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PPARA gene polymorphisms modulate the association between physical activity and cardiometabolic risk

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Abstract

Background and Aims—Habitual physical activity is understood to help prevent type 2 diabetes and atherosclerotic cardiovascular disease via beneficial effects on both metabolism and the vascular system. However, individuals do not have uniform cardiometabolic responses to physical activity. Here we explore the extent to which variation in the proliferator-activated receptor- α (*PPARA*) gene, which modulates carbohydrate and lipid metabolism, vascular function, and inflammation, predicts the overall cardiometabolic risk (CMR) profile of individuals engaging in various levels of physical activity.

Methods and results—917 unrelated, community volunteers (52% female, of Non-Hispanic European ancestry) aged 30–54 years, participated in the cross-sectional study. Subjects were genotyped for 5 single nucleotide polymorphisms in the *PPARA* gene, from which common haplotypes were defined. A continuous measure of CMR was calculated as an aggregate of 5 traditional risk factors: waist circumference, resting blood pressure, fasting serum triglycerides, HDL-cholesterol and glucose. Regression models were used to examine the main and interactive effects of physical activity and genetic variation on CMR. One common *PPARA* haplotype (H-23) was associated with a higher CMR. This association was moderated by daily physical activity ($B = -0.11$, $SE = 0.053$, $t = -2.05$, $P = 0.04$). Increased physical activity was associated with a steeper reduction of CMR in persons carrying the otherwise detrimental H-23 haplotype.

Conclusions—Variations in the *PPARA* gene appear to magnify the cardiometabolic benefits of habitual physical activity.

Keywords

Metabolic syndrome; cardiometabolic risk; physical activity; gene-by-physical activity interaction

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Introduction

The metabolic syndrome represents a covarying cluster of risk factors that together increase the risk for type II diabetes and cardiovascular disease (1). Obesity, aging, physical inactivity, unhealthy diet, insulin resistance and genetics are all potential causes underlying cardiometabolic disruptions, which often require multiple treatment strategies since no single medication can treat this condition (2). Hence lifestyle related changes are recommended as the primary treatment strategy (1). Regular aerobic physical activity has salutary effects on the individual components (3) and the syndrome (4).

Metabolic syndrome has a complex etiology determined by the interplay between genetic and non-genetic components. Heritability of the syndrome ranges between 10% – 30% (5) and several genes, mostly in energy metabolism pathways, have been implicated in its pathophysiology (5–6). The peroxisome proliferator-activated receptors (PPARs) are a family of ligand-activated nuclear transcription factors which are key mediators of energy homeostasis, lipid and glucose metabolism, vascular function and inflammation (7). The PPAR α isoform is encoded by the *PPARA* gene; is robustly expressed in tissues with elevated fatty acid catabolism and regulates transcription of multiple genes involved in glucose metabolism (8) and in transport and oxidation of fatty acids (e.g., *LPL*, *APOA1*) (9). Fibrates, which act as PPAR α agonists, have a well-established role in lowering triglycerides and in increasing HDL levels in humans (10). *PPARA* gene polymorphisms are associated with many cardiometabolic disturbances (8,11–14). Consequently, *PPARA* has emerged as an important candidate gene underlying the development of the metabolic syndrome. Since physical activity and PPAR α both affect lipid and glucose metabolism and vascular function understanding how they influence cardiometabolic risk (CMR) in relation to one another is important. We hypothesized that previously implicated genetic variation in *PPARA* (8,11–14) may jointly moderate the association between habitual physical activity and CMR.

Methods

Study Population

Participants were from the University of Pittsburgh Adult Health and Behavior registry (9) - a compendium of behavioral and biological measurements collected on mid-life community volunteers recruited via mass-mail solicitation from Southwestern Pennsylvania between 2001 and 2005. Exclusionary criteria included reported history of atherosclerotic cardiovascular disease, chronic kidney or liver disease, cancer treatment in the preceding year, major neurological disorders, schizophrenia or other psychotic illness, pregnancy and using insulin, glucocorticoid, antiarrhythmic, psychotropic, or prescription weight-loss medications. The study was approved by the University of Pittsburgh IRB and informed consent was obtained from participants prior to research. Data was collected in multiple laboratory sessions. Of the 1295 adults (30– 54 years of age) who were recruited, the current investigation included 936 individuals of European ancestry who were not using antihypertensive, lipid-lowering or diabetes medications, not missing any outcome or covariate data, had valid calls for 3 of 5 SNPs genotyped and scored above 80 on normed cognitive index scores.

Risk factor assessments

Participants arrived at the laboratory between 0730 and 1030 hours following an overnight fast, avoided exercise for 12 hours, alcohol for 24 hours and nicotine for 1 hour prior to arrival. In the first visit the project nurse completed a medical history and medication use interview, obtained measurements of height, weight and waist circumference (at the umbilicus). Subjects rested in a seated position for at least 10 minutes, after which two blood pressure (BP) measurements were obtained by trained staff using a mercury sphygmomanometer and appropriately-sized cuff. Resting BP was measured similarly on a second occasion, providing a total of 4 readings from which an average was calculated. Following phlebotomy, a portion of the blood sample was used to collect plasma which was stored at -80°C and used to determine fasting serum lipids and glucose levels using previously described protocol (15). A second portion of the blood sample was collected in EDTA treated tubes and stored at -80°C for DNA isolation. Smoking status was assessed as ever smoked vs. never smoked and used as a binary variable. Years of education was used as a surrogate measure of socioeconomic status.

Whereas the metabolic syndrome is customarily defined as present or absent based on 5 dichotomized risk factors, quantification of cardiometabolic risk from these same 5 risk factors as a continuous index has more power (4,16) and better predicts cardiovascular disease events (17). Accordingly, we defined a composite cardiometabolic risk (CMR) index for each individual using the components of the metabolic syndrome. For these computations, mean blood pressure (BP) was estimated using the formula $\{(\text{systolic BP} + 2 * \text{Diastolic BP})/3\}$, triglycerides and glucose values were log normalized, HDL-cholesterol was multiplied by -1 , waist circumference was untransformed. Each risk factor was mean standardized and a composite risk score was calculated as the mean of the 5 standardized risk factors and restandardized prior to statistical analysis.

Routine physical activity was assessed using the Paffenbarger Physical Activity Questionnaire (18). This eight question survey measures walking, stair-climbing and leisure time sports and exercise activities from which physical activity-related energy expenditure (kcal/week) is estimated. The scale was log normalized prior to statistical analysis. Details on the validity of this scale are provided in the Supplementary material.

Genotyping

Taqman SNP genotyping assays were used to genotype five *PPARA* SNPs: rs1800206 C>G (L162V), rs135542 A>G, rs135539 A>C, rs4253728 G>A, rs4253778 G>C, which have previously been associated with obesity, diabetes and cardiovascular risk (8,11–14). Genotypes were scored by allelic discrimination using the ABI 7900HT Fast Real-Time PCR system and the SDS 2.2 software (Applied Biosystems, Foster City, CA). To examine whether substantial genetic heterogeneity exists in the study population we examined a panel of 15 markers spread across the genome (rs1022106, rs1335995, rs1439564, rs1502812, rs1860300, rs548146, rs705388, rs715994, rs720517, rs722743, rs730899, rs734204, rs9059966, rs1328994, rs1485405). These markers were previously genotyped in all the participants who enrolled in the registry and have been used to control for genetic stratification in all studies from this cohort (9).

Genetic Analyses

Allele frequencies, Hardy Weinberg equilibrium and pairwise linkage disequilibrium (LD) were ascertained following standard methodology. Multilocus haplotypes were ascertained by PHASE v 2.0 (19). The program STRUCTURE (20) was used to evaluate genetic substructure in the sample of 936 individuals who were included in this analyses, using 15 genome-spanning SNPs and statistical methods as described in the supplementary section.

Statistical Analyses

Our primary analyses aimed to investigate the joint effect of previously implicated individual *PPARA* polymorphisms (8,11–14) on CMR, in relation to physical. Theoretical analyses have shown that haplotype based approaches provide several advantages over single locus based approaches when studying individual genes or localized genomic regions, especially in the presence of weak LD between neighboring loci (21) and may allow for epistatic interactions between loci (22). Since *PPARα* is known to affect lipid and glucose metabolism (7) we focused on examining the combination of known variants (i.e. haplotypes) that might better represent effects of the final gene product and used haplotypes as the primary unit of analysis. Prior to statistical analysis phenotypic outliers were identified as those points which were above or below mean ± 3 *standard deviation for either the normalized physical activity or the CMR scores and removed.

Statistical analyses were performed in SPSS v15 (SPSS, Inc., Chicago, IL). Bivariate or point biserial correlations between variables were tested. A hierarchical regression model was used to examine the proportion of variation in CMR explained by physical activity. Age, gender, smoking status and years of school were entered in step 1 and physical activity scores were entered in step 2. For examining the effect of haplotypes on CMR, we used hierarchical regression models in which age, gender, smoking status and years of school were entered in step 1 and haplotype status (presence vs. absence) was entered next. When a positive haplotype association was detected, a subsequent hierarchical regression model was used to test the interaction between haplotype and physical activity. In interaction models covariates, physical activity scores, haplotype and a haplotype-by-physical activity term were entered in steps 1–4. In secondary analyses each SNP was tested for association with CMR and each component of metabolic syndrome was tested for association with each haplotype and SNP using similar hierarchical regression models. False discovery rate (FDR) (23) method was used to control for multiple testing in primary haplotype analyses.

RESULTS

Sample characteristics

Of the 936 individuals selected for the study, only 917 remained after removing outliers. General characteristics of study subjects are shown in Table 1. Subjects were generally healthy with a mean BMI of 26.5. Metabolic syndrome was present in 17% of subjects (N=167), with higher prevalence in males than in females (27% vs. 10%; $P < 0.001$) and corresponding higher mean CMR score in males than in females (0.899 vs. -0.223; $P < 0.001$). Physical activity related energy expenditure ranged between 196 – 12326.73 kcal/week, with a mean of 2,559 kilocalories/week (Figure 1A), with more individuals at the

lower end of the activity spectrum. Physical activity correlated negatively with waist circumference, fasting serum triglyceride, glucose and BMI, and positively with higher HDL levels (Table 1). CMR showed a relatively normal distribution (Figure 1B) and in bivariate analysis, correlated inversely with physical activity ($r^2=-0.16$; $P<0.001$).

All loci conformed to the Hardy Weinberg equilibrium ($P>0.05$ for all) (Table 2). Allele and genotype frequencies are shown in Table 2. Pairwise LD values were relatively weak ($r^2<0.35$) and are shown in Supplementary Table 1. Twenty-four haplotypes were ascertained in the sample of which five occurred at $\geq 5\%$ frequencies and were used for statistical analyses (Table 3). No evidence of population stratification was detected in the sample (STRUCTURE inferred probabilities for 1, 2 and 3 groups were 0.998 vs. 0.827 and 0.838 respectively) and no further adjustments were made to control for population stratification.

Higher physical activity lowers cardiometabolic risk

CMR was positively associated with age ($B=0.012$, $SE=0.004$, $t=3.03$, $P=0.003$), and negatively associated with years of school ($B=-0.04$, $SE=0.01$, $t=-4.04$, $P<0.001$) and female gender ($B=-1.08$, $SE=0.05$, $t=-20.66$, $P<0.001$). Higher physical activity was associated with lower CMR after covariate adjustment ($B=-0.24$, $SE=0.04$, $t=-6.98$, $P<0.001$). All explanatory variables together explained 36% of variation in CMR of which 4% was explained by physical activity.

Main effects of haplotypes and genotypes on cardiometabolic risk

Main effects of haplotypes on CMR are summarized in Table 3. H-23 haplotype carriers ($N=373$) had higher CMR than non-carriers ($N=544$) ($B=0.15$, $SE=0.05$, $t=2.79$, $P=0.006$ (FDR adjusted $P=0.025$)). H-23 explained 1% of variation in CMR. This finding did not vary by gender.

In secondary analyses rs135542 was significantly associated with higher CMR ($B=0.12$, $SE=0.044$, $t=2.82$, $P=0.005$) following an additive model. No other SNPs were associated with CMR in this sample (Table 2).

Physical activity moderates the association between *PPARA* and CMR

A significant interaction was observed between haplotype H-23 and physical activity ($B=-0.14$, $SE=0.072$, $t=-2.02$, $P=0.04$; $R^2=2\%$). Although more physical activity was associated with lower CMR in both groups with and without H-23, the slopes differed significantly. As shown in Figure 2, physical activity covaried more strongly with CMR in individuals carrying at least one copy of H-23 ($B=-0.35$, $t=-6.07$, $P<0.001$, $R^2=6\%$) compared to those without this haplotype ($B=-0.19$, $t=-4.56$, $P<0.001$, $R^2=2\%$). Indeed, the high CMR profile associated with H-23 was absent in carriers who engaged in relatively high levels of physical activity.

In single locus analyses the interaction between SNP rs135542 and physical activity was significant ($B=-0.058$, $SE=0.027$, $t=-2.099$, $P=0.036$). The covariation of physical activity-

related energy expenditure with CMR was greatest among the GG homozygotes ($r=-0.27$), lower among heterozygotes ($r=-0.19$) and least among AA homozygotes ($r=-0.13$) ($p<0.05$).

Supplementary Analyses

Analyses of individual components of the metabolic syndrome showed a significant main effect ($B=0.15$, $t=2.45$, $P=0.014$) and a significant interactive effect between H-23 and physical activity ($B=-0.16$, $t=-1.9$, $P=0.05$) on DBP. H-23 significantly predicted SBP ($B=0.12$, $SE=0.06$, $t=1.96$, $P=0.05$), HDL ($B=-0.12$, $SE=0.06$, $t=-2.05$, $P=0.041$), waist circumference ($B=0.12$, $SE=0.06$, $t=2.09$, $P=0.036$) and triglycerides ($B=0.19$, $SE=0.06$, $t=3.34$, $P=0.001$) in models adjusted for age, gender, education, smoking and physical activity.

rs135542 was associated with DBP both as a main effect ($B=0.11$, $SE=0.5$, $t=2.34$, $P=0.02$) and in interaction with physical activity ($B=-0.13$, $SE=0.07$, $t=-1.91$, $P=0.05$) and had a main effect on triglycerides ($B=0.11$, $SE=0.05$, $t=2.45$, $P=0.02$) and waist circumference ($B=0.12$, $SE=0.04$, $t=2.67$, $P=0.008$).

DISCUSSION

The known effects of *PPARα* on glucose and lipid metabolism (7) clearly indicate a potential role of the *PPARA* gene as an important candidate for CMR. In our study of healthy middle-age adults we confirmed that a *PPARA* haplotype is associated with CMR and, may account for a portion of the heritability of the metabolic syndrome, and by extension, type 2 diabetes and atherosclerotic cardiovascular disease. Previous studies of *PPARA* have found that genetic variation affects multiple independent metabolic syndrome components directly (8,11–14), or in interaction with diet (12). This is the first report to demonstrate that *PPARA* variation also interacts with habitual physical activity to moderate the composite CMR. More significantly, the negative effects of the *PPARA* H-23 haplotype on CMR are attenuated by physical activity, to the extent that carriers of the apparently detrimental haplotype have that risk obliterated by physical activity related caloric expenditure at the upper end of the sample distribution.

Increased physical activity is well established as an important factor for lowering CMR (4). However, the health benefits of physical activity do not accrue consistently from person to person and interindividual variability has heritable determinants (24). An alternate interpretation of our results is that variation in *PPARA* significantly moderates the effects of physical activity on CMR factors, and thereby provides more specific evidence of a genetic basis underlying the inter-individual differences in the beneficial effects of physical activity. Presence of the otherwise detrimental H-23 haplotype appears to enhance the beneficial effects of higher levels of physical activity in lowering CMR risk. Understanding this interaction on a molecular level is challenging, given the multitude of pathways that may be affected by either the gene or by physical activity.

Metabolic syndrome involves accumulation of excess adipose tissue accompanied by lipotoxicity in the form of pathological infiltration of muscle, liver and pancreatic islet cells. Aerobic exercise counters this lipotoxicity by a healthy repartitioning of intramyocellular

lipids to increase those serving as fuel sources and decrease levels of other, potentially toxic, lipid metabolites (25). PPAR α activity both reduces circulating triglyceride concentrations and increases fatty acid oxidative metabolism. Hence, the activation of PPAR α and downstream gene expression by exercise very likely constitutes a significant aspect of the cardiometabolic effects of physical activity. Similarly, PPAR α is present in the vascular endothelium and smooth muscle, where it modulates endothelial responses and levels of inflammatory mediators in conjunction with exercise (26). Therefore, to the extent that we and others have observed associations between genetic variation in *PPARA* and individual differences in metabolic risk factors, it follows that this same genetic variation may influence the degree to which exercise mitigates the toxic cardiometabolic and vascular effects of excess adipose tissue. Several other genes (e.g., *PPARG*, *UCP3*) could similarly interact with modifiable health behaviors and should be investigated.

Most candidate gene studies of *PPARA* have only examined independent effects of individual *PPARA* SNPs (8,10–14) and shown that multiple variants in this gene could be responsible for the observed effects on CMR factors. Previous reports have suggested that for candidate gene studies a haplotype based approach has more power, as it is likely that the combination of alleles is more informative regarding the effect of final gene product (21,22), conceivably reflecting epistatic interactions between loci. Our results indicate that though the haplotype effect appears to be primarily driven by the rs135542 locus, it is not completely explained by this locus alone as seen by the higher B coefficient for the haplotype effect. Since the pattern of alleles in the gene ultimately informs the final gene product, examining the haplotype as the unit of analysis remains the only measure of assessing overall gene function in a genetic association model such as ours.

The intronic SNP rs135542 is located nearest to the 5' end of *PPARA* gene among all SNPs examined in this study and is in strong LD with 8 other intronic SNPs in the European sample in Hapmap. Presence of the G allele of rs135542 was associated with higher CMR, but this allele also appeared to respond most strongly to the effects of physical activity as individuals with two and one copy of G showed sequentially higher correlations between physical activity and CMR. Although the frequency of this allele is 20% in the sample, H-23 was the only haplotype that carried this allele at measurable proportions. While the specific functions of this SNP are unknown, our results suggest that this SNP in combination with other SNPs examined in this study are potentially important determinants of PPAR α functioning under normal physiological conditions. Failure to observe any independent effect of the functional L162V variant could be due to the fact that most previous reports on this locus were in clinical samples (8,12). It is possible that detecting significant effects of this SNP might require either a higher proportion of subjects with overtly disordered cardiometabolic parameters or disease states allowing greater pathogenic expression of the L162V minor allele. Neither of these conditions was satisfied in our sample of generally healthy and unmedicated adults.

The CMR used here was derived from the full distribution of the standard components of the metabolic syndrome and, thus, differs from the clinical, dichotomous classification of the metabolic syndrome (1) and has more statistical power (16) for detecting associations (17). The significant negative correlation between CMR and physical activity was as expected.

That waist circumference, triglycerides and glucose levels independently also covaried significantly with physical activity indicates that the computed CMR variable is able to encompass a combined effect of these covarying risk factors. While variants in *PPARA* gene have been associated with lower HDL levels, and higher triglycerides and waist circumference (8,10–14) in individuals who were obese or had insulin resistance or diabetes, our findings extend these observations to generally healthy individuals without any established diagnosis of disease, indicating that *PPARA* affects normative variation in CMR.

Although these results demonstrate an interesting gene-by-environment interaction the cross sectional nature of the present study limits the inference of causality in the data. Physical activity was assessed by a questionnaire and is thus an indirect measurement that may be prone to recall bias. However, estimates of physical activity generated by the Paffenbarger questionnaire has been validated against objective measures of physical activity and fitness (28) and predicts major cardiovascular events and total mortality (29) and is generally considered a reliable measure of habitual physical activity (See Supplementary section). Diet, another major determinant of CMR was not considered in the analysis and may have significant main and interactive effects with physical activity as shown in some previous studies (30), and could have confounded these results in untested manners. Thus, future studies which include diet as another modifying covariate are warranted. Finally, this association needs to be replicated in other normal cohorts and in populations of different races and health conditions prior to generalization of results. Nonetheless, the observation that normal physical activity interacts with variations in *PPARA* gene to influence CMR in generally healthy adults is a novel finding that hints at as yet unknown mechanisms by which a behavior (as part of the larger non-genetic environment) can alter genetic predisposition.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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List of Abbreviations

CMR	Cardiometabolic Risk
IRB	Institutional Review Board
HDL	High density lipoprotein
PPARA	Peroxisome proliferator activated receptor A
BP	Blood pressure
SNP	Single nucleotide polymorphism
PCR	Polymerase chain reaction

FDR	false discovery rate
F_{st}	Fixation index

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Figure 1. Distribution of Physical activity and Cardiometabolic Risk in sample

Distribution of physical activity measured in kilocalories/week and the computed cardiometabolic risk scores in the data (with outliers removed). A) Physical activity ranged between 196 kcal/week to 12326.73 kcal/week. Mean and quartile values are shown in the inset. B) Cardiometabolic scores ranged between -1.95 to 1.77. Mean and quartile values are shown in the inset.

Figure 2. *PPARA* haplotype moderates the association between physical activity and cardiometabolic risk

The relationship between physical activity-related energy expenditure and CMR scores in the two haplotype-based groups. X axis represents the log transformed physical activity related energy expenditure in kcal/week. The supplementary axis shows the corresponding raw values for comparison. The Y axis represents CMR scores adjusted for covariates. Crosses represent data points for H-23 carriers and circles represent data points for non carriers of H-23. Solid line represents individuals carrying the H-23 haplotype (N=386), broken line represents individuals lacking the haplotype (N=550). CMR is associated with physical activity in both groups; however, individuals carrying H-23 appear to receive greater benefit from increased physical activity that appears to mitigate the increased CMR otherwise associated with the presence of this haplotype.

Table 1

Participant Characteristics (mean and standard deviations) and bivariate correlations

	Total Data (N=936)	Test Data (N=917)	Physical Activity r	CMR r
Age (years)	44.3 (6.9)	44.2 (6.9)	−0.05 (NS)	0.055 (NS)
% Female [#]	52	52	−0.055 (NS)	−0.551**
Education (years)	16.1 (2.7)	16.1 (2.7)	0.056 (NS)	−0.081*
% Ex/Current-smoker [#]	80	80	−0.039 (NS)	−0.016 (NS)
Physical Activity (kcal/week)	2,524.2 (1793.6)	2559.4 (1786.8)		−0.162 **
SBP (mmHg)	114.7 (12.8)	114.5 (12.7)	−0.007 (NS)	0.598**
DBP (mmHg)	77.4 (9.1)	77.4 (9.1)	−0.063 (NS)	0.628**
Triglycerides (mg/dL)	120.8 (83)	118.4 (75.1)	−0.126**	0.686**
HDL-cholesterol (mg/dL)	54.2 (14.9)	54.3 (14.8)	0.147**	−0.709**
Glucose (mg/dL)	95.0 (14.9)	94.3 (10.9)	−0.086**	0.547**
Insulin (uU/ml)	12.6 (7)	12.4 (6.3)	−0.115**	0.537**
Waist circumference (cm)	89.9 (15.3)	89.6 (14.9)	−0.132**	0.798**
Hip circumference (cm)	41.3 (4.3)	41.3 (4.3)	−0.127**	0.572**
BMI	26.6 (5.3)	26.5 (5.1)	−0.13**	0.626**

Table shows correlations estimated using transformed physical activity and cardiometabolic risk scores. Total Data (N=936): Shows estimate for total data selected after applying study exclusionary characteristics and genotyping thresholds that were included in the current study. Test Data (N=917): Shows estimates for the final set of subjects included in statistical analysis after removal of outliers identified by applying a "Mean $\pm$ 3* Standard Deviation" cut off for normalized physical activity and cardiometabolic risk (CMR) score values. Correlations shown for normalized values of physical activity and CMR and with outliers removed.

Table 2

Genotype, minor allele frequencies and main effects of single loci on metabolic risk scores

SNP	Genotype (%)	MAF %	HWE (P)	Main Effects
rs135542				
AA	486 (53)	0.26	0.055 (0.81)	B = 0.12, SE = 0.04, t = 2.79, P = 0.005
AG	343 (37)			
GG	63 (7)			
rs135539				
AA	286 (31)	0.42	0.28 (0.59)	B = 0.043, SE = 0.04, t = 1.12, P = 0.25
AC	434 (47)			
CC	153 (17)			
rs4253728				
AA	74 (8)	0.27	1.67 (0.19)	B = 0.053, SE = 0.041, t = 1.28, P = 0.20
AG	338 (37)			
GG	478 (52)			
rs1800206				
CC	785 (86)	0.06	0.33 (0.56)	B = 0.02, SE = 0.08, t = 0.024, P = 0.98
CG	96 (11)			
GG	4 (<1)			
rs4253778				
CC	37 (4)	0.19	2.165 (0.14)	B = 0.018, SE = 0.01, t = 0.375, P = 0.71
CG	254 (28)			
GG	594 (65)			

MAF: Minor allele frequencies; HWE (P): Hardy-Weinberg statistic and associated P values. Main effects comparing three genotype groups following an additive model (first indicated genotype was considered baseline). Model P value for main effects are as shown. FDR corrected P for rs135542 is 0.025 and is >0.05 for other loci.

Table 3

Observed Haplotypes with their main effects on metabolic risk scores

Haplotype	Sequence	Frequency (%)	Main effect		
			B	SE	t
H-1	AAACG	9	0.001	0.001	-0.007
H-2	AAACC	10	-0.075	0.07	-1.09
H-5	AAGCG	31	-0.03	0.05	-0.58
H-13	AGGCG	14	-0.12	0.05	-2.03
H-23	GAGCG	23	0.15	0.053	2.8

Order of SNPs in haplotypes: rs135542 (A/G), rs135539 (A/C), rs4253728 (G/A), rs1800206 (C/G) and rs4253778 (G/C). False discovery rate adjusted P values shown.