**ARIC Manuscript Proposal # 1269r**

**PC Reviewed: _8_/21_/07** **Status: _A___** **Priority: __2_**
**SC Reviewed: _____** **Status: _____** **Priority: ____**

**1.a. Full Title**: *FTO*, Obesity, and Diabetes

**b. Abbreviated Title (Length 26 characters)**:

**2. Writing Group**:
Writing group members: Jan Bressler
Linda Kao
James Pankow
Eric Boerwinkle

Other investigators welcome

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
UTSCH
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: August – November 2007
Manuscript preparation: December 2007 – January 2008
Manuscript revision: February 2008
Manuscript submission: March 2008

**4. Rationale**:

Obesity is a major risk factor that confers increased susceptibility to type 2 diabetes mellitus, cardiovascular disease, hypertension, and stroke. Although the results of twin and adoption studies provide strong support for a genetic influence on body weight [1-3], mutations causing monogenic forms of obesity are rare in the population and genes reported to contribute to common obesity such as *GAD2*[4], and *INSIG2*[5] have not been consistently replicated. A widely used surrogate measure of obesity is body mass index (BMI) calculated as weight divided by height squared ($kg/m^2$) with individuals whose BMI>= 25 classified as overweight, and a BMI>=30 considered as an index of obesity.

Genetic variants in the fat mass and obesity associated (*FTO*) gene have recently been reported by two groups of investigators to be associated with BMI in children and adults[6], or in children and adults with severe obesity (BMI>=40)[7]. The function of the *FTO* gene is currently unknown. FTO was first described in the fused toes (*Ft*) mouse mutant generated by insertional mutagenesis in which a 1.6-Mb genomic region on chromosome 8 was deleted[8, 9]. Homozygosity for the *Ft* deficiency causes embryonic lethality and abnormal development including left/right asymmetry, while surviving heterozygotes show fused toes on the forelimbs and thymic hyperplasia due to defective apoptosis.

The deleted region encompasses the mouse orthologue of *FTO* (*Fto*) as well as five additional genes (*Fts*, *Ftm*, *Irx3*, *Irx5*, and *Irx6*) so that the *Ft* mouse cannot be considered as a suitable animal model for analysis of *Fto* function[10]. The *FTO* gene has been mapped to chromosome 16q12.2 and includes nine predicted exons (Genbank accession number NM 00108432).

Frayling et al.[6] identified the *FTO* rs9939609 single nucleotide polymorphism (SNP) as a result of a genome-wide association study carried out in the United Kingdom comparing 1,924 type 2 diabetes cases with 2,938 controls. The SNP was found to be strongly associated with diabetes both in the original set of cases and controls (OR=1.27, 95%CI =1.16 -1.37, $p=5 \times 10^{-8}$) and in a replication sample consisting of 3,757 type 2 diabetic individuals and 5,346 controls (OR=1.15, 95% CI=1.09-1.23, $p=9 \times 10^{-6}$). Adjustment for BMI in the replication sample abolished this association (OR=1.03, 95% CI=0.96-1.10, p=0.44), suggesting that the increased risk for diabetes was due to obesity. The association of the *FTO* SNP with BMI and the risk of being either overweight or obese under an additive model was then analyzed in an additional 38,759 white European participants in seven adult population-based studies and two childhood birth cohort studies. In a combined analysis, adults homozygous for the AA risk allele weighed about 3 kilograms more than low-risk TT allele carriers, and were significantly more likely to

be overweight (OR=1.38, 95% CI=1.26-1.52, p=4 x $10^{-11}$) or obese (OR=1.67, 95% CI=1.47-1.89, p=1 x $10^{-14}$).

An association between two other variants in the *FTO* gene (rs1421085 and rs17817449) and obesity was reported as an unexpected finding by Dina et al.[7] that was revealed during an effort to estimate the distribution of neutral SNPs in a case-control obesity sample of individuals from France and Canada. Both SNPs conferred a substantial risk of severe obesity (rs1421085, OR=1.56 95%CI 1.40-1.75, p=7.6 x $10^{-16}$, rs17817449, OR=1.56, 95%CI 1.40-1.75, p=1.44 x $10^{-15}$) that was replicated in a study of 537 Swiss adults and 541 anonymous donors.

A fourth *FTO* polymorphism (rs8050136) has also recently been shown to be associated with type 2 diabetes risk in another genome-wide association study[11] in which 1,161 Finnish type 2 diabetes cases and 1,174 Finnish normal glucose tolerant controls were genotyped.

We therefore propose to study the association of these four *FTO* polymorphisms with diabetes in the biracial prospective ARIC study. The rs9939609 SNP has recently been genotyped on the entire ARIC cohort, and genotyping of *FTO* SNPs rs1421085, rs17817449, and rs8050136 is currently in progress.

Since obesity is a well-established risk factor for type 2 diabetes [12, 13] the possibility that the risk for diabetes is influenced by an individual's *FTO* genotype and that disease susceptibility may be modified by obesity will also be addressed.

5. **Main Hypothesis/Study Questions**:

1. To estimate the frequency distribution of *FTO* gene variation in a population-based sample of whites and African-Americans.
2. To evaluate the independent effect of *FTO* gene variation on measures of body-size at visit 1 including body mass index (BMI), weight, height, 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 *FTO* gene variation on prevalent diabetes case status at visit 1 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, height, waist circumference, and waist-to-hip ratio modulates the independent effect of *FTO* gene variation on diabetes susceptibility. These analyses will be carried out using age, gender, and field center as covariates.

**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).**

Caucasian and African-American participants will be evaluated separately for analysis. The usual DNA restriction, ethnic group, and missing data exclusion criteria will be used.

The association of *FTO* genetic variation and diabetes or obesity will be analyzed individually for each of the four SNPs. The association between haplotypes within the *FTO* gene and diabetes or obesity will also be examined. For these analyses, haplotypes will be inferred and reconstructed using the PHASE software that was designed based on the statistical method developed by Stephens et al. for population-based samples[14]. A co-dominant model will be assumed.

In analysis models, measures of body size will be used as both categorical and continuous variables. Analysis of variance (ANOVA) will be performed to assess mean differences in measures of body size among individuals with different *FTO* genotypes. Multiple linear regression will be used to evaluate the association of the *FTO* SNPs with continuous measures of body size. 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 by gender. Prevalent diabetes case status will be defined using the derived variable DIABTS03 where diabetes is defined as a fasting glucose level that exceeds 126 mg/dl, a nonfasting glucose level>= 200 mg/dl, and/or a history of diabetes or treatment for diabetes. Multivariable logistic regression will be used to predict diabetes and obesity case status. The analysis of effect measure modification by obesity, BMI, or other measures of body size of any association between *FTO* genotypes or hapotypes and diabetes will be carried out by including interaction terms in the analysis models.

**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? ____ 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 ____ 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)?**

#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**)**

#1193 Association of an Insulin-Induced Gene 2 (INSIG2) polymorphism with diabetes and possible effect modification of obesity (Lead author: Jan Bressler, U.T. Houston Health Science Center)

There are no other manuscript proposals in ARIC investigating polymorphisms in the *FTO* 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.**

References


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