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Genetic Variations at *ABCG5/G8* Genes Modulate Plasma Lipids Concentrations in Patients with Familial Hypercholesterolemia

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Abstract

Objective—To investigate the association of four common single nucleotide polymorphisms (SNPs) at *ABCG5* (i7892A>G, i18429C>T, Gln604GluC>G, i11836G>A) and five at *ABCG8* (5U145T>G, Tyr54CysA>G, Asp19HisG>C, i14222T>C, and Thr400LysG>T) with plasma lipids concentrations and to explore the interaction between those SNPs and smoking in patients with FH.

Methods and Results—*ABCG5/G8* SNPs were genotyped in 500 subjects with genetic diagnosis of FH. Carriers of the minor A allele at the *ABCG5* i11836G>A SNP displayed significantly higher HDL-C concentrations ($P=0.023$) than G/G subjects. In addition, carriers of the minor G allele at the *ABCG5* Gln604GluC>G SNP had significantly lower VLDL-C ($P=0.011$) and lower TG ($P=0.017$) concentrations than homozygous C/C. Interestingly, a significant gene-smoking interaction was found, in which carriers of the minor alleles at *ABCG5* (i7892A>G, i18429C>T, i11836G>A) SNPs displayed significantly lower HDL-C, higher TC and higher TG respectively, only in smokers. On the other hand, non-smokers carriers of the minor alleles at *ABCG5* (i18429C>T and Gln604GluC>G) SNPs had significantly lower TG concentrations ($P=0.012$ and $P=0.035$) compared with homozygous for the major allele.

Conclusions—Our data support the notion that *ABCG5/G8* genetic variants modulate plasma lipids concentrations in patients with FH and confirm that this effect could be influenced by smoking. Therefore, these results suggest that gene-environmental interactions can affect the clinical phenotype of FH.

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DISCLOSURES

None of the authors had any conflict of interest.

Keywords

ABCG5/G8 polymorphisms; lipids; smoking; interaction; familial hypercholesterolemia

Familial hypercholesterolemia is an autosomal codominant inherited disorder of lipoprotein metabolism associated with the development of severe and premature coronary heart disease (CHD) (1). It is caused by mutations in the gene encoding the low-density lipoprotein receptor (LDLR) located in the short arm of the chromosome 19, and it is transmitted as an autosomal dominant character with a high penetrance. To date more than 800 mutations have been identified, and it has been estimated that there are 10,000,000 people with FH worldwide.

It is well known that despite being a monogenic disorder, the clinical expression of FH is highly variable in terms of the age of onset and severity, even in cases that share the same mutation (2). This fact suggests that other metabolic, environmental and genetic factors could play an important role in the development of atherosclerosis in FH.

ABCG5 and *ABCG8* are cholesterol half-transporters that have been identified to form heterodimers and function together to regulate cholesterol kinetics in humans (3–5). Furthermore, it has been recently shown the crucial role of *ABCG5* and *ABCG8* in promoting cholesterol excretion in vivo through the reverse cholesterol transport (RCT) pathway and their up-regulation by liver X receptor (LXR) agonists (6,7). Single nucleotide polymorphisms (SNPs) in these transporters have been associated with hypercholesterolemia (8–10). However, to date not many studies have examined the associations between *ABCG5/G8* polymorphisms with plasma lipids in patients with severe hypercholesterolemia. In this context, Miwa et al. (11) did not find significant associations with lipids in 100 Japanese patients with hypercholesterolemia. Another study examined the relationship between SNPs in the *ABCG8* gene (D19H and T400K) and plasma cholesterol concentrations in patients with heterozygous FH (12). Interestingly, although they did not find significant differences in plasma lipid concentrations, they showed that these genetic variants in the *ABCG8* gene may affect variation in cardiovascular risk.

On the other hand, cigarette smoking increases plasma LDL-C and TG levels and decreases HDL-C, and several studies have suggested gene-smoking interactions modifying these effects (13). In this regard, we have recently demonstrated that genetic variation at the *ABCG5/G8* genes modulates the effect of cigarette smoking on plasma HDL-C concentrations (14). This gene-environmental interaction could affect the clinical phenotype of FH but this effect has never been studied in patients with severe hypercholesterolemia. Therefore, our primary aim was to examine the association between nine *ABCG5/G8* polymorphisms with plasma lipids in patients with FH. In a next step, we explored the interaction between those SNPs and smoking status in the same population.

SUBJECTS AND METHODS

Subjects

The study population consisted of 500 unrelated subjects (244 women and 256 men) randomly selected from a Spanish FH longitudinal cohort study, supported by the “Fundación Española Hipercolesterolemia Familiar” (<http://www.colesterolfamiliar.com>) (15). All patients included in the study were heterozygous carriers for known functional mutations in the LDL receptor (*LDLR*) gene. The mean age ($\pm$ SD) was 43 ± 16.2 years. In order to homogenize the sample, patients with familial defective apolipoprotein B disorder were excluded from the analysis. A written informed consent was obtained from all participants before their inclusion in the cohort

and the protocol was approved by the ethic committee of the CEIC Fundación Jiménez Díaz (Madrid).

Laboratory methods

Blood samples were obtained after 12 h fasting. Total cholesterol (TC) and TG were determined by enzymatic techniques (16,17). Very low density lipoprotein cholesterol (VLDL-C) was determined from plasma TG (18). HDL-C was determined after precipitation with *phosphotungstate* (19). LDL-C concentration was calculated using the Friedewald formula (20). ApoA-I and B levels were determined by immunoturbidimetry (21).

Genetic analyses

The genotyping study was carried out in the Nutrition and Genomics Laboratory, at the Jean Mayer–US Department of Agriculture Human Nutrition Research Center on Aging. Genomic DNA was isolated from whole blood samples using standard methods. All subjects in this study were heterozygous carriers for known mutations in the LDLR gene associated with FH. The genetic diagnosis was made using a DNA-microarray (Progenika, Bilbao, Spain) as previously described (22). Four *ABCG5* SNPs (i7892A>G, i18429C>T, Gln604GluC>G, i11836G>A) and five *ABCG8* SNPs (5U145T>G, Tyr54CysA>G, Asp19HisG>C, i14222T>C, and Thr400LysG>T) were genotyped. SNPs were selected using two criteria: bioinformatics functional assessment and linkage disequilibrium (LD) structure. Computational analysis of *ABCG5/G8* SNPs (<http://www.ncbi.nlm.nih.gov/SNP/buildhistory.cgi>) ascribed potential functional characteristics to each variant allele. Given that SNP rs3806471 maps to the 5'-UTR of *ABCG8* but also lies around 216 bp upstream of the *ABCG5* mRNA start, this SNP sequence was analyzed by MAPPER (23), which identified an allele-specific farnesoid X receptor (FXR) (NR1H4) transcription factor binding site. Intronic SNPs were also analyzed with MAPPER and manually checked for altered mRNA splice donor and acceptor sites and transversions affecting the polypyrimidine tract near splice acceptors. Assessing LD structure at the *ABCG5/G8* loci facilitated the selection of tag SNPs representing different LD blocks.

Genotyping was performed using a TaqMan® assay with allele-specific probes on the ABIPrism 7900 HT Sequence Detection System (Applied Biosystems) according to routine laboratory protocols (24). The description of *ABCG5/G8* SNPs, probes, and sequences, as well as ABI assay-on-demand ID is presented in Table 1. Standard good laboratory practices were undertaken to ensure the accuracy of genotype data, including the inclusion of dummy duplicates.

Statistical analyses

Statistical Package for the Social Sciences (SPSS v 15.0, Chicago, IL, USA) was used for the statistical comparisons. A chi-square test was used to determine whether the genotype distribution followed Hardy-Weinberg equilibrium. Data were presented as means $\pm$ SD or means $\pm$ standard error for continuous variables and as frequencies or percentages for categorical variables. A logarithmic transformation was applied to measures of plasma TG to normalize the distribution of the data. Differences in mean values were assessed by using analysis of variance and unpaired t-tests. Furthermore, comparisons of frequencies between qualitative variables were carried out using the Chi-squared test. Potential confounding factors were age, gender, BMI, physical activity, smoking habits (current vs. never and past smokers), diabetes, HTA, lipid lowering drugs and cardiovascular disease (CVD). Potential interactions between *ABCG5/G8* and smoking in determining lipid values (as continuous variables) were tested using the variance test. In this regard, the nonsmokers were patients who reported that they had never smoked, not even a puff or reported that they had stopped smoking. In this context, past smokers were considered those patients who took more than one year without smoking. On the other hand smokers were considered those patients who smoked more than

ten cigarettes per day. Thus, we considered smoking habit into two categories: current vs never or past smokers. All the analyses were adjusted for potential confounders and $P < 0.05$ was considered to be significant.

Linkage disequilibrium and haplotype analysis

Analysis were performed using different R packages implementing statistical methods and algorithms for the analysis of genetic data (GNU) (25). The SNPs pairwise LD was evaluated by the measures of D' and r^2 , and the results plotted by using the *LDheatmap* package. For haplotype analysis, we estimated haplotype frequencies using maximum likelihood algorithm for a subset of SNPs selected on the basis of individual association with the phenotypes to ensure reasonable statistical power. Haplotype based hypothesis tests of generalised linear models (GLM) were conducted by use of prospective likelihood implemented in the *haplo.glm* function of the *haplo.stats* R package (26). The regression coefficient (β) represents the effect of the haplotype by phenotype interaction in which inferred haplotypes were considered as predictors, and the aforementioned confounding factors as covariates. Analyses were adjusted for gender, age, BMI, HDL-C levels, exercise, diabetes, treatment, CVD and smoking conditions as potential confounders. P values were further adjusted for multiple tests by a permutation test.

RESULTS

A total of 500 unrelated subjects (256 men and 244 women) with conclusive genetic diagnosis for FH were genotyped. In order to homogenize the analysis, only subjects with all lipid parameters available were included. Thus, complete demographic, biochemical and genotype data were available in 465 subjects.

Association of the *ABCG5/G8* SNPs with fasting plasma lipids

Genotype distributions did not deviate from Hardy-Weinberg expectations for all *ABCG5/G8* SNPs ($P > 0.05$). Given the low genotype frequencies of individuals homozygous for the minor alleles, and as the analysis did not suggest a recessive mode of action, we analyzed all SNPs using two genotype categories.

We found significant and novel association between genotypes *ABCG5_i11836G>A* SNP and HDL-C concentrations. Thus, carriers of the minor A allele showed significantly higher HDL-C ($P = 0.023$) than G/G subjects (Figure 1a). In addition, significantly lower plasma VLDL-C ($P = 0.011$) and TG ($P = 0.017$) concentrations were also observed in carriers of the minor G allele at *ABCG5_Gln604GluC>G* than C/C subjects (Figure 1b).

No other significant associations were found between all these SNPs explored and other plasma lipid variables. However, a similar trend was observed for *ABCG8_Asp19HisG>C* by which carriers of the minor C allele had lower VLDL-C ($P = 0.089$) and TG ($P = 0.089$) concentrations than G/G subjects (data not shown).

Interaction between *ABCG5/G8* SNPs and smoking on lipid levels

In the next step, we examined whether the interaction between smoking and the polymorphisms at the *ABCG5/G8* genes determines plasma lipid response. Demographic and biochemical characteristics according to the smoking status are shown in Table 2.

As expected, smokers displayed higher TC, LDL-C, TG, VLDL-C and ApoB and lower HDL-C and ApoA-I concentrations than nonsmokers. However, smokers had a lower prevalence of CVD with respect to nonsmokers (11/128, 8.1% vs 54/337, 14.8%) (Table 2). In this context, it is essential to note that smokers were younger than nonsmokers (40 vs 44; $P = 0.005$).

For *ABCG8* SNPs a significant interaction between *ABCG8_5U145T>G* and smoking was found for TG ($P=0.033$) and VLDL-C ($P=0.020$) (data not shown). In this context, smokers carriers of the minor G allele had significantly higher VLDL-C ($P=0.04$) concentrations and a trend toward higher values of TG (4.67 ± 0.05 vs. 4.60 ± 0.04 , respectively; $P=0.11$) compared to T/T subjects. Conversely, no significant differences across genotypes were found in nonsmokers ($P=0.796$ and $P=0.867$, respectively). No other significant gene-smoking interactions were found in other examined *ABCG8* SNPs.

For *ABCG5* (i7892A>G, i18429C>T, i11836G>A, Gln604GluC>G) SNPs strong interactions were found between genetic variants and smoking (Table 3). Interestingly, smokers carriers of the minor G, T and A alleles at *ABCG5* (i7892A>G, i18429C>T, i11836G>A) SNPs displayed significantly lower HDL-C ($P=0.042$), and higher TC ($P=0.040$) and TG ($P=0.035$) respectively, compared with smokers homozygous for the major allele. In contrast, it is essential to note that no significant differences were demonstrated in nonsmokers (Figures 2a, and 2b).

On the other hand, non-smokers carriers of the minor T and G alleles at *ABCG5* (i18429C>T and Gln604GluC>G) SNPs had a protective effect towards significantly lower TG concentrations ($P=0.012$ and $P=0.032$, respectively) than homozygous for the major allele. However, this effect was lost if they were smokers (Figure 2c and 2d).

It is essential to note that of the five significant interactions, four *ABCG5* (i7892A>G, i18429C>T, i11836G>A and Gln604GluC>G) SNPs (table 3) and *ABCG8_5U145T>G* were related with TG concentrations. To understand the combined effects of genetic variants at *ABCG5/G8*, we conducted haplotype analysis using a subset of *ABCG5/G8* SNPs according to their association with the phenotypes as individual variants. The pattern of pairwise LD between the nine SNPs is presented in Supplemental Data 1. Given that all pairwise linkage disequilibrium correlation coefficient (r^2) were < 0.80 , we concluded that no LD block was found on SNP structure of our population. For further haplotype analysis, we selected four SNPs (i7892A>G, i18429C>T, i11836G>A and Gln604GluC>G) which showed a statistical association with TG plasma levels in single marker analysis, and 5U145T>G because of the moderate LD linkage disequilibrium observed with i7892A>G.

There were eight haplotypes with frequencies ranking from 1.9 % to 13 % accounting for 45.5 % of all haplotypes in this population (Supplemental Data 2). After adjustment for covariates, carriers of the haplotype G-T-A-G-G showed significantly lower TG concentrations ($\beta=-1.8374$, $P=0.0007$).

We selected the aforementioned SNPs for haplotype analysis in relation to smoking status (Supplemental Data 3). In smokers, there were eight haplotypes with frequencies from 3 % to 14 % accounting for 67 % of all haplotypes. For individual haplotypes, carriers of C-T-A-G-T showed significantly higher plasma TG levels ($\beta=0.8166$, $P=0.0026$). Among nonsmokers, there were seven haplotypes with frequencies ranking from 2 % to 16 % accounting for 64 % of all haplotypes. For individual haplotypes, carriers of C-T-G-G-G showed significantly lower plasma TG levels ($\beta=0.2432$, $P=0.0016$). All associations reached significance even after correcting for multiple testing.

DISCUSSION

This study provides novel evidences supporting that genetic variation at *ABCG5/G8* genes modulates plasma lipids in FH patients. Moreover, this study confirms previous data suggesting that the effect of these genetic variants on plasma lipids is influenced by smoking status.

The ABCG5 and ABCG8 proteins play an essential role in hepatobiliary cholesterol transport (3–6). In this regard, the current study showed a significant and new association between a common *ABCG5*_i11836G>A SNP and plasma HDL-C, in which carriers of the minor A allele displayed significantly higher concentrations than G/G subjects. In addition, carriers of the minor G allele at *ABCG5*_Gln604GluC>G SNP displayed lower VLDL-C and lower TG concentrations than C/C subjects (similar trend was observed for carriers of the minor C allele at *ABCG8*_Asp19HisG>C SNP for VLDL-C and TG). This pattern of associations has not been previously described.

Despite the importance of *ABCG5/G8* transporters in the regulation of cholesterol kinetics in humans, not many studies have examined the associations between *ABCG5/G8* polymorphisms with plasma lipids in patients with familial hypercholesterolemia. In this context, Koeijvoets et al. (12) explored the effect of two common *ABCG8* (D19H and T400K) SNPs on lipids and CVD in 2,012 patients with heterozygous FH but did not find significant associations with plasma lipid concentrations. Interestingly, they showed that variants at the *ABCG8* gene may affect cardiovascular risk. In our study, although the prevalence of CVD was not the main purpose, there were no significant differences in CVD across *ABCG5/ABCG8* SNPs. In this context, it is noteworthy that our population is still too young for suffer cardiovascular disease (mean age was 43 years). This is consistent with previously reported findings in other populations with heterozygous familial hypercholesterolemia in which has been demonstrated that age is an important risk factor for CVD (27–29). On the other hand, Miwa et al. (11) reported no significant associations between three *ABCG5/G8* (Q604E, C54Y and T400K) SNPs and serum lipid concentrations in 100 Japanese primary hypercholesterolaemic patients. Consistent with both studies (11,12) we did not find associations between D19H, T400K and C54Y SNPs and plasma lipid concentrations. However, in contrast to Miwa et al. our data showed association between Q604E SNP and VLDL-C and TG concentrations. These differences may be due to small sample size, as well as to differences between both population. Additionally, Santosa et al. (10) examined the association of four SNPs at *ABCG5* (Q604E, i7892, i18429 and M216) and four *ABCG8* (C54Y, D19H, i14222 and T400K) with plasma lipids concentrations in 35 young women with mildly hypercholesterolemia. They found that C54Y and Q604E SNPs were associated with the response of cholesterol metabolism to weight loss. In the current study, the same SNPs were studied in our population but did not reveal such association between those two SNPs and TC concentrations. In contrast, our analyses showed association between the genotypes *ABCG5*_Gln604GluC>G SNP and VLDL-C and TG concentrations. However, it is important to consider the differences in the design of these studies, sample size and subject population included.

These novel associations provide strong evidence in support of the important role of ABCG5/G8 transporters in the regulation of plasma lipid kinetics in humans and resemble the importance of these genes in the last steps of the reverse cholesterol transport (RCT) (14). In our study, the presence of the *ABCG5*_i11836G>A SNP was associated with higher HDL-C. The mechanism underlying the modulation of genetic variants at *ABCG5/G8* genes on HDL-C levels is undefined. However, several studies in mice have demonstrated that liver X-receptor-mediated (LXR) activation of the RCT requires ABCG5/G8 (5–7,30). Therefore, the mechanism through which this particular SNP was associated with HDL-C could be due to up-regulation by LXR that will lead towards an increase of HDL-C concentrations across the activation of the RCT pathway. On the other hand, it has been demonstrated that the effect of *ABCG5/G8* genetic variants on HDL-C concentrations is dependent on *ABCA1* (ATP-binding cassette transporters A1). These findings may help to explain the associations between ABCG5/G8 polymorphisms and HDL-C (31). Additionally, we found another novel association in which carriers of the minor allele at Gln604GluC>G SNP displayed lower VLDL-C and TG concentrations. Recently, it has been demonstrated that the activation of LXR induces hypertriglyceridemia in animals (32–34) and that farnesoid X receptor (FXR)-null

mice lead to hypertriglyceridemia (35). In this context, the association between *ABCG5*_Gln604GluC>G with lower VLDL-C and TG could be explained across their up-regulation by FXR or the inactivation of LXR. These interesting mechanisms could provide novel evidence supporting the hypothesis that *ABCG5/G8* expression is regulated by several transcription factors, such as the LXR and FXR, as it has been demonstrated in mice (4,36).

Another interesting observation from our data was that genetic variation at the *ABCG5/G8* genes modulates the effect of cigarette smoking on plasma lipid concentrations in patients with FH. Interestingly, our data revealed that carriers of the minor G, T, A, G alleles at *ABCG5/G8* (i7892A>G, i18429C>T, i1836G>A, 5U145T>G) SNPs displayed significantly lower HDL-C, higher TC, higher TG and higher VLDL-C respectively, only in the group of smokers volunteers. It is essential to note that carriers of the minor alleles at the aforementioned polymorphisms are related with serum levels of atherogenic lipoproteins only in presence of cigarette smoking. On the other hand, non-smokers carriers of the minor T and G alleles at *ABCG5* (i18429C>T and Gln604GluC>G) SNPs had a protective effect towards significantly lower TG concentrations than homozygous for the major allele. Conversely, this protective effect was not observed in smokers. It is noteworthy that smoking is related with a different effect. In line with these observations, we have previously shown that genetic variation at the *ABCG5/G8* genes modulates the effect of cigarette smoking on plasma HDL-C concentrations (14). In contrast to this study, we found other novel interactions between *ABCG5/G8* genetic variants and smoking status in which other plasma lipids concentrations were modified, not only HDL-C, but the special characteristics of our population makes difficult any comparison to other non-FH populations. In this regard, it is essential to note that of the five significant interactions, four *ABCG5* (i7892A>G, i18429C>T, i1836G>A and Gln604GluC>G) SNPs (table 3) and *ABCG8*_5U145T>G were related with TG concentrations. Consistent with these findings, the haplotype effect appeared to be modulated by smoking habit. Interestingly, smokers carriers of C-T-A-G-T showed significantly higher plasma TG levels. Among nonsmokers, carriers of C-T-G-G-G showed significantly lower plasma TG levels. The mechanism underlying the observed interactions is unknown. However, it is known that cigarette smoking increases plasma TC and TG levels and decreases HDL-C, and several studies have already demonstrated gene-smoking interactions modifying these effects (13). On the other hand, it has been demonstrated in mice that the interaction between FXR- and LXR-mediated stimuli regulate expression of liver *ABCG5/G8* (36). Therefore, from our results we could then hypothesize that smoking decreases the expression of *ABCG5/G8* genes, through an inactivation of the LXR/FXR pathway that will lead towards the detriment of RCT in smokers, resulting in changes in plasma lipids concentrations, especially TG and HDL-C. Obviously, we need to be cautious before extrapolating our conclusions to other population and replication of our findings is essential.

In summary, our study demonstrates that *ABCG5* and *ABCG8* genetic variants modulate plasma lipids concentrations in patients with FH and confirms that the effect is influenced by smoking. Therefore, these results could help to explain the differences in the susceptibility to CHD in FH and support the notion that gene-environmental interactions can affect the clinical phenotype in these patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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1A) *ABCG5*_i11836 (HDL-C; $P=0.023$) 1B) *ABCG5*_Q604E (TG; $P=0.017$) **Figure 1.**

Association of the *ABCG5* SNPs with fasting plasma lipids. Values are mean $\pm$ standard error. P values were adjusted for age, gender, BMI, physical activity, diabetes, HTA, smoking habits, lipid lowering drugs and CVD.

Figure 1A) *ABCG5*_i11836 (HDL-C; $P=0.023$)

Figure 1B) *ABCG5*_Q604E (TG; $P=0.017$)

2A) *ABCG5*_i7892-smoking*(P* for interaction=0.039) **2B) *ABCG5*_i11836-smoking***(P* for interaction=0.030) **2C) *ABCG5*_i18429-smoking***(P* for interaction=0.047) **2D) *ABCG5*_Gln604Glu-smoking***(P* for interaction=0.038) **Figure 2.**

Interaction between *ABCG5* SNPs and smoking on lipid levels. Values are mean $\pm$ standard error. P values were adjusted for age, gender, BMI, physical activity, diabetes, HTA, lipid lowering drugs and CVD

Figure 2A) Interaction *ABCG5*_i7892-smoking for HDL-C

Figure 2B) Interaction *ABCG5*_i11836-smoking for TG

Figure 2C) Interaction *ABCG5*_i18429-smoking for TG

Figure 2D) Interaction *ABCG5*_Gln604Glu-smoking for TG

Table 1Description of *ABCG5/ABCG8* SNPs and probes.

Common name	dbSNP ID	Gene position	Minor allele (Frequency)	Probes or ABI assay-on demand ID
<i>ABCG5_i11836G>A</i>	Rs4289236	intron 5	A (0.19)	C__26135659_10
<i>ABCG5i_18429G>A</i>	Rs4148189	intron 10	A (0.10)	C__30437456_10
<i>ABCG5_i7892T>C</i>	Rs4131229	intron 4	C (0.37)	C__27530462_10
<i>ABCG5_Gln604GluC>G</i>	Rs6720173	exon 13	G (0.18)	VIC: CCTTCACTCAAGGAATT FAM: CTTCACTGAAGGAATT
<i>ABCG8_5U145A>C</i>	Rs3806471	exon 1	C (0.30)	C__26135644_10
<i>ABCG8_Tyr54CysA>G</i>	Rs4148211	exon 2	G (0.32)	VIC: CCTCTACCTGGTAGTTGAG FAM: CTACCTGGCAGTTGAG
<i>ABCG8_Asp19HisG>C</i>	Rs11887534	exon 1	C (0.05)	C__26135643_10
<i>ABCG8_i14222A>G</i>	Rs6709904	intron 6	G (0.10) A	VIC: TTGTTTCCCTACTGAGTCTG FAM: TTTCCCTACCGAGTCTG
<i>ABCG8_Thr400LysC>A</i>	Rs4148217	exon 8	(0.24)	VIC: ATCAGCGTCTTAAACTG FAM: AGCGTCGTAAACTG

SNPs names were designed by their positions relative to the transcription initiation site.

Table 2

Demographic and biochemical characteristics by smoking status.

	Nonsmokers (n=337)	Smokers (n=128)	P
Age (year)	44 ± 0.6	40 ± 1.0	0.005
BMI (Kg/m²)	25.8 ± 0.6	26.6 ± 0.3	0.096
Weight (Kg)	71.4 ± 0.3	71.7 ± 0.5	0.647
Waist circ. (cm)	84.8 ± 0.4	85.4 ± 0.7	0.470
CVD n (%)	54 (14.8)	11 (8.1)	0.031
Systolic blood pressure, mmHg	125 ± 0.8	123 ± 1.4	0.131
Diastolic blood pressure, mmHg	74 ± 0.5	73 ± 0.8	0.086
TC (mg/dL)	266 ± 3.7	279 ± 6.2	0.072
TG	4.51 ± 0.02	4.63 ± 0.03	0.004
LDL-C (mg/dL)	198 ± 3.6	213 ± 6.0	0.033
HDL-C (mg/dL)	47 ± 0.6	42 ± 1.0	<0.001
VLDL-C (mg/dL)	19 ± 0.4	22 ± 0.7	0.001
ApoA-I (mg/dL)	149 ± 1.3	143 ± 2.2	0.033
ApoB (mg/dL)	128 ± 2.1	141 ± 3.5	0.002
On lipid lowering treatment, n (%)	289 (79.6)	87 (64.9)	0.001

All values are mean ± standard error. TG: logarithm of triglycerides

Table 3

Plasma lipid profiles by *ABCG5* SNPs according to smoking status.

NONSMOKERS					SMOKERS		<i>P</i>
	A/A (n=139)	A/G+G/G (n=198)	A/A (n=53)	A/G+G/G (n=75)			
G5_17892							
TC	266.3 ± 5.9	266.4 ± 4.8	266.2 ± 9.5	289.5 ± 8.0	0.112		
TG	4.55 ± 0.03	4.47 ± 0.02	4.56 ± 0.05	4.68 ± 0.04	0.013		
LDL-C	197.4 ± 5.7	198.5 ± 4.7	199.1 ± 9.3	223.9 ± 7.8	0.095		
HDL-C	46.6 ± 1.0	48.6 ± 0.8	44.6 ± 1.6	41.2 ± 1.3	0.030		
VLDL-C	20.1 ± 0.7	18.9 ± 0.5	20.8 ± 1.1	23.6 ± 0.9	0.022		
G5_i18429							
TC	266.9 ± 4.2	264.1 ± 7.8	270.2 ± 7.5	299.5 ± 10.6	0.042		
TG	4.53 ± 0.02	4.41 ± 0.04	4.61 ± 0.04	4.67 ± 0.06	0.047		
LDL-C	198.0 ± 4.1	198.1 ± 7.6	204.6 ± 7.3	232.0 ± 10.3	0.077		
HDL-C	47.9 ± 0.7	47.3 ± 1.3	43.5 ± 1.2	40.8 ± 1.8	0.472		
VLDL-C	19.9 ± 0.5	17.6 ± 0.9	22.4 ± 0.9	22.8 ± 1.3	0.173		
G5_i11836							
TC	264.2 ± 4.8	269.8 ± 6.0	275.1 ± 7.6	289.3 ± 10.4	0.574		
TG	4.53 ± 0.02	4.47 ± 0.03	4.59 ± 0.04	4.70 ± 0.05	0.039		
LDL-C	196.4 ± 4.7	200.7 ± 5.9	209.9 ± 7.4	220.9 ± 10.2	0.646		
HDL-C	46.3 ± 0.8	50.1 ± 1.0	42.9 ± 1.3	42.3 ± 1.7	0.092		
VLDL-C	20.0 ± 0.5	18.4 ± 0.7	21.4 ± 0.9	24.3 ± 1.2	0.014		
G5_Q604E							
TC	268.4 ± 4.3	260.4 ± 7.3	278.2 ± 7.6	283.4 ± 10.5	0.400		
TG	4.55 ± 0.02	4.39 ± 0.04	4.62 ± 0.04	4.65 ± 0.05	0.038		
LDL-C	199.0 ± 4.2	195.1 ± 7.2	211.3 ± 7.4	218.2 ± 10.2	0.477		
HDL-C	48.0 ± 0.7	47.2 ± 1.2	43.3 ± 1.3	41.3 ± 1.8	0.637		

	NONSMOKERS		SMOKERS		<i>P</i>
	A/A (n=139)	A/G+G/G (n=198)	A/A (n=53)	A/G+G/G (n=75)	
G5 i7892					
VLDL-C	20.1 ± 0.5	17.3 ± 0.8	22.8 ± 0.9	22.0 ± 1.2	0.265

All values are mean ± standard error. *P* for interaction. *P* values were adjusted for age, gender, BMI, physical activity, diabetes, HTA, lipid lowering drugs and CVD