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ORIGINAL ARTICLE

Genetic determinants of dabigatran safety (*CES1 gene rs2244613* polymorphism) in the Russian population: multi-ethnic analysis

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Abstract

This study was aimed to investigate the prevalence of the *CES1 gene* (*c.1168-33A > C, rs2244613*) polymorphism among 12 different ethnic groups living in Russia to provide a basis for future clinical studies concerning genetic determinants of dabigatran safety. The study involved 1630 apparently healthy, unrelated, and chronic medication-free volunteers of both genders from 12 different ethnic groups in Russia: 136 Russians, 90 Avars, 50 Dargins, 46 Laks, 120 Kabardians, 112 Balkars, 244 Ossetians, 206 Mari, 204 Mordvinians, 238 Chuvashes, 114 Buryats and 70 Nanays. Genotyping was performed by using real-time polymerase chain reaction-based methods. The allelic prevalence of the ethnic groups was compared with Caucasus population participating in the RE-LY study. Statistically significant differences for the following gene polymorphism were found between all ethnic groups and RE-LY participants. Based on obtained results, it can be assumed that patients of all ethnic groups living in Russia taking dabigatran have a lower risk of bleeding.

Keywords *CES1* · *Rs2244613* · Dabigatran · Ethnicity · Pharmacogenetics

Introduction

Despite recent progress towards prevention, risk factor identification, and treatment of cardiovascular diseases (CVD), these diseases have remained the leading cause of death globally in the last years [1]. WHO's annual World Health Statistics reports that 17.5 million deaths are attributed to CDV [2]. In Russia CVD mortality remains quite high. Venous thromboembolic events (VTE) are the third leading cause of acute CVD mortality [3]. Atrial fibrillation (AF) and coronary heart disease (CHD) are associated with highly increased risk of death or thromboembolic events [4]. CHD at the same time is one of the most frequent cause of the development of AF itself [5]. Moreover, venous thromboembolism can be developed in patients after total knee arthroplasty.

Until recently, vitamin K antagonists (VKA) were the main modality for the venous thromboembolism treatment and prevention as well as systemic thromboembolism and strokes prevention in patients with AF. Warfarin is currently the most widely used anticoagulant from the VKA group, strongly recommended for the treatment and secondary prevention of venous thromboembolic diseases by all latest clinical guidelines [5]. Although effective, warfarin has important limitations, such as narrow therapeutic range, a need for international normalized ratio (INR) monitoring, a large number of drug–drug interactions, a slow onset of action, and a wide intraindividual variability in drug response [6, 7]. The major genetic determinants involved in the metabolism of warfarin are the CYP2C9 enzyme and the *VKORC1 gene* [8]. Single nucleotide polymorphisms (SNP) of the *CYP2C9* and *VKORC1 genes* have shown a significant effect on warfarin dosing variations and its safety parameters [6–9].

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Another treatment modality for these patients may be non-vitamin K antagonist oral anticoagulant (NOAC), such as dabigatran [10]. Dabigatran is a relatively new reversible direct thrombin inhibitor with low molecular weight.

As dabigatran is a polar molecule, to enhance oral bioavailability it is used as prodrug dabigatran etexilate (DE) (Boehringer Ingelheim International GmbH) [11, 12]. DE has a low bioavailability of 3–7%. After oral intake the plasma levels of DE peak within 1–2 h [12]. DE is metabolized by the carboxylesterases 1 and 2 (CES1, CES2). Shi et al. [13] show that the DE metabolism is much more dependent on the CES1 activity rather than on the CES2 activity, which allows us to consider the CES1 as a main enzyme in the DE biotransformation. Significant interindividual variability in both pharmacokinetics and pharmacodynamics of DE have been consistently reported. The individual variation in the efficacy and tolerability of many drugs metabolized by CES1 is considerable [14–19], which can be explained by a large number of the *CES1 gene* variants [20, 21]. Despite the large number of drugs metabolized by CES1 (oseltamivir, enalapril, simvastatin, clopidogrel, dabigatran etexilate, capecitabine, etc.), data on its clinical significance are still limited. *Rs2244613* is the most studied CES1 gene's SNP affected the dabigatran safety nowadays. The pharmacogenetic studies on *rs2244613* could assist in the development of the optimal anticoagulant therapy for non-valvular AF patients with a high risk of bleeding treatment and VTE prevention in total knee arthroplasty patients.

However, the current situation of racial and ethnic homogeneity of NOACs clinical trials patients [22] creates a problem in adequate extrapolation the study's results on other population's representatives and ethnic groups. These aspects are especially relevant to a such multinational country as Russia, and its regions as the Caucasus, the Volga region, Siberia and the Far East regions.

The present study was aimed to investigate the prevalence of the *CES1 gene rs2244613* polymorphism among 12 different ethnic groups living in Russia, providing a basis for future clinical studies concerning genetic determinants of dabigatran safety.

Materials and methods

Ethics

The study has been performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Russian Medical Academy of Continuous Professional Education of the Ministry of Health of the Russian Federation, Moscow. Written informed consent in Russian language was obtained from all participants before entering the study. According to the informed consent terms all study results could be used for scientific purposes without uncovering personal identifiers.

Study population

The study involved 1630 apparently healthy, unrelated, and chronic medication-free volunteers of both genders from the different ethnic groups living in Russia. 136 Russians (Irkutsk), 90 Avars, 50 Dargins, 46 Laks, 120 Kabardians, 112 Balkars, 244 Ossetians, 206 Mari, 204 Mordvinians, 238 Chuvashes, 114 Buryats and 70 Nanais were enrolled in the study. Belonging to a particular ethnic group was determined as described in the literature, a generally accepted self-identification method [23].

The prevalence of allelic variants of *CES1 rs2244613* was compared with similar indices of the Caucasian population participating in the RE-LY study [24] as the most complete work on the pharmacogenetics study for *rs2244613*.

Genotyping

A venous blood sample (4 mL) was collected from all participants in EDTA (ethylenediaminetetraacetic acid) tubes and kept on ice during transportation to the laboratory. Genomic DNA was extracted from the peripheral blood by using 'DNA-EKSTRAN-1' (ZAO Syntol, Russia) according to the manufacturer's protocol. Gene carriage was determined by real-time polymerase chain reaction (PCR) using kits 'GenTest CES1' (OOO Nomotek, Russia). The genotypes were determined with a TaqMan Single-Nucleotide Polymorphism Genotyping Assay kit and TaqMan Universal PCR Master Mix (Applied Biosystems, USA), according to the manufacturer's instructions, with an ABI PRISM® Sequence Detector 7000 (Applied Biosystems). Genotype polymorphism were detected using Real-Time CFX96 Touch (Bio-Rad Laboratories, Inc.). The cycling program consisted of preliminary denaturation at 95 °C for 3 min, followed by 50 cycles of denaturation at 95 °C for 10 s, annealing at 60 °C for 30 s.

Statistics

Genetic frequencies were tested for deviations from the Hardy–Weinberg equilibrium through Pearson $\chi 2$-test. Differences in allele frequencies between ethnic groups were measured using Fisher's exact test. A p-value < 0.05 was considered statistically significant. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) 22.0 (IBM Corporation, USA) and GraphPad InStat (GraphPad Software, USA).

Results

Allele and genotype frequencies observed in Russians, Avars, Dargins, Laks, Kabardians, Balkars, Ossetians, Mari, Mordovians, Chuvashes, Buryats and Nanays are presented in Table 1.

Upon testing for Hardy–Weinberg equilibrium, departures from equilibrium were detected only in Nanais. Results for the corresponding pairwise comparisons of allele frequencies are summarized in Table 2.

As Russians are the most numerous ethnic group in Russia the results from other ethnics were compared with Russians first. The genotype frequencies of the *rs2244613* in

Table 1 Genotype and allele frequencies for *CES1 gene rs2244613* polymorphism in observed ethnic groups

Ethnic groups	N	Frequency	Genotype			Minor allele frequency, %	Hardy–Weinberg equilibrium test	
			AA	AC	CC		χ^2	p
Russians	136	Obs	70	55	11	28.3	0.0018	0.999
		Exp	69.9	55.2	10.9			
		%	51.5	40.4	8.1			
Caucasus								
Avars	90	Obs	34	43	13	38.3	0.008	0.996
		Exp	34.2	42.6	13.2			
		%	37.8	47.8	14.4			
Dargins	50	Obs	18	24	8	40.0	3.944×10^{-31}	1
		Exp	18.0	24.0	8.0			
		%	36.0	48.0	16.0			
Laks	46	Obs	24	17	5	29.3	0.504	0.777
		Exp	23.0	19.1	4.0			
		%	52.2	37.0	10.9			
Kabardians	120	Obs	40	64	16	40.0	1.304	0.521
		Exp	43.2	57.6	19.2			
		%	33.3	53.3	13.3			
Balkars	112	Obs	48	54	10	33.0	0.733	0.693
		Exp	50.2	49.6	12.2			
		%	42.9	48.2	8.9			
Ossetians	244	Obs	134	87	23	27.3	2.614	0.2707
		Exp	129.1	96.8	18.1			
		%	54.9	35.7	9.4			
Volga Region								
Mari	206	Obs	75	96	35	40.3	0.226	0.8933
		Exp	73.4	99.1	33.4			
		%	36.4	46.6	17.0			
Mordovians	204	Obs	113	78	13	25.5	0.022	0.9892
		Exp	113.3	77.5	13.3			
		%	55.4	38.2	6.4			
Chuvashes	238	Obs	97	104	37	37.4	1.228	0.5411
		Exp	93.3	111.4	33.3			
		%	40.8	43.7	15.5			
Siberia and the Far Eas								
Buryats	114	Obs	17	52	45	62.3	0.159	0.9234
		Exp	16.2	53.6	44.2			
		%	14.9	45.6	39.5			
Nanais	70	Obs	3	40	27	67.1	6.254	0.0438
		Exp	7.56	30.89	31.56			
		%	4.3	57.1	38.6			

Obs. observed genotype, *Exp*. expected genotype

Table 2 Pairwise comparison of allele frequencies for *CES1 gene rs2244613* polymorphism in the twelve tested populations (Fisher's exact test, p-value)

Ethnic group	Russians	Avars	Dargins	Laks	Kabardians	Balkars	Ossetians	Mari	Mordovinians	Chuvashes	Buryats	Nanais
Russians	–	0.0309	0.0332	0.8940	0.0066	0.2811	0.7997	0.0014	0.4264	0.0129	< 0.0001	< 0.0001
Avars	–	–	0.7992	0.1798	0.7625	0.2957	0.0077	0.7150	0.0023	0.8568	< 0.0001	< 0.0001
Dargins	–	–	–	0.1322	1.0000	0.2573	0.0156	1.0000	0.0062	0.6509	0.0003	< 0.0001
Laks	–	–	–	–	0.0767	0.5958	0.7034	0.0577	0.4346	0.1557	< 0.0001	< 0.0001
Kabardians	–	–	–	–	–	0.1241	0.0007	1.0000	0.0001	0.5153	< 0.0001	< 0.0001
Balkars	–	–	–	–	–	–	0.1308	0.0729	0.0521	0.2736	< 0.0001	< 0.0001
Ossetians	–	–	–	–	–	–	–	< 0.0001	0.5946	0.0009	< 0.0001	< 0.0001
Mari	–	–	–	–	–	–	–	–	< 0.0001	0.4073	< 0.0001	< 0.0001
Mordovians	–	–	–	–	–	–	–	–	–	0.0002	< 0.0001	< 0.0001
Chuvashes	–	–	–	–	–	–	–	–	–	–	< 0.0001	< 0.0001
Buryats	–	–	–	–	–	–	–	–	–	–	–	0.3717
Nanais	–	–	–	–	–	–	–	–	–	–	–	–
Comparison with RE-LY study participants												
RE-LY	< 0.0001	< 0.0001	< 0.0001	0.0094	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0005	< 0.0001	< 0.0001	< 0.0001

Russians were: AA 70 (51.5%), 55 AC (40.4%) and 11 CC (8.1%). The minor allele frequency was 28.3%, which is not similar compared with other European populations (16.1%) according to http://www.pharmgkb.org [25] and RE-LY study (18.1%) [24].

Russians live relatively evenly throughout Russia, while the populations in North Caucasus are distinguished by a more compact area of residence. The North Caucasus region provides a great opportunity to investigate the influence of geography on the genetic structure of human populations [26, 27]. Lingua franca absence and religious dissociation also determines the genetic isolation of the Caucasian ethnics.

The Republic of Dagestan is located in the eastern part of the North Caucasus region of Russia. There are 26 out of 50 autochthonous Caucasus ethnic groups live in Dagestan. They are characterized by large genetic differences between populations living in a relatively small area [26, 28]. However, a comparison of *rs2244613* marker alleles frequency in the three ethnic groups of Dagestan (Avars, Dargins, Laks) showed no statistically significant differences between the groups ($p > 0.05$). *Rs2244613* prevalence in Avars and Dargins was the higher compared with Russians and accounted for 40.0% ($p = 0.0309$) and 38.3% ($p = 0.0332$), respectively. There were no statistically significant differences between Russians and Laks (Tables 1, 2).

Abkhaz-Adyghe language speaking populations live on the vast territories from Black Sea coast to Central Caucasus. One of those Kabardins live in Kabardino-Balkarian republic within Russia. The second largest ethnic group in the republic are the Balkarians, the Turkic-speaking population [29]. Despite the relative genetic isolation due to the language and cultural differences between Kabardians and Balkarians, there were no statistically significant differences in the *rs2244613* allele frequency between groups (Table 2). *Rs2244613* allele frequency differs from Russians only with Kabardians ($p = 0.0066$). The frequency of the *C* minor allele in Kabardians was 40.0% versus 28.3% in Russians.

Another Caucasus ethnic group included in our study was Ossetians living on the northern and southern slopes of the Greater Caucasus Range. No significant differences were found between Ossetians and Russians in *rs2244613* allele frequency ($p > 0.05$). Whereas, the comparison with other investigated Caucasian ethnic groups revealed statistically significant differences with Avars ($p = 0.0077$), Darginians ($p = 0.0156$) and Kabardians ($p = 0.0077$) (Table 2). The frequency of the variant rs2244613 allele was approximately the same as Russians − 27.3%, but it was lower than in Avars, Dargins and Kabardians (Table 1).

Another ethnically diverse region of Russia is the Volga region. On the of Volga river basin territory live ethnics of Turkic and Finno-Ugric language groups predominantly belonging to the Caucasus race. The

comparison of distribution of *rs2244613* allele frequencies in the Mari, Mordovians and Chuvash with Russians in the current study showed statistically significant differences only in Mari ($p = 0.0014$) and Chuvash ($p = 0.0129$). The allele frequency among the Mari and Chuvash was higher (40.3% and 37.4%, respectively) than in Russians (28.3%). Comparing Mari, Mordovia and Chuvash with each other revealed the significant differences between Mordovians vs. Mari ($p < 0.0001$) and Mordovians vs. Chuvash ($p = 0.0002$). Mordovian ethnic group has a lower percentage of *rs2244613* marker carriage 25.5%, which is close to the frequency in Russians (Table 1).

The comparison of the marker frequency between the ethnic groups of the Volga region and Caucasus showed the significant differences in the Mari and Chuvash groups in comparison only with Ossetians ($p < 0.0001$ and $p = 0.0009$, respectively), whereas in Mordovians statistically significant differences were observed with Avars ($p = 0.0023$), Dargins ($p = 0.0062$), and Kabardians ($p = 0.0001$) (Table 2).

Siberia and the Far East regions possess unique demographic characteristics of ethnic heterogeneity. The disproportionate distribution of small populations across an extensive stretch of territory (average population density of approximately 3 individuals per 1 km^2) explain why some autochthonous groups remain anthropologically, linguistically and genetically different from each other. Buryats is the indigenous population of the Republic of Buryatia in South Siberia. The Nanais is indigenous ethnic minority of the Far East. Both ethnics belong to the Mongoloids. There were no statistically significant differences in *rs2244613* prevalence between Buryats and Nanais, but such differences were found in comparison with all other included ethnic groups (Table 2). High level of *rs22244613* allele prevalence (62.3% in Buryats and 67.1% in Nanais) compared with other ethnic groups suggest the significant *rs22244613* difference between the Mongoloid and Caucasus race. This statement correlates with the data on *rs2244613* allele frequency among Chinese in previously published results (60%) [30].

Overall, we compared the frequency of *rs2244613* variant in studied ethnic groups with RE-LY study [24] Caucasian race participants. Statistically significant differences were found in all groups (Table 2). This fact requires special attention due to the large difference in the *rs2244613* variant frequency in RE-LY participants compared to the ethnic groups in Russia. For Caucasus race groups the frequency was accounted for 22.5% in Mordovians and 40.3% in Mari; for Mongoloids race groups the frequency was accounted for 62.3% in Buryats and for 67.1% in Nanais versus 18.3% in RE-LY. In Russians, the minor allele frequency was 28.3%, which is 1.5 times higher than in RE-LY participants.

Discussion

Despite the huge progress in pharmacogenetic studies and the growing role of pharmacogenetic technologies in the routine clinical practice in Europe and in the United States, the problem of the application of the results of such studies in populations that differ from European and American ones remains unresolved. This is a key point since the reported frequency of alleles and genotypes for one population may not be applicable to another population.

Ethnic drug susceptibility is associated with genetic characteristics of drugs biotransformation enzymes [9, 22]. The gene encoding such enzymes are characterized by high ethnic heterogeneity and the reported genetically determined pharmacokinetic and pharmacodynamic characteristics of the one ethnic group may not be extrapolated to another. For instance, the hepatic enzyme CYP2C19 promotes the metabolism of a large number of drugs (benzodiazepines, some proton pump inhibitors, clopidogrel). The *CYP2C19 gene* as well as many other members of the cytochrome P450 superfamily [9, 28] is also highly polymorphic. It has more than 25 known allele variants and it is characterized by high ethnic heterogeneity [31]. The frequency of *CYP2C19*2* allele variants that causes resistance to clopidogrel treatment is 54% in Pakistanis, 11.5% in Tunisians [32] and only 5.61% in Thai population [33]. Another example of interethnic differences is the *CYP2C9 gene* [34, 35] that determine the warfarin sensitivity. Carriage of the *CYP2C9*2* and *CYP2C9*3* variants is associated with reduced isoenzyme activity and increased concentration of warfarin with an excessive risk of bleeding and hypocoagulation [6, 7]. These findings indicate that pharmacogenetic testing is as a new tool to assess the treatment efficacy and safety.

Cytochrome P450 variations in different ethnic populations may help us to suppose such variations in genes encoding enzymes that metabolize NOACs, in particular dabigatran. The data of the leading resource on pharmacogenetics (http://www.pharmgkb.org) confirm this argument [25].

Dabigatran is one of the therapeutic alternatives to warfarin in the strokes and systemic thromboembolism prevention in patients with non-valvular AF. Dabigatran has more favorable drug–drug interaction profile, wide therapeutic range, fixed-dosing regimens, and does not require the INR monitoring. However, the problem of interindividual variability of dabigatran concentration followed by the risks of hemorrhagic complications remains unsolved. Idarucizumab has been recently approved as a direct dabigatran-specific antidote. Nevertheless, it does not provide complete hemostasis in some cases [36].

As we noted above the CES1 is the main DE biotransformation enzyme. The *CES1 gene* in humans is located in

Table 3 Genotype, allele frequencies of the *CES1* gene *rs2244613* polymorphism in previously published studies

Race (ethnicity)	Diseases	N	Genotype, %			Allele frequencies, %		References
			AA	AC	CC	A	C	
Caucasian	nAF[a]	1490	67.2	29.4	3.4	81.9	18.1	RE-LY [23]
Caucasian (Italians)	nAF	90	57.6	40.2	2.2	77,7	22.3	Dimatteo et al. [25]
Caucasian (New Zealanders)	nAF	52	73.1	23.1	3.8	84.7	15.3	Chin et al. [43]
Caucasian (Swiss)	onco[b]	143	x	x	x	82	18	Hamzic et al. [19]

[a]*nAF* non-valvular atrial fibrillation

[b]*onco*. capecitabine treated oncological diseases

the *16q13-q22.1* locus. Over the past decade, an enormous amount of data on single-nucleotide polymorphisms of the *CES1 gene* has appeared in the NCBI database [37]. In a RE-LY sub-study [24] genotyping was performed in 2944 Caucasian patients with AF and for thromboembolic events taking dabigatran. Plasma dabigatran concentration was assessed in 1490 patients. The results showed that the carriage of *CES1 gene's* polymorphism *c.1168-33A > C (rs2244613)* occurred in 38.2% of patients and was associated with lower dabigatran trough concentrations (Css_{min}). In heterozygote patients (AC) carrying 1 minor allele the Css_{min} value was lower by 15%, while in homozygotes (CC)—by 28% compared to non-carriers. Polymorphism *c.257 + 885T > C (rs8192935)* carriage was associated with a decrease in the adjusted peak concentrations (Css_{max}) of dabigatran by 12% ($p = 3.2 \times 10^{-8}$), but not Css_{min}. The CES1 SNP *rs2244613* was significantly associated with the decrease in the number of major bleeding, while carriage of *rs8192935* did not affect the risk of bleeding. The bleeding risk is consistent with the levels of the reduced trough dabigatran concentrations in *rs2244613* carriers [24]: the decrease of Css_{min} by 15% corresponded to a 27% decrease in the relative risk of bleeding which was standardized for dabigatran dose, patient's age, gender, CHADS2 score, concomitant use of aspirin and creatinine clearance. The comparison of the treatment regimens (warfarin or dabigatran) and *rs2244613* carriage shows statistically significant reduction in the number of bleedings in *rs2244613* carriers ($p = 5.2 \times 10^{-5}$) taking dabigatran compared to warfarin patients. However, no statistically significant difference was observed between the two treatment groups in noncarriers ($p = 0.65$). An important point is that *CES1* SNP *rs2244613* was not associated with the frequency of ischemic events or systemic embolism ($p = 0.34$) as the main efficacy index [6, 24, 38].

Another study [39] of 92 Caucasian patients with AF showed the significantly positive correlation between *CES1* SNP *rs8192935* and decreased dabigatran trough concentration levels. Dabigatran trough concentration levels decreased by 3% in heterozygotes (CT) and by 11% in homozygotes (TT) ($p = 0.055$ and $p = 0.033$, respectively).

The polymorphism *c.428G > A (rs71647871)* encodes the-loss-of-function *CES1* variant [13]. This allelic variant is related to the decrease in the activation level of DE in liver cells in vitro. However, up to now there has been no detailed investigation of this polymorphism in patients taking DE. *Rs2244613* and *rs8192935* were found to be unrelated to DE activation and *CES1* expression and activity [13].

Overall, the *CES1 gene rs2244613* polymorphism can affect the biotransformation of dabigatran and its concentration [11, 15, 20, 24, 39, 40], which in turn can determine the safety profile. Several studies reported a correlation between the dabigatran plasma concentration and its anticoagulant effect [41, 42].

These studies highlight the potential using of pharmacogenetics studies as an important tool in personalized anticoagulant therapy and the selection of NOACs, such as dabigatran. However, most of the pharmacogenetics studies on dabigatran generally and studies on *rs2244613* particularly were conducted with European ancestry participants (see Table 3).

As we can see, the *rs2244613* prevalence among Caucasian ethnic groups in Russia from our study is higher in comparison with the frequency of this gene among Caucasians from the studies in the Table 3. This fact confirms the problem of extrapolation the study's results from one population to another. At the same time, it was shown in [44] that the *rs2244613* polymorphism is equally common in both healthy and CVD patients. This result suggests the absence of connection between polymorphism carriage and the disease.

The results show that minor allele of the *CES1* SNP polymorphism *rs2244613* was associated with decreased dabigatran concentration and a lower risk of bleeding in dabigatran-treated patients. This polymorphism plays a key role in the interindividual variability of dabigatran concentration and possibly determines ethnic differences in sensitivity to

dabigatran. This assumption can serve as a basis for the creation of national pharmacotherapy guidelines, which will take into account the region's ethnicity. Studies on ethnic drug sensitivity are especially relevant for Russia with its various racial-ethnic groups regions (the Caucasus, the Volga region, Siberia and the Far East). Data obtained from this study could help to assess the priority of implementation of genotyping in ethnic diverse regions in Russia.

Conclusion

The study has shown the *CES1 gene* SNP *rs2244613* frequency heterogeneity in different ethnic groups in Russia. Based on these findings and the RE-LY conclusions [24] about *rs2244613* carriage association with dabigatran concentration and the risk of bleeding we can suppose that patients taking dabigatran in ethnic groups living in Russia have a lower risk of bleeding.

Future prospective pharmacogenetics studies regarding the dabigatran pharmacokinetics and drug response in different ethnic groups are required to clarify the effect of *rs2244613* on the efficacy and safety of dabigatran and to develop a personalized approach to dabigatran patients treatment.

Study limitations

The number of subjects included into each ethnic group was limited by the capabilities of the recruitment center. It is need to be considered that possible small sample size might be insufficient to estimate allele frequency and genotype distribution fully. The recruitment was carried out firstly in highly multiethnic regions of the Caucasus and the Volga regions following by Siberia and the Far East regions. Russians was recruited as the most numerous ethnic group.

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Compliance with ethical standards

Conflict of interest The work was carried out with the financial support of OOO Boehringer Ingelheim (Moscow, Russian Federation). All the authors report no conflicts of interest in this work.

References

1. Alwan A (2011) Global status report on noncommunicable diseases 2010 Introduction. In: A. Alwan, editor. Global status report on noncommunicable diseases 2010. World Health Organization, Geneva. pp VII–IX. https://www.who.int/nmh/publications/ncd_report2010/en/. Accessed 26 Aug 2018
2. World Health Organization website. http://www.who.int/cardiovascular_diseases/ru. Accessed 26 Aug 2018
3. Goldhaber SZ, Bounameaux H (2012) Pulmonary embolism and deep vein thrombosis. Lancet 379(9828):1835–1846. https://doi.org/10.1016/S0140-6736(11)61904-1
4. Napalkov DA, Sokolova AA, Rodionov AV (2016) Atrial fibrillation and ischemic heart disease: how to combine antiplatelet and anticoagulant therapy, depending on the clinical situation? Ration Pharmacother Cardiol 2(21):191–195. https://doi.org/10.20996/1819-6446-2016-12-2-191-195 (In Russ)
5. Kirchhof P, Benussi S, Kotecha D et al (2016) 2016 ESC Guidelines for the management of atrial fibrillation developed in collaboration with EACTS. Eur J Cardiothorac Surg 50(5):1–88. https://doi.org/10.1093/ejcts/ezw313
6. Ross S, Pare G (2013) Pharmacogenetics of antiplatelets and anticoagulants: a report on clopidogrel, warfarin and dabigatran. Pharmacogenomics 14(13):1565–1572. https://doi.org/10.2217/pgs.13.149
7. Sychev DA, Kazakov RE, Otdelenov VA, Prokofiev AB (2013) Applications of pharmacogenetic testing for personalization of therapy with oral anticoagulants in Russia. Ration Pharmacother Cardiol 9(5):525–531. https://doi.org/10.20996/1819-6446-2013-9-5-525-531
8. Eriksson N, Wadelius M (2012) Prediction of warfarin dose: why, when and how? Pharmacogenomics 13(4):429–440. https://doi.org/10.2217/pgs.11.184
9. Johnson JA (2008) Ethnic differences in cardiovascular drug response: potential contribution of pharmacogenetics. Circulation 118(13):1383–1393. https://doi.org/10.1161/CIRCULATIONAHA.107.704023
10. Mazur-Bialy AI, Zdebska K, Wypasek E, Undas A (2013) Repeated bleeding complications during therapy with vitamin K antagonists in a patient with the VKORC1*2A and the CYP2C9*3/*3 alleles: genetic testing to support switching to new oral anticoagulants. Thromb Res 131(3):279–280. https://doi.org/10.1016/j.thromres.2012.12.008
11. Blech S, Ebner T, Ludwig-Schwellinger E et al (2008) The metabolism and disposition of the oral direct thrombin inhibitor, dabigatran, in humans. Drug Metab Dispos 36:386–399. https://doi.org/10.1124/dmd.107.019083
12. Stangier J, Eriksson BI, Dahl OE et al (2005) Pharmacokinetic profile of the oral direct thrombin inhibitor dabigatran etexilate in healthy volunteers and patients undergoing total hip replacement. J Clin Pharmacol 45:555–563. https://doi.org/10.1177/0091270005274550
13. Shi J, Wang X, Nguyen JH, Bleske BE et al (2016) Dabigatran etexilate activation is affected by the CES1 genetic polymorphism G143E (rs71647871) and gender. Biochem Pharmacol 119:76–84. https://doi.org/10.1016/j.bcp.2016.09.003
14. Zhu HJ, Wang X, Gawronski BE et al (2013) Carboxylesterase 1 as a determinant of clopidogrel metabolism and activation. J Pharmacol Exp Ther 344:665–672. https://doi.org/10.1124/jpet.112.201640
15. Shi J, Wang X, Eyler RF et al (2016) Association of oseltamivir activation with gender and carboxylesterase 1 genetic polymorphisms. Basic Clin Pharmacol Toxicol 199:555–561. https://doi.org/10.1111/bcpt.12625
16. Nemoda Z, Angyal N, Tarnok Z et al (2009) Carboxylesterase 1 gene polymorphism and methylphenidate responsein ADHD. Neuropharmacology 57:731–733. https://doi.org/10.1016/j.neuropharm.2009.08.014
17. Wang X, Wang G, Shi J et al (2016) CES1 genetic variation affects the activation of angiotensin-converting enzyme inhibitors. Pharmacogenomics J 16:220–230. https://doi.org/10.1038/tpj.2015.42

18. Tarkiainen EK, Tornio A, Holmberg MT et al (2015) Effect of carboxylesterase 1 c.428G> A single nucleotide variation on the pharmacokinetics of quinapril and enalapril. Br J Clin Pharmacol 80:1131–1138
19. Hamzic S, Kummer D, Milesi S et al (2017) Novel genetic variants in carboxylesterase 1 predict severe early onset capecitabine-related toxicity. Clin Pharmacol Ther 102(5):796–804. https://doi.org/10.1111/bcp.12667
20. Merali Z, Ross S, Pare G (2014) The pharmacogenetics of carboxylesterases: CES1 and CES2 genetic variants and their clinical effect. Drug Metabol Drug Interact 29(3):143–151. https://doi.org/10.1515/dmdi-2014-0009
21. Rasmussen HB, Bjerre D, Linnet K et al (2015) Individualization of treatments with drugs metabolized by CES1: combining genetics and metabolomics. Pharmacogenomics 16(6):649–665. https://doi.org/10.2217/pgs.15.7
22. Jackson LR, Peterson ED, Okeagu E, Thomas K (2015) Review of race/ethnicity in non-vitamin K antagonist oral anticoagulants clinical trials. J Thromb Thrombolysis 39(2):222–227. https://doi.org/10.1007/s11239-014-1145-7
23. Tang H, Quertermous T, Rodriguez B et al (2005) Genetic Structure, Self-Identified Race/Ethnicity, and Confounding in Case-Control Association Studies. Am J Hum Genet 76(2):268–275. https://doi.org/10.1086/427888
24. Pare G, Eriksson N, Lehr T et al (2013) Genetic determinants of dabigatran plasma levels and their relation to bleeding. Circulation 127:1404–1412. https://doi.org/10.1161/CIRCULATIONAHA.112.001233
25. PharmGKB website: https://www.pharmgkb.org/variant/PA166155037/overview. Accessed 1 Sept 2018
26. Caciagli L, Bulayeva K, Bulayev O et al (2009) The key role of patrilineal inheritance in shaping the genetic variation of Dagestan highlanders. J Hum Genet 54(12):689–694. https://doi.org/10.1038/jhg.2009.94
27. Yunusbayev B, Kutuev I, Khusainova R et al (2006) Genetic structure of Dagestan populations: a study of 11 Alu insertion polymorphisms. Hum Biol 78(4):465–476. https://doi.org/10.1353/hub.2006.0059
28. Mirzaev KB, Sychev DA, Ryzhikova KA et al (2017) Genetic polymorphisms of cytochrome P450 enzymes and transport proteins in a Russian population and three ethnic groups of Dagestan. Genet Test Mol Biomarkers 21(12):747–753. https://doi.org/10.1089/gtmb.2017.0036
29. Balanovsky OP (2017) Gene pool of Europe. The Partnership of Scientific Publications, Moscow (In Russ.)
30. Gu ZC, Ma XW, Zheng XY et al (2018) Left atrial appendage thrombus formation in a patient on dabigatran therapy associated with ABCB1 and CES-1 genetic defect. Front Pharmacol 9:491. https://doi.org/10.3389/fphar.2018.00491
31. Golukhova EZ, Grigoryan MV, Ryabinina MN (2014) Modern aspects of clopidogrel pharmacogenetics and its clinical relevance. Creat Cardiol 3:39–52. (In Russ.)
32. Rehman KU, Akhtar T, Sabar MF, Tariq MA (2015) Allele frequency distribution of CYP2C19*2 allelic variants associated with clopidogrel resistance in cardiac patients. Exp Ther Med 10(1):309–315. https://doi.org/10.3892/etm.2015.2493
33. Sukasem C, Tunthong R, Chamnanphon M et al (2013) CYP2C19 polymorphisms in the Thai population and the clinical response to clopidogrel in patients with atherothrombotic-risk factors. Pharmgenomics Pers Med 6:85–91. https://doi.org/10.2147/PGPM.S42332
34. Sychev DA, Shuev GN, Suleymanov SS et al (2017) Comparison of CYP2C9, CYP2C19, CYP2D6, ABCB12017, and SLCO1B1 gene-polymorphism frequency in Russian and Nanai populations. Pharmgenomics Pers Med 10:93–99. https://doi.org/10.2147/PGPM.S129665
35. Romodanovsky DP, Khapaev BA, Ignatiev IV et al (2010) Frequencies the «slow» allele variants of the genes coding isoenzymes of cytochrome P450 CYP2D6, CYP2C19, CYP2C9 in Karachaevs and Circassians. Biomedicine 2:33–37 (In Russ.)
36. Dager WE, Banares L (2017) Reversing the anticoagulation effects of dabigatran. Hosp Pract 45(2):29–38. https://doi.org/10.1080/21548331.2017.1298389
37. NCBI website: https://www.ncbi.nlm.nih.gov/snp/?term=CES1. Accessed 14 Sept 2018
38. Baker WL, Johnson SG (2016) Pharmacogenetics and oral antithrombotic drugs. Curr Opin Pharmacol 27:38–42. https://doi.org/10.1016/j.coph.2016.01.008
39. Dimatteo C, D'Andrea G, Vecchione G et al (2016) Pharmacogenetics of dabigatran etexilate interindividual variability. Thromb Res 144:1–5. https://doi.org/10.1016/j.thromres.2016.05.025
40. Sychev DA, Levanov AN, Shelekhova TV et al (2018) The impact of ABCB1 (rs1045642 and rs4148738) and CES1 (rs2244613) gene polymorphisms on dabigatran equilibrium peak concentration in patients after total knee arthroplasty. Pharmgenomics Pers Med 11:127–137. https://doi.org/10.2147/PGPM.S169277
41. Reilly PA, Lehr T, Haertter S et al (2014) The effect of dabigatran plasma concentrations and patient characteristics on the frequency of ischemic stroke and major bleeding in atrial fibrillation patients: the RE-LY Trial (Randomized Evaluation of Long Term Anticoagulation Therapy). J Am Coll Cardiol 63:321–328. https://doi.org/10.1016/j.jacc.2013.07.104
42. Eriksson BI, Dahl OE, Buller HR et al (2005) A new oral direct thrombin inhibitor, dabigatran etexilate, compared with enoxaparin for prevention of thromboembolic events following total hip or knee replacement: the BISTRO II randomized trial. J Thromb Haemost 3:103–111. https://doi.org/10.1111/j.1538-7836.2004.01100.x
43. Chin PK, Wright DF, Zhang M et al (2014) Correlation between trough plasma dabigatran concentrations and estimates of glomerular filtration rate based on creatinine and cystatin C. Drugs R D 14(2):113–123. https://doi.org/10.1007/s40268-014-0045-9
44. Mirzaev KB, Osipova DV, Kitaeva EYu et al (2018) CES1 gene polymorphism effect on the antiplatelet activity of clopidogrel. Clin Pharmacol Ther 27(5):96–100. https://doi.org/10.32756/0869-5490-2018-5-96-100 (In Russ)

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