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Combined influence of *LDLR* and *HMGCR* sequence variation on lipid-lowering response to simvastatin

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Abstract

Objectives—Although statins are efficacious for lowering LDL-cholesterol (LDLC), there is wide inter-individual variation in response. We tested the extent to which combined effects of common alleles of *LDLR* and *HMGCR* can contribute to this variability.

Methods and Results—Haplotypes in the *LDLR* 3′-untranslated region (3UTR) were tested for association with lipid-lowering response to simvastatin treatment in the Cholesterol and Pharmacogenetics (CAP) trial (335 African-Americans and 609European-Americans). *LDLR* haplotype 5 (L5)was associated with smaller simvastatin-induced reductions in LDLC, total cholesterol, non-HDL cholesterol, and apolipoprotein B (P=0.0002–0.03)in African-Americans, but not European-Americans. The combined presence of L5 and previously described *HMGCR* haplotypes in African-Americans was associated with significantly attenuated apoB reduction(−22.4±1.5% N=89) both compared to noncarriers (−30.6±1.5% N=78, P=0.0001) and to carriers of either individual haplotype (−28.2±1.1% N=158, P=0.001). We observed similar differences when measuring simvastatin-mediated induction of LDLR surface expression using lymphoblast cell lines (P=0.03).

Conclusions—We have identified a common *LDLR* 3UTR haplotype that is associated with attenuated lipid-lowering response to simvastatin treatment. Response was further reduced in individuals with both *LDLR* and previously described *HMGCR* haplotypes. Previously identified racial differences in statin efficacy were partially explained by increased prevalence of these combined haplotypes in African-Americans.

Keywords

LDLR; HMGCR; statin; LDL-cholesterol; pharmacogenomics

Statins are prescribed for the prevention and treatment of cardiovascular disease (CVD) and work primarily to lower LDL-cholesterol (LDLC) [1–4]. Despite widespread use of statins,

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lipid-lowering response is variable and many patients fail to meet lipid-lowering goals [5–6]. Variation in response is influenced by multiple factors including age, gender, and racial ancestry [7]. For example, African-Americans have on average a lesser response to a given statin dose in comparison to European-Americans [7]. While common sequence variants in several candidate genes including *HMGCR* and *APOE* have been associated with alterations in lipid-lowering response to statin treatment [8–11], each of these associations accounts for a relatively small portion of the total variance in statin response. Hence the genetic contribution to variability in statin-mediated lipid reductions likely results from multiple polymorphisms with small individual effects.

Statins are competitive inhibitors of HMG-CoA reductase (HMGCR), the enzyme that catalyzes the rate-limiting step in cholesterol biosynthesis, and genetic variation in *HMGCR* has been associated with attenuated lipid-lowering response to statin treatment [8–9, 12–13]. In the Cholesterol and Pharmacogenetics (CAP) study, we found that certain *HMGCR* haplotypes were associated with smaller simvastatin-mediated lipid reductions in African-Americans but not in European-Americans [9] and that genetically-influenced *HMGCR* alternative splicing contributed substantially to reduced statin response[14].

Although reduced cholesterol synthesis may contribute to statin-mediated reduction of LDL-cholesterol (LDLC), the primary mechanism for this effect is increased plasma LDL clearance due to secondary up-regulation of LDL receptor (*LDLR*) expression[15]. Variation at the *LDLR* locus is known to influence LDLC levels [16–17]. Rare loss of function *LDLR* mutations are causative for familial hypercholesterolemia and the functional severity of these mutations has been associated with statin efficacy [18–20]. Common *LDLR* DNA polymorphisms also have been associated with inter-individual variation in LDLC as well as other lipid and lipoprotein traits [16, 21–24] and these variants are thought to mediate changes in LDLR protein expression or regulation. There is recent evidence that the 3′-untranslated region (3UTR) of *LDLR* is required for berberine-mediated LDLC reduction through a mechanism that increases *LDLR* mRNA stability [25]. Single nucleotide polymorphisms (SNPs) in the 3UTR have been associated with *in vivo* LDLC levels as well as *in vitro LDLR* mRNA stability [26] and variation in this region may also influence statin-mediated lipid reduction [27]. To further explore this latter possibility, we assessed the contribution of common sequence variations in the *LDLR* 3UTR to statin-induced changes in lipids and lipoproteins in participants of the CAP trial. We also determined the combined influence of *HMGCR* and *LDLR* DNA variation on simvastatin-mediated lipid reductions.

Methods

Study population

The CAP trial enrolled 944 participants in a 6-week simvastatin trial (40mg/d) that was designed to examine genetic factors affecting simvastatin-mediated changes in lipids and lipoproteins [7]. This trial is registered at clinicaltrials.gov (NCT00451828). Participants were healthy adult volunteers who self-reported either African-American (N=335) or European-American ancestry (N=609). They were recruited at either the University of California, Los Angeles School of Medicine (Los Angeles, CA) or at San Francisco General Hospital (San Francisco, CA). Informed consent was obtained and approved by the Institutional Review boards at those institutions and included approval for future genetic studies related to statin efficacy. Baseline health and demographic information were obtained at enrollment. Fasting plasma was collected at two pre-treatment time points (screen visit and enrollment visit) and at two post-treatment time points (4 weeks and 6 weeks of treatment). Lipids and lipoproteins were measured at all four time points. Total cholesterol, triglyceride, apolipoprotein B and HDL-cholesterol were measured using an Express 550 Plus analyzer (Ciba Corning, Oberlin, Ohio) in a laboratory that was monitored for consistency by the

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Centers for Disease Control-National Heart, Lung, and Blood Institute standardization program as described previously 7. LDL-cholesterol was calculated by the Friedewald equation 28. Because total cholesterol, HDLC, and triglycerides were not significantly different between screen and enrollment, the average of these two measurements was used as the pretreatment value in order to minimize technical variation. For the same reason, the average of four and six week measurements was used as the post-treatment value. ApoB was only measured at enrollment and after six weeks.

Identification of tagSNPs and haplotypes

DNA variants in the *LDLR* 3UTR (2539bp, chr19:11,241,995–11,244,534 on the GRCh37 reference sequence) were identified by Sanger resequencing of genomic DNA samples derived from 24 African-Americans (AA) and 23 European-Americans (EA) from the Coriell repository. Overlapping segments were PCR amplified and sequenced using 5% BDT v3.1 sequencing chemistry (Applied Biosystems) in MJ Tetrad PTC 225 thermocyclers, and chromatograms were generated from these reaction products on ABI3730 capillary sequencers. Sequences were base-called using Phred and assembled into contigs using Phrap. All sequence contigs were assembled and screened using Polyphred and, for confirmation, all variant sites were visually inspected using Consed. We identified twenty-three variable sites (fifteen with minor allele frequency greater than 5%) and these were mapped using the University of California, Santa Cruz Golden Path human genome assembly as the reference sequence. Six tagSNPs were identified using LD select ($r^2 > 0.80$ and MAF > 0.05)29[–30] and these were genotyped in all CAP participants using a combination of MassArray technology (rs1433099, rs7254521, rs2738467, rs17249057; Sequenom, San Diego, CA) and Taqman assays (rs14158 and rs5742911; Applied Biosystems, Foster City, CA). The genotyping data for rs17249057 failed quality control and were not included in subsequent analyses. However, rs17249057 was in linkage disequilibrium with a second genotyped SNP, rs14158 (r^2=0.72 in EA and r^2=1.00 in AA). All other SNPs were in Hardy-Weinberg equilibrium as measured by χ^2-test ($P<0.05$). SNP frequencies are listed in Supplementary Table I. No *LDLR* 3UTR tagSNPs were significantly associated with statin-mediated changes in lipid traits following correction for multiple testing.

Haplotypes were inferred separately in African-American and European-American participants of the CAP trial using PHASE2.0 [31]. Six common haplotypes (Table 1, MAF>0.05) were inferred and these were tested for associations with statin-mediated lipid reduction. Cladogram analysis was performed using TCS v1.21 [32]. Global association of *LDLR* 3UTR haplotypes were assessed by analysis of covariance both within the entire CAP population and separately in African-Americans and in European-Americans.

Quantification of LDLR surface protein expression in lymphoblast cell lines

Lymphoblast cell lines (LCLs) were derived from all CAP participants by Epstein-Barr virus transformation of lymphocytes isolated from blood samples collected at enrollment visit [33–]35. LCLs were grown at 37°C (95% O_2, 5% CO_2) in RPMI 1640 media (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT), 500 U/ml penicillin/streptomycin, and 2nmol/L GlutaMAX (Invitrogen). Simvastatin was provided by Merck Inc. (Whitehouse Station, NJ) and was 98% converted to active form (beta-hydroxy simvastatin acid, SVA) by heating to 50°C for 1 hour in 0.1N NaOH prior to use 36. SVA concentrations were quantified by liquid chromatography-tandem mass spectrometry using a Kromasil C18 column (Keystone Scientific, Bellfonte, PA) on a Shimadzu LC-10AD HPLC system (Shimadzu, Columbia, MD) connected to a Waters Micromass Quattro LC triple quadrupole mass spectrometer (Waters, Milford, MA) 37. LCLs (N=193) derived from African-Americans were normalized to a uniform cell density and exposed to 2μM

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simvastatin or sham buffer for twenty-four hours 14. LDLR protein was quantified following exposures aspreviously described 25. Briefly, 4×10^6 cells were incubated with a monoclonal LDLR antibody diluted 1:50, washed, and subsequently incubated with anti-IgG-FITC labeled antibody diluted 1:400. An isotype-matched normal mouse IgG diluted 1:50 was used to quantify background fluorescence. All antibodies were purchased from Santa Cruz Biosciences(Santa Cruz, CA). Fluorescent intensity in 10,000 gated events was measured on a BD FACS Caliber.

Statistical analysis

Associations of haplotypes with lipids, lipoproteins, and LDLR expression were analyzed by analysis of covariance using the dominant model and including age, sex, BMI, and smoking status as covariates. Associations were repeated after additional adjustment for *HMGCR* haplotypes H2 and H7, which have been previously associated with attenuated simvastatin response in this population [9]. To ensure that analyses were not influenced by nonnormal trait distributions, we also tested for associations using nonparametric analyses. Because these did not change any of the statistical findings, the results are reported using parametric analysis. For SNP analyses and haplotype analyses described in Table 2, correction for multiple testing was performed using QVALUE [38]. All statistical analyses were performed using JMP version 7.0 software (SAS, Cary, NC).

Results

Association of LDLR 3UTR L5 haplotype with in vivo simvastatin-mediated lipid reductions and in vitro LDLR expression

LDLR 3UTR haplotypes were inferred based on tagSNPs selected to cover DNA variation across the entire 3UTR including the AU-rich elements (ARE) and alu-rich regions (Figure 1A). For all six haplotypes, allelic prevalence was significantly different between African-Americans and European-Americans (Figure 1A). *LDLR* 3UTR haplotype 5 (*LDLR* L5) was inferred to contain the major allele at all tagSNPs and was more prevalent in African-Americans (Figure 1A and 1B). The alleles represented by this haplotype were also observed in the chimpanzee genome, indicating that they represent the ancestral alleles. Although individual SNPs were not associated with statin-mediated lipid changes in either the CAP population as a whole or within the African-Americans or European-Americans separately, haplotypes within this region were associated with statin-mediated changes in lipids and lipoproteins using an omnibus haplotype test. Within African-Americans, *LDLR* 3UTR haplotypes were globally associated with statin-mediated changes in apoB (global variance: r^2=0.07, P=0.0004), LDLC (r^2=0.04, P=0.04), total cholesterol (r^2=0.05, P=0.01), and nonHDL-cholesterol (r^2=0.05, P=0.0009) by omnibus haplotype test.

We next assessed the associations of individual *LDLR* 3UTR haplotypes with statin-mediated lipid changes. There was no evidence for an association of the *LDLR* 3UTR haplotypes with statin response among European-Americans. Among African-Americans, we observed a significant association of *LDLR* L5 with attenuated statin-mediated percent changes in TC, apoB, and nonHDLC (Table 1). ApoB reductions were lower in both homozygous *LDLR* L5 carriers (−19.2±3.8mg/dL apoB N=14, P=0.02) and in heterozygous *LDLR* L5 carriers (−21.4±1.4mg/dL apoB N=114, P<0.0001) in comparison to noncarriers (−29.2±1.1mg/dL apoB N=207, P-value for trend: 0.0004). Similar associations were observed for TC, LDLC and nonHDLC(data not shown). Associations of *LDLR* L5 with attenuated statin-mediated changes in all four traits remained significant after adjusting for the previously identified *HMGCR* haplotypes (H2 and H7) (Table 1) [9]. *LDLR* L5carriers demonstrated a nonsignificant trend towards reduced pre-treatment and elevated post-treatment concentrations for all four traits (Table 2). In European-Americans, no association

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of *LDLR* L5 was observed with either pre-treatment lipids or statin-mediated changes in these traits (Table 2).

To identify the basis for these findings, we tested for an association of *LDLR* L5 with change in expression of LDLR surface protein in response to *in vitro* simvastatin exposure in lymphoblast cell lines derived from African-American CAP participants. LDLR surface protein expression was significantly induced in response to simvastatin exposure (1.45±0.06 fold change, N=193, *P*=0.002) in a manner that was correlated with *in vivo* statin-mediated decrease in apoB in the individuals from whom these cell lines were derived (Figure 2A). LDLR surface protein expression was not associated with *LDLR* L5 following exposure to either sham buffer or simvastatin(Figure 2B and 2C). However, LDLR induction was significantly reduced in cell lines derived from *LDLR* L5 carriers in comparison to noncarriers (Figure 2D).

Combined influence of LDLR L5 and HMGCR H2/H7 haplotypes on in vivo and in vitro simvastatin response

We have previously observed that, similar to the findings for the *LDLR* L5 haplotype reported here, *HMGCR* H2 and H7 haplotypes were also associated with reduced LDL response to simvastatin in African Americans. This raises the possibility of additive effects of *LDLR* L5 and *HMGCR* H2 or H7(H2/H7)on simvastatin response. Indeed, we observed that carriers of both haplotypes (L5+H2/H7) had significantly attenuated LDLC reductions in comparison to carriers of only the *LDLR* L5 haplotype (*P*=0.05) and in comparison to carriers of neither haplotype (*P*=0.0002, Figure 3A) with a similar trend observed in comparison to *HMGCR* H2/H7 only carriers (*P*=0.06). Carriers of both alleles also demonstrated significantly lower simvastatin-mediated apoB reductions in comparison to *HMGCR* H2/H7 only carriers (*P*=0.03) and in comparison to carriers of neither haplotype ((*P*<0.0001, Figure 3B) with a nonsignificant trend in comparison to *LDLR* L5 carriers (*P*=0.09). We tested for association of the combined L5+H2/H7 haplotype with *in vitro* LDLR induction following simvastatin exposure (Figure 3C) and found that response was significantly reduced in L5+H2/H7 carriers compared to *HMGCR* H2/H7 only carriers and noncarriers. No difference in LDLR induction was detected between noncarriers and carriers of either individual haplotype. This indicates that the association of *LDLR* L5 with attenuated LDLR induction (Figure 2) is due predominantly to the combined L5+H2/H7 carriers.

We next assessed whether inclusion of both haplotypes into a combined model would be predictive of statin response in African-Americans. We categorized individuals by lipid-lowering response as carriers of 2 haplotypes (both *LDLR* L5 and *HMGCR* H2/H7), 1 haplotype(either the *LDLR* L5 or *HMGCR* H2/H7 haplotypes) or 0 haplotypes (neither haplotype). We observed a significant additive association of *LDLR*/*HMGCR* haplotypes with statin-induced reduction of LDLC and apoB (Figure 4) as well as TC and nonHDLC (data not shown). In support of these observations, *in vitro* LDLR protein induction in response to simvastatin was reduced in combined L5+H2/H7 carriers (0.74±0.09A.U. N=57 two haplotype carriers vs. 1.02±0.06 A.U. N=97 one haplotype carriers vs. 0.97±0.10A.U. N=39 noncarriers, *P*=0.03). These associations were not observed in European-American CAP participants (data not shown), in whom the prevalence of the combined haplotypes was significantly lower (N=89 African-Americans (14.5% prevalence) vs. N=3 European-Americans(<1.0% prevalence), *P*<0.0001). The previously observed differences between African-Americans and European-Americans in simvastatin-mediated LDLC reduction (−35.7±4.4% AA vs. −38.8±4.4% EA, *P*=0.001) [7] were reduced to nonsignificance by inclusion of both *HMGCR* and *LDLR* haplotypes into the model (−35.6±4.2% AA vs. −36.9±4.2% EA, *P*=0.23), but not by individual inclusion of either *HMGCR* or *LDLR* haplotypes alone.

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Discussion

In this study, we have found that DNA variation in the 3UTR of *LDLR* is associated with diminished effects of statin on *in vivo* lipid reductions and on *in vitro* LDLR induction. We further demonstrated that there is a combined influence on statin response of the L5 *LDLR* 3UTR haplotype and the *HMGCR* haplotypes that we have previously shown to be associated with statin efficacy, such that carriers of both haplotypes had significantly attenuated statin-mediated changes in LDLC and LDLR in comparison to either noncarriers or carriers of individual haplotypes. Furthermore, these effects were more evident in African-Americans than in European-Americans. Although this may be the result of differences between African-Americans and European-Americans in the underlying sequence variation that is tagged by these haplotypes, it is more likely the result of differences in haplotype prevalence. Indeed, these haplotypes were significantly more prevalent in African-Americans than in European-Americans for both *HMGCR* (32% AA vs. 2% EA, $P<0.0001$) [9] and *LDLR* (21% AA vs. 3% EA, $P<0.0001$). Interestingly, both *LDLR* and *HMGCR* haplotypes were specifically represented by the ancestral alleles at all tagSNPs, suggesting that they represent the ancestral haplotypes [9]. Furthermore, the combination of both *LDLR* and *HMGCR* haplotypes was most strongly associated with attenuated response and this combined genotype was practically absent in the European-Americans within this study population (prevalence <1%).

The *LDLR* 3UTR haplotypes describe common variation that spans the entire *LDLR* 3UTR including the proximal AU-rich elements (AREs) and the distal Alu-rich region (ARR). The AREs have been shown to be involved in mediating the LDLC reduction induced by the herbal product, berberine [25]. This region promotes mRNA decay and binds to multiple proteins including several involved in mRNA processing and splicing 39–40. In addition to the ARE region, *LDLR* L5 is also represented by the ancestral alleles of SNPs located within the ARR. This region is responsible for transcriptional stability in response to protein kinase C (PKC) activation [39]. The ARR is a rapidly mutagenized genomic region but the influence on LDLR function of SNPs within this region has not been previously studied 41–42. The present study has demonstrated that the *LDLR* 3UTR ancestral haplotype, which is most prevalent in African-Americans, is associated with attenuated statin response. Because *LDLR* L5 was represented by ancestral alleles at all tagSNPs, these data suggest that multiple SNPs within the region may modify functionality of this region. Further studies are necessary to determine the functional influence of individual SNPs within the haplotype on statin response.

Previous *LDLR* 3UTR association analyses focusing on the ARE region have demonstrated that the ancestral haplotype within this region was associated with increased mRNA stability, but contrary to expectation, with increased LDLC in European-American populations [26–27]. Association with increased LDLC may reflect linkage disequilibrium between this region and other functional regions of the *LDLR* gene. Within our extended *LDLR* 3UTR haplotype analysis, the ancestral tagSNPs within the ARE region are represented by three haplotypes: *LDLR* L5, for which prevalence is low in European Americans (MAF=0.03), and *LDLR* L1 and L6, which are more common in European-Americans (MAF=0.22 for each). Both *LDLR* L1 and L6 are in linkage disequilibrium with an exonic SNP (rs688) that induces *LDLR* alternate splicing and is associated with increased LDLC [43]. This suggests that while the ARE region may influence *LDLR* mRNA stability, the previously described association of this region with increased LDLC in European-Americans may reflect the functional consequences of variation outside of the 3UTR. In contrast, *LDLR* L5 was not in linkage disequilibrium with rs688 or any other *LDLR* SNPs identified by genome-wide association study (GWAS) to be associated with LDL-cholesterol (Supplementary Table II) [16, 21–24, 29, 31, 43–45]. Furthermore, we saw no

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indication that any of these GWAS-identified SNPs were associated with statin-medicated LDLC reduction within our own GWA analysis, although they were all associated with untreated LDLC [46]. Studies to assess the influence of *LDLR* L5 on mRNA stability and processing are ongoing.

Both *LDLR* L5 and the *HMGCR* H2/H7 haplotypes were independently associated with reduced simvastatin response specifically in African-American but not European-American CAP participants. The combination of these haplotypes was strongly associated with diminished statin response such that *LDLR* L5 + *HMGCR* H2/H7 carriers exhibited significantly attenuated lipid reductions in comparison to carriers of either individual haplotype. Reduced induction of LDLR surface protein was also observed in simvastatin-exposed lymphoblast cell lines derived from carriers of both haplotypes in comparison to cell lines derived from carriers of individual haplotypes. The contribution of these individual haplotypes to the magnitude of variation in statin response was small, with either haplotype explaining 1–4% of the variance in lipid response traits in African-Americans. However, these contributions are additive, with the combined haplotype state accounting for 3–6% of the variation in lipid-lowering simvastatin response in African-Americans. Indeed, presence of both haplotypes diminished LDLC reduction by 17.0% and apoB reduction by 27.4% in African-Americans, suggesting the need for a greater statin dosage requirement in African-American carriers, since a doubling of dose yields a ~6% further reduction in LDLC [47–48]. In contrast, the combined haplotypes described less than 1% of the variance in statin response in European Americans, for whom the prevalence of the combined haplotype was much smaller (<1.0%). These data suggest that the effect of racial ancestry on lipid-lowering statin response is caused in part by differential prevalence of these *LDLR* and *HMGCR* haplotypes. Indeed, the effect of racial ancestry on lipid-lowering response to simvastatin was reduced to nonsignificance following adjustment for these haplotypes. In conclusion, these data suggest that the dissimilar simvastatin response observed between African-Americans and European-Americans is due in part to the differential prevalence of multiple sites of genetic variation that have independent but compounded effect on statin efficacy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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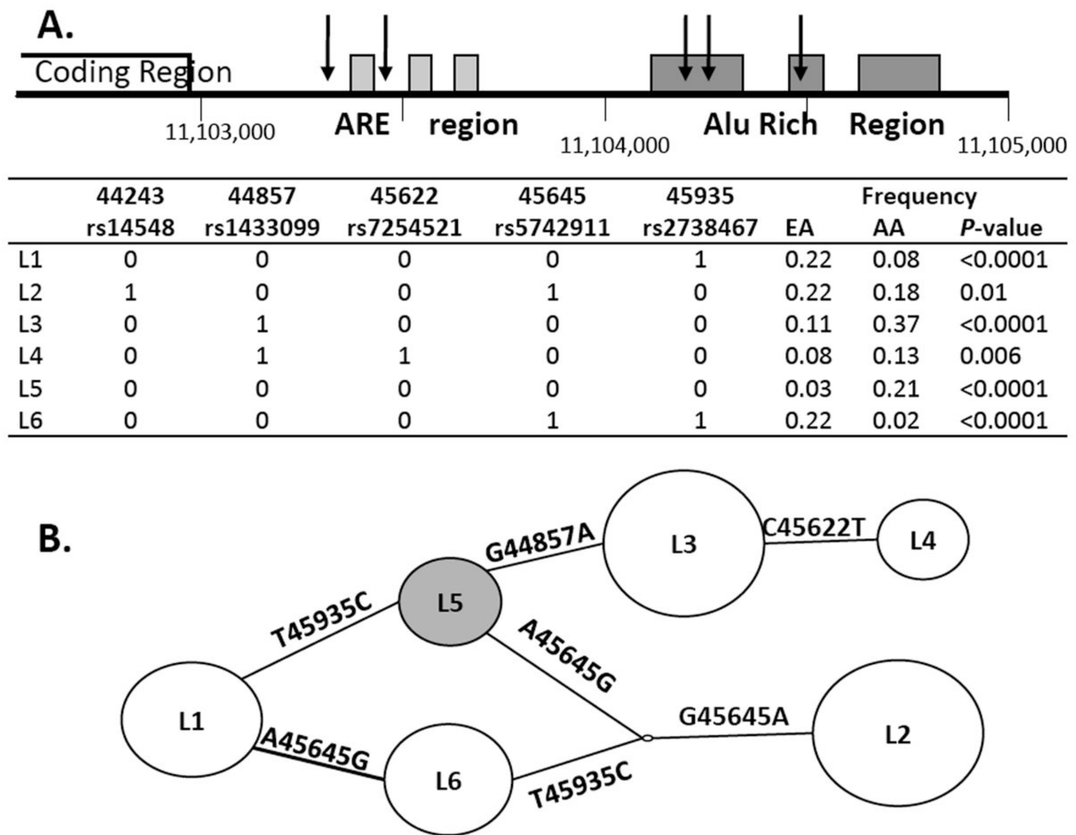

	44243 rs14548	44857 rs1433099	45622 rs7254521	45645 rs5742911	45935 rs2738467	Frequency EA	Frequency AA	*P*-value
L1	0	0	0	0	1	0.22	0.08	<0.0001
L2	1	0	0	1	0	0.22	0.18	0.01
L3	0	1	0	0	0	0.11	0.37	<0.0001
L4	0	1	1	0	0	0.08	0.13	0.006
L5	0	0	0	0	0	0.03	0.21	<0.0001
L6	0	0	0	1	1	0.22	0.02	<0.0001

Figure 1. *LDLR* 3UTR SNPs and haplotypes

A. Inclusion of *LDLR* 3UTR tagSNPs in inferred haplotypes. Schematic representation of *LDLR* 3UTR is shown with five tagSNPs indicated by arrows. Chromosomal position labeled is based on genome assembly March 2006. Major alleles are designated by 0 (G, 44243; G, 44857; C, 45622; A, 45645; C, 45935) and minor alleles by 1. B. Cladogram of *LDLR* 3UTR haplotypes. *LDLR* 3UTR haplotype 5 (L5) is, shown in grey, was represented by the ancestral allele at all five tagSNPs.

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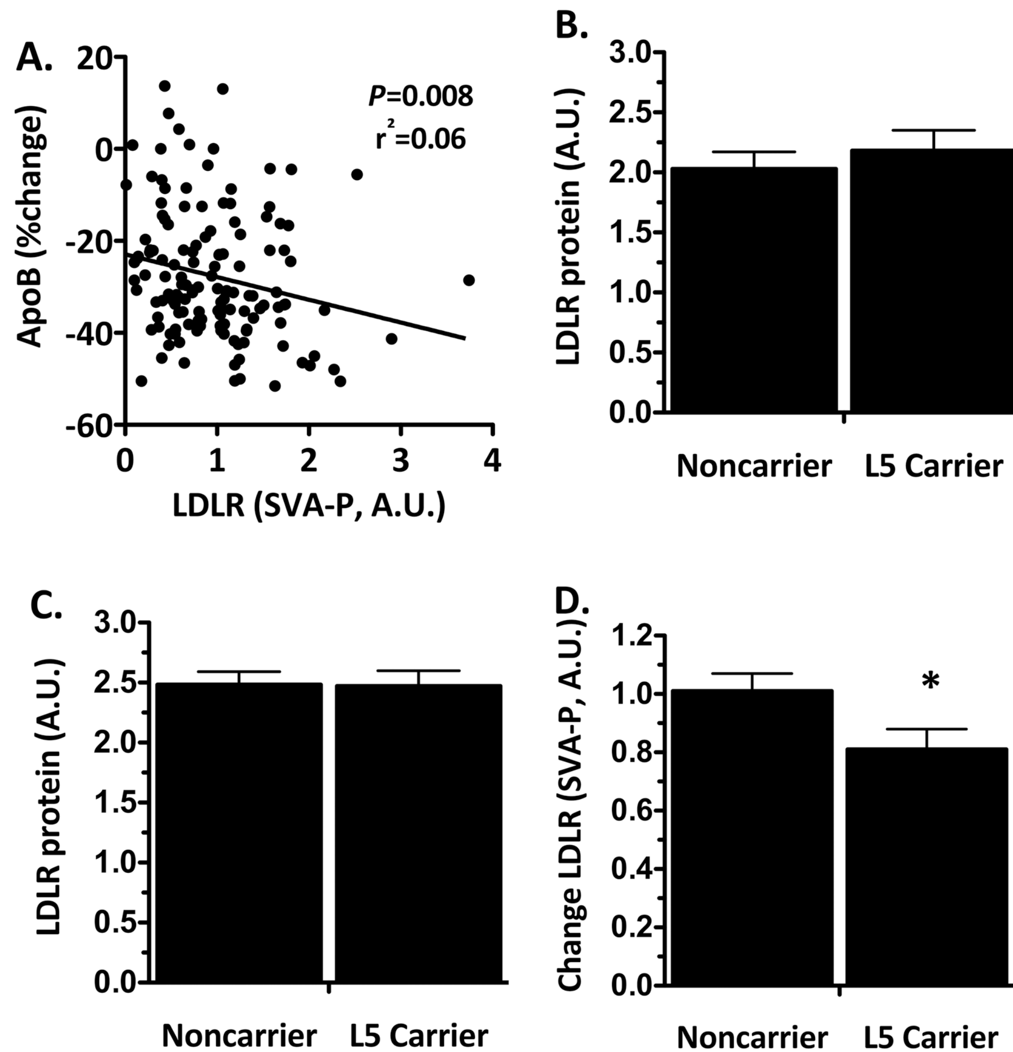

Figure 2. Correlation of simvastatin-mediated induction in *in vitro* LDLR surface expression with *in vivo* apolipoprotein B reduction and association with *LDLR* L5 haplotype

(A) Correlation of *in vitro* LDLR surface protein induction (simvastatin treated – placebo treated, A.U.) and *in vivo* apolipoprotein B reductions (simvastatin – baseline, % change) in response to simvastatin exposure. LDLR surface protein expression was measured by FACS following 24 hour exposure to (B) sham buffer or (C) 2μM simvastatin in lymphoblast cell lines derived from 109 *LDLR* L5 noncarrier and84 *LDLR* L5 carrier African-American CAP participants.(D) Statin-mediated change in LDLR protein expression is presented as LDLR expression following simvastatin exposure minus LDLR expression following sham exposure(*P*=0.03 adjusted for age, sex, BMI and smoking status).

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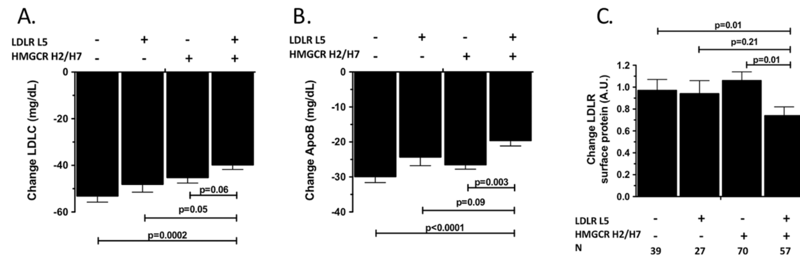

Figure 3. Combined influence of *LDLR* L5 and *HMGCR* H2/H7 haplotypes on *in vivo* lipid-lowering response and *in vitro* LDLR protein response to simvastatin exposure

(A) LDLC and (B) apolipoprotein B were measured before and after simvastatin treatment in 324 African-American CAP participants including 78 noncarriers, 39 *LDLR* L5 only carriers, 119 *HMGCR* H2/H7 only carriers, and 89 *LDLR* L5 + *HMGCR* H2/H7 carriers. (C)LDLR surface protein was measured following 24 hour exposure with 2mM simvastatin or sham buffer in 193 lymphoblast cell lines derived from African-American CAP participants. Associations adjusted for age, sex, BMI and smoking status. P-values for trends are (A) 0.002, (B) <0.0001, and (C) 0.05.

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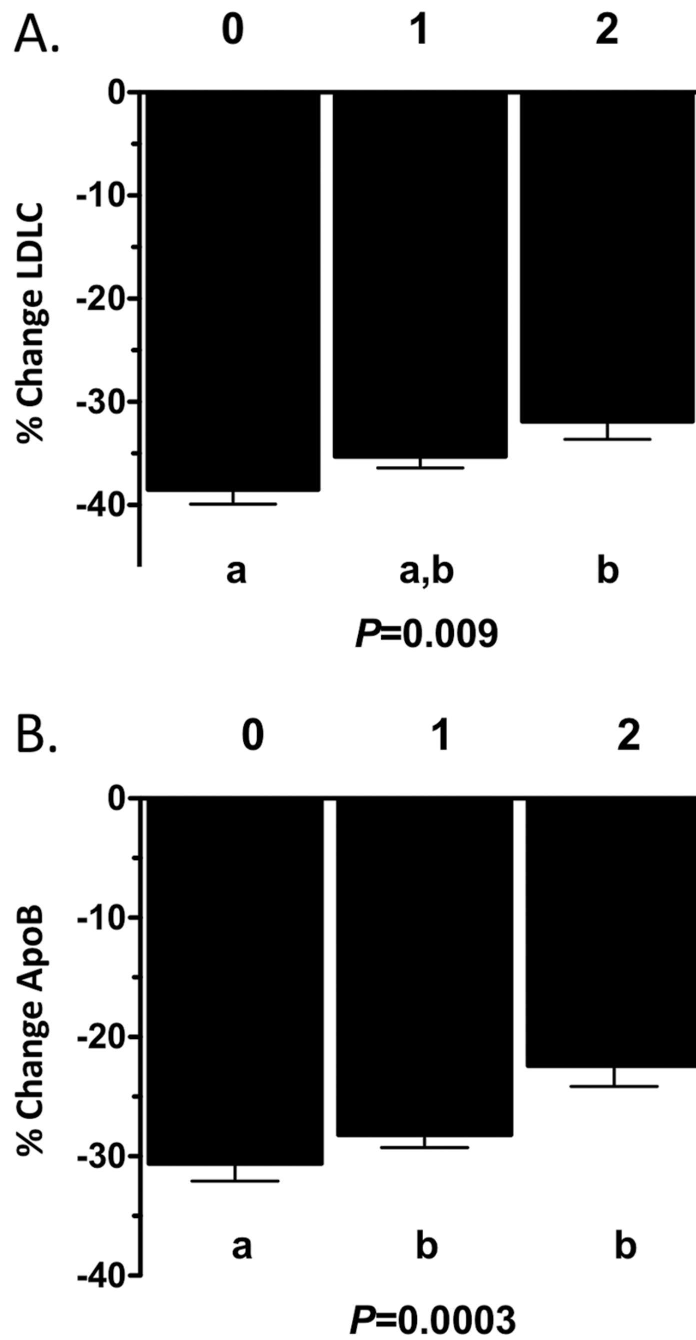

Figure 4. Association of combined haplotypes on (A) LDLC and (B) apoB change in response to simvastatin treatment

0, noncarriers (N=78); 1, LDLR L5 only or HMGCR H2/H7 only carriers (N=158); 2, LDLR L5 + HMGCR H2/H7 carriers (N=89). Associations adjusted for age, sex, BMI, and smoking status.

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Table 1

Associations of LDLR 3UTR haplotypes with statin-mediated percent change inlipidsin African-Americans.

		N	LDL-cholesterol	Total cholesterol	apoB[†]	nonHDLC[†]
LDLR L1	Noncarriers	286	−35.7±4.7	−24.8±3.4	−27.2±0.9	−33.8±4.4
	Carriers	49	−39.3±5.1	−27.5±3.7	−29.3±2.0	−37.2±4.8
	q-value[*]		0.23	0.11	0.36	0.23
LDLR L2	Noncarriers	223	−35.7±4.7	−24.6±3.4	−26.9±1.0	−33.6±4.4
	Carriers	112	−37.2±4.9	−26.7±3.5	−29.0±1.4	−35.7±4.5
	q-value[*]		0.53	0.11	0.22	0.28
LDLR L3	Noncarriers	140	−35.6±4.8	−24.7±3.5	−25.9±1.2	−33.5±4.5
	Carriers	195	−36.2±4.7	−25.2±3.4	−28.8±1.0	−34.4±4.4
	q-value[*]		0.57	0.44	0.10	0.50
LDLR L4	Noncarriers	256	−36.1±4.7	−25.0±3.4	−27.7±0.9	−34.2±4.4
	Carriers	79	−35.7±4.9	−25.6±3.6	−27.4±1.6	−33.8±4.6
	q-value[*]		0.58	0.44	0.71	0.66
LDLR L5	Noncarriers	207	−36.9±4.7	−25.9±3.4	−29.8±1.0	−35.2±4.4
	Carriers	128	−33.5±4.8	−22.8±3.5	−23.9±1.3	−31.1±4.5
	q-value[*]		0.12	0.03	0.001	0.03
LDLR L6	Noncarriers	320	−36.1±4.7	−25.2±3.4	−28.0±0.9	−34.2±4.4
	Carriers	15	−33.7±5.9	−21.2±4.2	−20.9±3.5	−31.1±5.5
	q-value[*]		0.56	0.16	0.10	0.42

[*] Associations were adjusted for age, sex, BMI, smoking status and *HMGCR* H2/H7 haplotype. Q-values represent P-values following adjustment for multiple testing within each phenotype.

[†] Abbreviations: apoB; apolipoprotein B; nonHDLC, nonHDL-cholesterol.

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Table 2

Association of *LDLR* 3UTR haplotype 5 (L5) with lipids in African-American and European-American CAP participants

Values, reported in mg/dL, are mean±SEM for change adjusted for age, sex, BMI, smoking status, and *HMGCR* haplotype.

		N	Baseline	Treatment	Change	Percent change
African-Americans						
ApoB	Noncarriers	207	87.1±6.4	64.7±1.3	−28.3±1.0	−29.8±1.0
	Carriers	128	83.9±6.5	67.9±1.6	−21.7±1.3	−23.9±1.3
	p-value		0.13	0.09	<0.0001	0.0002
TC	Noncarriers	207	206.0±11.2	152.0±9.3	−54.9±8.1	−25.9±3.4
	Carriers	128	206.3±11.5	158.3±9.6	−47.7±8.3	−22.8±3.5
	p-value		0.96	0.07	0.02	0.008
LDLC	Noncarriers	207	132.6±11.1	83.4±8.7	−49.2±7.5	−36.9±4.7
	Carriers	128	130.0±11.4	86.1±9.0	−43.9±7.7	−33.5±4.8
	p-value		0.49	0.45	0.03	0.03
nonHDLC	Noncarriers	207	151.7±11.9	97.8±9.6	−53.8±8.0	−35.2±4.4
	Carriers	128	148.1±12.2	101.7±9.8	−46.4±8.2	−31.1±4.5
	p-value		0.38	0.23	0.006	0.006
European-Americans						
ApoB	Noncarriers	567	95.7±1.8	68.6±1.4	−27.2±1.2	−27.5±1.0
	Carriers	40	102.3±3.8	72.7±2.9	−30.3±2.7	−27.6±2.2
	p-value		0.07	0.21	0.23	0.96
TC	Noncarriers	567	211.3±2.7	153.4±2.2	−58.0±1.9	−26.9±0.7
	Carriers	40	218.6±5.9	158.0±4.6	−60.6±4.0	−26.8±1.5
	p-value		0.20	0.28	0.49	0.70
LDLC	Noncarriers	567	132.0±2.5	76.6±1.8	−55.4±1.7	−41.6±0.9
	Carriers	40	136.9±5.4	77.9±3.9	−59.0±3.7	−41.8±2.0
	p-value		0.34	0.73	0.30	0.90
nonHDLC	Noncarriers	567	158.9±2.8	99.0±2.1	−59.8±1.9	−37.2±0.9
	Carriers	40	167.1±6.2	104.3±4.5	−62.8±4.0	−36.2±1.9
	p-value		0.16	0.22	0.4	0.45