1a. Full Title: Association of ADORA2B SNPs with subclinical atherosclerosis and interaction with caffeine intake: Replication effort

b. Abbreviated Title: ADORA2B SNPs and subclinical atherosclerosis

2. Writing Group:
Kari North, University of North Carolina, ARIC investigator
Ani Manichaikul, University of Virginia, MESA investigator
Stephen S. Rich, University of Virginia, MESA investigator
Mike Nalls, National Institute on Aging, HANDLS investigator

Other investigators welcome

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal.

Corresponding/senior author (if different from first author correspondence will be sent to both the first author & the corresponding author):

3. Timeline (The following are estimates. Please see project phases and timeline below for more details):
   Individual cohort statistical analyses: September 30, 2013
   Consortium meta-analyses: October 15, 2013
   Manuscript preparation: October 31, 2013
   Manuscript submission: November 15, 2013

4. Rationale: Diabetes is associated with atherosclerosis. Previous studies have demonstrated that blocking adenosine receptors with the non-selective antagonist BW-1433 or by A₂B-R-selective antagonists such as 9-methyladenines and ATL-801 reduce insulin resistance in diabetic mice primarily by enhancing hepatic insulin action. In addition, there is an association between SNPs in the A₂B-R gene, Adora2b, and pro-inflammatory cytokines such as IL-6, that are influenced by diabetes status (4). Our recent investigations show that A₂B-R blockade with ATL-801 is associated with improved glucose disposal and reduced atherosclerosis in ApoE(−/−) mice fed a western diet.
We recently performed analyses in the Multi-Ethnic Study of Atherosclerosis (MESA) to association of SNPs in \textit{ADORA2B} with measures of subatherosclerosis (CAC and IMT). As caffeine is a known inhibitor of adenosine receptors, we further hypothesized the effects of \textit{ADORA2B} SNPs may be attenuated in individuals with greater consumption of caffeine. To examine this hypothesis, we performed analyses for SNPs statistically significantly associated with subclinical atherosclerosis to further investigate evidence of caffeine as a modifier of these SNPs’ effects on subclinical atherosclerosis.

In the current effort, we propose to replicate the association results seen in the discovery cohort, MESA. Results from participating replication cohorts will be incorporated in a manuscript together with findings from MESA.

5. Main Hypotheses/Study Questions:

Based on results from MESA, we hypothesize \textit{ADORA2B} SNPs are associated with measures of subclinical atherosclerosis (coronary artery calcium [CAC] presence/absence, common carotid IMT). The SNPs of interest from MESA are summarized in Table 1, below.

Table 1: SNP effects and corresponding traits of interest by race/ethnic group, based on analyses in MESA as the discovery cohort. All SNPs shown here surpassed the suggestive threshold of \( \hat{\beta} = 0.1/(\text{effective number of independent SNPs}) \) for the \textit{ADORA2B} gene region.

<table>
<thead>
<tr>
<th>SNP ID</th>
<th>Trait</th>
<th>Race/ethnic group</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs758857</td>
<td>Common carotid IMT</td>
<td>White</td>
</tr>
<tr>
<td>rs758858</td>
<td>Common carotid IMT</td>
<td>African American</td>
</tr>
<tr>
<td>rs2779205</td>
<td>Coronary artery calcium (presence/absence)</td>
<td>African American</td>
</tr>
</tbody>
</table>

In addition to SNP main effects, the biology of adenosine receptors leads us to hypothesize the SNPs with statistically significant main effects (shown in Table 1) are modified by caffeine consumption.

6. Design and Analysis:

7.a. Will the data be used for non-CVD analysis in this manuscript?

X Yes

___ 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?

X 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 current investigation is a replication effort for selected SNPs in the candidate gene ADORA2B. To our knowledge, there are no other ARIC proposals investigating the effects of ADORA2B SNPs on subclinical atherosclerosis.

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 (AS #2006.03 & 2007.02)
__ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* __________ __________)

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.