1. **Full Title:** Interactions of Consumption of Processed Meat with Beta Cell-Related Genetic Loci and Fasting Blood Glucose and Insulin in Individuals of European Descent
   
   **Abbreviated Title (Length 26 characters):** Processed meat x beta cell function-related loci

2. **Writing Group:** the lead author is from the CHS cohort team—information listed below: Amanda M. Fretts, Ph.D. (postdoctoral fellow with David Siscovick)
   
   Writing group members: ARIC authors are listed below (others are welcome); in lieu of a full author list (TBD), other participating cohorts and estimated sample sizes are given in the table.
   
   ➔ Jennifer A. Nettleton, Kari E. North, James S. Pankow + others welcome!!

<table>
<thead>
<tr>
<th>PARTICIPATING COHORT</th>
<th>ARIC</th>
<th>MESA</th>
<th>Framingham</th>
<th>CHS</th>
<th>Family HS</th>
<th>GOLDN</th>
<th>Health ABC</th>
<th>GLACIER</th>
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<td>~N</td>
<td>800</td>
<td>2,305</td>
</tr>
</tbody>
</table>

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

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**ARIC author** to be contacted if there are questions about the manuscript and the first author does not respond or cannot be located (this must be an ARIC investigator).

*Name:* **AS LISTED ABOVE**

3. **Timeline:**
   
   **Cohort-specific data analyses:** March 1, 2012
   
   **Meta-analysis:** April 1, 2012
   
   **Manuscript drafting complete:** July 1, 2012
4. **Rationale:**
Nitrosamines found in processed meats have been shown to have a toxic effect on beta cells [1-5] and may promote the development of diabetes. The purpose of this analysis is to examine the association of processed meat intake with fasting glucose/insulin levels, and determine if SNPs related to beta cell function interact with processed meat intake to influence fasting glucose/insulin levels.

Type 2 diabetes is a leading cause of morbidity and mortality. In 2010, 11.3% of American adults aged 20 years+ had diabetes and 35% had impaired fasting glucose/pre-diabetes [6]. The rise in the global burden of diabetes is attributable, at least in part, to recent changes in lifestyle, including a sedentary lifestyle and diets high in fat, carbohydrates, processed/refined foods, and total calories that increase the risk of overweight/obesity. Whereas several genes have been identified that are related to the incidence of type 2 diabetes, the proportion of risk attributable to these genes remains quite small, possibly due in part to gene*environment interactions. However, few studies have detected gene*diet interactions in relation to diabetes risk [7-13], and more studies are needed to better understand the complex relationship of genes and dietary factors with diabetes-related phenotypes.

There has been recent interest in the association of processed meat and unprocessed red meat with diabetes development. Several studies show a consistent strong, positive association of processed meat intake and diabetes [14-21]. On the other hand, the association of unprocessed red meat with diabetes risk is less clear, with much smaller [19] or even no associations in several studies [14-16]. To date, only one published study has examined the interaction of meat intake and genes with diabetes risk [12]. In that study, the authors’ primary interest was in assessing the interaction of a western dietary pattern and SNPs related to diabetes risk, but in secondary analyses, the interactions of genetic risk score (GRS)*processed meat and GRS*unprocessed red meat were assessed. The GRS was calculated using 10 single nucleotide polymorphisms (SNPs) that were associated with diabetes risk in genome wide association studies (GWAS). Both processed meat and unprocessed red meat showed significant interaction with GRS in relation to diabetes risk. Additional studies are needed to further explore candidate gene*diet interactions for processed and unprocessed red meat intake in relation to glucose/insulin traits.

As diabetes is a heterogeneous disorder characterized by variable degrees of impaired insulin secretion and insulin resistance, it is likely that multiple genetic abnormalities at several loci are associated with diabetes-related phenotypes. These genes encode proteins that may predispose individuals to diabetes by altering beta cell function/insulin secretion or promoting cellular insulin resistance. Moreover, there are several possible biological pathways by which processed meat intake may interact with genetic variants to influence diabetes risk. Processed meats are rich in additives and preservatives, including sodium nitrate, which could influence diabetes risk. Nitrosamines are present in processed meats at manufacturing or formed by interactions of amino acids and nitrates within the body. Nitrosamines have been shown
to have a toxic effect on beta cells and may promote the development of diabetes [1-5]. Additionally, processed meats are high in advanced glycation end products (AGES). AGES are formed in the heating and processing of meats and have been shown to influence inflammation and oxidative stress, both risk factors for insulin resistance [1, 2, 22, 23]. Because many of the known diabetes genetic variants might affect beta cell function or insulin resistance, individuals who consume processed meat regularly may have a higher risk of diabetes if they carry these variants.

Using available diet and genetic data from studies that are part of the CHARGE GxE Nutrition Working Group, we propose to investigate associations of SNP*processed meat interactions in relation to diabetes-related phenotypes. We hypothesize that processed meat intake is associated with higher levels of fasting glucose/insulin, and that SNPS related to beta cell function interact with processed meat intake to influence diabetes-related phenotypes. To explore other possible mechanisms (other than beta cell function-nitrates) by which SNPs and dietary intake of processed meats might interact to influence diabetes-related traits, we will also look at SNPS associated with insulin resistance. Additionally, we will investigate the associations of SNP*unprocessed red meat interactions in relation to diabetes-related phenotypes. Because unprocessed red meats are not a major source of nitrates and most studies in the literature do not show a relationship between unprocessed red meats and diabetes phenotypes, we do not expect to find an interaction of unprocessed red meat intake with SNPS associated with beta cell function in relation to fasting glucose/insulin. If we find a positive association in the unprocessed red meat analyses, this may suggest that another biological pathway (rather than nitrates) may be driving the association.

References can be found on the last pages of this proposal.

5. Main Hypothesis/Study Questions:

Project Aims

Main Effects Models 1-3
Determine the magnitude of the association of processed meat intake, unprocessed red meat intake or combined processed meat and unprocessed red meat intake with diabetes-related phenotypes (fasting glucose, log-transformed fasting insulin) using multiple linear regression.
- Adjustment for covariates will be based on factors that have been shown to be associated with diet, insulin, or glucose, or of clinical interest, in hierarchical models. Variables of interest include demographic variables (age, sex, education, field center, population structure), health behaviors (smoking, alcohol use, physical activity), dietary factors (total calories, unprocessed red meat, fish (not fried—in cohorts where this can be ascertained, as defined in previous projects), vegetables (not including white potatoes or legumes—as defined in previous projects), fruit (not including fruit juice—as defined in previous projects), whole grains (as defined in previous projects), sugar-sweetened beverages (soda and sugar-sweetened artificially fruit flavored drinks—as defined in previous projects), nuts, saturated fat, and BMI.

Main Effects Model 4-5
Investigate if SNPS previously shown to be associated with beta cell function or insulin resistance (as defined by HOMA-B & HOMA-IR) are associated with diabetes-related phenotypes (fasting glucose, log transformed fasting insulin) using multiple linear regression.
We will consider 2 genetic risk scores (GRS) as the primary exposures of interest for these analyses. These scores will be created based on the 36 SNPS related to fasting glucose/HOMA-B (GRS-fg) and the 9 SNPS related to insulin resistance/HOMA-IR (GRS-IR) in previous GWAS. We expect the gene scores will be associated with fasting glucose and insulin because each of the SNPS of interest were discovered based on their association with diabetes-related phenotypes.

Interaction Models (6-9)
Investigate if SNPS previously shown to be associated with beta cell function or insulin resistance (as defined by HOMA-B & HOMA-IR) interact with processed meat intake or unprocessed red meat intake in relation to diabetes-related phenotypes (fasting glucose, log transformed fasting insulin) using multiple linear regression.

We will test the interaction of intake of processed meat or unprocessed red meat with the 2 GRS. As above, these scores will be created based on the 36 SNPS related to fasting glucose/HOMA-B (GRS-fg) and 9 SNPS related to insulin resistance/HOMA-IR (GRS-IR).

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).

Methods
Sample: Cohorts that are part of the CHARGE GxE Nutrition Working Group with measures of processed meat intake, unprocessed red meat intake, fasting glucose, fasting insulin, and genotype data without diabetes will be included in the analysis.

Exclusions: Participants with implausible dietary data (based on cohort-specific criteria), prevalent diabetes, missing genotype, diet or fasting glucose/insulin measures, or of non-European-descent will be excluded from analyses.

Dependent Variables:
(1) fasting glucose (mg/dl)
(2) log transformed fasting insulin (mg/dl)

Independent variables:
Meat intake will be determined based on participant responses to food frequency questionnaires, multiple 24-hour diet recalls, or 7-day food diaries. Processed meat and unprocessed red meat intake will be defined based on cohort-specific variables available, as described in the attached excel spreadsheet. The SNPS used in this analysis have been shown to be associated with beta cell function or insulin resistance in previous GWAS (see attached excel spreadsheet).

Main Effects Analyses Exposures
(1) processed meat intake (servings per day): in ARIC = hot dog/ sausage, salami/ bacon/ liver
(2) unprocessed red meat intake (servings per day): in ARIC = hamburger/ sandwich with meat/ main dish with meat
(3) total: unprocessed red meat + processed meat intake (servings per day) in ARIC = sum of above line items (servings/day)
(4) GRS-fg
(5) GRS-IR
Interaction Analyses Exposures

(6) GRS-fg*processed meat intake (number of risk alleles summed across 36 fasting glucose-related SNPS from previous GWAS of fasting glucose)
(7) GRS-IR*processed meat intake (number of risk alleles summed across 9 insulin resistant-related SNPS from previous GWAS of insulin resistance)
(8) GRS-fg*unprocessed red meat intake (number of risk alleles summed across 36 fasting glucose-related SNPS from previous GWAS of fasting glucose)
(9) GRS-IR*unprocessed red meat intake (number of risk alleles summed across 9 insulin resistant-related SNPS from previous GWAS of insulin resistance)

Analysis Plan
*These analyses will be done in a series of steps (1-3)

Step 1
Main Effects Analysis 1-- intake of processed meat with log transformed fasting insulin or fasting glucose—2 tests using multiple linear regression; p<0.025
- Model 1a: Covariates include sex, age (continuous), total energy intake (kcal/day, continuous), field center, population substructure
- Model 1b: Model 1a covariates, education (continuous), smoking (current former, never), drinks/week (continuous), physical activity (hours/week), unprocessed red meat intake (continuous), fish intake (continuous), fruit intake (continuous), vegetable intake (continuous), whole grain intake (continuous), sugar-sweetened beverages (continuous), nuts (continuous), saturated fat (continuous)
- Model 1c: Model 1b covariates + BMI (continuous)

Main Effects Analysis 2-- intake of unprocessed red meat with log transformed fasting insulin or fasting glucose—2 tests using multiple linear regression; p<0.025
- Model 2a: Covariates include sex, age (continuous), total energy intake (kcal/day, continuous), field center, population substructure
- Model 2b: Model 2a covariates, education (continuous), smoking (current former, never), drinks/week (continuous), physical activity (hours/week), processed meat intake (continuous), fish intake (continuous), fruit intake (continuous), vegetable intake (continuous), whole grain intake (continuous), sugar-sweetened beverages (continuous), nuts (continuous), saturated fat (continuous)
- Model 2c: Model 2b covariates + BMI (continuous)

Main Effects Analysis 3-- intake of (unprocessed red meat+ processed meat) intake with log transformed fasting insulin or fasting glucose—2 tests using multiple linear regression; p<0.025
- Model 3a: Covariates include sex, age (continuous), total energy intake (kcal/day, continuous), field center, population substructure
- Model 3b: Model 2a covariates, education (continuous), smoking (current former, never), drinks/week (continuous), physical activity (hours/week), fish intake (continuous), fruit intake (continuous), vegetable intake (continuous), whole grain intake (continuous), sugar-sweetened beverages (continuous), nuts (continuous), saturated fat (continuous)
- Model 3c: Model 2b covariates + BMI (continuous)

Main Effects Analysis 4--GRS-fg with fasting glucose or log transformed fasting insulin—2 tests using multiple linear regression; p<0.025
- Model 4: Covariates include sex, age (continuous), field center, population substructure
Main Effects Analysis 5-- GRS-IR with fasting glucose or log transformed fasting insulin—2 tests using multiple linear regression; p<0.025
  - Model 5: Covariates include sex, age (continuous), field center, population substructure

*If we find a similar effect size in the association of unprocessed red meat or processed meat with fasting glucose/log-transformed insulin, we will combine the exposures unprocessed red meat & processed meat for subsequent analyses (i.e.,—we will only have 2 interaction analyses instead of 4 interaction analyses)

Step 2
Interaction Analysis 1—GRS-fg*processed meat with log transformed fasting insulin or fasting glucose--2 tests using multiple linear regression; p<0.025
  - Model 1: Covariates include GRS-fg, processed meat, sex, age (continuous), field center, population substructure, total energy intake (kcal/day, continuous)

Interaction Analysis 2—GRS-IR*processed meat with log transformed fasting insulin or fasting glucose--2 tests using multiple linear regression; p<0.025
  - Model 2: Covariates include GRS-IR, processed meat, sex, age (continuous), field center, population substructure, total energy intake (kcal/day, continuous)

Interaction Analysis 3—GRS-fg*unprocessed red meat with log transformed fasting insulin or fasting glucose--2 tests using multiple linear regression; p<0.025
  - Model 3: Covariates include GRS-fg, unprocessed meat, sex, age (continuous), field center, population substructure, total energy intake (kcal/day, continuous)

Interaction Analysis 4—GRS-IR*unprocessed red meat with log transformed fasting insulin or fasting glucose--2 tests using multiple linear regression; p<0.025
  - Model 4: Covariates include GRS-IR, unprocessed meat, sex, age (continuous), field center, population substructure, total energy intake (kcal/day, continuous)

Step 3—(secondary analyses)
*If we find evidence of an interaction of GRS*processed meat or GRS*unprocessed red meat, in secondary analyses, we’d like to assess the interaction of each single SNP* processed meat or unprocessed red meat in relation to fasting glucose/log-transformed insulin to better understand potential biological mechanisms. If we do not find evidence of an interaction of GRS*processed meat or GRS*unprocessed red meat, step 3 will be skipped

Secondary Analysis 1—interaction between intake of processed meat and single SNPS (each SNP included in the GRS)—44 tests using multiple linear regression; p<0.0011
  - Model 1: Covariates include SNP of interest, processed meat, sex, age (continuous), field center, population substructure, total energy intake (kcal/day, continuous)

Secondary Analysis 2 —interaction between intake of unprocessed red meat and single SNPS (each SNP included in the GRS)—44 tests using multiple linear regression; p<0.0011
  - Model 2: Covariates include individual SNP of interest, unprocessed red meat, sex, age (continuous), field center, population substructure, total energy intake (kcal/day, continuous)

Data Sharing
For descriptive tables, each cohort to provide cohort-specific:
- sex (% female)
- age (mean, SD, median, minimum value, maximum value, interquartile range (IQR))
kcal/day (mean, SD, median, minimum value, maximum value, IQR)
smoking (% never, %former, % current)
alcohol (drinks/day) (mean, SD, median, minimum value, maximum value, IQR)
BMI (kg/m$^2$) (mean, SD, median, minimum value, maximum value, IQR)
education (years) (mean, SD, median, minimum value, maximum value, IQR)
physical activity (hours/week) (mean, SD, median, minimum value, maximum value, IQR)
unprocessed red meat intake (servings/day) (mean, SD, median, minimum value, maximum value, IQR)
processed meat intake (servings/day) (mean, SD, median, minimum value, maximum value, IQR)
saturated fat intake (% calories) (mean, SD, median, minimum value, maximum value, IQR)
fasting glucose (mg/dL) (mean, SD, median, minimum value, maximum value, IQR)
fasting insulin (mg/dL) (mean, SD, median, minimum value, maximum value, IQR)
frequency of effect allele for each of the 36 SNPS included in the GRS-fg and 9 SNPS included in the GRS-IR.

Each cohort to provide beta regression coefficient and robust standard errors for the following:

**Step 1**
1. Main Effects Analysis 1: Main Effects of processed meat with log transformed fasting insulin or fasting glucose
   - 1 exposure, 2 outcomes, 3 models → 6 regression coefficients + robust SE
2. Main Effects Analysis 2: Main Effects of unprocessed red meat with log transformed fasting insulin or fasting glucose
   - 1 exposure, 2 outcomes, 3 models → 6 regression coefficients + robust SE
3. Main Effects Analysis 3: Main Effects of combined unprocessed red meat and processed meat intake with log transformed fasting insulin or fasting glucose
   - 1 exposure, 2 outcomes, 3 models → 6 regression coefficients + robust SE
4. Main Effects Analysis 4: Main Effects of GRS-fg with fasting glucose or log transformed fasting insulin
   - 1 exposures, 2 outcomes, 1 model → 2 regression coefficients + robust SE
5. Main Effects Analysis 5: Main Effects of GRS-IR with fasting glucose or log transformed fasting insulin
   - 1 exposures, 2 outcomes, 1 model → 2 regression coefficients + robust SE

**Step 2**
6. Interaction Analysis 1: GRS-fg*processed meat with log transformed fasting insulin or fasting glucose
   - 1 exposure, 2 outcomes, 1 model → 2 regression coefficients + robust SE
7. Interaction Analysis 2: GRS-IR*processed meat with log transformed fasting insulin or fasting glucose
   - 1 exposure, 2 outcomes, 1 model → 2 regression coefficients + robust SE
8. Interaction Analysis 4: GRS-fg*unprocessed red meat with log transformed fasting insulin or fasting glucose
   - 1 exposure, 2 outcomes, 1 model → 2 regression coefficients + robust SE
(9) Interaction Analysis 5: GRS-IR*unprocessed red meat with log transformed fasting insulin or fasting glucose
- 1 exposure, 2 outcomes, 1 model → 2 regression coefficients + robust SE

Step 3
(10) Secondary Analysis 1: interaction between intake of processed meat and single SNPs
- 44 exposure, 2 outcomes, 1 model → 88 regression coefficients + robust SE

(11) Secondary Analysis 2: interaction between intake of unprocessed red meat and single SNPs (each SNP included in the GRS)
- 44 exposure, 2 outcomes, 1 model → 88 regression coefficients + robust SE

Summary/conclusion
Whether variation in genes related to beta cell function or insulin resistance influence the relationship of processed meat intake and diabetes-related phenotypes is largely unknown. We will assess the association of the interaction of SNPs known to be associated with beta cell function or insulin resistance and processed meat intake and unprocessed red meat intake with diabetes-related phenotypes. This investigation may reveal that genetic variation may influence the association of meat intake with diabetes-related phenotypes and provide clues to the mechanism underlying the association of dietary intake of processed or unprocessed red meat with diabetes traits.

<table>
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<tr>
<th>Nearest Gene</th>
<th>SNP</th>
<th>Chrom</th>
<th>Effect Allele</th>
<th>Mechanism</th>
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<tr>
<td>PROX1</td>
<td>rs340874</td>
<td>1</td>
<td>C</td>
<td>Gene expressed in the pancreas and human islet cells, and related to beta cell development [24]</td>
</tr>
<tr>
<td>G6PC2</td>
<td>rs560887</td>
<td>2</td>
<td>C</td>
<td>Encodes a protein (IGRP) that controls the set point for glucose-stimulated insulin secretion in the beta cells [32]</td>
</tr>
<tr>
<td>GCKR</td>
<td>rs780094</td>
<td>2</td>
<td>C</td>
<td>Encodes the regulatory protein glucokinase—which enhances insulin secretion from beta cells [33, 34]</td>
</tr>
<tr>
<td>ADCYS</td>
<td>rs11708067</td>
<td>3</td>
<td>A</td>
<td>Encodes adenylate cyclase 5, which is a catalyst in a process that results in transcription of the proinsulin gene and insulin secretion [24]</td>
</tr>
<tr>
<td>SLC2A2</td>
<td>rs11920090</td>
<td>3</td>
<td>T</td>
<td>Encodes GLUT2 that transports glucose into beta-cells &amp; stimulates insulin secretion [24]</td>
</tr>
<tr>
<td>GCK</td>
<td>rs4607517</td>
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<td>A</td>
<td>Encodes glucokinase; serves as the glucose sensor that controls the set point for insulin secretion [31, 35]</td>
</tr>
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<td>DGKB-TMEM195</td>
<td>rs2191349</td>
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<td>T</td>
<td>Gene expressed in the pancreas and human islet cells and influences insulin secretion [24]</td>
</tr>
<tr>
<td>SLC30A8</td>
<td>rs13266634</td>
<td>8</td>
<td>A</td>
<td>Encodes the ZnT-8 transporter responsible for transporting zinc into the insulin secretion granules within the beta cells; affects insulin storage &amp; secretion [31, 36]</td>
</tr>
<tr>
<td>GLIS3</td>
<td>rs7034200</td>
<td>9</td>
<td>A</td>
<td>Gene expressed in the pancreas and human islet cells, and related to beta cell development [24]</td>
</tr>
<tr>
<td>ADRA2A</td>
<td>rs10885122</td>
<td>10</td>
<td>G</td>
<td>Impairment of docking of insulin granule &amp; controls insulin release; beta cell dysfunction [24, 37, 38]</td>
</tr>
<tr>
<td>TCF7L2</td>
<td>rs7903146</td>
<td>10</td>
<td>T</td>
<td>Encodes transcription factors produced in the beta cell; a protein product of TCF7L2 (TCF4) contains the DNA binding domain GLP-1. GLP-1 stimulates insulin secretion in the pancreatic beta cell. TCF4 trans-activates the gene that encodes GLP-1. The gene works in the beta cell itself by modulating beta cell mass &amp; it may cause a defect in insulin processing [31]</td>
</tr>
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<td>MTNR1B</td>
<td>rs10830963</td>
<td>11</td>
<td>G</td>
<td>Mediates the inhibiting effect of melatonin on insulin secretion [39]</td>
</tr>
<tr>
<td>MADD</td>
<td>rs7944584</td>
<td>11</td>
<td>A</td>
<td>Contributes to beta cell mass and insulin secretion [24]</td>
</tr>
<tr>
<td>FADS1</td>
<td>rs174550</td>
<td>11</td>
<td>T</td>
<td>Involved in biosynthesis of unsaturated fatty acids. A product of this process enhances glucose-mediated insulin release &amp; beta-cell dysfunction [38] [24]</td>
</tr>
<tr>
<td>CRY2</td>
<td>rs11605924</td>
<td>11</td>
<td>A</td>
<td>Related to circadian rhythmicity, and associated with fasting glucose in humans [24]</td>
</tr>
<tr>
<td>Gene</td>
<td>SNP</td>
<td>Chrom</td>
<td>Effect</td>
<td>Allele</td>
</tr>
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<td>-----------</td>
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</tr>
<tr>
<td>FAM148B/C2CD4B</td>
<td>rs11071657</td>
<td>15</td>
<td>A</td>
<td>Gene expressed in the pancreas and human islet cells, but molecular mechanism of action is unknown [40]</td>
</tr>
<tr>
<td>THADA</td>
<td>rs7578597</td>
<td>2</td>
<td>C</td>
<td>Reduced beta cell mass [38]</td>
</tr>
<tr>
<td>ADAMTS9</td>
<td>rs4607103</td>
<td>3</td>
<td>T</td>
<td>Peripheral insulin resistance [38]</td>
</tr>
<tr>
<td>IGF2BP2</td>
<td>rs4402960</td>
<td>3</td>
<td>T</td>
<td>IFG2 mRNA binding protein; pancreatic development [31]</td>
</tr>
<tr>
<td>ZBED3</td>
<td>rs4457053</td>
<td>5</td>
<td>G</td>
<td>Unknown mechanism [38]</td>
</tr>
<tr>
<td>CDKAL1</td>
<td>rs7754840</td>
<td>9</td>
<td>C</td>
<td>Involved in the cyclin-dependent kinase 5 inhibitor pathway, and influence beta cell regeneration [31]</td>
</tr>
<tr>
<td>TP53INP1</td>
<td>rs896854</td>
<td>8</td>
<td>T</td>
<td>Unknown mechanism [38]</td>
</tr>
<tr>
<td>CDKN2A/B</td>
<td>rs10811661</td>
<td>9</td>
<td>T</td>
<td>Involved in the cyclin-dependent kinase 5 inhibitor pathway, and influence beta cell regeneration [31]</td>
</tr>
<tr>
<td>CDC123, CAMKID</td>
<td>rs12779790</td>
<td>10</td>
<td>A</td>
<td>Reduced beta cell mass [38]</td>
</tr>
<tr>
<td>HHEX</td>
<td>rs1111875</td>
<td>10</td>
<td>C</td>
<td>Encoded transcription factors produced in the beta cell and involved in pancreatic development [31]</td>
</tr>
<tr>
<td>KCNQ1</td>
<td>rs231362</td>
<td>11</td>
<td>G</td>
<td>Decreased incretin secretion [38]; influences expression of CDKN1C, a gene which regulates beta cell development [36]</td>
</tr>
<tr>
<td>CENTD2</td>
<td>rs1552224</td>
<td>11</td>
<td>A</td>
<td>Impaired beta cell function [38]</td>
</tr>
<tr>
<td>MTNR1B</td>
<td>rs1387153</td>
<td>11</td>
<td>T</td>
<td>Mediates the inhibiting effect of melatonin on insulin secretion [39]</td>
</tr>
<tr>
<td>PRC1</td>
<td>rs8042680</td>
<td>15</td>
<td>A</td>
<td>Unknown mechanism [38]</td>
</tr>
<tr>
<td>JAZF1</td>
<td>rs864745</td>
<td>7</td>
<td>A</td>
<td>Beta-cell dysfunction [41]</td>
</tr>
<tr>
<td>TSPAN8, LGR5</td>
<td>rs7961581</td>
<td>12</td>
<td>T</td>
<td>Possibly beta cell dysfunction [38]</td>
</tr>
<tr>
<td>IRS1</td>
<td>rs7578326</td>
<td>2</td>
<td>A</td>
<td>Related to beta cell function and mass [42]</td>
</tr>
<tr>
<td>HNF1B</td>
<td>rs757210</td>
<td>17</td>
<td>A</td>
<td>Beta-cell dysfunction [41]</td>
</tr>
<tr>
<td>KCNJ11</td>
<td>rs5219</td>
<td>11</td>
<td>T</td>
<td>Encodes the islet ATP-sensitive potassium channel Kir6.2, thereby regulating beta cell depolarization and the trigger of insulin release [31]</td>
</tr>
<tr>
<td>WFS1</td>
<td>rs4689388</td>
<td>C</td>
<td>C</td>
<td>Encodes wolframin, a protein that regulates calcium transport in the endoplasmic reticulum. Regulates pancreatic beta cell development and final beta cell mass [31]</td>
</tr>
<tr>
<td>HNF1A</td>
<td>rs7957197</td>
<td>12</td>
<td>T</td>
<td>Acts as transcription factor regulating pancreatic beta cell development and final beta cell mass [31]</td>
</tr>
</tbody>
</table>

**SNPS associated with insulin resistance/HOMA-IR in meta-analysis of GWAS [24]**

<table>
<thead>
<tr>
<th>Nearest Gene</th>
<th>SNP</th>
<th>Chrom</th>
<th>Effect</th>
<th>Allele</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCKR</td>
<td>rs780094</td>
<td>2</td>
<td>C</td>
<td>Encodes the regulatory protein glucokinase— which enhances insulin secretion from beta cells [33, 34]</td>
<td></td>
</tr>
<tr>
<td>IGF1</td>
<td>rs35767</td>
<td>15</td>
<td>G</td>
<td>Encodes insulin-like growth factor 1. Related to glucose homeostasis [24]</td>
<td></td>
</tr>
<tr>
<td>COBLL1/GRB14</td>
<td>rs7607980</td>
<td>2</td>
<td>T</td>
<td>Unknown mechanism</td>
<td></td>
</tr>
<tr>
<td>IRS1</td>
<td>rs2943634</td>
<td>2</td>
<td>C</td>
<td>Related to beta cell function and mass [42]</td>
<td></td>
</tr>
<tr>
<td>PDGFC</td>
<td>rs4691380</td>
<td>4</td>
<td>C</td>
<td>Unknown mechanism</td>
<td></td>
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<tr>
<td>PEPD</td>
<td>rs8182584</td>
<td>19</td>
<td>T</td>
<td>Unknown mechanism</td>
<td></td>
</tr>
<tr>
<td>PPP1R3B</td>
<td>rs4841132</td>
<td>8</td>
<td>A</td>
<td>May be involved in regulating glycogen synthesis in the liver and skeletal muscle [43]</td>
<td></td>
</tr>
<tr>
<td>LYPLAL1</td>
<td>rs2785980</td>
<td>4</td>
<td>T</td>
<td>Unknown mechanism</td>
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</tr>
<tr>
<td>UHRF1BP1</td>
<td>rs4646949</td>
<td>6</td>
<td>T</td>
<td>Unknown mechanism</td>
<td></td>
</tr>
</tbody>
</table>
7.a. Will the data be used for non-CVD analysis in this manuscript?

*Fasting glucose and insulin are the primary outcomes*

b. If Yes, is the author aware that the file ICTDER03 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

(This file ICTDER03 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?  Yes

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER03 must be used to exclude those with value RES_DNA = “No use/storage DNA”? Yes

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](http://www.cscc.unc.edu/ARIC/search.php)  Yes

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)?

*Examples of others from the CHARGE Nutrition working group:*

1534 “Interactions between whole grain intake and genotype with respect to fasting glucose concentrations in multiple cohorts within the CHARGE & MAGIC consortia”

1577 “Interactions between zinc intake and SNPs and their impact on fasting blood glucose levels in multiple cohorts within the CHARGE and MAGIC consortia”

1675 “Low density lipoprotein receptor related protein 1, fatty acids and anthropometric traits”

1656 “Genome-wide association analysis of macronutrient intake”

1738 “Interaction between a multi-factorial diet score and genetic loci for fasting glucose and insulin”

1779 “Meta-analysis: FTO and MC4R genes, Dietary Intakes and Obesity”

11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data?  Yes

*GWAS via STAMPEDE & GENEVA, #2006.03  
Interactions between Diet and Genes Related to Risk of Type II Diabetes, #2007.12*

11.b. If yes—is the proposal a primarily the result of an ancillary study (numbers 2007.12 and 2006.03)  
*ARIC is one of 10 cohort studies contributing data to the CHARGE/MAGIC-based meta-analysis. Since this work is a product of CHARGE which utilizes GWA data, ancillaries related to STAMPEDE & GENEVA are also acknowledged.*
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.

*The lead author is aware of, and will comply with, this stipulation.*

REFERENCES