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## Genetic coding variants in the niacin receptor, *hydroxyl-carboxylic acid receptor 2 (HCAR2)*, and response to niacin therapy

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### Abstract

**Objectives**—Niacin has been used for seven decades to modulate plasma lipids, but its mechanism of action is still unclear. We sought to determine whether variants in the niacin receptor gene, hydroxycarboxylic receptor 2 (*HCAR2*) are associated with lipid response to treatment.

**Methods**—Coding variants, rs7314976 (p.R311C) and rs2454727 (p.M317I), were genotyped in 2067 participants from the *Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides and Impact on Global Health Outcomes* (AIM-HIGH) trial. AIM-HIGH was a randomized, placebo controlled trial to assess the effect of extended release (ER) niacin in patients with cardiovascular disease aggressively treated with low density lipoprotein (LDL-C) lowering therapy.

**Results**—We did not find an association of p.R311C or p.M317I with change in LDL-C, triglycerides (TG) or high-density lipoprotein cholesterol (HDL-C) at one year in groups receiving placebo or ER niacin. In white subjects, the reduction in lipoprotein(a) [Lp(a)] in response to niacin was greater in homozygous carriers of the major 317M allele (-22.7%; p=0.005) compared with minor allele carriers (-15.3%). This was directionally consistent in the black participants. Upon combining both groups, the reduction in Lp(a) in response to niacin was significantly greater

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**Conflict of Interest:** RLD has consulted for Catabasis Pharmaceuticals, Inc., the maker of two novel niacin pro-drugs. All other authors have no conflicts.

in the homozygous major allele carriers (-23.0%;  $p=0.003$ ) compared with minor allele carriers (-15.2%).

**Conclusions**—Understanding the genetic contribution to variation in response to niacin therapy, including Lp(a) reduction, could uncover mechanisms by which niacin decreases Lp(a), an important independent risk factor for cardiovascular disease.

### Keywords

Niacin; dyslipidemia; pharmacogenetics; lipoprotein (a); lipids; statins; HCAR2; GPR109A

## Introduction

Niacin's potent lipid-modifying properties were first described in normal and hypercholesterolemic subjects in 1955 by Altschul and colleagues [1]. Niacin favorably modulates the lipoprotein profile by reducing total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), triglycerides (TG) and raising high density lipoprotein cholesterol (HDL-C) [2]. Niacin is one of few drugs that also significantly lowers lipoprotein (a) (Lp(a)) [3], identified as an independent risk factor for cardiovascular disease [4,5]. In the pre-statin era, niacin was the first pharmacologic agent shown to reduce the incidence of nonfatal myocardial infarction and cardiac death [6,7]. Niacin has also demonstrated beneficial effects on arterial plaque regression in combination with statin therapy [8,9]. However in recent clinical outcomes trials, including *The Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides and Impact on Global Health Outcomes* (AIM-HIGH) trial, the addition of extended release (ER) niacin to intensive LDL-C lowering therapy did not reduce atherothrombotic events compared with LDL-C lowering therapy alone [10,11].

Pharmacological effects of niacin are not well understood. Most prominently, niacin stimulates the hydroxyl-carboxylic acid receptor 2 (HCAR2, also known as GPR109A and the niacin receptor) located on the surface of adipocytes, resulting in the anti-lipolytic effect of the drug [12]. The reduction in free fatty acids returning to the liver may reduce the assembly and secretion of very low density lipoproteins (VLDL), [2] however additional mechanisms may be operative [13]. Since HCAR2 is not expressed in the liver, *in vitro* studies suggest that niacin may inhibit TG synthesis by directly inhibiting diacylglycerol acyltransferase-2, the key enzyme for TG synthesis [14]. Niacin may increase HDL-C via reduction of HDL-apoA-I catabolism [15] and inhibition of cholesteryl ester transfer protein (CETP) [16]. The mechanism of Lp(a)-lowering with niacin is unknown.

There is a growing body of literature regarding additional anti-inflammatory effects of niacin that are both dependent [17-20] and independent [21] of HCAR2. This receptor is expressed in various immune cells of the body such as macrophages, neutrophils, and epidermal Langerhans cells [17,18]. In an experimental model of mice deficient of *HCAR2*, Lukasova showed that niacin reduced the progression of atherosclerosis by a *HCAR2* mediated effect on bone-marrow derived cells, independent of its lipid lowering effect [17].

The effects of niacin on lipids and cardiovascular risk are variable. Genetic variation in *HCAR2* could influence niacin's effects on plasma lipids, Lp(a), or cardiovascular events, but this has not been tested [22]. First we sequenced *HCAR2* in 294 healthy subjects to discover novel coding variants. We identified two commonly occurring coding single nucleotide polymorphisms (SNPs): rs7314976 (C931T, p.R311C) and rs2454727 (G951A, p.M317I) occurring in the c-terminal tail. However, the functionality of these SNPs is unknown. The primary objective of this study was to determine whether these variants were associated with the change in LDL-C, HDL-C, TG and Lp(a) following treatment with ER Niacin in the AIM-HIGH study. The secondary objective was to determine whether the *HCAR2* variants were associated with cardiovascular events in the trial and differentially modulated by treatment group.

## Methods

### Ethics Statement

AIM-HIGH participants provided written informed consent and all research as conducted according to the principles outlines in the Declaration of Helsinki. The protocol was approved by the Institutional Review Boards (IRB) at all participating clinical sites. The genetic sub-study of AIM-HIGH was approved by the IRB at the University of Pennsylvania.

### Healthy cohort study

This preliminary study recruited healthy volunteers to a University of Pennsylvania inpatient Clinical and Translational Research Center protocol to investigate the genomic basis of the flushing and metabolic responses to pharmacological doses of niacin. Healthy men and non-pregnant, non-lactating women age 18-45, with BMI 18-30 kg/m<sup>2</sup>, and of self-reported white or black race were included. Here we report the results of the *HCAR2* gene sequencing in 294 subjects. The study was approved by the University of Pennsylvania Institutional Review Board (NCT00953667). All subjects provided written informed consent.

### AIM-HIGH cohort

The AIM-HIGH study (NCT00120289) design and baseline characteristics of the participants have been previously published [10]. Briefly, the trial tested whether ER niacin 1500-2000 mg per day added to intensive statin therapy, as compared with statin therapy alone, would reduce the risk of cardiovascular events in patients with established atherosclerotic cardiovascular disease and atherogenic dyslipidemia (low levels of HDL-C, elevated TG, and small dense particles of LDL-C). Of the total 3414 AIM-HIGH participants randomized into the trial, 2067 had provided DNA and had complete phenotype data to perform the current analysis.

### Laboratory Methods

**Analytical Measurements**—Lipid and Lp(a) measurements were performed at the Northwest Lipid Metabolism and Diabetes Research Laboratory at the University of Washington as previously described [10]. Analysis of Lp(a) was performed by a monoclonal

anti-body-based enzyme-linked immunoadsorbent assay (ELISA) as previously published [23].

**Sequencing of HCAR2 in the healthy cohort study**—DNA was extracted from buffy coat samples using a QIA Symphony SP instrument (Qiagen, Inc. Valencia, CA). Due to the high degree of homology of *HCAR2* and *HCA3*, being 95% identical on the protein level [24], we employed a two-step process for sequencing using a nested primer approach. We sequenced the 5′ untranslated region (UTR) and the coding sequence (Figure 1).

Polymerase chain reaction (PCR) amplification and sequencing of the single exon region of *HCAR2* were carried out with the following primers for amplification: F, ACATGACATAAAGGCAGGCGT; R, TTCTTGCGATGGTTATTTAAGGAG. PCR reactions were amplified in a total volume of 25 μL using 100 ng of genomic DNA, 0.4 μM of each forward and reverse primer, ready-to-go PCR beads (Amersham) while undergoing denaturation at 94° C for 2 minutes, followed by 35 cycles (94° C for 30 seconds, 62° C for 30 seconds, and 72° C for 90 seconds), and an extension at 72° C for 7 minutes. The unincorporated nucleotides and primers were removed by incubation with ExoSAP-IT (USB Corp, Cleveland, OH) for 15 minutes at 37°C followed by enzyme inactivation at 80°C for 15 minutes prior to sequencing. Sequencing was performed at GENEWIZ, Inc. (South Plainfield, NJ) with the following primers: F, GATGCCGATCCAGAATGGCGG; F, CACCACACAGACACACACCTCC; R, CCGCCATTCTGGATCGGCATC; R, TTCTTGCGATGGTTATTTAAGGA. Sequences were aligned using Sequencher software (GeneCodes Corp., Ann Arbor, MI). Allelic variations noted from multiple alignments were verified by inspecting chromatograms. Linkage disequilibrium blocks in each race group were identified using Haploview 4.2 [25].

**Genotyping in AIM-HIGH**—DNA was extracted from buffy coat samples using the FlexiGene DNA Kit (Qiagen Inc, Valencia, CA). We employed a two-step process for genotyping using a nested primer approach. First we amplified the *HCAR2* gene region with the primers described above. Single nucleotide polymorphisms in *HCAR2*, rs7314976 and rs2454727, were genotyped in AIM-HIGH participants using by TaqMan Allelic discrimination Assay (Applied Biosystems, Foster City, CA, USA). Subjects were excluded from the analysis if they reported race other than white or black (n=27). The *HCAR2* p.R311C and p.M317I variants were successfully genotyped in 2050 and 2067 individuals, respectively. The TaqMan genotyping call rate was 96% with an error rate of 8% for p.R311C and 6% for p.M317I as determined on 51 DNA samples genotyped in duplicate. In all cases, discordant calls were caused on the pair being classified as a genotype call and the other member of the pair being classified as failure to genotype. All SNPs were consistent with Hardy-Weinberg equilibrium ( $p > 0.2$ ). Subjects were excluded from the analysis if they were missing outcome data leaving 1994 white and 73 black individuals for the baseline lipoprotein analysis and 1818 white and 58 black individuals for the 1 year lipoprotein analysis. The CV event analysis was performed in 1994 white and 73 black individuals for whom event data were available.

**Statistical analysis**—The primary outcomes were defined as the percent change in plasma concentrations of LDL-C, HDL-C, TG and Lp(a) from baseline to 1 year after

treatment with either statin + placebo or statin + ER niacin. Secondarily, we also examined the baseline pre-treatment plasma concentrations of the lipid traits. Baseline TG and Lp(a) were log-transformed before analysis because of non-normal distribution. The percent changes in lipids and Lp(a) at 1 year were normally distributed. We employed linear regression analysis to test the dominant effect [26] of *HCAR2* genotype on baseline lipid and Lp(a) values and on the percent change in lipids and Lp(a) at one year after randomization, adjusting for age, sex, body mass index (BMI) and baseline lipid values. The one year lipid and Lp(a) outcomes were evaluated separately in the niacin and placebo groups. The analyses were first performed separately for each race group to avoid concerns of population stratification. Since there was no race  $\times$  treatment interaction in the percent change in lipid and Lp(a), we also reported the combined results. Cox regression models were fit to determine the hazard of experiencing a cardiovascular event including *HCAR2* genotype, age, sex, race and BMI in the model. Kaplan-Meier survival curves for time to CV event were created for the *HCAR2*M317I variant. Cardiovascular events were defined as the composite of death from coronary heart disease, nonfatal myocardial infarction, ischemic stroke, hospitalization for an acute coronary syndrome, or symptom-driven coronary or cerebral revascularization [10]. For the primary outcome, the Bonferroni correction was applied to account for multiple comparisons, based on 8 tests (2 SNPs  $\times$  4 lipid traits), so that each association was claimed at the level of 0.006. A post-hoc power analysis was performed for the change in Lp(a) outcome in the niacin group. With 916 patients, we had 74% power to detect the differences in means we observed by M317I genotype at an alpha level of 0.006. Analysis was performed using STATA version 13.1 (College Station, TX).

## Results

We sequenced the single exon *HCAR2* gene in 294 healthy subjects. The summary of the variation is listed in Table 1. Figure 1 provides a schematic of the *HCAR2* gene structure and the location of the SNPs identified by the sequencing study. Six polymorphic sites were identified, two of these resulted in amino acid changes. The two most common coding variants C931T (p.R311C) and G951A (p.M317I) occurred at lower frequency in the black subjects (Table 1). These variants were predicted to be benign by SIFT and PolyPhen [27]. The coding variants were in linkage disequilibrium (LD) ( $r^2=0.96$  for whites and  $r^2=1.0$  for blacks). One major LD block was identified in each race group (Figure S1). To determine whether variation in *HCAR2* was associated with the lipid response to niacin, we chose to genotype these two commonly occurring coding SNPs in the AIM-HIGH cohort.

The clinical and demographic characteristics for the AIM-HIGH population that provided DNA during the course of the study (n=2067) as compared with the whole cohort are provided in Table 2. There was no difference between baseline lipid values seen in the genetic subgroup compared to the larger AIM-HIGH study cohort [10,23]. In the complete cohort, as previously published, the addition of ER niacin to statin treatment resulted in a 25% increase in HDL-C compared with 10% in the placebo group at two years ( $p<0.001$ ). Niacin further decreased LDL-C by 12.0% and triglyceride concentrations by 28.6% as compared to 5.5% and 8.1% in the placebo group, respectively [10]. In a separate analysis of AIM-HIGH, niacin significantly decreased Lp(a) levels by 21% in the statin + ER niacin group compared with 5.9% in the statin + placebo group ( $p<0.05$ ) [23].

Table 3 provides associations of *HCAR2* p.R311C and p.M317I with baseline LDL-C, HDL-C, TG and Lp(a) in the AIM-HIGH cohort combined. The minor allele frequency of *HCAR2* SNPs in the white subjects was p.R311C (MAF 0.17) and p.M317I (MAF 0.39), consistent with our sequencing study in the healthy cohort and to what has been previously reported in dbSNP. The frequency of these variants was lower in the black subjects; p.R311C (MAF 0.06) and p.M317I (MAF 0.16). There were no significant associations with baseline lipid values.

We then tested whether each SNP was associated with the change in lipids from baseline to 1 year after randomization. The percent changes in LDL-C, HDL-C and TG were not significantly associated with *HCAR2* variants in either persons of white (Table 4) or black (Table 5) ancestry. Interestingly, the mean reduction in Lp(a) in response to niacin was significantly greater in homozygous carriers of the 317M major allele ( $-22.7 \pm 35\%$ ;  $p=0.005$ ) than in carriers of the minor allele ( $-15.3 \pm 39.4\%$ ) (Table 4, Figure 2). This was directionally consistent in the small number of black participants (Table 5), and upon combining both race groups the robust response of Lp(a) to niacin in the 317M homozygous major allele carriers was statistically significant ( $p=0.003$ ). Additionally, the reduction in Lp(a) and 317M genotype was evaluated in an interaction model and there was a significant treatment  $\times$  genotype effect ( $p$ -interaction=0.005). The Lp(a) lowering effect due to niacin did not follow an additive model, since a similar decrease was observed in 317I minor allele carriers. There were minimal changes in Lp(a) in the placebo group and no differences by *HCAR2* genotype (Tables 4,5).

We then tested whether the cardiovascular event rate was different by *HCAR2* genotype by Cox regression modeling. *HCAR2* genotype did not increase the hazard of a CV event in either the placebo or niacin group (Table 6, Figure S2).

## Discussion

In this study we evaluated whether two coding variants in *HCAR2* (p.R311C and p.M317I) were associated with lipid and Lp(a) response to niacin in the AIM-HIGH trial. Polymorphisms in *HCAR2* did not predict the change in LDL-C, HDL-C or TG after one year of niacin therapy. The reduction in Lp(a) in response to niacin was significantly greater in the homozygous carriers of the 317M major allele compared with minor allele carriers. Our findings confirm a previous report suggesting that niacin's lipid effect is independent of the *HCAR2* receptor.[13] However, this is the first study to suggest that activation of the *HCAR2* receptor by niacin may mediate Lp(a) lowering. Neither *HCAR2* variant was associated with cardiovascular events in our analysis. This study is the first large association study of these variants and the first pharmacogenetic study of niacin therapy.

The functionality of the p.R311C and p.M317I variants in *HCAR2* is not well known. The first SNP results in an arginine to cysteine substitution at amino acid 311, and the latter SNP results in a methionine to isoleucine substitution at amino acid 317. Prediction tools [27] to identify pathogenic variants such as PolyPhen and SIFT have categorized these variants as benign. However, these tools rely heavily on sequence conservations among species and may not result in reliable predictions for variants located in a residue that is not present in the



majority of species, as is the case for *HCAR2*. In addition, many functional prediction tools do not account for the location of the variants, which may have a substantial impact if they occur in a critical region of the protein or are involved with protein-protein interactions [27]. The p.R311C and p.M317I variants are not located in the transmembrane regions of the receptor and therefore are not involved with niacin binding [28]. Rather, they are found in the c-terminal tail, which has been demonstrated to be functionally important for facilitating the interactions with various cellular proteins regulating receptor desensitization and endocytosis in several GPCR family members [29]. In vitro experiments involving mutations of structural motifs in the c-terminal region of *HCAR2* found that this region plays an important role in the regulation of the receptor surface localization and specifically the 315-328 mutant, the region of the M317I variant, exhibited a significant decrease in niacin-mediated internalization and arrestin3 translocation [30]. While mutagenesis experiments for the specific coding variants we describe have not been published in the peer-reviewed literature, patent literature from Arena Pharmaceuticals has shown that the 317I variant results in reduced ERK 1/2 phosphorylation and mitogen activated protein kinase (MAPK) activation in cell based assays [31]. If this is the case, individuals with this polymorphism would be expected to have reduced ERK 1/2 phosphorylation in response to niacin therapy.

Lipoprotein (a) is an atherogenic particle that has been associated with increased risk of coronary disease and stroke [4,5]. Lp(a) consists of an LDL-like particle bound to a plasminogen-like glycoprotein, apolipoprotein (a) [32]. Lp(a) levels are highly genetically influenced, with copy-number variation within the kringle IV- type 2 repeat of apolipoprotein (a) explaining 40% of the variation [33]. The number of kringle IV-2 repeats are inversely correlated with Lp(a) levels [33]. The contribution of Lp(a) on CVD risk is not well understood, but due to its structural composition it may contribute to both atherosclerosis and thrombosis [32].

In this study we found significantly greater reduction in Lp(a) in response to niacin in homozygous carriers of the 317M major allele compared with carriers of the minor allele. It is unknown how signaling through HCAR2 receptor may influence plasma Lp(a) concentrations. Lp(a) is found in atherosclerotic plaques and involved with macrophage foam cell formation similar to LDL [32]. The HCAR2 receptor is also found on macrophage and niacin acting via HCAR2 has been shown to stimulate cholesterol efflux by increasing ABCA1, ABCG1 and CD36 expression [17,34,35]. Carriers of the major allele may have enhanced ability to efflux lipid from foam cells. Alternatively, niacin may also work to lower Lp(a) via anti-inflammatory mechanisms. Lp(a) via its apo(a) component has been shown to promote oxidative and inflammatory actions on the vascular wall [32,36]. The apo(a) domain was found to increase IL-8 mRNA and protein expression in macrophage via the oxidized phospholipid (oxPL) modification of Lp(a) which facilitated the interaction of Lp(a) with CD36 and macrophage toll-like receptor [37]. Niacin also has anti-inflammatory effects that have been shown to be dependent on HCAR2 receptor activation on immune cells including macrophages, monocytes, neutrophils and dermal dendritic cells [17,20,38,39]. Niacin activation of HCAR2 inhibited monocyte chemotactic protein-1 (MCP-1) induced macrophage recruitment into atherosclerotic plaques; this effect was abolished in HCAR2-deficient mice [17]. Chemokine signaling via MCP-1 is a key component of immune cell recruitment into the atherosclerotic vessel wall, and niacin may

reduce atherosclerosis by interfering with intracellular signaling pathways induced through chemokine receptors, which are also mediated through Gi-type G-proteins [17,40]. Since the *HCAR2* 317I variant causes reduced intracellular signaling as shown by diminished ERK1/2 phosphorylation and MAPK activation, it is conceivable that this variant cannot activate the necessary signaling pathways to interfere with macrophage chemotaxis and therefore would exhibit a reduced anti-inflammatory and anti-atherosclerotic response when exposed to niacin. However, we were not able to show a difference in cardiovascular events rates by *HCAR2* genotype, likely due to the low number of events in the overall trial.

While we performed the genetic evaluation within the context of a prospective, randomized placebo-controlled design with longitudinal follow-up of lipids and cardiovascular events, this study has several limitations. We did not have access to another cohort of chronic niacin treatment to replicate our findings, thus our findings would require replication. It would be worth investigating whether these findings could be replicated in the HPS2-THRIVE study which employed a similar trial design [11]. The overall number of cardiovascular events was low in this study, limiting our ability to evaluate the effect of *HCAR2* variants on these outcomes. The two coding variants we investigated are not found on genome wide arrays, likely due to 95% sequence homology of *HCAR2* with *HCAR3*[24], and therefore have not been associated with lipid traits in prior genome-wide association studies [41,42]. We employed a two -step process in order to successfully amplify the SNP region, and this added step may limit high throughput genotyping in larger cohorts. Despite high homology to *HCAR2*, *HCAR3* has ~1000-fold less affinity for nicotinic acid and has been identified as a receptor for 3-OH-octanoic acid [43,44]. Additionally further work is required to clarify the role of Lp(a) levels as a contributor to cardiovascular risk in the black population. It is known that blacks have a higher Lp(a) levels than the white population [45], but these higher levels have not been consistently shown to translate into a higher risk of cardiovascular events in blacks [46,47]. Consensus statements from the European Atherosclerosis Society states that individuals are at increased risk for cardiovascular disease at levels >30 mg/dL (approximately equivalent to 75 mmol/L by newer assays)[48], but these cut-points may not apply to individuals of black or Asian ancestry [49]. We also observed higher Lp(a) levels at baseline in the AIM-HIGH trial, but the greatest benefit in terms of Lp(a) lowering due to niacin was observed in homozygous carriers of the 317M allele in both race groups. It is still unclear from the literature what is the optimal level of Lp(a) reduction required in black individuals to achieve cardiovascular risk reduction.

In conclusion, we observed differential Lp(a) lowering based on genotype at the M317I variant in response to niacin. We did not find an association, however, between coding variants in *HCAR2* and the change in plasma LDL-C, HDL-C or TG with niacin treatment. Understanding the genetic contribution to variation in response to niacin therapy, including Lp(a) reduction, could uncover mechanisms by which niacin decreases Lp(a), an important independent risk factor for cardiovascular disease.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.



## Acknowledgments

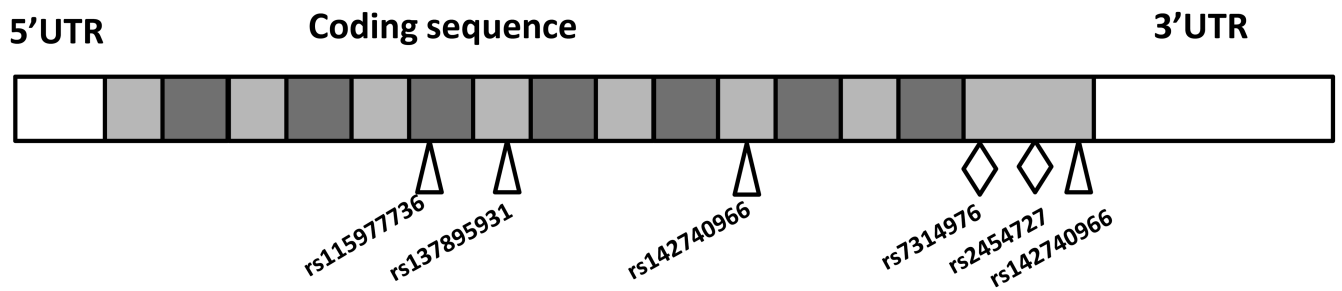
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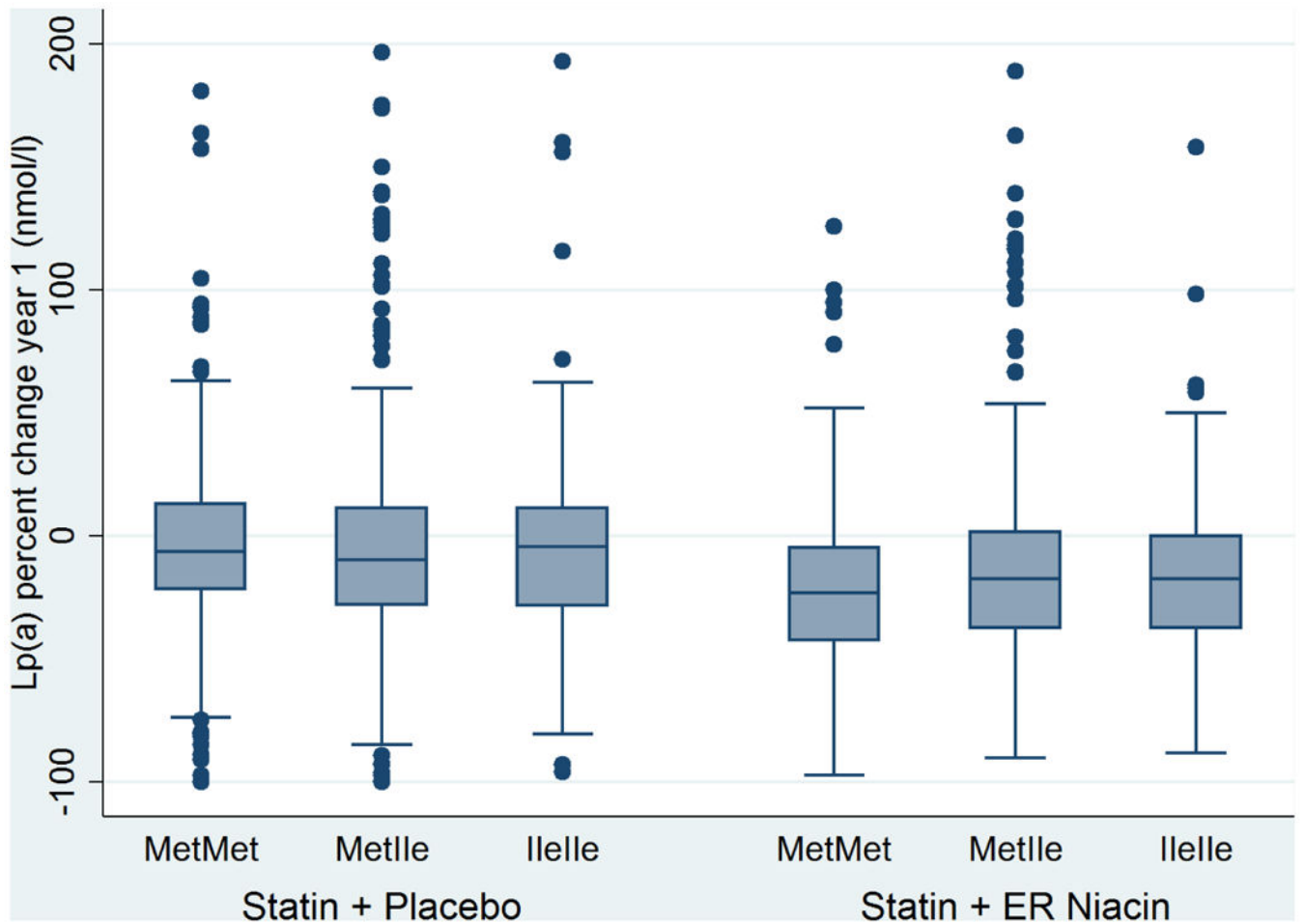
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**Figure 1.**

Schematic of *HCAR2* gene structure. The 5' untranslated region (UTR) and all 1092 nucleotides of the coding region were sequenced in healthy subjects. The location of nonsynonymous single nucleotide polymorphisms (SNPs) identified in this study is denoted by diamonds, while the synonymous SNPs are denoted by the triangles. The transmembrane domains of the translated protein are depicted by dark grey boxes.



**Figure 2.**

Change in Lipoprotein (a) levels by *HCAR2* (rs2454727) genotype in white subject from the AIM-HIGH trial. The reduction in Lp(a) in response to niacin was greater in homozygous 317M carriers compared with 317I carriers ( $p=0.0053$  in the niacin group;  $p=0.37$  in the placebo group;  $p$ -interaction =0.005). The boxes on the boxplots represent the 75<sup>th</sup> percentile, median, and 25<sup>th</sup> percentile.

**Table 1**  
**Summary of variation identified in HCAR2 by sequencing the healthy cohort (n=294)**

Rs. No.	Chr: bp <sup>1</sup>	Base Position	Alleles	AA position	AA change	MAF EA	MAF AA	SIFT <sup>2</sup>	PolyPhen <sup>2</sup>
rs115977736	12:122702960	324	T/C	108	Ala-Ala	0.000	0.039	-	-
rs137895931	12:122702726	558	C/T	186	Phe-Phe	0.005	0.000	-	-
rs142740966	12:122702531	753	G/C	251	Arg-Arg	0.000	0.005	-	-
rs7314976	12:122702353	931	C/T	311	Arg-Cys	0.161	0.023	0.26	0.015
rs2454727	12:122702333	951	G/A	317	Met-Ile	0.385	0.147	0.19	0
rs142025654	12:122702303	981	G/A	327	Thr-Thr	0.000	0.025	-	-

<sup>1</sup> Build GRCh38.p10

<sup>2</sup> SIFT and PolyPhen scores obtained from [www.ensembl.org](http://www.ensembl.org); both of these variants were predicted to be tolerated by SIFT and benign by PolyPhen

MAF= minor allele frequency

EA= European Americans

AA= African-Americans



**Table 2**  
**Clinical and Demographic Characteristics of the AIM-HIGH participants**

mean± sd median (IQR)	Genetic subgroup		Total AIM-HIGH study	
	Statin + Placebo (n=1020)	Statin + ER Niacin (n=1047)	Statin + Placebo (n=1696)	Statin + ER Niacin (n=1718)
Age (years) mean± sd	64.0 ± 8.7	64.6 ± 8.7	63.7 ± 8.7	63.7 ± 8.8
Sex, n (%) female	164 (16.1)	158 (15.1)	251 (14.8)	253 (14.7)
Race, n (%) Black	35 (3.4)	38 (3.6)	49 (2.9)	68 (4.0)
Baseline LDL-C (mg/dL)	74.6 ± 22.2	73.5 ± 22.0	75.8 ± 24.3	76.2 ± 25.7
Baseline HDL-C (mg/dL)	35.1 ± 5.6	34.6 ± 5.6*	35.3 ± 5.9	34.8 ± 5.9*
Baseline TG (mg/dL)	162 (133-215)	166 (131-217)	162 (128-218)	164 (127-218)
Baseline Lp(a) (nmol/L)	32 (13-118)	36 (14- 132)	32.7 (13.1-122.6)	36.1 (13.5-126.6)

Lipid traits reported as mean (sd); Triglycerides and Lp(a) reported as median (interquartile range).

\* HDL-C levels were nominally lower in the statin + ER niacin group at baseline p=0.04 in the genetic subgroup and in the whole AIM-HIGH cohort as previously reported.

LDL-c= low-density lipoprotein cholesterol, HDL-c= high-density lipoprotein cholesterol, TG=triglycerides, Lp(a)= lipoprotein (a)

**Table 3**  
**Association of HCAR2 variants with baseline lipid values in participants from the AIM-HIGH study**

SNP	Trait	White Ancestry (MAF 0.17)				Black Ancestry (MAF 0.06)			
		ArgArg (n=1359)	ArgCys (n=553)	CysCys (n=65)	p-value	ArgArg (n=64)	ArgCys (n=9)	CysCys (n=0)	p-value
p-R311C	LDL-C (mg/dL)	74.1 ± 22.2	74.0 ± 21.7	70.0 ± 21.3	0.49	82.2 ± 24.5	70.6 ± 19.9	n/a	0.15
	HDL-C (mg/dL)	34.7 ± 5.5	35.0 ± 5.6	35.3 ± 5.9	0.81	35.7 ± 6.0	35.0 ± 3.8	n/a	0.97
	TG (mg/dL)	162 (131-216)	167 (131-218)	169 (145-211)	0.46	157 (120-205)	148 (120-182)	n/a	0.24
	Lp(a)(nmol/l)	33 (13-123)	32 (13-135)	31 (15-74)	0.88	84 (41-148)	51 (40-67)	n/a	0.54
SNP	Trait	White Ancestry (MAF 0.39)				Black Ancestry (MAF 0.16)			
		MetMet (n=737)	MetIle (n=928)	IleIle (n=329)	p-value	MetMet (n=50)	MetIle (n=22)	IleIle (n=1)	p-value
p-M317I	LDL-C (mg/dL)	74.0 ± 22.6	74.0 ± 21.4	73.4 ± 22.3	0.74	81.9 ± 24.7	76.8 ± 22.8	110.0	0.47
	HDL-C (mg/dL)	34.7 ± 5.4	34.8 ± 5.7	35.1 ± 5.6	0.50	36.4 ± 5.7	33.7 ± 5.5	40.0	0.24
	TG (mg/dL)	163 (132-222)	164 (131-215)	168 (134-211)	0.67	155± (125-203)	172 (116-204)	122	0.59
	Lp(a)(nmol/l)	32 (13-107)	32 (13-140)	31 (13-116)	0.31	65 (37-135)	97 (51-203)	30	0.14

Lipid traits reported as mean ± sd; TG and Lp(a) reported as median (interquartile range). TG and Lp(a) were log-transformed prior to analysis. P-values were calculated using a dominant model adjusting for age, sex, race and body mass index.

LDL-c= low-density lipoprotein cholesterol, HDL-c= high-density lipoprotein cholesterol, TG=triglycerides, Lp(a)= lipoprotein (a), MAF= minor allele frequency

**Table 4**  
**Association of HCAR2 variants with percent change in lipid values at one year by treatment in the White Participants**

SNP	Trait	Statin + Placebo				Statin + ER Niacin				p-value
		ArgArg (n=614)	ArgCys (n=253)	CysCys (n=32)	p-value	ArgArg (n=624)	ArgCys (n=256)	CysCys (n=24)	p-value	
<b>p.R311C</b>	LDL-C (mg/dL)	-0.8 ± 32.8	-0.4 ± 28.2	6.3 ± 28.3	0.96	-3.5 ± 36.7	-2.1 ± 41.0	-1.8 ± 28.5	0.55	
	HDL-C (mg/dL)	9.3 ± 15.4	11.9 ± 16.0	11.9 ± 16.9	0.049	28.2 ± 24.9	26.4 ± 21.8	27.9 ± 21.1	0.23	
	TG (mg/dL)	2.4 ± 40.2	0.7 ± 38.3	2.4 ± 51.7	0.98	-21.4 ± 38.7	-24.0 ± 37.8	-24.0 ± 30.5	0.49	
	Lp(a)(nmol/l)	-2.9 ± 41.5	-6.7 ± 41.2	-15.2 ± 33.5	0.069	-19.7 ± 37.7	-13.8 ± 39.3	-20.4 ± 34.2	0.049	
<b>SNP</b>	<b>Trait</b>	<b>MetMet (n=344)</b>	<b>MetHe (n=412)</b>	<b>IleIle (n=146)</b>	<b>p-value</b>	<b>MetMet (n=327)</b>	<b>MetHe (n=441)</b>	<b>IleIle (n=148)</b>	<b>p-value</b>	
<b>p.M317I</b>	LDL-C (mg/dL)	-2.4 ± 31.9	0.06 ± 31.9	3.1 ± 29.1	0.23	-3.7 ± 39.1	-2.6 ± 37.4	-3.5 ± 35.2	0.58	
	HDL-C (mg/dL)	9.7 ± 15.3	9.8 ± 16.2	11.4 ± 14.5	0.47	28.2 ± 25.4	27.9 ± 23.5	26.1 ± 22.8	0.62	
	TG (mg/dL)	2.0 ± 39.1	1.8 ± 38.5	3.3 ± 46.2	0.96	-20.9 ± 38.4	-23.0 ± 38.6	-22.5 ± 36.2	0.50	
	Lp(a)(nmol/l)	-3.1 ± 37.2	-5.0 ± 44.0	-5.8 ± 41.1	0.37	-22.7 ± 35.2	-15.2 ± 40.1	-15.8 ± 37.3	0.0053	

Lipid traits reported as mean (sd) percent change from baseline to one year. P-values were calculated from a dominant model adjusting for age, sex, body mass index and baseline lipid levels.

**Table 5**  
**Association of HCAR2 variants with percent change in lipid values at one year by treatment in the Black Participants**

SNP	Trait	Statin + Placebo				Statin + ER Niacin			
		ArgArg (n=26)	ArgCys (n=1)	CysCys (n=0)	p-value	ArgArg (n=25)	ArgCys (n=6)	CysCys (n=0)	p-value
<b>p.R311C</b>	LDL-C (mg/dL)	2.0 ± 36.0	56.6	n/a	0.29	-20.0 ± 29.8	-6.1 ± 25.4	n/a	0.39
	HDL-C (mg/dL)	5.6 ± 17.0	14.8	n/a	0.81	24.2 ± 24.5	28.8 ± 19.1	n/a	0.76
	TG (mg/dL)	-3.8 ± 35.0	4.1	n/a	0.91	-18.2 ± 45.6	-42.7 ± 27.3	n/a	0.18
	Lp(a)(nmol/l)	-2.8 ± 29.1	-8.0	n/a	0.85	-17.9 ± 48.2	-28.8 ± 29.1	n/a	0.63
<b>SNP</b>	<b>Trait</b>	<b>MetMet (n=20)</b>	<b>MetHe (n=7)</b>	<b>HeHe (n=0)</b>	<b>p-value</b>	<b>MetMet (n=18)</b>	<b>MetHe (n=12)</b>	<b>HeHe (n=1)</b>	<b>p-value</b>
<b>p.M317I</b>	LDL-C (mg/dL)	2.7 ± 37.4	7.9 ± 37.7	n/a	0.30	-15.7 ± 32.4	-18.5 ± 25.9	-31.8	0.57
	HDL-C (mg/dL)	6.7 ± 17.9	3.7 ± 13.8	n/a	0.65	23.8 ± 28.0	26.8 ± 16.5	27.5	0.55
	TG (mg/dL)	-3.9 ± 37.3	-2.6 ± 26.2	n/a	0.86	-14.9 ± 52.9	-33.4 ± 23.6	-41.0	0.21
	Lp(a)(nmol/l)	-2.0 ± 32.9	-5.7 ± 11.3	n/a	0.93	-28.5 ± 30.9	-1.2 ± 57.8	-77.6	0.11

Lipid traits reported as mean (sd) percent change from baseline to one year. P-values were calculated from a co-dominant model adjusting for age, sex and body mass index.

**Table 6**  
**Cardiovascular events by *HCAR2* genotype in AIM-HIGH participants**

SNP	Genotype	Statin + placebo (n=1,020)	HR	95% CI	p-value	Statin + ER Niacin (n=1,047)	HR	95% CI	p-value
p.R311C	Arg/Arg	121 (17.3%)	0.87	0.65, 1.17	0.38	114 (15.7%)	0.99	0.74, 1.32	0.94
	Arg/Cys	41 (14.6%)				49 (17.3%)			
	Cys/Cys	5 (13.5%)				2 (7.1%)			
p.M317I	Met/Met	69 (17.8%)	0.91	0.73, 1.14	0.42	65 (16.8%)	1.06	0.85, 1.32	0.61
	Met/Ile	71 (14.3%)				72 (14.5%)			
	Ile/Ile	24 (14.4%)				34 (20.7%)			

HR= hazard ratio from Cox regression adjusted for age, sex, race, BMI