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Candidate Genes from Molecular Pathways Related to Appetite Regulatory Neural Network and Adipocyte Homeostasis and Obesity: the Coronary Artery Risk Development in Young Adults (CARDIA) Study

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Abstract

Background—Appetite regulatory neural network and adipocyte homeostasis molecular pathways are critical to long-term weight maintenance. Genetic variation in these pathways may explain variability of obesity in the general population.

Aims—The associations of four genes in these pathways (leptin (LEP), leptin receptor (LEPR), neuropeptide Y2 receptor (NPY2R) and peptide YY (PYY)) with obesity-related phenotypes were examined among participants in the CARDIA Study. Participants were 18-30 years old upon recruitment (1985-86). Weight, BMI and waist circumference were measured at baseline and at years 2, 5, 7, 10, 15, and 20. Genotyping was conducted using tag SNPs that characterize the common pattern of genetic variation in these genes. Race-specific linear regression models were used to examine associations of the various SNPs with obesity-related measurements, controlling for sex and age. The overall association based on the 7 repeated anthropometric measurements was tested with GEE. False discovery rate was used to adjust for multiple testing.

Results—In African-Americans, SNPs across the LEP gene demonstrated significant overall associations with obesity-related phenotypes. The associations between rs17151919 in LEP gene with weight tended to increase with time (SNP × time interaction p=0.0193). The difference in

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weight levels associated with each additional minor allele ranged from 2.6 kg at entry to 4.8 kg at year 20. Among African-American men, the global tests indicated that SNPs across the NPY2R gene were also associated with waist circumference measurements (p=0.0462). In Caucasians, SNPs across the LEP gene also tended to be associated with weight measurements (p=0.0471) and rs11684664 in PYY gene was associated with obesity-related phenotypes (p= 0.010-0.026) in women only.

Conclusions—Several SNPs in the LEP, NPY2R and PYY but not the LEPR genes were associated with obesity-related phenotypes in young adults. The associations were more prominent for the LEP gene and among African-Americans.

Keywords

association study; genes; weight; BMI; waist circumference

Introduction

In Western societies the epidemic of obesity over the past 25 years represents a major threat to the health of the public. Increased incidence of obesity in young adults is of particular concern, because it is likely to lead to long-term consequences. Obesity is influenced by lifestyle characteristics as well as by a clear genetic component that may exert influence over time and in response to environmental exposures (Hunt et al. 2002; Mutch & Clement 2006; Bauer et al., 2009). However, the identification of the specific genetic factors underlying the susceptibility to obesity is far from complete (Hofker & Wijmenga 2009).

Adipocyte homeostasis and appetite regulatory neural networks are important pathways for body weight regulation. Several studies have reported a strong relationship between leptin gene expression and obesity in humans and animals (Maffei et al., 1995; Considine et al. 1996; Patel et al., 2008;). In addition, leptin resistance associated with the expression of leptin receptors has been proposed as a potential mechanism for the development of human obesity and may lead to obesity-associated disorders such as: hyperinsulinism, diabetes, hypertension and cardiovascular diseases (Sader et al., 2003; Patel et al., 2008).

Mutations in LEP and LEPR genes resulting in obesity, insulin resistance, and diabetes in animals (Zhang et al., 1994; Chen et al., 1996) and in humans (Montague et al. 1997; Clement et al., 1998), have been described. However, these mutation are rare and the causes of the genetic predisposition for the majority of the human obesity cases remain unexplained. Several common single nucleotide polymorphisms (SNPs) involved in the regulation of LEP and LEPR have been investigated and associations with circulating leptin concentrations and other obesity phenotypes have been reported (Hager et al., 1998; Li et al., 1999; Jiang et al., 2004). Various neuropeptides, such as neuropeptide Y (NPY) and peptide YY (PYY) that are expressed within the hypothalamus and together regulate energy balance (Friedman & Halaas 1998), have been implicated in the development of obesity. Both human and animal studies have demonstrated that NPY2R and PYY have an important role in appetite regulation and in the development of obesity (Ding et al., 2005; Ma et al., 2005; Siddiq et al., 2007; Campbell et al. 2007; Ahituv et al., 2006; Shih et al., 2009).

The secular trend in obesity over the past two decades may have resulted from secular environmental effects, including changes in dietary and physical activity habits, and/or from age-limited developmental effects of genes. The CARDIA cohort has been followed with multiple examinations over the past two decades and thus provides a unique cohort to examine whether common genetic variation in candidate genes from adipocyte homeostasis and appetite regulatory neural network pathways, contributes importantly to the development of obesity phenotypes during young adulthood.

Methods

The details of the Coronary Artery Risk Development in Young Adults (CARDIA) study have been described elsewhere (Friedman et al., 1988). In brief, CARDIA is a population-based study, initiated in 1985, to investigate the evolution of cardiovascular risk factors in a large cohort of African-American and Caucasian adults aged 18–30 years. At baseline (1985–1986), 5115 eligible participants aged 18–30 were recruited (51% response rate) from 4 urban areas including Chicago, IL; Birmingham, AL; Minneapolis, MN; and Oakland, CA with similar representations with respect to age (18–24, 25–30 years), ethnicity (African American, Caucasian) and education (high school or less, greater than high school) groups. Participants were examined at baseline and re-examined at six follow-up visits, year 2 (1987–1988), year 5 (1990–1991), year 7 (1992–1993), year 10 (1995–1996), year 15 (2000–2001) and year 20 (2005-2006) with retention rates of 90%, 86%, 81%, 79% 74% and 72%, respectively.

The CARDIA database includes standardized repeated measures of demographic and socioeconomic information, lifestyle characteristics, metabolic, physiological and obesity phenotypes. Height and weight were measured at all clinic examinations while participants stood wearing light clothing and without shoes. Waist circumference was measured at the minimum abdominal girth midway between the iliac crest and the xiphoid process with the participant standing. Height was measured to the nearest 0.5 cm using a vertical ruler and weight was measured to the nearest 0.2 kg by using a calibrated balance beam scale. Body mass index was calculated as the ratio of weight (kg) to standing height (m) squared (kg/m^2).

For this report, eligible participants were 2,129 African-Americans and 2,173 Caucasians (overall 4,302) who consented to isolation of genomic DNA at year 10 and for whom genotype data were available from the year 20 examination. Single nucleotide polymorphisms (SNPs) in the Leptin (LEP), Leptin Receptor (LEPR), Peptide YY (PYY), and Neuropetide Y2 Receptor (NPY2R) genes were chosen based on linkage disequilibrium (LD) data, as well as publicly available genotype and haplotype information (The International HapMap Consortium 2005; Hinds et al. 2005). Within each race, a minimal set of tagSNPs was selected based on pairwise LD relationships (r^2), as described by Carlson et al (2004). Briefly, bins of SNPs are created based on a specified r^2 threshold; and then one SNP is selected to represent the remainder of SNPs in that bin. In this study, we used an r^2 threshold of 0.8 and minimum allele frequency of 0.05 Moreover, polymorphisms of known or hypothesized functional significance based on published information were also selected.

Genotyping for the 37 SNPs, 7 in LEP, 16 in LEPR, 5 in PYY and 9 in NPYR2 was performed using the TaqMan assay (Applied Biosystems, Foster City, CA) as previously described (Fornage et al., 2004). Primer and probes are available from the authors upon request. Polymorphism genotyping in the CARDIA study adheres to a rigorous quality control (QC) program, which includes barcode identification of samples, robotic sample handling, and blind replicate genotype assessment on 5% of the total sample.

It is well-established that the prevalences of cardiovascular-related risk factors vary between African-American and Caucasian population groups (Hutchinson et al., 1997; Sharma et al., 2004). The underlying genetic architecture may also vary between these different populations. Studies have shown that if the frequencies of both marker alleles and phenotype-traits differ between the admixed populations, results may be vulnerable due to confounding by population substructure. Therefore, all analyses were conducted separately for African-Americans and Caucasians.

Statistical Methods

The following dependent variables were included in the analyses: weight, BMI and waist circumference. Departures of allele frequencies from the Hardy-Weinberg equilibrium were tested to identify potential bias in the genotypic distributions. For each gene, we used multivariate linear models to estimate the long-term longitudinal and the cross-sectional year specific associations between genotypes and anthropometric phenotypes. For each SNP a single predictor coded as 0, 1, or 2 (representing the number of copies of the minor allele) was created. These predictors were then assessed with the linear models in order to estimate the change in phenotype levels, after controlling for other characteristics. Age as a continuous variable and sex coded as a dichotomous variable (male/female) were used as covariates.

The generalized estimating equation (GEE) method (Liang & Zeger 1986) was used to examine the longitudinal data in the 20-year follow-up for long-term association between genotypes and dependent anthropometric phenotypes. A typical linear regression model used is as follows:

$$y_{it}=\beta_0+\beta_1 SNP_i+\beta_2 t+\beta_3 SNP_i * t+\beta_4 Cov1_{it}+\beta_5 Cov2_{it}+ \ldots\ldots +e_{it}$$

where t = 0, 2, 5, 7, 10, 15, 20, y_{it} = the obesity-related phenotype for the ith individual at year t, Cov_{it} = covariate for the ith individual and year t and, e_{it} = error term. The SNP is time independent while the covariates are time-dependent. Coefficient β_1 represents the covariate-adjusted mean change in phenotype levels associated with one additional minor allele at year 0. Coefficient β_2 is the annual change in the dependent variable for homozygous for the common allele (SNP=0) and β_3 measures the annual change associated with one additional minor allele. Coefficients β4 and β5 are used for the relevant covariates. In our initial analyses, β_2 and β_3 were set to zero and in these reduced models β_1 presents the overall differences in obesity-related phenotypes among participants with different genotypes over a period of 20 years. In additional models, estimation of β_2 and β_3 were used to examine the interaction of SNP effect with time on dependent variables.

A WALD test which tests several regression parameters simultaneously was used to examine the 20-years overall associations of all available SNPs in a specific gene with the outcome variables (Diggle P.J., et al. 2002). Global test p-values are presented, in the cases where one or more of the SNPs within a gene had shown long-term significant association with the outcome variable. For those SNPs found to be significantly associated with phenotypic changes over the 20-year period of observation, we tested whether observed genetic effects were homogeneous or changed significantly throughout the follow-up period.

Estimates are presented as regression coefficients (βs) with 95% confidence intervals (CIs). All statistical analyses were carried out with the use of STATA version # (Stata Corp, College Station, TX). Two-tailed *P* values < 0.05 were considered significant and false discovery rate (FDR) was calculated to account for multiple statistical testing (Benjamini & Hochberg 1995). The generalized estimating equation (GEE) method was used to examine the longitudinal data for association between genotypes and dependent anthropometric phenotypes. As a result, imputation is not necessary to deal with missing data (Twisk & de Vente 2002). This is due to the fact that subjects with incomplete data are not excluded from the analyses; and, if one or more of the measurements are missing for a particular participant, the remaining available measurements are used in the analyses. In addition, there were no differences in baseline and follow-up measurements of obesity-related phenotypes between the CARDIA participants who did and did not have available genetic information.

Results

Baseline characteristics of CARDIA participants are summarized in Table 1. Caucasian men weighed more for their height and had a considerably larger waist circumference than Caucasian women, but African-American women had a greater BMI levels than African-American men. Table 2 summarizes the distribution characteristics of the obesity-related phenotypes during 20 years of follow-up. All 3 phenotypes increased considerable over the period and the changes were more prominent among African-Americans. At year 20, a considerably higher proportion of African-Americans (51%) than Caucasians (27.5%) was classified as obese (BMI ≥30.0).

Allele frequencies for the four investigated candidate genes are shown in Suppl. Table. Allele frequencies at the LEP and PYY genes tended to differ between African-Americans and Caucasians. In contrast, for most of the SNPs in the LEPR and NPY2R genes, the allele frequencies were similar in both race groups.

Genotype distributions for 6 SNPs (4 in African-Americans and 2 SNPs in Caucasians) deviated from Hardy–Weinberg equilibrium. When adjusted for multiple comparisons, only the departure from HWE for rs17376826 in the NPY2R gene was significant in African-Americans (*P*-value ≤0.00068, the critical value after Bonferroni correction for the number of multiple tests conducted). In addition, none of our "top hits" in African-Americans and Caucasians were among the 6 SNPs not in Hardy–Weinberg equilibrium, before the adjustment for multiple comparisons, and thus could not affect the interpretation of our findings. Next, we used a GEE model to examine the long-term associations between multiple SNPs at the four candidate genes and anthropometric variables (Table 3). In African-Americans, the global tests showed that the LEP gene was significantly associated with weight and waist. ($P = 0.0092 – 0.0189$). For the multivariate GEE model that included all leptin SNPs simultaneously, two (rs4731427 and rs17151919) were significantly associated with all three anthropometric measures. For example, the long-term differences in mean levels associated with each additional minor allele in rs17151919, were 3.9 kg for weight, 1.12 kg/m^2 for BMI, and 2.2 cm for waist circumference. In African-American men only, another leptin SNP was significantly associated with waist circumference. The long-term difference in waist levels associated with each additional minor allele in rs28954369, was 9.0 cm in men and -0.4 cm in women. In African-American men, the global test of the long-term associations between genotypes in the NPY2R gene tended to be significant for waist circumference (p=0.0462). Four single SNPs were associated with weight and waist circumference and two were significantly associated with BMI. In addition, rather than testing for associations with BMI over time, we also examined the genetic associations between all common tag SNP with weight after an adjustment for height. In African-Americans, the later model for LEP gene was somewhat improved (Global p = 0.0382). In Caucasians, both models showed similar long-term SNP associations.

In Caucasians, the global tests of the long-term association between all common tag SNP genotypes in the LEP gene was significant for weight ($P = 0.047$). Among women, the associations were highly significant for waist circumference ($P = 0.0001$). The multivariate GEE indicated that rs2167270 and rs17151913 were significantly associated with weight (a long-term, 20-year, increase of 1.8 kg and decrease in 5.1 kg was associated with each additional minor allele in rs2167270 and rs17151913, respectively). An additional SNP (rs28954369) was associated with all three anthropometric measures among women only. Another PYY SNP (rs1684664) was associated with all three anthropometric measures in Caucasian women. A single NPY2R SNP (rs17376826) was associated with BMI and waist variables and the association tended to homogeneous across gender groups. The models

testing the global and the SNP specific associations between the genes and weight adjusted for height showed similar results to the models testing the associations with BMI over time.

Table 4 presents the sex- and age-adjusted single SNP associations over time ($P \leq 0.05$) for those SNPs which were found to be globally associated with the anthropometric phenotypes either for the total group or for one of the gender groups. Among African-Americans, the rs17151919 and rs4731427 SNPs in the LEP gene were associated with weight at all visits. The difference in weight levels associated with each additional minor allele in rs17151919 ranged from 2.6 kg at entry to 4.8 kg at year 20. (*P* value for rs17151919 × time interaction = 0.0193). The rs4731427 SNP was consistently associated with waist circumference over time. Yet, the overall association from GEE analysis had only borderline significance with waist circumference (P=0.0136, q=0.095). Both SNPs were not consistently associated with BMI over time.

Among Caucasians, a single SNP (rs2167270) in the LEP gene was consistently associated with all three anthropometric measures across the visits during the 20-year study period, however, after a correction for multiple comparisons the statistical significance was lost.

As shown in Table 2 weight increased significantly over the 20-year period. Weight-change was considerably greater in African-Americans (average = 16.6 kg in males and 18.2 kg in females) than among Caucasians (average = 13.2 kg in males and 11.8 kg in females). Our results showed that both in African-Americans and Caucasians (in men and women), global tests of the associations between genotypes in the LEP and LEPR genes were not significantly associated with 20-year changes in weight measurements. Yet, several single SNPs in LEPR (rs3828033 and rs1137101a in African-Americans and rs3828033 and rs6696954 in Caucasians) were associated with weight-change.

Leptin concentrations were measured on a sub-sample of 500 participants at Year 2 and Year 10. In African-Americans, none of the LEP SNPs gene was associated with levels and longitudinal change of leptin. In Caucasians, one single SNP (rs2167270) was associated with levels at Year 2 (β=2.523; 95%CI 0.41-4.64). This SNP was consistently associated with all three anthropometric measures across the visits and after further adjustment for BMI the association between is rs2167270 and Year 2 leptin levels was not significant. Interestingly, several SNPs in LEPR were significantly associated with leptin levels. For example, in African-Americans, rs3828033 and rs1137101 were associated with leptin levels measured at Year 2, Year 10 and with long-term average value. In Caucasians, none of the SNPs in LEPR were associated with leptin levels measured at Year 2, but 2 SNPs (rs312145690 and rs4655802) were significantly associated with leptin levels at Year 10 and with long-term average values. These associations between LEPR SNPs and leptin levels did not change after further adjustment for BMI.

Discussion

In this prospective study, we found long-term associations between multiple SNPs at the leptin gene and multiple anthropometric measures that reflect obesity. Among African-Americans, two LEP SNPs (rs4731427 and rs17151919) were significantly associated with weight, BMI and waist circumference; and, for weight the effects tended to increase over time. Among Caucasians, long-term associations between two common SNPs in LEP gene (rs2167270 and rs17151913) were significantly associated with weight; and, among women another LEP SNP (rs28954369) was found to be associated with all three anthropometric measures.

A number of studies have previously shown that common SNPs in the LEP gene are associated with obesity (Hager et al., 1998; Li et al., 1999; Jiang et al., 2004). In the Family

Heart Study, several LEP SNPs, including A19G (rs2167270) and a common haplotype (49%), were associated with obesity and BMI (Jiang et al., 2004). The SNPs in this haplotype are predicted to modify transcription-factor binding sites, and, thus, any one of them may change the transcription of LEP. Similar to our findings, the association between A19G SNP and obesity/BMI in the Family Heart Study was limited to men.

Other studies have reported an association between G-2548A in the promoter of LEP and obesity (Li et al., 1999; Jiang et al., 2004; Maffei et al., 1995; Le Stunff et al., 2000; Mammes et al., 2000). This observation is in agreement with the association between the -2548 SNP and leptin levels observed in a sample of unrelated French Caucasians (Mammes et al., 2000) and with the observed association of low leptin levels and weight gain in a study group of Pima Indians (Ravussin et al., 1997). Yet, the result was not confirmed in other populations (Hodg et al., 1998). If the observed association is confirmed, it would mean that the G-2548A substitution either is located in a regulatatory site specific for LEP and not yet identified, or in LD with a mutation creating a regulating site in regions not yet explored. For example, in the study reported by Li et al. (1999), there was strong LD between A19G (rs2167270) and -2548 SNP, however, in another study the LD between these sites was relatively low (Li et al., 1999). Racial differences in degree of LD and in allelic frequencies, such as in the A19G SNP (Li et al., 1999), may explain in part the heterogeneity in the strength of associations across studies.

Some studies have observed associations between SNPs in the LEPR gene and obesity phenotypes (Chagnon et al., 2000). However, in accordance with our lack of findings, results of two meta-analyses reported no significant associations between polymorphisms of LEPR and obesity levels (Paracchini et al., 2005).

Interestingly, in the present study, among both African-Americans and Caucasians, the association between SNPs within the LEP gene and anthropometric measures tended to increase during the 20 years of follow-up. Age and gender may serve as aggregate measures for a number of environmental factors, such as smoking and hormonal levels, that might be expected to change over the individual's lifetime. The long exposure to environmental variables may influence the expression of genes involved in determining phenotype levels. A number of studies have shown that longitudinal changes in body weight are associated with polymorphisms in various genes (van Rossum et al., 2002; Fox et al., 2005). In addition, numerous studies have demonstrated significant associations between longitudinal changes in body weight and subsequent risk of CHD (Lissner et al., 1991; Hamm et al., 1989).

In the present study a large increase in weight over the 20-year period was observed, both in African-Americans and Caucasians. While, no associations between polymorphisms of LEPR and weight levels were observed, several SNPs in LEPR (rs3828033 and rs1137101a in African-Americans and rs3828033 and rs6696954 in Caucasians) were associated with weight change. Recently, an examination of longitudinal changes in body composition of Australian women has shown that the LEPR gene may only exert a small effect on body mass or composition at any given time, however, over several years, the effects of genetic variation in this gene on adiposity become apparent (de Silva et al., 2001). Our findings suggest that LEPR may influence changes in weight over 20-year period.

In the present investigation, among African-Americans men, global tests between genotypes in NPY2R gene tended to be significantly associated with waist circumference. Four SNPs (rs17304901, rs2234759, rs10212868 and rs12641982) were associated with weight and waist variables and two (rs10212868 and rs12641982) were associated with BMI. Among

Caucasians, a single NPY2R SNP (rs17376826) was associated with BMI and waist variables.

Among French Caucasian obese and non-obese non-diabetic controls, a significant association has been shown between a 5′ variant (rs6857715) in the NPY2R gene and obesity (Siddiq et al., 2007). In this study, rs6857715 was in strong LD with several other 5′ SNPs including with rs2234759 (D′=0.946) that in the present report is associated with obesity among African-American men. Additional studies have also reported associations between SNPs located in the 5′ region or in the coding area of the NPY2R gene and obesity. For example, in Pima Indians, the 5′ SNP rs6857715 was associated with obesity (Ma et al., 2005). Additionally, in a large study of Danish Caucasian subjects other common variants (rs12649641, rs2342676 and rs6857530) in the 5′ region of NPY2R were associated with obesity (Torekov et al., 2006). Results from in silico analysis showed that rs2342676 SNP disrupts the site GATA-binding factor 3 (GATA-3). It has been shown that this binding factor has an essential role in brain development and is abundantly expressed in the central nervous system and thus this binding site disruption might influence the promoter activity of the NPY2R leading to less appetite inhibition and risk for development of obesity (Asnagli et al., 2002). In addition, common SNPs in exon 2 (585T>C and 936T>C) that were in strong LD, were found to be associated with obesity in Caucasians (Lavebratt et al., 2006).

Among Caucasian women in this report, a single SNP (rs1684664) in PYY gene was associated with the anthropometric measures. In French Caucasian obese and non-obese subjects, rs162430 located in intron 6 of the PYY gene was associated with childhood obesity (Siddiq et al., 2007), and in Pima Indians, this SNP was found to be associated only with severe obesity among men (Ma et al., 2005). In the present study, rs162430 SNP was not related with the anthropometric measures. This could be due to the fact that obesity is not just purely defined by weight, BMI and waist but it is a complex phenotype and it could be due to an influence of the variant on other obesity traits.

Other studies have examined the association between two common polymorphisms in the PYY gene and obesity phenotypes (Lavebratt et al., 2006; Torekov et al., 2005; Hung et al., 2004). The Arg72Thr polymorphism located five residues downstream of the proteolysis-amidation site essential for proper C-terminal processing to mature PYY (rs1058046) and the IVS3 + 68C>T in intron 3 are in tight linkage disequilibrium. In a population-based case-control study of overweight and obesity among Danish Caucasians, the PYY Arg72 variant was associated with overweight and type 2 diabetes and those found to be homozygous for Arg72Arg had lower circulating PYY levels (Torekov et al., 2005). In contrast, studies among individuals aged 40 to 65 years in the U.K. (Hung et al., 2004) and among Swedish men suggested no association between PYY rs1058046 and obesity (Lavebratt et al., 2006).

In summary, the present study suggests that common LEP sequence variants are associated with obesity-related phenotypes in the general population. In addition, the associations between SNPs within the LEP gene and anthropometric measures tended to increase over the 20 years of follow-up, suggesting either an increasing expression of the obese phenotype over time or possible gene × environment interaction effects in the determination of these phenotypes. These interactions will need to be examined in larger sample studies. Our study also suggests that NPY2R and PYY genes may have modest effects on obesity-related phenotypes. Additional studies are required to confirm the present study's associations and to further elucidate the pathways through which variation in these genes exert their effects on the development of obesity in the general population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Baseline Characteristics of CARDIA Participants by Race and Gender

	Caucasians		African-Americans	
Characteristic [a]	**Men (n = 1016)**	**Women (n = 1157)**	**Men (n = 891)**	**Women (n = 1238)**
Age, y	25.5 (3.4)	25.5 (3.4)	24.3(3.7)	24.5 (3.9)
Education ≤ high school, %	6.4	4.8	16.1	10.1
Diabetes, %	0.5	0.4	0.6	0.9
Hypertension, %	3.9	1.6	5.7	5.0
Hypercholesterolemia, %	4.7	3.6	4.5	4.4
Current smoking, %	25.2	25.9	35.7	30.3
Physical activity score	509.7 (296.1)	404.5 (263.2)	523.7 (341.9)	276.2 (224.0)
Alcohol intake (ml/day)	18.2 (23.7)	8.9 (15.8)	17.4 (30.5)	5.1 (12.2)
Weight (kg)	77.2 (12.7)	62.9 (12.7)	77.0 (15.3)	69.7 (18.6)
Body mass index, (kg/m^2)	24.3 (3.5)	23.1 (4.4)	24.5 (4.4)	26.0 (6.6))
Waist (cm)	83.0 (8.8)	72.0 (9.4)	80.7 (10.2)	77.0 (13.4)
Systolic blood pressure (mm Hg)	114.3 (10.3)	104.8 (9.3)	115.7 (10.60	108.3 (10.0)
Diastolic blood pressure (mm Hg0	70.8 (9.4)	66.3 (8.4)	70.7 (10.5)	67.5 (9.6)
Total cholesterol (mg/dl)	176.6 (33.5)	176.1 (31.3)	176.4 (34.8)	179.2 (33.3)
Triglyceride (mg/dl)	88.4 (66.40	70.5 (42.1)	70.1 (40.6)	63.7 (32.8)
LDL-C (mg/dl)	111.9 (31.0)	105.8 (28.9)	108.9 (32.8)	111.5 (31.5)
HDL-C, (mg/dl)	47.0 (11.0)	56.2 (13.0)	53.5 (13.5)	55.0 (12.7)
Glucose (mg/dl)	84.7 (9.9)	81.3 (13.8)	83.7 (16.3)	80.2 (14.3)
Insulin (uU/ML)	9.3 (6.0)	9.3 (6.8)	10.8 (8.7)	13.4 (9.1)

[a] Values are expressed as percent or as mean (SD).

Table 2

Obesity-related phenotypes of CARDIA participants by Race and Visit

	Caucasians				African-Americans			
Visit [a]	**N**	**Weight (kg)**	**BMI (kg/m^2)**	**Waist (cm)**	**N**	**Weight (kg)**	**BMI (kg/m^2)**	**Waist (cm)**
Baseline	2173	69.3 (14.5)	23.7 (4.1)	77.1 (10.7)	2129	72.7 (17.6)	25.4 (5.8)	78.5 (12.3)
Year 2	2096	71.3 (15.3)	24.3 (4.4)	79.1 (11.6)	1937	75.0 (18.5)	26.3 (6.2)	80.9 (13.0)
Year 5	2076	73.3 (16.4)	24.9 (4.7)	80.5 (12.2)	1886	78.8 (20.2)	27.5 (6.7)	83.7 (13.5)
Year 7	1972	75.4 (17.3)	25.5 (5.1)	82.2 (13.3)	1781	81.4 (20.7)	28.2 (6.9)	86.0 (14.8)
Year 10	1983	76.9 (18.1)	26.0 (5.4)	83.3 (13.8)	1887	84.0 (21.7)	29.1 (7.2)	88.1 (15.2)
Year 15	1905	80.3 (19.6)	27.2 (5.9)	87.5 (14.8)	1704	88.2 (22.4)	30.5 (7.4)	91.8 (15.8)
Year 20	1870	81.9 (19.5)	27.9 (6.5)	89.8 (15.0)	1615	90.1 (23.0)	31.3 (7.6)	94.5 (15.9)

[a] Values are expressed as mean and SD.

Table 3

Longitudinal Association Analyses (GEE) between Multiple SNPs in Candidate Gene and Anthropometric Phenotypes in Young Adults

			African-Americans - Total Group	African-American Men	African-American Women
Variable	**Gene**	**SNP**	**$\beta^{a,b}$ 95% CI^{a} (for each additional copy of Variant allele)**	**$\beta^{a,c}$ 95% CI^{a} (for each additional copy of Variant allele)**	**$\beta^{a,c}$ 95% CI^{a} (for each additional copy of Variant allele)**
Weight	LEP	rs4731427	2.959 (0.95 – 4.97)	3.808 (0.85 – 6.76)	2.427 (-0.31 – 5.16)
		rs17151919	3.872 (1.18 – 6.56)	3.332 (-0.53 – 7.19)	4.044 (0.43 – 7.66)
	Global P-Value		0.0092	0.0118	0.1890
	NPY2R	rs17304901	1.822 (-1.23 – 4.88)	4.643 (0.16 – 9.13)	0.221 (-3.82 – 4.26)
		rs2234759	3.485 (-1.77 – 8.74)	6.866 (0.27 – 13.46)	1.707 (-5.54 – 8.96)
		rs10212868	-3.982 (-9.12 – 1.16)	-7.429 (-14.00 - -0.86)	-2.170 (-9.16 – 4.82)
		rs12641982	4.159 (-1.08 – 9.40)	8.255 (1.32 – 15.20)	1.857 (-5.09 – 8.81)
	Global P-Value		0.6292	0.0693	0.9956
BMI	LEP	rs4731427	0.837 (0.18 - 1.5)	0.982 (0.14 - 1.83)	0.784 (-0.19 - 1.76)
		rs17151919	1.115 (0.2 - 2.04)	0.997 (-0.09 - 2.09)	1.145 (-0.17 - 2.46)
	Global P-Value		0.0590	0.0525	0.3719
	NPY2R	rs10212868	-1.415 (-3.05 - 0.22)	-2.114 (-3.93 - -0.30)	-1.021 (-3.37 - 1.33)
		rs12641982	1.294 (-0.39 - 2.98)	2.087 (0.15 - 4.03)	0.844 (-1.54 - 3.23)
	Global P-Value		0.6777	0.1556	0.9698
Waist	LEP	rs28954369	0.93 (-3.37 - 5.23)	8.975 (1.47 - 16.48)	-0.357 (-5.18 - 4.47)
		rs4731427	2.095 (0.72 - 3.47)	2.489 (0.49 - 4.49)	1.886 (-0.004 - 3.78)
		rs17151919	2.235 (0.48 - 3.99)	2.115 (-0.47 - 4.70)	2.23 (-0.12 - 4.58)
	Global P-Value		0.0189	0.0128	0.1795
	LEPR	rs4655802	1.343 (0.08 - 2.61)	0.431 (-1.31 - 2.17)	1.814 (0.05 - 3.58)
	Global P-Value		0.5380	0.9154	0.4029
	NPY2R	rs17304901	0.900 (-1.18 - 2.98)	3.369 (0.50 - 6.23)	-0.555 (-3.38 - 2.27)
		rs2234759	2.394 (-1.11 - 5.89)	4.387 (0.53 - 8.24)	1.416 (-3.55 - 6.39)
		rs10212868	-2.980 (-6.41 - 0.45)	-5.353 (-9.20 - -1.50)	-1.742 (-6.54 - 3.06)
		rs12641982	3.013 (-0.50 - 6.52)	5.447 (1.39 - 9.51)	1.744 (-3.09 - 6.58)
	Global P-Value		0.6269	0.0462	0.9845

			African-Americans - Total Group	African-American Men	African-American Women
Variable	**Gene**	**SNP**	**$\beta^{a,b}$ 95% CI[a] (for each additional copy of Variant allele)**	**$\beta^{a,c}$ 95% CI[a] (for each additional copy of Variant allele)**	**$\beta^{a,c}$ 95% CI[a] (for each additional copy of Variant allele)**
			Caucasians - Total Group	Caucasian Men	Caucasian Women
Variable	Gene	SNP	$\beta^{a,b}$ 95% CI[a] (for each additional copy of Variant allele)	$\beta^{a,c}$ 95% CI[a] (for each additional copy of Variant allele)	$\beta^{a,c}$ 95% CI[a] (for each additional copy of Variant allele)
Weight	LEP	rs2167270	1.797 (0.36 – 3.23)	2.880 (0.92 – 4.84)	0.904 (-1.15 – 2.95)
		rs17151913	-5.102 (-10.09 - -0.11)	-4.302 (-12.51 – 3.90)	-5.707 (-10.81 - -0.60)
		rs28954369	-1.081 (-2.68 – 0.52)	0.548 (-1.80 – 2.89)	-2.47 (-4.64 - -0.29)
	Global P-Value		0.0471	0.0932	0.1541
	PYY	rs1684664	0.804 (-0.30 – 1.91)	-0.330 (-1.99 – 1.33)	1.692 (0.20 – 3.18)
	Global P-Value		0.5890	0.4921	0.4050
BMI	LEP	rs2167270	0.435 (-0.04 - 0.91)	0.756 (0.20 - 1.31)	0.169 (-0.56 - 0.90)
		rs28954369	-0.498 (-1.0 - 0.01)	0.183 (-0.48 - 0.85)	-1.070 (-1.82 - -0.32)
	Global P-Value		0.1701	0.1461	0.0931
	NPY2R	rs17376826	-0.743 (-1.46 - -0.03)	-0.815 (-1.65 - 0.02)	-0.657 (-1.79 - 0.48)
	Global P-Value		0.6086	0.1598	0.9423
	PYY	rs1684664	0.371 (0.17 - 0.73)	-0.035 (-0.49 - 0.42)	0.687 (0.16 - 1.21)
	Global P-Value		0.3384	0.3466	0.2342
Waist	LEP	rs2167270	0.958 (-0.1 - 2.02)	2.021 (0.63 - 3.41)	0.068 (-1.48 - 1.62)
		rs17151913	-3.06 (-6.97 - 0.86)	-1.29 (-7.15 - 4.56)	-5.049 (-10.05 - -0.05)
		rs28954369	-0.653 (-1.81 - 0.50)	0.792 (-0.83 - 2.41)	-1.854 (-3.48 - -0.23)
	Global P-Value		0.2078	0.1430	<0.0001
	NPY2R	rs17376826	-1.597 (-3.13 - -0.06)	-1.711 (-3.81 - 0.39)	-1.45 (-3.69 - 0.79)
	Global P-Value		0.4290	0.3307	0.9635
	PYY		0.650 (-0.15 - 1.44)	-0.234 (-1.34 - 0.87)	1.322 (0.20 - 2.44)
	Global P-Value	rs1684664	0.5754	0.5354	0.3032

[a] All available SNPs in specific gene were included in the model and regression coefficient (β) and 95% confidence interval (95% CI) are presented for those found to be significantly associated with the anthropometric variables. Regression coefficients represent overall differences in obesity-related phenotypes between genotypes in 20 years for each additional copy of the minor allele, relative to subjects homozygous for the more common allele.

[b] Estimates are adjusted for sex and age.

[c] Estimates are adjusted for age.

Table 4

Associations between Candidate Gene SNPs and Anthropometric Variables in Young Adults

					African-Americans -Total Group			African-American Men	African-American Women
Trait	**Gene**	**SNP**	**Year of Measurement**	**Frequency[a] (%)**	**β[b,c] 95% CI[b] (for each additional copy of Variant allele)**	***P* Value**	**FDR q Value**	**β[b,d] 95% CI[b] (for each additional copy of Variant allele)**	**β[b,d] 95% CI[b] (for each additional copy of Variant allele)**
Weight	LEP	rs17151919	0	8.73	2.65 (0.83, 4.49)	0.0045	0.0312	2.31 (-0.32, 4.94)	2.84 (0.34, 5.34)
			2		2.68 (0.65, 4.70)	0.0096	0.0478	2.51 (-0.44, 5.47)	2.74 (-0.01, 5.48)
			5		2.71 (0.46, 4.97)	0.0182	0.0810	1.50 (-1.90, 4.91)	3.34 (0.35, 6.34)
			7		3.24 (0.86, 5.63)	0.0078	0.0271	3.11 (-0.43, 6.65)	3.26 (0.05, 6.46)
			15		4.13 (1.48, 6.79)	0.0023	0.0160	2.98 (-1.18, 7.13)	4.83 (1.37, 8.30)
			20		4.81 (1.99, 7.62)	0.0008	0.0057	4.97 (0.08, 9.86)	4.73 (1.29, 8.17)
			β estimated from GEE		3.12 (0.96, 5.27)	0.0046	0.0319	2.54 (-0.54, 5.61)	3.42 (0.52, 6.33)
		rs4731427	0	15.61	1.71 (0.27, 3.15)	0.0199	0.0695	1.95 (-0.01, 3.91)	1.55 (-0.51, 3.60)
			2		2.01 (0.41, 3.61)	0.0137	0.0478	2.21 (0.02, 4.40)	1.86 (-0.40, 4.11)
			5		2.06 (0.28, 3.85)	0.0231	0.0810	2.94 (0.39, 5.51)	1.50 (-0.98, 3.91)
			7		2.71 (0.84, 4.58)	0.0044	0.0271	4.27 (1.65, 6.90)	1.52 (-1.09, 4.14)
			10		3.50 (1.57, 5.42)	0.0004	0.0026	4.59 (1.82, 7.37)	2.68 (0.03, 5.33)
			15		2.64 (0.54, 4.74)	0.0139	0.0485	7.24 (0.11, 6.45)	2.14 (-0.68, 4.95)
			β estimated from GEE		2.24 (0.54, 3.88)	0.0097	0.0341	2.99 (0.39, 5.59)	1.70 (-0.54, 3.93)
BMI	LEP	rs17151919	15	8.73	1.00 (0.13, 1.87)	0.0242	0.1691	0.78 (-0.4, 1.96)	1.10 (-0.12, 2.33)
			20		1.12 (0.19, 2.05)	0.0179	0.1251	1.31 (-0.09, 2.72)	1.01 (-0.22, 2.24)
		rs4731427	7	15.61	0.67 (0.04, 1.29)	0.0357	0.2497	1.02 (0.27, 1.77)	0.40 (-0.53, 1.33)
			10		0.99 (0.35, 1.63)	0.0025	0.0173	1.08 (0.28, 1.88)	0.92 (-0.02, 1.87)
			β estimated from GEE		0.57 (0.02, 1.13)	0.0422	0.2125	0.66 (-0.07, 1.4)	0.51 (-0.28, 1.30)
Waist	LEP	rs17151919	0	8.73	1.85 (0.12, 3.57)	0.0356	0.1244	2.33 (-0.12, 4.79)	1.50 (-0.87, 3.86)
			20		2.15 (0.20, 4.10)	0.0306	0.2139	2.47 (-0.97, 5.92)	1.97 (-0.39, 4.33)
			β estimated from GEE		1.48 (0.09, 2.88)	0.0365	0.1278	1.56 (-0.51, 3.62)	1.43 (-0.43, 3.28)
		rs4731427	2	15.61	1.42 (0.30, 2.53)	0.0129	0.0902	1.76 (0.31, 3.2)	1.16 (-0.46, 2.79)

					African-Americans -Total Group			African-American Men	African-American Women
Trait	**Gene**	**SNP**	**Year of Measurement**	**Frequency[a] (%)**	**β[b,c] 95% CI[b] (for each additional copy of Variant allele)**	***P* Value**	**FDR q Value**	**β[b,d] 95% CI[b] (for each additional copy of Variant allele)**	**β[b,d] 95% CI[b] (for each additional copy of Variant allele)**
			5		1.67 (0.48, 2.85)	0.0058	0.0403	2.22 (0.59, 3.86)	1.28 (-0.37, 2.94)
			7		1.84 (0.50, 3.19)	0.0072	0.0506	2.44 (0.63, 4.24)	1.39 (-0.54, 3.33)
			10		2.12 (0.76, 3.48)	0.0023	0.0159	2.10 (0.19, 4.00)	2.14 (0.24, 4.04)
			15		1.60 (0.12, 3.09)	0.0346	0.2419	2.03 (-0.24, 4.29)	1.26 (-0.71, 3.23)
			β estimated from GEE		1.48 (0.30, 2.65)	0.0136	0.0952	1.81 (0.04, 3.58)	1.24 (-0.32, 2.81)
					Caucasians - Total Group			Caucasian Men	Caucasian Women
Trait	Gene	SNP	Year of Measurement	Frequency[a] (%)	β[b,c] 95% CI[b] (for each additional copy of Variant allele)	*P* Value	FDR q Value	β[b,d] 95% CI[b] (for each additional copy of Variant allele)	β[b,d] 95% CI[b] (for each additional copy of Variant allele)
Weight	LEP	rs17151913	β estimated from GEE	0.3	3.12 (0.96, 5.27)	0.0046	0.0319	2.54 (-0.54, 5.61)	3.42 (0.52, 6.33)
		rs2167270	0	33.0	0.81 (0.004, 1.61)	0.0490	0.3430	1.01 (-0.19, 2.20)	0.63 (-0.46, 1.72)
			2		1.08 (0.20, 1.95)	0.0159	0.1112	1.50 (0.23, 2.78)	0.72 (-0.48, 1.91)
			5		1.04 (0.09, 2.00)	0.0322	0.2255	1.58 (0.21, 2.95)	0.59 (-0.73, 1.92)
			7		1.51 (0.47, 2.56)	0.0044	0.0310	2.08 (0.64, 3.52)	1.01 (-0.49, 2.51)
			10		1.39 (0.29, 2.49)	0.0131	0.0918	1.77 (0.26, 3.27)	1.07 (-0.52, 2.65)
			15		1.59 (0.36, 2.81)	0.0112	0.0781	2.34 (0.62, 4.06)	0.98 (-0.76, 2.72)
			20		1.50 (0.29, 2.72)	0.0490	0.1048	1.86 (0.17, 3.56)	1.22 (-0.50, 2.94)
			β estimated from GEE		1.23 (0.25, 2.20)	0.0135	0.0942	1.67 (0.31, 3.04)	0.85 (-0.53, 2.24)
BMI	LEP	rs2167270	2	36.04	0.30 (0.02, 0.58)	0.0372	0.1300	0.40 (0.04, 0.77)	0.21 (-0.20, 0.62)
			7		0.47 (0.13, 0.80)	0.0069	0.0290	0.59 (0.19, 1.00)	0.35 (-0.18, 0.88)
			10		0.39 (0.03, 0.75)	0.0326	0.2282	0.48 (0.05, 0.91)	0.32 (-0.24, 0.88)
			15		0.47 (0.07, 0.88)	0.0219	0.1531	0.70 (0.21, 1.19)	0.28 (-0.34, 0.90)
			β estimated from GEE		0.339 (0.02, 0.66)	0.0357	0.1251	3.69 (0.69, 6.70)	0.26 (-0.23, 0.74)
		rs28954369	2	8.29	-0.58 (-1.08, -0.08)	0.0239	0.1300	-0.34 (-0.99, 0.31)	-0.77 (-1.51, -0.03)
			5		-0.66 (-1.21, -0.12)	0.0173	0.1212	-0.11 (-0.82, 0.6)	-1.10 (-1.91, -0.29)
			7		-0.81 (-1.41, -0.21)	0.0083	0.0290	-0.27 (-1.01, 0.47)	-1.26 (-2.18, -0.34)

					African-Americans -Total Group			African-American Men	African-American Women
Trait	**Gene**	**SNP**	**Year of Measurement**	**Frequency[a] (%)**	**β[b,c] 95% CI[b] (for each additional copy of Variant allele)**	***P* Value**	**FDR q Value**	**β[b,d] 95% CI[b] (for each additional copy of Variant allele)**	**β[b,d] 95% CI[b] (for each additional copy of Variant allele)**
			β estimated from GEE		-0.57 (-1.03, -0.11)	0.0158	0.1104	-0.14 (-0.75, 0.47)	0.26 (-0.23, 0.74)
Waist	LEP	rs2167270	7	36.04	0.94 (0.15, 1.73)	0.0192	0.1346	1.15 (0.12, 2.19)	0.75 (-0.42, 1.93)
			10		0.84 (0.01, 1.66)	0.0461	0.3229	1.02 (-0.06, 2.1)	0.68 (-0.55, 1.9)
			15		1.03 (0.11, 1.95)	0.0273	0.1909	1.26 (0.00, 2.52)	0.85 (-0.47, 2.17)
			20		0.95 (0.02, 1.87)	0.0441	0.2114	1.29 (0.04, 2.53)	0.66 (-0.68, 2.01)
			β estimated from GEE		0.73 (0.03, 1.42)	0.0396	0.2599	3.69 (0.69, 6.70)	0.51 (-0.49, 1.51)

[a] Frequency of the minor allele.

[b] Regression coefficient (β) and 95% confidence interval (95% CI) are for each additional copy of the minor allele, relative to subjects homozygous for the more common allele. Regression coefficients from GEE represent overall differences in obesity-related phenotypes between genotypes in 20 years.

[c] β estimates are adjusted for sex and age.

[d] β estimates are adjusted for age.