Research Article

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Pharmacogenomics

Pharmacogenetic loci for rosuvastatin are associated with intima-media thickness change and coronary artery disease risk

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Aim: Polymorphisms at *LPA*, *LDLR*, *APOE*, *APOC1*, *MYLIP* and *ABCG2* are attractive targets for assessment of their impact on lipid-lowering therapy with rosuvastatin. The present study investigated whether polymorphisms at these genes are associated with the risk of coronary artery disease (CAD) development, and reduction of atherogenic lipids and carotid intima-media thickness (CIMT) in CAD patients, taking rosuvastatin. **Materials & methods:** 190 CAD patients and 1697 subjects were enrolled in pharmacogenetic and genetic association study, respectively. SNP genotyping was done using the MassARRAY-4 system. **Results:** *MYLIP* rs6924995, rs3757354, *APOC1* rs445925, *LDLR* rs6511720, *APOE* rs7412, *ABCG2* rs2199936, rs1481012 variants were significantly associated with CAD susceptibility ($p = 0.016$, 0.0003, <0.0001, <0.0001, 0.013, 0.016, 0.0035, respectively), as well as with CIMT regression (except *ABCG2* variants; $p = 0.05$, 0.039, 0.039, 0.016, 0.0065), and changes in plasma lipids during rosuvastatin therapy. **Conclusion:** The studied polymorphisms possess pleiotropic effects on plasma lipids and CIMT, CAD susceptibility, and determine lipid-lowering response to rosuvastatin.

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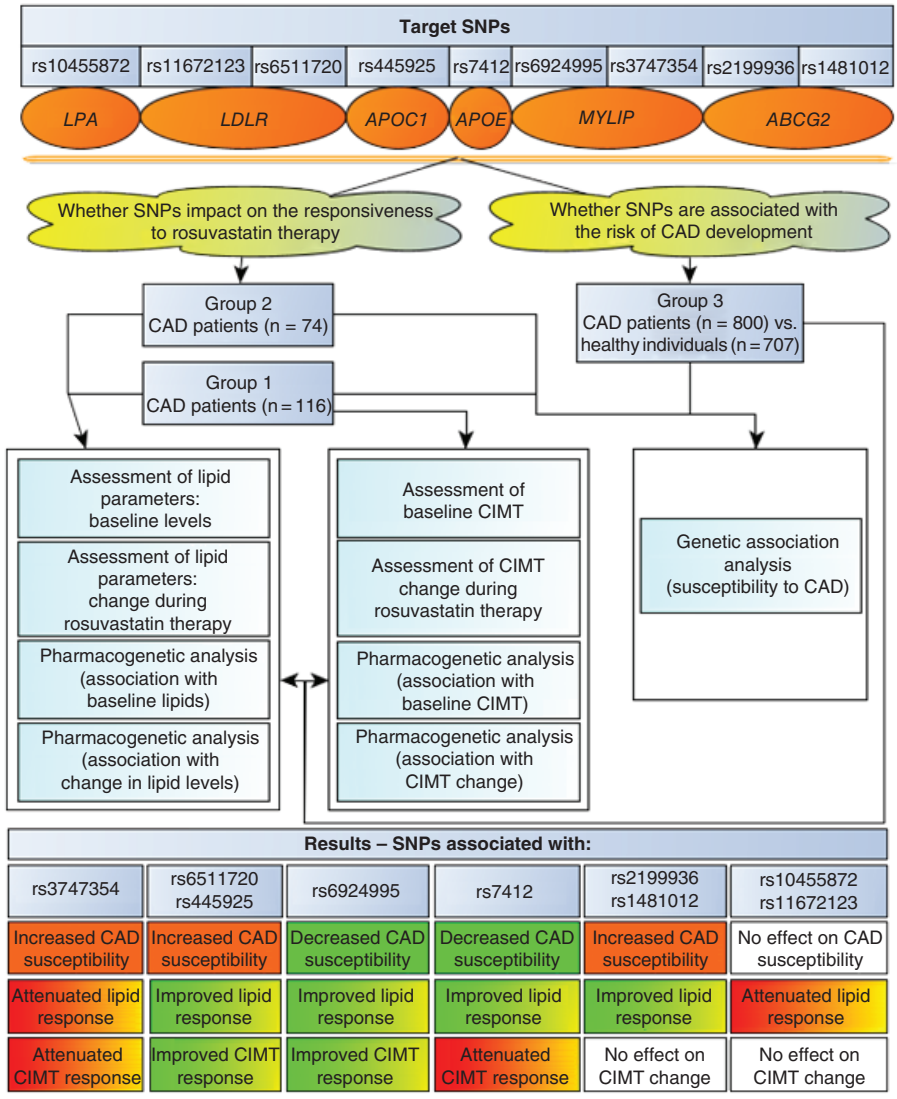
Keywords: carotid intima-media thickness • coronary artery disease • lipids • pharmacogenetics • polymorphism • risk • rosuvastatin

Graphical abstract:



The crucial role of cardiovascular disease (CVD) in world mortality is well known. CVD causes death of more than 4 million people in Europe each year [1]. Coronary artery disease (CAD) plays an important role in the structure of cardiovascular disorders. It is well established that the risk of CAD is determined by genetic and environmental factors [2,3], and coronary atherosclerosis is the main pathological process underlying the disease [4]. The association between CAD and carotid intima-media thickness (CIMT) is well established. CIMT is measured by ultrasound imaging [5], and the increase in CIMT is considered as a risk factor for CAD [6–8]. Furthermore, CIMT is associated with the severity and extent of coronary artery stenosis in patients with CAD [9]. It is well known that the main cause of atherosclerosis is dyslipidemia [10] that is the object for the treatment of CAD patients with statins. Statins are known to be variable in hypolipidemic effect, however rosuvastatin surpasses the statins of previous generations [11]. Several clinical trials have shown positive effects of rosuvastatin treatment on both CIMT regression [12] and atherosclerotic cardiovascular disease [13,14]. Despite the beneficial effects of rosuvastatin on plasma lipids, some patients respond to the treatment poorly. In particular, 6.4 to 12.7% of patients treated with rosuvastatin do not attain the target lipid levels even after 1 year of treatment [15,16]. It has been observed that genetic factors are responsible for the distribution, biotransformation and pharmacodynamics of statins, thereby influencing drug response [17–19]. Single nucleotide polymorphisms (SNPs) in *LPA, APOE, LDLR* genes were found to affect the efficacy of rosuvastatin therapy [18,19]. Further studies, including the JUPITER trial,

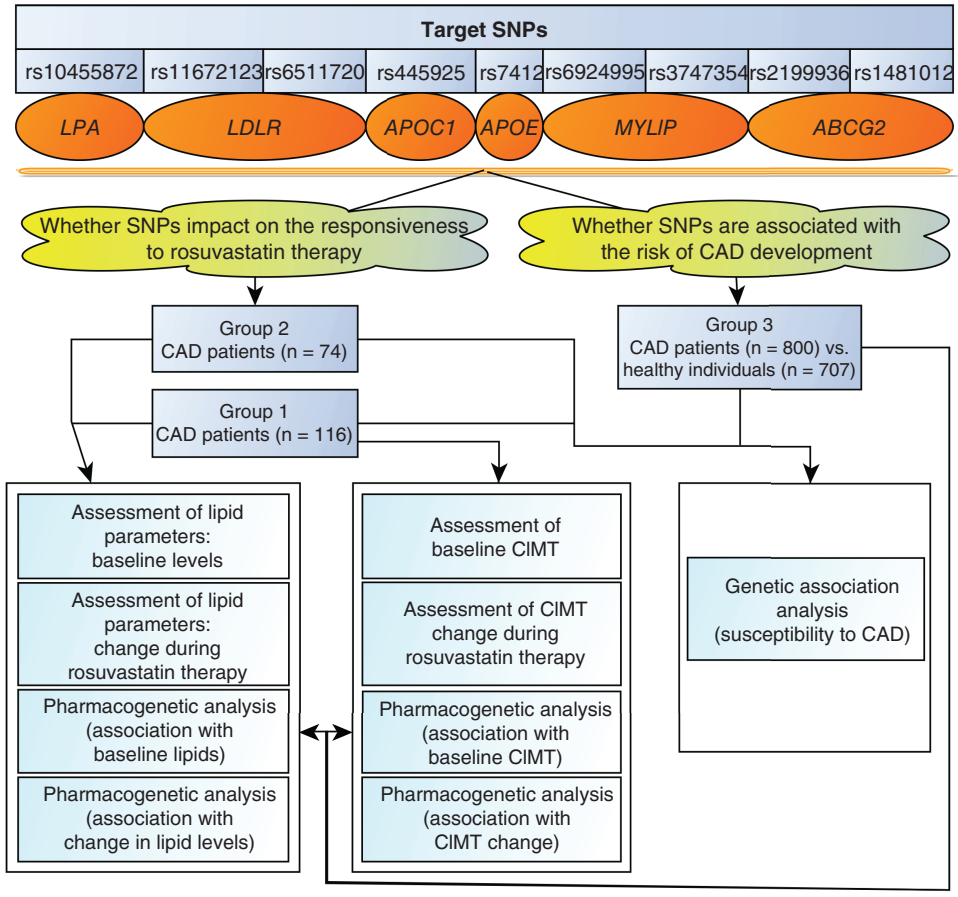


Figure 1. Study design.
CAD: Coronary artery disease; CIMT: Carotid intima-media thickness; LDL-C: Low-density lipoprotein cholesterol; SNP: Single nucleotide polymorphism; TC: Total cholesterol.

the largest pharmacogenetic study conducted on rosuvastatin, has shown that *ABCG2, LDLR* and *MYLIP* gene polymorphisms are also significant contributors to the drug efficacy [20–25]. It is known that *LPA, APOE, LDLR* and *APOC1* gene polymorphisms have been extensively investigated concerning the risk of CAD [18,26–32], as well as CIMT and carotid atherosclerosis [33–35]. No studies have so far been done to investigate the association between the known 'pharmacogenetic SNPs' affecting rosuvastatin treatment and susceptibility to CAD, as well as the effects of the polymorphisms on carotid intima-media thickness in patients receiving rosuvastatin. Thus, the present study was designed to evaluate whether 'pharmacogenetic SNPs' are associated with the risk of CAD and responsible for regression of carotid intima-media thickness in patients on rosuvastatin treatment.

Materials & methods

Study design

The present study consisted of two parts, pharmacogenetic and genetic association analysis of susceptibility to CAD development (Figure 1). After approval by the Institution's ethics committee and written informed consent, patients were enrolled in pharmacogenetic study of rosuvastatin efficacy. The patients did not take statins before inclusion into the study. All patients were prescribed a low-fat diet and rosuvastatin in an initial dose of 5 mg daily to attain target lipid levels: total cholesterol (TC) <4.0 mmol/l and low-density lipoprotein cholesterol (LDL-C) <1.8 mmol/l. Lipid levels were controlled every 4 weeks during rosuvastatin treatment. Patients who attained target lipid levels continued to take the prescribed dose. Patients, who did not attain target lipid levels, underwent

Table 1. Baseline parameters of the pharmacogenetic study population.

Baseline parameter	Value in group 1 (n = 116)	Value in group 2 (n = 74)
Age (years)	61.0 ± 7.25	52.8 ± 6.00
Body mass index (kg/m^2)	28.77 ± 4.18	27.46 ± 3.88
Hypertension (%)	97.5	72.0
Past myocardial infarction (%)	57.6	*N/A*
Systolic blood pressure (mmHg)	131.1 ± 8.1	*N/A*
Diastolic blood pressure (mmHg)	74.9 ± 4.4	*N/A*
Total cholesterol (mmol/l)	5.28 (4.61; 6.03)	6.10 (5.90; 6.60)
LDL-C (mmol/l)	3.27 (2.70; 4.08)	4.2 (3.88; 4.69)
HDL-C (mmol/l)	1.06 (0.97; 1.28)	1.01 (0.94; 1.20)
TG (mmol/l)	1.68 (1.22; 2.37)	1.68 (1.59; 1.83)
CIMT, maximum (mm)	0.80 (0.60; 1.00)	*N/A*
CIMT, mean (mm)	0.70 (0.55; 0.85)	*N/A*

Data are expressed as mean ± standard deviation or as median (Q1; Q3).
CAD: Coronary artery disease; CIMT: Carotid intima-media thickness; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; N/A: Not available; TG: Triglycerides.

an increase of the dose gradually to 10, 20 and 40 mg daily. Lipids were analyzed after 1, 6 and 12 months, CIMT – after 6, and 12 months after the enrollment.

Study populations

A total of 116 Russian patients with CAD (stable angina pectoris grade II–III) and dyslipidemia were enrolled in the pharmacogenetic study. Diagnosis of CAD was confirmed by qualified cardiologists according to Canadian Cardiovascular Society grading of angina pectoris, ECG stress tests (treadmill test), and 24-h (Holter) ECG monitoring. TC levels higher than 4.0 mmol/l, LDL-C levels higher than 1.8 mmol/l were considered as criteria for dyslipidemia. The study population included both men and menopause women in the ratio of 73 to 27%. The mean age of the patients was 61.0 ± 7.25 years (mean ± standard deviation), for men 60.07 ± 7.63 years, for women 63.61 ± 5.36 years. BMI was 28.77 ± 4.18, for men 28.21 ± 4.1, for women 30.33 ± 4.06. According to the grade of angina pectoris, 22.2% of patients were with grade II, 77.8 % with grade III. 57.6% of patients had a history of myocardial infarction. Arterial hypertension was diagnosed in the majority of patients (97.5%). Additionally, DNA samples and phenotypic data from 74 patients with the same diagnosis as a part our previous pharmacogenetic study [36] were included in pharmacogenetic study with the purpose to extent the sample size for estimating effects of the SNPs on baseline lipids and their change during rosuvastatin therapy. Parameters of study populations are given in Table 1. Exclusion criteria for the patients in the pharmacogenetic study were: statin intolerance, familial hypercholesterolemia, hypothyroidism, hepatic dysfunction, accompanied by elevation of aspartate- and alanine aminotransferase levels higher than threefold of upper normal limit, myopathy, including its episodes in history, an elevation of serum creatine kinase level higher than fivefold of upper normal limit, stable angina grade IV and acute coronary syndrome.

Clinical chemistry & ultrasound measurements

For the measurement of lipid levels, blood was taken from the cubital vein of patients in the morning after 12-h fasting. TC, TG levels were measured by enzymatic method using automatic analyzer «Vitalab Flexor E» (Vital Scientific, Netherlands) using reagents of «Analyticon® Biotechnologies AG» (Germany). HDL-C level was measured by the direct assay without precipitation. To calculate LDL-C levels, we used Friedewald's equation [37]. Duplex ultrasound of carotid arteries was performed in B-mode using high-resolution linear sensor on the «MyLab™40» system (Esaote, Netherlands). CIMT was measured bilaterally at a distal third of common carotid artery at a distance of 1–1.5 cm proximal to the bifurcation along the posterior wall. CIMT was measured as the distance between the first and second echo-positive lines [5]. We used two parameters of carotid intima-media thickness: mean CIMT (mean value of all measurements on right and left sides) and maximum CIMT (the maximum value obtained from all measurements on both sides). A criterion for atherosclerotic plaque was a focal thickening of the vessel wall by more than 50% compared with surrounding sections of the vessel wall, or a focal thickening of intima-media more than 1.5 mm protruding into the lumen of the vessel.

Genetic association analysis

DNA samples and phenotypic characteristics of 1697 unrelated Russian individuals – 990 patients with coronary artery disease (including patients from the pharmacogenetic study cohort (both group 1 and group 2, total n = 190; CAD patients from group 3, n = 800) with mean age of 59.9 ± 8.8 years, mean BMI - 29.8 ± 5.4 kg/m^2, with concomitant arterial hypertension in 96.5% of patients) and 707 relatively healthy residents of Central Russia (with mean age of 60.4 ± 8.1 years, mean BMI - 27.0 ± 4.5 kg/m^2) recruited during previous studies between 2003 and 2018 [36,38–43] and deposed in the biobank of Research Institute for Genetic and Molecular Epidemiology of Kursk State Medical University, were used for genetic association study of SNPs with the risk of CAD. Genes and polymorphisms involved in lipid metabolism and rosuvastatin pharmacokinetics were selected from PubMed publications. Gene polymorphisms included in the study such as rs10455872 of *LPA*, rs11672123 and rs6511720 of *LDLR*, rs7412 of *APOE*, rs445925 of *APOC1*, rs6924995 and rs3757354 of *MYLIP*, rs2199936 and rs1481012 of *ABCG2* were previously investigated for hypolipidemic effects of rosuvastatin [18–20,22,24,25,44], excepting rs445925 of the *APOC1* gene that was known to be associated with both coronary artery disease and CIMT [35]. SNPs rs10455872 of *LPA*, rs7412 of *APOE*, rs2199936, and rs1481012 of *ABCG2* for the pharmacogenetic study were selected since they were associated with rosuvastatin-induced LDL-C reduction at genome-wide significance level in the JUPITER study [20], and their minor allele frequencies (MAF) were higher than 0.05 in European populations. Polymorphisms at the *LDLR* gene were selected because they showed the effects on lipids on rosuvastatin therapy in the JUPITER study and were justified [20] as having a significant association with the outcome and involved in lipid metabolism (i.e. 'biologically selected candidate SNPs'). The *MYLIP* rs6924995 variant was found to be associated with pharmacogenetic effects of rosuvastatin at sub-genome-wide level in JUPITER, and was highlighted to be an intriguing marker that should be investigated in further pharmacogenetic studies of statin treatment. So, the above variant was selected as having a pronounced effect on lipid metabolism [20,25], whereas SNP rs3757354 at the same gene was selected as associated with LDL-C levels [44]. *APOC1* variant has not so far been tested for association with reduction in lipid levels by rosuvastatin, and attracted our attention because of having associations with atherosclerosis, CAD and CIMT [26,34,35], as well as with lipid-lowering effect of atorvastatin [24]. SNPs rs10455872, rs6511720, rs445925, rs7412 have been found to be also associated with CAD susceptibility [30–32,35,45,46].

Extraction of DNA was performed from venous blood samples using phenol-chloroform method and precipitation with ethanol [47]. Genotyping was performed using iPLEX technology on the MassARRAY 4 system (Agena Bioscience, USA). Software MassARRAY Assay Design Suite (https://agenacx.com) was used to select a primer set and to design a multiplex panel for SNP genotyping. The sequences of primers are available on request. For some studied SNPs, particularly for polymorphisms in *APOE* and *APOC1* genes, there was a low genotyping call (Supplementary Tables 1–3) due to a poor quality of some DNA samples and/or imperfect co-amplification of these variants with other markers within the multiplex panel.

Statistical analysis

Normality of quantitative traits was tested with Kolmogorov-Smirnov test. Since lipid and CIMT values deviated from the normal distribution, they were expressed as median, first and third quartiles (Me, Q1; Q3). The change in both lipid and CIMT values was determined by Wilcoxon matched pairs test. Spearman rank correlation test was used for correlation analysis between the traits. The distribution of genotype frequencies in accordance with Hardy-Weinberg equilibrium was evaluated with Fisher's exact test. Linear regression analysis was used to detect effects of genotypes on log-transformed baseline lipid levels, CIMT and their change during the treatment. Codominant, dominant, recessive, overdominant and log-additive models were tested for genotype–phenotype associations. Associations were adjusted for covariates such as sex, age, body mass index (BMI) and rosuvastatin dose. The association of alleles and genotypes with CAD risk was measured by odds ratio (OR) and 95% confidence intervals (95% CI) [48] using logistic regression analysis after adjustment for sex, age, and BMI. The data were analyzed using Statistica for Windows 10.0 (StatSoft Inc., USA) and SNPStats tool (https://snpstats.net) [49]. p-value less than 0.05 was considered statistically significant.

Results

Change in lipid parameters & CIMT during the therapy

As it can be seen from Table 2, a significant decrease in TC, LDL-C, HDL-C, TG levels (among 190 participants, group 1 and group 2), maximum and mean CIMT (among 116 participants, group 1) occurred during rosuvastatin

Table 2. Baseline levels of total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides and carotid intima-media thickness and their change in coronary artery disease patients during rosuvastatin therapy (group 1 and group 2).

Parameter	Baseline value	1 month of therapy		6 months of therapy		12 months of therapy	
		Value	Change	Value	Change	Value	Change
TC, mmol/l	5.86 (5.00; 6.30)	3.77 (3.32; 4.11)	-1.27† (-1.89; -0.70)	3.54 (3.21; 3.82)	-2.15† (-2.70; -1.58)	3.48 (3.20; 3.71)	-2.18† (-2.78; -1.60)
LDL-C, mmol/l	3.87 (3.00; 4.36)	2.30 (1.76; 3.16)	-1.14† (-1.82; -0.74)	1.69 (1.45; 1.80)	-2.09† (-2.70; -1.34)	1.64 (1.45; 1.75)	-2.16† (-2.76; -1.46)
HDL-C, mmol/l	1.04 (0.96; 1.21)	1.10 (1.00; 1.28)	0.04§ (-0.07; 0.15)	1.18 (1.00; 1.34)	0.06†(-0.07; 0.22)	1.20 (1.04; 1.36)	0.09† (-0.03; 0.27)
TG, mmol/l	1.68 (1.41; 2.01)	1.56 (1.20; 1.80)	-0.08† (-0.46; 0.01)	1.48 (1.16; 1.70)	-0.19† (-0.58; -0.05)	1.41 (1.10; 1.65)	-0.26† (-0.68; -0.07)
Maximum CIMT¶, mm	0.80 (0.60; 1.00)	N/A	N/A	0.70 (0.50; 0.80)	-0.10† (-0.20; 0)	0.60 (0.50; 0.80)	-0.10† (-0.25; 0)
Mean CIMT¶, mm	0.70 (0.55; 0.85)	N/A	N/A	0.65 (0.52; 0.80)	-0.05‡ (-0.15; 0.07)	0.60 (0.52; 0.75)	-0.07† (-0.15; 0.02)

†$p < 0.0001$ for the change in the parameter compared with baseline value.
‡$p = 0.025$ for the change in the parameter compared with baseline value.
§$p < 0.01$ for the change in the parameter compared with baseline value.
¶Data are available only for group 1 (n = 116).
Data are expressed as median (first; third quartile).
CIMT: Carotid intima-media thickness; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; N/A: Not applicable; TC: Total cholesterol; TG: Triglycerides.

treatment. In particular, a decrease in TC levels was -23.88% (-32.47; -12.28) on the 1st month ($p < 0.0001$), -38.05% (-43.84; -31.03) on the 6th month ($p < 0.0001$), and -38.19% (-44.84; -32.50) on the 12th month of rosuvastatin treatment ($p < 0.0001$). A change in LDL-C was -34.18 (-46.67; -19.68) % on the 1st month ($p < 0.0001$), -55.99 (-62.37; -46.86) % on the 6th month ($p < 0.0001$), and -57.07 (-64.05; -47.45) % on the 12th month of rosuvastatin treatment ($p < 0.0001$). The change in HDL-C was 3.08 (-6.67; 15.00) % on the 1st month ($p < 0.01$), 5.59 (-6.43; 21.90) % on the 6th month ($p < 0.0001$), and 9.21 (-2.94; 25.00) % on the 12th month of rosuvastatin treatment ($p < 0.0001$). Triglyceride level change was -5.56 (-26.35; 0.62) % on the 1st month ($p < 0.0001$), -12.36 (-30.37; -3.23) % on the 6th month ($p < 0.0001$), and -16.13 (-33.33; -4.49) % on the 12th month of the treatment ($p < 0.0001$). The change in maximum CIMT on the 6th month of the therapy was -0.10 (-0.20; 0) mm ($p < 0.0001$), and on the 12th month it was -0.10 (-0.25; 0) mm ($p < 0.0001$). The change in mean CIMT on the 6th month of rosuvastatin therapy was -0.05 (-0.15; 0.07) mm ($p = 0.025$), and on the 12th month of the study it was -0.07 (-0.15; 0.02) mm ($p < 0.0001$). Target levels of TC and LDL-C were attained in 96.9% of patients during the whole period of observation (12 months). Median dose of rosuvastatin, which allowed the patients to attain target TC and LDL-C levels was 20 (10; 20) mg daily. During the whole period of observation, 8 patients experienced progressing of angina pectoris, and 1 patient developed atrioventricular block that required pacemaker implantation.

Since it is known that regression of CIMT is proportional to the degree of decrease in LDL-C levels leading to a decrease in the rate of CAD complications [50], we carried out correlation analysis between the quantitative parameters. There was a positive correlation between absolute change in TC after 12 months of therapy and the change in mean CIMT after 6 months of therapy ($R = 0.19$, $p = 0.04$), whereas fractional change in TC after 12 months of therapy positively correlated with the change in maximum CIMT after 6 months of therapy ($R = 0.19$, $p = 0.04$). Moreover, absolute change in LDL-C after 1 month of therapy positively correlated with the change in maximal CIMT after 6 months of therapy ($R = 0.20$, $p = 0.02$). And finally, fractional change in LDL-C after 1 month of therapy positively correlated with the change both in maximal and mean CIMT after 6 months of rosuvastatin treatment ($R = 0.19$, $p = 0.04$, and $R = 0.23$, $p = 0.01$, respectively). Thus, the better the lipid-lowering effect of rosuvastatin (in terms of TC reduction) occurred at the end of 12 months of therapy, the better the decrease in CIMT occurred during 6 months of follow-up. The better the lipid-lowering effect (in terms of LDL-C reduction) was seen after the first month of therapy, the better the CIMT decrease was observed in the next 6 months of observation.

Pharmacogenetic analysis of hypolipidemic effects of rosuvastatin

To find the associations between SNPs and baseline lipid levels we used the data of 792 CAD patients (both groups, that we included into the pharmacogenetic study, n = 190; and the data of 602 CAD patients from biobank with available parameters of blood lipids). The frequencies of genotypes were tested according to the Hardy-Weinberg equilibrium (HWE). For all the SNPs, except rs10455872 of *LPA* ($p = 0.042$), rs7412 of *APOE* ($p = 0.0004$),

Table 3. Associations of polymorphisms in genes involved in lipid metabolism and genes of membrane transporters with baseline low-density lipoprotein cholesterol levels.

Gene (SNP)	Genotype	Genotype frequencies		LDL-C	
		n	%	Me (Q1; Q3)	p_{adj} †
Genes involved in lipid metabolism					
LPA rs10455872	A/A	484	94.0	2.18 (1.51; 3.73)	**0.0002**
	A/G	28	5.4	2.32 (1.04; 3.64)	
	G/G	3	0.6	4.27 (4.03; 5.8)	
LDLR rs11672123	A/A	2	0.4	2.08 (0.63; 3.52)	**0.034**
	G/A	83	16.1	2.00 (1.3; 3.1)	
	G/G	429	83.5	2.3 (1.61; 3.87)	
LDLR rs6511720	G/G	434	84.4	2.19 (1.5; 3.68)	0.39
	G/T	78	15.2	2.47 (1.86; 3.89)	
	T/T	2	0.4	3.0 (1.19; 4.8)	
APOC1 rs445925	A/A	5	1.3	2.22 (1.9; 4.58)	**0.0002**
	G/A	105	26.1	2.9 (1.85; 4.06)	
	G/G	292	72.6	1.89 (1.3; 2.7)	
APOE rs7412	C/C	289	86.5	2.2 (1.48; 3.9)	0.066
	C/T	36	10.8	2.54 (1.78; 4.34)	
	T/T	9	2.7	2.2 (1.51; 2.99)	
MYLIP rs6924995	A/A	265	51.7	2.17 (1.46; 3.46)	**0.0033**
	A/G	204	39.8	2.19 (1.56; 4.01)	
	G/G	44	8.5	3.05 (2.0; 4.1)	
MYLIP rs3757354	C/C	221	43.0	2.46 (1.61; 3.88)	0.12
	C/T	236	45.9	2.15 (1.48; 3.61)	
	T/T	57	11.1	2.07 (1.48; 3.22)	
Genes of membrane transporters					
ABCG2 rs2199936	A/A	7	1.4	1.9 (1.51; 2.93)	0.56
	G/A	73	14.4	2.4 (1.7; 3.46)	
	G/G	425	84.2	2.2 (1.48; 3.85)	
ABCG2 rs1481012	A/A	424	82.6	2.2 (1.49; 3.86)	0.54
	A/G	82	16.0	2.26 (1.51; 3.1)	
	G/G	7	1.4	1.9 (1.51; 2.93)	

†p value, adjusted for sex, age and BMI.
LDL-C: Low-density lipoprotein cholesterol; Me (Q1; Q3): Median (first; third quartile); SNP: Single nucleotide polymorphism.

and rs2199936 of *ABCG2* (p = 0.029) genotype frequencies were in HWE. For all the calculations we used adjustment for age, sex and BMI. Baseline TC levels were associated with the only one SNP rs3757354 of *MYLIP* (p = 0.042, overdominant model). All associations with baseline TC level are given in Supplementary Table 4. The associations of baseline LDL-C levels with SNPs are presented in Table 3. Baseline LDL-C levels were higher in homozygotes for the minor allele rs10455872-G of *LPA* (p = 0.0002), in the carriers of the minor allele rs445925-A of *APOC1* (p = 0.0004), and in homozygotes for the minor allele rs6924995-G of *MYLIP* (p = 0.0033). The lower LDL-C levels were associated with *LDLR* rs11672123 variant (p = 0.034, log-additive model). No SNP showed an association with baseline HDL-C levels; and only *LPA* rs10455872 variant was associated with baseline TG levels, which were higher in homozygotes for the minor G allele (p = 0.017).

We analyzed the associations of SNPs with both absolute (in mmol/l) and fractional (%) TC and LDL-C reduction on 1, 6 and 12 months of treatment. The data of 190 patients were analyzed (group 1 and group 2). The results are shown in Figures 2 & 3. As it can be seen from Figure 2, carriers of allele rs10455872-G of *LPA* had an attenuated decrease in TC level after 6 (p = 0.018 and 0.022 for absolute and fractional reduction, respectively) and 12 months of treatment p = 0.0085 and 0.0052, respectively). Patients with allele rs11672123-A of *LDLR* showed an attenuated reduction in TC levels after 1 month of rosuvastatin treatment (p = 0.021 and 0.0024 for absolute and fractional reduction, respectively). Homozygotes for the minor allele rs6511720-T of *LDLR* experienced a more pronounced decrease in TC levels after 1 (p = 0.0008 and 0.02 for absolute and fractional reduction, respectively)

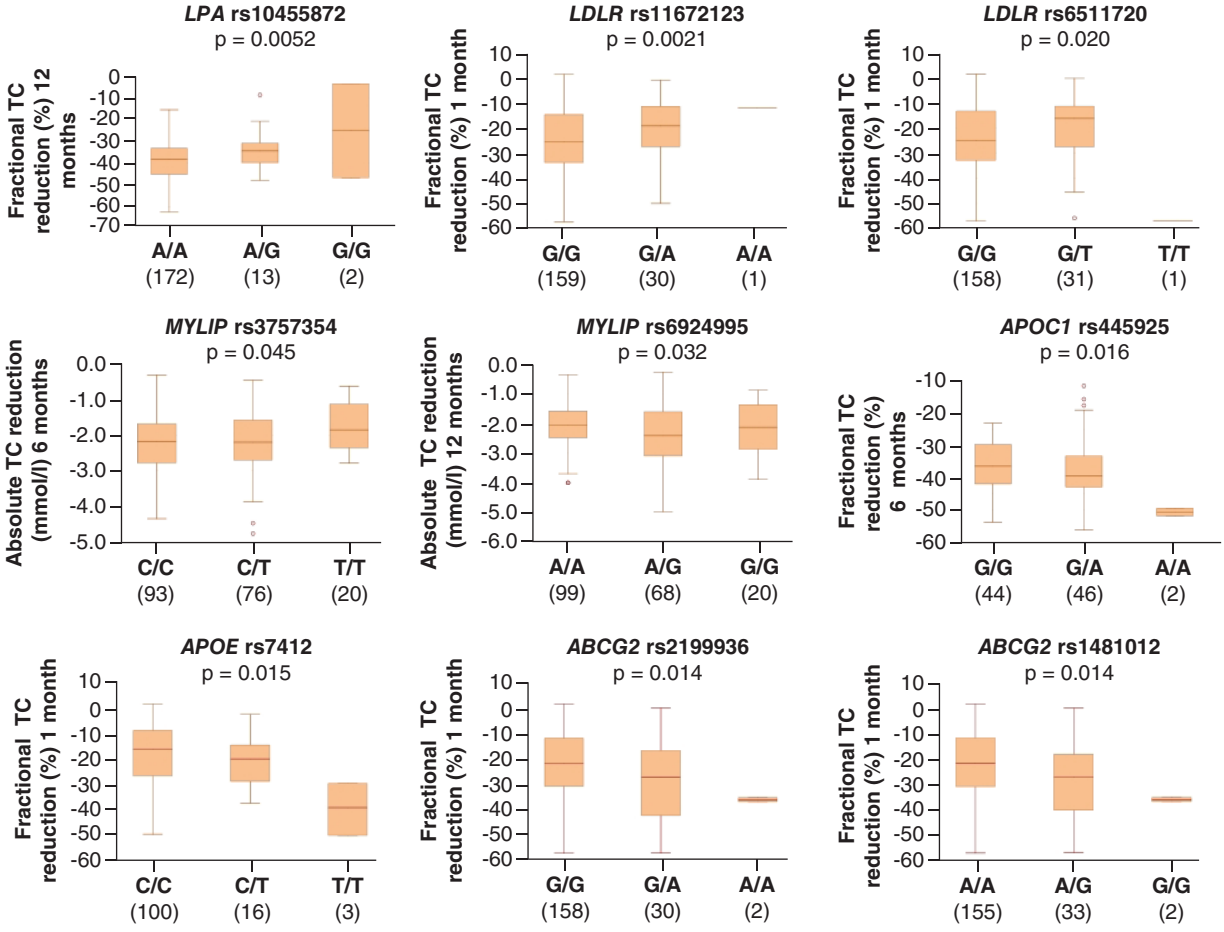


Figure 2. Distribution of total cholesterol reduction for single nucleotide polymorphisms in genes involved in lipid metabolism and genes of membrane transporters in coronary artery disease patients during rosuvastatin therapy. Horizontal line and boxes indicate the median and interquartile range of either absolute (mmol/l) or fractional TC (%) reduction as indicated. Values beyond 1.5-times the interquartile range are indicated with circles.

and 12 months (p = 0.045 for absolute reduction). The *MYLIP* gene polymorphisms such as rs6924995 and rs3757354 were associated with absolute reduction in plasma TC. Allele rs6924995-G showed a more pronounced TC reduction after 12 months (p = 0.032), whereas patients with genotype rs3757354-TT had an attenuated response to rosuvastatin treatment on both 1 and 6 months of observation (p = 0.034 and 0.045, respectively). The observed effects of SNPs did not depend on covariates such as sex, age, BMI and rosuvastatin dose. SNP rs445925 of the *APOC1* gene was associated with higher reduction in TC after 6 months of treatment (p = 0.022 and 0.016 for absolute and fractional reduction, respectively). A carriage of minor A allele was associated with more significant TC reduction on the 12th month of treatment (p = 0.031 for absolute reduction). Homozygotes rs7412-TT of *APOE* experienced a more pronounced TC reduction after 1 (p = 0.0022 and 0.015 for absolute and fractional reduction, respectively) and 12 months, however the effect of this SNP after 12 months of treatment depended on both BMI and drug dose (p = 0.037 for absolute reduction; the effect was present in patients with obesity and taking a 20 mg dose). Both variants in the *ABCG2* gene (rs2199936, rs1481012) were associated with better response to rosuvastatin treatment with an increase in the number of copies of the minor alleles (A and G, respectively) after 1 month of observation (rs2199936, p = 0.045 and 0.014 for absolute and fractional reduction, respectively; rs1481012, p = 0.014 for fractional reduction).

A significant reduction in the levels of LDL-C was observed in the patients with SNPs at *LPA, LDLR, APOC1, APOE* and *MYLIP* genes (Figure 3). Carriers of rs10455872-G allele of *LPA* showed an attenuated decrease in the

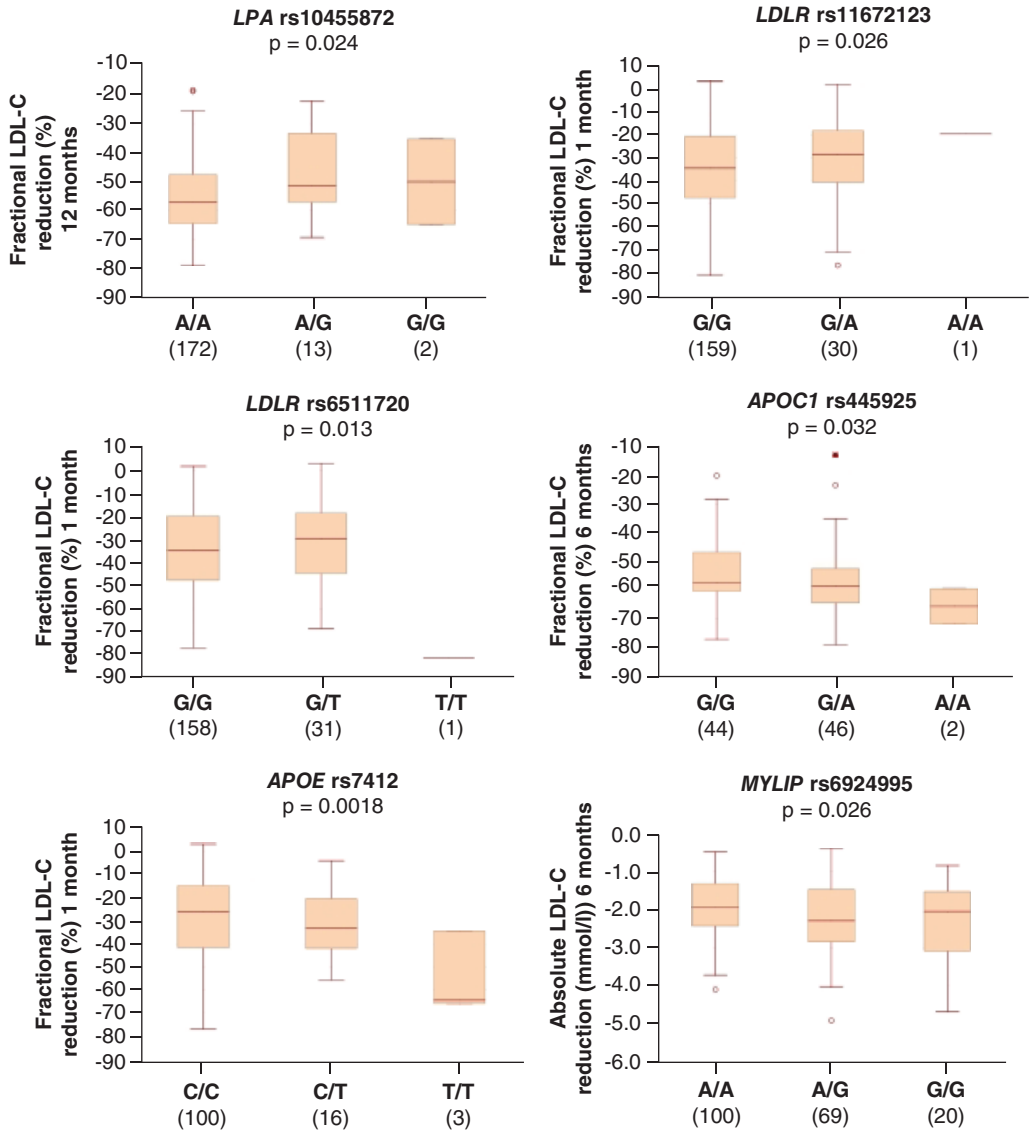


Figure 3. Distribution of low-density lipoprotein cholesterol reduction for single nucleotide polymorphisms in genes involved in lipid metabolism and genes of membrane transporters in coronary artery disease patients during rosuvastatin therapy. Horizontal line and boxes indicate the median and interquartile range of either absolute (mmol/l) or fractional LDL-C (%) reduction as indicated. Values beyond 1.5-times the interquartile range are indicated with circles.

levels of LDL-C after 6 (p = 0.031 and 0.03 for absolute and fractional reduction, respectively) and 12 months of treatment (p = 0.03 and 0.024 for absolute and fractional reduction, respectively). Similar effect of this SNP was observed in TC reduction. However, the effect of rs10455872 on LDL-C reduction was seen in CAD patients of 60 years, or older. The same effect was also observed in the carriers of rs11672123-A of *LDLR* regarding TC reduction on 6th month of rosuvastatin treatment (p = 0.011 and 0.026 for absolute and fractional reduction, respectively). Homozygotes for the minor allele rs6511720-T of *LDLR* demonstrated a more pronounced reduction in LDL-C levels after 1 month of treatment (p = 0.0014 and 0.013 for absolute and fractional reduction, respectively). SNP in *APOC1* was associated with LDL-C reduction at all control points. Homozygotes for the minor allele rs445925-A had a more pronounced effect after 1 month (p = 0.026 and 0.03 for absolute and fractional LDL-C reduction), after 6 (p = 0.032, association only with fractional LDL-C reduction), and 12 months of treatment

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(p = 0.05; association only with fractional LDL-C reduction). The effect became more pronounced with the increase in the number of copies of the minor A allele and was also dependent on sex (was observed in women) after 12 months of observation. Homozygotes for the minor allele rs7412-T of *APOE* were characterized by better LDL-C reduction after 1 (p = 0.0018 and 0.027 for absolute and fractional reduction, respectively) and 6 months (p = 0.043 - association only with absolute LDL-C reduction); in the latter case, effect was dependent on age and drug dose (was observed in patients of 60 years old and higher and taking 20 mg of rosuvastatin). Carriers for allele rs6924995-G of *MYLIP* had a more pronounced effect (absolute LDL-C reduction) after 6 and 12 months of treatment (p = 0.026 and 0.028, respectively). The association of rs3757354 of *MYLIP* became insignificant after adjustment for covariates (sex and drug dose, p = 0.056 for absolute reduction after 1 month of treatment). For SNPs in *ABCG2*, the association was found only after 6 months in terms of attenuated decrease in heterozygotes (p = 0.046 and 0.048, only for fractional reduction for rs2199936 and rs1481012, respectively).

An increase of HDL-C was associated with SNPs in *MYLIP* and *ABCG2* genes. The minor allele rs3757354-T of *MYLIP* was associated with a more pronounced increase in HDL-C after 1 (p = 0.012 and 0.045, for absolute and fractional HDL-C change, respectively), 6 (p = 0.024 and 0.042, respectively), and 12 months of observation (p = 0.043 for absolute HDL-C change). More pronounced growth of HDL-C concentration was observed with the increase in the number of copies of the minor allele rs6924995-G of *MYLIP* - after 1 month of treatment (p = 0.036) and it was dependent on age (for the patients of 60 years old and higher). Both variants in *ABCG2* were associated with attenuated growth in HDL-C after 1 (p = 0.0081 and 0.0045 for absolute and fractional change for rs2199936; p = 0.035 and 0.022 for absolute and fractional change for rs2199936) and 12 months of treatment (p = 0.012 and 0.0092; p = 0.0046 and 0.0039, respectively).

Triglyceride concentration change was associated with two SNPs - rs10455872 of *LPA* and rs6511720 of *LDLR*. Homozygotes for the minor allele rs10455872-G of *LPA* were characterized by a more pronounced reduction of TG levels after 1 month of treatment (p = 0.0091 and 0.038 for absolute and fractional reduction, respectively), dependent on age and sex (in males below 50 years old). The presence of the minor allele rs6511720-T of *LDLR* was associated with a better absolute decrease in TG after 1, 6 and 12 months of observation (p = 0.035, 0.05 and 0.043, respectively).

Pharmacogenetics of rosuvastatin impact on carotid intima-media thickness

For the assessment of the associations of studied SNPs with baseline CIMT the data of patients from group 1 (n = 116) and from biobank (with available mean CIMT data from group 3, n = 281; total 397 patients) were used. Baseline mean CIMT was associated only with *LDLR* rs6511720 polymorphism. TT genotype was associated with the higher CIMT value – 1.18 (1.05; 1.30) mm versus 0.60 (0.52; 0.78) mm in heterozygotes, and 0.61 (0.53; 0.80) mm in homozygotes for the major allele rs6511720-G (p = 0.0002, adjusted for sex, age and BMI).

We assessed the associations of SNPs with CIMT change. The data of 116 patients were analyzed (group 1). The results are shown in Figure 4. During 6 months of rosuvastatin therapy the better decrease in both maximum and mean CIMT was associated with heterozygous genotype rs445925-GA of *APOC1* gene (p = 0.005 for both parameters). Attenuated regression of maximum CIMT was associated with the presence of the minor allele rs7412-T of *APOE* (p = 0.0065). Homozygotes for the minor allele rs6511720-T of *LDLR* were characterized by more pronounced mean CIMT regression (p = 0.016), and the presence of minor allele rs3757354-T of *MYLIP* was associated with attenuated mean CIMT regression alongside the increase in the number of copies of the minor T allele (p = 0.039).

During 12 months of therapy a more pronounced both maximum and mean CIMT regression was associated with heterozygous genotype rs445925-GA of *APOC1* (p = 0.047 and 0.039 respectively for maximum and mean CIMT), and also was found in the carriers of rs6924995 variant with borderline significance (p = 0.05 and 0.051 respectively for maximum and mean CIMT). The levels of significance of all associations with CIMT change are presented after adjustment for age, sex, BMI and rosuvastatin dose.

Target SNPs were tested for associations with the presence of atherosclerotic plaques in the carotid basin. The data of 116 patients were analyzed, 33 of them had atherosclerotic plaques. Association was found for the patients with the genotype rs11672123-GA of *LDLR* (OR = 3.32 95% CI 1.12–9.87, p = 0.03, adjusted for age, sex, BMI). Also, this SNP showed an association with past myocardial infarction. The risk was higher in patients with rs11672123-GA genotype (OR = 4.51 95% CI 1.24–16.40, p = 0.013, adjusted for age, sex and BMI).

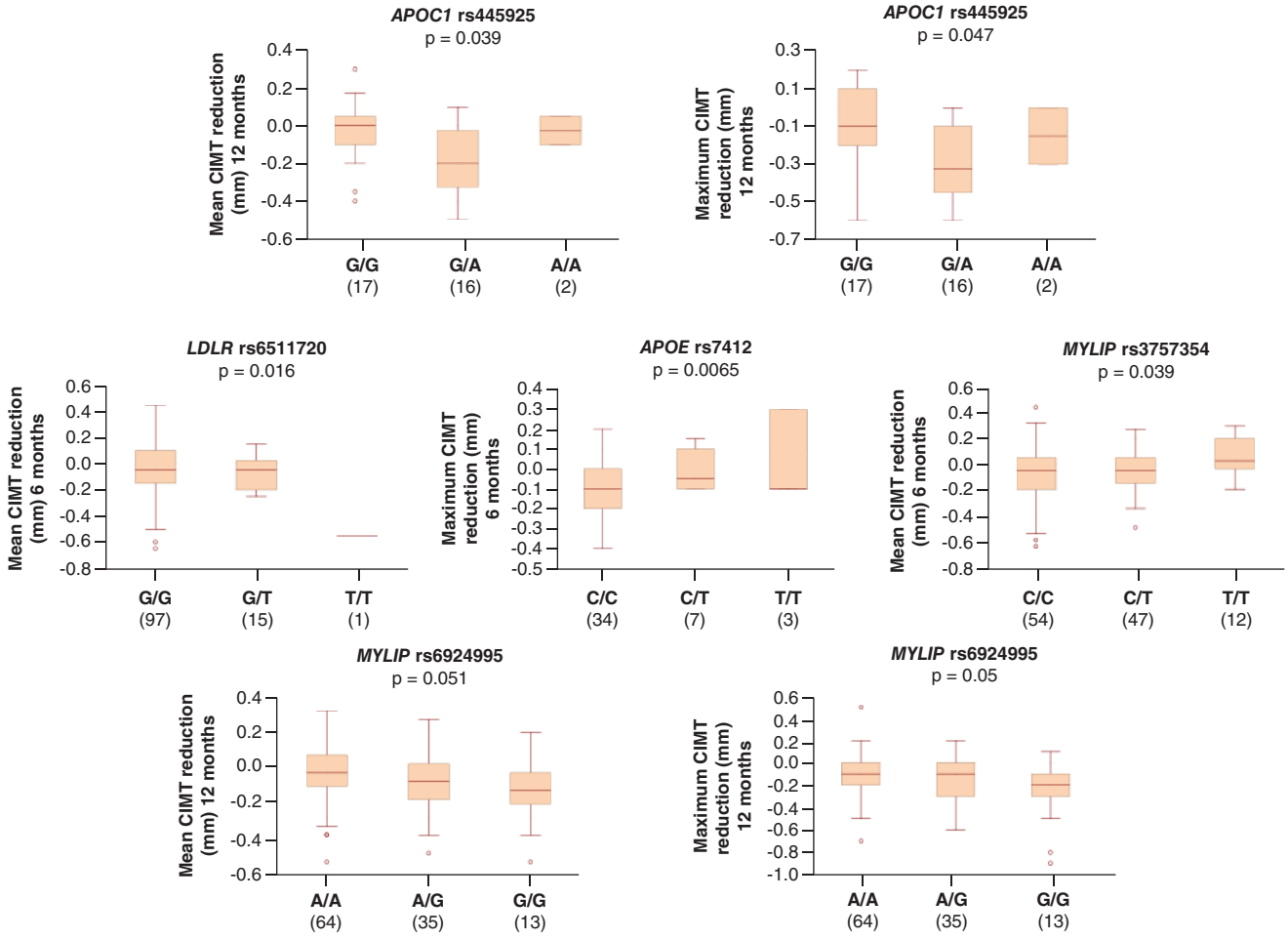

Figure 4. Distribution of carotid intima-media thickness reduction for single nucleotide polymorphisms in genes involved in lipid metabolism and genes of membrane transporters in coronary artery disease patients during rosuvastatin therapy. Horizontal line and boxes indicate the median and interquartile range of either maximum (mm) or mean (mm) CIMT reduction as indicated. Values beyond 1.5-times the interquartile range are indicated with circles.

Assessment of associations of target SNPs with the risk of CAD development

The analysis of the association of polymorphisms in genes involved in lipid metabolism and genes of membrane transporters with the risk of CAD development was carried out. The data of 1697 individuals were analyzed. The results are shown in Table 4. An increased risk of CAD was associated with the genotypes: rs6511720-GT of *LDLR* and rs445925-GA of *APOC1* ($p < 0.0001$ for both associations). Also, the risk was higher in the carriers of homozygous genotype for the rare allele rs3757354-T of *MYLIP* ($p = 0.0003$). The lower risk of CAD was characteristic of the carriers of the minor allele rs7412-T of *APOE* and of individuals with rs6924995-AG genotype of *MYLIP* ($p = 0.013$ and 0.016, respectively). Among the genes of membrane transporters, an increase in the risk of CAD development was associated with homozygotes for the variant alleles rs2199936-A and rs1481012-G of *ABCG2* ($p = 0.016$ and 0.0035, respectively).

Furthermore, significant differences in the frequencies of *LDLR, MYLIP* and *ABCG2* haplotypes were identified between CAD patients and controls: global haplotype association p-value was <0.0001, 0.0023 and <0.0001, respectively. In particular, haplotype rs11672123G-rs6511720T of the *LDLR* gene was associated with an increased risk of coronary artery disease (OR = 1.95 95% CI 1.42–2.68, $p < 0.0001$). Haplotype rs6924995G-rs3757354T of *MYLIP* was associated with an increased risk of coronary artery disease (OR = 1.36 95% CI 1.06–1.76, $p = 0.016$), whereas haplotype rs6924995G-rs3757354C possessed a protective effect against the disease risk (OR = 0.79 95%

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Table 4. Genotype frequencies of polymorphisms of genes involved in lipid metabolism and genes of membrane transporters in coronary artery disease patients and healthy individuals of Central Russia.

Gene (SNP)	Genotype, allele	Healthy (n = 707), n (%)†	Patients (n = 990), n (%)†	OR (95% CI)‡	p-value
Genes involved in lipid metabolism					
LPA rs10455872	A/A	661 (93.5)	922 (93.1)	1.00	0.13
	A/G	46 (6.5)	64 (6.5)	1.00 (0.67–1.47)	
	G/G	0 (0)	4 (0.4)	*N/A* (0.00-*N/A*)	
LDLR rs11672123	G/G	573 (81)	827 (83.6)	1.00	0.17
	G/A	130 (18.4)	156 (15.8)	0.83 (0.65–1.08)	
	A/A	4 (0.6)	6 (0.6)	1.02 (0.29–3.65)	
LDLR rs6511720	G/G	652 (92.3)	840 (85.1)	1.00	**<0.0001**
	G/T	50 (7.1)	143 (14.5)	**2.22 (1.58–3.11)**	
	T/T	4 (0.6)	4 (0.4)	0.77 (0.19–3.11)	
APOC1 rs445925	G/G	358 (84)	625 (73.6)	1.00	**<0.0001**
	G/A	60 (14.1)	213 (25.1)	**2.27 (1.64–3.13)**	
	A/A	8 (1.9)	11 (1.3)	0.97 (0.37–2.51)	
APOE rs7412	C/C	80 (74.1)	474 (84.5)	1.00	**0.013**
	C/T	25 (23.1)	74 (13.2)	**0.51 (0.30–0.87)**	
	T/T	3 (2.8)	13 (2.3)	0.56 (0.15–2.13)	
MYLIP rs6924995	A/A	345 (48.8)	522 (52.9)	1.00	**0.016**
	A/G	315 (44.5)	382 (38.7)	**0.80 (0.66–0.98)**	
	G/G	47 (6.7)	83 (8.4)	1.17 (0.80–1.72)	
MYLIP rs3757354	C/C	357 (50.5)	453 (45.8)	1.00	**0.0003**
	C/T	309 (43.7)	430 (43.5)	1.09 (0.89–1.34)	
	T/T	41 (5.8)	106 (10.7)	**2.04 (1.39–3.00)**	
Genes of membrane transporters					
ABCG2 rs2199936	G/G	609 (86.1)	819 (84)	1.00	**0.016**
	G/A	96 (13.6)	143 (14.7)	1.11 (0.84–1.47)	
	A/A	2 (0.3)	13 (1.3)	**4.78 (1.07–21.26)**	
ABCG2 rs1481012	A/A	575 (81.4)	809 (81.9)	1.00	**0.0035**
	A/G	130 (18.4)	166 (16.8)	0.91 (0.71–1.17)	
	G/G	1 (0.1)	13 (1.3)	**9.14 (1.20–69.92)**	

†Absolute number and % of patients with presented genotypes.
‡Odds ratio (95% confidence interval) adjusted for sex and age.
N/A: Not applicable; SNP: Single nucleotide polymorphism.

CI 0.63–0.99, p = 0.039). Haplotypes rs2199936A-rs1481012G and rs2199936G-rs1481012G of *ABCG2* were associated with an increased (OR = 1.29 95% CI 1.00–1.67, p = 0.049) and a decreased (OR = 0.23 95% CI 0.11–0.48, p = 1.0×10^{-4}) risk of CAD, respectively.

Table 5 shows a summary of the results of SNP function annotation. As it is shown in Table 5, the studied gene polymorphisms are significant quantitative trait loci (QTL) such as mRNA expression (eQTL), methylation (mQTL) and histone modification (hQTL). Impact of SNPs on gene expression (i.e., data on eQTLs) in arteries and blood cells is a subject of the great interest for better understanding the observed genotype-phenotype associations. No significant eQTLs were observed in arteries or aorta tissues. An allele A of SNP rs11672123 located at 5.2 kb 5′ of the *LDLR* gene was associated with increased expression levels of *C19orf66* (FDR = 0.0008), *CTC-510F12.4* (FDR = 0.01) and *SMARCA4* (FDR = 0.04) genes in blood. Polymorphism rs3757354 correlated with the levels of *MYLIP* (FDR = 1.4×10^{-7}) and *ATXN1* (FDR = 0.048) in blood neutrophils. Allele rs6511720-G is associated with decreased levels of *LDLR* in blood neutrophils (FDR = 0.0003). A polymorphism rs1481012 was found to be associated with decreased levels of *PPM1K-DT* (FDR = 0.003). Gene polymorphisms such as rs10455872 of *LPA*, rs6511720 of *LDLR*, rs445925 of *APOC1* and rs7412 of *APOE* were associated with protein levels in blood (pQTLs). Moreover, we have also found that SNPs such as rs11672123 and rs6511720 of *LDLR*, rs445925 of *APOC1*, rs7412 of *APOE*, rs6924995 and rs3757354 of *MYLIP* represent epigenetically regulated polymorphisms. Interestingly, SNPs rs11672123 and rs6511720 of the *LDLR* gene are located in enhancers that may affect gene

Table 5. Summary of functional annotation for the studied SNPs using various bioinformatics tools and resources.

Gene, location of SNP	SNP ID	Ref >Alt alleles	dbSNP function annotation[†,¶]	Number of SNPs in LD (r^2 ≥0.8)[‡]	Transcription factor binding sites			eQTL		pQTL[††]	Epigenetic regulation[‡]				GWAS hits (NHGRI-EBI)[¶]
					FuncPred[§]	HaploReg[‡]	VannoPortal[¶]	Whole blood[#]	Other tissues[¶]		mQTL[††]	Regulatory chromatin states from DNAse and histone ChIP-Seq[‡]			
												Histone modifications		DNAse	
												Promoter histone marks	Enhancer histone marks		
LPA	rs10455872	A >G	Intronic	2	–	4	14	–	4	Yes	13	–	–	–	36
LDLR (5.2 kb 5′ of *LDLR*)	rs11672123	G >A	Intergenic	–	–	–	19	2	–	–	–	2	18	8	–
LDLR	rs6511720	G >T	Intronic	48	–	–	21	–	2	Yes	21	20	10	44	124
APOC1 (2.3 kb 5′ of *APOC1*)	rs445925	G >A	Exonic[‡‡]	2	Yes	6	14	–	2	Yes	27	3	15	4	79
APOE	rs7412	C >T	Missense	–	–	2	17	3	1	Yes	24	1	4	–	167
MYLIP (13 kb 5′ of U3/RNU3)	rs6924995	A >G	Exonic[‡‡]	11	Yes	–	12	–	1	–	7	–	3	–	1
MYLIP (1.9 kb 5′ of *MYLIP*)	rs3757354	C >T	Upstream	4	Yes	6	4	–	–	–	5	23	1	41	14
ABCG2	rs2199936	G >A	Intronic	–	–	–	12	–	–	–	3	–	–	–	12
	rs1481012	A >G	Intronic	8	–	2	17	–	–	–	6	–	–	–	12

[†]dbSNP: https://www.ncbi.nlm.nih.gov/snp/;
[‡]HaploReg v4.1: https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php.
[§]FuncPred: https://snpinfo.niehs.nih.gov/snpinfo/snpfunc.html.
[¶]VannoPortal: http://www.mulinlab.org/vportal/index.html.
[#]eQTLGen: https://www.eqtlgen.org/index.html.
[††]QTLbase: http://www.mulinlab.org/qtlbase/index.html.
[‡‡]Noncoding transcript exon variant.
Date of access: 28 September 2021. Description of these data is presented in the body of the article.

expression levels in aorta. In particular, polymorphism rs11672123 of *LDLR* is located within enhancers associated with histone marks such as H3K4me1 and H3K27ac. SNP rs6511720 represents a regulatory polymorphism associated with histone marks such as H3K4me3 promoter and H3K27ac enhancer. The studied polymorphisms excepting rs11672123 were found to be involved the sequence-specific regulation of gene expression through DNA methylation. Almost all SNPs are located within transcription factor binding sites (TFBS). In addition, SNPs excepting rs11672123 represent GWAS hits, in other words, tagged SNPs associated with human traits. In particular, the rs10455872-A allele is associated with decreased levels of the LPA protein in blood (FDR = 7.0×10^{-165}), allele rs6511720-T of *LDLR* is associated with decreased blood levels of the APOB protein (FDR = 6.0×10^{-11}), allele rs445925-T of *APOC1* is associated with decreased (FDR = 6.9×10^{-27}), and the rs7412-T allele of *APOE* is associated with increased expression of the APOE protein in blood (FDR = 4.3×10^{-137}). Thus, the functional annotation showed that the studied SNPs possess the regulatory potential determined by a variety of mechanisms (i.e. SNP-created TFBS and epigenetic modifications) through which these polymorphisms may modulate expression of genes involved in the regulation of lipid homeostasis.

Discussion/conclusion

The present study was the first to show the genetic contribution to the effect of rosuvastatin in terms of reduction of CIMT in patients with coronary artery disease. Furthermore, we have found for the first time that genetic polymorphisms responsible for lipid-lowering effects of rosuvastatin such as rs6924995 and rs3757354 of *MYLIP*, rs2199936 and rs1481012 of *ABCG2* are significantly associated with the risk of CAD. In addition, SNP rs445925 of *APOC1*, that have previously been found to be associated with CAD, carotid atherosclerosis and CIMT [26,34,35], has shown the association with lipid-lowering effect of rosuvastatin. For *LDLR* rs11672123 variant, previously determined to be associated with the lipid-lowering effect of rosuvastatin, we have found its association with carotid atherosclerotic plaques in CAD patients and past myocardial infarction. The results for the studied SNPs are discussed below, taking into account the function of genes and their polymorphisms.

LPA gene encodes apolipoprotein A, a part of lipoprotein A (Lp (a)), which is associated with thrombotic processes, atherosclerotic plaque formation; elevated levels of ApoA serve as a risk factor for atherosclerosis and CAD [2,18,27,29]. Allele rs10455872-G of the *LPA* gene is associated with the elevated levels of lipoprotein(a) [51], apolipoprotein A1 [52] (due to the smaller size of ApoA and, consequently, its hyperproduction), total cholesterol [53] and LDL-C [53], as well as with the risk of coronary artery disease [54]. Our findings of better hypolipidemic response (TC and LDL-C decrease) to rosuvastatin therapy in the carriers of genotype rs10455872-AA are consistent with the results of pharmacogenomic genome-wide association studies on statin therapy [24,55–57]. In particular, D.I. Chasman with co-workers have observed that the subjects with genotypes rs10455872-AG + GG showed a decreased response to rosuvastatin as compared with genotype rs10455872-AA [20]. The data suggest that subjects with allele rs10455872-A experienced a better response to rosuvastatin treatment because the allele is initially associated with the decreased levels of atherogenic lipoprotein(a), whereas the effect of rs10455872-G allele is probably explained by an inability of statins to decrease Lp (a) level, and consequently, to decrease LDL-C included into Lp (a) particles, which level there is higher in carriers of rs10455872-G allele [58]. The effect of the variant might be related to binding of transcription factors such as CTCF, GABPA, PBX3, KLF4, ZBTB33, ZNF274, MEF2B, NFYB, CEBPB, FOXA3, MYC, XRCC1, NFYA and THAP1 that have been *in silico* predicted by GWAS4D [59] (data are available at VannoPortal: http://www.mulinlab.org/vportal/index.html).

LDLR gene encodes low-density lipoprotein receptor, which plays a crucial role in lipid metabolism, transporting LDL particles into the cell, thereby providing a hypolipidemic effect. In the use of statins, the above process enhances due to a decrease in plasma cholesterol concentration and subsequent increase in the expression of LDL receptors [18,60]. We have found that patients with the rs11672123-GG *LDLR* genotype (wild type homozygotes) had higher levels of LDL-C in plasma and showed a better reduction in the lipid levels (TC and LDL-C) in response to rosuvastatin therapy than patients with alternative genotypes, what is consistent with other studies [20,44]. As it has been mentioned above, the rs11672123-A allele is associated with increased expression levels of *C19orf66*, *CTC-510F12.4*, and *SMARCA4* genes in the whole blood. C19orf66 and SMARCA4 are transcription factors that may modulate gene expression by chromatin remodeling, and the relationship of their levels with a carriage of allele rs11672123-A of *LDLR* might be explained by location of the polymorphism within enhancer associated with histone modifications such as H3K4me1 and H3K27ac. We have found that polymorphism rs11672123 of *LDLR* is in strong linkage disequilibrium with SNP rs6511720 (D' = 0.987). The rs6511720-GT genotype of *LDLR* was found to be associated with the risk of coronary artery disease in our study, and also, patients with TT genotype

showed a better reduction of plasma TC and LDL-C (which is consistent with the data, observed by Chasman and co-workers [20]), as well as a decrease of CIMT after 6 months of rosuvastatin therapy, than carriers of alternative genotypes. The minor T allele works as an enhancer, activating LDLR promotor, which consequently stimulates LDL-receptor expression [32]. This mechanism can contribute to enhanced lipid-lowering effect of rosuvastatin in the carriers of rs6511720-T allele.

APOC1 gene encodes apolipoprotein C1, which acts as an inhibitor of lipoprotein binding with LDL-receptor and influences the function of apolipoprotein E (ApoE) [61,62]. The carriers of the minor allele rs445925-A had higher baseline LDL-C levels, but patients with genotype rs445925-AA responded better to rosuvastatin therapy than carriers of alternative genotypes in our study. This finding is consistent with the results of meta-analysis that showed that patients with allele rs445925-A had a better response to atorvastatin and pravastatin in comparison with subjects with allele rs445925-G [24,56], but the effect was not studied for rosuvastatin before. Moreover, we also revealed that a carriage of heterozygous genotype rs445925-GA was associated with increased risk of coronary artery disease in our population, and patients with this genotype have shown a better decrease in CIMT after 1 year of therapy with rosuvastatin. Meta-analysis of genome-wide association studies performed by partners of the CHARGE consortium in European populations has identified that allele rs445925-A is associated with higher CIMT and higher CAD risk, and, notably, the effect on CIMT was independent of APOE ε variants, despite the location of this SNP in the region, that includes *APOE, APOC1, APOC2* and *APOC4* [35]. In our study there was no association with baseline CIMT, but the revealed association in the carriers of the minor allele rs445925-A with higher baseline LDL-C level could partially explain the higher CAD risk and higher CIMT.

APOE gene encodes apolipoprotein E, which takes part in the clearance of chylomicrons, very-low density lipoproteins and LDL, being a part of them [18,19]. In the present study, SNP rs7412 of *APOE* was associated with the reduced risk of coronary artery disease, and also it was found that patients with genotype rs7412-TT response better to rosuvastatin therapy than the patients with alternative genotypes. However, patients with genotype rs7412-CC showed a better decrease in CIMT after 6 months of therapy by rosuvastatin. Polymorphism rs7412 is a missense mutation associated with amino acid change (Arg176Cys) in apolipoprotein E, and allele rs7412-T is associated with increased levels of total cholesterol [63], HDL cholesterol [64] and also with decreased levels of LDL-C [57], lipoprotein (a) [64] as well as with longevity (age >90th survival percentile) [65]. The presence of the minor allele rs7412-T in genotype (in combination with wild-type T-allele of rs429358, which forms the haplotype ε2) determines the synthesis of the E2 isoform, which has a reduced affinity for the LDL receptor. This leads to a decrease in the liver uptake of lipid particles rich in triglycerides, which increases the expression of LDL receptors on the surface of liver cells and, consequently, increases the catabolism of LDL particles and reduces their level in the blood [46]. Our finding of better hypolipidemic response to rosuvastatin treatment in patients with genotype rs7412-TT of *APOE* is consistent with the results of multiethnic pharmacogenetics study conducted by Oni-Orisan with colleagues who have observed that subjects with T allele had better response (change in LDL-C) to statins [57].

MYLIP gene (also known as *IDOL*) encodes myosin regulatory light chain interacting protein (MYLIP), also known as an inducible degrader of LDL receptor (IDOL), which regulates intracellular level of cholesterol in hepatocyte. With an increase in the level of cholesterol, transcriptional induction of IDOL occurs by sterol-dependent liver X receptor, and then IDOL causes destruction of LDL receptor [66]. Polymorphisms rs6924995 and rs3757354 of *MYLIP* were in a weak linkage disequilibrium with SNP rs6511720 (D' = 0.174) and associated with baseline TC (a higher level was associated with rs3757354-CT genotype), baseline LDL-C (a higher level was associated with rs6924995-GG genotype), CAD risk (higher in rs3757354-TT individuals, lower in rs6924995-AG individuals), and also with TC, LDL-C, and CIMT response to rosuvastatin therapy. In the literature, there are data on the association of rs3757354 variant with total cholesterol, and with LDL-C [50,67,68], but no information on CAD risk. During rosuvastatin treatment, the weakened lipid-lowering effect in homozygotes for the minor allele rs3757354-T was accompanied by a co-directional weakening of CIMT regression; an association with both lipid-lowering effect and CIMT regression was also true for the second studied variant rs6924995. Association of the above SNP with LDL-C in response to rosuvastatin confirms the finding of Chasman with co-workers [20]. The mentioned polymorphism is located within RP1–13D10.2, a processed pseudogene. Mitchel with co-authors showed the mechanism of the effect of rs6924995, that it affects *LDLR* expression and LDL uptake, but does not influence *MYLIP* expression [25].

ABCG2 gene encodes ATP-binding cassette super-family G member 2, which is involved in rosuvastatin pharmacokinetics, providing its excretion by enterocytes into the intestinal lumen (i.e., reducing absorption) and by the liver cells into bile [18,19]. Polymorphisms rs2199936 and rs1481012 of *ABCG2* were in strong negative linkage

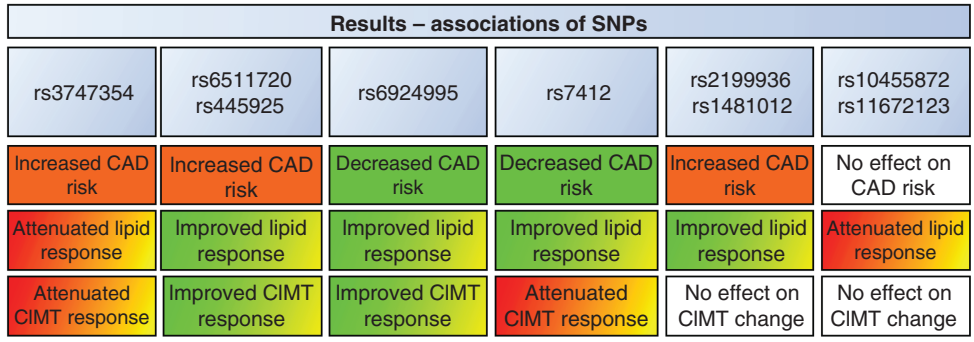

Figure 5. Results of the study: associations of single nucleotide polymorphisms with coronary artery disease risk, change in lipid levels and carotid intima-media thickness in coronary artery disease patients during rosuvastatin therapy.
CAD: Coronary artery disease; CIMT: Carotid intima-media thickness; SNP: Single nucleotide polymorphism.

disequilibrium with each other (D' = 0.996) and have shown similar effects on the risk of coronary artery disease and reduction of plasma TC during rosuvastatin therapy. It is known from the literature that SNPs rs2199936 and rs1481012 are associated with metabolic traits such as blood urate levels [69], body mass index [70] and other phenotypes (http://www.mulinlab.org/vportal/index.html). These associations point out broad substrate specificity and the role of ATP-binding cassette transporter ABCG2 in excreting from cells a broad variety of physiological compounds, toxins and xenobiotics [71,72]. Interestingly, polymorphisms at the *ABCG2* gene may influence its transport capacity and substrate recognition [72] that may play a role in the excretion rate of drugs like rosuvastatin. In this context, ABCG2 along with other ABC-transporters has been shown to be a capable statin efflux pump [73], and *ABCG2* haplotypes have been found to differentially affect the pharmacokinetics of statins such as simvastatin, atorvastatin and rosuvastatin [74]. Only one study reported the relationship between the above polymorphisms in *ABCG2* and response to statin therapy, however, it is unknown which alleles at the SNPs are responsible for these effects on the transporter [20]. Our findings suggest that a better reduction of total plasma cholesterol in the response to rosuvastatin in patients with genotype rs2199936-AA and rs1481012-GG may be attributed to a suspected decrease in the activity of ABCG2 in excreting rosuvastatin from cells. In contrast, higher risk of coronary artery disease in subjects with rs2199936-AA and rs1481012-GG, than in subjects with alternative genotypes, may be explained by the potential involvement of ABCG2, like many other ABC-transporters, in the pathogenesis of atherosclerosis due to their participation in cholesterol metabolism and the regulation of endothelial function, vascular inflammation, platelet production and aggregation [75].

The study has several limitations, and the first one is not so high sample size, which led to the low rate of minor allele homozygotes, and the second one is a low call rate for some SNPs (mostly in *APOE* and *APOC1*). And also, some limitation is a small difference in the effect of polymorphisms between genotypes (for example in the analysis of the effect of *MYLIP* rs6924995 SNP on TC reduction), nevertheless, it has reached the needed significance level, but influenced the statistical power of analyses – for the associations of the loci in *LPA, APOC1*, and *MYLIP* with the change in lipid levels the power did not reach the level of 0.8. The statistical power for the analyses made in the study is presented in Supplementary Table 5. According to above mentioned limitations, further studies with higher sample sizes in other populations of the world are needed to receive more precise results.

Summing up, the polymorphisms of genes involved in lipid metabolism and genes of membrane transporters, involved in rosuvastatin pharmacokinetics, on the one hand, are associated with the risk of CAD development, and on the other hand – with hypolipidemic effect and change of CIMT in CAD patients during rosuvastatin therapy (Figure 5). In this way, increased CAD risk, attenuated both lipid-lowering and CIMT regression effect was associated with the presence of *MYLIP* rs3757354 variant; increased CAD risk, but improved both lipid-lowering and CIMT regression effect was associated with *LDLR* rs6511720 and *APOC1* rs445925 variants. Then, decreased CAD risk and improved both lipid-lowering and CIMT regression effect was associated with *MYLIP* rs6924995 SNP; decreased CAD risk, improved lipid-lowering effect, but attenuated CIMT regression was characteristic of *APOE* rs7412 polymorphism. And finally, increased CAD risk in combination with improved lipid-lowering effect

and no effect on CIMT change was associated with *ABCG2* rs2199936 and rs1481012 variants; absence of effect on CAD risk and CIMT regression, but only attenuated lipid-lowering response was associated with *LPA* rs10455872 and *LDLR* rs11672123 variants.

Future perspective

Further studies with larger sample sizes are needed to elucidate the mechanisms by which the genetic variants influence the changes in the vascular wall during rosuvastatin therapy and to make it more relevant to use the risk alleles found in the study as biomarkers to predict the drug response. It would be of high importance to establish the associations of the genotype with carotid and coronary plaque burden change during the lipid-lowering therapy, taking into account the rapid development of technologies for the precise visualization of atherosclerotic plaques.

Summary points

- Although genetic variants at *LPA* (rs10455872), *LDLR* (rs11672123 and rs6511720), *APOC1* (rs445925), *APOE* (rs7412), *MYLIP* (rs6924995 and rs3757354), *ABCG2* (rs2199936 and rs1481012) were known to influence an inter-individual response to the lipid-lowering treatment with rosuvastatin, their effects on the regression of carotid intima-media thickness have not been yet investigated.
- In this study, *LDLR* rs6511720, *APOC1* rs445925, *MYLIP* rs6924995 and rs3757354 variants were associated with more pronounced carotid intima-media thickness (CIMT) regression in coronary artery disease patients taking rosuvastatin.
- *APOE* rs7412 and *MYLIP* rs3757354 variants were associated with attenuated CIMT regression in patients with coronary artery disease treated with rosuvastatin.
- Single nucleotide polymorphisms, associated with the more pronounced intima-media thickness regression (in *LDLR*, *APOC1*, and rs6924995 in *MYLIP*), were also associated with better lipid-lowering effect of rosuvastatin.
- The polymorphisms associated with the lipid-lowering effect of rosuvastatin showed an influence on the susceptibility to coronary artery disease development.
- Individuals with *MYLIP* rs6924995-AG genotype were characterized by a decreased incidence of coronary artery disease – OR was 0.8 (90% CI: 0.66–0.98).
- *MYLIP* rs3757354-TT genotype (homozygotes for the rare allele) was associated with an increased incidence of coronary artery disease – OR was 2.04 (90% CI: 1.39–3.00).
- Homozygotes for the minor allele of both *ABCG2* rs2199936 and rs1481012 polymorphisms showed an increased risk of coronary artery disease development – ORs were 4.78 (90% CI: 1.07–21.26) and 9.14 (90% CI: 1.20–69.92), respectively.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/pgs-2021-0097

Author contributions

Each co-author participated sufficiently in the work to take responsibility for the content, and that all those who qualify are listed. Authorship credit is based on (a) substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND (b) drafting the work or revising it critically for important intellectual content; AND (c) final approval of the version to be published; AND (d) agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Ethical conduct of research

The authors state that they have obtained approval of Kursk State Medical University ethics committee (11 May 2015) and have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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