1.a. Full Title: Association of polymorphisms in obesity susceptibility genes with obesity-related inflammation and body mass index

b. Abbreviated Title: Obesity polymorphisms, inflammation, and BMI

2. Writing Group (list individual with lead responsibility first)

Writing Group Members: Tiana Garrett, Kari E. North, Nora Franceschini, Jim Pankow, Sherita Golden, Eric Boerwinkle Kelly Volcik, Nena Matijevic (others welcome)

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

First author: Tiana Garrett, PhD
University of North Carolina-Chapel Hill
Department of Epidemiology
Bank of America Center
137 E. Franklin Street, Suite 306
CB# 8050
Chapel Hill, NC 27514
Phone: (919) 966-4564
Fax: (919) 966-9800
Email: taga@unc.edu

Corresponding/senior author (if different from first author correspondence will be sent to both the first author & the corresponding author):
Kari E. North, PhD
University of North Carolina-Chapel Hill
Department of Epidemiology
Bank of America Center
137 E. Franklin Street, Suite 306
CB #8050
Chapel Hill, NC 27514
Phone: (919) 966-2148
Fax: (919) 966-9800
Email: kari_north@unc.edu
3. Timeline: 4-6 months

4. Rationale:

Obesity is a worldwide epidemic and public health challenge. Modifiable behavioral factors such as low physical activity and excess caloric intake are known contributors to obesity; however, it is unknown how genetic predisposition influences obesity. Recent population studies have identified polymorphisms in genes associated with obesity using candidate genes, genome-wide linkage and genome-wide association studies (GWAS). Seven of the genes identified are fat mass and obesity associated (FTO), melanocortin-4 receptor (MC4R), glucosamine-6-phosphate deaminase 2 (GNPDA2), mitochondrial carrier protein 2 (MTCH2), SH2B adaptor protein 1 (SH2B1), neuronal growth factor 1 (NEGR1), and neurrxin 3 (NRNX3). Some of these genes encode proteins that are expressed in the brain and are responsible for various obesity-associated physiological functions. For example, the FTO and MC4R genes play a role in controlling food intake and fat consumption. The SH2B1 gene regulates metabolic signaling of insulin and leptin, which affects food intake and energy homeostasis. Expression of the GNPDA2 gene (also known as GABRG1) modulates addiction and reward signals that could affect satiation in eating behavior. Although specific roles for NEGR1 and NRNX3 in obesity have not been identified, these genes affect neuronal development and synaptogenesis, respectively. The function of MTCH2 is largely unknown.

One copy of the genetic variants can enhance an individual’s susceptibility for increased body mass index (BMI). In a study of ~39,000 Europeans, individuals lacking one or two copies of a specific FTO gene variant had a higher BMI compared to individuals without this trait. The association between the FTO gene and BMI was replicated in nine cohorts of white European adults, two studies in European children, and two diabetic populations. Variants in the MC4R gene affect fat mass and body weight contributing to obesity risk as well as have an additive effect with the FTO gene. Recently, genetic variants in SH2B1, MTCH2, NEGR1, and NRNX3 were shown associated with BMI in large European populations.

Obesity is associated with the persistent infiltration of activated leukocytes (mostly monocytes and macrophages) in adipose tissue, which results in chronic low-grade inflammation, although the relationship between inflammation and obesity has not been fully characterized. Generally, a small number of macrophages reside in adipose tissue. Studies have shown that macrophages invade adipose tissue as obesity increases, and this infiltration positively correlates with increasing BMI and percent body fat. Macrophages may infiltrate adipose tissue for various reasons, including clearance of necrotic adipocytes, usage of local fatty acids, and chemotactic response to tissue hypoxia and hypertrophy. When stimulated, these macrophages secrete pro-inflammatory molecules (i.e., TNF-α, IL-6, MCP-1) and acute phase reactants to signal the recruitment of more macrophages, thus creating a chronic inflammatory state. Work by Bruun et al. (2006) showed that diet and exercise reduce macrophage infiltration and low-grade inflammation in the adipose tissue of obese individuals.

The effect of genetic variants on the relationship between inflammatory markers and BMI requires further investigation. Since obesity is associated with low-grade inflammation, one or more variants in obesity susceptibility genes may influence the
levels of inflammatory markers typically found associated with adiposity. In addition, carrier status of these genetic variants may modify the relationship between BMI and systemic inflammatory markers.

Inflammation plays a major role in several obesity-related diseases, including hypertension and type 2 diabetes. Acute phase reactants such as fibrinogen and white blood cell count, and to a lesser extent, von Willebrand factor and albumin, are well-characterized markers of inflammation in population studies, available on the ARIC cohort members at baseline (and the first cohort re-examination). High plasma levels of fibrinogen, white blood cells, and von Willebrand factor were found correlated with weight and with weight gain in middle-aged adults in the ARIC study14.

5. Main Hypothesis/Study Questions:

Study Question: The hypotheses to be tested in this study posit that one or more variants in well-established obesity susceptibility genes modify the association between inflammation and BMI.

Specific Aim 1: To examine the relationship between inflammatory markers (fibrinogen, white blood cell count, von Willebrand factor, and albumin) and BMI in individuals enrolled in the Atherosclerosis Risk in Communities (ARIC) study. (Descriptive information similar to that of Duncan et al. 14, restricted to the population included in this analysis).

Specific Aim 2: To evaluate the association between carrier status of polymorphisms in seven obesity susceptibility genes encoding FTO, MCR4, NEGR1, GNPDA2, MTCH2, SH2B1 and NRXN3 and inflammatory markers (fibrinogen, white blood cell count, von Willebrand factor, and albumin) in individuals enrolled in the ARIC study, controlling for anthropometric measures.

Specific Aim 3: To determine if carrier status of polymorphisms in obesity susceptibility genes modifies the relationship between inflammatory markers (fibrinogen, white blood cell count, von Willebrand factor, and albumin) and BMI in individuals enrolled in the ARIC study.

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

Aim 1 Methods: We propose to examine the association of aforementioned inflammatory markers with BMI in women and men enrolled in the entire ARIC cohort (N = 15,792), after excluding the individual with RES DNA = “No use/storage DNA” and those without baseline information on inflammatory markers or anthropometric measurements. BMI will be used as an interval scale measurement and also categorized in quartiles and conventional groupings (< 25 kg/m², normal; 25-<30 kg/m², overweight; ≥ 30 kg/m², obese) for analysis. Inflammatory markers (fibrinogen, white blood cell count, von Willebrand factor, and albumin) will be used as an interval scale measurement and also categorized into quartiles based on gender- and ethnicity-specific cut points. Linear
regression will be used to analyze BMI as a continuous outcome. We will use polytomous logistic models by comparing quartiles of inflammatory markers and BMI quartiles or levels, with the lowest quartile as the referent.

**Aim 2 Methods:** The genetic associations between each obesity susceptibility gene variant and inflammatory markers will be analyzed for its potential role in obesity and obesity-related inflammation. To determine the effect of obesity susceptibility genes on inflammatory markers we will use additive genetic models (1 degree of freedom) and linear regression models for quantitative inflammatory outcomes or logistic regression for binary categories of inflammatory markers, in race-stratified analysis.

**Aim 3 Methods:** To study the effect measure modification of obesity susceptibility genes on the association among inflammatory markers and BMI, we will test interaction terms at an alpha=0.10. We will use additive genetic models (1 degree of freedom) and linear regression for quantitative traits or logistic models for qualitative traits. All models will be race-stratified.

Final multivariate models will be adjusted for demographic and lifestyle covariates associated with obesity as identified from literature review and directed acyclic graph (DAG) (i.e., age, ethnicity, ARIC field center, educational level, physical activity, smoking status, alcohol consumption, diabetes, and fasting insulin).

7.a. Will the data be used for non-CVD analysis in this manuscript? _____ Yes  **X** 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 to contact lead authors of these proposals for comments on the new proposal or collaboration)?
None

11. 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.


