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*Original Article*

# Utility of genetic variants to predict prognosis in coronary artery disease patients receiving statin treatment

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**Abstract:** Statins are widely used drugs for lowering low-density lipoprotein cholesterol (LDL-C) and can prevent cardiovascular events. This study aimed to evaluate the influence of single nucleotide polymorphisms (SNPs) and their cumulative effects on the prognosis of coronary artery disease (CAD) patients treated with statins. Sixteen SNPs were genotyped in 785 CAD patients receiving statin therapy, and their associations with clinical features and prognosis of patients were investigated. Four SNPs (rs2296651, rs11206510, rs8192870, and rs1801133) were significantly associated with complications of CAD ($P$<0.05). Four SNPs (rs8192870, rs4149056, rs12916, and rs2231142) affected blood lipid levels ($P$<0.05). Furthermore, rs1801133 showed a weak but significant association with fasting plasma glucose ($P$ = 0.033). Survival analyses showed that rs11206510 (adjusted HR = 1.891, 95% CI: 1.188-3.010, $P$ = 0.007) and rs1801133 (adjusted HR = 1.499, 95% CI: 1.141-1.971, $P$ = 0.004) were independently associated with an increased risk of major cardiovascular events, and exhibited cumulative effect on even-free survival (adjusted HR = 1.810, 95% CI: 1.179-2.802, $P$ = 0.007). In conclusion, rs11206510 and rs1801133 were independent risk factors for clinical outcome in CAD patients treated with statins.

**Keywords:** Statin, coronary artery disease, prognosis, major cardiovascular event

## Introduction

Coronary artery disease (CAD) is the leading cause of death worldwide [1]. Elevated low-density lipoprotein cholesterol (LDL-C) is the most common cause of CAD. Effective reduction of LDL-C can prevent the progress of atherosclerosis, and thereby reduce the incidence of mortality caused by CAD. Statins, inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR), are the most efficient drugs used to reduce LDL-C and cardiovascular events. However, individual differences existin the effects of statins on the prognosis of CAD, and the underlying mechanism remains unclear.

The lipid-regulating mechanisms of statins is mainly through competitively inhibiting HMG-CoA reductase, a key enzyme in the cholesterol synthesis, to reduce the intracellular free cholesterol, which stimulate upregulation of low density lipoprotein receptor (LDLR) on the surface of hepatocytes, accelerate plasma LDL-C uptake, and thus reduce plasma LDL-C level [2]. The reduction of LDL-C by each mmol/L can reduce major cardiovascular events by approximately 20% [3]. Furthermore, statins mitigate inflammation by modulating the expression of genes involved in inflammatory and immune responses [4-6], which further reduce the risk of major cardiovascular events. Although statins are the most widely used lipid-lowering drugs, apparent individual differences exist in the lipid-lowering effects of these drugs. It has been demonstrated that such individual differences in drug response are associated with genetic variants in enzymes and transport proteins involved in drug metabolism and transport, and drug targets. Single nucleotide poly-

**Table 1.** Baseline clinical characteristics of the study patients

| General characteristics | All (n = 785) |
|---|---|
| Age, years | 63.7 ± 10.8 |
| Male, % | 67.4 |
| Non-smokers, % | 51.6 |
| HP, % | 64.3 |
| DM, % | 21.0 |
| SBP, mmHg | 132.9 ± 19.2 |
| DBP, mmHg | 80.2 ± 11.0 |
| TC, mmol/L | 4.7 ± 1.0 |
| TG, mmol/L | 1.8 ± 1.0 |
| HDL-C, mmol/L | 1.2 ± 0.3 |
| LDL-C, mmol/L | 2.8 ± 0.8 |
| ApoA, mg/L | 1.4 ± 0.2 |
| ApoB, mg/L | 0.9 ± 0.2 |
| Lp(a), mg/L | 0.3 ± 0.2 |
| FG, mmol/L | 5.7 ± 2.2 |

SBP, systolic blood pressure. DBP, diastolic blood pressure. TG, triglycerides. TC, total cholesterol. HDL-C, high density lipoprotein cholesterol. MI, myocardial infarction. HP, hypertension. DM, diabetes mellitus. FPG, fasting plasma glucose.

morphisms (SNPs) affect the pharmacokinetics and pharmacodynamics of statins and thus influences the clinical efficacy and safety of statins. In this regard, numerous studies have been conducted to clarify the pharmacogenetics of statins [7-10], but there are no report concerning the effects of SNPs on the prognosis of CAD patients administered with statins.

In the present study, we evaluated the effects of 16 SNPs related to the efficacy and toxicity of statins on the prognosis of CAD patients receiving statins. SNPs, which are found to be related to the prognosis of CAD patients treated with statins, may be served as biomarkers to guide personalized therapy to more effectively manage CAD complicated with hyperlipemia.

## Materials and methods

### *Patients*

A total of 785 CAD patients, consisting of 530 males and 255 females and with mean age of 63.7 ± 10.8 years, were enrolled from Taizhou People's Hospital between October 2009 and July 2013. All patients exhibited narrowing (≥50%) in at least one of three major coronary arteries (left anterior descending branch, left circumflex artery, and right coronary artery). Patients with a history of myocardial infarction (MI), coronary revascularization or stroke were not included for analysis in this study. All the patients were administered with statins for lipid-lowering effects based on conventional *therapy*. The primary endpoint was the first occurrence of major cardiovascular events (MI, stroke, heart failure, hospitalization for unstable angina, arterial revascularization, and all-cause death) after enrollment. All study subjects were unrelated ethnic Han Chinese and gave written informed content. The study was approved by the Ethnic Committee of Taizhou People's Hospital. Three ml of venous blood was drawn for DNA extraction.

### *Genotyping*

A total of 16 SNPs that have been reported to be associated with statin response were selected (Table S1). All SNPs are common genetic variants with minor allele frequency (MAF) ≥0.05 in the Chinese populations in dbSNP. Genomic DNA was extracted from peripheral blood leukocytes using DNA Extraction Kit (Promega, USA) according to the manufacture's protocol. Genotyping was performed using the ligase detection reaction-polymerase chain reaction (LDR-PCR) method. To evaluate the accuracy of genotyping, 20 samples were randomly selected from the whole study population and re-genotyped using direct sequencing. The LDR-PCR results were 100% concordant with sequencing.

### *Statistical analyses*

All continuous variables were expressed as mean ± standard deviation, and categorical data as percentages or absolute numbers unless otherwise indicated. Chi-square test was used to determine whether genotype distribution was in agreement with Hardy-Weinberg equilibrium (HWE). Unpaired Student's T-test and analysis of variance (ANOVA) were performed to compare differences of means between genotypes. The association of SNPs and complications of CAD was examined by odds ratios (OR) and 95% confidence intervals (CI) estimated using unconditional logistic regression model. Cox proportional hazard regression model was used to calculate hazard ratios (HR) with 95% confidence intervals (CI). The cumula-

**Table 2.** Significant associations between polymorphisms and MI, DM, and HP

| SNP | MI | | DM | | HP | |
|---|---|---|---|---|---|---|
| | OR (95% CI) | *P* value | OR (95% CI) | *P* value | OR (95% CI) | *P* value |
| rs13279522 | 0.968 (0.692-1.355) | 0.850 | 1.077 (0.841-1.381) | 0.557 | 0.933 (0.755-1.154) | 0.522 |
| rs708272 | 1.062 (0.756-1.490) | 0.730 | 1.038 (0.810-1.331) | 0.768 | 0.880 (0.711-1.089) | 0.241 |
| rs5882 | 1.089 (0.789-1.503) | 0.604 | 1.093 (0.860-1.389) | 0.467 | 1.091 (0.890-1.337) | 0.401 |
| rs2231142 | 0.816 (0561-1.187) | 0.288 | 1.111 (0.848-1.455) | 0.445 | 0.965 (0.770-1.209) | 0.755 |
| rs4149056 | 1.137 (0.687-1.880) | 0.618 | 0.941 (0.655-1.350) | 0.740 | 1.025 (0.757-1.386) | 0.875 |
| rs2306283 | 1.277 (0.826-1.974) | 0.272 | 0.923 (0.680-1.252) | 0.605 | 1.001 (0.775-1.293) | 0.993 |
| rs2296651 | 1.753 (1.006-3.055) | 0.047 | 0.930 (0.587-1.472) | 0.756 | 0.896 (0.605-1.327) | 0.585 |
| rs693 | 0.978 (0.452-2.116) | 0.956 | 0.523 (0.263-1.039) | 0.064 | 1.073 (0.661-1.741) | 0.776 |
| rs1801133 | 1.374 (0.981-1.926) | 0.065 | 0.980 (0.761-1.262) | 0.875 | 1.318 (1.059-1.640) | 0.014 |
| rs12916 | 0.981 (0.704-1.368) | 0.981 | 0.843 (0.658-1.080) | 0.176 | 1.136 (0.922-1.399) | 0.232 |
| rs688 | 0.866 (0.562-1.335) | 0.515 | 1.170 (0.847-1.615) | 0.341 | 1.103 (0.835-1.457) | 0.492 |
| rs11206510 | 2.273 (1.280-4.038) | 0.005 | 1.887 (1.025-3.474) | 0.042 | 1.319 (0.858-2.027) | 0.206 |
| rs8192870 | 0.998 (0.679-1.466) | 0.991 | 1.323 (1.004-1.742) | 0.047 | 0.993 (0.779-1.265) | 0.954 |
| rs20455 | 0.988 (0.718-1.360) | 0.942 | 0.920 (0.726-1.157) | 0.492 | 1.137 (0.930-1.391) | 0.212 |

**Table 3.** Significant associations between polymorphisms and HP, FPG, and plasma lipid levels

| SNP | Variable measured | Effect | *P* value |
|---|---|---|---|
| rs8192870 | HDL | AA: 1.1 ± 0.3; AC: 1.2 ± 0.4; CC: 1.1 ± 0.3 | 0.025 |
| rs4149056 | ApoA | CC: 1.2 ± 0.2; AC: 1.4 ± 0.2; TT: 1.3 ± 0.2 | 0.017 |
| rs12916 | TG | CC: 1.9 ± 0.9; CT: 1.7 ± 0.9; TT: 1.9 ± 1.2 | 0.012 |
| rs1801133 | FPG | CC: 1.2 ± 0.2; AC: 1.4 ± 0.2; TT: 1.3 ± 0.6 | 0.033 |
| rs2231142 | HDL | AA: 1.1 ± 0.3; AC: 1.1 ± 0.3; CC: 1.2 ± 0.4 | 0.018 |
| | LDL | AA: 2.4 ± 0.7; AC: 2.8 ± 0.9; CC: 2.8 ± 0.8 | 0.001 |

tive effect of risk SNPs on EFS was determined by counting the number of unfavorable genotypes each patient carried and using those without any unfavorable genotypes as the reference group. A level of $P<0.05$ was considered as statistically significant. All statistical analyses were performed using SPSS version 19.0 (SPSS, IL, USA).

## Results

*Association of SNPs with complications of CAD*

The patient characteristics were summarized in **Table 1**. During the follow-up, MI occurred in 79 of 785 (10.1%) patients. Two SNPs (rs328 and rs3798220) deviated from HWE and thus were excluded from further analysis. **Table 2** showed the results of the association analysis for 14 SNPs and complications of CAD. Both rs2296651 and rs11206510 were significantly related to MI ($P<0.05$). The A allele of rs2296651 (adjusted OR = 1.753, 95% CI: 1.006-3.055, $P = 0.047$) and the C allele of rs11206510 (adjusted OR = 2.273, 95% CI: 1.280-4.038, $P = 0.005$) were associated with an increased risk of MI. Both rs11206510 and rs8192870 showed a weak but statistically significant association with DM ($P<0.05$). The C allele of rs11206510 (adjusted OR = 1.887, 95% CI: 1.025-3.474, $P = 0.042$) and the A allele of rs8192870 (adjusted OR = 1.323, 95% CI: 1.004-1.742, $P = 0.047$) were related to an increased risk of DM. Furthermore, rs1801133 was significantly associated with HP. Patients with the CT and TT genotypes of rs1801133 have a higher risk of HP than those with GG genotype (adjusted OR = 1.898, 95% CI: 1.170-3.078, $P = 0.014$).

*Effects of SNPs on HP, FPG, and plasma lipid levels*

We also evaluated whether SNPs influenced HP, FPG, and plasma lipid levels. LDL-C lowering by statins was influenced by rs2231142 (**Table 3**). The C allele of rs2231142 was related to significantly less LDL-C lowering response to statins ($P = 0.001$). The CC genotype of rs2231142 and the AC genotype of rs8192870

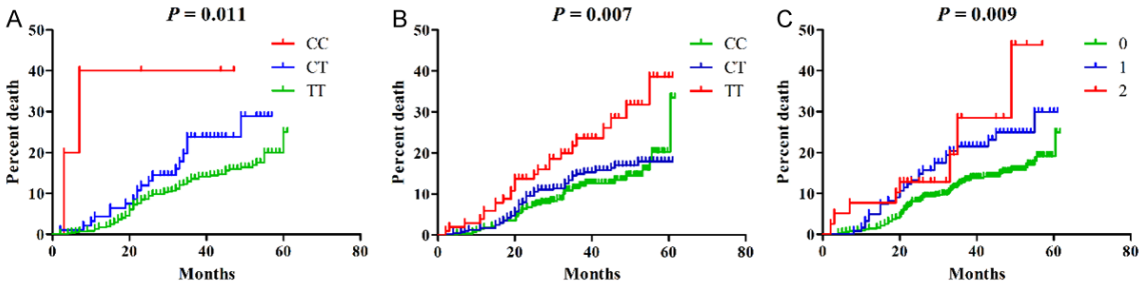


**Figure 1.** Cumulative hazard curves for EFS by genotypes. A. rs11206510. B. rs1801133. C. Cumulative effect of unfavorable genotypes of rs11206510 and rs1801133 on OS.

**Table 4.** Associations between SNPs and event-free survival of CAD patients

| SNP | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| | HR (95% CI) | *P* value | HR (95% CI) | *P* value |
| rs13279522 | 0.958 (0.741-1.238) | 0.742 | 0.873 (0.662-1.152) | 0.336 |
| rs708272 | 0.877 (0.679-1.133) | 0.316 | 0.806 (0.621-1.047) | 0.106 |
| rs5882 | 1.075 (0.839-1.376) | 0.568 | 1.019 (0.784-1.325) | 0.887 |
| rs2231142 | 0.829 (0.622-1.105) | 0.201 | 0.895 (0.656-1.220) | 0.482 |
| rs4149056 | 1.064 (0.730-1.552) | 0.746 | 1.052 (0.711-1.554) | 0.804 |
| rs2306283 | 1.106 (0.800-1.530 | 0.541 | 1.124 (0.794-1.590) | 0.510 |
| rs2296651 | 1.359 (0.875-2.110) | 0.172 | 1.5189 (0.948-2.431) | 0.082 |
| rs693 | 1.123 (0.604-2.087) | 0.714 | 1.140 (0.607-2.143) | 0.684 |
| rs1801133 | 1.402 (1.083-1.815) | 0.010 | 1.499 (1.141-1.971) | 0.004 |
| rs12916 | 1.012 (0.784-1.306) | 0.928 | 1.060 (0.812-1.383) | 0.667 |
| rs688 | 0.890 (0.639-1.240) | 0.491 | 0.844 (0.596-1.197) | 0.342 |
| rs11206510 | 1.792 (1.146-2.802) | 0.010 | 1.891 (1.188-3.010) | 0.007 |
| rs8192870 | 0.941 (0.702-1.261) | 0.683 | 0.952 (0.695-1.304) | 0.759 |
| rs20455 | 1.044 (0.817-1.334) | 0.733 | 1.056 (0.818-1.363) | 0.678 |

were associated with higher HDL level (*P* = 0.018 and 0.025, respectively). Patients carrying the AC genotype of rs4149056 and the CT genotype of rs12916 displayed high level of ApoA (*P* = 0.017) and low level of plasma TG (*P* = 0.012), respectively. Furthermore, it is interesting that rs1801133 influenced FPG level in CAD patients (*P* = 0.033).

*Effects of SNPs on event-free survival (EFS)*

We then assessed whether any of 14 SNPs were associated with EFS. Both rs11206510 (HR = 1.792, 95% CI: 1.146-2.802, *P* = 0.010) and rs1801133 (HR = 1.402, 95% CI: 1.083-1.815, *P* = 0.010) were associated with an increased risk of major cardiovascular events in CAD patients treated with statins (**Figure 1**). These effects remained significant even after adjusted for age, sex, DM, MI, SBP, DBP, TC, TG, HDL-C, LDL-C and FPG (rs11206510, HR = 1.891, 95% CI: 1.188-3.010, *P* = 0.007; rs1801133, HR = 1.499, 95% CI: 1.141-1.971, *P* = 0.004) (**Table 4**).

We further investigated the cumulative effects of rs11206510 and rs1801133 on EFS. The unfavorable genotypes were defined as CC and CT genotypes for rs11206510 and TT genotype for rs18011-33. Cox regression analysis revealed a multivariable-adjusted HR of 1.810 (95% CI: 1.179-2.802, *P* = 0.007) for major cardiovascular events in patients carried with 1 risk genotype. Although patients with 2 risk genotypes had the highest risk of major cardiovascular events, the different disappeared after adjusted for age, sex, DM, MI, SBP, DBP, TC, TG, HDL, LDL and FPG (adjusted HR = 3.172, 95% CI: 0.963-10.448, *P* = 0.058).

## Discussion

Recent studies have revealed that the individual difference in statins response is a result of both genetic and environmental factors [7-13]. Genetic variants in genes encoding enzymes and proteins involved in drug metabolism and transport, and drug target proteins were the primary genetic determinants influencing the lipid-lowering effects of statins on individual

patients [14, 15]. In this study, we examined whether 16 established genetic variants for statins response predispose to major cardiovascular events in CAD patients receiving statins. We found that both rs11206510 and rs1801133 showed an increased risk of developing major cardiovascular events, and exhibited cumulative effect on EFS.

Dyslipidemia involves multiple regulatory pathways. HMGCR catalyzes the conversion of HMG-CoA to mevalonate, a key intermediate for catalyzing cholesterol biosynthesis, which affects cholesterol level in the body [16]. Statins, extensively used agents for lipid-lowering in clinical practice, are also known as HMGCR inhibitors. HMGCR inhibitors are clinically proven to reduce plasma levels of TC, TG, LDL, and VLDL and increase plasma HDL. The rs12916 is located at the 3'UTR of HMGCR, and its T allele is associated with low HMGCR expression in the liver [17]. The lipid-lowering effects of statins on patients carrying CC genotype are poorer than those carrying CT and TT genotypes [18], indicated that HMGCR expression mediate LDL-C signals. In the present study, rs12916 was not related to LDL-C level, but affected TG level. rs8192870, located in the first intron of the cytochrome P450, family 7, subfamily A, polypeptide 1 (CYP7A1) gene, is associated with the progression of primary biliary cirrhosis [19]. Our study also showed that the risk for DM dramatically increased in CAD patients carrying the AA genotype of rs8192870. CYP7A1 is a rate-limiting enzyme for bile acid synthesis from cholesterol, which can influence the cholesterol content in hepatocytes, as well as the efficacy of statins. Jiang et al. [20] found that rs8192870 was associated with LDL-C response to atorvastatin treatment. The lipid-lowering effects on individuals carrying the AA genotype of rs8192870 are the most apparent following atorvastatin treatment. However, in this study, we found that rs8192870 was not related to LDL-C level but associated with HDL level. This phenomenon occurred probably because LDL-C levels in this study were measured after the administration of statins in an inconsistent duration.

Although drug metabolic enzymes are considered important determinants for statins disposition, the effects of drug transporters on the disposition and efficacy of statins are more crucial [21, 22]. Solute carrier organic anion transporter family, member 1B1 (SLCO1B1) is predominantly expressed on the basolateral membrane of human hepatocytes, by which hepatocytes uptake various endogenous substances and drugs such as statins and methotrexate from the portal system, and then metabolize and eliminate them [8, 23, 24]. Therefore, SLCO1B1 significantly influenced the distribution of drugs in the body, and changes in its activity will increase the toxicity and side effects of various drugs, including statins and methotrexate [25, 26]. The rs4149056 leads to a change of valine to alanine at codon 174 and a reduced transport function of SLCO1B1 [27, 28], and is associated with side effects of statins and methotrexate [29, 30]. In the present study, we found that rs4149056 was associated with plasma ApoA level. Given that statins can elevate the level of ApoA [31], SLCO1B1 variants may influence the regulatory effects of statins on ApoA. Further study is warranted to validate this results. ATP-binding cassette sub-family G member 2 (ABCG2) is a P-glycoprotein that transports exogenous substances out of the cells. rs2231142, causing a reduced transport function of ABCG2, is not only associated with the susceptibility of complex diseases, but also markedly affects the pharmacokinetics of drugs such as statins and methotrexate [21, 22, 26, 32, 33]. The AA genotype of rs2231142 elevates the systemic exposure of statins and increases the lipid-lowering effects [7, 34]. Our findings confirmed that rs2231142 is significantly correlated with the levels of HDL and LDL, and indirectly proved that rs2231142 affects the metabolism of statins.

Proprotein convertase subtilisin/kexin type 9 (PCSK9) regulates LDLR level in the liver through post-transcriptional mechanism [35]. Overexpression of PCSK9 leads to reduced LDLR level in the liver, which further decreases the LDLR-mediated LDL-C intake and thereby affects cholesterol level, as well as increases plasma LDL-C level [35-37]. Evolocumab, a monoclonal antibody to PCSK9, can effectively lower plasma LDL-C in patients with statin intolerance [38]. In 2008, Cristen et al. [39] first reported that rs11206510, located 20 kb upstream of PCSK9, was associated with blood lipid levels and CAD. Recent study has shown that rs11206510 is related to increased MI risk

in patients with vascular diseases following additional administration with statins, but not in those who have never been administrated with statins [40]. In the present study, rs11206510 was associated with not only DM risk but also an increased risk of major cardiovascular events of CAD patients receiving statins. These findings indicate that rs11206510 is an independent prognostic factor for CAD patients after long-term statin administration.

Elevated homocysteine level contributes to oxidative stress and thus causes endothelial dysfunction, and is considered to be a risk factor for cardiovascular disease [41]. The T allele of rs1801133 may reduce the enzyme activity of methylenetetrahydrofolate reductase (MTHFR) and increase plasma homocysteine level [42, 43], and thus is closely associated with increased risks of many diseases, including cardiovascular diseases [44, 45]. However, differences exist in the relationship between rs1801133 and CAD [42, 44], which require further verification. Furthermore, the CC genotype of rs1801133 has been reported to reduce the risk for CAD in individuals receiving lipid-lowering treatment [41]. In this study, we found that the TT genotype of rs1801133 was an independent factor for CAD patients treated with statins, which may be caused by high homocysteine because TT genotype carriers have higher homocysteine levels in patients on statin treatment [46].

In conclusion, our findings provide evidence that genetic variants are not only associated with complications of CAD, but also influence the prognosis in CAD patients treated with statins. Given that genetic heterogeneity, there is also a need to confirm our findings by further large-scale studies. In the future, genetic variants may exert their potential as a biomarker to guide the administration of statins to CAD patients, which will provide an enhanced scientific basis for CAD treatment.

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## Disclosure of conflict of interest

None.

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**Table S1.** Candidate genes and SNPs included in the study

| Gene | Symbol | SNP | Minor allele | MAF* | HWE* |
|---|---|---|---|---|---|
| DnaJ (Hsp40) homolog, subfamily C, member 5 beta | DNAJC5B | rs13279522 | C | 0.41 | 0.68 |
| Cholesteryl ester transfer protein | CETP | rs708272 | T | 0.40 | 0.68 |
| | | rs5882 | G | 0.45 | 0.19 |
| ATP-binding cassette sub-family G member 2 | ABCG2 | rs2231142 | A | 0.29 | 0.72 |
| Solute carrier organic anion transporter family, member 1B1 | SLCO1B1 | rs4149056 | C | 0.13 | 0.16 |
| | | rs2306283 | T | 0.20 | 0.47 |
| Solute carrier family 10 (sodium/bile acid cotransporter), member 1 | SLC10A1 | rs2296651 | A | 0.08 | 0.48 |
| Lipoprotein lipase | LPL | rs328 | G | 0.09 | 0.04 |
| Apolipoprotein B precursor | APOB | rs693 | T | 0.05 | 0.18 |
| Methylenetetrahydrofolate reductase | MTHFR | rs1801133 | T | 0.39 | 0.41 |
| 3-hydroxy-3-methylglutaryl-Coenzyme A reductase | HMGCR | rs12916 | T | 0.44 | 0.86 |
| Low-density lipoprotein receptor | LDLR | rs688 | T | 0.17 | 0.20 |
| Proprotein convertase subtilisin/kexin type 9 | PCSK9 | rs11206510 | C | 0.07 | 0.85 |
| Cytochrome P450, family 7, subfamily A, polypeptide 1 | CYP7A1 | rs8192870 | A | 0.24 | 0.96 |
| Lipoprotein, Lp(a) | LPA | rs3798220 | C | 0.09 | 0.01 |
| Kinesin like protein 6 | KIF6 | rs20455 | C | 0.47 | 0.08 |

*MAF: minor allele frequency. HWE: Hardy-Weinberg equilibrium.