Published in final edited form as:

Pharmacogenomics. 2010 July ; 11(7): 959–971. doi:10.2217/pgs.10.58.

Physiogenomic analysis of statin-treated patients: domain-specific counter effects within the *ACACB* gene on low-density lipoprotein cholesterol?

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Abstract

Aim—Administered at maximal dosages, the most common statins – atorvastatin, simvastatin and rosuvastatin – lower low-density lipoprotein cholesterol (LDLC) by an average of 37–57% in patients with primary hypercholesterolemia. We hypothesized novel genetic underpinnings for variation in LDLC levels in the context of statin therapy.

Materials & methods—Genotyping of 384 SNPs in 202 volunteers from a lipid outpatient clinic was accomplished and LDLC levels obtained from chart records. The SNPs were distributed across 222 genes representing physiological pathways such as general metabolism, cholesterol biochemistry, cardiovascular function, inflammation, neurobiology and cell proliferation. We discovered significant associations with LDLC levels for the rs34274 SNP ($p < 0.0002$) and for rs2241220 ($p < 0.008$) in the acetyl-coenzyme A carboxylase β (*ACACB*) gene. When corrected for multiple testing, the false-discovery rate associated with rs34274 was 0.076 (significance threshold: 0.10) and for rs2241220 the false-discovery rate was 0.93 (not significant). The acetyl coenzyme A carboxylase β enzyme synthesizes malonyl coenzyme A, an essential substrate for hepatic fatty acid synthesis and an inhibitor of fatty acid oxidation.

Results—The SNPs were in weak linkage disequilibrium ($D' = 0.302$). Minor alleles at these sites demonstrate opposing influences on LDLC; the C>T substitution at rs34274 is a risk marker and the C>T substitution at rs2241220 a protective marker for LDLC levels. These SNPs hypothetically influence enzymatic activity through different mechanisms, rs34274 through the PII promoter and rs2241220 via alteration of the protein's responsiveness to allosteric influence.

Conclusion—Physiogenomic evidence suggests a novel link between LDLC levels and the regulation of fatty acid metabolism. The findings complement previously discovered novel SNP relationships to myalgia (pain) and myositis (serum creatine kinase activity). By genotyping for

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

myositis, myalgia and LDLC levels, a physiogenomic model may be developed to help clinicians maximize effectiveness and minimize side effects in prescribing statins.

Keywords

acetyl coenzyme A carboxylase β ; LDL cholesterol; low-density lipoprotein cholesterol; physiogenomics; statin efficacy

Statins are selective, competitive inhibitors of HMG CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis. Administered at maximum dosages, the most common statins – atorvastatin, simvastatin, rosuvastatin and pravastatin – lower low-density lipoprotein cholesterol (LDLC) by 37–57% in patients with primary hypercholesterolemia [1–4]. The magnitude of the LDLC response differs according to phenotypic, demographic and as yet unexplained characteristics [5].

Although approximately 50% of the variability in plasma LDLC is estimated to be owing to inheritance [6], only a small number of common and multiple rare gene variants that contribute to the phenotype are known [6–8]. Pharmacogenetic studies of LDLC lowering associated with statin therapy have focused mainly on genes in cholesterol synthetic, lipoprotein lipid transport and pharmacokinetic pathways, showing that SNPs in genes of cholesterol metabolism, such as *HMGCR* [9–12], and lipoprotein transport, such as *APOE* [12–21] and *LIPC* [22], can influence the statins' ability to lower LDLC levels. Variants in pharmacokinetic genes, such as *SLCO1B1*, which encodes the organic ion transporter protein 1B1, and *CYP7A1*, which encodes the CYP7A1 protein, can also affect LDLC lowering with statins [23,21]. Recent findings have begun to extend the repertoire of gene variants associated with statin efficacy to new mechanisms of drug action. The *KIF6* gene codes for a cytoskeletal protein involved in intracellular transport of protein complexes, membrane organelles and mRNA [24]. The Trp719Arg substitution in the protein enhances the efficacy of statin therapy, apparently through pleiotropic effects [25]. In the absence of statin therapy, variants in genes such as *APOE* [26–28], *APOB* [27,28], *NPC1L1* [29], *PSCK9* [28,30], *CELSR2* [27,28], *PSRC1* [27], *SORT1* [27,28] and *LDLR* [6,27,28] affect LDLC. As baseline LDLC, to some extent, predicts the magnitude of LDLC lowering with statins, there may be overlap in the genes that regulate LDLC metabolism and statin-mediated LDLC lowering.

No comprehensive analysis has yet identified an association between genetic variations and statin-induced LDLC lowering in patients typical of clinical practice. Physiogenomics is a medical application of sensitivity analysis and systems engineering that defines a new paradigm in the genetic analysis of complex human phenotypes [31]. Sensitivity analysis is the study of the dependence of a system on changes in its components [32]. In physiogenomics, SNPs provide the variable components of genes, and analysis of the relationship between that variation and the physiological response provides information regarding which genes play important roles in the physiological process [31,33]. This approach has been advanced in both human clinical studies [34–39] and animal models [33,40,41]. The associated gene markers are combined into SNP ensembles, harnessing their combined predictive power to estimate functional variability among individuals similarly treated [33,42].

Our previous physiogenomic studies have generated hypothetical mechanisms related to statin-induced myositis [36] and myalgia [38]. Here, in a cohort of 202 subjects receiving statin therapy and genotyped for an array consisting of 384 SNPs distributed across physiological pathways represented by 222 genes, physiogenomic analysis was employed to further investigate gene associations to LDLC in patients receiving statin therapy.

Physiogenomic analysis provides new evidence associating an intronic variant near the *ACACB* mitochondrial binding domain, rs34274, and a SNP near the cAMP-dependent phosphorylation site, rs2241220, to LDLC-lowering in patients receiving statin therapy.

Materials & methods

■ Study design

This is a cross-sectional study investigating genetic factors predicting LDLC in patients receiving statin therapy. Duration of exposure to statins was 4 weeks or more.

■ Subjects

A total of 207 men and women who were treated with statin therapy for hyperlipidemia were recruited from outpatient lipid clinics at the University of California at San Francisco (San Francisco, CA, USA). Of these, 202 had been genotyped and had complete clinical data (Table 1). Each subject signed a written consent, approved by the University of California, San Francisco Committee on Human Research, for blood collection and review of medical and laboratory records, as well as testing of blood samples for discovery of gene mutations and polymorphisms. Concurrent medications and supplements were recorded, including those that increase myopathy risk such as gemfibrozil [43,44], niacin [45], ezetimibe [46] and amiodarone [45], or decrease the metabolism of atorvastatin and simvastatin through CYP3A4 inhibition (e.g., antifungals, macrolides, HIV protease inhibitors, nefazodone, ciclosporin, verapamil and amiodarone) [47].

■ Laboratory analysis

Blood for DNA was either collected prospectively or retrieved from routine clinical analysis. Samples were collected into tubes containing either ethylenediamine tetra-acetic acid or citrate for DNA extraction. The DNA was extracted from leukocytes in 3.5 ml of whole blood using a DNA isolation kit (Puregene Gentra®, Qiagen, CA, USA).

■ Low-density lipoprotein cholesterol

Low-density lipoprotein cholesterol was measured at least 4 weeks after initiation of statin therapy. Blood was drawn after a 10-h fast. Cholesterol and triglyceride contents of plasma and lipoproteins were determined by automated chemical analysis [48]. High-density lipoprotein cholesterol was measured after precipitation of apoB-containing lipoproteins with dextran sulfate and magnesium [49]. LDLC was calculated using the Friedewald equation when the triglyceride was less than 400 mg/dl [50], or when triglyceride exceeded 400 mg/dl, very-low-density lipoprotein cholesterol (density [d] < 1.006 g/ml) was prepared by ultracentrifugation [51] and LDLC calculated as total cholesterol minus the sum of high-density lipoprotein cholesterol plus very-low-density lipoprotein cholesterol, after determination of very-low-density lipoprotein cholesterol from the very-low-density lipoprotein (d < 1.006 g/ml) fraction. Standards were provided by the Centers for Disease Control.

■ Gene selection & genotyping technology

The physiogenomic array comprises 384 SNPs distributed across 222 genes, representing various physiological pathways: energy homeostasis, adiposity, apolipoproteins and receptors, fatty acid and cholesterol metabolism, lipases, receptors, cell signaling and transcriptional regulation, growth factors, drug metabolism, vascular signaling, endothelial dysfunction, coagulation and fibrinolysis, vascular inflammation, cytokines, neurotransmitter axes (serotonin, dopamine: cholinergic, histamine and glutamate) and behavior (satiety). We searched public databases (dbSNP and Ensembl) for validated SNPs

with known allele frequencies for mixed or Caucasian populations. We selected SNPs with a minor allele frequency of between 10 and 30%, avoiding higher allele frequencies because such SNPs are more likely to be phenotypically neutral. We also selected SNPs by haplotype block, avoiding pairs with high linkage of disequilibrium scores [201]. A complete listing of all genes and SNPs can be found online (see Online Appendix, www.futuremedicine.com/doi/suppl/10.2217/pgs.10.58/suppl_file/Online_supplement.doc).

Of note, the physiogenomic array included 122 SNPs in 65 lipid metabolic and statin pharmacogenetic genes. The lipid metabolic and statin pharmacogenetic genes queried included: APOA1, APOA2, APOA4, APOA5, APOC1, APOB, APOC2, APOC3, APOC4, APOF, APOE, APOH, APOL1–APOL5, APOM, LDLR, SCARB1, SCARB2, ABCG5, ACE, CETP, FABP2, FASN, CYP3A4, CYP3A5, CYP7A1, LIPA, LPC, LIPE, LIPG, LPL, PPARA, RARA, RARB, RARG, RXRA, RXRG, CPT1A, CPT2, ADRA1A, ADRA2A, ADRA2B, ADRB1, ADRB2, ADRB3, MC3R, MC4R, MDH1, POMC, PRKAA1, PRKAA2, PRKAB, PRKAG1, PRKAG2, ACACA, ACACB, ACAT1, ACAT2, MTP, PON1, UCP2 and UCP3.

Genotyping was performed using the Illumina (CA, USA) BeadArray™ platform and the GoldenGate™ assay [52,53].

■ Genotyping quality

Genotyping quality was assessed using two methods, and both yielded excellent results. First, the comparison of a molecular gender marker provided by the Illumina platform with the demographic gender showed 100% agreement. This check assesses errors in the assignment of samples and/or clinical phenotypes. Second, the scores assigned to genotype calls by the Illumina GenCall™ software were used to control genotyping quality. A tenth percentile of GenCall scores of 0.3 or higher was required over all SNPs for a sample to pass quality control. Most samples passed by a wide margin of 0.5 or better. Of the samples that we attempted to genotype, 97% yielded a passing score. Positive and negative control samples were run and checked to monitor genotyping accuracy. Hardy–Weinberg equilibrium was analyzed for each SNP to guard against potential assay problems.

■ Statistical analysis & physiogenomic associations

Physiogenomics is a biomedical application of sensitivity analysis, an engineering discipline concerned with how variation in the input of a system leads to changes in output quantities [32,33]. Physiogenomic analysis is a two-stage process consisting of: first, association screening for genetic markers or haplotypes and physiological characteristics that have an influence on the disease status of the patient or the progression to disease (in this case, LDLC concentration); and second, model building for the dependence of response on the markers [33]. Marker discovery is accomplished through sensitivity analysis, wherein many SNPs are assessed simultaneously for their ability to predict a phenotype using locally weighted scatter plot smooth regression. Locally weighted scatter plot smooth regression is a method to smooth data using a locally weighted linear regression [54,55]. Significant independent SNPs and other nongenetic characteristics serve as predictor variables for the phenotype(s) of interest in multivariate regression.

In the present context, the end point LDLC has a continuous distribution. Covariates were analyzed using multiple linear regression and the stepwise procedure. Age, gender, ethnicity, statin, statin dose, body mass and height were each included as potential covariates. Of these, age ($p < 0.0002$), gender ($p < 0.005$), statin type ($p < 0.01$), statin dose ($p < 0.03$) and height ($p < 0.08$) were associated with LDLC levels and controlled for in the SNP association analysis.

To test for SNP association, a multivariate linear model was constructed, including the significant covariates and the SNP genotype. SNP genotype was coded quantitatively as a numerical variable indicating the number of minor alleles: 0 for major homozygotes, 1 for heterozygotes and 2 for minor homozygotes. The F-statistic p-value for the SNP variable was used to evaluate the significance of association. To account for the multiple testing of 384 SNPs, we calculated adjusted p-values (q-values) using the false-discovery rate (FDR) procedure [57].

Results

■ Study population

Atorvastatin was administered to 65% of the 202 patients (Table 1), and 35% received simvastatin, rosuvastatin or pravastatin therapy. Concurrent with statin therapy, 50.8, 8.4, 30.0 and 7.4% received ezetimibe, a fibric acid derivative, niacin and/or fish oil, respectively (Table 1). Patients were reported to take an average of 3.1 units of other non-lipid-lowering medications, of which the most common were angiotensin-converting enzyme inhibitors or angiotensin receptor blockers. No patients received strong CYP3A4 inhibitors, such as ritonavir and itraconazole or other antifungals. Three patients (1.5%) received the less potent CYP3A4 inhibitor diltiazem. Of the 17 patients receiving fibric acid derivative therapy, 14 received fenofibrate (Tricor®; Abbott Laboratories, IL, USA); also, seven received atorvastatin, one received simvastatin, eight received rosuvastatin and one received pravastatin. Although concurrent fibric acid therapy increases the plasma concentration of atorvastatin, rosuvastatin and simvastatin by up to twofold [57–60], and of pravastatin by two- to four-fold [61], fibric acid therapy was unrelated to LDLC levels in the present study and thus was not used a covariate.

■ Low-density lipoprotein cholesterol phenotype

The mean LDLC concentration was 103.7 ± 48.9 (standard deviation) mg/dl (Figure 1). For roughly half of the patients, the LDLC level was close to the common clinical target goal of 100 mg/dl for LDLC.

■ Physiogenomic associations

Of 222 genes queried, 13 were associated with LDLC lowering at a significance level of a p-value of less than 0.05 (Table 2). The strongest associations were the *ACACB* SNPs rs34274 ($p < 0.0002$) and rs2241220 ($p < 0.008$). The minor allele frequency for rs34274 was 0.138, and for rs2241220, 0.202 in our cohort, which is mixed with respect to ethnogeographic origin. Such frequencies are expected to give a minor allele frequency of 0.10 and 0.125, respectively, for Central Europeans living in Utah (CEU) populations, of 0.178 and 0.272, respectively, for Han Chinese, and of 0.653 and 0.242, respectively, for Yoruban populations (HapMap, Build36). For our cohort, the SNPs were in weak linkage disequilibrium ($D' = 0.302$).

The positive β -coefficient for rs34274 in the regression equation to predict LDLC defines the rs34274 SNP in the *ACACB* promoter region as a protective marker owing to the association of the minor allele T to decreased LDLC levels. The rs2241220 SNP in exon 33 of *ACACB* is considered a risk marker because the presence of a C>T substitution is associated with increased LDLC levels. After correcting for the FDR, the significance levels for rs34274 and rs2241220 were 0.076 and 0.93, respectively. Figure 2A depicts the significant association to LDLC lowering through *ACACB* SNP rs34274, and Figure 2B, the trend toward association through rs2241220. None of the four *ACACA* SNPs in the physiogenomic array, including rs2946342 at intron 1; rs2229416 which is a synonymous SNP at exon 13; rs4795180, at intron 31; and rs2053670 at intron 50, were found to be

significantly related to LDLC levels. rs2229416 and rs4795180 serve as examples in Figure 2.

■ Predictive model

We predicted LDLC concentration from the nongenetic factors age, gender, dose, heritage, and drug using multiple linear regression (Figure 3; $R^2 = 24.8\%$). In Figure 3A, predicted LDLC concentration, using the nongenetic factors age, gender, dose, heritage and drug as predictor variables, is plotted against actual LDLC in 202 patients. Figure 3B shows predicted versus actual LDLC, using LDLC predicted from age, gender, ethnogeographic origin, drug, dose and number of minor alleles at rs34274. Figure 3C shows the same using the number of minor alleles at rs2241220. Figure 3D shows predicted versus actual LDLC, using LDLC predicted from age, gender, ethnogeographic origin, drug, dose, rs34274 and rs2241220. Two patients were missing genotyping data for rs2241220, accounting for 200 patients in Figure 3C & D. Incorporation of either *ACACB* SNP alone significantly increased the proportion of the variance in LDLC to 28%, and inclusion of both SNPs increased the regression model's predictive power to 35.0% of the total variance.

Discussion

Here, we report the results of a cross-sectional approach that evaluated genetic associations with LDLC using lipid clinic outpatients who were also statin therapy recipients. The approach is novel owing to its focus on patients treated with statins. Large genome-wide studies, as well as cross-sectional studies, have included many patients not receiving statin therapy [7,8,28,62]. These studies identify SNP markers innate to LDLC levels in the broader population [7,8,28,62]. By contrast, others have focused on the change in LDLC obtained by subtracting baseline minus intervention LDLC levels. That approach is limited to distinguishing SNPs that serve as markers for statin effect [11,63]. Whether SNP markers identified using either approach are generalizable to patients receiving statin therapy is not clear. Our cross-sectional approach carries the expectation that the findings readily apply to the population receiving statin-based therapy to decrease LDLC and reduce cardiovascular disease risk. Such an approach can discover relationships in terms of both disease susceptibility (baseline) and drug response (LDLC change).

New associations between LDLC and two SNPs in the *ACACB* gene with opposing influence on LDLC were discovered, as discussed later. However, we recognize several limitations to the study. Not all genes and not all pathways were represented in the study. After our study began, several genes, *SORT1* [62], *HMGCR* [10,11] and *PSCK9* [62], were discovered to be associated with baseline LDLC levels or changes. These genes were not queried directly in our study. For a cross-sectional design, the sample size is relatively limited. Finally, some LDLC data were missing owing to statin-induced side effects that caused discontinuation of the medication prior to lipid measurement. For these reasons, replication will be required in larger cohorts.

We employed the physiogenomics array to identify novel gene associations with LDLC concentrations during statin therapy. Genotyping of 384 SNPs in 202 volunteer patients receiving statin therapy revealed physiogenomic associations to LDLC levels through two *ACACB* SNPs, rs34274 ($p < 0.0002$; FDR = 0.073) and rs2241220 ($p < 0.008$; FDR = 0.93). The significance threshold for the present study was 0.10. Given the low FDR for rs2241220, a validation set is needed to test whether the SNPs exert a significant influence on the LDLC level.

Of interest are the novel and independent opposing effects on LDLC levels owing to polymorphisms within a single gene. The rs34274 SNP in the *ACACB* promoter region is

considered a risk marker owing to the association of its minor allele with increased LDLC levels. The rs2241220 SNP, a synonymous SNP in exon 33 of *ACACB*, is considered a protective marker because the presence of its minor allele is associated with decreased LDLC levels. Neither SNP induces an alteration in the amino acid sequence of the protein product.

The *ACACB* gene spans 130 kb at chromosomal location 12p24.11. It has an open reading frame of 7343 bases with 52 exons encoding acetyl coenzyme-A (CoA) carboxylase β (ACCB β), a protein consisting of 2458 amino acids [64,65] and molecular weight 275–280 kDa [66,67]. The protein includes a mitochondrial binding sequence near the N-terminal for amino acids 1–215 (corresponding to exon 1), a 5'-AMP-dependent protein kinase phosphorylation site spanning amino acids 216–221, an ATP-binding region at amino acids 456–466, a biotin-binding site at amino acids 927–931 (corresponding to exons 17 and 18), a cAMP-dependent protein kinase phosphorylation site at amino acids 1333–1336 (corresponding to exons 27–29) and the acyl-CoA-binding region at amino acids 2069–2096 [64]. Allosteric binding of citrate to an unknown number of the more than 100 lysine side chains enables dimerization and polymerization to more highly active enzymatic forms [68].

Acetyl-CoA carboxylase β catalyzes the formation of malonyl-CoA from acetyl CoA [68]. Malonyl-CoA allosterically inhibits carnitine palmitoyltransferase-1, decreasing β -oxidation of fatty acids and, in lipogenic tissues, increases fatty acid synthesis and elongation by serving as an essential substrate [68]. A counterpart to ACCB β , acetyl carboxylase α (ACCA α) is found in the hepatic cytosol. Both are potential targets for obesity, diabetes and dyslipidemia treatment [65].

The *ACACB* gene has three promoter regions, P-O, PI and PII, which produce exons 1a, 1b, and 1c, respectively [69], which connect to the common exon 2 in mRNA after splicing. Different transcriptional controls for the expression of ACCB β exist in cardiac/skeletal muscle compared with liver [69]. In the liver, fatty acid oxidation provides acetyl-CoA for production of ketone bodies during periods of fasting, and feeding upregulates hepatic ACCB β through activation by sterol regulatory binding element protein (SREBP)-1 [67]. In muscle, ACCB β activity is rapidly regulated via phosphorylation by AMP-activated protein kinase fatty acid oxidation [69]. A change in tissue ACCB β mass via transcriptional regulation may be of greater importance in the liver, whereas phosphorylation/dephosphorylation is the major control in skeletal and cardiac muscle [69,70].

Our findings lead us to consider new hypotheses through which SNPs in *ACACB* might affect LDLC. Increased expression of ACCB β activity could increase fatty acid synthesis and chain elongation [68]. Dietary saturated fatty acids (12:0, 14:0 and 16:0) raise LDLC through decreased LDL receptor activity [71]. Fatty acids are known to regulate the nuclear receptors of the PPAR family α , β , γ 1 and γ 2; RXR α , LXR α , and HNF-4 α and HNF-4 γ ; and bHLH-L, SREBP, ChREBP and MLX [72–76].

The rs34274 SNP in intron 1 is approximately 3 kb upstream from exon 2 and within 5kb of the PII promoter found 8 kb upstream from exon 2. The PII promoter harbors sterol response elements that are -62 to -42 bp relative to exon 1b [77]. Feeding controls hepatic ACCB β expression at least in part through a SREBP-1 transcription factor [69]. SNPs near promoter regions may affect enhancer/promoter activity and gene expression. Some 23 SNPs in the region including rs34274 comprise a haplotype block (HapMap reference; see Figure 4). The haplotypes could differentially affect the interactions with binding factors. We hypothesize that the minor allele alters *ACACB* expression through a promoter-related effect that increases LDLC through fatty acid-regulated mechanisms.

Acetyl coenzyme A carboxylase β occurs as highly active dimers and polymers [68], whose formation is controlled allosterically through the binding of citrate to lysine. The rs2241220 SNP in exon 33 is synonymous but lies within 22 kb of seven SNPs, which are nonsynonymous. Two of these reside among exons 30–36, which harbor a region of strong linkage disequilibrium. One of these, A4448G (rs17848825) located on exon 32, 1.6 kb centromeric to rs2241220 [78,101], produces a Lys1480Arg change, eliminating a citrate-binding site. Other amino acid sequence alterations besides lysine>arginine can be disruptive to enzyme conformation, and a non-synonymous SNP in linkage disequilibrium with rs2241220 could lead to conformational changes that alter enzyme activity.

In contrast to *ACACB* SNPs, SNPs that are found in the *ACACA* gene were not associated with LDLC levels (Figure 3). Although functionally similar, the protein products of *ACACB* and *ACACA*, which are ACC α and ACC β , respectively, derive from genes on chromosomes 12 and 17, respectively. The product of *ACACA*, ACC α , is found in the hepatic cytosol, and its high activity, relative to the product of *ACACB*, ACC β [68], suggests an important hepatic metabolic role relative to fatty acid synthesis. Yet in mice specifically lacking hepatic *ACACA*, ACC β activity compensates [79], and the mice have no apparent physiological complications. Although distinct physiological roles for ACC α and ACC β are not certain, our results point to a more important role in LDLC metabolism for *ACACB* compared with that of *ACACA*.

The integration of multiple SNP effects and clinical phenotypes is a cornerstone of physiogenomic analyses [32,51]. A related approach based on integration of known risk factors with novel genetic markers is being explored to improve the prediction of cardiovascular disease susceptibility [80]. We examined the combined effect of adding the *ACACB* SNPs on the model to predict LDLC levels (Figure 3). The nongenetic factors of age, gender, baseline LDLC and statin dosage were shown to explain 24.8% of the variance in LDLC (Figure 3A). Incorporating either of the *ACACB* SNPs alone into the model significantly increased the variance explained by 3–4% (Figure 3B & C). Simultaneous incorporation of both SNPs further enhanced the predictive model, increasing the variance to 35%. The significant incremental refinement to the model achieved with one then two SNPs is consistent with the interpretation that the SNPs exert independent effects, strengthening the plausibility of the separate molecular bases for each SNP hypothesized earlier.

To our knowledge, this is the first report of an association between the *ACACB* gene and LDLC levels. As baseline LDLC predicts the magnitude of LDLC lowering that will result from statin therapy, it may be expected that there is overlap in the genes that regulate cholesterol and LDL metabolism (disease susceptibility) and statin-mediated LDLC lowering (efficacy). Recent association studies in large cohorts have confirmed loci at *APOB*, *APOE*–*APOC1*–*APOC4*–*APOC2*, *LDLR*, *HMGCR* and *PCSK9* [7,27,28], and discovered intergenic SNPs in the chromosomal regions 1p13 (near *CELSR2*, *PSRC1* and *SORT1*) [28] and 19p13 (near *CILP2* and *PBX4*) [7], which are associated with LDLC in patients with elevated cholesterol. Genome-wide studies have shown that common, noncoding SNPs in HMG-CoA reductase are significantly associated with LDLC levels but that the effect sizes are relatively small – a 5% difference in LDL level [7]. The addition of genotype scoring, consisting of summing the number of risk markers among the eleven SNPs just mentioned, has significantly improved risk classification in cardiovascular disease-prone subjects in large cohorts [8].

Conclusion

In summary, the finding of novel associations in *ACACB* with LDLC levels demonstrates the utility of the physiogenomic approach in discovering new associations to LDLC behavior embedded in pathways so far uninvestigated. The growing number of pharmacogenetic and pharmacogenomic investigations of LDLC levels, with and without statins, have focused largely on the genes directly involved in cholesterol synthesis [9,11], LDL particle handling, intracellular lipoprotein packaging and transport [8], traditional lipid metabolic genes [9,11] and statin pharmacokinetics [23,21]. None have comprehensively queried the widest ranging aspects of energy metabolism to include basic biochemical pathways, subtle components of which may significantly affect LDLC. By examining genes in such pathways, our study has discovered novel links to LDLC levels.

Executive summary

- Statins lower low-density lipoprotein cholesterol (LDLC) levels by an average of 37–57% in patients with primary hypercholesterolemia, but genetic determinants of LDLC-lowering efficacy have not been fully characterized.
- Physiogenomic analysis was used to investigate 384 preselected SNPs in relation to LDLC levels in 202 patients receiving statin therapy.
- We discovered novel, bidirectional associations between SNPs in the *ACACB* gene and LDLC levels. The rs32374 SNP is a risk marker associated with an 18-mg/dl increase in LDLC per minor allele, and rs2241220 is a protective marker associated with a 12-mg/dl decrease.
- The SNP associations will require confirmation in larger populations.
- The bidirectionality of these associations with respect to LDLC concentration is consistent with an integrative role of the *ACACB* gene with respect to lipid metabolic pathways.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Financial & competing interests disclosure

This research has been funded by NIGMS grant # 4R 44 GM085201-02. Gualberto Ruano, Alan Wu and Mohan Kocherla are full time employees of Genomas, Inc. Richard L Seip and Theodore R Holford are consultants to Genomas, Inc. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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Figure 1.
Distribution of low-density lipoprotein cholesterol.

Figure 2. Physiogenomic plots

Associations with low-density lipoprotein cholesterol lowering through (A) *ACACB* rs34274 and (B) *ACACB* rs2241220, and absence of associations through (C) *ACACA* rs4795180 and (D) *ACACA* rs2229416. Each circle represents a subject (genotype), with the horizontal axis specifying the low-density lipoprotein cholesterol, and the vertical axis the genotype. Bottom circles: homozygous for major allele; middle circles: heterozygous; top circles: homozygous for minor allele. A locally weighted scatter plot smooth fit of the allele frequency as a function of low-density lipoprotein cholesterol (curved line) is shown.

Figure 3. Prediction of low-density lipoprotein cholesterol lowering

Prediction is carried out using **(A)** the factors age, gender, ethnogeographic origin, drug and dosage; **(B)** the same factors and rs34274; **(C)** the same factors and rs2241220; and **(D)** the same factors and rs34274 and rs2241220.

Figure 4. *ACACB* gene

(A) Promoter region and (B) exons 27 to 35 (not to scale). Regions that encode known features of the protein are marked (C) Acyl-CoA binding region. The locations of the rs34274 SNP, which is a risk marker for low-density lipoprotein cholesterol levels, and rs2241220 SNP, which is a protective marker, are shown. Each is found within a distinct haplotype block.

A: ATP-binding site; B: Biotin-binding site; M: Mitochondrial binding site; P_a: 5'-AMP-dependent protein kinase phosphorylation site; P_c: cAMP-dependent protein kinase phosphorylation site.

Table 1

Patient characteristics (n = 202).

Characteristic	Mean $\pm$ standard deviation or percentage of total
General	
Number of men/women	142/60
Ethnogeographic origin:	
– Caucasian	162
– African–American	6
– Asian	19
– Hispanic	2
– Other	13
Mean age (years)	59 $\pm$ 14
Mean BMI	27.4 $\pm$ 7.4
Statin administered	
– Atorvastatin	65%
– Simvastatin	14%
– Rosuvastatin	16%
– Pravastatin	6%
Number of concurrent medications (by category)	4.14 $\pm$ 1.88
Other antihyperlipidemic	1.04 $\pm$ 0.74
– Gemfibrozil or fibric acid derivative	8.4%
– Ezetimibe	50.8%
– Niacin or niaspan	30.0%
– Fish oil	7.4%
All others	3.09 $\pm$ 1.40
– Angiotensin-converting enzyme inhibitor or angiotensin receptor blockers	23.2%
– α - or β -blocker	23.2%
– Antiplatelet	8.9%
– Warfarin	1.0%
– Antihyperglycemic	6.4%
– Thyroid medication	3.9%

Table 2

Gene associations for low-density lipoprotein cholesterol with $p \leq 0.05$.

SNP	Symbol	p-value	Coeff.	FDR	SNP type	Gene name
rs34274	<i>ACACB</i>	2×10^{-4}	18.2	0.076068	Intron 1	Acetyl-coenzyme A carboxylase β
rs2241220	<i>ACACB</i>	0.008	-11.94	0.934963	Exon 32, L1582L	Acetyl-coenzyme A carboxylase β
rs2033447	<i>RARB</i>	0.014	12.8	0.991425	Intron 2 (MT)	Retinoic acid receptor β
rs903361	<i>ADORA1</i>	0.026	9.542	0.999787	Intron 2, tag SNP 1	Adenosine A1 receptor
rs1800545	<i>ADRA2A</i>	0.03	13.07	0.999954	5'-UTR	Adrenergic α -2A-receptor
rs745075	<i>MTP</i>	0.035	11.94	0.999991	intron 13	Microsomal triglyceride transfer protein (large polypeptide, 88 kDa)
rs2293445	<i>PRKAG1</i>	0.039	8.492	0.999998	Intron 6 (MT)	Protein kinase, AMP-activated, γ 1 noncatalytic subunit
rs1355920	<i>CHRNA7</i>	0.039	-11.12	0.999998	Intron 1	Cholinergic receptor, nicotinic, α -polypeptide 7
rs2515449	<i>MCPHI</i>	0.041	19.82	0.999999	Intron 9	Microcephaly, primary autosomal recessive 1
rs4784642	<i>GNAO1</i>	0.041	8.358	0.999999	~2.1 kbp upstream	Guanine nucleotide-binding protein (G-protein), α -activating activity polypeptide O
rs2020933	<i>SLC6A4</i>	0.043	13.02	0.999999	Intron 1	Solute carrier family 6 (neurotransmitter transporter, serotonin), member 4
rs281432	<i>ICAM1</i>	0.045	-7.848	1	Intron 2	Intercellular adhesion molecule 1 (CD54), human rhinovirus receptor
rs1356413	<i>PIK3CA</i>	0.047	-19.72	1	Intron 16	Phosphoinositide-3-kinase, catalytic, α -polypeptide
rs439401	<i>APOE</i>	0.05	-7.456	1	~1.5 kbp downstream	Apolipoprotein E

Coeff.: Indicates the average increase (or decrease) in the low-density lipoprotein concentration for each copy of the minor allele, when low-density lipoprotein cholesterol is adjusted for the covariates age, gender and drug; FDR: False-discovery rate; SNP type: Location of the SNP relative to the transcriptional start of the gene; SNP: D6SNP identifier for each locus; Symbol: HUGO gene symbol.