**ARIC Manuscript Proposal # 1193**

**PC Reviewed: _10/_27_/06** **Status: __A___** **Priority: __2__**
**SC Reviewed: _10/_27_/06** **Status: ___A__** **Priority: __2__**

**1.a. Full Title**: Association of an Insulin-Induced Gene 2 (*INSIG2*) Polymorphism with Diabetes and Possible Effect Modification of Obesity

**b. Abbreviated Title (Length 26 characters)**: ***INSIG2*, Obesity, and Diabetes**

**2. Writing Group**:
Writing group members: Jan Bressler
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I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. __x___ **[please confirm with your initials electronically or in writing]** JB


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**3. Timeline**: Statistical analyses: October-January 2007
Manuscript preparation: January – March 2007
Manuscript revision: April 2007
Manuscript submission: May 2007

**4. Rationale**:

Lipid homeostasis in animals is regulated by the sterol-dependent cleavage of sterol regulatory element-binding protein 1 (SREBP1) and sterol regulatory element-binding protein 2 (SREBP2), membrane-bound transcription factors that control the expression of genes involved in the synthesis of cholesterol, fatty acids, triglycerides, and phospholipids in the liver and other organs. Proteolytic release of SREBPs from the cell membrane requires the presence of SREBP cleavage-activating protein (SCAP) that contains a sterol-sensing domain and forms a complex with the SREBPs. In cells lacking sterols, SCAP transports SREBPs from the endoplasmic reticulum (ER) to the Golgi complex where the SREBPS are activated through cleavage by site-1 protease and site-2 protease before subsequent entry into the nucleus [1-5]. The product of the insulin-induced gene 2 (*INSIG2*) is a 225 amino acid protein containing six membrane-spanning helices. If sterols are available, INSIG2 blocks lipid synthesis by preventing the activation of SREBPs by SCAP so that the SREBPs are retained in the ER and transcription of target genes declines[6].

INSIG2 also regulates the level of cholesterol contained in cell membranes by binding to the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA reductase) which catalyzes the rate-limiting step in cholesterol biosynthesis. Following interaction with INSIG2 and INSIG1 (insulin-induced gene 1), a protein highly expressed in liver with 59% amino acid identity to INSIG2, HMG CoA reductase is ubiquitinated and rapidly degraded in the proteasome[7-10]. In order to establish the role of insig-2 in lipogenesis *in vivo*, Takaishi et al. infected Zucker diabetic fatty rats with recombinant adenovirus containing the insig-2 cDNA and found that insig-2 overexpression resulted in decreased levels of triacylglycerols in the liver and plasma when compared to uninfected control diabetic rats [11].

Herbert et al. identified a genetic variant (rs7566605) 10 kb upstream of the *INSIG2* gene associated with obesity as assessed by a BMI>=30 kg/m2 in participants in the Framingham Heart Study[12]. This finding was subsequently replicated in four of five additional populations including individuals of Western European ancestry, African-Americans, and children. Since the CC genotype conferring susceptibility was found to be present in about 10% of the individuals studied, the authors speculated that although the rs7566605 single nucleotide polymorphism (SNP) has a moderate influence on the risk for obesity (pooled odds ratio (OR) of case-control studies = 1.22, 95% confidence interval (CI) = 1.05-1.42, P value = 0.0080) there could be a considerable impact on public health due the high frequency of the allele in the population. However, the absence of an association between the rs7566605 SNP and BMI levels when DNA samples from the Nurses Health Study cohort were genotyped suggests that the SNP may be associated with obesity in some but not all populations. The results of linkage analyses in both humans[13] and mice[14] also suggest that the *INSIG2/Insig2* region may harbor a

quantitative trait locus for obesity. We therefore propose to study the association of the *INSIG2* polymorphism with obesity in the biracial prospective ARIC study. The 7566605 SNP has recently been genotyped on the entire ARIC cohort.

Since obesity is a well-established risk factor for non-insulin dependent diabetes mellitus (NIDDM)[15, 16], the possibility that the risk for diabetes is influenced by an individual's *INSIG2* genotype and that disease susceptibility may be modified by obesity will also be addressed.

## References


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13. Deng, H. W. et al. A genomewide linkage scan for quantitative-trait loci for obesity phenotypes. Am J Hum Genet 70, 1138-51 (2002).
14. Cheverud, J. M. et al. Quantitative trait loci for obesity- and diabetes-related traits and their dietary responses to high-fat feeding in LGXSM recombinant inbred mouse strains. Diabetes 53, 3328-36 (2004).
15. Harris, T. et al. Body mass index and mortality among nonsmoking older persons. The Framingham Heart Study. Jama 259, 1520-4 (1988).
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**5. Main Hypothesis/Study Questions**:

1. To estimate the frequency distribution of *INSIG2* gene variation in a population-based sample of whites and African-Americans
2. To evaluate the independent effect of *INSIG2* gene variation on measures of body size including body mass index (BMI), weight, waist circumference, and waist-to-hip ratio in a race-specific manner. Age, gender, and field center will be included as covariates.
3. To evaluate the independent effect of *INSIG2* gene variation on prevalent diabetes case status in a race-specific manner. Age, gender, and field center will be included as covariates.
4. To evaluate whether obesity as assessed by various measures of body size including BMI, weight, waist circumference, and waist-to-hip ratio modulates the independent effect of *INSIG2* gene variation on diabetes susceptibility. These analyses will be carried out using age, gender, and field center as covariates.

**6. Data (variables, time window, source, inclusions/exclusions)**:

Caucasian and African-American participants will be evaluated separately for this analysis. The usual DNA restriction, ethnic group, and missing data exclusion criteria will be used. In analysis models, BMI will be used as both a categorical and a continuous variable. Division into categories of BMI will be carried out based on standard criteria where an individual with a BMI>=25 $kg/m^2$ is considered overweight, a BMI>=30 $kg/m^2$ is considered as a measure of obesity, while those individuals with a BMI>=40 $kg/m^2$ are considered morbidly obese. Waist-to-hip ratio will be analyzed separately for males and females after division into quartiles in controls by gender. Logistic regression will be used to predict prevalent diabetes case status.

**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 tocontact lead authors of these proposals for comments on the new proposal or collaboration)?**

#796 Resistin gene polymorphisms and association with insulin resistance and diabetes in the ARIC study (Lead author: Fred Brancati, U.T. Houston Health Science Center)

#1116 Association of Uncoupling Protein 2 with diabetes and possible effect modification of obesity (Lead author: Suzette J. Bielinski, University of Minnesota**)**

There are no other manuscript proposals in ARIC investigating polymorphisms in the *INSIG2* gene and their relationship to either obesity or diabetes.

**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* AS#1995.07)**
**___ 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/

**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.**