While stroke incidence has been decreasing since the 1990s for whites, this trend has not been observed for blacks[1]. Stroke incidence is not only higher for African Americans, it occurs at a younger age, resulting in substantial morbidity and indirect costs through lost earnings[2]. Sickle cell trait (SCT), the heterozygous form of sickle cell anemia, may confer additional stroke risk to African Americans. Sickle cell anemia is an inherited hemoglobinopathy, characterized by misshapen red blood cells, acute pain, and vaso-occlusive crisis. Sickle cell anemia is a well-established risk factor for stroke, conveying a relative risk of 2.7 for African Americans aged 35-64, compared to those with normal hemoglobin[3]. On the other hand, sickle cell trait, which has a prevalence of 8% in African Americans[4], is generally considered a benign carrier state. However, numerous case reports have described stroke in carriers of SCT with no precipitating risk factors for cerebrovascular disease[5-15]. While SCT is a debatable risk factor for stroke and cerebrovascular disease[16, 17], to date, no prospective epidemiological studies have investigated this association.

SCT may plausibly contribute to cerebral small vessel disease and stroke through the complementary actions of hypercoagulability and hypoperfusion. A 2-fold higher prevalence of pulmonary embolism and venous thrombosis has been observed in carriers of SCT[18, 19], suggesting elevated coagulation activation. Indeed, laboratory assays of SCT carriers have shown increased markers of coagulation (prothrombin fragment 1+2, thrombin-antithrombin complex, and d-dimer)[20]. Under conditions of exertion, dehydration, and high altitude, erythrocytes of SCT carriers sickle and polymerize[21-23]. The sickling deformation is known to expose phosphatidylserine on the cell membrane surface, which facilitates enzymatic complexes and coagulation[24]. Subclinical brain infarctions (SBI) of the small penetrating cerebral arteries, related to hyalinosis, microatheroma and thrombosis[25, 26], are likewise associated with markers of coagulation[27]. Though asymptomatic, SBI are predictive of stroke[28, 29] and cognitive decline[30, 31]. A higher prevalence of SBI has been noted in patients with sickle cell anemia, compared to African Americans with normal hemoglobin[32]; however, SBI has not been investigated in individuals with SCT.

Abnormal blood rheology of SCT carriers results in hypoperfusion of the skeletal muscle tissue[33], and likely the cerebral tissue. Laboratory assays of SCT carriers indicate increased blood viscosity[33], abnormal erythrocyte adhesion to the vascular endothelium[34], and decreased red cell rigidity[33], all of which contribute to microvascular occlusion and are amplified in the setting of hypoxia and exertion[33, 35]. In SCT carriers, muscle biopsies show decreased capillary density[36]; the result of endothelial apoptosis due to capillary obstruction by microthrombi[36, 37]. Similarly, cerebral white matter lesions (WML) are characterized by diffuse areas of hypoperfusion and chronic ischemia[38], with markedly decreased capillary density[39]. Though asymptomatic, WML are predictive of stroke[29] and cognitive decline[30, 31], and have been detected by MRI screenings of healthy pediatric SCT carrriers[40].

We propose to examine the association of SCT with the prevalence, incidence, and progression of stroke/TIA, and small vessel disease (SBI and WML) in African American participants of the ARIC cohort. A subset of the ARIC cohort was prospectively imaged by MRI at 3 visits: 1993-1995, 2004-2006, and 2011-2013. Imaging was performed on 926 African Americans ≥ 55 years at the first exam, with 858 expected in the final exam. Stroke and TIA have been identified in the ARIC study by annual, standardized questionnaires with a diagnostic algorithm[41], and hospital surveillance[42]. SBI and WML have been characterized from MRI scans by standardized definitions. SCT trait genotyping has been funded by ancillary study 2010.16, and is ongoing at the central research laboratory. Genotyping is expected to be completed by Fall 2013, and we have been given express permission by the ancillary study to use this variable.

5.   Main Hypothesis/Study Questions:

Is sickle cell trait associated with stroke and TIA?
We hypothesize that African American participants with SCT will have a higher prevalence of stroke /TIA at the baseline visit, and a higher incidence of stroke and TIA over 25 years of follow up.

Is sickle cell trait associated with white matter lesions (leukoaraiosis)?
We hypothesize that African American participants with SCT will have a greater severity of WML at the baseline MRI (1993-1995), and a greater increase in the volume of WML between MRI 2 (2004-2006) and MRI 3 (2011-2013).

Is sickle cell trait associated with subclinical brain infarctions (lacunes)?
We hypothesize that African American participants with SCT will have a higher prevalence of SBI at the baseline MRI, and longitudinally over the course of all 3 MRI visits.

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).

Sickle cell trait will be defined by heterozygosity for hemoglobin S (single nucleotide polymorphism rs334). Our study will be restricted to African-American participants with either heterozygous SCT or normal hemoglobin; thus, participants with known or detected homozygosity for hemoglobin S, hemoglobin C, or concomitant presence of hemoglobin S and C will be excluded. Hemoglobin S is being genotyped in ancillary study 2010.16. Hemoglobin C (rs33930165) will be imputed.

To examine associations between SCT and the prevalence and incidence of stroke/TIA, we will include all African Americans in the ARIC cohort with blood sample available for genotyping. For the purposes of this analysis, stroke will include thrombotic brain infarction, cardioembolic stroke, subarachnoid hemorrhage, and intracerebral

hemorrhage. Stroke symptoms resolving in less than 24 hours will be considered TIAs. Prevalence differences for stroke/TIA associated with SCT will be assessed at the baseline visit. Hazard ratios for incident stroke/TIA will be analyzed by Cox regression. Associations between sickle cell trait and stroke or TIA will be adjusted to control for the ARIC field center, and the traditional risk factors for stroke (age, gender, blood pressure, smoking, diabetes, hypercholesterolemia, and atrial fibrillation)[1], using covariates from the baseline visit.

Associations between SCT and cerebral small vessel disease (white matter lesions and subclinical brain infarctions) will be determined in a subset of African Americans participating in brain MRI. Due to technological advances, WML were measured by a grading system for MRI 1 (1993-1995), and volumetrically for MRI 2 (2004-2006) and MRI 3 (2011-2013). Differences in mean WML grade associated with SCT will be determined from MRI 1, using ANCOVA. The longitudinal increase in WML volumes between MRI 2 and MRI 3 associated with SCT will be assessed by ANCOVA. The analysis of white matter lesions will account for age, gender, blood pressure, smoking[31, 38]

Baseline prevalence ratios for subclinical brain infarctions associated with SCT will be analyzed by Poisson regression at MRI 1. Longitudinal prevalence ratios will be analyzed with Poisson regression and generalized estimator equations, and will include all 3 MRI exams. SBI will be classified as either small (3-7mm) or large (8-20mm), for separate analyses. The analysis of subclinical brain infarctions will control for factors related to hyalinosis (age, gender, blood pressure, smoking, and diabetes)[43] for the small (3-7mm) infarcts, and will be adjusted for gender, blood pressure, smoking, and LDL cholesterol[43] for large (8-20mm) infarcts, as these are thought to arise from distinct etiologies.

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 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?
   __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

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)?

Ancillary study # 2010.16 Sickle cell trait: a risk factor for kidney disease?
We have contacted the lead authors (Abhijit Kshirsagar , Vimal Derebail, and Nigel Key) and are collaborating with them on this proposal.

Manuscript proposal #2150 Sickle cell trait and venous thromboembolism.
This is a validation study for research previously conducted by one of our collaborators (Nigel Key).

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*** 2010.16**)**
    ___ **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/

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.cscc.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.

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