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Frequency of ABCG2 421C>A Genetic Variant and The Efficiency of Treatment with Rosuvastatin in Kazakh Male Patients

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Abstract

Rosuvastatin is one of the most effective lipid-lowering drugs. Nevertheless its activity varies in different populations. Important role in this belongs to ABCG2. The objective of this study was to determine the impact of ABCG2 genotype on therapeutic effect of rosuvastatin in Kazakh population. This study included 82 Kazakh patients with metabolic disorder undergoing 10 -20 mg/day of rosuvastatin therapy for 12 weeks. Our findings indicated that people with 421AA genotype had higher response to the drug. The frequency of minor alleles in the studied group was 29.5%.

Keywords: ABCG2 Gene polymorphism; Frequency; Rosuvastatin

Introduction

Rosuvastatin is a hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitor commonly used for the treatment of metabolic disorders. Rosuvastatin administration resulted in a significant reduction of low density lipoprotein (LDL-C) a moderate increase in high density lipoprotein (HDL-C) and a decrease in serum triglycerides.

However, achieving the desired effect equally for all patients is not always possible as this can be influenced by features of the endogenous metabolism of drugs. Therefore, the question remaining is whether the gene polymorphism influences cholesterol levels in patients receiving statins.

Currently, interest in rosuvastatin is increasing since it is one of the most effective statin which makes it an attractive target for further investigations. The pharmacokinetics of rosuvastatin exhibit substantial inter-subject variability. It is known that polymorphisms play an important role in the pharmacokinetics of rosuvastatin in European and Asian populations [1,2]. There are some reports showing the relationship between ABCG2 421C>A and the total cholesterol (TC), HDL-C and LDL-C. ABCG2 transporter. A polymorphic locus ABCG2 421C>A (rs2231142) reduces the activity of ABCG2. This polymorphism was found to play an important role in the rosuvastatin pharmacokinetics in healthy Chinese males [3]. The frequency of this polymorphism depends on ethnicity [4]. In our work we investigated the association of this polymorphism with therapeutic effect of rosuvastatin in Kazakh male patients.

Material and Methods

Sample collection

In general, 82 samples from unrelated patients of the Kazakh nationality with metabolic disorders were collected. Patients were recruited from the Medical Centre Hospital of the President's Affairs Administration of the Republic of Kazakhstan (Astana) and introduced to 10 to 20 mg/day prescribed drug for three months. Patients taking beta-blockers and diuretics were excluded. All patients were informed on main provisions of the research and signed informed consent to participate in the study. Study was approved to proceed by the local Ethics committee of the Center for Life Sciences of Nazarbayev University (Protocol No 17 of 25 March 2015). Fasting blood samples (4-5 ml) were taken and analyzed for lipid profile (Total cholesterol, Low-density lipoprotein (LDL-C), High-density lipoprotein (HDL-C)).

DNA extraction

DNA was extracted using Wizard® genomic DNA purification kit (Promega Corporation, Madison, WI, USA).

Genotyping ABCG2 (rs2231142) polymorphism was determined by real-time polymerase chain reaction (PCR) using TaqMan allelic discrimination system (Drug Metabolism Genotyping Assays Applied Biosystems, ID 1447487, Applied Biosystems; Foster City, CA). PCR contained 12.5 ul universal Master Mix (2×) (Life Technologies, Carlsbad, CA, USA), 1,25 ul TaqMan Drug Metabolism Genotyping Assay (20 ×, Applied Biosystems, Foster City, CA, US) and 3 ul DNA (20 ng) diluted in DNA/RNA free water. The thermal cycling protocol was as described below: initial cycle at 10 min a 95°C followed by 50 cycles at 92°C for 15s, 60°C for 90 sec using standard conditions for real-time system (Life Technologies).

Statistical analysis

ANOVA was used for the analysis of the potential effect of ABCG2 variant on individuals with metabolic disorders.

Results

Table 1 shows demographic and clinical characteristics of recruited patients with metabolic disorders. Patients were divided into two groups depending on the daily dose (Table 2). All patients were on the therapy with rosuvastatin. The average age of patients was 53.6 years. The study consisted of only male subjects of Kazakh nationality. Concentrations of TC ($p<0.0001$), LDL-C ($p<0.01$) were lower than the baseline levels following lipid-lowering therapy. Also, HDL-C concentrations increased significantly after rosuvastatin treatment ($p<0.01$, (Table 3)).

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Parameter	Individuals
	n=82
Demographics	
Age (years)	53.6 ± 2.5
BMI (kg/m^2)	28.4 ± 3.9
Baseline lipids	
TC (mmol/L)	5.82 ± 1.07
LDL-C (mmol/L)	3.73 ± 0.5
HDL-C (mmol/L)	1.35 ± 0.4
TG (mmol/L)	2.08 ± 0.7
Clinical characteristics	
Arterial hypertension, n (%)	53 (65%)
Diabetes mellitus, n (%)	41 (50%)
Metabolic syndrome, n (%)	52 (63%)
Values expressed as mean ± standard deviation. n, number of subjects; BMI, body mass index; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.	

Table 1: Clinical and demographic characteristics of the patient.

Parameter	Individuals with 10 mg/ day rosuvastatin	Individuals with 20 mg/ day rosuvastatin
	n-41	n-41
Age (years)	53.7 ± 2.6	53.5 ± 2.5
BMI (kg/m^2)	27.9 ± 2.9	28.9 ± 4.8
TC (mmol/L)	5.90 ± 0.7	6.60 ± 0.7
LDL-C (mmol/L)	3.67 ± 0.1	3.8 ± 0.9
HDL-C (mmol/L)	1.29 ± 0.3	1.44 ± 0.4
TG (mmol/L)	1.93 ± 0.9	2.22 ± 0.3
Clinical characteristics		
Arterial hypertension, n (%)	16 (19.5)	37 (45.1)
Diabetes mellitus, n (%)	12 (14.6)	29 (35.4)
Metabolic syndrome, n (%)	16 (19.5)	36 (43.9)
Values expressed as mean ± standard deviation. n, number of subjects; BMI, body mass index; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides.		

Table 2: Baseline characteristics of two groups.

Parameter	Baseline (mmol/L)	Treatment (mmol/L)	Change (mmol/L)	*p*-Value
Baseline and post-treatment with rosuvastatin (10 mg/day, 12 weeks)				
TC	5.90 ± 0.7	5.03 ± 0.4	-0.87 ± 0.6	< 0.0001
LDL-C	3.65 ± 0.2	3.14 ± 0.2	-0.50 ± 0.2	< 0.01
HDL-C	1.37 ± 0.1	1.69 ± 0.2	0.32 ± 0.2	< 0.02
TG	1.93 ± 0.9	1.33 ± 0.6	-0.60 ± 0.4	< 0.03
Baseline and post-treatment with rosuvastatin (20 mg/day, 12 weeks)				
TC	6.60 ± 0.7	5.10 ± 0.4	-1.50 ± 0.7	< 0.01
LDL-C	4.0 ± 0.6	3.2 ± 0.6	-0.89 ± 0.5	< 0.01
HDL-C	1.32 ± 0.1	1.72 ± 0.1	0.39 ± 0.2	< 0.01
TG	2.22 ± 0.3	1.71 ± 0.2	-0.50 ± 0.2	< 0.0006

Table 3: Serum lipid levels at baseline and after the treatment with rosuvastatin (10 and 20 mg/day for 12 weeks).

Parameter	Basal condition	Genotypes		
		c.421CC	c.421CA	c.421AA
Baseline and post-treatment with rosuvastatin (10 mg/day, 12 weeks)				
		(n = 26)	(n = 11)	(n = 4)
TC	5.90 ± 0.7	-0.53 ± 0.4*	-1.32 ± 0.1*	-1.81 ± 0.5
LDL-C	3.65 ± 0.2	-0.40 ± 0.2	-0.67 ± 0.2	-0.71 ± 0.2
HDL-C	1.37 ± 0.1	0.26 ± 0.2	0.40 ± 0.3*	0.46 ± 0.2*
TG	1.93 ± 0.9	-1.39 ± 0.5*	-0.58 ± 0.2	-0.48 ± 0.4*
Baseline and post- treatment with rosuvastatin (20 mg/day, 12 weeks)				
		(n = 20)	(n = 13)	(n = 8)
TC	6.60 ± 0.7	-0.96 ± 0.5*	-1.82 ± 0.1*	-2.32 ± 0.2*
LDL-C	4.0 ± 0.6	-0.54 ± 0.5*	-1.22 ± 0.4*	-1.37 ± 0.2*
HDL-C	1.32 ± 0.1	0.36 ± 0.2	0.42 ± 0.1	0.45 ± 0.1
TG	2.22 ± 0.3	-0.84 ± 0.1*	-0.58 ± 0.1*	-0.84 ± 0.1*

*p-Value < 0.03
TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides.

Table 4: Genotype and allele frequencies of ABCG2 c.421C>A genotype (rs2231142).

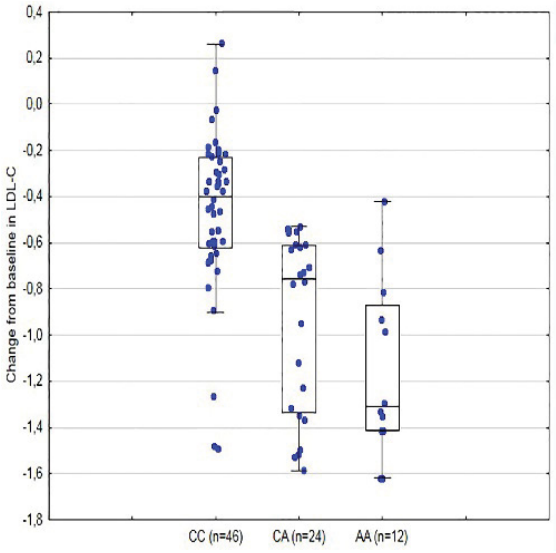

Figure 1: Change in LDL-C levels from baseline after rosuvastatin (both dose) treatment, stratified by ABCG2 421C>A.

Analysis of the data in (Table 4) shows the association between genotype ABCG2 421C>A and reduction of TC and LDL-C levels. Also, gene-dose effect in selected group was observed. Patients with heterozygous allele (CA) and homozygous (AA) LDL-C reduced in value compared to the wild type (Figure 1) ($p< 0.0000001$)). This dependence was observed in the first treatment group (10 mg/day) as well as the second (20 mg/day). The frequency in the general study group of allele C of 70, 5%, allele A 29.5%.

Discussion

In this study, we identified SNP in ABCG2 gene associated with LDL-C levels in Kazakh patients. The results of previous studies by Tomlinson et al. [5] investigated 305 Chinese patients with hypercholesterolemia and Keskitalo et al. [2] studied 660 healthy Finnish volunteers identified higher incidence of the A allele and genotype CA. The results of our study were consistent with these findings. Table 4 shows that the polymorphism of ABCG2 c.421C>A highly associated with effective reduction of TC and LDL-C ($p<0.03$). In contrast, concentrations of HDL-C increased from CC allele to AA allele. Among Kazakh population, the variant allele appears to be very common with 29.5% frequency (Figure 2). A report from Jada et al. [6] for Chinese population (90) showed frequency of allele A 0.28, for Malays (85) 0.27, for Indians (94) 0.15. de Jonq et al. [7] published similar data on Han Chinese population frequency 0,34, for American Caucasian (88) 0.12, Canadian (330) 0.11, [8] European Caucasian

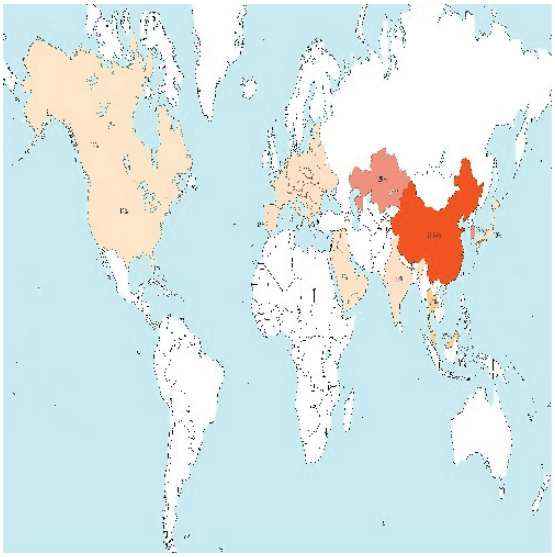

Figure 2: Distribution of the ABCG2 c.421C>A genotype.

(84) 0.11, African American (94) 0.05, African (sub-Sahara) (938) 0.01. Japan population (100) 0.1 [9], Korean population (469) 0.28 [10], Middle Eastern 0.05 [11], Vietnamese (140) 0.31, Portuguese (135) 0.08. Polymorphism ABCG2 421C>A shows a clear differentiation between the different ethnic groups. The data from Kazakh population show that they belong to the Asian group. Results are comparable with the genotype frequency distribution in Korean and Chinese populations. According to the data above it can be stated that the genotypes frequency distribution of ABCG2 421C>A increases from Africa to Oceania.

Interestingly, CA and AA genotypes in the study group was associated with a good response to rosuvastatin therapy. Moreover, patients with minor allele demonstrate the greatest response. This result is also consistent with the data from other population groups [2,3,5].

Polymorphisms in the ATP-binding cassette (ABC) efflux transporter ABCG2 421C>A have been associated with higher rosuvastatin concentration. Zhou et al. showed that the carrier genotype ABCG2 421AA max and AUC (0-∞) increased by 40.2% and 52.0%, respectively, in comparison with carrier genotypes 421CC and 421CA [12]. Lee et al. [13] showed that the average concentration of blood plasma rosuvastatin and its metabolites were higher for 41 and 63% in patients with genotype ABCG2 421AA than in patients with 421CA and for 99% higher than in patients with 421CC genotype.

Conclusion

In conclusion, this study shows that the carriage of ABCG2 421AA allele is the most common nonsynonymous mutation in Kazakh population similar to Chinese population frequency. ABCG2 421C>A remains as an important intersubject variability in the therapeutic effect of rosuvastatin in Kazakh population.

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