1.a. Full Title: Association of stroke high risk SNPs with cardiovascular risk factors.

b. Abbreviated Title (Length 26 characters): Stroke SNPs and risk factors

2. Writing Group:
   Writing group members: F Suri, T Mosley, M Fornage, W Tang, A Folsom
   I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. _____ [please confirm with your initials electronically or in writing]

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   Address:

   Phone:             Fax:             E-mail:

3. Timeline:
   Three months

4. Rationale:
   A recent CHARGE meta-analysis identified an association of two unsuspected SNPs on chromosome 12p13 with total, ischemic and atherothrombotic stroke. These SNPs are located in region of the NINJ2 gene and may have a role in transcription of this protein. NINJ2 is a cell surface adhesion molecule that has been noted to have some role in peripheral nerve injury and hence belongs to the ‘nerve injury induced protein’ family.\(^1\) Although it is also noted to be highly expressed in hematopoietic and lymphatic tissues, no clinical association was known to exist.
It is possible that these SNPs are mainly a predictor for the development of an already known cardiovascular risk factor and do not have an association with stroke independent of that risk factor. It is also possible that these SNPs are independently associated with atherosclerosis (as measured by carotid intima-media thickness) or retinal vascular disease and this may explain their association with stroke. It is therefore imperative to determine if there is any association between these SNPs and any known cardiovascular risk factors or surrogate markers of cardiovascular disease. Cardiovascular risk factors that can potentially be considered to have a genetic component include hypertension, hyperlipidemia, diabetes and left ventricular hypertrophy (as marker of long-standing hypertension). Surrogate markers of cardiovascular disease studied in ARIC study include retinal microvascular disease and carotid intima media thickness.

In addition to these two SNPs, 9 other SNPs were identified in CHARGE meta-analysis to have high association with stroke (listed below). The r^2 for rs11833579 and rs12425791 ranges from 0.561 to 0.751 in different populations.

<table>
<thead>
<tr>
<th>SNP</th>
<th>chromosome</th>
<th>coordinate</th>
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<tr>
<td>rs17429019</td>
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<td>190727839</td>
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<td>rs11615969</td>
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<td>rs2318308</td>
<td>14</td>
<td>65460852</td>
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</table>

We propose to study the association of these 11 SNPs with known cardiovascular risk factors and CVD surrogate markers. Gene-environmental interaction has always been a theory for the development of these risk factors and is noted in at least one recent study. We also propose to study the interaction of these SNPs with known environmental cardiovascular risk factors for the development of diabetes, hypertension, hyperlipidemia, left ventricular hypertrophy, carotid intima media thickness and retinal microvascular disease.

5. Main Hypothesis/Study Questions:
Eleven SNPs identified in CHARGE meta-analysis to be highly associated with stroke are also independently associated with known cardiovascular risk factors and surrogate markers.

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).

Study design: Cross-sectional.
Inclusion criteria:
1. All ARIC participants with Affymetrix GeneChip® SNP Array 6.0 genotyping
2. Cardiovascular risk factors (listed below) available at baseline
3. Retinal examination available (only for subset analysis of retinal vascular disease)
4. Carotid doppler ultrasound at visit 1 available (only for subset analysis of carotid intima media thickness)

**Outcome:**
Status of cardiovascular risk factors at baseline including
1. Hypertension (elevated systolic blood pressure or use of antihypertensive medication)
2. Systolic blood pressure
3. Diastolic blood pressure
4. Diabetes
5. Hyperlipidemia (elevated cholesterol or use of lipid lowering medication)
6. Total cholesterol, mg/dl
7. HDL-cholesterol, mg/dl
8. LDL-cholesterol, mg/dl
9. ECG, left ventricular hypertrophy

Retinal Vascular disease: Evidence of “any retinopathy” defined as blot hemorrhages, flame shaped hemorrhages, microaneurysms, soft exudates (cotton-wool spots), hard exudates, macular edema, intraretinal microvascular abnormalities, venous beading, new vessels at the disc or elsewhere, vitreous hemorrhage, disc swelling, and laser photocoagulation scars (as defined in Wong et al 2001).

Carotid Intima Media Thickness (Visit 1): Mean of combined thickness of the intimal and medial layers of the far walls of three different segments (common carotid artery, carotid bifurcation, and internal carotid artery) from both sides will be used as continuous variable.

**Independent Variables of Interest**
Eleven SNPs identified to have high association with stroke in CHARGE meta-analysis

**Other Variables**

<table>
<thead>
<tr>
<th>Variable</th>
<th>ARIC variable</th>
<th>Visit</th>
<th>Usage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Age</td>
<td></td>
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<tr>
<td>2. Gender</td>
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</tr>
<tr>
<td>3. Race</td>
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<td>Categorical: White, African American</td>
</tr>
</tbody>
</table>

| 4. Body mass index | Continuous |
| 5. Smoking status  |            |
| 6. Alcohol consumption |        |

**Analysis**
1. Univariate analysis between minor allele for each SNP and cardiovascular risk factors will be performed
2. Regression (logistic or linear) analysis for association of each independent minor allele to cardiovascular risk factor adjusted for age, gender, race, body mass index, smoking status, alcohol consumption.
3. Regression (logistic or linear) analysis for association of each independent minor allele to cardiovascular risk factor adjusted for age, gender, race, body mass index, smoking status, alcohol consumption including interaction for body mass index, smoking status and alcohol consumption.
4. Separate logistic regression analysis for association of each allele to retinal vascular disease adjusted for cardiovascular disease risk factors
5. Separate linear regression analysis for association of each allele to carotid intima media thickness adjusted for cardiovascular disease risk factors
6. Analysis will be stratified by race

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  
    _x_ 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

   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

    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:

    ___ Yes

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)?
    1390  Fornage et. al., Genome-wide association study of incident stroke in the CHARGE consortium

11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data?
    ___ Yes

   b. If yes, is the proposal
    _x_  A. primarily the result of an ancillary study (list number
    _2006.03____)
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

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.

References