Variants in the Adiponectin Gene and Serum Adiponectin: The Coronary Artery Development in Young Adults (CARDIA) Study

Christina L. Wassel[1], **James S. Pankow**[2], **David R. Jacobs Jr**[2,3], **Michael W. Steffes**[4], **Na Li**[5], and **Pamela J. Schreiner**[2]

[1]Department of Family and Preventive Medicine, University of California San Diego, La Jolla, California, USA

[2]Department of Epidemiology, University of Minnesota, Minneapolis, Minnesota, USA

[3]Department of Nutrition, University of Oslo, Oslo, Norway

[4]Department of Lab Medicine and Pathology, University of Minnesota, Minneapolis, Minnesota, USA

[5]Department of Biostatistics, University of Minnesota, Minneapolis, Minnesota, USA.

Abstract

Circulating adiponectin is involved in the atherosclerotic process and has been associated with cardiovascular disease as well as obesity, insulin resistance, metabolic syndrome, and type 2 diabetes. The adiponectin gene (*ADIPOQ*) encodes the circulating protein adiponectin and affects its expression. Only a small proportion of all known *ADIPOQ* polymorphisms have been investigated in relation to circulating adiponectin concentrations. Using data from 3,355 African-American and white men and women aged 33–45 at the year 15 examination from the Coronary Artery Development in Young Adults (CARDIA) Study the association between 10 single-nucleotide polymorphisms (SNPs) within *ADIPOQ* and serum adiponectin was examined using linear regression. SNPs were chosen based on a tagSNP approach. Models were stratified by self-reported race to control for population stratification, and Bonferroni corrected for multiple comparisons. *ADIPOQ* SNPs rs17300539 ($P < 0.0001$), rs182052 ($P = 0.0013$), rs822393 ($P = 0.0005$), rs9882205 ($P = 0.0001$), and rs3774261 ($P = 0.0001$) were strongly associated with serum adiponectin concentrations in whites. In general, there was a dose–response relationship of adjusted mean adiponectin concentrations across genotypes. Only one SNP, rs17300539 was marginally associated with serum adiponectin concentrations ($P = 0.0087$) in African Americans. Significant interactions were found between waist and rs182052 ($P = 0.0029$) and between rs9882505 and smoking ($P = 0.001$) in whites. Many *ADIPOQ* SNPs have not yet been examined, and additional studies are needed to determine whether these may be functional variants.

INTRODUCTION

Adiponectin is a 247-amino acid, 30 kDa protein secreted by adipocytes that circulates at high concentrations in the blood (1). Adiponectin is involved in the atherosclerotic process by stimulating the production of nitric oxide in endothelial cells (2) and suppressing the expression of cellular adhesion molecules by inhibiting endothelial nuclear factor-κB signaling through the activation of cAMP protein kinase C (3–6). It also inhibits the proliferation and migration of smooth muscle cells by binding to platelet derived growth factor and promotes plaque stability in more advanced stages of atherosclerosis by increasing the expression of a tissue inhibitor of matrix metal-loproteinases in macrophages (1). A number of studies have evaluated the association between circulating adiponectin and coronary heart disease or cardiovascular disease outcomes with some showing higher levels of adiponectin associated with decreased risk (7–11) whereas other studies show an increased risk (12–14). There is some evidence that adiponectin concentrations may relate to the stage of cardiovascular disease; higher adiponectin levels may be an attempt to slow progression of the disease (15) and may account for discrepant findings of these studies. It is well established that adiponectin has also been linked to obesity, insulin resistance, type 2 diabetes, and the metabolic syndrome which are risk factors for cardiovascular disease (16).

The adiponectin gene (*ADIPOQ*) encodes the circulating protein adiponectin and is expressed primarily in the adipose tissue of various organs, but it is expressed in vascular tissue as well (17). Only a small proportion of all known *ADIPOQ* polymorphisms have been investigated in relation to circulating adiponectin concentrations and it is likely that functional variants responsible for these observed associations have yet to be discovered or studied (18). Several studies have examined the association of single-nucleotide polymorphisms (SNPs) and haplotypes of *ADIPOQ* with circulating adiponectin concentrations, but these studies have had mixed results and have concentrated on a small number of SNPs, usually within one ethnic group (19–24). A review and meta-analysis by Menzaghi *et al.* (18) note that although evidence for the association of a few *ADIPOQ* SNPs with circulating adiponectin is strong, there is still much variation in *ADIPOQ* that has not been examined.

Thus, this study examined the association of 10 SNPs in *ADIPOQ* chosen by a tagSNP method with serum adipo nectin in whites and African Americans from Coronary Artery Risk Development in Young Adults (CARDIA) study. Given circulating adiponectin's likely involvement in the pathophysio logy of adiposity, interactions of *ADIPOQ* SNPs with BMI, waist circumference, as well as age, sex, and smoking status were evaluated.

METHODS AND PROCEDURES

Participants

The CARDIA study is a prospective cohort study examining the determinants and trends in risk factors of heart disease development starting in young adulthood. Between 1985 and 1986 5,115 black and white men and women aged 18–30 years were recruited from four US centers—Birmingham, AL; Chicago, IL; Minneapolis, MN; and Oakland, CA. Details of inclusion and exclusion criteria can be found elsewhere (25). Follow-up examinations

occurred during 1987–1988 (year 2), 1990–1991 (year 5), 1992–1993 (year 7), 1995–1996 (year 10), and 2000–2001 (year 15), with 74% of the surviving cohort participating in the year 15 examination. The CARDIA participants attending the year 15 clinic visit consented to isolation of genomic DNA from a blood sample obtained at the year 10 and year 15 examinations. A total of 3,355 year 15 participants had serum adiponectin assayed as part of the Young Adult Longitudinal Trends in Antioxidants (YALTA) ancillary study and comprised the total sample size for this study.

ADIPOQ SNP selection and genotyping

Polymorphisms spanning *ADIPOQ* were selected from the International HapMap Project database (HapMap Phase I release) based on the estimated pairwise linkage disequilibrium (LD), r^2, between all common SNPs in HapMap, i.e., SNPs with minor allele frequency >10%, as described by Carlson *et al.* (26). Briefly, the algorithm bins SNPs for which pairwise LD exceeds a threshold r^2 (0.80 in this case). For each racial group (Centre d'Etude du Polymorphisme Humain and Yoruban populations), tagSNPs were selected from each bin so as to constitute a minimal set of highly informative markers while minimizing redundant data. In addition, tagSNPs with potential functional relevance were included where available. When a full set of tagSNPs was not available due to poor coverage in HapMap, additional SNPs were selected from the literature. Through this process, *ADIPOQ* SNPs rs17300539, rs182052, rs822393, rs822395, rs2036373, rs9882205, rs2241766, rs1501299, rs3774261, rs17366743, rs4686804, and rs1063539 were chosen to be genotyped. Two of these SNPs, rs1501299 and rs4686804, could not be designed in the multiplex assays, leaving 10 total SNPs that were genotyped.

The selected *ADIPOQ* tagSNPs were genotyped using a multiplex matrix-assisted laser desorption/ionization time-of-flight mass spectrometry method (Sequenom, San Diego, CA). Polymorphism genotyping in the CARDIA study adheres to a rigorous quality control program, which includes barcode identification of samples, robotic sample handling, and blind replicate genotype assessment on 5% of the total sample. The geno-type call rate was 96.7%, with a maximum missing per SNP of 8.62%.

Data collection

Information on gender, race, age, and smoking status was collected via interview and questionnaire at the year 15 examination. Smoking was categorized into current vs. never/former. Weight was measured to the nearest 0.2 pound, rounding down, using a calibrated balance beam scale. BMI was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured once to the nearest 0.5 cm with a Gulick Plus II measurement tape, in a location laterally midway between the iliac crest and the lowest lateral portion of the rib cage and anteriorly midway between the xiphoid process of the sternum and the umbilicus. Blood was drawn by venipuncture and stored as serum at −70 °C. Serum adiponectin was measured via radioimmunoassay using a polyclonal rabbit antibody and purified recombinant adiponectin standards (Linco Research, St Charles, MO). The assay had a range of 0.20–40 mg/l and an interassay coefficient of variation of 7–9%.

Statistical analysis

Pairwise LD (r^2) was calculated among the SNPs and LD plots were generated using Haploview V4.0 (27). Hardy–Weinberg equilibrium tests were conducted using the exact test within each race group, and SNPs with substantial deviation from Hardy–Weinberg equilibrium ($P < 0.001$) were excluded. Baseline characteristics for each ethnic group were compared across groups by t-test, χ^2-test or Wilcoxon test as appropriate.

Linear regression models were used to assess the association of SNPs with natural log transformed serum adiponectin. Analyses were stratified by self-reported race to control for population stratification. Associations of each SNP with serum adiponectin were evaluated first by two degree of freedom tests in models adjusting for age, sex, and field center. Additional adjustment for smoking, waist circumference, and BMI was performed. African-American models were also additionally adjusted for African ancestry to further control for population stratification. Unadjusted and adjusted geometric means for each genotype of the SNP were examined for individual SNPs with two degree of freedom $P < 0.05$. A Bonferroni correction was used to adjust for multiple comparisons within each race group, $a = 0.05/10 = 0.005$.

Interactions of *ADIPOQ* SNPs with age, sex, BMI, waist circumference, and smoking status were examined on an additive scale. Interactions were not corrected for multiple testing. With the exception of sex and smoking status, all covariates in interactions with *ADIPOQ* were modeled as continuous variables. All analyses were conducted using SAS V9.1 (SAS Institute, Cary, NC).

RESULTS

All SNPs were in Hardy–Weinberg equilibrium ($P > 0.001$) except for rs9882205 among African Americans with a $P < 0.0001$ (**Supplementary Table S1** online), so this SNP was eliminated from further analysis in African Americans. The LD plots showed little LD among the SNPs in either race group (**Figure 1**). As estimation of haplotypes in the presence of low LD can result in high levels of haplotype inaccuracy (28), estimation of haplotypes was not attempted. African Americans and whites significantly differed on age, gender, smoking status, waist circumference, BMI, and serum adipo nectin concentrations at the year 15 examination (**Table 1**).

Figure 2 shows the results of single SNP association tests for both race groups and all outcomes adjusted for age, sex, and center. In some cases (SNPs rs2241766 and rs1063539 in African Americans, and rs17366743 in whites) where the rare homozygote group had <10 participants, this group was combined with the heterozygote group in order to obtain more stable estimates of association. Among whites, rs17300539, rs182052, rs822393, rs9882205, and rs3774261 were strongly significantly associated with serum adiponectin concentrations with $P < 0.005$ or –log(P value) of 2.3 (**Figure 2**). There was some indication of associations of rs822395 and rs2036373 with serum adiponectin, although these associations did not reach the $P < 0.005$ Bonferroni threshold of significance (**Figure 2**). Unadjusted and adjusted geometric mean adiponectin concentrations among whites are shown in **Table 2**. For most of the significant SNPs, there appeared to be a dose– response relationship across

the genotypes. In particular, rs17300539, rs9882205, and rs3774261 had the largest between genotype differences in mean adiponectin concentrations. Further adjustment for waist circumference, BMI, and smoking did not substantially change these results (data not shown). The amount of variance explained (R^2) with only age, sex, and center in the model was 21%. Adding the five SNPs that were significantly associated with adiponectin in whites, 24% of the variance in adiponectin was accounted for.

Rs17300539 was marginally associated with serum adipo nectin in African Americans in models adjusted for age, sex, and center, $P = 0.0087$. The geometric mean adiponectin concentration for those with the AG genotype was 9.5 mg/l (95% CI (7.8, 11.5)) whereas those with the GG genotype had a mean of 7.3 mg/l (95% CI (7.0, 7.5)). No African-American participants had the AA genotype of rs17300539. The amount of variance explained using age, sex, center, and percent African ancestry was 9%; adding rs17300539 resulted in 11% of the variation in adiponectin levels explained. Further adjustment for percent African ancestry, waist circumference, BMI, and smoking did not substantially change these results (data not shown). No other statistically significant SNP associations were observed among African Americans.

Among whites, there was a significant interaction of waist and rs182052 in their association with serum adiponectin, $P = 0.0029$. Those with the AA genotype had 15.6% lower (95% CI (−21.3%, −9.4%) in serum adiponectin per standard deviation increase in waist circumference, while those with the AG genotype had a 22.8% lower (−25.4%, −20.1%) and those with the GG genotype had a 16.9% lower (−19.7, −14.0) serum adiponectin concentrations. There was also a significant interaction of rs9882205 and smoking among whites ($P = 0.001$). Smokers with the GG genotype had a geometric mean adiponectin concentration of 11.1 mg/l (95% CI (10.7, 11.5)), whereas those with the AG and AA genotypes had significantly lower mean adiponectin concentrations, 8.7 mg/l (95% CI (7.9, 9.6)) and 6.5 mg/l (95% CI (5.1, 8.2)), respectively. No other interactions of *ADIPOQ* SNPs and age, sex, BMI, or waist circumference were significant.

DISCUSSION

In this study, SNPs rs17300539, rs182052, rs822393, rs9882205, and rs3774261 within *ADIPOQ* were strongly associated with serum adiponectin concentrations in whites. In general, there was a dose–response relationship of mean adiponectin concentrations across genotypes. This study adds to the growing body of literature suggesting that rs17300539 is strongly associated with serum adiponectin levels in whites (21,22), and confirms the likely importance of four other SNPs associated with adipo nectin levels in whites that had previously been identified (22). Confirmatory studies such as the present study are necessary to ensure that the associations observed are true rather than spurious.

We identified only one SNP, rs17300539, in African Americans that had a marginally significant association with serum adiponectin. This is the first large scale study, to our knowledge, to examine the association of *ADIPOQ* SNPs with serum adiponectin levels in African Americans. Most SNPs associated with serum adiponectin levels in whites were not associated with serum levels in African Americans, with the exception of rs17300539, which

had a marginal association. It is possible that this SNP is a functional variant or is in LD with another SNP that is a functional variant among both African Americans and whites. Differences in SNP findings between the whites and African Americans in this study could be due to different patterns of LD within race, i.e., it could be that none of these SNPs are in LD with functional SNPs of *ADIPOQ* in African Americans, but may be in LD among the whites.

A significant interaction of rs182052 and waist circumference was found in the association with serum adiponectin in whites. Although this interaction makes sense biologically given the evidence that adiponectin plays a role in central adiposity (16), it should be interpreted with caution given the number of interactions examined, and should be confirmed in independent samples in future studies. A significant interaction of rs9882205 with smoking was also found in whites. Smoking is associated with lower adiponectin concentrations (29), and cessation of smoking results in increased serum adiponectin concentrations, both in healthy subjects (30) and those with existing coronary heart disease (31).

Many previous studies have examined the influence of *ADIPOQ* SNPs on circulating adiponectin concentrations, and as much as 70% of the variation in adiponectin concentrations is thought to be due to variants in *ADIPOQ* (18). Two studies most similar to ours in sample size and *ADIPOQ* variants genotyped were performed among 1,727 whites (22) and 922 French whites (21). Heid *et al.* (22) found 9 of 15 tagSNPs, including rs822387, rs17300539, rs822388, rs182052, rs9882205, rs2241766, rs1501299, rs3774261, and rs4686804, significantly associated with circulating adiponectin concentrations. In the Vasseur *et al.* study (21), those with the A allele of rs17300539 had significantly higher adiponectin concentrations as compared to those with the G allele, $P < 0.0001$. In addition, the T allele of rs2241766 was significantly associated with higher adiponectin levels. A meta-analysis of *ADIPOQ* SNPs from the literature with greater than 2,000 cumulative participants concluded that fairly strong evidence exists for rs17300539 and rs2241766 influencing adiponectin concentrations, but there is far less evidence for rs1501299 (18). Among whites in our study, rs17300539, rs182052, rs822393, rs9882205, and rs3774261 were strongly associated with serum adiponectin concentrations. These results are consistent in direction and magnitude with Heid *et al.* (22) for all SNPs genotyped in both studies except rs2241766. The study by Heid *et al.* was very similar to ours not only in sample size and ethnic composition, but also in the tagSNP method used to choose the *ADIPOQ* SNPs. Similar to Vasseur *et al.* (21), our study found a strong association of rs17300539 with serum adiponectin, with increasing number of A alleles associated with higher adiponectin levels.

The most significant associations observed were between *ADIPOQ* SNPs rs17300539, rs182052, rs822393, rs9882205, and rs3774261 and serum adiponectin in whites. SNP rs17300539 lies in the promoter region of *ADIPOQ*, whereas rs182052, rs822393, rs9882205, and rs3774261 lie in intron regions of the gene. Introns are non-coding regions of a gene; however, there is evidence that introns of the protein-coding gene transcripts can affect gene expression by repressing translation or cleaving RNA transcripts (32). In particular, rs3774261 is an intronic enhancer, and thus could affect protein levels via enhancing transcription. Variants in the promoter region of *ADIPOQ* also affect circulating adiponctin concentrations by enhancing *ADIPOQ* promoter activity(18). We might have

expected that either rs2241766 in exon 2 or rs17366743 in exon 3 to be associated with serum adiponectin given that they both lie in coding regions of the gene. However, rs17366743 is a rare mis-sense mutation (His-Tyr) so there could have been a lack of power to detect any associations of this SNP and adiponectin concentrations unless effects were large. Although rs2241766 lies in a coding region, it is a synonymous (Gly/Gly) SNP, which would not likely affect the adiponectin protein.

There are several important strengths of this study, but also some limitations. Using the tagSNP approach to choose SNPs enables the capturing of the variation across the adiponectin gene. Very few of the previous *ADIPOQ*-serum adiponectin studies have used a tagSNP approach to examine associations or have examined these associations in multiple ethnicities. On the other hand, some SNPs that were chosen with the tagSNP approach were not able to be genotyped because they could not be designed in the multiplex assays. It is also possible that there are important SNPs with minor allele frequencies of less than 0.10 used to choose the tagSNPs in this study. Other regions of *ADIPOQ* where SNPs were not genotyped could affect serum adiponectin concentrations, particularly in the exon or promotor regions of the gene. Despite using a tagSNP approach, this still does not ensure all known gene variation was captured.

We found variants of *ADIPOQ* associated with serum adiponectin concentrations in whites. This study replicates findings of earlier studies such as Heid *et al.* and Vasseur *et al.* that found the same *ADIPOQ* SNPs associated with serum adiponectin concentrations in whites. This article makes the novel contribution of evaluating tagSNPs of *ADIPOQ* with serum adiponectin levels in African Americans. In contrast to whites, we found only one *ADIPOQ* variant to be associated with serum adiponectin concentrations in African Americans, an association that was of only modest strength. There are still many *ADIPOQ* variants that have not been examined; so far only a small proportion of them have been studied (18) even in relation to serum adiponectin. Further, studies are needed to determine whether SNPs that replicate well are functional variants or if other SNPs may in fact be the functional variants.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

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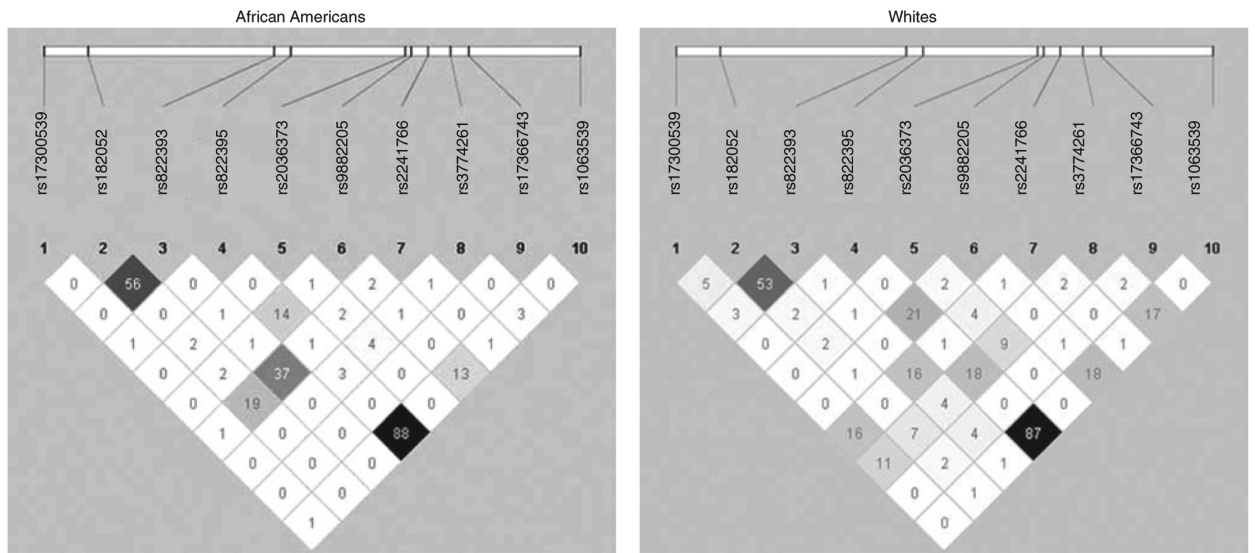
Figure 1.
Pairwise linkage disequilibrium (r^2) for adiponectin gene SNPs. Linkage disequilibrium plots by race group. These plots show the pairwise correlation (r^2) for each tag single-nucleotide polymorphism (tagSNP) chosen in this study.
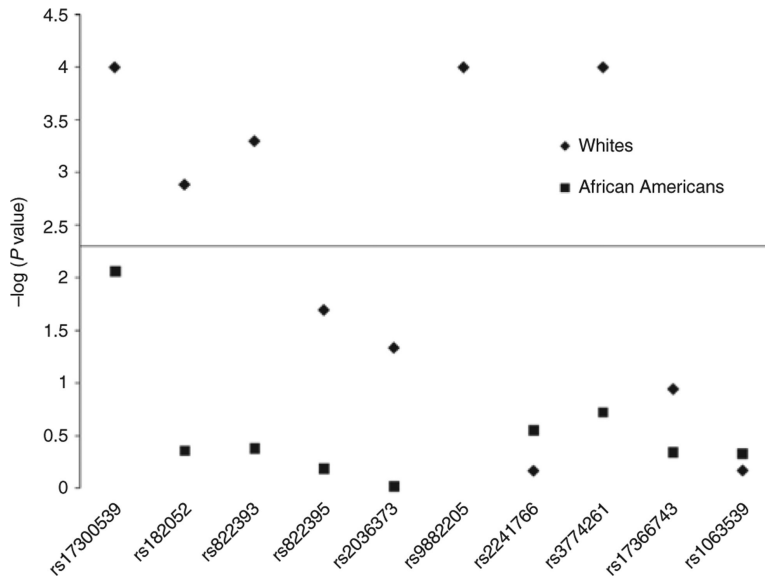
Figure 2.
Results of two degree of freedom tests for adiponectin gene SNPs and serum adiponectin. The –log(P values) for each two degree of freedom test of each single-nucleotide polymorphism (SNP) with serum adiponectin concentrations by race. Each SNP is along the x axis with the corresponding –log(P value) on the y axis. The solid horizontal line represents the –log(0.0050) Bonferroni significance threshold, and the black diamonds represent the P values for whites whereas black squares show the results for African Americans.

Table 1

Coronary Artery Risk in Young Adults (CARDIA) characteristics from the year 15 (2000–2001) examination by race group

Characteristics	White ($n = 1,772$)	African Americans ($n = 1,583$)	P value[a]
Age, years	40.5 ± 3.4	39.5 ± 3.8	<0.001
Gender			
Male	838 (47.3)	652 (41.2)	<0.001
Female	934 (52.7)	931 (58.8)	
Field center			
Birmingham	372 (21.0)	465 (29.4)	<0.001
Chicago	441 (24.9)	364 (23.0)	
Minneapolis	533 (30.1)	299 (18.9)	
Oakland	426 (24.0)	455 (28.7)	
Current smoking	287 (16.2)	441 (27.9)	<0.001
BMI, kg/m^2	27.2 ± 5.9	30.6 ± 7.4	<0.001
Waist circumference, cm	87.5 ± 14.7	92.0 ± 15.7	<0.001
Serum adiponectin, mg/l[b]	11.0 (7.2, 16.0)	7.2 (5.0, 11.0)	<0.001

[a]P values by χ^2, Wilcoxon or t-test as appropriate.

[b]Median (quartile 1, quartile 3).

Table 2

Associations of single adiponectin gene (*ADIPOQ*) SNPs with serum adiponectin concentrations (mg/l) among whites (*n* = 1,772)

	Unadjusted[a]		Demographic adjusted[b]	
	Means (95% CI)	*P* value	Means (95% CI)	*P* value
rs17300539				
GG (*n* = 1,429)	10.3 (10.0, 10.6)	ref	10.3 (10.0, 10.5)	ref
AG (*n* = 264)	12.8 (11.9, 13.6)	<0.0001	12.8 (12.1, 13.6)	<0.0001
AA (*n* = 20)	15.3 (12.0, 19.4)	0.0014	15.7 (12.7, 19.5)	0.0001
rs182052				
GG (*n* = 750)	11.2 (10.7, 11.6)	ref	11.1 (10.8, 11.6)	ref
AG (*n* = 780)	10.2 (9.8, 10.6)	0.0016	10.2 (9.9, 10.6)	0.0008
AA (*n* = 201)	9.9 (9.2, 10.7)	0.0082	10.1 (9.4, 10.8)	0.01
rs822393				
CC (*n* = 951)	11.1 (10.7, 11.5)	ref	11.1 (10.8, 11.5)	ref
CT (*n* = 656)	10.3 (9.8, 10.7)	0.0066	10.2 (9.8, 10.6)	0.0005
TT (*n* = 101)	9.7 (8.7, 10.8)	0.02	9.8 (8.9, 10.8)	0.02
rs822395[c]				
AA (*n* = 696)	10.5 (10.1, 11.0)	ref	10.5 (10.1, 10.9)	ref
AC (*n* = 700)	10.7 (10.3, 11.2)	0.11	10.7 (10.3, 11.1)	0.44
CC (*n* = 197)	11.5 (10.6, 12.4)	0.05	11.8 (11.0, 12.6)	0.02
rs2036373[d]				
GG/GT (*n* = 231)	10.5 (10.2, 10.8)	ref	10.5 (10.3, 10.8)	ref
TT (*n* = 1,518)	11.3 (10.5, 12.2)	0.07	11.3 (10.6, 12.1)	0.05
rs9882205[c]				
GG (*n* = 874)	11.1 (10.7, 11.5)	ref	11.1 (10.7, 11.5)	ref
AG (*n* = 635)	10.0 (9.6, 10.5)	0.001	10.1 (9.7, 10.5)	0.0002
AA (*n* = 133)	9.7 (8.9, 10.7)	0.01	9.6 (8.8, 10.4)	0.0016
rs2241766[c]				
TT (*n* = 1,306)	10.5 (10.2, 10.9)	ref	10.6 (10.3, 10.9)	ref
GT (*n* = 346)	10.4 (9.8, 11.0)	0.6	10.4 (9.8, 10.9)	0.52
GG (*n* = 22)	12.8 (10.1, 16.1)	0.11	11.2 (9.1, 13.8)	0.57
rs3774261				
GG (*n* = 656)	10.0 (9.5, 10.4)	ref	10.0 (9.7, 10.4)	ref
CG (*n* = 783)	10.8 (10.3, 11.2)	0.0083	10.8 (10.4, 11.1)	0.0086
CC (*n* = 307)	11.7 (10.9, 12.4)	<0.0001	11.6 (10.9, 12.2)	<0.0001
rs17366743				
TT (*n* = 1,645)	10.6 (10.3, 10.8)	ref	10.6 (10.3, 10.8)	ref
CT (*n* = 103)	11.4 (10.2, 12.7)	0.17	11.4 (10.4, 12.6)	0.11
rs1063539				
GG (*n* = 1,349)	10.7 (10.3, 11.0)	ref	10.7 (10.4, 11.0)	ref

	Unadjusted[a]		Demographic adjusted[b]	
	Means (95% CI)	*P* value	Means (95% CI)	*P* value
CG (*n* = 371)	10.4 (9.8, 11.0)	0.41	10.4 (9.9, 10.9)	0.38
CC (*n* = 26)	12.1 (9.7, 15.0)	0.27	10.5 (8.6, 12.7)	0.83

[a]Geometric means.

[b]Geometric means, adjusted for age, sex, and center.

[c]These SNPs were genotyped on ~200 fewer CARDIA participants than the remaining SNPs.

[d]GG and GT genotypes combined due to rare homozygotes, i.e., < *n* = 10 in the rare homozygote group.