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The effects of *ABCG5/G8* polymorphisms on HDL cholesterol concentrations depend on *ABCA1* genetic variants in the Boston Puerto Rican Health Study

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Abstract

Background and aims—ATP-binding cassette transporters G5/G8 (ABCG5/G8) are associated with HDL-C concentrations. To assess whether the effect of *ABCG5/G8* genetic variants on HDL-C concentrations is dependent on ATP-binding cassette transporters A1 (*ABCA1)*, we studied potential interactions between single nucleotide polymorphisms (SNPs) at *ABCG5/G8* (i7892T>C, 5U145A>C, T54CA>G, T400KC>A) and *ABCA1* (i27943G>A, i48168G>A, K219RG>A, i125970G>C, 3U8995A>G) genes with HDL-C concentrations.

Methods and Results—*ABCG5*/*G8* and *ABCA1* SNPs were genotyped in 788 subjects (228 men and 560 women) who participated in the Boston Puerto Rican Health Study. Biochemical measurements were determined by standard procedures. Genotyping was performed using TaqMan® assays according to routine laboratory protocols. Significant gene-gene interactions for HDL-C were found between *ABCG8* (5U145A>C, T54CA>G, T400KC>A) SNPs and *ABCA1*_ i48168G>A genetic variant (*P*=0.009, *P*=0.042 and *P*=0.036, respectively), in which carriers of the 5U145C and 54C alleles, and homozygotes for the T400 allele at *ABCG8* genetic variants displayed lower HDL-C concentrations than homozygotes for the 5U145A and T54 alleles, and heterozygotes for the 400K allele at *ABCG8* SNPs, only if they were also homozygous for the minor allele (A) at the aforementioned *ABCA1* SNP.

Conclusions—The gene-gene interactions reported in the present study support the hypothesis that the effect of *ABCG5/G8* genetic variants on HDL-C concentrations is dependent on *ABCA1* expression. Replication of these analyses to further populations, particularly with low HDL-C, is clearly warranted.

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Keywords

ATP binding cassette transporters; HDL-cholesterol; gene-gene interaction; pathway; reverse cholesterol transport

INTRODUCTION

Low concentrations of high-density lipoprotein cholesterol (HDL-C) are associated with increased risk for coronary heart disease (CHD) (1). One of the most likely mechanisms by which low HDL-C promotes atherosclerosis is through the impairment of cholesterol clearance from arteries to the liver via reverse cholesterol transport (RCT) (2). This is a process whereby HDL participates in efflux of cholesterol from peripheral tissues, transport in the plasma, uptake in the liver, and excretion into bile.

Excretion of cholesterol from enterocytes into the intestinal lumen and their excretion into the bile is mediated by ATP-binding cassette transporters G5 and G8 (ABCG5/G8), which play a key role in cholesterol clearance through the RCT pathway (3,4). In humans, mutations in either of these genes cause the genetic disease sitosterolemia (5), characterized by increased plasma concentrations of plant sterols and increased risk for premature development of atherosclerosis. In mice, impairment of this pathway has been associated with reduced biliary cholesterol secretion and enhanced cholesterol absorption with low HDL-C concentrations (6).

The ATP-binding cassette transporter 1 (ABCA1) mediates the efflux of cholesterol from macrophage foam cells, particularly, the efflux of cholesterol and phospholipids onto lipid-poor apolipoprotein A–I, contributing to the first steps of RCT. In addition, ABCA1 is primarily responsible for the initiation of HDL particle formation, principally in the liver and the small intestine (7,8). Mutations in the *ABCA1* gene cause Tangier disease, characterized by the almost complete absence of plasma HDL and increased risk for CHD (9). In mice, deficiency of *ABCA1* decreases plasma HDL-C concentrations, whereas its overexpression increases HDL-C concentrations and protects against atherosclerosis (10, 11). Given that both genes are involved in the RCT pathway, potential interactions between their variants on plasma HDL-C concentrations are of interest.

Expression of *ABCG5/G8* and *ABCA1* genes is regulated, in part, by the liver X receptor (LXR) group of transcription factors (12,13). In mice, global LXR activation by synthetic agonists has been shown increased plasma HDL-C concentrations through the upregulation of *ABCA1* (14–18). Based on these observations, this pathway appears to be required for normal HDL-C homeostasis, where accumulation of HDL-C due to defects in *ABCG5/G8* can be compensated by an inhibition of HDL synthesis. In contrast, enhanced HDL-C elimination by *ABCG5/G8* overexpression can be regulated by an increase in HDL synthesis. We recently reported significant associations between several common *ABCG5/G8* single nucleotide polymorphisms (SNPs) (i7892T>C, 5U145A>C, T54CA>G, and T400KC>A) and HDL-C, where carriers of the minor alleles at those SNPs who were also homozygotes for the T400 allele had lower HDL-C concentrations (19). These data raise the possibility that these *ABCG5/G8* variants may be associated with reduced *ABCA1*

expression. To date, no large population studies examining potential interactions between common polymorphisms at *ABCG5/G8* and *ABCA1* genes with HDL-C concentrations have been reported. Therefore, given the importance of these three genes on HDL-C metabolism through the RCT pathway, the aim of the present study was to investigate potential interactions between common polymorphisms at these genes in relation to HDL-C concentrations.

MATERIALS AND METHODS

Participants

Complete demographic, biochemical and genotype data were available for 788 participants (228 men and 560 women). These participants aged 45–75 years, were recruited from the Boston Puerto Rican Center for Population Health Disparities to participate in the Boston Puerto Rican Health Study, a longitudinal cohort study on stress, nutrition, health, and aging. The design of the study was approved by the Institutional Review Board of Tufts Medical Center and all participants provided informed consent. The detailed design and methodology of the study have been described previously (19).

Data Collection

Information on socio-demographics, health status, history, and behavior was collected by home interview administered by bilingual interviewers. CHD was defined as a positive response to the question “Have you ever told by a physician that you had heart attack or angina”. Anthropometrical and blood pressure (BP) measurements were collected using standard methods. Weight was measured with a beam balance and height with fixed stadiometer. BMI was calculated as weight in kilograms divided by the square of height in meters. BP was measured with an oscillometric device (Dinamap Pro Series 100, GE Medical Systems) while participants were seated and had rested for at least five minutes. Reported systolic and diastolic BP values were the mean of two measurements. Smoking and alcohol intake were determined by questionnaire. Physical activity was estimated as a score based on the Paffenbarger questionnaire of the Harvard Alumni Activity Survey (20).

Using American Diabetes Association criteria, participants were classified as having diabetes if fasting plasma glucose concentration was ≥1.38 mmol/L or use of insulin or other diabetes medication was reported.

Laboratory methods

Blood samples were drawn after fasting overnight. Total cholesterol was measured using a cholesterol esterase cholesterol oxidase reaction on an Olympus AU400e autoanalyzer (Olympus America Inc., Melville, NY). The same reaction was used to measure HDL-C after precipitation of non-HDL cholesterol with magnesium/dextran. Low-density lipoprotein cholesterol (LDL-C) was measured by use of a homogeneous direct method (LDL Direct Liquid Select Cholesterol Reagent; Equal Diagnostics). Triglycerides (TG) were measured by a glycerol-blanked enzymatic method on the Olympus AU400e centrifugal analyzer (Olympus America Inc., Melville, NY).

Genetic analyses

DNA was extracted from blood samples and purified using commercial Puregene reagents (Gentra Systems) following the manufacturer's instructions. The set of polymorphisms in *ABCG5/G8* (i7892T>C, rs4131229; 5U145A>C, rs3806471; T54CA>G, rs4148211; T400KC>A, rs4148217) and *ABCA1* (i27943G>A, rs2575875; i48168G>A, rs4149272; K219RG>A, rs2230806; i125970G>C, rs2297404; 3U8995A>G, rs363717) genes were selected for genotype analysis. *ABCG5/G8* SNPs were selected according to their associations with HDL-C levels in a recent report (19). *ABCA1* SNPs were selected using the criteria of bioinformatics functional assessment and linkage disequilibrium (LD) structure. Computational analysis of *ABCA1* SNPs (http://www.ncbi.nlm.nih.gov/SNP/buildhistory.cgi) ascribed potential functional characteristics to each variant allele. Intronic SNPs were also analyzed with MAPPER (21) to uncover potential allele-specific transcription factor binding sites and manually checked for altered mRNA splice donor and acceptor sites and transversions affecting the poly-pyrimidine tract near splice acceptors. Assessing LD structure at the *ABCA1* locus facilitated the selection of tag SNPs representing different LD blocks. Additionally, for the i48168G>A polymorphism, analysis by MAPPER indicated a potential allele-specific binding site for the cartilage paired-class homeoprotein 1 (CART1) transcription factor, whose motif appears enriched in certain genes involved in cholesterol metabolism (JL, LDP, JO, personal data). Genotyping was performed using TaqMan® assays with allele-specific probes on the ABIPrism 7900 HT Sequence Detection System (Applied Biosystems) according to routine laboratory protocols (22). The description of the SNPs, primers, probes, sequences, as well as ABI assay-on-demand ID is presented in Supplemental Table 1.

Statistical analyses

SPSS software (version 16.0) was used for statistical analyses. Data were presented as means±SE for continuous variables and as frequencies or percentages for categorical variables. Differences in mean values were assessed using analysis of variance and unpaired t-tests. Categorical variables were compared with the Pearson chi-square or Fisher's exact tests. Potential confounding factors were age, gender, BMI, physical activity, smoking habit (current vs. never and past smokers), alcohol consumption (current *vs*. never and past drinkers), medications (treatment for hypertension, diabetes, hyperlipidemia, use of hormone therapy by women), and prior CHD. All analyses were further adjusted for population admixture estimated using the program STRUCTURE 2.2 (see below). Potential interactions between genotypes for HDL-C values (as continuous variables) were tested using the analysis of variance test. Pairwise LD between SNPs was estimated as correlation coefficient (R) using the Helixtree software package (Golden Helix). Two-sided *P* values <0.05 were considered statistically significant.

Population admixture

Population admixture was estimated based on the genotypes of 100 ancestral informative markers (AIMs) using two programs: STRUCTURE 2.2 and IAE3CI, with reference to three ancestral populations: European settlers, native Taíno population, a panel of 100 AIMs was

found to be necessary to properly estimate ancestral proportions by using a combination of simulated and applied data.

RESULTS

Characteristics of participants and genotype frequencies are shown in Table 1. BMI was significantly lower in men who also displayed lower total cholesterol, LDL-C, and HDL-C levels than women. In addition, men were more likely to smoke and drink alcohol compared to women. No significant differences in other examined variables were observed between men and women.

For all *ABCG5*/*G8* and *ABCA1* polymorphisms examined, there was no departure from Hardy-Weinberg equilibrium ($P>0.05$). Pairwise LD in correlation coefficients of all nine SNPs is presented in Supplemental Table 2. Given that all pairwise LD was <0.80, all SNPs were retained for further analysis. Except for *ABCA1* SNPs i27943G>A, i48168G>A, genetic variants were analyzed using two genotype categories on account of the low genotype frequencies of homozygotes for the minor allele.

As reported previously (20), *ABCG5/G8* SNPs (i7892T>C, 5U145A>C, T54CA>G, T400KC>A) were significantly associated with HDL-C concentrations (*P* values ranging from <0.001 to 0.025); carriers of the minor alleles at the aforementioned polymorphisms and homozygotes for the T400KC allele displayed lower concentrations (Figure 1). We also examined the associations between *ABCA1* SNPs and lipids without observing any significant association (Supplemental Table 3).

We next examined HDL-C concentrations depending on the interaction between genotypes (Table 2). A significant interaction between *ABCG8*_5U145A>C and *ABCA1*_i48168G>A SNP for HDL-C concentrations a trend towards lower concentrations than homozygotes for the major allele (in mmol/L) (1.11±0.03 vs.1.10±0.02 vs. 1.17±0.02; $P=0.118$), only if they were carriers of the minor alleles at both *ABCA1* SNPs. In contrast, within homozygotes for the major allele at *ABCG8*_5U145A>C, a trend towards higher HDL-C concentrations was seen among *ABCA1*_ i48168G>A) genotype categories (1.21±0.03 vs. 1.24±0.02 vs. 1.16±0.02; $P=0.148$) (Figure 2).

Similarly, a significant interaction between *ABCG8* T54CA>G and *ABCA1*_i48168G>A for HDL-C was found ($P=0.042$). Within carriers of the 54C allele at *ABCG8*, AA subjects at the *ABCA1*_i48168A>G showed lower concentrations compared to GG participants (in mmol/L) (1.11±0.03 vs. 1.16±0.02), whereas higher HDL-C concentrations were seen in minor allele carriers at *ABCA1* if they were homozygotes for the T54 allele at ABCG8 (1.25±0.02 vs. 1.17±0.02). However, these differences were not significant ($P>0.2$ for both). Finally, a significant interaction between *ABCG8*_T400KC>A and *ABCA1*_i48168G>A SNP was also found for HDL-C concentrations ($P=0.036$). Although no significant differences were found among genotypes, A allele carriers at *ABCA1* SNP displayed lower HDL-C concentrations compared to GG participants, only if they were homozygotes for the T400 allele at *ABCG8* (in mmol/L) (1.11±0.02 vs. 1.18±0.02; $P>0.2$) whereas higher concentrations were observed in AA subjects compared to GG participants within carriers of

the 400K allele (1.22±0.03 vs. 1.15±0.03; *P*=0.179) (Figure 2). Importantly, these interactions remained significant after further adjustment for TG (*P* values ranging from 0.003 to 0.030) (data not shown). No significant interactions were found for LDL-C and TG concentrations according to the presence of *ABCG5/G8* and *ABCA1* polymorphisms (Supplemental Tables 4,5).

DISCUSSION

The present study provides the first evidence of a significant gene-gene interaction between common *ABCG5/G8* and *ABCA1* polymorphisms for HDL-C concentrations. These findings may help to explain prior reported associations between common *ABCG5/G8* polymorphisms and HDL-C (19). In contrast to the well-documented association between *ABCG5/G8* polymorphisms and LDL-C (23,24), association with HDL-C concentrations has been highly inconsistent (23–27). Except for Acalovschi *et al.* (26), who reported low HDL-C concentrations in carriers of the Gln604 allele at *ABCG5*, no statistically significant associations between *ABCG5/G8* polymorphisms and HDL-C concentrations have been reported from previous studies (23–25,27). Therefore, while results from earlier studies are inconclusive, the present study provides consistent and compelling evidence of the involvement of *ABCG5/G8* genes in HDL metabolism through their interaction with *ABCA1*.

Significant interactions between *ABCG8* (5U145A>C, T54CA>G, T400KC>A) and *ABCA1*_i48168G>A SNPs for HDL-C concentrations were found. The fact that HDL-C concentrations within *ABCG8* genotypes decreased only in carriers of the minor alleles at *ABCA1*_i48168G>A SNP, raises the possibility that unaffected activity of this intronic *ABCA1* SNP is important for the expression of gain of function variants in *ABCG8*. In support of this possibility, we demonstrate an interaction between several polymorphisms located on different genes, functionally linked through the RCT pathway, and whose effects on the phenotype of serum HDL-C concentrations are in the same direction.

In addition, this study examines the effects of *ABCA1* genotype on lipids, particularly on HDL-C concentrations. Consistent with previous data (28–30), we did not find any significant association between common *ABCA1* variants and HDL-C concentrations. However, when the gene-gene interaction with the *ABCG5/G8* polymorphisms was considered, we observed significant associations between *ABCA1*_i48168G>A polymorphism and HDL-C depending on *ABCG5/G8* genotypes. In this regard, *ABCA1*_i48168G>A had the potential to influence the effects of *ABCG8* SNPs (5U145A>C, T54CA>G, and T400KC>A) on HDL-C. These findings suggest that determinants of steady-state HDL-C cholesterol concentrations depending on *ABCA1* variants may differ in function of *ABCG8* genetic variation.

Importantly, *ABCG5/G8* and *ABCA1* are direct target genes for the ligand-activated transcription factors LXR (12,13). A plausible mechanism for the observed associations may result from the LXR-mediated upregulation of those genes. In mice, global LXR activation by synthetic agonists has been shown to increase plasma HDL-C concentrations through the induction of *ABCA1* expression (14–18) and therefore, the promotion of the RCT. Indeed,

increased *ABCA1* activity can protect against atherosclerosis by mediating the translocation of phospholipids to apolipoprotein A–I generating nascent HDL particles that are primed for further cholesterol efflux (11). Therefore, the observed epistatic effect between *ABCG8* and *ABCA1* genes supports the hypothesis that increased HDL-C concentrations are associated with their overexpression through stimulation of the RCT pathway.

Interestingly, the SNP *ABCG8*_5U145A>C maps within a transcription factor binding motif for farnesoid X receptor (FXR) (NR1H4), and it is therefore possible that binding of this transcription factor in an allele-specific manner may upregulate expression of *ABCG5* or *ABCG8* or both and thus, potentially counteract the accelerated loss of cholesterol from the body with a compensatory increase in HDL-C synthesis via upregulation of *ABCA1*. The potential mechanisms whereby the SNP *ABCA1*_i48168G>A, within intron 5, may contribute to the observed interactions are unknown. The reported presence of transcriptional enhancers and other regulatory elements containing LXR motifs in intronic regions of the *ABCA1* gene (16) could alter either mRNA splicing or control of gene expression and explain our findings. On the basis of these observations, it is possible that binding of these transcription factors in an allele-specific manner may increase plasma HDL-C concentrations through the upregulation of *ABCA1* expression. Therefore, we may surmise that these interactions result in recruitment of coactivators to the liganded LXR, with a subsequent increase in the transcription of both genes.

These interactions may have consequences in terms of therapeutic LXR activation as a novel treatment strategy against atherosclerosis by increasing HDL-C levels and stimulating RCT through the upregulation of *ABCA1* (31,32). However, the utility of LXRs as pharmacological targets has been compromised by their effect on expression in mice of hepatic lipogenic genes leading to hypertriglyceridemia and fatty liver. Therefore, identification of selective LXR agonists that activate genes in a tissue-specific manner, to exploit the beneficial aspects of LXR activation while avoiding unwanted side effects, is currently warranted.

Although there are no studies in humans examining the association between *ABCG5/G8* and/or *ABCA1* expression and atherosclerosis, several results from animal models are consistent with an anti-atherogenic effect of their overexpression by promoting the RCT pathway (31,32). Overall, the results in the present study provide a good example that the epistatic effect of *ABCG5/G8* and *ABCA1* variants on plasma HDL-C concentrations may modulate the risk of atherosclerosis through the RCT pathway. Therefore, our findings have wide-ranging implications for health initiatives targeted at reducing CHD risk.

One of the main limitations in the present study is the lack of measurement of apolipoprotein A–I, HDL particle size, as well as plasma RCT whose measurements might assist in a deeper evaluation of potential mechanisms involved in this gene-gene interaction. Secondly, the prevalence of CHD was self-reported, and this may be subject to recall bias. Finally, despite the fact that all statistical analyses performed in this study were adjusted for population admixture to avoid spurious associations, replication in other ethnic populations, particularly those with low HDL-C concentrations, is clearly warranted.

In conclusion, the data and analyses presented here demonstrate that several polymorphisms in *ABCG5/G8* and *ABCA1* genes, both of which are functionally linked through the RCT, interact in determining HDL-C concentrations. Importantly, the gene-gene interactions described here support the hypothesis that the upregulation of *ABCG5/G8* genes may depend on *ABCA1* expression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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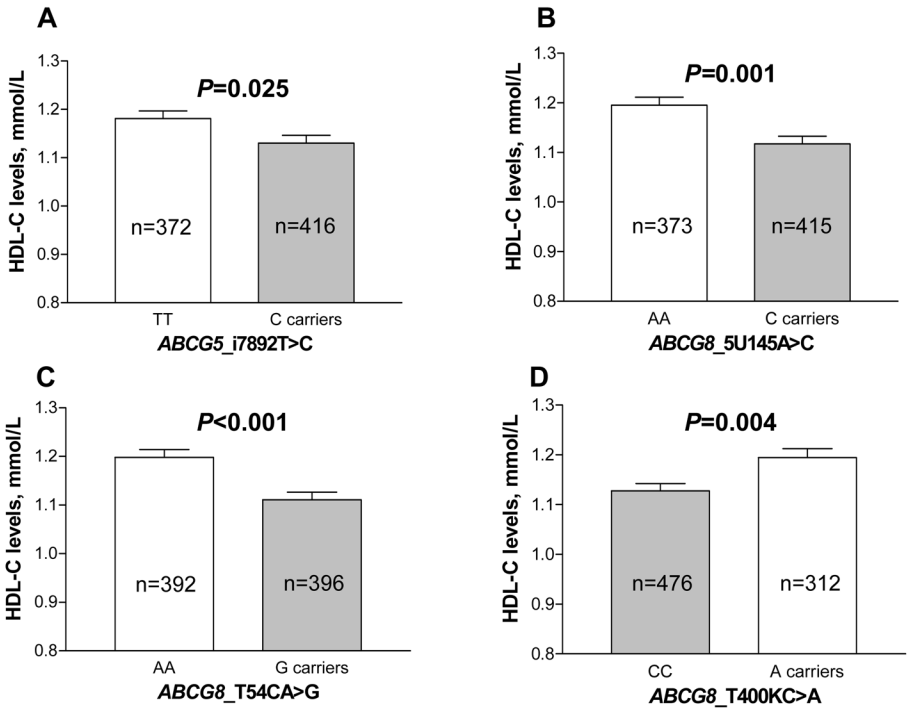

Figure 1.

Adjusted HDL-cholesterol concentrations depending on *ABCG5* (i7892T>C) (A) and *ABCG8* (5U145A>C (B), T54CA>G (C), T400KC>A (D)) polymorphisms. Values are mean±SD.

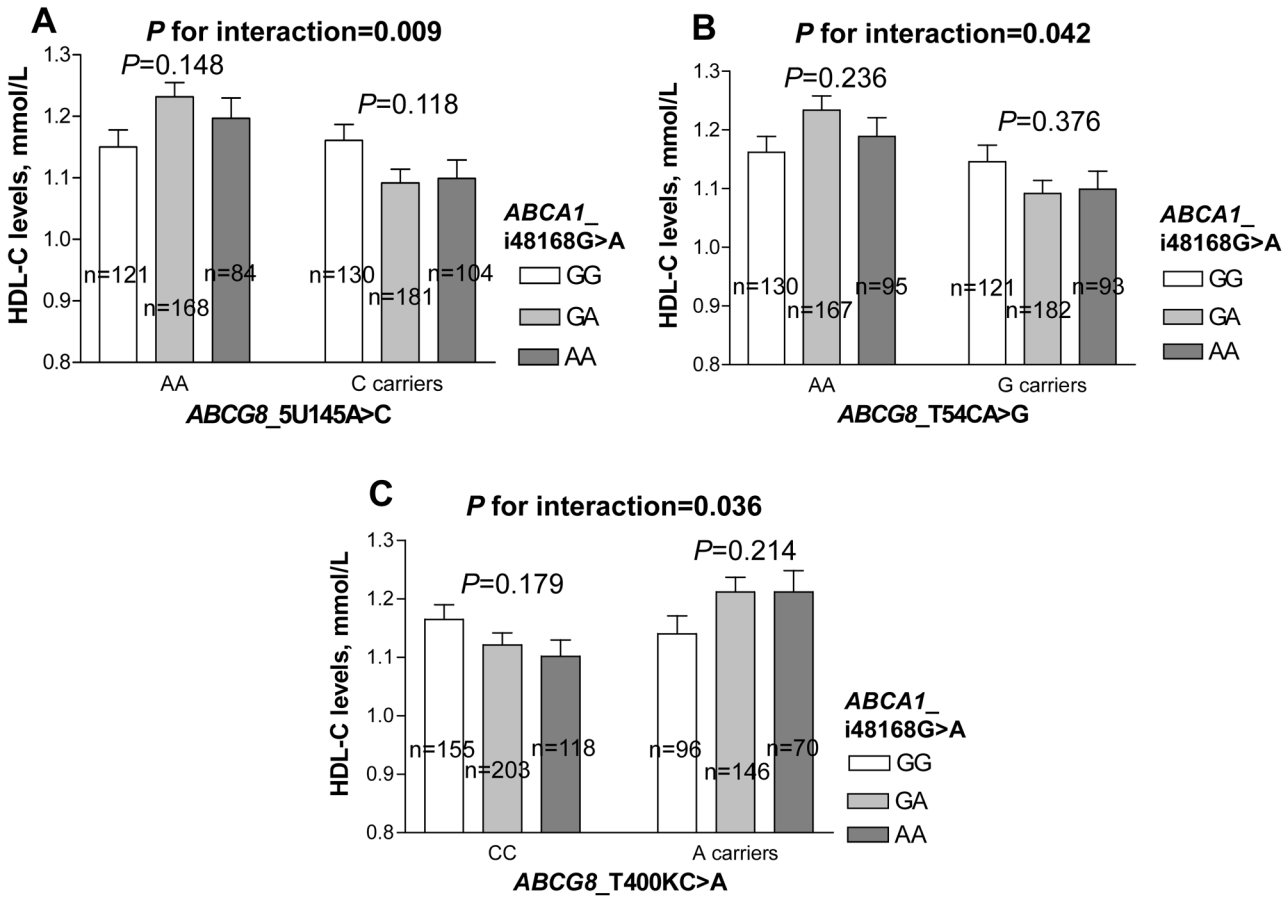

Figure 2.

Adjusted HDL-cholesterol concentrations depending on the interactions between *ABCG8*_5U145A>C (A), *ABCG8*_T54CA>G (B), and *ABCG8*_T400KC>A (C) polymorphisms and *ABCA1*_ i48168G>A genetic variant. Values are mean±SD.

Table 1

Demographic, biochemical, and genotypic characteristics in participants.

	Men (n=228)	Women (n=560)	All (n=788)
Age, years (range)	57 (45–75)	58 (45–75)	58 (45–75)
Body mass index, kg/m^2*	29.6±5.4	33.0±6.7	32.0±6.5
Systolic blood pressure, mmHg*	138.1±1.2	134.4±0.8	135.5±18.4
Diastolic blood pressure, mmHg*	83.1±0.7	80.3±0.5	81.1±10.8
Total cholesterol, mmol/L*	4.47±1.12	4.92±1.07	4.79±1.10
LDL cholesterol, mmol/L*	2.53±0.90	2.89±0.89	2.75±0.90
HDL cholesterol, mmol/L*	1.04±0.32	1.22±0.32	1.17±0.33
Triglycerides, mmol/L	2.07±0.95	1.79±0.82	1.87±0.92
Current smokers, n (%)*	77 (34)	117 (21)	194 (25)
Current drinkers, n (%)*	113 (50)	182 (33)	295 (37)
Physical activity score	32.0±4.9	31.3±4.2	31.5±4.4
On diabetic treatment, n (%)	82 (36)	203 (26)	285 (36)
On beta-blockers treatment, n (%)	115 (50)	295 (53)	410 (52)
Prior coronary heart disease, n (%)	52 (23)	105 (19)	157 (20)
Polymorphisms, n (%)			
*ABCG5*_i7892T>C			
TT	110 (48)	262 (47)	372 (47)
TC+CC	118 (52)	298 (53)	416 (53)
*ABCG8*_5U145A>C			
AA	111 (49)	262 (47)	373 (47)
AC+CC	117 (51)	298 (53)	415 (53)
*ABCG8*_T54CA>G			
AA	110 (48)	282 (50)	392 (50)
AG+GG	118 (52)	278 (50)	396 (50)
*ABCG8*_T400KC>A			
CC	147 (65)	329 (59)	476 (60)
CA+AA	81 (36)	231 (41)	312 (40)
*ABCA1*_i27943G>A			
GG	76 (33)	157 (28)	233 (30)
GA	108 (48)	281 (50)	389 (49)
AA	44 (19)	122 (22)	166 (21)
*ABCA1*_i48168G>A			
GG	80 (35)	171 (31)	251 (32)
GA	97 (43)	252 (45)	349 (44)
AA	51 (22)	137 (24)	188 (24)
*ABCA1*_K219RG>A			
GG	92 (40)	188 (34)	280 (36)
GA+AA	136 (60)	372 (66)	508 (64)

	Men (n=228)	Women (n=560)	All (n=788)
*ABCA1*_i125970G>C			
GG	201 (88)	509 (91)	710 (90)
GC+CC	27 (12)	51 (9)	78 (10)
*ABCA1*_3U8995A>G			
AA	148 (65)	342 (61)	490 (62)
AG+GG	80 (35)	218 (39)	298 (38)

All values are mean±SD.

* Statistically significant differences between men and women.

Table 2

Adjusted plasma HDL-C concentrations (in mmol/L) depending on *ABCG5/G8* and *ABCA1* SNPs.

	***ABCG5*_i7892T>C**		***P*** *	***ABCG8*_5U145A>C**		***P*** *	***ABCG8*_T54CA>G**		***P*** *	***ABCG8*_T400KC>A**		***P*** *
***ABCA1*_i27943G>A**	TT (n=372)	TC+CC (n=416)		AA (n=373)	AC+CC (n=415)		AA (n=392)	AG+GG (n=396)		CC (n=476)	CA+AA (n=312)	
GG (n=233)	1.20±0.02	1.16±0.02	0.816	1.19±0.02	1.18±0.02	0.070	1.20±0.02	1.17±0.02	0.184	1.17±0.02	1.20±0.03	0.688
GA (n=389)	1.19±0.02	1.12±0.02		1.21±0.02	1.10±0.02		1.21±0.02	1.10±0.02		1.13±0.02	1.19±0.02	
AA (n=166)	1.20±0.03	1.14±0.03		1.24±0.03	1.11±0.03		1.24±0.03	1.11±0.03		1.14±0.02	1.22±0.03	
***ABCA1*_i48168G>A**												
GG (n=251)	1.18±0.02	1.15±0.02	0.619	1.16±0.02	1.17±0.02	**0.009**	1.17±0.02	1.16±0.02	**0.042**	1.18±0.02	1.15±0.03	**0.036**
GA (n=349)	1.21±0.02	1.14±0.02		1.24±0.02	1.10±0.02		1.25±0.02	1.10±0.02		1.13±0.02	1.22±0.02	
AA (n=188)	1.19±0.03	1.12±0.03		1.21±0.03	1.11±0.03		1.20±0.03	1.11±0.03		1.11±0.02	1.22±0.03	
***ABCA1*_K219RG>A**												
GG (n=280)	1.19±0.02	1.15±0.02	0.393	1.20±0.02	1.14±0.02	0.357	1.20±0.02	1.14±0.02	0.243	1.14±0.02	1.22±0.02	0.471
A carriers (n=508)	1.20±0.02	1.13±0.01		1.21±0.01	1.12±0.01		1.22±0.01	1.11±0.01		1.14±0.01	1.19±0.02	
***ABCA1*_i125970G>C**												
GG (n=710)	1.20±0.01	1.14±0.01	0.713	1.21±0.01	1.13±0.01	0.885	1.21±0.01	1.12±0.01	0.495	1.14±0.01	1.21±0.01	0.060
C carriers (n=78)	1.20±0.05	1.12±0.04		1.21±0.04	1.12±0.04		1.22±0.04	1.09±0.04		1.19±0.04	1.12±0.05	
***ABCA1*_3U8995A>G**												
AA (n=490)	1.20±0.02	1.14±0.01	0.699	1.20±0.02	1.14±0.01	0.134	1.20±0.01	1.13±0.02	0.257	1.14±0.01	1.20±0.02	0.997
G carriers (298)	1.20±0.02	1.13±0.02		1.23±0.02	1.11±0.02		1.22±0.02	1.10±0.02		1.14±0.02	1.20±0.02	

Values are mean±SE. *P* values were adjusted for age, gender, BMI, physical activity, smoking habit, alcohol consumption, medications, prior coronary heart disease, and population admixture.

* *P* values for interaction between *ABCG5/G8* and *ABCA1* SNPs.