1a. **Full Title**: Sickle Cell Trait (SCT) as a Contributing Factor to Increased Cardiovascular and Stroke Risk Disparity in African Americans

b. **Abbreviated Title (Length 26 characters)**: SCT effect on CVD and CVA risk

2. **Writing Group**:
   Writing group members: Hyacinth I. Hyacinth, MD, MPH; Myriam Fornage, PhD; Robert J. Adams, MD, MS; Leonard E. Egede, MD, MS; Daniel T. Lackland, DrPH; Mark S. Kindy, PhD; Eric Boerwinkle, PhD; Thomas H. Mosley, PhD; Austin Hughes, PhD; Gregory L. Burke, MD, MS

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

**First author**: Hyacinth I. Hyacinth, MD, MPH
   Address: Medical University of South Carolina, Stroke Center, 19 Hagood Avenue, Suite 501, Charleston, SC 29425
   Phone: 843-876-5931      Fax: 843-792-2484
   E-mail: hyacinth@musc.edu

**ARIC author** to be contacted if there are questions about the manuscript and the first author does not respond or cannot be located (this must be an ARIC investigator).

Name: Thomas H. Mosley, PhD
   University of Mississippi Medical Center
   Jackson, MS
   Phone: (601) 984-2763      Fax: (601) 815-3422
   E-mail: tmosley@umc.edu

3. **Timeline**:
   August: Apply for and possibly obtain approval from ARIC and MESA to use data set for meta-analysis
September/October: 1. Complete and submit application to include Jackson Heart Study data in meta-analysis

October/November: Carryout preliminary analysis on ARIC and MESA data individually and then in a meta-analysis while awaiting approval from Jackson Heart Study. Also writing group meet to discuss preliminary results.

November/December: Independent analysis of Jackson heart study data and later incorporation into meta-analysis.

January, 2014: Writing group meets to discuss results and direction of manuscript and potential journals to submit to

January/February: Draft manuscript prepared for circulation among authors for comments and critical reviews

March/April: submission of draft manuscript to journal for review

This timeline is by no means definitive and could be longer or shorter depending on the how fast certain components of this writing project moves.

4. Rationale:
Sickle cell disease results from a mutation in the beta-globin gene of the hemoglobin molecule, resulting in the formation of hemoglobin S (HbS) which polymerizes in conditions of dehydration and/or acidosis to formation of tactoids [1]. These deform the cells giving them a characteristic sickle shape. The heterozygous form of sickle cell disease referred to as sickle cell trait (SCT) or HbAS has one normal β-globin gene and one sickle Hb gene. The homozygous or HbSS genotype is associated with a global activation and increased plasma levels of prothrombotic mediators/factors [2] and with a 300 fold increase in stroke risk among children with SCD compared with non-SCD [3].

We reason that SCT (A/T mutation or single nucleotide polymorphism [SNP]), although expected to be clinically silent, might be associated with increased risk for stroke and cardiovascular diseases among African Americans. This is because, despite having a “subclinical” course, the presence of SCT has been associated with a 30 fold increase in the risk for sudden death among military recruits [4], a 2 fold increased risk for venous thrombembolism (VTE) and approximately a 4 fold increased risk for pulmonary embolism (PE) [5] compared to healthy age race and sex matched controls. Among African Americans, the population attributable risk for VTE due to HbAS is about 6 – 9% [5].

Further evidence shows that baseline D-dimer, thrombin-antithrombin (TAT) complex, prothrombin fragment 1.2 (F1.2) and c-reactive protein (CRP) levels in healthy ambulatory SCT subject at steady state was significantly higher compared with age, race and sex matched HbAA subjects [6,7]. High levels of these procoagulating factors have been associated with increased stroke risk and larger infarct volume in the general population [8]. An elevated CRP level is usually indicative of ongoing or acute inflammatory response. Thrombosis and inflammation are two pathological processes
well documented to be associated with the pathobiology of stroke development in sickle and non-sickle cell patients. Epidemiological studies have concluded that the rate of stroke in African Americans (AAs) is higher than in White patients and the reasons for this remain unclear [9]. While there are some differences in classical risk factors for stroke in terms of the prevalence between these two groups, it is not clear if these differences explain the marked difference in stroke incidence. One possible contribution to increased risk in AAs could be an unrecognized contribution to risk by the presence of SCT or A/T SNP.

5. Main Hypothesis/Study Questions:
Taken together, we believe that with ongoing background inflammation and increased predisposition to thrombophilia, individuals with SCT will have a higher stroke incidence and/or prevalence adjusted for age and sex. We hypothesized that stroke and cardiovascular disease prevalence will be higher among patients with SCT adjusted for age and sex. And we expect a clustering of the this SNP among African Americans with stroke if our hypothesis is correct.

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).

To test our hypothesis, we are proposing a meta-analysis of all the African American individuals in the MESA-SHARE (GWAS), ARIC and Jackson Heart Study (who have consented to genetic studies) for the frequency of the A/T mutation which is the rs7112844 SNP in the HBB gene, and to investigate the association between this SNP and incident stroke and/or cardiovascular event. The combination of these cohorts in a meta-analysis is expected to provide an anticipated sample size of around 10,000 individuals with phenotype and SNP data. Thus we are requesting data from the SNP direct genotyping analysis or imputation. Also we will request corresponding phenotype data (incident stroke or cardiovascular event) for the same individual.

**Phenotype:** Incident ischemic stroke/hemorrhagic stroke and cardiovascular events. Prevalent strokes and cardiovascular events at baseline will be excluded.

**Genotypes:** Directly genotyped or imputed data will be used. For imputed data, a quality metrics of imputation is also requested (e.g., R^2) so that only adequately imputed genotypes will be used (R^2>0.3).

- Analyses will be carried out separately in each study (ARIC, MESA, and JHS) and meta-analyzed using an inverse-variance weighted method.

In each study run GWAS, an additive genetic model will be used. A Cox model will be used to evaluate the association between SNP and time to first incident ischemic stroke adjusting for covariates (see below).

- Participants are right censored at death or at the time of their last follow-up examination or health status update when they were known to be stroke-free.

- Persons are also right-censored when they have an alternative type of stroke (hemorrhagic or unknown).
Covariate adjustment:
Age, Gender, study site, and principal components of ancestry (the first 4 are generally sufficient but we will evaluate up to 10).

Meta-analysis: We will use a fixed effect inverse variance weighted meta-analysis, using METAL. If evidence of heterogeneity is found, we will use a random effects model.

Based on information gathered so far, there are about 13 SNPs at different location on the HBB locus. Since there is evidence of epistatitical interaction between SNPs on the same locus, we will use a log-linear model/method [10] to test for the effects on stroke and CHD of two-way and higher interactions among SNPs in the HBB gene.

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.csc.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)?
GWAS of Stroke conducted by Dr. Fornage and Mosley, who are co-authors on this manuscript.

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

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

X B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)*_2009.12; 2007.07;

2013.03__________ __________ __________)

*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.