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Variants of the *CD36* Gene and Metabolic Syndrome in Boston Puerto Rican Adults

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Abstract

Objective—Puerto Ricans experience a high prevalence of several chronic conditions, including metabolic syndrome. Genetic variants of the *CD36* gene have been associated with metabolic syndrome. We aimed to determine the association between 6 single nucleotide polymorphisms (SNPs) for *CD36* and metabolic syndrome and its components in Puerto Ricans (45-75 y) living in the Greater Boston area.

Methods—Associations between each SNP, metabolic syndrome and its components were examined using multivariate logistic regression models. Haplotype trend regression analysis was used to determine associations between haplotypes and metabolic syndrome.

Results—For two SNPs of *CD36* (rs1049673 and rs3211931), homozygous subjects of the minor allele (G and T, respectively) were associated with a higher likelihood of metabolic syndrome (odd ratio (OR) (95% confidence interval (CI): 1.89 (1.0, 3.5) and 1.77 (1.0, 3.1), respectively) relative to carriers of the major allele. Although *CD36* haplotypes were not significantly associated with metabolic syndrome overall (global significance, P=0.23), one haplotype (G-C-C vs. C-C-C (reference haplotype) was marginally associated (P=0.049).

Conclusion—SNPs of *CD36* were associated with metabolic syndrome in Puerto Ricans. Prospective studies should further explore the role of *CD36* variants in the development of this condition.

Keywords

Puerto Rican; Hispanic; *CD36*; metabolic syndrome

Introduction

Puerto Ricans, the second largest US Hispanic group (1), experience a higher prevalence of several chronic conditions, including type 2 diabetes (2) and systolic hypertension (3), relative to other ethnic groups. Metabolic syndrome, characterized by insulin resistance,

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dyslipidemia, hypertension and central adiposity (4), can increase risk of cardiovascular disease and type 2 diabetes (5-7).

Although few studies have focused on Puerto Ricans, metabolic syndrome has been reported to be prevalent among older Puerto Rican adults living in the US (50%) (8). It has been reported that there is a strong genetic component involved in susceptibility to metabolic syndrome (9). Yet, few studies have examined the influence of genetic variants on metabolic syndrome, especially for Puerto Ricans in the US.

CD36 variants have been associated with type 2 diabetes, metabolic syndrome and abnormalities in triglyceride and serum fatty acid concentrations (10-12). Studies of spontaneous hypertensive rats (SHR) indicate that quantitative trait loci (QTL) for chromosome 4, likely due to *CD36* gene variants, were associated with metabolic syndrome components (13,14). In humans, the *CD36* gene, located on chromosome *7 q11.2*, is encoded by 15 exons and plays a role in long-chain fatty acid (LCFA) transport (15). *CD36* also functions as a receptor for native lipoproteins (16), oxidized high-density lipoprotein (HDL) (17) and oxidized low-density lipoprotein (LDL) (15). Genome-wide linkage scans have identified nearby regions of chromosome 7 that are associated with features of metabolic syndrome, such as triglyceride concentrations, HDL-cholesterol, and triglyceride/HDL ratio (18,19).

Changes in the expression and function of a major fat transporter, such as *CD36*, may influence fatty acid metabolism (20). Higher free fatty acids have been associated with insulin resistance (21) and accumulation of fat and fat metabolites, which may increase the risk of metabolic syndrome (20). Thus, it is likely that variants of *CD36* may influence risk. This study aimed to determine the allele frequencies of six single nucleotide polymorphisms (SNPs) in the *CD36* gene in a sample of Puerto Rican adults living in Massachusetts, and to examine associations of these SNPs and haplotypes with metabolic syndrome.

Methods

Study population

This study included self-identified Puerto Ricans aged 45-75 y, from the Boston Puerto Rican Health Study, a longitudinal cohort examining relationships between chronic disease, diet and stress. Participants were recruited from the Greater Boston area through door-to-door enumeration of high-Hispanic dense areas identified using 2000 Census data, through media advertisements and community events, and through referrals from community members. One participant per household was randomly invited to participate in the study. Exclusion criteria included inability to answer questions due to serious illness, plans to move from the Boston area within two years or having a Mini Mental State Examination (MMSE) score ≤10. All procedures for this study were in accordance with the Institutional Review Board at Tufts Medical Center guidelines; all subjects provided informed consent. Participants with complete genotype and metabolic syndrome data were included in this analysis (n=1178).

Data collection and variable definitions

A home interview was conducted in the participant's language of preference (Spanish or English) and included questionnaires on health and health behaviors, socio-demographics, and diet. Anthropometrics and blood pressure were obtained during the interview. On the day following the interview, or as soon as possible thereafter, the study phlebotomist collected fasting biological samples at home.

Blood samples were collected in a serum separator tube and spun down using an onsite centrifuge. Samples were kept cold and brought to the laboratory where plasma was separated within 4 h; serum was isolated and then frozen at -80°C for further analysis. Serum glucose and plasma lipid concentrations were measured on the Olympus AU400e (Olympus America Inc., Melville, NY). Serum glucose was analyzed with a kinetic reaction with Olympus Glucose Reagents (OSCR6131). Plasma HDL-cholesterol and triglyceride concentrations were analyzed using EDTA plasma with enzymatic endpoint reactions with Olympus HDL-cholesterol Reagents (OSR6195) and Olympus Triglyceride Reagents (OSR6033), respectively.

Anthropometric measures were taken in duplicate as follows: weight was obtained using a clinical scale (Toledo Weight Plate, Model I5S, Bay State Systems Inc. Burlington, MA), standing height with a SECA 214 Portable Stadiometer, and waist circumference with an anthropometric tape. Body mass index (BMI) was calculated as weight (kg) divided by height (m^2). Blood pressure was obtained using an electronic sphygmomanometer (Dinamap™ Model 8260, Critikon, Tampa, FL) at three time points during the interview; the average of the second and third reading was used.

A modified Paffenbarger questionnaire of the Harvard Alumni Activity Survey (22) assessed physical activity as a score, which was categorized as sedentary (score <30), light (score ≥ 30 to <40), moderate (score ≥ 40 to <50) or heavy physical activity (score ≥50). Smoking and alcohol use were determined through questionnaire and categorized as never, current or past.

Metabolic syndrome, was defined as having at least three of the following five criteria: waist circumference ≥102 cm for men or ≥88 cm for women, elevated triglycerides ≥150 mg/dl or drug treatment for elevated triglycerides, low HDL-cholesterol (<40 mg/dl for men and <50 mg/dl for women) or drug treatment for low HDL-cholesterol, high blood pressure (systolic ≥130 mm Hg or diastolic ≥85 mm Hg) or drug treatment for hypertension, and elevated fasting glucose ≥100 mg/dl or drug treatment for elevated glucose (4). Metabolic syndrome and its individual components were examined dichotomously.

Selection and Genotyping of Single Nucleotide Polymorphisms

SNPs were selected for genotyping based on published associations (11,12) and linkage disequilibrium to sample different genetic blocks. SNPs rs3173804, rs13230419 and rs7755 (30215 G > A) have been associated with metabolic syndrome (11), and rs13246513 with metabolic syndrome and HDL- cholesterol (11); all of these SNPs are tagged by rs3211931. Additionally, rs1761667 and rs1049673 have been associated with free fatty acid concentrations, especially in men (12). SNPs were selected to cover the other LD blocks, balancing LD values of CEU and YRI populations to incorporate approximately 84% of the genetic composition of our sample (23). Minor allele frequencies (MAF) for each SNP had to be above 0.1 for inclusion in this analysis.

QIAamp® DNA Blood Mini Kits (*Qiagen, Hilden, Germany*) were used to isolate DNA from buffy coats of nucleated cells of samples according to standard procedures. Genotyping for the SNPs was performed using pre-designed TaqMan® assays from Applied Biosystems (Applied Biosystems, Foster City, CA). DNA (10 *ng*) was diluted, aliquoted and added from 96-well plates into 384-well plates using a rapidplate robotic system. The TECAN robotic system mixed DNA with TaqMan Universal PCR Master Mix, assay, and water. Allelic discrimination was completed using Applied Biosystems 7900. Microsoft ® Excel macros were used for creating plate records with allele base codes and for data cleaning. The DNA sample plates included blinded no-template controls and DNA sample replicates that were routinely checked by laboratory technicians. The genotype error rate was estimated at <1%.

The genotype success rates were as follows: rs1761667 (95.8%, n=1295), rs1049673 (96.2%, n=1300), rs1953299 (93.6%, n=1266), rs3211816 (95.7%, n=1294), rs3211931 (95.9%, n=1296) and rs7807607 (95.6%, n=1293).

Population admixture

Population admixture was calculated using STRUCTURE 2.2 with reference to three ancestral populations including European, Native Americans, and West Africans estimated from a panel of 100 ancestral informative markers for Hispanic populations (23).

Linkage disequilibrium and haplotype analysis

Pairwise linkage disequilibrium (LD) was estimated using the HelixTree program (GOLDEN Helix, Bozeman, MN) for all six SNPs. LD was estimated as correlations coefficients (r^2) with corresponding P-values. Haplotype frequencies were estimated using the expectation-maximization algorithm (24) for SNPs that were selected based on significant (P <0.05) or trended (P <0.2) associations between the SNP and metabolic syndrome and a pair-wise LD between the SNPs of $r^{\wedge 2}$ <0.85. The most frequent haplotype was selected as the reference haplotype. Haplotype trend regression analysis, as implemented in HelixTree, was used to examine associations between haplotypes and metabolic syndrome adjusting for potential confounders as described below.

Statistical analyses

Statistical analyses were performed using SAS (version 9.2, SAS Institute, Cary, NC) and Microsoft ® Excel. Allele frequencies were calculated and chi-square statistics were used to test Hardy-Weinberg Equilibrium (HWE) for each SNP. Implausible values of continuous variables were excluded; i.e., triglycerides >1700 mg/dl (n=2), waist circumference >180 cm (n=1) and HDL-cholesterol <10 mg/dl (n=1). Sample means and frequencies were examined for selected basal characteristics using t-tests and Pearson chi-square analyses, respectively. Multivariate logistic regression models examined associations between each SNP and prevalence of metabolic syndrome, and of the dichotomous individual components of metabolic syndrome. Model 1 was adjusted for age, sex, educational attainment, alcohol and smoking use, population admixture, and physical activity; model 2 was adjusted for variables in model 1 and BMI. Indicator variables were used to include individuals with missing data. Results for analyses using three genotypes were similar to those observed when we combined carriers for major allele. Thus, we present results for carriers of the major allele compared with subjects homozygous for the minor allele. Models were examined for effect modification by sex (25,26). None of the interactions were significant; therefore, analyses were performed using the full sample. Multivariate logistic regression models were further adjusted for multiple comparisons using a Bonferroni adjustment (adjusted P-value <0.008). In secondary analyses, interactions between total dietary fat intake and each SNP were tested using multivariate logistic regression adjusted for the specified models (listed above) and total energy.

Results

Six SNPs of the *CD36* gene were genotyped; MAFs ranged from 0.28 to 0.50 (Table 1). All SNPs were in HWE. Mean age was similar between men and women (57 vs. 58 y, respectively) (Table 2). Women were less physically active (score: 31 vs. 33%, P <0.001) and less likely to be currently smoking or consuming alcohol relative to men (21 vs. 34%, P<0.001 and 35 vs. 50%, P<0.001, respectively). Women had significantly higher prevalence of metabolic syndrome than men (78 vs. 66%, P <0.001). On average, women had higher BMI (33 vs. 30 kg/m^2, P <0.001) and HDL-cholesterol concentrations (47 vs. 40 mg/dl, P <0.001), and significantly lower blood pressure (systolic: 135 vs.138 mmHg,

P=0.02; diastolic: 80 vs. 83 mmHg, P <0.001) relative to men. Crude dietary analyses revealed no significant interactions between dietary fat intake and *CD36* variants (data not shown).

The following SNPs were in strong LD: rs1761667 and rs7807607 (r^2=0.89), rs1761667 and rs1953299 (r^2= 0.92), rs1049673 and rs3211931 (r^2= 0.97), and rs1953299 and rs7807607 (r^2= 0.85, Table 3). Associations between *CD36* SNPs and metabolic syndrome were examined based on a recessive model (Table 4). For SNP rs1049673, homozygotes for the minor allele (G) were twice as likely to have metabolic syndrome compared with carriers of the major allele (C) (OR, (CI): 1.89 (1.0, 3.5)). Homozygotes for the minor allele (T) also showed a higher likelihood of metabolic syndrome relative to carriers of the major allele (C) (1.77 (1.0, 3.1)) for SNP rs3211931. These associations were no longer statistically significant after adjusting for multiple comparisons (P>0.008 for both). For individual metabolic syndrome components, homozygotes for the minor allele (G) of SNP rs1049673 showed higher likelihood of elevated fasting serum glucose (≥100 mg/dl) or treatment for elevated glucose after adjusting for potential confounders including BMI (1.7 (1.04, 2.8)), (data not shown). This result was not significant after adjusting for multiple comparisons (P>0.008). There were no other significant results between the SNPs and individual metabolic syndrome components.

We conducted haplotype analysis for a subset of *CD36* SNPs to examine the combined effects of several variants in this gene, and to increase statistical power for detecting associations between SNPs and phenotypes. We selected three SNPs based on their individual associations with metabolic syndrome. There were eight haplotypes with frequencies ranging from 5 to 36% accounting for all haplotypes in this population (Table 5). *CD36* haplotypes were not significantly associated with metabolic syndrome in this population (at the global level, P=0.23). For individual haplotypes, carriers of the haplotype G-C-C had lower likelihood of metabolic syndrome compared to the reference haplotype C-C-C after adjusting for all covariates (β= -7.34, P= 0.049). Other haplotypes did not display significant associations with metabolic syndrome.

Discussion

There were significant associations between *CD36* variants and metabolic syndrome in Puerto Rican adults living in Massachusetts. One haplotype consisting of three SNPs was significantly associated with metabolic syndrome after adjusting for potential confounders. For individual SNPs, subjects who were homozygous for the minor allele (G) for SNP rs1049673 or for the minor allele (T) for SNP rs3211931, had higher likelihood of metabolic syndrome, although strong LD for these SNPs suggests that these may not be completely independent, and thus should not be interpreted separately. A few studies have reported associations between *CD36* polymorphisms and diabetes (10,27) and more recently metabolic syndrome (11). A study in African Americans found that five intronic SNPs were associated with increased likelihood, and those with the minor allele of a coding SNP decreased likelihood of metabolic syndrome (11). A study in the CEU population of non-Hispanic whites of western European decent from HapMap, found that several SNPs (rs3173804, rs13246513, rs13230419, and rs7755) were in strong LD (r^2: 0.967-1.00) with SNP rs3211931—the latter was significantly associated with metabolic syndrome in our study.

Given the many functions of *CD36* including LCFA transport (15), changes in *CD36* expression and protein may lead to several disturbances including insulin resistance and dyslipidemia (10,20). Some studies have reported alterations in free fatty acid and triglyceride concentrations (12) with a common haplotype in *CD36*. More specifically, SNPs

rs1761667 and rs1049673 have been associated with elevated plasma free fatty acids (carriers of the common allele had higher concentrations compared with homozygotes for the minor allele) in white men without diabetes (12). We did not find significant associations between these SNPs and low HDL-cholesterol or elevated triglyceride concentrations. The lack of replication of such results may be due, in part, to the predominance of women and high prevalence of diabetes in our sample. Additionally, the high prevalence of metabolic syndrome in this population (~74%) may limit power to detect associations. In our study, haplotype analyses did not improve the power to detect associations between *CD36* variants and metabolic syndrome, suggesting that the selected SNPs do not have a synergistic effect on metabolic syndrome. The individual SNPs may be associated with the same unknown functional variant by LD (three SNPs in weak LD, r^2 = 0.39 and 0.4, respectively).

Other studies have reported significant associations between *CD36* variants and fasting glucose concentrations and measures of insulin resistance (10,27). The TT genotype of a C/T promoter polymorphism has been associated with higher fasting plasma glucose concentrations and homeostasis model assessment of insulin resistance (HOMA-IR) compared with other genotypes (10). We found an association between SNP rs1049673 and elevated fasting glucose, although this was not statistically significant after adjusting for multiple comparisons. Griffin et al (28) reported an increase in *CD36* surface expression in macrophages, which was associated with approximately a 10-fold increase in *CD36* mediated oxidized- LDL uptake. That, along with a more recent study reporting an increase in monocyte *CD36* expression and uptake of oxidized LDL from lipid induced insulin resistance, suggest a possible connection between atherosclerosis and insulin resistant states through *CD36* (29).

Animal models of insulin resistance have shown that defects in *CD36* were linked to disturbances associated with metabolic syndrome (13). In humans, *CD36* deficiency has been associated with hyperlipidemia, and elevated fasting glucose concentrations and blood pressure (30). *CD36* may play a role in increased uptake of triglycerides in skeletal muscle, especially for obese individuals and those with type 2 diabetes (31), and higher free fatty acids can elicit insulin resistance (21). It has been suggested that *CD36* gene variants may influence metabolic syndrome through an ectopic accumulation of fat and fat metabolites because of impaired fat storage in adipose tissue, and alterations in fat metabolism (20). Because the majority of *CD36* polymorphisms do not result in deficiency, it is likely that variants of this gene influence lipid and glucose metabolism, thereby contributing to the development of metabolic syndrome (27).

This study has several limitations. SNPs were selected based on information from other studies, mainly conducted in non-Hispanics; thus, the selected SNPs may not be the most appropriate for this population. Gene-gene or gene-environment interactions may also partly explain the lack of association between several of the selected *CD36* variants and metabolic syndrome. The high prevalence of metabolic syndrome in this sample, a serious health issue in this population, may have also played a role in the marginal or lack of associations observed. Future studies should investigate possible interactions between variants of this gene and other genetic or environmental factors. Finally, results from this study may not be applicable to other ethnic populations, as the estimated MAF of the selected *CD36* variants are particular to our Puerto Rican sample; variations in MAF of disease-associated gene polymorphisms could translate into different outcomes across ethnic groups (32). Studies performed in other populations may confirm the associations observed here.

In conclusion, this is one of the first studies to examine associations of *CD36* variants in Puerto Rican adults, a high-risk urban population. We show that variants of the *CD36* gene are significantly associated with metabolic syndrome in this population. These findings may

have larger implications, as metabolic syndrome can increase the risk of cardiovascular disease and type 2 diabetes. Future studies should examine the possible role of *CD36* variants in the development of metabolic syndrome in Puerto Ricans as well as other ethnic populations.

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Table 1

Allele and genotype frequencies for six *CD36* SNPs in 1178 Puerto Rican adults

Accession number	Distance from TSS*	Gene Region	ABI assay ID	Minor allele frequency
rs1761667	13417	Intron 1	C___8314999_10	0.46
rs1049673	74828	Intron 2	C___8315317_20	0.31
rs1953299	34103	Intron 1	C___1803805_10	0.50
rs3211816	46468	Intron 3	C__31374634_20	0.28
rs3211931	66651	Intron 8	C__30064430_10	0.33
rs7807607	-4637	Upstream	C__1803845_10	0.47

*TSS=Transcription start site, in base pairs

Table 2

Sample characteristics for 1178 Boston Puerto Rican adults aged 45 to 75 y[1,2]

Sample Characteristic	Men[1] (n=348)	Women[1] (n=830)	P-value
Age (y)	56.6 ± 0.42	57.5 ± 0.26	0.08
Physical activity score (%)	32.5 ± 0.31	31.1 ± 0.14	<0.001
Body mass index (kg/m^2)	29.6 ± 0.27	32.8 ± 0.24	<0.001
Fasting serum glucose (mg/dl)	123.6 ± 3.0	121.6 ± 1.8	0.57
Triglycerides (mg/dl)	172.0 ± 6.6	159.0 ± 3.6	0.09
HDL-cholesterol (mg/dl)	40.3 ± 0.66	46.8 ± 0.42	<0.001
Systolic blood pressure (mmHg)	137.6 ± 0.99	134.7 ± 0.66	0.02
Diastolic blood pressure (mmHg)	82.9 ± 0.59	80.3 ± 0.36	<0.001
Waist circumference (cm)	101.6 ± 0.71	101.5 ± 0.53	0.86
Sex (%)	29.5	70.5	<0.001
< 8th grade education	44.2	49.5	0.10
Smoking			
Never	30.9	50.7	<0.001
Past	34.7	28.3	
Current	34.4	21.0	
Alcohol use			
Never	7.3	38.9	<0.001
Past	42.9	25.8	
Current	49.9	35.3	
Metabolic syndrome[3] (%)	65.5	78.1	<0.001
Type 2 diabetes (%)	40.3	40.4	0.98

[1] Sample sizes differed due to missing data as follows: BMI: (n= 345 men, 822 women), fasting glucose (n= 347 men, 827 women), triglycerides and HDL-cholesterol (n= 347 men), systolic and diastolic blood pressure (n= 343 men, 809 and 809 women, respectively), waist circumference (n= 346 men, 823 women), education (n=346 men), smoking (n=343 men, 823 women) and alcohol use (n=345 men) and type 2 diabetes (n=345 men, and 825 women).

[2] Significant differences for continuous variables were estimated using t-tests and for categorical variables using Pearson chi-square tests

[3] Defined as at least 3 of the following 5: waist circumference ≥102 cm for men or ≥88 cm for women, elevated triglycerides ≥150 mg/dl or drug treatment for elevated triglycerides, low HDL-cholesterol (<40 mg/dl for men and <50 mg/dl for women) or drug treatment for low HDL-cholesterol, high blood pressure (systolic ≥130 mm Hg or diastolic ≥85 mm Hg) or drug treatment for hypertension, or elevated fasting glucose ≥100 mg/dl or drug treatment for elevated glucose.

Table 3

Linkage disequilibrium for genotyped SNPs of the *CD36* gene

Linkage Disequilibrium (LD)[1]	**rs1761667**	**rs1049673**	**rs1953299**	**rs3211816**	**rs3211931**	**rs7807607**
rs1761667		0.46	0.92	0.56	0.47	0.89
rs1049673	9.71^{-63}		0.41	0.39	0.97	0.40
rs1953299	2.79^{-243}	2.47^{-49}		0.61	0.44	0.85
rs3211816	2.34^{-91}	2.15^{-46}	7.26^{-107}		0.40	0.56
rs3211931	7.85^{-64}	6.26^{-268}	2.92^{-56}	3.04^{-48}		0.43
rs7807607	5.22^{-227}	2.58^{-48}	4.61^{-207}	5.64^{-93}	4.70^{-53}	

[1]Pairwise LD for six SNPs as correlation coefficient (r^2) displayed above the diagonal and the corresponding P-values below the diagonal

Table 4

Odds ratio (95% confidence interval) of metabolic syndrome for *CD36* variants, in 1178 Puerto Rican adults[1,2]

SNP	Carriers for major allele	Homozygous for minor allele	P-value
rs1761667	AA + AG	GG	
Model 1	1.0	1.30 (0.90, 1.9)	0.17
Model 2	1.0	1.33 (0.89, 2.0)	0.16
rs1049673	CC + CG	GG	
Model 1	1.0	1.65 (0.94, 2.9)	0.08
Model 2	1.0	1.89 (1.0, 3.5)	0.04
rs1953299	TT + GT	GG	
Model 1	1.0	1.08 (0.77, 1.5)	0.66
Model 2	1.0	1.08 (0.75, 1.6)	0.67
rs3211816	GG + AG	AA	
Model 1	1.0	1.25 (0.72, 2.2)	0.43
Model 2	1.0	1.32 (0.73, 2.4)	0.36
rs3211931	CC + CT	TT	
Model 1	1.0	1.52 (0.90, 2.6)	0.12
Model 2	1.0	1.77 (1.0, 3.1)	0.05
rs7807607	CC + CT	TT	
Model 1	1.0	1.34 (0.92, 1.9)	0.13
Model 2	1.0	1.45 (0.97, 2.2)	0.07

[1] Model 1: Logistic regression analysis adjusted for age, sex, smoking, drinking, physical activity and educational attainment, and population admixture

[2] Model 2: Logistic regression analysis adjusted for age, sex, smoking, drinking, physical activity and educational attainment, and population admixture and BMI

Table 5

Associations between *CD36* haplotypes and metabolic syndrome in 1178 Puerto Rican adults[1, 2, 3, 4]

		Frequency	Coefficient B	Odds Ratio	P-value
G-T-T	Model 1	0.13	0.45	1.58	0.21
	Model 2	0.13	0.55	1.74	0.16
G-T-C	Model 1	0.07	0.14	1.15	0.84
	Model 2	0.07	-0.13	0.88	0.86
G-C-T	Model 1	0.06	0.47	1.59	0.82
	Model 2	0.06	-0.81	0.44	0.71
G-C-C	Model 1	0.05	-6.02	0.002	0.08
	Model 2	0.05	-7.31	0.0007	0.05
C-T-T	Model 1	0.07	-0.97	0.38	0.39
	Model 2	0.07	-0.99	0.37	0.42
C-T-C	Model 1	0.06	1.32	3.76	0.52
	Model 2	0.06	1.99	7.28	0.35
C-C-T	Model 1	0.21	-0.19	0.83	0.57
	Model 2	0.21	-0.16	0.85	0.66
C-C-C	Model 1	0.36	Reference		
	Model 2	0.36	Reference		

[1] Three SNPs in the haplotypes were arranged in the following order: rs1049673, rs3211931, and rs7807607

[2] Global association for haplotypes with metabolic syndrome for model 1 was P=0.45, and P=0.23 for model 2

[3] For odds of metabolic syndrome, haplotypes were compared with reference haplotype C-C-C

[4] Model 1 was adjusted for sex, age, education, alcohol use, smoking, physical activity, and population admixture, and model 2 for all variables in model 1 and BMI