

ARIC Manuscript Proposal # 1193

PC Reviewed: 10/27/06

Status: A

Priority: 2

SC Reviewed: 10/27/06

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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
Linda Kao
Eric Boerwinkle

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

First author: Jan Bressler

Address: Human Genetics Center
UTHSCH School of Public Health
1200 Herman Pressler
Houston, TX 77030

Phone: 713-500-9919

Fax: 713-500-0900

E-mail: jan.bressler@uth.tmc.edu

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

Address: Human Genetics Center
UTSCH School of Public Health
1200 Herman Pressler
Houston, TX 77030

Phone: 713-500-9800

Fax: 713-500-0900

E-mail: Eric.Boerwinkle@uth.tmc.edu

- 3. Timeline:** Statistical analyses: October-January 2007
Manuscript preparation: January – March 2007
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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 nucleus¹⁻⁵. 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 declines⁶.

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 proteasome⁷⁻¹⁰. 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 rats¹¹.

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 Study¹². 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 humans¹³ and mice¹⁴ 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

1. Brown, A. J., Sun, L., Feramisco, J. D., Brown, M. S. & Goldstein, J. L. Cholesterol addition to ER membranes alters conformation of SCAP, the SREBP escort protein that regulates cholesterol metabolism. *Mol Cell* 10, 237-45 (2002).
2. Brown, M. S. & Goldstein, J. L. A proteolytic pathway that controls the cholesterol content of membranes, cells, and blood. *Proc Natl Acad Sci U S A* 96, 11041-8 (1999).
3. Edwards, P. A., Tabor, D., Kast, H. R. & Venkateswaran, A. Regulation of gene expression by SREBP and SCAP. *Biochim Biophys Acta* 1529, 103-13 (2000).
4. Goldstein, J. L., Rawson, R. B. & Brown, M. S. Mutant mammalian cells as tools to delineate the sterol regulatory element-binding protein pathway for feedback regulation of lipid synthesis. *Arch Biochem Biophys* 397, 139-48 (2002).
5. Horton, J. D., Goldstein, J. L. & Brown, M. S. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest* 109, 1125-31 (2002).
6. Yabe, D., Brown, M. S. & Goldstein, J. L. Insig-2, a second endoplasmic reticulum protein that binds SCAP and blocks export of sterol regulatory element-binding proteins. *Proc Natl Acad Sci U S A* 99, 12753-8 (2002).
7. Goldstein, J. L. & Brown, M. S. Regulation of the mevalonate pathway. *Nature* 343, 425-30 (1990).
8. Yang, T. et al. Crucial step in cholesterol homeostasis: sterols promote binding of SCAP to INSIG-1, a membrane protein that facilitates retention of SREBPs in ER. *Cell* 110, 489-500 (2002).
9. Ravid, T., Doolman, R., Avner, R., Harats, D. & Roitelman, J. The ubiquitin-proteasome pathway mediates the regulated degradation of mammalian 3-hydroxy-3-methylglutaryl-coenzyme A reductase. *J Biol Chem* 275, 35840-7 (2000).
10. Sever, N. et al. Insig-dependent ubiquitination and degradation of mammalian 3-hydroxy-3-methylglutaryl-CoA reductase stimulated by sterols and geranylgeraniol. *J Biol Chem* 278, 52479-90 (2003).
11. Takaishi, K., Duplomb, L., Wang, M. Y., Li, J. & Unger, R. H. Hepatic insig-1 or -2 overexpression reduces lipogenesis in obese Zucker diabetic fatty rats and in fasted/refed normal rats. *Proc Natl Acad Sci U S A* 101, 7106-11 (2004).
12. Herbert, A. et al. A common genetic variant is associated with adult and childhood obesity. *Science* 312, 279-83 (2006).
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).
16. Must, A. et al. The disease burden associated with overweight and obesity. *Jama* 282, 1523-9 (1999).

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/m² is considered overweight, a BMI ≥ 30 kg/m² is considered as a measure of obesity, while those individuals with a BMI ≥ 40 kg/m² 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? ☒ 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? ☒ 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? ☒ 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"? ☒ 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> ☒ 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)?

#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? ☒ Yes ☐ No

11.b. If yes, is the proposal

☒ **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.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.