International Heart and Vascular Disease Journal • Volume 8, № 25, March 2020

Journal of the Cardioprogress Foundation

LEADING ARTICLE

Pharmacogenetic analysis of the association between polymorphic variant rs2199936 of the ABCG2 gene and the effectiveness of rosuvastatin therapy in patients with coronary heart disease

Kononov S. I.[1], Mal G. S.[1], Churilin M. I.[1], Azarnova Yu. E.[1], Klesova E. Yu.[1], Bikanova M. A.[1], Samko G. N.[2], Polonikov A. V.[1]

[1] Kursk State Medical University of the Ministry of Healthcare of the Russian Federation, Kursk, Russia.
[2] Transnistrian State University named after T. G. Shevchenko, Tiraspol, Republic of Moldova.

Authors:

Stanislav I. Kononov*, M.D., Ph.D., assistant professor of the Department of Pharmacology of Kursk State Medical University of the Ministry of Healthcare of the Russian Federation, Kursk, Russia.
Galina S. Mal, M.D., doctor of sciences, professor, head of the Department pf Pharmacology of Kursk State Medical University of the Ministry of Healthcare of the Russian Federation, Kursk, Russia.
Mikhail I. Churilin, M.D., assistant professor of the Department of Infectious Diseases and Epidemiology of Kursk State Medical University of the Ministry of Healthcare of the Russian Federation, Kursk, Russia.
Yulia E. Azarova, M.D., Ph.D., docent of the Department of Biological Chemistry of Kursk State Medical University of the Ministry of Healthcare of the Russian Federation, Kursk, Russia.
Elena Yu. Kolesova, engineer biotechnologist of the Laboratory of Biochemical Genetics and Metabolomics of the National Research Institute of Genetic and Molecular Epidemiology of Kursk State Medical University of the Ministry of Healthcare of the Russian Federation, Kursk, Russia.
Marina A. Bikanova, Ph.D., junior researcher of the National Research Institute of Genetic and Molecular Epidemiology of Kursk State Medical University of the Ministry of Healthcare of the Russian Federation, Kursk, Russia.
Galina N. Samko, Ph.D., head of the Department of Public Health and Health Organization with the course of infectious diseases of the Transnistrian State University named after T.G. Shevchenko, Tiraspol, Republic of Moldova.

* Corresponding author. Tel.: +79606808414. E-mail: ck325@yandex.ru

Alexey V. Polonikov, M.D., doctor of sciences, professor of the Department of Biology, Medical Genetics and Ecology, the director of the National Research Institute of Genetic and Molecular Epidemiology of Kursk State Medical University of the Ministry of Healthcare of the Russian Federation, Kursk, Russia.

Objective. *To determine the association between polymorphic variant rs2199936 of the ABCG2 gene and levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL cholesterol), its dynamics and rosuvastatin dosage in order to achieve target lipid levels in patients with coronary heart disease (CHD).*

Materials and methods. *The study included 217 patients, residents of Central Russia with CHD, II-III functional classes of stable angina aged 40-70 years. Patients received titrated dosages of rosuvastatin from 5 mg / day to 10-20-40 mg in order to achieve target TC and LDL cholesterol levels. The duration of follow-up was 12 months. The association between rs2199936 carriage and lipid levels was established using linear regression analysis; and with rosuvastatin dosage using logistic regression analysis.*

Results. *The decrease of TC and LDL cholesterol levels was significant at the end of 1, 6 and 12 months of follow-up (p <0.0001 for all periods). No genotype associations with initial lipid levels were found. Carriage of variant allele A was associated with more pronounced decrease of TC level according to 1-month follow-up, in both absolute values (mmol / l, p = 0.045) and percentage of initial level (p = 0.014). Lipid metabolism targets achievement was associated with low rosuvastatin doses (5-10 mg / day), G / A genotype (OR = 0.20 95% CI 0.06-0.62, p = 0.0029) and carriage of variant allele A (OR = 0.33 95% CI 0.13-0.82, P = 0.014).*

Conclusion. *This study established the role of polymorphic variant rs2199936 of the ABCG2 gene in the individual drug response to rosuvastatin treatment in patients with coronary heart disease. Thus, genetic factors contribute to the ability to achieve lipids target levels during rosuvastatin treatment.*

Key words: *pharmacogenetics, rosuvastatin, coronary heart disease, cholesterol, ABCG2, polymorphism.*

Conflict of Interest: None declared.

Received: *19.12.2019* **Accepted:** *26.01.2020*

Introduction

Nowadays it has been established that pharmacological therapy needs personalization due to its unequal effectiveness and the possibility of adverse effects in different patients [1]. Rosuvastatin has been widely used for the management of patients with coronary heart disease (CHD) due to its sufficient hypolipidemic effect in order to reach modern lipid target levels in patients with high cardiovascular risk [2, 3]. However, rosuvastatin has individual effect due to genetically determined characteristics. Pharmacokinetics of this medication is affected by membrane transporter proteins that, along with endogenous substrates transport rosuvastatin. In this regard, ATP-binding membrane transporter of the G2 family that is involved in pharmacological effect of rosuvastatin rather than in other medication from statin group is very remarkable [4, 5]. This protein is encoded by the ABCG2 gene that has significant polymorphism. Carriage of the polymorphic variant rs2199936 of this gene according to pharmacogenetic studies is associated with rosuvastatin lipid-lowering effect increase compared with low-density lipoprotein cholesterol (LDL cholesterol) [4, 6, 7]. We performed pharmacogenetic analysis of polymorphic variant of indicated gene on the effectiveness of rosuvastatin therapy in order to reach target lipid levels among residents of Central Russia, and also established the association between medication dosage needed to achieve target levels and genetic component.

Materials and methods

The study included 217 patients of Slavic origin (self-identification), residents of Central Russia with CHD: with functional classes (FC) II-III of stable angina according to Canadian Cardiovascular Society classification aged from 40 to 70 years. Patients had dyslipidemia, established according to V revision of National recommendations for the diagnosis and management of lipid metabolism disturbances [8], with total cholesterol (TC) level over 4 mmol/l, LDL-cholesterol over 1.8 mmol/l. The sample included men (73%) and women (during menopause). Average age of patients at the time of inclusion in the study was 61.0

± 7.25 years (M ± standard deviation). The study included patients who did not receive statins on regular basis. According to Helsinki Declaration, patients signed informed consent to participate in the study, and Regional Ethics Committee at the Kursk State Medical University of the Russian Ministry of Health approved the study. The exclusion criteria were: individual intolerance to statins, adverse effects during treatment, conditions and risk factors that contribute to their development: increased aspartate aminotransferase and alanine aminotransferase levels over three normal limits, alcoholism, hypothyroidism, myopathy, as well as the history of this condition, including the case of medications adverse effect. Patients with chronic kidney disease with creatinine clearance less than 60 ml/min, as well as patients with concomitant chronic heart failure above IIA class according to Vasilenko-Strazhesko classification, were excluded.

All patients had their genotype and lipid profile determined via blood test. All patients received rosuvastatin starting from 5 mg per day and started a diet with reduction of saturated fats, trans fats and simple carbohydrates. After 1 month of treatment, the lipid profile was determined. In case required targets of TC and LDL-cholesterol were not reached in patients with very high cardiovascular risk [8], higher doses of statins were prescribed (10–20–40 mg sequentially with its increase and control of the lipid profile once a month) until targets were achieved. Patients who achieved target lipid levels continued to receive rosuvastatin in current dosage. Lipids were controlled after 6 and 12 months of the therapy. The level of TC was determined by direct enzymatic assay using "Vitalab Flexor E" automatic analyzer (Netherlands), the concentration of LDL cholesterol was calculated using the Friedewald formula.

5 ml of venous blood was collected for molecular genetic studies (in tubes with 0.5 M EDTA). DNA was isolated using standard two-stage phenol-chloroform extraction with ethanol precipitation method. Multiplex genotyping was performed using MassARRAY 4 genetic analyzer (Agena Bioscience, USA).

Statistical analysis. The normality of the distribution was assessed using Kolmogorov-Smirnov test, the distribution of TC and LDL cholesterol was different from normal, the indicators are presented as median (first to third quartiles). The significance of changes in lipid concentrations during therapy was determined using Wilcoxon signed rank test for paired comparisons. Statistical analysis of obtained data was performed using Statistica 10.0 software. Fisher exact test was used to analyze the frequency distribution of genotypes and its testing for Hardy-Weinberg equilibrium compliance. DNA samples and phenotypic data of 100 patients with CHD from Research Institute of Genetic and Molecular Epidemiology of the Kursk State Medical University who participated in a pharmacogenetic study were used in order to assess the association between genotype and lipid metabolism and to increase study significance [9]. The associations between the polymorphic variant rs2199936 of the ABCG2 gene with lipid metabolism parameters and its dynamics were established using linear regression analysis. The level of statistical significance was established using logarithmic transformation variables. An adjustment for body mass index and medication dosage were made. The association between genotype and medication dosage was evaluated in 115 patients included in the study using logistic regression analysis adjusted for gender, age, body mass index with the calculation of the odds ratio (OR) and 95% confidence interval (95% CI). Regression analysis was performed using the SNPStats tool (https://www.snpstats.net; Spain). A p value less than 0.05 was considered significant.

Results

We compared allele frequencies of the studied polymorphic variant in the population of Central Russia with Europe and East Asia populations due to the presence of pharmacogenetic studies of rosuvastatin in these populations [6, 10, 11, 12]. Allele frequencies were compared with data obtained in the 1000 Genomes Project (1000 Genomes Project, Phase 3) [13]. The frequency of the minor allele A (MAF) in studied population was 0.083 and did not differ significantly (p = 0.6372) from European population (MAF = 0.094), but differed (p<0.00001) from East Asian population (MAF = 0.6372). The frequencies of genotypes of polymorphic variant rs2199936 in studied population for did not deviate from Hardy-Weinberg equilibrium (p = 0.8030).

Carriage of the rs2199936 polymorphic variant was not associated with initial TC and LDL cholesterol levels. The results of the analysis are presented in table 1.

The association between studied polymorphic variant and TC level during rosuvastatin treatment in patients with CHD was established during 1-month follow-up (Table 2). Thus, carriage of the minor allele A was associated with more pronounced hypolipidemic effect of rosuvastatin, expressed both in abso-

Table 1. **The association between polymorphic variant rs2199936 of the ABCG2 gene and initial TC and LDL cholesterol level in patients with CHD**

Gene	Genotype	The frequency of genotypes		TC		LDL-cholesterol	
		n	%	Me (Q1-Q3)	P_{cor}*	Me (Q1-Q3)	p_{cor}*
ABCG2 rs2199936	A/A	2	0,9	5,84 (5,18-6,5)	0,43	3,66 (3,1-4,21)	0,16
	G/A	32	14,7	5,67 (4,75-6,14)		3.51 (2,73-4,16)	
	G/G	183	84,3	5,9 (5,28-6,3)		3.95 (3,22-4,49)	

* The level of statistical significance

Table 2. **The association between polymorphic variant rs2199936 of the ABCG2 gene and TC and LDL cholesterol levels in patients with CHD during rosuvastatin treatment**

Gene (SNP)	Genotype	The frequency of genotypes		Δ TC, 1-month therapy			
		n	%	Mmol/l	%		
				Me (Q1-Q3)	p_{cor}*	Me (Q1-Q3)	p_{cor}*
ABCG2 rs2199936	A/A	2	0,9	-2,12 (-2,40... -1,83)	**0,045**	-36,12 (-36,92... -35,33)	**0,014**
	G/A	32	14,7	-1,56 (-2,34...- 0,94)		-27,13 (-42,40... -16,67)	
	G/G	183	84,3	-1,18 (-1,84 ...-0,68)		-21,89 (-30,82... -11,86)	

* The level of statistical significance with an adjustment for gender, age, body mass index, TC – total cholesterol in blood plasma, LDL-cholesterol – low density lipoprotein cholesterol.

Table 3. **Initial TC and LDL-cholesterol levels in patients with CHD and its dynamics after 1, 6 and 12 months of rosuvastatin treatment (Me (Q1-Q3))**

Parameter	Initial level, mmol/l	1-month therapy		6-months therapy		12-months therapy	
		Δ*, mmol/l	p†	Δ*, mmol/l	p†	Δ*, mmol/l	p†
TC	5,28 (4,61-6,03)	-1,57 (-2,21...-0,94)	**<0,0001**	-1,92 (-2,56...-1,30)	**<0,0001**	-1,93 (-2,49...-1,38)	**<0,0001**
LDL-cholesterol	3,27 (2,70-4,08)	-1,34 (-1,96...-0,85)	**<0,0001**	-1,69 (-2,40...-1,15)	**<0,0001**	-1,62 (-2,29...-1,12)	**<0,0001**

* The dynamics of the parameter to initial level,
† The level of the dynamic's significance of the parameter to initial level according to Wilcoxon signed rank test for paired comparisons, TC – total cholesterol in blood plasma, LDL-cholesterol – low density lipoprotein cholesterol.

Table 4. **The frequencies of alleles and genotypes of polymorphic variant rs2199936 of the ABCG2 gene in patients with low and high doses of rosuvastatin.**

gene (SNP)	genotype, allele	Low dose, n (%)[1]	High dose, n (%)[1]	OR (95 % CI)[2]	p
ABCG2 G>A rs2199936	G/G	39 (69,6)	53 (89,8)	1,00	**0,0029**
	G/A	16 (28,6)	5 (8,5)	**0,20 (0,06-0,62)**	
	A/A	1 (1,8)	1 (1,7)	0,72 (0,03-15,43)	
	A	18 (16,1)	7 (5,9)	**0,33 (0,13-0,82)**	**0,014**

[1] Absolute value and % of patients with indicated genotypes / the frequency of variant allele,
[2] Odds ratio (95 % confidence interval) with an adjustment for gender, age, body mass index.

lute (mmol / l) and in relative (percentage of initial level) values. TC level was not associated with genetic component (p = 0.54 and 0.47 for the dynamics in absolute and relative values during 6 months follow-up, respectively, p = 0.42 and 0.23, respectively, during 12 months follow-up).

The analysis of the association between polymorphic variant rs2199936 and LDL cholesterol level during rosuvastatin treatment showed statistically significant association between genotype and LDL cholesterol level in percentage of the initial level (Δ, %) during 6-months follow-up. The association between the effect during other periods of follow-up were not statistically significant (p=0.28 and 0.14, respectively, for absolute and relative values during 1-month follow-up; p=0.31 and 0.23, respectively, during 12 months follow-up). Initial levels of TC and LDL cholesterol and its dynamics in patients with CHD during rosuvastatin treatment are presented in table 3.

We also estimated the association between the polymorphic variant rs2199936 of the ABCG2 gene and medication dosage needed to achieve target TC and LDL cholesterol levels. Patients were divided into 2 groups: with low rosuvastatin dosages (5 and 10 mg/day, 48%) and with high rosuvastatin dosages (20–40 mg/day, 51.3%). Results of the analysis are presented in table 4.

Patients with heterozygous variant rs2199936 (genotype G / A) more often achieved lipid metabolism target levels with low dosages of studied medication (OR = 0.20 95% CI 0.06-0.62, P = 0.0029) Carriage of the variant A allele was associated with low dosages

of rosuvastatin needed to achieve target lipid levels (OR = 0.33–95% CI 0.13–0.82, P = 0.014).

Discussion

ABCG2 (ATP-binding cassette transporter G2) belongs to the family of translocation ATP-binding proteins and is involved in the transport of both endogenous substances and xenobiotics, including medications. ABCG2 is expressed in enterocytes and provides active transport of rosuvastatin into the intestinal lumen that decreases drug absorption from the gastrointestinal tract. The transporter is also expressed in hepatocytes, where provides excretion of rosuvastatin in bile [4, 5, 7, 14]. Initially, the ABCG2 transporter was known as the breast cancer multidrug resistance protein in chemotherapy. Later it was established that it is also a hepatic excretory transporter, and hepatic is responsible for the excretion of rosuvastatin [6]. The transporter is encoded by the ABCG2 gene that is characterized by polymorphic variants that affect transport protein activity. Carriage of the rs2231142 variant leads to the synthesis of protein with low transport function, and enterocytes excrete rosuvastatin slowly, therefore, the absorption of rosuvastatin, its bioavailability and plasma concentration increases [10]. Carriage of the rs2231142 variant is also associated with enhanced lipid-lowering effect of rosuvastatin [4], that also occurs in patients with rs2199936 polymorphism genotype, that was associated with lipid-lowering effect and had the highest level of significance in the genome-wide association JUPITER study (Justification for Use Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin). The last single nucleotide polymorphism (SNP) is in linkage disequilibrium with rs2231142 according to the HapMap project (r2 = 0.81 in North and West European population) [6], and, therefore, the mechanism of the rs2199936 variant influence on protein can probably be similar by functional effect to rs2231142 variant. In the present study, the carriage of rs2199936 was associated with lipid-lowering effect of rosuvastatin in patients with CHD, while the previous JUPITER study confirmed the association of genotype in patients for primary prevention. We established significant associations of rs2199936 with TC level during 1-month therapy with absolute and relative dynamics of this indicator. Moreover, in current study, we used an approach that is similar to clinical practice. Patients underwent a gradual dose titration, that ensured the achievement of TC and LDL-cholesterol target levels, after that we established contribution of genetic factors into treatment results. Polymorphic variant rs2199936 of the ABCG2 gene that was the most significant predictor of enhanced hypolipidemic response in the JUPITER study, proved its significance in patients with CHD in our study.

Conclusion

This pharmacogenetic study established individual drug response to rosuvastatin treatment in patients with CHD, II-III FC of stable angina. The results of the study show the significance of genotyping of polymorphic variant rs2199936 of the ABCG2 gene for prognosing the response to hypolipidemic rosuvastatin treatment in patients with CHD.

We have concluded that:

We established the association between lipid-lowering effect of rosuvastatin on TC level and carriage of the polymorphic variant rs2199936 of the ABCG2 gene. The presence of the minor allele A in the genotype led to hypolipidemic effect increase.

Initial total cholesterol and low-density lipoprotein cholesterol levels were independent of the carriage of the polymorphic variant rs2199936 of the ABCG2 gene.

The presence of the G / A genotype in the polymorphic variant rs2199936, as well as the carriage of the variant A allele, was associated with low doses of rosuvastatin (5-10 mg / day) needed to achieve target levels of total cholesterol and low-density lipoprotein cholesterol in patients with coronary heart disease.

Conflict of interests: None declared.

References

1. Sychev DA, Kazakov RE, Otdelenov VA, et al. Applications of pharmacogenetic testing for personalization of therapy with oral anticoagulants in Russia. Ration Pharmacother Cardiol. 2013;9(5):525-531. Russian.
2. Arutyunov AG, Arutyunov GP. Correction of cardiovascular risk with statins. Challenges and unsolved issues at the current stage. Russian Heart Journal. 2015;14(4):193–212. Russian.
3. Urazgildeeva SA, Malygina OF. Features of dyslipidemia management in patients with type 2 diabetes mellitus. Meditsinskiy Sovet. 2016;3:48–53. Russian
4. Alfonsi JE, Hegele RA, Gryn SE. Pharmacogenetics of Lipid-Lowering Agents: Precision or Indecision Medicine? Curr Atheroscler Rep. 2016;18(5):24.

5. Birmingham BK, Bujac SR, Elsby R, et al. Impact of ABCG2 and SLCO1B1 polymorphisms on pharmacokinetics of rosuvastatin, atorvastatin and simvastatin acid in Caucasian and Asian subjects: a class effect? Eur J Clin Pharmacol. 2015;71(3):341-55.
6. Chasman DI, Giulianini F, MacFadyen J, et al. Genetic determinants of statin-induced 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(2):257-64.
7. Soko ND, Masimirembwa C, Dandara C. Pharmacogenomics of Rosuvastatin: A Glocal (Global+Local) African Perspective and Expert Review on a Statin Drug. OMICS. 2016;20(9):498-509
8. Aronov DM, Arabidze GG, Ahmedzhanov NM et al. Diagnostics and correction of lipid metabolism disorders for the prevention and treatment of atherosclerosis. Russian recommendations 5th revision. Atherosclerosis and Dyslipidemias. 2012;4(96). S1:2-32. Russian.
9. Zvyagina MV, Mal' GS, Bushueva OYu, et al. Estimating the effectiveness of hypolipidemic therapy with rosuvastatin in patients with coronary heart disease depending on the genotype of lipoprotein lipase. Russian Journal of Experimental and Clinical Pharmacology. 2016;79(1):15-19. Russian.
10. Lee HK, Hu M, Lui SSh, et al. Effects of polymorphisms in ABCG2, SLCO1B1, SLC10A1 and CYP2C9/19 on plasma concentrations of rosuvastatin and lipid response in Chinese patients. Pharmacogenomics. 2013;14(11):1283-94.
11. 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;5:5068.
12. Chu AY, Giulianini F, Barratt BJ, et al. Differential Genetic Effects on Statin-Induced Changes Across Low-Density Lipoprotein-Related Measures. Circ Cardiovasc Genet. 2015;8(5):688-95.
13. 1000 Genomes Project Consortium, Auton A, Brooks LD, Durbin RM, et al. A global reference for human genetic variation. Nature. 2015;526(7571):68-74.
14. Mo W, Zhang JT. Human ABCG2: structure, function, and its role in multidrug resistance. Int J Biochem Mol Biol. 2012;3(1):1-27.