1.a. Full Title: Association of polymorphisms in peptide-YY genes with body mass index, sex, and ethnicity

b. Abbreviated Title: Peptide-YY polymorphisms, BMI, and ethnicity

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

Writing Group Members: Kim Brownley, Kari E. North, Keri Monda, others welcome

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

First author: Kim Brownley, PhD
University of North Carolina-Chapel Hill
Department of Psychiatry
242 Medical School Wing C
CB# 7175
Chapel Hill, NC 27514
Phone: (919) 966-5276
Fax: (919) 966-0708
Email: kbrownle@med.unc.edu

Corresponding/senior author (if different from first author correspondence will be sent to both the first author & the corresponding author):

3. Timeline: 4-6 months

4. Rationale:

Nearly 65% of U.S. adults are overweight, more than 30% are obese and the prevalence of obesity is on the rise [1]. Non-Hispanic blacks are disproportionately affected, with black women suffering the highest rates of obesity (~50%) overall [2]. Compared with white women, black women gain weight at an earlier age, lose less weight with traditional diet and exercise modification, and are especially vulnerable to obesity-related cardiovascular and metabolic complications such as hypertension and diabetes [3-5]. Reasons for increased risk of obesity and its co-morbid conditions in black women are not fully understood; however, recent work by our group [6] and others
suggests differences in gastrointestinal peptides involved in short-term appetite regulation may play a role.

Peptide-YY (PYY) is a naturally occurring peptide secreted in the distal gastrointestinal tract in response to food intake [9-11]. In circulation, PYY is found in two main forms, PYY3-36 and PYY1-36. PYY3-36, the predominant circulating form, is a selective agonist for the Y2 receptor (Y2R) [12], which is highly expressed on neuropeptide Y (NPY) neurons in the arcuate nucleus of the hypothalamus, a key brain area involved in homeostasis and regulation of food intake [13]. Peripheral injection in rats and mice inhibits food intake, and infusion of postprandial concentrations of PYY3-36 significantly decreases food intake and subjective ratings of hunger in humans [14, 15]. Endogenous fasting and postprandial levels of PYY3-36 are significantly lower in obese compared with lean individuals [14], in women relative to men [16] and in blacks compared with whites [7, 8]. Our group has shown that PYY levels are significantly lower in obese black women compared with normal weight black women and with obese and normal weight white women [17].

In a recent study [18], two rare PYY sequence variants (L73P and IVS2 + 32delG) and three rare Y2R missense mutations (L40F, F87I, and A172T) were observed in 101 hyperphagic, severely obese subjects but not in 100 normal weight white control subjects. Two common single nucleotide polymorphisms (SNPs), R72T and IVS3 + 68C>T (in PYY) were in tight linkage disequilibrium but showed no association with BMI in a large white population. In addition, two SNPs (585T>C and 936T>C, in the Y2R) were in tight linkage disequilibrium and men who were homozygous for the rarer variant had significantly lower BMI and waist-to-hip ratio (WHR).

Another study [19] sequenced PYY gene coding exons and splice sites in a large cohort of extremely obese (n=379) and lean (n=378) individuals. In total, three rare non-synonymous variants were identified, only one of which, PYY Q62P, exhibited familial segregation with body mass. Through serendipity, previous studies based on cell culture revealed this precise variant to have altered receptor-binding selectivity in vitro. Using mouse peptide injection experiments, it was further demonstrated that while the wild-type PYY peptide reduced food intake, the mutant PYY 62P had an insignificant effect in reducing food intake in vivo. Taken together, these results support the assertion that rare sequence variants within PYY can influence human susceptibility to obesity.

A third study [20] in 83 extremely obese Pima Indians identified three untranslated region (UTR) SNPs, one missense (Ala172Thr) substitution, and two silent substitutions in the Y2R coding region. Eight additional SNPs in the 5′ UTR of Y2R were identified from public databases and genotyped in 489 full-heritage unrelated adult Pima Indians (362 severely obese and 127 non-diabetic, non-obese subjects) for association analysis. The PYY variants were not associated with obesity, whereas four variants from two haplotype blocks in Y2R were marginally associated (p = 0.054-0.067) with obesity. However, when restricted to men (n = 167, 100 obese and 67 lean), the PYY variants and two SNPs in Y2R that were in complete linkage disequilibrium were significantly associated with severe obesity (p = 0.001 and p = 0.002, respectively). These findings suggest that the PYY-Y2R pathway may influence body weight through a sex-specific mechanism, but this finding requires confirmation in other populations.

A fourth study [21] found a significant association for a 5′ variant (rs6857715) in the NPY2R gene with both severe adult obesity (p=0.002) and childhood obesity
This significant association was further supported by a pooled allelic analysis of all obese cases (adults and children, n=928) vs. the control subjects (n=938) (p=0.0004, odds ratio=1.3, 95% CI 1.1–1.5). Quantitative trait analysis of BMI and WHR was performed and significant association was observed for SNP rs1047214 in NPY2R with an increase in WHR in the severely obese children (co-dominant model p=0.005, recessive model p=0.001). Association was also observed for an intron 3 variant (rs162430) in the PYY gene with childhood obesity (p=0.04). No significant associations were observed for PPY variants. Only one rare variant in the NPY2R gene (C-5641T) was not found in lean individuals and this was found to co-segregate with obesity in one family.

Taken together, the above studies suggest that one or more variants in PYY and Y2R may influence body mass index. However, whether effect modification by gender or sex is important is still unclear. This work follows from our earlier work (ARIC MS #1368) for the initial analyses on the genome-wide SNP data (~1,000,000 SNPs) available on the ARIC sample through its collaboration with the Broad Institute.

5. Main Hypothesis/Study Questions:

Study Question: Are genetic variants in PYY and Y2R gene variants associated with obesity related traits?

Specific Aim 1: To evaluate the association between polymorphisms in PYY and Y2R genes and obesity related traits.

Specific Aim 2: To test the interaction effect between SNPs in PYY and Y2R and sex on baseline adiposity traits.

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

Subjects and Sample size:
The usual DNA consent restriction and missing data exclusion criteria will be used. These analyses will be restricted to the white participants in ARIC. We plan to expand our analyses to African Americans as the genotyping data become available. Use of GWAS data in African-Americans will follow CARE procedures.

Definitions and treatment of variables
Genotype: Polymorphisms within or near the PYY or Y2R gene. See Table 1 (below) for listing of individual SNPs.

Table 1. List of imputed PYY and NPY2R SNPs

<table>
<thead>
<tr>
<th>SNP ID</th>
<th>Gene</th>
<th>Coded allele</th>
<th>Noncoded allele</th>
<th>Imputation ratio</th>
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Phenotype measures: BMI, waist circumference (WC), and waist-hip ratio (WHR) will be defined as quantitative traits. Outcome variables will likely be transformed into z-scores prior to analyses.

Covariates: Models will be minimally adjusted for age, sex, field center, and smoking status. Principal components will be controlled for in models to account for population substructure.

Representation of Genetic Variants from the previous literature: While the rare genetic variants previously interrogated in the literature will not be available (the Affymetrix chip did not contain these variants and accurate imputation is not currently available for rare variants), there are some important variants from the previous literature that we will capture. For example, proxies (rs > 0.80) for rs458924, rs162431, and rs61612861 from the Siddiq et al. paper are available [21]. In addition, both rs1058046 and rs2700320 were measured and interrogated in previous work [19, 21].

Analysis strategy / statistical analysis
Additive models will be used to estimate the main effect of each SNP on the obesity related phenotype. Secondly, the interaction between SNPs and sex on adiposity traits will be considered. Models will contain both the main effects of SNP and sex as well as
the interaction term for SNP*sex. We will seek replication of results within our existing collaboration with the CHARGE Consortium. Beta coefficients and p-values as well as other necessary data (strand, etc) will be shared with collaborators. No primary data will be shared. Meta-analysis based on both p-values, as well as potentially effect estimates, will be run.

Multiple testing: We will control for multiple testing using the Bonferroni correction on an overall $\alpha=0.05$.

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.
References


