**1.a. Full Title:** Genetic variations in CCAAT/enhancer binding protein-α, sterol regulatory binding transcription factor-1, and peroxisome proliferator activated receptor-2 and insulin resistance and type 2 diabetes

**b. Abbreviated Title (Length 26 characters):** CEBPα, SREBF1, PPARγ2 and type 2 diabetes

**2. Writing Group:**

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

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**3. Timeline:** Genotype data collection will begin immediately upon approval. Data analysis will begin by August 2003, and drafts of the manuscripts will be distributed for internal circulation by December 2005.

**4. Rationale:**

Obesity is a major risk factor for the development of insulin resistance and type 2 diabetes; however, the exact mechanism in which obesity leads to these two conditions is
unclear. Three possible mechanisms as to how obesity leads to insulin resistance and type 2 diabetes have been suggested: (1) free fatty acid secreted from adipocytes can block insulin action (Randle/portal hypothesis), (2) ectopic fat storage in various organs including the liver and muscle, can cause lipotoxicity, (3) adipocyte-derived molecule, such as, leptin and adiponectin, can affect insulin action. Any of these three mechanisms may counteract normal insulin-mediated glucose uptake, hepatic regulation of glucose output, and insulin secretion, thus leading to the development of insulin resistance and type 2 diabetes. Therefore, we propose to study the associations between insulin resistance and type 2 diabetes and genetic variations in three transcription factors that are important in the expressions of genes involved in the aforementioned mechanisms. The candidate genes proposed are: CCAAT/enhancer binding protein-α (CEBPα), sterol regulatory element binding transcription factor-1 (SREBF1), and peroxisome proliferator activated receptor-gamma2 (PPARγ2) [genotype data have already been collected by Dr. Bray].

CEBPα is a transcription factor comprised of 408 amino acids. It has a bipartite DNA-binding domain consisting of positively-charged basic region for DNA binding and a leucine zipper in the C terminus for dimerization. CEBPα belongs to the CEBP family, which has six members. Of these, CEBPβ and CEBPδ are expressed in the early stage of adipogenesis and activate the expression of PPARγ. CEBPα is involved in the differentiation of granulocytes, hepatocytes, and adipocytes. Its mRNA is expressed in the liver, adipose tissue, intestine, lung, adrenal gland, placenta, ovary, and peripheral blood mononuclear cells. The gene encoding CEBPα is located on chromosome 19q13.1 and is intronless with 2,796 base pairs. Knockout mice for CEBPα have failures in glycogen storage, activation of gluconeogenesis and lipid accumulation and have abnormal expression of several genes that are transactivated by CEBPα, including glycogen synthase.

SREBF1, otherwise known as adipocyte differentiation and determination factor-1, is a member of the basic helix-loop-helix-leucin zipper transcription factor family with three isoforms (SREBF1a, 1b, and 1c). It is comprised of 1,147 amino acids (22 exons). The gene encoding SREBF1 is located on chromosome 17p.11.2, within the Smith-Magenis syndrome region. SREBF1 is expressed in the hepatoma cell line, adrenal gland, ovary, kidney, brain, white fat, and muscle. SREBF1 activates genes involved in lipid metabolism, including those encoding LDL receptors and HMG CoA synthase. Transgenic mice with overexpression of SREBF1 showed lowered mRNA of CEBP α, PPAR γ, and leptin in adipose tissue with fatty liver. Gene expression of SREBF1 is reduced in people with diabetes but not in people with obesity. A few studies have been performed to examine the associations between genetic variations in SREBF1 and various lipid measurements in human populations. The -36delG polymorphism of SREBF1 has been shown to be associated with increased total cholesterol and LDL-cholesterol among people with high risk of cardiovascular disease and to be associated with response to fluvastatin in a clinical trial setting. Among HIV-positive individuals, the 322C/G polymorphism was associated with change in total cholesterol and triglyceride after initiation of protease inhibitors. Similar to CEBPα, no studies in
humans have examined the association between genetic variations in SREBF1 and insulin resistance or type 2 diabetes.

PPARγ2 is a transcription factor that is expressed predominantly in adipose tissues, and is believed to have a critical role in adipogenesis and insulin action.17,18 A nonconservative substitution at codon 12 (Pro12Ala) was identified19 and, unlike C/EBPα and SREBF1, several association studies have been conducted to examine the associations between the Pro12Ala variant and adiposity or type 2 diabetes. One of the more consistent findings is the association of the Ala12 allele and insulin sensitivity20-23 and protection from type 2 diabetes24,25. In Dr. Brancati’s ancillary study of candidate genes and type 2 diabetes in 1800 African-American ARIC participants, the Pro/Ala genotype was associated with greater insulin sensitivity among non-obese individuals. Although the three transcription factors (or combinations of these transcription factors) are required for terminal adipocyte differentiation and for expressions of each other and other genes involved in lipid metabolism, no study has examined their interactions in conferring risk for insulin resistance or type 2 diabetes. To fill this gap, we propose the study with to investigate the association between the genetic variations in CEBPα, SREBF1, and PPARγ2 and type 2 diabetes in the ARIC study.

In addition to investigating type 2 diabetes and insulin resistance, we propose to investigate the association between the following three adipocytokines and the genetic variations in the proposed three transcription factor genes: interleukin 6 (IL-6), leptin, and adiponectin, all of which are secreted from adipocytes and could exert their functions as endocrines.26 Plasma IL-6 level has been reported to be positively correlated with adiposity and negatively associated with insulin action in non-diabetic Pima Indians27 and can predict incident diabetes in the ARIC study28 and in another population29. Similarly, leptin is positively associated with adiposity30-33 and is negatively associated with insulin sensitivity34. Contrary to IL-6 and leptin, adiponectin is shown to be negatively associated with insulin resistance35 and adiposity36,37. Hypoadiponectinemia is shown to predict future development of diabetes in the ARIC study38 and in several other populations39-43. There is evidence that these molecules’ expressions are regulated with PPARγ244 or PPARγ agonist45,46, members of the CEBP family47-49, and SREBF150-52. Thus, we hypothesize that genetic variations in the three transcription factors are associated with the level of adipocytokines. Moreover, if there is any association between the risk of type 2 diabetes and the genetic variations in the three transcription factors, the adipocytokines may be at least one of the mediators for such an association.

5. Main Hypothesis/Study Questions:

a) Genetic variations in CEBPα and SREBF1 are associated with prevalent and incident type 2 diabetes, obesity, and markers of insulin resistance, including the HOMA index, at least partially mediated through adipocytokines.

b) Genetic variations in CEBPα, SREBF1, and PPARγ2 synergistically interact with each other to confer risks of type 2 diabetes, obesity, insulin resistance, and adipocytokines.

c) Genetic variations in CEBPα, SREBF1, and PPARγ2 interact with dietary intake of fatty acid for the risks of type 2 diabetes, obesity, insulin resistance, and adipocytokines.
6. Data (variables, time window, source, inclusions/exclusions):

   We will collect genotype data in ~9000 ARIC participants included in Dr. Brancati’s ancillary study “NIDDM Susceptibility Genes in a Biracial Cohort”. They include: (a) all African Americans who gave the consent for genotype analysis, (b) all white cases with diabetes, and (c) a random sample of white without diabetes. The DNA samples have already been sent by Dr. Boewinkle’s laboratory to Dr. Shuldiner’s laboratory at the University of Maryland. The exact polymorphisms to be genotyped will be selected from ones studied in previous literatures as well as ones from public databases. We anticipate genotyping 5 to 10 SNPs in CEBPA and SREBF1 for haplotype-based analyses.

   Other variables needed and already available at the local center are: center, age, gender, race, dietary intake of nutrients, diabetes status and the time of its development, insulin, glucose, anthropometry, blood pressure, lipid profiles. Data for additional variants within the genes targeted for study will be provided by Drs. Bray and North. Adipocytokine data for IL-6, leptin, and adiponectin will be provided by Dr. Pankow derived from his ancillary study, “Inflammatory Precursors of Diabetes”.

   Dr. Asao will perform data analysis with the following steps: estimate allele and genotype frequencies, check Hardy-Weinberg equilibrium for allele and genotype frequencies, evaluate associations between the genetic variations and the outcomes of interests with stratification by degree of adiposity, sex, and race using logistic regression models and survival analysis, examine modes of inheritance, assign haplotypes using expectation-maximization algorithm, and explore gene-gene and gene-environment interactions by regression models. Data analyses including adipocytokines incorporate with case-cohort design, which is the design of the ancillary study “Inflammatory Precursors of Diabetes”.

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

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. _____ 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)?

1. In general,
   a. Diabetes Genes in African Americans I: Case-Control Studies (Brancati, F): Manuscript Proposal #357
   b. NIDDM Susceptibility Genes in a Biracial Cohort (Brancati, F): Ancillary Study #1996.02

   The current proposal (#357Gr) is an extension of #357 and is conducted within the scope of the Ancillary Study #1996.02.

2. Regarding PPAR\(\gamma_2\) genotype and additional variants in the targeted genes,
   a. Gene-environment interaction in CVD (Boerwinkle, E [originally Bray, M]): Ancillary Study # 1995.07

   Dr. Bray has agreed to provide PPAR\(\gamma_2\) genotype and to be a co-author of the current proposal.

3. Regarding adipocytokine,
   a. Inflammatory precursors of diabetes (Pankow, J): Ancillary Study # 1995.09
   b. IL-6, acute phase proteins and incident diabetes mellitus (Ancillary study) (Duncan, B): Manuscript Proposal #853
   c. Adiponectin, complement component 3, leptin and incident diabetes mellitus (Ancillary study) (Duncan, B): Manuscript Proposal #976
   d. Adiponectin, complement component 3, leptin and incident diabetes mellitus (Ancillary study) (Duncan, B): Manuscript Proposal #976C

   Dr. Pankow has agreed to provide adipocytokine variables and to be a co-author of the current proposal.

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* 1996.02, 1995.07)
   x B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* 1995.09)

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.
Reference List


