Short communication

Frequency distribution of polymorphisms of *CYP2C19*, *CYP2C9*, *VKORC1* and *SLCO1B1* genes in the Yakut population

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Abstract

Allele frequencies of single nucleotide polymorphisms (SNPs) are variable among different populations; therefore the study of SNPs in ethnic groups is important for establishing the clinical significance of the screening of these polymorphisms. The main goal of the research is to study the polymorphisms of *CYP2C9*, *CYP2C19*, *VKORC1*, and *SLCO1B1* in Yakuts. Genomic DNA from 229 Yakut subjects were analyzed by real-time polymerase chain reaction (PCR) (*SLCO1B1* +521T > C, *VKORC1* -1639G>A, *CYP2C19* +681G>A, +636G>A, *CYP2C9* +430C>T, +1075A>C). Genotype frequencies of polymorphisms in the population of the Yakuts were more characteristic of the Asian population. The results have been included in the software application "Lekgen" that we developed for the interpretation of pharmacogenetic testing. The data of our study obtained on frequency carriers of polymorphisms of genes *SLCO1B1*, *CYP2C19*, *CYP2C9*, *VKORC1* among the Yakuts may be useful in developing recommendations for a personalized therapy.

Keywords: Polymorphism; Genotyping; Pharmacogenetics; Yakuts

INTRODUCTION

Currently, all over the world, there is a high incidence of adverse reactions to medications. Evidence suggests that drug therapies not accounting for interindividual differences are ineffective in 30-60% of patients (1,2). Personalized medicine is an approach based on patient-oriented methods of treatment and diagnostics. Personalized medicine allows us to use modern molecular genetic technologies to personalize the use of drugs. This will make the therapy safe, efficient and cost-effective. Applying the principles of personalized medicine will improve clinical outcomes for patients and help to achieve more effective use of health care resources (3). The most promising tool for clinical practice of personalized medicine is the pharmacogenetic testing that can identify the individual's genetic characteristics that contribute to the pharmacological response (4,5). Some of the genes complexes determining drug metabolism are cytochrome P450 (CYP) genes. CYP P450 is a superfamily of enzymes that plays an important role in the metabolism and elimination of a wide range of medications (6). The CYP2C enzymes account for the metabolism of approximately 20% of therapeutic drugs (7). The main CYP2C isoforms, namely CYP2C9 (50% of the total CYP2C) and CYP2C19 (16% of the total CYP2C) are homologous and share more than 80% amino acid sequence identity (8,9). CYP2C19 is responsible for the metabolism of a range of clinically important drugs, namely clopidogrel, proton pump inhibitors, diazepam, imipramine, fluoxetine, tolbutamide, voriconazole. CYP2C19*2 and CYP2C19*3 alleles are responsible for the poor metabolizer phenotype (10). CYP2C9 is involved in the metabolism of drugs including warfarin, losartan, tolbutamide, phenytoin, nonsteroidal anti-inflammatory drugs, and antidepressants. CYP2C9*2 and CYP2C9*3 alleles cause defective CYP2C9 catalytic activity (10,11). Numerous groups of researchers have found that the vitamin K epoxide reductase

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complex 1 polymorphisms correlate with the therapeutic warfarin dose (12). Solute carrier organic anion transporter family member 1B1 (SLCO1B1) transports statins and metabolites from the blood stream into the cells of the liver. SLCO1B1*5 allele have demonstrated significant pharmacokinetic influence (13,14). It is well known that pharmacogenetic relevant alleles occur with varying frequencies among different ethnic populations (15). The study of single nucleotide polymorphisms (SNPs) in different ethnic groups is important to establish clinical relevance of screening for these SNPs to the evaluation of the efficacy and safety of pharmacotherapy for individuals around the world.

Yakuts are a population isolate in Asia and live in the North-Eastern part of Siberia in the Russian Federation. Yakuts emigrated from southern to northern Siberia in the 13th or 14th century AD and their population has expanded rapidly to >450 000 people in a restricted area. Yakuts are a population isolate that can provide a unique model for genetic studies (16,17). The genetic difference is one of the main causes of inter-individual differences in response to pharmacotherapy. Assessment of inter-individual differences in the genotype frequencies is very important for the Russian Federation because of its high multinationality. The purpose of this research is to study polymorphisms of genes associated with the metabolism of drugs. To achieve the goal, we analyzed the distribution of 6 polymorphisms at *CYP2C9*, *CYP2C19*, *VKORC1*, and *SLCO1B1* genes in individuals of the Yakut ethnicity living in North-Eastern Siberia. Also the results of this study will be used for the correct interpretation of pharmacogenetic testing using our software application

"Lekgen" (Limited Liability Company "GeneDiag", Yakutsk, Russia).

MATERIALS AND METHODS

Subject

Whole blood samples from 229 unrelated healthy individuals comprising 120 males and 109 females were obtained from the biological sample bank of Limited Liability Company "GeneDiag" (Yakutsk, Russia) and sampling carried out from a Yakut ethnicity subjects (Asian ethnic origin) from the Yakutia (North-Eastern Siberia, Russia). The mean age was 38.5 ± 7.7 years (range: 21-57 years).

The main exclusion criteria were standard for blood donors in the Russian Federation. Research was performed in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) and was approved by the local ethics committee of the FSBI "Yakut Scientific Centre of Complex Medical Problems". All individuals provided informed consent before the study was carried out.

Genotyping methods

Genomic DNA was isolated from blood of healthy individuals using phenol-chloroform extraction methods (18). Six polymorphisms in *SLCO1B1*, *CYP2C19*, *CYP2C9*, and *VKORC1* genes (Table 1) were selected according to their effect on enzyme activity (19-21). Genotyping polymorphisms were conducted by real-time polymerase chain reaction (PCR). All of the probes used in this study were specifically designed to detect the SLCO1B1*5, VKORC1 haplotype*2, *CYP2C19*2*, *3*, CYP2C9*2, *3 alleles (LLC "TestGen", Ulyanovsk, Russia). The primers and probes for these assays are commercially available.

Table 1. Characterization of the polymorphisms.

Gene	SNP	rs	Drug
SLCO1B1	SLCO1B1*5, +521T>C	rs4149056	statins
CYP2C19	CYP2C19*2, +681G>A CYP2C19*3, +636G>A	rs4244285 rs4986893	clopidogrel, proton-pump inhibitors,voriconazole
CYP2C9	CYP2C9*2, +430C>T CYP2C9*3, +1075A>C	rs1799853 rs1057910	warfarin
VKORC1	VKORC1 haplotype*2 (A), -1639G>A	rs9923231	

Statistical analysis

Deviation from Hardy-Weinberg Equilibrium (HWE) was tested using the online calculator (22). Allele frequencies of various genotypes were compared using the χ^2 test. *P*-value of $\leq$ 0.05 was considered statistically significant.

RESULTS

The determination of genotype frequencies of the SNPs in question showed compliance of this index to the HWE, indicating the representativeness of the sample. The frequencies of the genotypes of polymorphisms investigated in Yakuts are shown in Table 2. Data on SNPs of previous studies were used to compare the frequency distribution of genotypes in the Yakut population with other populations. The comparative analysis of the genotype frequencies of the polymorphism *SLCO1B1 + 521T > C* between groups consisting of individuals of the Yakut and the Russian nationality found statistically significant differences ($\chi^2 = 25.52$, *P* < 0.001). The comparison of the frequencies of genotypes *CYP2C19 + 681G > A* polymorphism found a significant difference between the Yakuts and the Chinese ($\chi^2 = 45.42$, *P* < 0.001), and also between the Yakuts and the Russians ($\chi^2 = 8.94$, *P* < 0.05).

The analysis of the frequency distribution of genotypes of *CYP2C19 + 636G > A* polymorphism found similar frequencies in groups of the Yakuts and the Chinese. The difference between *CYP2C19 + 636G > A* genotype frequencies among the Yakuts and Russians ($\chi^2 = 6.39$, *P* < 0.05) as well as between the Yakuts and the Finnish ($\chi^2 = 10.76$, *P* < 0.001) were statistically significant. The comparison of the frequencies of *CYP2C9 + 430C > T* genotypes found a statistically significant difference both between groups of the Yakuts and the Chinese ($\chi^2 = 15.9$, *P* < 0.001), as well as between groups of the Yakuts and the Finns ($\chi^2 = 8.52$, *P* < 0.05). At that, it found no significant differences between the frequencies of the SNP in the Yakuts and the Russians. The study of *CYP2C9 + 1075A > C* polymorphisms in the groups established no significant differences in the frequency of genotypes. There is some frequency difference detected in the polymorphism *VKORC1 - 1639G > A* between the Yakut and Chinese groups ($\chi^2 = 9.26$, *P* < 0.05), and between the Yakut and Russian groups ($\chi^2 = 62.15$, *P* < 0.001).
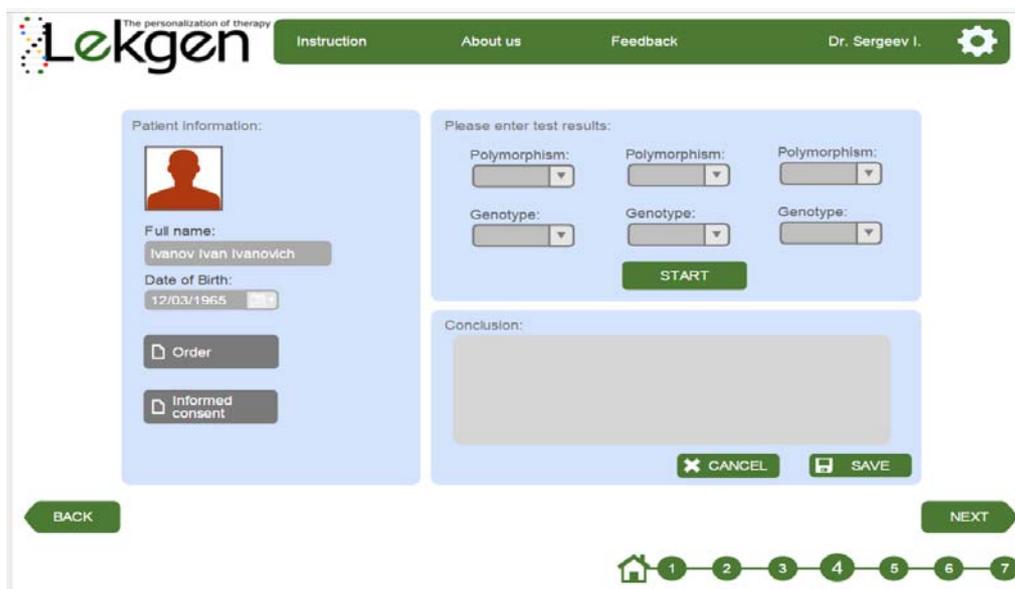
DISCUSSION

It is known that the SNPs frequency distribution can vary greatly between different ethnic groups. As a consequence, the pharmacogenetic test may be clinically significant in the regions where there is a high frequency of the detected allelic variant of the ethnic groups living in the territory. In regions where there is a low frequency of the detectable SNP, the introduction of the pharmacogenetic test will be less relevant. The results obtained in this study of the genotype frequencies of polymorphisms in the population of the Yakuts were more characteristic of the Asian population. Frequencies of minor alleles of polymorphisms in the *CYP2C19 + 681G > A* and *CYP2C9 + 430C > T* in the sample we studied were somewhere in the middle between the frequencies in the populations of Asia and Europe. The genotyping work to identify the distribution of genotype frequencies of SNPs associated with a change in pharmacological response is an important condition for the correct interpretation of pharmacogenetic testing using our software application "Lekgen".

The desktop software application "Lekgen" automatically generates evaluations on the basis of pharmacogenetic test results for drugs of 11 pharmacological groups. The interpretation is based on the algorithms provided by the PharmGKB (33). The software application we developed will reduce the time and labor intensiveness for employees, and as a consequence, will increase the efficiency of their work. The software tool is easy to use due to the structured data, simplicity of navigation and user-friendly menu system, the user manual, based on conventional terms, the possibility of using templates (Fig. 1).

Table 2. Genotype frequencies of *SLCO1B1*, *CYP2C19*, *CYP2C9*, and *VKORC1* gene polymorphisms in different ethnic groups.

SNP	Genotype	Ethnic groups				Reference
		Asian	Asian	Caucasian	Caucasian	
SLCO1B1 +521T>C	TT	87 (87)	80 (160)	61 (349)	63.9 (299)	Shuev *et al.* (23); Mei *et al.* (24); Pasanen *et al.* (25)
	TC	12 (12)	18 (36)	32.5 (186)	31.8 (149)	
	CC	1 (1)	2 (4)	6.5 (37)	4.3 (20)	
CYP2C19 +681G>A	GG	69 (158)	42.6 (243)	79 (229)	67.6 (297)	
	GA	25.8 (59)	47.7 (272)	19.3 (56)	29 (128)	
	AA	5.2 (12)	9.7 (55)	1.7 (5)	3.4 (15)	Hilli *et al.* (26); Peng *et al.* (27); Sychev *et al.* (28); Makeeva *et al.* (29)
CYP2C19 +636G>A	GG	93.9 (215)	89.3 (509)	87 (100)	87.7 (386)	
	GA	6.1 (14)	10.7 (61)	0 (0)	12.5 (55)	
	AA	0 (0)	0 (0)	0 (0)	1.8 (8)	
CYP2C9 +430C>T	CC	91 (81)	99.5 (213)	87.9 (80)	77.6 (347)	
	CT	7.9 (7)	0.5 (1)	9.9 (9)	21 (94)	
	TT	1.1 (1)	0 (0)	2.2 (2)	1.3 (6)	Hilli *et al.* (26); Ivashenko *et al.* (30); Miao *et al.* (31); Zhuang *et al.* (32); data from the 1000 Genomes Project (phase_3:FIN population)
CYP2C9 +1075A>C	AA	87.6 (78)	162 (91)	87.9 (80)	87.4 (388)	
	AC	11.2 (10)	16 (9)	11 (10)	12.1 (54)	
	CC	1.1 (1)	0 (0)	1.1 (1)	0.45 (2)	
VKORC1 -1639G>A	GG	1.1 (1)	0.6 (1)	49.5 (45)	48.5 (48)	
	GA	31.5 (28)	15.7 (28)	28.6 (26)	41.4 (41)	
	AA	67.4 (60)	83.7 (149)	22 (20)	10 (10.1)	

Fig.1. The interface of "Lekgen" software application.

CONCLUSION

The data obtained in our study on frequency carriers of polymorphisms of genes *SLCO1B1*, *CYP2C19*, *CYP2C9*, *VKORC1* among the Yakuts may be useful in developing recommendations for a personalized therapy and risk stratification of adverse drug reactions for each region of the Russian Federation.

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