RESEARCH ARTICLE

Influence of 6 genetic variants on the efficacy of statins in patients with dyslipidemia

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Background: Patients with dyslipidemia are often treated with statins to reduce lipids and hence cardiovascular risk, but treatment response is variable, partly due to genetic factors.

Methods: We studied the influence of 6 gene variants (APOE c.526C > T (APOE2), APOE c.388T > C (APOE4), SLCO1B1 c.521T > C, CYP3A4 c.-392G > A, HMGCR c.1564-106A > G, and LPA c.3947 + 467T > C) on statin efficacy assessing 2 indicators: the percent reduction in total cholesterol (TC) and non-HDL cholesterol (non-HDL), as well as the achievement of therapeutic goals. The study was performed in a group of patients (n = 100) without previous pharmacological treatment. Multiple regression models were used to calculate the percentage of explanation in response variability added by every variant to a basal model constructed with significant non-genetic control variables.

Results: The most influential variant was HMGCR c.1564-106A > G (rs3846662), and carriers showed a significantly lower reduction in TC and non-HDL. This variant is related to an alternative splicing involving exon 13, which is also regulated by lipid concentrations in patients without the variant. Concerning therapeutic goals, HMGCR c.1564-106A > G hindered the achievement of TC targets on patients.

Conclusions: The HMGCR c.1564-106A > G variant was associated with less statin efficacy to decrease cholesterol.

KEYWORDS

dyslipidemia, genetic variants, non-HDL cholesterol, statins, total cholesterol

1 | INTRODUCTION

Cardiovascular disease is a leading cause of death in the world population. High serum total cholesterol (TC) and LDL cholesterol (LDL-c) are 2 of the most important modifiable risk factors for cardiovascular disease, and there is strong evidence that lowering TC and LDL-c prevents atherothrombotic cardiovascular events.¹⁻⁷

Changes in life habits such as giving up smoking, reducing alcohol intake, or practicing more physical exercise are usually recommended to reduce cholesterol or triglycerides in patients with dyslipidemia. However, these changes are often not enough to reach desirable lipid concentrations, and a pharmacological intervention is required.

Currently, over 100 million people worldwide are treated with statins to reduce cholesterol and hence cardiovascular risk.⁸ There are different types of statins, but all of them lower cholesterol concentrations by the same molecular mechanism: They inhibit the key enzyme of the endogenous cholesterol synthesis pathway, 3-hydroxy-3-methylglutaryl-CoA reductase. Due to enzyme inhibition, intracellular cholesterol concentration decreases and consequently, cells express a higher number of LDL cholesterol receptors in their membrane to internalize circulating LDL. Finally, LDL circulating concentrations are reduced, with beneficial consequences on cardiovascular risk.

However, the effects of statins are not homogenous among patients, not even in those treated with the same statin at the same

dose. Moreover, some patients develop secondary effects such as myalgia or myopathy, but others do not. These 2 aspects suggest that there could be a genetic influence on the efficacy of statin treatment. Thus, we aimed to investigate it through the study of some gene variants, all of them related to lipid metabolism or to pharmacokinetics-pharmacodynamics of statins. The 5 genes and 6 variants were as follows: *APOE* (rs7412 and rs429358), *HMGCR* (rs3846662), *SLCO1B1* (rs4149056), *CYP3A4* (rs2740574), and *LPA* (rs10455872).

APOE variants (*APOE2* c.526C > T, rs7412, and *APOE4* c.388T > C, rs429358) have already been related to lipid concentration and cardiovascular risk⁹⁻¹²; besides, apoE has a paramount implication in lipoprotein metabolism. The chosen *HMGCR* variant (c.1564-106A > G) is related to a functional polymorphism that produces an RNA without exon 13, resulting in an enzyme with an inactive catalytic site, and a likely loss of sensibility for statin binding.^{13,14} *SLCO1B1* codes for a transport protein essential for the entry of statins to the hepatocytes. The variant c.521T > C produces a loss of activity of the protein and it is known to be related to the development of secondary effects of statin treatment.¹⁵⁻¹⁷ *CYP3A4* codes for an isoform of P450 cytochrome, and it is involved in the initial metabolism of statins, reducing their bioavailability. The variant (c.-392G > A) inhibits the binding of a repressor to the promoter, so there is an over-expression of the protein and a greater degradation of the drug.¹⁸ *LPA* codes for lipoprotein (a), presently considered as an emerging risk factor because it is an atherogenic protein related to low-density lipoprotein. Some authors described an association between the variant (*LPA* c.3947 + 467T > C) and the response to statins.¹⁹⁻²²

2 | MATERIALS AND METHODS

2.1 | Study population

All patients were attended in the vascular risk unit (VRU) of the Bellvitge University Hospital between 1994 and 2012. Exclusion criteria for this study were as follows: initial serum TC concentration (TCi) lower than 5.2 mmol/L or higher than 13 mmol/L, second visit after more than 1-year, concomitant treatment with interfering drugs and other hypolipemiant drugs. Final population consisted of 100 patients; none had been treated before starting the study.

Patients received different statins at different doses, and conversion tables were employed to standardize all statin doses to simvastatin (<https://www.fda.gov/Drugs/DrugSafety/ucm256581.htm>).

2.2 | Genotyping and biochemical analysis

DNA was extracted from peripheral blood with the High Pure™ PCR Template Preparation Kit (Roche, Germany). For *CYP3A4* analysis, DNA was amplified by PCR employing the primers 5'-GGACAGCCATAGAGACAAGTCA-3' and 5'-CTTTCCTGCCCTGCACAG-3' followed by RFLP (restriction

fragment length polymorphism), with Pst I restriction enzyme. *SLCO1B1*, *HMGCR*, and *LPA* variants were analyzed by real-time PCR using TaqMan allelic discrimination assays (Life Technologies, CA, USA). In the case of *APOE* variants, some samples were analyzed by PCR-RFLP,²³ and then the technology was replaced by real-time PCR with TaqMan assays, after checking the interchangeability of methods.

Serum total cholesterol (TC) and cholesterol from HDL (HDL) were measured in a Cobas 711 analyzer (Roche Diagnostics), and serum cholesterol excluded from HDL (non-HDL) was calculated (non-HDL = TC - HDL).

2.3 | Treatment efficacy indicators

Two aspects were studied as follows: first, the percent change of TC and non-HDL cholesterol between the first and the second visit to VRU (%C = [(concentration visit 2 - concentration visit 1)/concentration visit 1] × 100), considering that a negative number meant a lipid reduction; second, the achievement of therapeutic goals, defined as reaching serum TC concentrations ≤5.2 mmol/L in the last visit to the VRU, and 4.2 mmol/L for non-HDL.

2.4 | Control variables

Every patient was surveyed in each visit to the hospital and the data recorded were as follows: body mass index (BMI), smoking pattern (present smoker, nonsmoker, and former smoker), physical exercise (hours/wk), alcohol intake (g/d), diabetes (yes/no) (DM), hypertension (yes/no) (HT), and previous ischemic event (yes/no) (I). Date and treatment changes were also registered. With this information, the control variables constructed were as follows: percent change in BMI (Δ BMI), total change in alcohol intake (Δ A), exercise practice (Δ E) and smoking habits (Δ S), and mean daily statin dose (D). Sex, age, DM, I and HT were also considered as control variables.

These variables have been related to circulating lipid concentrations and cardiovascular risk. The influence of the genetic variants on statin efficacy was evaluated independently and together with these variables.

Before introducing nongenetic variables in the model, a correlation study was carried out to evaluate the relationship between them. In case of high correlation between 2 variables, only one of them (the best related to lipid change) was included in the model.

2.5 | Statistics

The influence of the genetic variants on statin efficacy was assessed by constructing several regression models in several steps:

1. Linear regressions for quantitative variables and comparisons of means for qualitative variables were firstly used to assess the influence of each control variable on the indicator. Variables showing significant influence ($P < .05$) were selected, and a multiple regression model (basal model) was constructed with

TABLE 1 Patient initial data

Variable (units)	Median (range)	Proportions (%)
Age (y)	45.8 (43.6; 48.0) ^a	
BMI (kg/m ²)	27.9 (19.8; 37.0)	
Physical exercise practice (h/wk)	2 (0; 14)	
Alcohol intake (g/d)	0 (0; 130)	
Srm—Cholesterol; c.sust (mmol/L)	6.9 (5.3; 11.8)	
Srm—Cholesterol non-HDL; c.sust (mmol/L)	5.6 (3.4; 10.2)	
Sex (%F/%M)		33/67
Smoking pattern (% smokers/%nonsmokers/% former smokers/% ND)		34/22/25/19
DM (yes/no/ND)		13/81/6
HT (yes/no/ND)		19/75/6
I (yes/no/ND)		16/68/16

BMI, body mass index; DM, diabetes mellitus; F, female; HT, hypertension; I, previous ischemic event; M, male; ND, no data available.

^aMean and confidence interval 95% range: minimum and maximum value.

them. The R^2 of this model represents the percentage of the change in the indicator that is explained by the variables.

- The influence of individual genetic variants was independently studied comparing the value of the indicator between carriers and noncarriers of each SNP employing a X^2 test. Those showing the most significant influence were selected.
- The selected genetic variants were individually included into the basal model, creating the expanded model, and a new R^2 was determined. The difference between both R^2 represented the additional explanation of changes in the indicator that could be attributed to the genetic variant.

For qualitative indicators (therapeutic goals achievement), the model was similar, but logistic regressions and proportion comparison tests were employed. The significance level was raised from .05 to .1 because none of the variants reached the .05 level, but there were 2 well-defined groups: One of them included variants with an associated near-significance P around .1, while the rest had a much higher P value.

3 | RESULTS

The correlation study only showed a moderate correlation between the variables DM and I ($\rho = .570$ and $P = .001$). The variable I also presented low correlation with another 2 variables: D ($\rho = .294$ and $P = .007$) and ΔS ($\rho = .268$ and $P = .034$). In consequence, I was not included in the models.

Table 1 shows the initial data of the patients. Mean TC concentration was, by inclusion criteria, higher than 5.2 mmol/L, and mean non-HDL was higher than the discriminant value (4.2 mmol/L), as expected for patients who require lipid-lowering treatment. Most patients (67%) were men, as it could be expected according to the relationship between dyslipidemia and gender. About one-third of the patients were active smokers, and only few patients were diabetic

or hypertensive or had suffered an ischemic event before the study. In a minority of cases (between 6% and 19%), there were no data available for these variables.

To build up regression models based on nongenetic control variables with a significant influence on the percent reduction in TC and non-HDL cholesterol, individual regressions were calculated for each variable. Table 2 shows the R^2 and P value obtained in the regression studies for quantitative variables, and the P value obtained in comparison tests for qualitative variables. The variables finally selected for constructing the TC and non-HDL basal models are in bold. TC model presented an R^2 of 27.1% and non-HDL cholesterol model an R^2 of 22.2% (Table 4).

On the other hand, a X^2 test was developed to compare the indicators between carriers and noncarriers of the gene variants. The results are shown in Table 3. Only 1 gene variant was selected to study its possible influence on statin efficacy, the HMGR c.1564-106A > G variant (bold in Table 3), because it had a clearly lower and almost significant P value. The variant showed a harmful effect: Carriers showed lower reductions of TC and non-HDL with statin therapy.

Therefore, the expanded model was constructed with the selected control variables and the HMGR c.1564-106A > G variant. Table 4 shows the R^2 of the basal and the expanded models for TC and non-HDL as well as all the variables included and the results of the multiple regression tests. The R^2 of the TC expanded model was 36.6%, meaning that the genetic variant added a 9.5% of the explanation. For non-HDL, the expanded model explained a 30.4% of the variation, so the variant added an 8.2%.

Concerning to therapeutic goals achievement, as shown in Table 5, only 1 control variable could be selected for each basal model: the initial concentration of total cholesterol (TCi; $P = .005$) and of non-HDL cholesterol (non-HDLi), respectively, in the last case because it was also the only control variable whose significant level was lower than .1 ($P = .094$). However, no basal model was

Variables (units)	TC			non-HDL		
	B (IC 95%)	R ²	P	B (IC 95%)	R ²	P
Age (y)	0.105 (-0.201; 0.411)	0.005	.497	-0.072 (-0.485; 0.340)	0.001	.728
Statin daily dose (mg)	-0.243 (-0.338; -0.148)	0.209	<.001	-0.273 (-0.398; -0.149)	0.175	<.001
ΔBMI (kg/m ²)	0.030 (-0.175; 0.235)	0.003	.766	-0.020 (-0.324; 0.283)	0.001	.893
ΔE (h/wk)	-1.477 (-2.653; -0.302)	0.094	.015	-1.467 (-3.018; 0.084)	0.059	.063
ΔA (g/d)	-0.248 (-0.770; 0.274)	0.015	.346	-0.127 (-0.815; 0.560)	0.002	.712
Sex (M/F)			.526			.497
DM (yes/no)			.770			.871
HT (yes/no)			.519			.794

ΔA, changes in alcohol intake; B (IC 95%), coefficient B with confidence interval 95%; DM, diabetes mellitus; ΔE, changes in physical exercise practice; F, female; HT, hypertension; ΔIMC, percentage change in body mass index; M, male; non-HDL, non-HDL cholesterol; P, associated probability; R², coefficient of determination; TC, total cholesterol.

constructed, as there was no sense in constructing it with only 1 variable.

In the case of the TC model, the variants HMGCR c.1564-106A > G and APOE2 c.526C > T were selected in the initial phase of comparisons of proportions (Table 6) but when they were included with TCi in a multiple regression to build the expanded model, the only 1 reaching the predefined significance level was HMGCR c.1564-106A > G ($P = .034$). Carriers reached the TC goal to a lesser extent (41%) than noncarriers (60%), so these data were in agreement with those obtained for quantitative indicators. Even though, it is worth remarking that in case of confirmation of the results, the presence of the variant APOE2 c.526C > T seemed to be beneficial to TC objective achievement (75% carriers reached the TC objective vs 43% noncarriers). Table 7 shows the expanded model. The variant added a 4.6% of explanation to the model constructed only with TCi.

Regarding non-HDL, none of the gene variants reached the predefined significance level (Table 6), so the expanded model was not constructed. HMGCR c.1564-106A > G and APOE2 c.526C > T, while not reaching significance level ($P = .223$ and $P = .274$, respectively), showed the same trend for non-HDL and for CT: HMGCR variant seemed to be harmful and APOE variant beneficial.

4 | DISCUSSION

Statins are the most widely employed drugs for cholesterol lowering. Different studies have quantified the efficacy of statins by

TABLE 2 Influence of the control variables on the percent reduction in TC and non-HDL

measuring the reduction in LDL cholesterol, which varies between 25% and 50%.^{22,24-26} This cholesterol-lowering effect has shown relatively large interindividual differences that have been related with different patient's conditions, treatment adherence, and genetic factors.

The efficacy of statin treatment is generally evaluated by LDL-c and TC reductions, but in this study also, the non-HDL cholesterol reduction was considered. Non-HDL cholesterol includes the cholesterol of all the pro-atherogenic Apo B100-containing lipoproteins, and is feasible in those cases where LDL-c could not be estimated because of high triglyceride concentration.

Some other studies have already evaluated non-HDL cholesterol concentrations in patients treated with statins, demonstrating the high relationship between its concentration and cardiovascular diseases.²⁷ Also, as the National Lipid Association guideline proposed non-HDL goals both for patients at very high cardiovascular risk and high cardiovascular risk, some recent studies employ the percent reduction in non-HDL to evaluate treatment efficacy.²⁸ This US association considers non-HDL concentration as a primary therapy goal, although European guides still consider it as a secondary target.^{28,29}

In our study, none of the patients had been treated with lipid-lowering drugs before the basal sample was taken and all of them presented initial cholesterol concentrations between 5.2 and 13 mmol/L. We observed mean reductions of 25% and 31% in TC and non-HDL cholesterol, respectively. The higher absolute reductions of non-HDL cholesterol in comparison with those of TC can be explained because statin effects include a decrease in LDL

TABLE 3 Comparison in percent reductions of TC and non-HDL between carriers and noncarriers of the 6 gene variants

Gene variants	Carriers (n) Noncarriers (n) P value	%C TC	%C non-HDL
APOE2 c.526C > T	NC (CC, n = 95)	-23.8 (-63.6; 19.3)	-28.6 (-70.5; 27.3)
	C (n = 5)	-24.5 (-40.5; -17.6)	-28.6 (-37.3; 23.4)
	P	.524	.554
APOE4 c.388T > C	NC (TT, n = 77)	23.8 (-27.5; -20.2)	-28.7 (-33.5; -24.0) [†]
	C (n = 23)	-21.1 (-27.8; -14.3)	-27.3 (-35.7; -18.8) [†]
	P	.585	.955
SLCO1B1 c.521T > C	NC (TT, n = 68)	-23.2 (-27.1; -19.4)	-28.0 (-32.9; -23.1) [†]
	C (n = 32)	-23.1 (-28.6; -17.6)	-29.2 (-36.6; -21.8) [†]
	P	.914	.845
CYP3A4 c.-392G > A	NC (CC, n = 67)	-23.6 (-27.6; -19.7)	-29.3 (-34.4; -24.2) [†]
	C (n = 2)	-44.8 and -20.7	-53.4 and -29.0
	P	.512	.397
HMGCR c.1564-106A > G	NC (AA, n = 27)	-28.3 (-33.5; -23.1)	-34.9 (-41.7; -28.1) [†]
	C (n = 73)	-21.6 (-25.5; -17.7)	-26.4 (-31.5; -21.3) [†]
	P	.098	.097
LPA c.3947 + 467T > C	NC (TT, n = 77)	-23.1 (-27.0; -19.2)	-27.3 (-32.4; -22.3) [†]
	C (n = 23)	-20.9 (-26.9; -15.0)	-29.9 (-37.9; -22.0) [†]
	P	.752	.380

[†]cases normally distributed.

C, carriers; %C, percentage change; NC, noncarriers; non-HDL, non-HDL cholesterol; P, associated probability; TC, total cholesterol.

cholesterol but also an increase in HDL cholesterol, and HDL is included within CT, but not within non-HDL cholesterol.

To explain the observed differences in statin response among patients, we selected several plausible nongenetic variables, and genetic variants with a likely influence on relevant features of genes involved in statins and lipid metabolism. Two of the genes (*SLCO1B1* and *CYP3A4*) code for proteins essential for statins pharmacokinetics, and *HMGCR* is crucial for statins pharmacodynamics. *APOE* is

tightly involved in cholesterol metabolism, and *LPA* was selected because recent studies have demonstrated the association between one of its variants and LDL cholesterol response to atorvastatin.³⁰

Three of our chosen variants (those in *SLCO1B1*, *CYP3A4*, and *LPA*) did not explain any difference in statin response. The *SLCO1B1* c.521T > C variant is well related to the risk of side effects of statin treatment, but conflicting results have been reported about its influence on the lipid-lowering effects of statins. In some works, the C

TABLE 4 Basal and expanded model for the percent reduction in TC and non-HDL

Variables (units)	TC				non-HDL			
	Basal model ($R^2 = 0.271$)		Expanded model ($R^2 = 0.366$)		Basal model ($R^2 = 0.222$)		Expanded model ($R^2 = 0.304$)	
	B (IC 95%)	P	B (IC 95%)	P	B (IC 95%)	P	B (IC 95%)	P
Constant	-14.306 (-19.552; -9.059)	<.001	-23.766 (-31.490; -16.041)	<.001	-17.191 (-24.309; -10.072)	<.001	-28.518 (-39.083; -17.954)	<.001
D (mg)	-0.238 (-0.353; -0.122)	<.001	-0.232 (-0.339; -0.124)	<.001	-0.286 (-0.439; -0.133)	<.001	-0.283 (-0.428; -0.138)	.001
ΔE (h/wk)	-1.416 (-2.453; -0.380)	.008	-1.656 (-2.635; -0.677)	.001	-1.404 (-2.804; -0.003)	.050	-1.666 (4.249; 26.009)	.016
HMGCR c.1564-106A > G (AA vs AG + GG)			12.511 (4.606; 20.416)	.002			15.129 (4.249; 26.009)	.007

B (IC 95%), coefficient B with confidence interval 95%; D, statin daily dose; ΔE, changes in physical exercise practice; non-HDL, non-HDL cholesterol; P, associated probability; R^2 , coefficient of determination; TC, total cholesterol.

Variables (units)	TC			non-HDL		
	OR (IC 95%)	R ²	P	OR (IC 95%)	R ²	P
Age (y)	1.013 (0.975; 1.052)	0.05	.515	1.024 (0.984; 1.066)	0.016	.239
TCi/ non-HDLi (mmol/L)	0.582 (0.399; 0.848)	0.091	.005	0.735 (0.513; 1.053)	0.032	.094
D (mg)	1.009 (0.995; 1.023)	0.016	.217	1.009 (0.994; 1.024)	0.015	.255
ΔIMC (kg/m ²)	0.886 (0.704; 1.114)	0.152	.299	0.942 (0.821; 1.080)	0.142	.391
ΔE (h/wk)	0.932 (0.801; 1.084)	0.014	.361	0.924 (0.791; 1.079)	0.017	.319
ΔA (g/d)	1.039 (0.964; 1.121)	0.019	.315	1.039 (0.965; 1.118)	0.019	.312
Sex (M/F)	0.555 (0.236; 1.305)		.175	0.796 (0.338; 1.875)		.602
DM (yes/no)	2.314 (0.712; 7.525)		.155	1.628 (0.452; 5.858)		.453
HT (yes/no)	1.029 (0.375; 2.891)		.956	0.714 (0.253; 2.013)		.523

ΔA, changes in alcohol intake; D, statin daily dose; DM, diabetes mellitus; ΔE, changes in physical exercise practice; F, female; HT, arterial hypertension; ΔIMC, percentage change in body mass index; M, male; non-HDL, non-HDL cholesterol; non-HDLi, initial non-HDL concentration; OR, odds ratio with confidence interval 95%; P, associated probability; R², coefficient of determination; TC, total cholesterol; TCi, initial total cholesterol concentration.

allele was associated with lower reductions of non-HDL cholesterol, but in others, this effect was not observed,³¹⁻³³ as is the case in our study. CYP3A4 variant causes a reduction in statins bioavailability, and some authors have related it to lower reductions of LDL cholesterol after statin treatment,³⁴ although we could not find it relevant. LPA codes for lipoprotein (a), which is presently considered

TABLE 6 Comparison in therapeutic goals achievement between carriers and noncarriers of the 6 gene variants

Gene variants	TC		non-HDL	
	OR (IC 95%)	P	OR (IC 95%)	P
APOE2 c.526C > T	5.171 (0.557; 48.034)	.111	3.265 (0.351; 30.390)	.274
APOE4 c.388T > C	1.421 (0.558; 3.623)	.460	1.041 (0.391; 2.773)	.936
SLCO1B1 c.521T > C	0.726 (0.310; 1.699)	.459	0.990 (0.413; 2.370)	.981
CYP3A4 c.-392G > A	1.161 (0.070; 19.349)	.917	0.800 (0.048; 13.368)	.876
HMGCR c.1564-106A > G	0.480 (0.195; 1.178)	.106	0.563 (0.222; 1.427)	.223
LPA c.3947 + 467T > C	1.380 (0.542; 3.510)	.498	1.444 (0.540; 3.860)	.463

CT, total cholesterol; non-HDL, non-HDL cholesterol; OR, odds ratio with confidence interval 95%; P, associated probability.

TABLE 5 Influence of the control variables on the therapeutic goals achievement

as a nonconventional risk factor. Some authors described an association between the variant (LPA c.3947 + 467T > C) and response to statins.⁴⁻⁷ A recent GWAS published by Postmus et al,³⁵ which included data from 40 000 subjects treated with different statins, showed the association of this genetic variant with LDL cholesterol reductions. Our data did not confirm this association, maybe due to the small number of participants (n = 100).

Among the gene variants included in our study, that in HMGCR (c.1564-106A > G, rs3846662) appeared to have a greater influence on TC and non-HDL cholesterol decrease with a harmful effect. The presence of the variant drove to a smaller reduction in TC (-28.3% vs -21.6%) and in non-HDL (-34.9% vs -26.4%). This fact has a translation into the efficacy of the treatment, as our data show that it seems easier to reach the therapeutic goal for rs3846662 variant noncarriers.

There is no consensus about the influence of rs3846662 on statin response in the literature. While some studies have recently related this variant to the presence of hypercholesterolemia, but not with statin efficacy,³⁶ others have related it to statin efficacy only in

TABLE 7 Expanded model for TC therapeutic goals achievement

Variables (units)	OR (IC 95%)	P
TCi (mmol/L)	0.539 (0.356; 0.815)	.003
HMGCR c.1564-106A > G	0.321 (0.113; 0.916)	.034

OR, odds ratio with confidence interval 95%; P, associated probability; TCi, initial total cholesterol concentration.

women³⁷ or in general population.³⁸ Some of these works have proposed a way to explain the influence. *HMGCR* gene codes for the key enzyme of the endogenous synthesis of cholesterol, and rs3846662 variant is related to the production of an alternatively spliced RNA without exon 13.³⁸ The corresponding defective protein has an altered catalytic site making the binding to statins more difficult³⁸ and hindering these drugs effect on lipid lowering. Exon 13 skipping impairs enzymatic activity.³⁹ Some studies have demonstrated that all cells have both the complete protein (*HMGCR*+) and the defective 1 (*HMGCR*-) in different proportions, and people/cells without rs3846662 variant present a higher (*HMGCR*-) proportion.^{14,40} Besides, there is an extra regulation of these proportions in noncarriers (AA genotype): When circulating lipids decrease, the fraction of (*HMGCR*+) increases and that of (*HMGCR*-) decreases. This regulation has not been observed in carriers (AG and GG genotypes).⁴⁰

The other variant which showed some influence on achieving goals was *APOE2* c.526C > T, but in the expanded model, it did not reach the defined significance level. Given that the minor allele, T, has a very low frequency, it is possible that with a higher population the effect could have been demonstrated. Anyway, homozygosis for this variant has been related to disbetalipoproteinaemia, although some authors have also related the presence of 1 allele to lower LDL concentrations, as well as to lower risk of cardiovascular disease.^{41,42} Our results agree with some published data like those of Mega et al,⁴³ which showed higher efficacy of statin treatment for carriers of *APOE2* c.526C > T variant. Moreover, Postmus et al³⁵ also demonstrated the relationship between *APOE* loci and statin LDL cholesterol reductions.

It is very important to remark that statin efficacy is not fully clarified by the presence or absence of genetic polymorphisms. In fact, all the studied SNPs in published data show relative low explanation of the different efficacy of statins between patients. Some other aspects such as epigenetic modifications, intra-individual or environmental factors, or biochemical pathways should be considered in future studies.

In conclusion, our results show a modest relation between the presence of the *HMGCR* rs3846662 variant and a worse response to statins, and are consistent with recently published proposals of molecular ways to explain it. However, it would be advisable to confirm our results in a larger population.

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REFERENCES

1. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III) final report. *Circulation*. 2002;106:31-43.
2. Banerjee A. A review of family history of cardiovascular disease: risk factor and research tool. *Int J Clin Pract*. 2012;66:536-543.
3. Wierzbicki SA. New directions in cardiovascular risk assessment: the role of secondary risk stratification markers. *Int J Clin Pract*. 2012;66:622-630.
4. Kannel WB, McGee DL. Diabetes and cardiovascular disease: the Framingham study. *JAMA*. 1979;241:2035-2038.
5. Stamler J, Wentworth D, Neaton JD. Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? Findings in 356,222 primary screens of the multiple risk factor intervention trial (MRFIT). *JAMA*. 1986;256:2823-2828.
6. Lipid Research Clinics Program. The lipid research clinics coronary primary prevention trial results. I: reduction in the incidence of coronary heart disease. *JAMA*. 1984;251:351-364.
7. Lipid Research Clinics Program. The lipid research clinics coronary primary prevention trial results. II: the relationship of reduction in incidence of coronary heart disease to cholesterol lowering. *JAMA*. 1984;251:365-374.
8. Simons J. The \$10 billion pill. *Fortune*. 2003;147:58-62.
9. Getz GR, Reardon CA. Apolipoprotein E as a lipid transport and signaling protein in the blood, liver, and artery wall. *J Lipid Res*. 2009;50(Suppl):S158-S161.
10. Li H, Dhanasekaran P, Alexander ET, Rader DJ, Phillips MC, Lund-Katz S. Molecular mechanisms responsible for the differential effects of apoE3 and apoE4 on plasma lipoprotein cholesterol levels. *Arterioscler Thromb Vasc Biol*. 2013;33:687-693.
11. Khan TA, Shah T, Prieto D, et al. Apolipoprotein E genotype, cardiovascular biomarkers and risk of stroke: systematic review and meta-analysis of 14,015 stroke cases and pooled analysis of primary biomarker data from up to 60,883 individuals. *Int J Epidemiol*. 2013;42:475-492.
12. Bennet AM, Di Angelantonio E, Ye Z, et al. Association of apolipoprotein E genotypes with lipid levels and coronary risk. *JAMA*. 2007;298:1300-1311.
13. Medina MW. The relationship between *HMGCR* genetic variation, alternative splicing, and statin efficacy. *Discov Med*. 2010;9:495-499.
14. Burkhardt R, Kenny EE, Lowe JK, et al. Common SNPs in *HMGCR* in micronesians and whites associated with LDL-cholesterol levels affect alternative splicing of exon13. *Arterioscler Thromb Vasc Biol*. 2008;28:2078-2084.
15. Yoshida K, Takano J, Ishizu Y, et al. Sugiyama Y direct and rapid genotyping of *SLCO1B1* 388A>G and 521T>C in human blood specimens using the SmartAmp-2 method. *AAPS J*. 2013;15:618-622.
16. Maeda K, Sugiyama Y. Impact of genetic polymorphisms of transporters on the pharmacokinetic, pharmacodynamic and toxicological properties of anionic drugs. *Drug Metab Pharmacokinet*. 2008;23:223-235.
17. Niemi M, Pasanen MK, Neuvonen PJ. Organic anion transporting polypeptide 1B1: a genetically polymorphic transporter of major importance for hepatic drug uptake. *Pharmacol Rev*. 2011;63:157-181.
18. Becker ML, Visser LE, van SR, Hofman A, Uitterlinden AG, Stricker BH. Influence of genetic variation in *CYP3A4* and *ABCB1* on dose decrease or switching during simvastatin and atorvastatin therapy. *Pharmacoepidemiol Drug Saf*. 2010;19:75-78.
19. Chasman DI, Giulianini F, MacFadyen J, Barratt BJ, Nyberg F, Ridker PM. Genetic determinants of statin low-density lipoprotein cholesterol reduction: the Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) trial. *Circ Cardiovasc Genet*. 2012;5:257-264.
20. Colhoun HM, Betteridge DJ, Durrington PN, et al. Primary prevention of cardiovascular disease with atorvastatin in type 2 diabetes in the Collaborative Atorvastatin Diabetes Study (CARDS): multicentre randomised placebo-controlled trial. *Lancet*. 2004;364:685-696.

21. Sever PS, Dahlöf B, Poulter NR, et al. Prevention of coronary and stroke events with atorvastatin in hypertensive patients who have average of lower than average cholesterol concentrations, in the Anglo-Scandinavian Cardiac Outcomes Trial Lipid Lowering Arm (ASCOT-LLA): a multicenter randomized controlled trial. *Drugs*. 2004;64(Suppl 2):43-60.
22. Sheperd J, Blauw GJ, Murphy MB, et al. Pravastatin in elderly at risk of vascular disease (PROSPER): a randomised controlled trial. *Lancet*. 2002;360:1623-1630.
23. Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. *J Lipid Res*. 1990;31:545-548.
24. Downs JR, Clearfield M, Weis S, et al. Primary prevention of acute coronary events with lovastatin in men and women with average cholesterol levels: results of AFCAPS/TexCAPS. Air Force/Texas Coronary Atherosclerosis Prevention Study. *JAMA*. 1998;279:1615-1622.
25. Shepherd J, Cobbe SM, Ford I, et al. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. West of Scotland Coronary Prevention Study Group. *N Engl J Med*. 1995;333:1301-1307.
26. Ridker PM, Danielson E, Fonseca FA, et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med*. 2008;359:2195-2207.
27. Boekholdt SM, Arsenault BJ, Mora S, et al. Association of LDL cholesterol, non-HDL cholesterol, and apolipoprotein B levels with risk of cardiovascular events among patients treated with statins: a meta-analysis. *JAMA*. 2012;307:1302-1309.
28. Bays HE, Jones PH, Orringer CE, Brown WV, Jacobson TA. National lipid association annual summary of clinical lipidology 2016. *J Clin Lipidol*. 2016;10:S1-S43.
29. Catapano AL, Graham I, De Backer G, et al. 2016 ESC/EAS guidelines for the management of dyslipidaemias. *Eur Heart J*. 2016;37:2999-3058.
30. Deshmukh HA, Colhoun HM, Johnson T, et al. Genome-wide association study of genetic determinants of LDL-c response to atorvastatin therapy: importance of Lp(a). *J Lipid Res*. 2012;53:1000-1011.
31. Donnelly LA, Doney AS, Tavendale R, et al. Common nonsynonymous substitutions in SLCO1B1 predispose to statin intolerance in routinely treated individuals with type 2 diabetes: a go-DARTS study. *Clin Pharmacol Ther*. 2011;89:210-216.
32. Zhang W, Chen BL, Ozdemir V, et al. SLCO1B1 521T->C functional genetic polymorphism and lipid-lowering efficacy of multiple-dose pravastatin in Chinese coronary heart disease patients. *Br J Clin Pharmacol*. 2007;64:346-352.
33. Martin NG, Li KW, Murray H, Putt W, Packard CJ, Humphries SE. The effects of a single nucleotide polymorphism in SLCO1B1 on the pharmacodynamics of pravastatin. *Br J Clin Pharmacol*. 2012;73:303-306.
34. Kajinami K, Brousseau ME, Ordovas JM, Schaefer EJ. CYP3A4 genotypes and plasma lipoprotein levels before and after treatment with atorvastatin in primary hypercholesterolemia. *Am J Cardiol*. 2004;93:104-107.
35. Postmus I, Trompet S, Deshmukh HA, et al. Pharmacogenetic meta-analysis of genome-wide association studies of LDL cholesterol response to statins. *Nat Commun*. 2014;28:5068.
36. Angelini S, Rosticci M, Massimo G, et al. Relationship between lipid phenotypes, overweight, lipid lowering drug response and kif6 and hmg-coa genotypes in a subset of the Brisighella heart study population. *Int J Mol Sci*. 2017;19:pii: E49.
37. Leduc V, Bourque L, Poirier J, Dufour R. Role of rs3846662 and HMGCR alternative splicing in statin efficacy and baseline lipid levels in familial hypercholesterolemia. *Pharmacogenet Genomics*. 2016;26:1-11.
38. Medina MW, Gao F, Ruan W, Rotter JI, Krauss RM. Alternative splicing of 3-hydroxy-3-methylglutaryl coenzyme A reductase is associated with plasma low-density lipoprotein cholesterol response to simvastatin. *Circulation*. 2008;118:355-362.
39. Medina MW, Gao F, Naidoo D, et al. Coordinately regulated alternative splicing of genes involved in cholesterol biosynthesis and uptake. *PLoS One*. 2011;6:e19420.
40. Medina MW, Gao F, Naidoo D, et al. Coordinately regulated alternative splicing of genes involved in cholesterol biosynthesis and uptake. *PLoS One*. 2011;29:e19420.
41. Gofman JW. Diet and lipotropic agents in atherosclerosis. *Bull N Y Acad Med*. 1952;28:279-329.
42. <http://www.ncbi.nlm.nih.gov/SNPandGENE/database>
43. Mega JL, Morrow DA, Brown A, Cannon CP, Sabatine MS. Identification of genetic variants associated with response to statin therapy. *Arterioscler Thromb Vasc Biol*. 2009;29:1310-1315.

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