1.a. **Full Title:** Association of the human membrane glycoprotein plasma cell differentiation antigen (PC1), insulin receptor substrate 2 (IRS2), and inositol polyphosphate phosphatase-like 1 (INPPL1) genotypes with insulin resistance and type 2 diabetes in African Americans and whites from the Atherosclerosis Risk in Communities Study

1.b. **Abbreviated title:** PC1, IRS2, and INPPL1 genotypes, insulin resistance, and type 2 diabetes

2. **Writing group:**
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3. **Timeline:**
   Genotype data collection will begin immediately upon approval. Data analysis will begin by March 2003, and drafts of the manuscripts will be distributed for internal circulation by September 2003.

4. **Rationale:**
   Insulin resistance is a major characteristic of most patients with type 2 diabetes. Strong evidence exists for genetic predispositions to both insulin resistance and type 2 diabetes. Therefore, we propose to examine three candidate genes, PC1, IRS2, and SHIP2, involved in insulin signaling pathway for their associations with insulin resistance and type 2 diabetes in the ARIC study. Many groups have reported that insulin receptor tyrosine kinase activity, the initial step of insulin signaling, is impaired in muscle, fat, and other tissues in several types of insulin resistant, including obese, lean, diabetic and non-diabetic, patients\(^1\text{-}^5\). However, most cases of insulin resistance are not due to alterations in the insulin receptor gene. Several other potential biochemical defects have been proposed to account for insulin resistance, including alterations in PC1. PC1 is a class II transmembrane glycoprotein that is located both on plasma membranes and in the endoplasmic reticulum and has multiple biochemical functions, including phosphodiesterase I and nucleotide pyrophosphatase activities\(^6\text{-}^7\). PC1 levels are higher in muscle, fat, and fibroblasts of insulin resistant individuals\(^8\text{-}^10\) and of type 2 diabetic
individuals\textsuperscript{11} compared to controls; furthermore, when overexpressed, it is an inhibitor of insulin receptor tyrosine kinase activity, which in theory would lead to insulin resistance\textsuperscript{11}. The Lys121Gln variant of PC1 was identified and has been shown to be associated with insulin resistance\textsuperscript{12,13}. Although this variant is not associated with altered expression of PC1, the skin fibroblasts derived from heterozygous carriers of the variant had reduced insulin receptor tyrosine kinase activity when compared with carriers of the wild-type allele\textsuperscript{12}. Since the exact function of this variant and the importance of other potential functional variants in PC1 are not well understood, we propose to obtain genotype data on 5 SNPs, including Lys121Gln, in PC1, estimate haplotypes of these 5 SNPs, and examine the association between the PC1 haplotypes and insulin resistance and type 2 diabetes in the ARIC Study.

Several biological processes are mediated by IRS proteins, including mitogenesis, protein synthesis, and glucose transport\textsuperscript{14}. IRS2 is a protein phosphorylated by the insulin receptor and connects signaling between proteins with Src homology-2 domains and insulin receptor\textsuperscript{15,16}. Mice which are homozygous for the absence of the gene have profound insulin resistance in both skeletal muscle and liver and severe type 2 diabetes\textsuperscript{17}. In certain mice, chronic hyperinsulinemia down regulates the mRNA for IRS2 and stimulates production of sterol regulatory element-binding protein 1c (SREBP1c), a transcription factor that activates fatty acid synthesis\textsuperscript{18}. The cycle of insulin resistance that results from the down regulation of IRS2 and the insulin sensitivity that results from elevated lipogenesis leads to perpetual hyperinsulinemia and insulin resistance in lipodystrophis and ob/ob mice. In addition, IRS2-deficient mice have been shown to develop diabetes because of both inadequate beta-cell proliferation and insulin resistance in the liver\textsuperscript{19}. Recently, these same IRS2-deficient mice have also been shown to have greater adiposity associated with increased serum leptin levels, suggesting IRS2 deficiency can result in leptin resistance, fatty liver, obesity, and ultimately type 2 diabetes\textsuperscript{20}. Several variations in the IRS2 gene have been identified; however, the associations with insulin resistance or type 2 diabetes have been mostly negative\textsuperscript{21-23}. The most promising variant appeared to be the Gly1057Asp variant, which was shown to be associated with type 2 diabetes with an interaction with obesity – the Asp allele is protective in nonobese but detrimental in obese\textsuperscript{24}. Similar to PC1, since the exact function of this variant and the importance of other potential functional variants in PC1 are not well understood, we propose to obtain genotype data on 5 SNPs, including Gly1057Asp, in IRS2, estimate haplotypes of these 5 SNPs, and examine the association between the IRS2 haplotypes and insulin resistance and type 2 diabetes in the ARIC Study.

Inositol phosphatases can hydrolyze the important second messenger inositol triphosphate (IP3). INPPL1 belongs to the family of inositol triphosphate phosphatases\textsuperscript{25} and has been shown to play a significant role in regulation of phosphatidylinositol 3-prime-kinase signaling by insulin\textsuperscript{56}. It is highly expressed in human heart, skeletal muscle, and placenta. In mice, absence of SHIP2 (homolog of INPPL1c in human) led to increased insulin sensitivity which resulted in severe hypoglycemia and perinatal death\textsuperscript{27}. Adult mice which were heterozygous of the SHIP2 mutation had increased glucose
tolerance, insulin sensitivity, and increased glycogen synthesis in skeletal muscle. A deletion in the 3’untranslated region containing the adenylate-rich element (ARE) motif was identified in INPPL1 and was shown to be associated with type 2 diabetes. Furthermore, this deletion resulted in increased expression of reporter mRNA and protein in vitro. Therefore, we propose to obtain genotype data the 16-bp deletion in INPPL1 and examine this variant’s association with insulin resistance and type 2 diabetes in the ARIC Study.

5. Main Issues/Hypotheses to be addressed:
   a. Variations (genotypes and estimated haplotypes) in PC1, IRS2, and INPPL1 are associated with:
      a. Prevalent type 2 diabetes in the ARIC study
      b. Incident type 2 diabetes in the ARIC study
      c. Measures of insulin sensitivity/resistance, including fasting insulin, glucose-to-insulin ratio, and HOMA index, in non-diabetic participants at visit 1 of the ARIC study
   Sex- and race-specific associations will be examined; however, we do not believe true causal associations would differ between these groups.
   b. Haplotypes associated with increased insulin resistance are hypothesized to have stronger associations with type 2 diabetes in non-obese individuals than in obese individuals.

6. Data (variables, timeline, source, inclusion/exclusion):
   Genotype data will be collected in all ARIC participants included in Brancati’s ancillary study genetics of obesity, insulin resistance and type 2 diabetes in a biracial cohort (this study includes: (a) all African Americans with DNA (and consent) and defined diabetes status at baseline of the study, (b) all white prevalent and incident diabetic cases, and (c) a random sample of white non-diabetic controls).
   All analyses (cross-sectional or prospective) will assume the following steps:
   1. Hardy-Weinberg equilibrium among genotypes will be checked by calculating expected frequencies of genotypes and using the chi-square goodness-of-fit test.
   2. All analyses will first be stratified by ethnicity to test for interaction. If no interaction is detected, pooled analyses will be performed.
   3. Genotype will be coded as 0 (zero copies of the candidate allele), 1 (one copy of the candidate allele), or 2 (two copies of the candidate allele). An additive genetic model will be assumed unless indicated otherwise by results of the analysis or unless the allele frequency of a given candidate variant is low, in which case, a dominant model combining the risk of heterozygotes and homozygotes will be used. We will establish multiple associations using multiple linear regression models or multiple logistic regression models to fit better models that explain the separation of the genotype groups and to control for the effects of potential confounders.
   4. A Bayesian-based method by Matthew et al. (PHASE v.1.2) will be used to estimate and assign individual specific probabilities of haplotypes (within a candidate gene) for each individual. The specifications for the proposed study will be for biallelic markers only.
5. Analysis of haplotypes will be performed in a similar manner as analysis of genotype except the independent variable will be haplotypes instead of genotypes. Each individual will be modeled using all of his/her possible haplotype combinations with weights according to the probability of each haplotype. An overall omnibus likelihood ratio test for all haplotypes having a null effect will be conducted.

6. Secondary analyses will examine diabetes-related traits such as obesity. We will further examine the interactions among candidate genes, diabetes, and environmental factors including physical activity and body-mass index. We will examine the presence of detectable gene-gene and gene-environment interactions first by stratification and then by standard regression techniques. Interactions hypothesized in the literature will be considered first followed by interactions between genes in the same biologic pathways.

7.a. Will the data be used for non-CVD analysis in this manuscript?  _X_ Yes  ___No

7.b. If Yes, is the author aware that the file ICTER02 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 ICTDER01 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 ICTDER01 must be used to exclude those with the 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://bios.unc.edu/units/cscj/ARIC/stdy/studymem.html](http://bios.unc.edu/units/cscj/ARIC/stdy/studymem.html)

  _X_ Yes  _____ No

Reference List


