

**ARIC Manuscript Proposal # 1540**

**PC Reviewed:** 7/14/09  
**SC Reviewed:** \_\_\_\_\_

**Status:** A  
**Status:** \_\_\_\_\_

**Priority:** 2  
**Priority:** \_\_\_\_\_

**1.a. Full Title:**

**b. Abbreviated Title (Length 26 characters):** Association of fasting glucose  
GWAS candidate SNPs with CVD: The Atherosclerosis Risk in Communities Study

**2. Writing Group:**

Writing group members: Laura Rasmussen-Torvik, Aaron Folsom, James Pankow, Mandy Li, Linda Kao, Anna Kottgen, David Couper, Eric Boerwinkle, Suzette Bielinski for the ARIC Diabetes GWAS working group

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

*Eric Boerwinkle has not yet responded to our request to be a coauthor. All other coauthors have given their approval.*

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**3. Timeline:** SNP typing on all Caucasians (from GWAS studies) anticipated to be available by July 1. Analysis completed Sept 1. Draft to coauthors Oct 1. Draft to publications committee Nov 1.

#### 4. Rationale:

It is well-established that fasting glucose is associated with many cardiovascular disease phenotypes. Fasting glucose level has been shown to be prospectively associated with myocardial infarction and stroke (1-3). In cross sectional analyses, fasting glucose level has been shown to be associated with intima-media thickness (IMT) (4, 5).

Additionally, although few studies have examined the association of PAD and fasting glucose level per se, multiple studies have shown incident or prevalent diabetes to be associated with incident PAD (6-8). However, it remains unclear if there is actually a casual relationship between fasting glucose and atherosclerotic disease; it is possible that the associations seen with fasting glucose are due to derangements caused by the progression of diabetes (defined by fasting glucose levels) or due to confounding with one of the many CVD risk factors associated with fasting glucose level such as insulin resistance or hyperinsulinemia.

Several recent genome-wide association studies have identified SNPs associated with fasting glucose at genome-wide levels of significance ( $p < 5 \times 10^{-8}$ ) (9-11). SNPs in three genes (*MTNR1B*, *G6PC2*, *GCK*) have been shown to be reproducibly associated with fasting glucose at genome-wide levels of significance. SNPs in these three genes are also associated with fasting glucose (at genome-wide levels of significance) in the ARIC study (data in preparation, see manuscript proposal # 1468). Interestingly, SNPs in these three genes have not been found to be significantly associated with diabetes in early GWAS studies (12).

The recent discovery of these multiple genetic variants reproducibly associated with fasting glucose now provides the opportunity to examine the association between fasting glucose and CVD using mendelian randomization. The concept of mendelian randomization refers to the random allocation of alleles at the time of gamete formation. Therefore, a genetic variant of interest should not be associated with known and unknown confounding factors in association analyses (13). Given this, if a genetic variant is found to be reliably associated with a trait (here fasting glucose) the association of this trait with disease can be tested by examining the direct association of the genetic variant with disease. Using mendelian randomization we hope to examine the association of fasting glucose with atherosclerotic disorders in the absence of significant confounding by examining the association of SNPs in *MTNR1B*, *G6PC2*, and *GCK* with incident CVD, stroke, prevalent PAD, and IMT measurements.

The ARIC study is ideally suited for such an analysis because of the multiple measurements of atherosclerotic disease available in the dataset. There are also large numbers of genotyped individuals and CVD events (for incidence analyses) which is critical, given the relatively low percentage of fasting glucose variance accounted for by SNPs in *MTNR1B*, *G6PC2*, and *GCK*.

## 5. Main Hypothesis/Study Questions:

We hypothesize that fasting glucose level is causally related to CVD and atherosclerotic diseases and thus that we will see an association between fasting glucose risk score and several measurements of atherosclerotic disease in ARIC.

Question 1: Is a fasting glucose genetic risk score (made up of risk alleles from SNPs from *MTNR1B*, *G6PC2*, and *GCK*) significantly associated with incident CHD in the ARIC study?

Question 2: Is a fasting glucose genetic risk score (made up of risk alleles from SNPs from *MTNR1B*, *G6PC2*, and *GCK*) significantly associated with incident stroke in the ARIC study?

Question 3: Is a fasting glucose genetic risk score (made up of risk alleles from SNPs from *MTNR1B*, *G6PC2*, and *GCK*) significantly associated with prevalent (visit 1) PAD in the ARIC study?

Question 4: Is a fasting glucose genetic risk score (made up of risk alleles from SNPs from *MTNR1B*, *G6PC2*, and *GCK*) significantly associated with IMT measurements in ARIC study?

## 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).

### Question 1—

Study design-- Prospective study of CHD incidence

Inclusion-- Caucasian participants in ARIC with completed cleaned GWAS genotyping

Exclusions—Individuals with prevalent CVD at visit 1. Individuals with prevalent diabetes at visit 1 (the association between the fasting glucose risk score and fasting glucose has been shown to be strongest in those without prevalent diabetes)\*.

Exposure variable—A fasting glucose genetic risk score. We will use the SNP with the lowest p-value for each region identified by the MAGIC consortium's Meta-analysis of fasting glucose GWAS (in submission). The SNPs are as follows: rs1803096 for *MTNR1B*; rs560887 for *G6PC2*; rs4607517 for *GCK*. All these genotypes are available through the ARIC GWAS genotypes. For each individual the number of risk (glucose-increasing) alleles will be summed over all three SNPs to create the score. Values of the

score will range from 0-6. This score is highly significantly associated with visit 1 fasting glucose level ( $p = 9.24 \times 10^{-41}$ ).

Outcome variable -- Incident CHD (measured through 2005) [we will use variable in\_05s which included CHD death, MI, and silent MI measured with EKG]

Covariates—age, sex, field center

Analysis plan--Cox regression will be used to calculate hazard ratios of CHD by fasting glucose genetic risk score.

### **Question 2—**

Study design-- Prospective study of stroke incidence

Inclusion-- Caucasian participants in ARIC with completed cleaned GWAS genotyping

Exclusions—Individuals with prevalent CVD at visit 1. Individuals with prevalent diabetes at visit 1\*.

Exposure variable—A fasting glucose genetic risk score (see above)

Outcome variable -- Incident ischemic stroke (measured through 2005) [ we will use variable in05isc which includes definite and probable ischemic strokes]

Covariates—age, sex, field center

Analysis plan--Cox regression will be used to calculate hazard ratios of ischemic stroke by fasting glucose genetic risk score.

### **Question 3—**

Study design—Cross-sectional study of prevalent PAD

Inclusion--Caucasian participants in ARIC with completed cleaned GWAS genotyping

Exclusions—Individuals with prevalent CVD at visit 1. Individuals with prevalent diabetes at visit 1\*. Individuals with insufficient information to determine PAD diagnosis at visit 1.

Exposure variable— A fasting glucose genetic risk score (see above)

Outcome variable -- Prevalent PAD defined as ABI < .9 or self-report of intermittent claudication at visit 1.

Covariates—age, sex, field center

Analysis plan--Logistic regression will be used to calculate odds ratios of prevalent PAD by fasting glucose genetic risk score.

#### **Question 4—**

Study design—Cross-sectional study of IMT thickness

Inclusion--Caucasian participants in ARIC with completed cleaned GWAS genotyping

Exclusions—Individuals with prevalent CVD at visit 1. Individuals with prevalent diabetes at visit 1\*. Individuals without any carotid IMT measurements at visit 1 or individuals who had undergone an endarterectomy prior to visit 1.

Exposure variable— A fasting glucose risk score (see above)

Outcome variable -- all-site mean IMT measurement (from ultrasound) at visit 1

Covariates—age, sex, field center

Analysis plan--Linear regression will be used to estimate adjusted mean increase in IMT per 1 increase in fasting glucose genetic risk score.

Although the assumption is that confounding should not be of concern in these genetic analysis, common CVD risk factors will be investigated as confounders or possible effect modifiers. These covariates include total cholesterol, HDL, SBP, prevalent LVH, antihypertensive med use, ethanol intake, current cigarette use and cigarette years, and triglycerides (all measured at visit 1)).

If fasting glucose genetic risk score is associated univariately with any CVD outcome, an additional model will be run including visit 1 fasting glucose in the model to see to what account the actual measure of fasting glucose accounts for the fasting glucose genetic risk score / CVD association.

\* Primary analyses will exclude prevalent diabetics. However, we will perform secondary sensitivity analyses for all outcomes including diabetics in the sample and additionally perform analyses testing if diabetic status is an effect modifier of the SNP / outcome association.

**7.a. Will the data be used for non-CVD analysis in this manuscript?**     Yes  
 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 ICTDER03 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

**8.c. If yes, is the author aware that the participants with RES\_DNA = 'not for profit' restriction must be excluded if the data are used by a for profit group?**  
\_\_\_\_\_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)?**

**MS #1468** Genetic variants are associated cross-sectionally and longitudinally with multiple measures of fasting glucose: the ARIC Study". Dr. Rasmussen-Torvik is the lead author on both papers and there are many coauthors in common for the two proposals. This proposal included no cardiovascular endpoints.

**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\* \_\_\_\_\_)**  
\_\_\_\_\_ **B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)\* \_\_\_\_\_)**  
\_\_\_\_\_

**2006.03 Stampede and Geneva Genotyping in Caucasians**

\*ancillary studies are listed by number at <http://www.csc.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.**

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