ARIC Manuscript Proposal # 1705

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1.a. Full Title: Sequence Variation in *FTO* and Cognitive Decline: The Atherosclerosis Risk in Communities Study

b. Abbreviated Title (Length 26 characters): *FTO* and Cognition

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3. Timeline: Statistical analyses: October 2010 – December 2010
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4. Rationale:

Obesity is a major risk factor that confers increased susceptibility to type 2 diabetes mellitus, cardiovascular disease, hypertension, and stroke. 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 $\geq$ 25 classified as overweight, and a BMI $\geq$ 30 considered as an index of obesity.

Genetic variants in the fat mass and obesity associated (*FTO*) gene have been reported to be associated with BMI[1-3] and diabetes[1, 4, 5] in children and adults of European descent with variable replication in other ethnicities. *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[6, 7]. 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 was not a suitable animal model for analysis of *Fto* function[8]. Introduction of an *Fto* null mutation in mice by homologous recombination resulted in postnatal growth retardation, loss of white adipose tissue, and increased energy expenditure.[9] In humans, duplication of a chromosomal region that includes *FTO* has been reported to result in obesity and mental retardation although increased expression of *FTO* was not confirmed in an analysis of an immortalized lymphocyte cell line derived from this patient.[10, 11] Affected individuals with a null mutation in *FTO* identified in a large Palestinian Arab consanguineous multiplex family were characterized by postnatal growth retardation, microcephaly, cardiac defects, psychomotor delay, functional brain deficits and structural brain malformations, as well as postnatal lethality occurring from 1-30 months

of age.[12] The *FTO* gene has been mapped to chromosome 16q12.2 and includes nine predicted exons (Genbank accession number NM 00108432).

Frayling et al.[1] 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 were 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, $p=4 \times 10^{-11}$) or obese (OR=1.67, 95% CI=1.47-1.89, $p=1 \times 10^{-14}$). 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.[2] that was revealed during an effort to estimate the distribution of neutral SNPs in a case-control study of French adults. Both SNPs conferred a substantial risk of severe obesity (rs1421085, OR=1.56 95% CI 1.40-1.75, $p=7.6 \times 10^{-16}$, rs17817449, OR=1.56, 95%CI 1.40-1.75, $p=1.44 \times 10^{-15}$) that was replicated in a study of 537 Swiss adults and 541 anonymous donors. A fourth *FTO* polymorphism (rs8050136) was subsequently shown to be associated with type 2 diabetes risk in another genome-wide association study[5] in which 1,161 Finnish type 2 diabetes cases and 1,174 Finnish normal glucose tolerant controls were genotyped.

The association of these four *FTO* polymorphisms with obesity and diabetes has recently been studied in the biracial prospective ARIC study. For white participants, all four *FTO* variants described above were associated with an increased risk of both obesity and diabetes, while only rs1421085 conferred a more modest susceptibility to obesity in African-Americans when compared to whites but was found to be protective against diabetes.[13] In addition, genetic variants in *FTO* were implicated as determinants of waist circumference in a recent genome-wide association study performed by the CHARGE consortium that included ARIC study participants.[14]

FTO is highly expressed in human fetal and adult brain,[1, 2] predominantly in the cerebellar cortex as well as in the hypothalamus, temporal lobe, and parietal lobe. In accordance with previous evidence that obese individuals showed structural differences in the frontal lobes, anterior cingulate gyrus, hippocampus, and thalamus when compared to healthy elderly subjects whose weight was in the normal range,[15] Ho et al. found that carriers of at least one copy of the *FTO* rs3751812 minor allele had significantly reduced brain volume in the frontal lobes (- 8%) and occipital lobes (-12%) when compared to average volumes observed in the general population (p = 0.0013) in a study of 206 cognitively normal subjects scanned by MRI. However, when regression models were

adjusted for age, sex, and BMI, there were no statistically significant alterations in any of the brain regions associated with carrying at least one *FTO* risk allele so that the brain atrophy associated with the *FTO* polymorphism may have been mediated by BMI.[16] Since a relationship between brain atrophy and diminished cognitive function has previously been demonstrated in the ARIC study,[17] the goal of this proposal is to determine whether cognitive status and cognitive decline are associated with *FTO* genotype.

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

1. To evaluate the independent effect of *FTO* gene variation on three separate measures of cognitive status (DWRT, DSST, and WF) administered at visit 2 and visit 4 in a race-specific manner. Age, gender, and field center will be included as covariates.

2. To evaluate the independent effect of *FTO* gene variation on 6-year cognitive change between visits 2 and visit 4 assessed in the entire cohort using three neurocognitive tests (DWRT, DSST, and WF). Age, gender, and field center will be included as covariates.

3. To evaluate whether obesity as assessed by various measures of body size including BMI, weight, height, waist circumference, subcutaneous skinfold measures (triceps and subscapular) and waist-to-hip ratio modulates the independent effect of *FTO* gene variation on cognition. 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).

<u>Cognitive measurements (dependent variable)</u>

The Delayed Word Recall Test (DWR), Digit Symbol Substitution Test (DSS), and Word Fluency Test (WF) are available from Visit 2 (1990-1992, labeled cognitive assessment 1 [CA1], whole cohort), Visit 3 (1993-1995, labeled CA2, Forsyth and Jackson MRI subset), Visit 4 (1996-1998, labeled CA3, whole cohort), and in participants in the ARIC Brain MRI study (2004-2006, labeled CA4, Forsyth and Jackson Brain MRI study subset).

For the subset (N = 1,134) of participants enrolled in the ancillary ARIC Brain MRI study a more extensive battery of neuropsychological tests was administered (2004-2006, CA4). From this battery, 5 domains of cognitive functioning were derived through principal components factor analysis. The factors to be examined in the current study are: (1) Global Mental Status, (2) Memory, (3) Psychomotor Speed, (4) Verbal Fluency, and (5) Executive Function.

Data analysis plan

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. Other exclusion criteria will include history of stroke or TIA prior to Visit 2 and incident stroke. We will not analyze those individuals with the lowest 5% of scores on the cognitive tests at CA1 to exclude those with possible preclinical dementia.

The association of *FTO* genetic variation and cognition will be analyzed individually for each of the four SNPs. The association between haplotypes within the *FTO* gene and cognitive status 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[18]. A co-dominant model will be assumed. The *FTO* variant (rs3751812) examined by Ho et al. as part of the Alzheimer's Disease Neuroimaging Inititative and associated with regional brain atrophy[16] is in strong linkage disequilibrium with all of the genotyped SNPs in whites (r^2 = 0.979 (rs9939609); 0.979 (rs8050136); 0.979 (rs17817449); and 0.958 (rs1421085)) and with rs1421085 (r^2 = 1.00) in African-Americans.[19] Analysis of variance (ANOVA) will be performed to assess mean differences in cognitive test scores among individuals with different *FTO* genotypes. Multiple linear regression will be used to evaluate the association of the *FTO* SNPs with cognitive scores considered as continuous measures in cross-sectional analyses. Cognitive change will be analyzed as a continuous variable defined as the difference between CA3 test score and CA1 test score (6-year change) for each of the three cognitive tests. A categorical measurement of cognitive impairment, defined as those falling below the 20th percentile of scores for each of the cognitive tests, will also be analyzed using multivariable logistic regression to predict case status. In analysis models, measures of body size will be used as both categorical and continuous variables. Division into categories of BMI will be carried out based on standard criteria where an individual with a BMI $\geq$ 25 kg/m^2 is considered overweight, a BMI $\geq$ 30 kg/m^2 is considered as a measure of obesity, while those individuals with a BMI $\geq$ 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.

The analysis of effect measure modification by obesity, BMI, or other measures of body size of any association between *FTO* genotypes or hapotypes and cognitive status will be carried out by including interaction terms in the analysis models.

Other variables of interest:

In aims 1-3 above, we will determine whether any observed relationships are independent of cardiovascular risk factors and potential confounding factors. These factors will include but are not limited to:

Visit 1- Education, gender, exam center, physical activity (Baecke).

Visit 2- Age, *APOE* genotype, fasting blood sugar, history of diabetes, fasting glucose, systolic blood pressure, diastolic blood pressure, smoking pack years,

hypertension status, antihypertensive medications, systolic blood pressure and diastolic pressure, BMI, carotid IMT (right and left sides), alcohol consumption, total cholesterol, LDL-c, HDL-c, triglycerides, Lp(a).

Depression as assessed as Vital Exhaustion at CA1 and the CES-D score at CA4. CNS medications (antidepressants, neuroleptics, antianxiolytics, benzodiazepines, antiepileptics) at each visit (CA1, CA2, CA3, CA4).

Incident stroke

Limitations of study:

A limitation of the study is the possibility of selection bias introduced because of differences between those subjects who did and did not participate in the Brain MRI study. To address this issue, baseline characteristics and clinical outcomes will be compared for the two groups.

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)?

#924 Apolipoprotein E genotype, cardiovascular risk factors, and cognitive decline in a middle-aged cohort: the Atherosclerosis Risk in Communities Study (Lead author: Cindy K. Blair, University of Minnesota, Minneapolis, MN)

#1269 FTO, Obesity, and Diabetes (Lead author: Jan Bressler, University of Texas Health Science Center at Houston, Houston, TX)

#1358 Interaction between FTO genotype and physical activity level on adiposity: The Atherosclerosis Risk in Communities (ARIC) Study (Lead author: Ellen Demerath, University of Minnesota, Minneapolis, MN)

#1363 *PCSK9* sequence variation and cognitive decline (Lead author: Jan Bressler, The University of Texas Health Science Center at Houston, Houston, TX)

#1407 Interaction between FTO and dietary patterns in relation to diabetes and obesity in the Atherosclerosis Risk in Communities (ARIC) Study (Lead author: Jennifer Nettleton, University of Texas Health Science Center at Houston, Houston, TX)

#1521 Association of polymorphisms in obesity susceptibility genes with obesity-related inflammation and body mass index (Lead author: Tianna Garrett, University of North Carolina, Chapel Hill, NC)

There are no other manuscript proposals in ARIC investigating polymorphisms in the *FTO* gene and their relationship to cognition.

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

1. Frayling, T. M. et al. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science 316, 889-94 (2007).
2. Dina, C. et al. Variation in FTO contributes to childhood obesity and severe adult obesity. Nat Genet 39, 724-6 (2007).
3. Scuteri, A. et al. Genome-Wide Association Scan Shows Genetic Variants in the FTO Gene Are Associated with Obesity-Related Traits. PLoS Genet 3, e115 (2007).
4. Scott, L. J. et al. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science 316, 1341-5 (2007).
5. Saxena, R. et al. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science 316, 1331-6 (2007).
6. van der Hoeven, F. et al. Programmed cell death is affected in the novel mouse mutant Fused toes (Ft). Development 120, 2601-7 (1994).
7. Peters, T., Ausmeier, K. & Ruther, U. Cloning of Fatso (Fto), a novel gene deleted by the Fused toes (Ft) mouse mutation. Mamm Genome 10, 983-6 (1999).
8. Peters, T., Ausmeier, K., Dildrop, R. & Ruther, U. The mouse Fused toes (Ft) mutation is the result of a 1.6-Mb deletion including the entire Iroquois B gene cluster. Mamm Genome 13, 186-8 (2002).
9. Fischer, J. et al. Inactivation of the Fto gene protects from obesity. Nature 458, 894-8 (2009).
10. Stratakis, C. A. et al. Anisomastia associated with interstitial duplication of chromosome 16, mental retardation, obesity, dysmorphic facies, and digital anomalies: molecular mapping of a new syndrome by fluorescent in situ hybridization and microsatellites to 16q13 (D16S419-D16S503). J Clin Endocrinol Metab 85, 3396-401 (2000).
11. van den Berg, L. et al. Investigation of a patient with a partial trisomy 16q including the fat mass and obesity associated gene (FTO): fine mapping and FTO gene expression study. Am J Med Genet A 152A, 630-7.
12. Boissel, S. et al. Loss-of-function mutation in the dioxygenase-encoding FTO gene causes severe growth retardation and multiple malformations. Am J Hum Genet 85, 106-11 (2009).
13. Bressler, J., Kao, W. H., Pankow, J. S. & Boerwinkle, E. Risk of type 2 diabetes and obesity is differentially associated with variation in FTO in whites and African-Americans in the ARIC study. PLoS One 5, e10521.
14. Heard-Costa, N. L. et al. NRXN3 is a novel locus for waist circumference: a genome-wide association study from the CHARGE Consortium. PLoS Genet 5, e1000539 (2009).
15. Raji, C. A. et al. Brain structure and obesity. Hum Brain Mapp 31, 353-64.
16. Ho, A. J. et al. A commonly carried allele of the obesity-related FTO gene is associated with reduced brain volume in the healthy elderly. Proc Natl Acad Sci U S A 107, 8404-9.
17. Mosley, T. H., Jr. et al. Cerebral MRI findings and cognitive functioning: the Atherosclerosis Risk in Communities study. Neurology 64, 2056-62 (2005).

18. Stephens, M., Smith, N. J. & Donnelly, P. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 68, 978-89 (2001).
19. A haplotype map of the human genome. Nature 437, 1299-320 (2005).