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Association of gene variants with lipid levels in response to fenofibrate is influenced by metabolic syndrome status

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Abstract

Objective—Fenofibrate therapy reduces serum triglycerides (TG) and increases high-density lipoprotein-cholesterol (HDL-C) and thus addresses the atherogenic dyslipidemia associated with metabolic syndrome (MetS). Our hypothesis is that genetic factors contribute to the variability of lipid response to fenofibrate differently in subjects with MetS and without MetS.

Methods—We investigated the association in 25 candidate genes with lipid responses to a 3-weeks trial on fenofibrate in subjects with and without MetS. We employed growth curve mixed models to generate the response phenotypes to fenofibrate in TG, HDL-C, and low-density lipoprotein-cholesterol (LDL-C) and examined the genetic associations accounting for family dependencies.

Results—After correcting for multiple testing ($p < 0.05$) and accounting for significant differences in the association effect sizes between subjects with and without MetS ($p < 0.05$), variants of *APOA5* (rs662799) and *APOE* (rs429358) were associated with HDL-C and LDL-C responses in MetS subjects, while *APOA4* (rs675) was associated with TG response in non-MetS subjects. There was also suggestive evidence that MetS may interact with *APOA4* ($p = 0.017$), *APOA5* ($p = 0.06$), and *APOE* ($p = 0.09$) to the variation to lipid responses.

Conclusions—Genetic effects that contributed to the variability of lipid responses to fenofibrate may differ in subjects with and without MetS. This research may provide guidance for more personalized and effective therapies.

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1. Introduction

Fenofibrate is a peroxisome proliferate-activated receptor (*PPAR*) agonist that improves lipid profiles, particularly reducing triglyceride (TG) levels and increasing high density lipoprotein-cholesterol (HDL-C) levels [1]. Fenofibrate improves the components of dyslipidemia in metabolic syndrome (MetS) patients including a shift from small, dense low density lipoprotein-cholesterol (LDL-C) particles to larger, more buoyant LDL-C particles [2].

Clinical trials have suggested that the absolute benefits of fenofibrate are likely to be greater in improving the lipid profile in the presence of MetS [3,4]. The patients with primary dyslipidemia, MetS or type 2 diabetes (T2D) treated with fenofibrate therapy were associated with changes from baseline of -25% to -59% in TG levels, -33% to +1% in LDL-C levels, and 1% to 34% in HDL-C levels [2]. In addition, a post-hoc analysis of clinical trials has suggested that fenofibrate was more effective in reducing cardiovascular risk in patients with MetS or T2D [4,5]. The largest effect of fenofibrate to reduce cardiovascular disease risk was detected in patients with marked dyslipidemia, features of the MetS, in whom a significant 27% relative risk reduction was found in the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study [4]. Another study reported that pharmacological treatment with fenofibrate in patients with MetS was able to reverse conditions leading to the labeling of patients with MetS in 24% of cases after 12 months of therapy, which may indicate a useful therapy for MetS patients [6]. These clinical trial findings have yielded substantial evidence of MetS status influencing the changes from baseline lipid to fenofibrate treatment.

It is well known that the expression of several genes is modified in response to *PPAR* protein. Activation of *PPAR* gene increases lipolysis and plasma clearance of atherogenic TG-rich lipoproteins via activation of lipoprotein lipase (*LPL*) and apolipoprotein A5 (*APOA5*), reduces production of the lipoprotein lipase inhibitor apolipoproteins C3 (*APOC3*), and increases expression of fatty acid-binding protein 2 (*FABP2*). Increased activity of several enzymes related to beta-oxidation of fatty acids is also seen. *PPAR* activation also upregulates expression of several genes related to HDL-C metabolism including apolipoproteins A1 (*APOA1*) and A2 (*APOA2*), and ATP-binding-cassette transporter A1 (*ABCA1*) [2,5]. Moreover, it has been well documented that the individual response of lipids to fenofibrate is highly variable [2,5], and probably due in part to genetic components. Association between the *APOA5* variant with increased HDL-C levels in response to fenofibrate was found in the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) [7].

There is evidence that *PPAR* protein plays an important role in the pathogenesis of MetS, including insulin resistance, glucose intolerance or T2D, dyslipidemia, obesity, hypertension, atherosclerosis, and albuminuria [8]. In addition, genetic factors are likely to affect the risk for MetS [1,9,10]. It is possible that the effect sizes of genetic variants associated with lipid response to fenofibrate treatment may differ between subjects with and without MetS, and consequently these genetic effects may influence differentially the metabolic pathway of *PPAR* and its related-expression genes in MetS and non-MetS subsets.

Although there is a body of evidence of multiple genes involved in the pathway of lipid lowering by fenofibrate treatment and that genetic factors influence the risk for MetS, whether the MetS etiology modulates differential genetic factors to lipid responses to lowering drugs has not been reported in the literature as far as we are aware. Our objective in this study is to investigate whether selected gene variants, in 25 candidate genes involved

in lipid metabolism, are associated with changes in lipid levels in response to a 3-week fenofibrate treatment differently among subjects with and without MetS from the GOLDN study.

2. Material and Methods

2.1. Study Design

The GOLDN study is comprised of families selected through individuals who had participated in the NHLBI Family Heart Study at two field research centers, in Minneapolis, MN and Salt Lake City, UT [11]. Written informed consent was obtained from each participant at his/her screening visit. This protocol was approved by the Institutional Review Boards at the University of Minnesota, the University of Utah and Tufts University. A detailed description of the study design and data has been reported elsewhere [7]. Some of the criteria for subjects to participate in the study included: presence of at least two siblings in the family; fasting TG < 1500 mg/dL and not taking lipid-lowering medications for at least 4 weeks prior to the initial visit; aminotransferase and alanine transaminase concentrations within normal range; negative medical history for myocardial infarction, coronary bypass surgery, coronary angioplasty or PTCA; and negative history of liver, kidney, pancreas, or gall bladder disease [7]. Each subject took 160 mg of fenofibrate (TriCor®, Abbott Laboratories, Chicago, IL) per day for 3 weeks. Fasting blood was drawn twice, a day apart, before and after fenofibrate treatment.

2.2. Sample and Phenotypes

The sample included 428 men and 433 women distributed in 174 European ancestry families. Lipids were measured using the Roche/Hitachi 911 Automatic Analyzer (Roche Diagnostics Corporation). TG levels were measured by the glycerol blanked enzymatic method. Cholesterol was measured by cholesterol esterase, cholesterol oxidase reaction, and LDL-C was measured by a homogeneous direct method (LDL Direct Liquid Select™ Cholesterol Reagent, Equal Diagnostics, Exton, Pa). HDL-C was calculated after precipitation of non-HDL-C with magnesium/dextran. Individuals were categorized as having MetS based on their clinical measurements at the clinical visit before fenofibrate treatment. The MetS was defined according to the 2005 National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) guidelines [12].

We verified the distributions of TG, HDL-C and LDL-C levels for normality before and after deriving the respective response phenotypes to fenofibrate treatment. Log of TG levels was taken to better approximate normalization. The response phenotypes in TG, HDL-C and LDL-C to the fenofibrate treatment were derived by a growth curve mixed method using the SAS package to estimate individual slope characterizing the change in these phenotypes using two measures before fenofibrate treatment and two measures after that. The response phenotypes were only estimated if the subjects had at least one lipid measure before and after the fenofibrate treatment. The individual growth curve model used a regressive error variance component matrix. Age up to the third degree (age, age², and age³), sex, their interactions and clinical centers were also modeled as fixed effects, while the interactions of individual and family structure with time points were modeled as random effects. Finally, the derived phenotypes were standardized to a mean of 0 and a standard deviation of 1. The TG, HDL-C and LDL-C responses did not deviate grossly from normal. Details of growth curve and the distributions of the final response phenotypes are shown in the Supplement 1.

The maximum heritability estimates, calculated by variance components model, were implemented in the computer program SOLAR (<http://solar.sfbgenetics.org/>).

2.3. Genotyping

DNA was extracted from blood and purified using Puregene reagents (Gentra System) following the manufacturer's instructions. SNPs were genotyped by TaqMan (Applied Biosystems, Foster City, CA, USA). One hundred and nine SNPs, from 25 genes known to be involved in lipoprotein metabolism, were identified through the genetic association literature and additional SNPs within a gene were included from public databases such as dbSNP (<http://www.ncbi.nlm.nih.gov/SNP/>).

The genes are: *ABCA1*, ATP-binding cassette transporter, subfamily G, member 5 (*ABCG5*), ATP-binding cassette transporter, subfamily G, member 8 (*ABCG8*), *APOA1*, *APOA2*, apolipoprotein A-IV (*APOA4*), *APOA5*, apolipoprotein B (*APOB*), *APOC3*, apolipoprotein E (*APOE*), cholesterol-7- α -hydroxylase (*CYP7A1*), fatty acid-binding protein 1 (*FABP1*), *FABP2*, hepatic lipase (*LIPC*), endothelial lipase (*LIPG*), *LPL*, low-density lipoprotein-related receptor protein 1 (*LRP1*), microsomal triglyceride transport protein (*MTP*), nitric oxide synthase 3 (*NOS3*), PDZ domain-containing 1 (*PDZK1*), *PPAR*, peroxisome proliferate-activated receptor (*PPAR*), peroxisome proliferate-activated receptor, cofactor 1, (*PPARGC1*), scavenger receptor, class B, member 1 (*SCARB1*), WD and tetratricopeptide repeats 1 (*WDTCT1*). The 25 lipoprotein metabolism genes and their respective SNPs are present in Supplement 2a, and the TaqMan SNP probe sequences for variants of *APOA4*, *APOA5* and *APOE* are shown in Supplement 2b. *APOE* rs429358 and rs7412 give rise to three *APOE* isoforms E2, E3 and E4, which differ in their amino acid compositions. *APOE2* has Cys at 130 and 176 sites, *APOE4* has Arg at both sites, and *APOE3* has Cys130 and Arg176. The metabolic function of these genes as well as the quality control of the GOLDN genotype data were described elsewhere [13].

2.4. Statistical Analysis

A Student's t-test was used to compare mean phenotype responses in two subsets of the data defined by the presence or absence of MetS using NCEP criteria. To examine the association of genetic variants with response phenotypes, we employed additive mixed model regression analysis which accounts for correlations among family members using SAS®. Subsequently, associations were estimated separately in subjects with and without MetS. Benjamini-Hochberg false discovery rate (FDR) [14] was used to control for multiple hypothesis testing. P value of 0.005 was the cutoff adjusted p-value for 109 multiple tests using FDR at significance level of 0.05. The FDR p-value was named as q-value.

Tests of heterogeneity between the mean response levels in MetS and non-MetS groups were carried out using a t-test, comparing the estimated regression coefficients (and standard error, SE) between MetS and non-MetS subjects, and also accounting for family structure, as such:

$$T\text{-test:}(\beta_{\text{MetS}} - \beta_{\text{Non-MetS}}) / \text{SQRT}[(\text{SE}_{\text{MetS}})^2 * \text{DF}_{\text{MetS}} + (\text{SE}_{\text{Non-MetS}})^2 * \text{DF}_{\text{Non-MetS}}] / (\text{DF}_{\text{MetS}} + \text{DF}_{\text{Non-MetS}}),$$

where DF (degree of freedom)=number of subjects –number of families - 2. The significance level ($p_{\text{t-test}}$) was based on two-tailed T test (Q. Zhang, personal communication).

Latest, the interaction effect between MetS status and SNP was tested by examining the statistical significance of the main effects and interaction term, using mixed model that accounted for family structure, as such: $Y = \alpha + \beta_{\text{SNP}} + \beta_{\text{MetS status}} + \beta_{\text{SNP*MetS status}}$.

This interaction model was carried out only for the SNPs that showed significant associations with lipid response.

3. Results

3.1. Characteristic of Study Sample

Table 1 gives the characteristics of the studied sample. Forty percent of subjects were classified with MetS. As expected, the MetS subjects were older, heavier, and had higher mean plasma levels of TG and LDL-C and lower mean HDL-C levels, both before and after fenofibrate treatment, as compared to those without MetS. Compared to the baseline lipid measures, the mean levels of TG and LDL-C decreased by 39.8% and 10.2% in MetS, and by 32.3% and 19.6% in non-MetS subjects, respectively, while HDL-C increased by 8.9% in MetS and 3.5% in non-MetS, after fenofibrate treatment.

For the MetS and non-MetS subjects combined, the heritability estimates were significant ($p < 0.05$) for TG (0.32 ± 0.06), LDL-C (0.23 ± 0.07) and HDL-C (0.30 ± 0.07) responses, suggesting that genetic effects influence the variability of lipid responses to fenofibrate treatment.

3.2. Genetic Associations with Lipid Responses to Fenofibrate

The association results are reported if they met two criteria: (i) the empirical p values of mixed model regression were significant ($p < 0.05$) after adjustment for multiple tests (cutoff: $q\text{-value} < 0.005$); and, (ii) the effect sizes of mixed regression analysis (β s and SEs) differed significantly ($p_{t\text{-test}} < 0.05$) between MetS and non-MetS subjects. As shown in Table 2, there were four SNPs that satisfied both criteria, which suggest evidence of heterogeneity in association of genetic variants with lipid responses in these subsets. For the MetS subset, *APOA5* and *APOE* were significantly associated with lipid response to fenofibrate. Carriers of *APOA5* T>C substitution at -1131 (rs662799, MAF=0.07) showed significantly higher HDL-C response as compared with homogeneous CC-*APOA5* subjects ($q\text{-value} = 0.001$, phenotypic variance (r^2)=3.2%). The difference in the effect size of association between MetS versus non-MetS subjects was also significant ($p_{t\text{-test}} = 0.039$). Similarly, carriers and non-carriers of the *APOE* 130Cys>Arg (rs429358, MAF=0.17) showed a statistically significant different LDL-C response ($q\text{-value} = 0.001$, $r^2 = 3.1\%$, $p_{t\text{-test}} = 0.025$).

In non-MetS subjects, we found associations between *APOA4* and *APOE* variants with lipid responses to fenofibrate treatment (Table 2). There was a statistically significant differential decrease in TG responses ($q\text{-value} = 0.003$, $r^2 = 2.1\%$) between *APOA4* 347Thr>Ser(rs675, MAF=0.18) carriers and non-carriers. The effect size of association between MetS and non-MetS subjects was significant ($p_{t\text{-test}} = 0.002$). We also observed a significant differential increase in HDL-C change levels ($q\text{-value} = 0.0007$, $r^2 = 2.4\%$) between *APOE* 176Arg>Cys (rs7412, MAF=0.07) carriers and non-carriers, and the difference of association effects between subjects with and without MetS was significant ($p_{t\text{-test}} = 0.023$). The mean levels and standard errors of changes in lipid responses to fenofibrate by genotype classes and MetS status are shown in Supplement 3.

We also carried out association analysis by combining genotypes of homozygous for low allele frequency with heterozygous and the associations did not vanish (Supplement 4). It suggests that a small proportion of the population carrying these low frequency variants has a higher change lipid levels in response to fenofibrate treatment compared with the average population.

Further, we tested whether the MetS etiology interacted with these four genetic variants (Table 2, Supplement 5) to influence the lipid response to fenofibrate treatment. There was a significant evidence that Met etiology modulated *APOA4* 347Thr>Ser to the TG response ($p = 0.017$). Also, there were borderline interaction associations between MetS status and *APOE* 130Cys>Arg to LDL-C response ($p = 0.061$), and between MetS status and *APOE*

176Arg>Cys to HDL-C response ($p=0.090$). However, MetS did not show interaction effect with *APOA5* T>C (rs662799, $p=0.187$) to influence the HDL-C response.

4. Discussion

We evaluated the association between lipid response to fenofibrate and 109 genetic polymorphisms in 25 candidate genes with established effects on lipid metabolism in subjects with or without the MetS. After accounting for multiple testing and significant differences in the association effect sizes between MetS and non-MetS subjects, we found evidence of association in lipid responses to fenofibrate treatment with *APOA5*, *APOA4* and *APOE* genes and MetS status.

Fenofibrate is a *PPAR* agonist, and its protein has been shown to play an important role in the pathogenesis of MetS including a critical role in lipid metabolism, a regulator of insulin sensitivity, and may contribute to the pathogenesis of obesity and implicated in vascular inflammation and blood pressure regulation [8]. Post-hoc analyses of clinical trials have shown that fibrate therapy was more effective in patients with MetS [1,2–5]. Pathophysiological conditions as a result of abnormal metabolic pathways in MetS patients occur mainly as a result of derangement in lipid metabolism, and fibrates are indicated to correct dyslipidemia by influencing these pathways [15]. Our results support the finding that fenofibrate was more effective in altering TG and HDL-C levels in MetS than in non-MetS subjects (39.8% vs. 32.3% decreasing TG, and 8.9% vs. 3.5% increasing HDL-C, respectively), as might be expected. However, fenofibrate was more effective in decreasing LDL-C levels in non-MetS subjects compared with MetS subjects (19.6% vs. 10.2%). The pathway by which fenofibrate affect the variation of lipoprotein may differ by MetS status.

Although there is a body of evidence that fenofibrate treatment improves lipid profile, there is significant interindividual variability in lipid response to fenofibrate, which may be mediated by genes. Further, the effect of those genes may differ in individuals with altered metabolic state of individuals with MetS as compared with subjects with normal metabolic profile. Our findings support the hypothesis that influence of genetic variants on lipid responses to fenofibrate depend on MetS status. There was evidence of association between lipid responses to fenofibrate treatment with variants of *APOA5* –1131T>C (rs662799) and *APOE* 130Cys>Arg (rs429358) in MetS subjects, and of *APOA4* 347Thr>Ser (rs675) and *APOE* 176Arg>Cys (rs7412) in non-MetS subjects.

These genes are well known to be involved in lipid metabolism. However, the genetic components of lipid response to fenofibrate and MetS pathway is poorly understood. *APOA4* variants have been linked to TG levels at baseline [16] and also in response to fenofibrate treatment [17]. However, this is the first report that the effect of *APOA4* variant associated with lipid response to fenofibrate treatment depends on the metabolic status of subjects with or without MetS. The precise function of *APOA4* protein is still unknown. It has been suggested that *APOA4* plays an important role in the metabolism of TG-rich lipoproteins. *APOA4* influences chylomicron synthesis, modulates the activation of *LPL* by apolipoprotein C-II, and activates lecithin-cholesterol acyltransferase [16,17]. We hypothesize that the missense *APOA4* 347Thr>Ser alter its protein structure and lead to altered gene function that interacts with either the abnormal metabolic state of individual or the genes that underlie *PPAR* pathway, thus contributing to a differential lipid response to fenofibrate in MetS subjects and non-MetS subjects.

Variants of *APOA5* have shown association with MetS [10,18–23] and baseline TG levels and HDL-C levels [7,10,24,25] in various populations. In addition, Cardona and collaborators [25] reported association between *APOA5* –1131T>C with decrease in TG response to fenofibrate in MetS Spanish patients. In the GOLDN study, carriers and non-

carriers of the *APOA5*–1131T>C had no significant different lipid response to fenofibrate in the whole sample [7], but the association of *APOA5*–1131T>C with HDL-C response was apparent only in MetS subjects. These findings suggest that the variability of lipid response to fenofibrate may be in part due to interactions between *APOA5* with gene(s) and/or environmental factors involved in the MetS pathway. It is known that *APOA5* is regulated by transcription factors known to affect triglyceride metabolism such as *PPAR* gene. The activation of *PPAR* increases lipolysis and plasma clearance of atherogenic TG-rich lipoproteins via activation of *LPL* and *APOA5* genes [2,5]. However, additional investigation will be necessary to understand how MetS status modulates differentially the metabolic pathway of *PPAR* protein and the expression of *APOA5* to contribute to the variability in lipid response- to fenofibrate treatment.

A few studies have reported the association between *APOE* alleles and response to fenofibrate treatment, though with conflicting results [26]. Variants of *APOE* were associated with changes in lipid levels treated with fenofibrate in patients with primary hypertriglyceridemia [27, 28] or mixed hyperlipidemia [27], but not in type 2 diabetic patients [29]. Our findings showed association of *APOE* variants with LDL-C response in MetS subjects and with HDL-C response in non-MetS subjects. For both results, the association effect sizes were significantly different in MetS versus non-MetS subjects. However, an interaction effect of MetS status was only suggested with *APOE* to LDL-C response, but not with HDL-C response to fenofibrate treatment. The lack of MetS interaction effect could be due to small power in the analysis, however other investigations will be necessary to endorse or reject this hypothesis. The *APOE* locus is considered to be one of the strongest genetic determinants of LDL-C variation in the general population [27], and its protein is essentially responsible for lipid and lipoprotein transport system [30]. Accelerated *APOE4* isoform containing lipoprotein clearance by the liver increases cholesterol and downregulates LDL receptors, consequently increasing the levels of total cholesterol, LDL-C and APOB [30]. *APOE* also plays an important role in the development of obesity and insulin resistance, which are components of MetS. Studies have also reported associations between MetS components and polymorphisms of *APOE* [30–31]. Despite the primary role of *APOE* to dyslipidemia, obesity, and insulin resistance in the development of the MetS, the functional interaction of MetS with *APOE* protein in the lipid-lipoprotein transport system and *PPAR* pathway remain unclear. We hypothesize that the amino acid composition changes in variants of *APOE* may modify the structure and properties of their proteins that interact differently with metabolic components and/or genes involved in the MetS pathway, and thus the subjects with and without MetS respond differentially to the fenofibrate treatment.

GOLDN is a unique study in that fenofibrate responses were assessed in families, including both normal and dyslipidemic subjects. The design of the GOLDN study was an important strategy, with a broad range of lipid phenotypes at baseline that differed from a traditional clinical trial, which provided a valuable resource for investigating genetic association with lipid responses to fenofibrate treatment. In GOLDN study, several of selected SNPs from the 25 genes known to be involved in lipoprotein metabolism are located in the coding region. These SNPs may alter the expression or the activity of a target protein and can have a real clinical relevance to predict drug side effects. Further studies will be necessary to replicate our findings in different populations. However, the feasibility of confirming results is a common challenge in pharmacogenetic studies because of the differences in the population characteristics, low allele frequency, small genetic effect sizes, or by chance. A prerogative study of participants undergoing intervention of fenofibrate with and without MetS may be needed to confirm/or not our findings of MetS-gene interaction contributing to the variability of lipid responses.

In summary, genetic factors seem to modulate lipid response to fenofibrate treatment. Our results show evidence that the genetic variants and MetS status contribute to individual susceptibility to lipid responses to fenofibrate. Although a genetic predisposition might be involved in the etiology of the MetS, acquired risk factors are readily modifiable by appropriate therapy. These findings might provide guidance for additional studies seeking to personalize the selection of effective therapy for patient with dyslipidemia and MetS status.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Characteristics of the Study Population by MetS Status

Covariates and Phenotypes	MetS [†]	Non-MetS [†]
Sample size	344	517
Sex (% of males)	53%	47%
Age (in years)	56.2±13.5	43.2±15.5
BMI (in kg/m ²)	31.7±5.0	26.3±4.8
TG (mg/dL)		
Before fenofibrate *	206.3±139.2	101.0±62.3
After fenofibrate *	124.3±65.4	68.4±33.7
% Response	-39.8	-32.3
HDL-C (mg/dL)		
Before fenofibrate *	40.3±10.4	50.9±13.2
After fenofibrate *	43.9±11.4	52.7±13.4
% Response	+8.9	+3.5
LDL-C (mg/dL)		
Before fenofibrate *	133.4±30.3	116.9±30.6
After fenofibrate *	119.8±30.0	94.0±27.8
% Response	-10.2	-19.6

* The values for TG, HDL-C, and LDL-C refer to the means ± standard deviations (SD) before and after fenofibrate treatment.

[†] These mean levels of TG, HDL-C and LDL-C were significantly ($p<0.05$) different between MetS and Non-MetS both before and after fenofibrate treatment (by T-test). The lipid levels from before and after fenofibrate were used to estimate the phenotype responses that were derived by growth curve mixed method.

Additive Mixed Model Regression Analysis Results for Single SNP, SNP and MetS Status Interaction, and T-test between MetS and Non-MetS subjects for Lipid Changes in Response to Fenofibrate Treatment

Table 2

<i>Gene</i>	<i>SNP</i>	<i>MAF</i>	<i>Type</i>	<i>Response</i>	<u>MetS (N=344)</u>		<u>Non-MetS (N=517)</u>		<u>T-test</u>		<u>Interaction SNP* MetS status</u>	
					<i>q-value</i> [*]	(SE)	<i>q-value</i> [*]	(SE)	<i>p-value</i> [†]		<i>p-value</i>	
<i>APOA4</i>	rs675	0.18	Exon-3, missense	TG	0.79	0.05 (0.21)	0.003	-0.50 (0.16)	0.002			0.017
<i>APOA5</i>	rs662799	0.07	5 near gene	HDL-C	0.001	0.89 (0.26)	0.50	0.23 (0.35)	0.039			0.061
<i>APOE</i>	rs7412	0.07	Exon-4, missense	HDL-C	0.22	0.32 (0.26)	0.0007	0.95 (0.27)	0.023			0.187
<i>APOE</i>	rs429358	0.17	Exon-4, missense	LDL-C	0.001	0.72 (0.22)	0.04	0.32 (0.15)	0.025			0.090

* q-value of 0.005 is the cutoff adjusted p-value for 109 multiple tests using FDR at significance level of 0.05.

† p-value of t-test refers to the comparison of response means between MetS and non-MetS subjects.