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LPL gene variants affect apoC-III response to combination therapy of statins and fenofibric acid in a randomized clinical trial of individuals with mixed dyslipidemia

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LPL gene variants affect apoC-III response to combination therapy of statins and fenofibric acid in a randomized clinical trial of individuals with mixed dyslipidemia[S]

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Abstract **ApoC-III is a proatherogenic protein associated with elevated triglycerides; its deficiency is associated with reduced atherosclerosis. Mixed dyslipidemia, characterized by elevated triglyceride and apoC-III levels and low HDL cholesterol level, with or without elevated LDL cholesterol, increases cardiovascular disease risk and is commonly treated with combined statin and fibrate therapy. We sought to identify single nucleotide polymorphisms (SNPs) associated with apoC-III level response to combination therapy with statins and fenofibric acid (FA) in individuals with mixed dyslipidemia. Participants (n = 1,250) in a multicenter, randomized, double-blind, active-controlled study examining response to FA alone and in combination with statin were genotyped for candidate SNPs. Multivariate linear regression and two-way ANOVA for percent change in apoC-III level were performed. SNPs in the lipoprotein lipase (*LPL*) gene region, rs1801177 ($P = 4.7 \times 10^{-8}$), rs7016529 ($P = 1.2 \times 10^{-6}$), and rs249 ($P = 4.1 \times 10^{-5}$), were associated with apoC-III response to combination therapy. A haplotype composed of the minor alleles of these SNPs, with 2% population frequency, was associated with an unexpected apoC-III increase in response to statins and FA. This is the first report to show that genetic variation within the *LPL* gene region can affect the response of apoC-III levels to combined statin and FA therapy.**—Brautbar, A., S. S. Virani, J. Belmont, V. Nambi, P. H. Jones, and C. M. Ballantyne. ***LPL* gene variants affect apoC-III response to combination therapy of statins and fenofibric acid in a randomized clinical trial of individuals with mixed dyslipidemia. *J. Lipid Res.* 2012.** 53: **556–560.**

Supplementary key words apolipoproteins • fibrates • genetic variants • HDL-C • pharmacogenetics

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ApoC-III is an important inhibitory cofactor for the hydrolysis of triglycerides (TGs) by LPL, and increased apoC-III levels are frequently observed in individuals with elevated TG levels (1). On the other hand, a partial deficiency in apoC-III level was shown to be associated with an improved lipid profile, including higher HDL-cholesterol (HDL-C), lower TG, and lower LDL-cholesterol (LDL-C) levels and reduced subclinical atherosclerosis (2, 3).

Fenofibric acid (FA) is a peroxisome proliferator-activated receptor-α agonist that reduces apoC-III and TG levels and increases apoA-I and HDL-C levels (4). In combination with statins, FA is indicated and frequently used to treat individuals with the mixed dyslipidemia phenotype, which is characterized by elevated TG and low HDL-C levels, with or without high LDL-C level (5, 6). Individuals with mixed dyslipidemia have an increased risk for coronary heart disease (CHD) events, and a recent clinical trial showed that fenofibrate in combination with statins may lower CHD event rates in individuals with type 2 diabetes and mixed dyslipidemia (7). Genetic variation may play an important part in the interindividual differences in the response of lipid and apolipoproteins to statin-fibrate combination therapy (8).

In this study, we examined the association between single-nucleotide polymorphisms (SNPs) and change in

Abbreviations: CHD, coronary heart disease; FA, fenofibric acid; HDL-C, HDL-cholesterol; LD, linkage disequilibrium; LDL-C, LDL-cholesterol; MAF, minor allele frequency; SNP, single-nucleotide polymorphism; TG, triglyceride.

The data used for the analysis described in this paper are available at Gene Expression Omnibus (GEO) database accession number GSE34945.

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apoC-III levels in response to the combination of FA and statins. We hypothesized that genetic variants may have a significant effect on the response of apoC-III to combination therapy with FA and statins in individuals with mixed dyslipidemia.

METHODS

Study population

The study population included adults who participated in one of three concurrent prospective, randomized, double-blind, phase 3 studies designed to examine the efficacy and safety of a new FA (Trilipix, Abbott Laboratories, Abbott Park, IL) used as monotherapy or combined with statin as has been previously published (9). In brief, men and women with TG ≥150 mg/dl, HDL-C <40 mg/dl for men or <50 mg/dl for women, and LDL-C ≥130 mg/dl were eligible to participate. In each study, participants were randomized into groups receiving FA (135 mg/d) alone, statin (rosuvastatin 10 or 20 mg/day, atorvastatin 20 or 40 mg/day, or simvastatin 20 or 40 mg/day) alone, or the combination of FA and statin. After a washout period of 6 weeks, participants received study treatment for 12 weeks. ApoC-III and lipid levels were assessed at the beginning and end of the treatment period. The study was approved by the Institutional Review Board of Baylor College of Medicine, and informed consent was obtained by Abbott Laboratories.

Selection of genes and SNPs

Genes related to TG, HDL-C, and apoC-III pathways were included (4, 10). Common (>5%) and less common (1–5%) tag SNPs were selected using the Genome Variation Server (http://gvs.gs.washington.edu/GVS/). Criteria for SNP selection were HapMap-CEU dataset, $r^2 \geq 0.8$ for each bin, minor allele frequency (MAF) ≥1%, and up to 10 Kb margin from gene boundary. Other SNPs identified in published genome-wide association studies that examined associations with HDL-C and TG were added (11–14). Thirty-four SNPs were used for correcting possible population stratification within the European-American population (15), and 350 SNPs (see Table 1 in reference 8 for the details of the examined SNPs) were used to examine possible effects on apoC-III response to therapy. SNPs were genotyped with the Golden Gate chemistry platform on an Illumina Bead Express system (Igenix, Seattle, WA). Only samples with call rate >90% were included, and SNPs were excluded if they had a call rate <95% or showed evidence of deviation from Hardy-Weinberg equilibrium at $P < 0.001$ using the exact test.

Statistical analysis

Statistical analysis was performed in R (16) using the *Rcmdr* GUI (http://cran.r-project.org/web/packages/Rcmdr/index.html) and PLINK (version v1.07, http://pngu.mgh.harvard.edu/purcell/plink/) (17). To avoid possible increased type I and type II errors caused by population stratification, analysis was limited to European-American Caucasian participants. Uncorrelated SNPs were identified by pruning out those with pairwise linkage disequilibrium (LD) $r^2 > 0.5$. The remaining SNPs (including all 34 ancestry-informative SNPs) were then used to cluster individuals based on identity-by-state analysis. The resulting identity-by-state data were used in multidimensional scaling, and four components were saved. No significant structure in the sample was detected.

A total of 1,233 individuals were included in the study. To enhance statistical power, treatment groups of the original studies were collapsed into three major therapy groups: FA alone (n = 226), statin alone (n = 586), and combination therapy with statin and FA (n = 421).

Multivariate linear regression for associations with percent change was conducted using PLINK for an additive model. Covariates included in the regression analysis were age, sex, body mass index, smoking, and diabetes. Two-way ANOVA was used to estimate percent change and mean differences between genotypes. Percent change was defined as the difference in trait level before and after treatment divided by the before-treatment level. SNPs were excluded from the analysis because of MAF <5% and missing >1% or MAF >5% and missing >5% (n = 14), Hardy-Weinberg equilibrium <0.001 (n = 30), and monomorphism (n = 2). Excluding the 34 SNPs selected for the stratification analysis, 304 out of 384 that were initially genotyped were used for association analysis. A P value of $<1.7 \times 10^{-4}$ was considered significant after Bonferroni correction for the number of SNPs included in the association analysis (304 after exclusions). Haplotypes inferred with expectation-maximization algorithm were examined using multivariate regression analysis including available covariates. The Wald test P value was calculated for each haplotype's partial regression coefficients.

RESULTS

From a total sample size of 2,684 individuals, 1,415 samples were excluded. Samples were excluded because of total call rate <95% (n = 66), duplicated DNA samples (n = 64), ethnicity other than European (n = 326), and participants with no apoC-III information available (n = 959). A total of 1,233 participants were included in the analysis.

Mean apoC-III level in the combination therapy group was 19 mg/dl at baseline and was reduced by a mean of 32% after therapy.

On multivariate regression analysis, three SNPs in the *LPL* gene, rs1801177 ($P = 1.1 \times 10^{-6}$), rs7016529 ($P = 3 \times 10^{-6}$), and rs249 ($P = 1.5 \times 10^{-4}$), had significant associations with percent change in apoC-III after the combination of statin and FA treatment. No significant associations were identified in the FA-only and statin-only groups between SNPs and percent change in apoC-III level in response to therapy (**Table 1**). rs249 and rs7016529 reside within introns of the *LPL* gene, whereas rs1801177 is a missense mutation in exon 2 that results in a nonconservative substitution of asparagine for aspartic acid.

A two-way ANOVA demonstrated significant differences between genotypes for rs1801177 ($P = 4.7 \times 10^{-8}$), rs7016529 ($P = 1.2 \times 10^{-6}$), and rs249 ($P = 4.1 \times 10^{-5}$) (**Table 2**). The minor alleles for each of the three SNPs demonstrated the same direction of effect on the apoC-III response to FA plus statins. Carriers of the minor alleles for rs1801177 and rs7016529 had a mean increase in apoC-III rather than the expected decrease after combination therapy with FA and statins, whereas carriers of the rs249 minor allele had around half of the decrease in apoC-III observed in participants who were homozygous for the wild-type allele. Significant LD was observed between rs1801177 and rs7016529 ($r^2 = 0.92$), and lesser LD was observed between rs249 and either rs1801177 or rs7016529 ($r^2 = 0.16$–0.17) (Supplementary Fig. I).

In the entire study population, SNPs rs1801177 ($P = 0.04$), rs7016529 ($P = 0.02$), and rs249 ($P = 0.008$) had

TABLE 1. Significant associations using multivariate regression analysis for percent change in apoC-III adjusted for age, sex, body mass index, smoking, baseline trait level, baseline triglyceride level, and diabetes by treatment group

				Combination therapy		Statin monotherapy		FA monotherapy	
Gene	SNP	MAF	Minor allele	P	Beta	P	Beta	P	Beta
LPL	rs1801177	2.0%	A	1.1×10^{-6}	37.1	0.59	2.2	0.67	−3.2
LPL	rs7016529	2.1%	G	3.0×10^{-6}	34.8	0.71	1.4	0.67	−3.2
LPL	rs249	7.5%	G	1.5×10^{-6}	15.6	0.3	2.2	0.08	−6.4

Based on NCBI Build 36.1; β, β coefficient; FA, fenofibric acid; MAF, minor allele frequency.

weak associations with baseline apoC-III levels, but the associations decreased for rs249 ($P = 0.09$) and disappeared for rs1801177 ($P = 0.8$) and rs7016529 ($P = 0.1$) after therapy. When haplotypes composed of SNPs rs1801177, rs7016529, and rs249 in the group receiving combination therapy were examined, the AGG haplotype (frequency = 2%), which consists of the minor allele of each marker, was associated with an increase in apoC-III in response to combination therapy ($P = 3.9 \times 10^{-7}$) (**Table 3**). The GAA haplotype, which consists of the wild-type alleles, was associated with a decrease in apoC-III ($P = 2.1 \times 10^{-4}$) after combination therapy with statin and FA as expected (Table 3).

Although baseline TG levels were modestly lower for carriers of the minor alleles of SNPs rs1801177, rs7016529, and rs249, the difference between carriers and noncarriers was not statistically significant (Supplementary Table I). Also, SNPs rs1801177, rs7016529, and rs249 were not associated with baseline HDL-C levels (Supplementary Table I). rs1801177 and rs7016529 were not associated with percent change in HDL-C or TG after FA-statin treatment, whereas rs249 had a weak association with percent change in TG in a regression analysis for the comparison between genotypes (Supplementary Table II). When examining the differences in percent change in TG across genotypes as a separate hypothesis by ANOVA, carriers of the minor allele for the SNPs rs1801177 and rs249 had significantly less percent change in TG in response to therapy compared with noncarriers (Supplementary Table III).

Although none of the haplotypes was associated with baseline levels of HDL-C or TG (Supplementary Table IV), the GAG and GAA haplotypes were associated with percent change in TG in the combination therapy group; none of the haplotypes was associated with percent change in HDL-C (Supplementary Table V).

DISCUSSION

In this study we identified three SNPs in the *LPL* gene that affect change in apoC-III level in response to FA and statin therapy in individuals with mixed dyslipidemia.

ApoC-III is a component of TG-rich lipoproteins such as chylomicrons and VLDL, HDL, and LDL; it serves as an inhibitor of LPL activity and therefore inhibits the catabolism of TG-rich lipoproteins (18). ApoC-III has been associated with hypertriglyceridemia (19, 20), increased cardiovascular risk (21), atherosclerosis in animal models (22), and subclinical atherosclerosis (increased coronary artery calcium) in humans (2).

Mixed dyslipidemia, characterized by elevated apoC-III and TG levels and low HDL-C level, with or without elevated LDL-C, is also associated with a higher incidence of cardiovascular events (7, 23–26) and is frequently treated with combination therapy to address the multiple lipid abnormalities of this phenotype. The SNPs identified in this study were associated with a paradoxical increase in apoC-III levels (SNPs rs1801177 and rs7016529) in response to combination therapy or impeded the apoC-III level decrease (SNP rs249) observed in participants with the wild-type alleles. This raises an important question regarding the clinical relevance of this observation. Fibrates as monotherapy improved clinical event outcomes in men with baseline nonHDL-C ⩾200 mg/dl in the Helsinki Heart Study (27) and in men with baseline HDL-C ⩽40 mg/dl in the Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial (28) but not in men and women with type 2 diabetes in the FIELD (Fenofibrate Intervention and Event Lowering in Diabetes) study (25). However, subgroup analysis of the FIELD participants with mixed dyslipidemia (TG ⩾150 mg/dl and HDL-C <40 mg/dl for men and <50 mg/dl for women) did demonstrate outcome benefit. A similar result was shown in the ACCORD

TABLE 2. Means of percent change in apoC-III after therapy for genotypes in the combination therapy group

SNP	Homozygous	Heterozygous	Wild-type	P*	P†
rs1801177	A/A	G/A	G/G	2.7×10^{-7}	4.7×10^{-8}
Mean change (%)	NI	13	−33		
rs7016529	G/G	G/A	A/A	7.8×10^{-7}	1.2×10^{-6}
Mean change (%)	NI	10	−33		
rs249	G/G	G/A	A/A	2.8×10^{-5}	4.1×10^{-5}
Mean change (%)	NI	−16	−34		

* Univariate regression analysis including copy number of the minor allele for percent change.
† Multiway ANOVA.
NI, none identified in the study.

TABLE 3. Haplotypes composed of SNPs rs1801177, rs7016529, and rs249 and their associations with apoC-III response to therapy in the group receiving the combination of statins and fenofibric acid, adjusted for age, sex, body mass index, smoking, baseline triglyceride levels, and diabetes

Haplotype	Frequency	Beta coefficient	P for percent change
AGG	0.02	40.1	3.9×10^{-7}
GAG	0.06	6.19	0.18
GAA	0.92	−15	2.1×10^{-4}

(Action to Control Cardiovascular Risk in Diabetes) trial, in which the combination of statin and fenofibrate failed to improve cardiovascular event outcomes in the overall study population of individuals with type 2 diabetes but seemed to improve outcomes in the subgroup with TG ⩾204 mg/dl and HDL-C ⩽34 mg/dl (7). Therefore, fibrate therapy appears to provide more benefit in individuals with persistent TG elevation and low HDL-C; however, a limitation of our study was the lack of clinical event outcome information.

Although apoC-III has been associated with atherosclerosis in human and animal models, only the Cholesterol and Recurrent Events trial (which examined the effect of pravastatin on CHD event rates in secondary prevention) showed that levels of apoC-III bound to VLDL and LDL were associated with CHD events (29). Additional evidence of the connection between apoC-III and atherosclerosis comes from an in vitro study suggesting that elevated apoC-III level causes vascular dysfunction (30). Thus, apoC-III reduction may be one of the pathways by which fibrates decrease atherosclerosis in the mixed dyslipidemia population. In this study, we have identified three SNPs that increase apoC-III levels or attenuate the expected reduction in apoC-III levels after statin-FA therapy. These are relatively rare SNPs, with a frequency of 2–7%. We hypothesize that, to some extent, the observed reduction in atherosclerotic event outcomes in individuals with mixed dyslipidemia after combination therapy of statin and FA may be attenuated or inhibited in carriers of the minor allele of SNPs rs1801177, rs7016529, and rs249; however, this hypothesis should be examined in a randomized clinical trial that includes event outcomes.

It is not possible to determine whether the SNPs we identified have a direct effect on the response of apoC-III levels to combination therapy. Fibrates are thought to reduce apo C-III by at least two mechanisms: enhanced clearance of TG-rich lipoproteins, which carry apo CIII, due to enhanced LPL activity, and direct effect on apoC-III levels by reduced transcription. Statins are thought to reduce apoC-III primarily by enhanced clearance of VLDL remnants due to up-regulation of the LDL receptor, which interacts with apoB and apoE. ApoC-III is postulated to inhibit LPL-mediated removal by several mechanisms, including displacement of apoE, which has a high affinity for negatively charged surfaces and thus would inhibit interaction with LPL, which resides on the cell surface; displacement of apoC-II, which directly activates LPL; and a possible direct protein-protein interaction between apoC-III and LPL (1). The fact that haplotypes GAA and GAG were associated with a negative trend for the percent change in TG (Supplementary Table III) suggests that these haplotypes reduce the functionality of LPL. We hypothesize that these haplotypes identify a genetic variant in which LPL has enhanced interaction with apoC-III, which would result in reduced activity of LPL, reduced clearance of TG-rich lipoproteins, increased apoC-III, and potentially increased risk for CHD. VLDL remants enriched in apoC-III would have more displacement of apoE and thus may have reduced binding of apoE to the LDL receptor and less reduction in apoC-III when the combination of fibrate and statin was given.

rs1801177 has been previously associated with atherosclerosis progression and CHD events. Although none of these studies examined apoCIII in relation to the SNP's effect on atherosclerosis and although the SNP was not associated with baseline apoC-III level in our analysis, it is possible that the interaction between the SNP and statin-FA combination therapy increases risk for atherosclerosis progression and CHD events through paradoxical elevation of apoC-III levels.

In summary, genetic variants in the *LPL* gene region were associated with attenuated decrease or paradoxical increase of apoC-III in response to statin-FA combination therapy in individuals with mixed dyslipidemia. We postulate that these genetic variants may attenuate the favorable effect of fibrates not only on apoC-III levels but also potentially on the cardiovascular outcome benefit observed in individuals with mixed dyslipidemia receiving combination therapy with FA and statins.

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