YAKUT MEDICAL JOURNAL 2(46)2014

VKORC1 and *CYP2C9* Genes Polymorphisms, Affecting the Sensitivity of Anticoagulant Therapy in Patients with Acute Cerebral Circulation Impairment

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ABSTRACT

Preliminary data of the frequency of genetic variants *VKORC1* and *CYP2C9*, affecting sensitivity to anticoagulant therapy, in Asian indigenous and Caucasian patients with acute ischemic stroke in Yakutia is presented in this article. The frequencies of increased warfarin sensitivity alleles *CYP2C9*2* (rs1799853), *CYP2C9*3* (rs1057910) and C1173T *VKORC1* (rs9934438) polymorphism's variants were determined in the group of 15 patients. *CYP2C9* genotyping revealed 3 heterozygous for *CYP2C9**1/*2. The allelic variant *CYP2C9*3* have not been identified in this group. *VKORC1* genotyping revealed 4 heterozygous for 1173CT *VKORC1*. There were 22.2% carrier with allele *CYP2C9*2* and 22.2% with T allele of C1173T *VKORC1* polymorphism among Asian indigenous patients. Genotyping of *VKORC1* and *CYP2C9*, including *CYP2C9*2* allele, is recommended for personified prevention of the cardioembolic stroke in Yakutia.

Keywords: *VKORC1* gene polymorphism, *CYP2C9* gene polymorphism, cardioembolic stroke, warfarin.

INTRODUCTION

The oral anticoagulants have used for the primary and secondary stroke prevention in the certain clinical situations. Warfarin, the most commonly prescribed anticoagulant, significantly reduces the risk of cardioembolic ischemic stroke and transient ischemic attacks if it received the adequate dose [2, 7]. In patients with atrial fibrillation the anticoagulant's efficiency for stroke

prevention is much higher than the one of the aspirin therapy (risk reduction 64 % and 22 %, respectively) [8]. The warfarin effect depends from many factors such the individual characteristics (e.g., body size, gender, race), diet, medication, liver and kidneys diseases, as well as other factors.

It has been established that the sensitivity to warfarin dependents on genetic factors, e.g. , genotype isoforms of CYP2C9, which codes for the cytochrome P450, and VCORC1, which codes one subunit of vitamin K epoxide reductase complex [9]. *VKORC1* gene mutations associated with the sensitivity to warfarin [10]. C1173T *VKORC1* polymorphism associated with increased sensitivity to warfarin. Carriers of the mutant 1173T allele require lower doses of warfarin compared to carriers of allele 1173C *VKORC1*, while the average daily dose is reduced by 43 % for homozygous 1173TT *VKORC1* and by 22 % for heterozygous 1173CT *VKORC1* [1]. It was found differences in genetic polymorphisms frequency that cause different sensitivity to warfarin. For example, African Americans are relative stability and Asians are relative sensitivity to warfarin [4]. The low dose haplotype of *VKORC1* leads to the rapid achievement of target INR, on the other hand, also leads to INR be more than 4 quickly and is associated with the hemorrhagic complications risk [6]. Currently *CYP2C9* genotypes are recognized the most clinically significant factors for the diagnosis of sensitivity to warfarin. Alleles *CYP2C9*2* and *CYP2C9*3* are associated with a slow metabolism of the warfarin [13]. The frequency of the "slow" *CYP2C9* alleles varies widely in different ethnic groups [3, 11]. *CYP2C9* gene polymorphisms also affect to the time INR achieve > 4 [6].

Racial differences in the frequency of "defect" alleles which affect warfarin metabolism require consideration in therapy. Thus, Asian patients must have lower average dose of warfarin, while patients of the black race - higher average dose for therapeutic INR levels [12].

Aims: To study the frequency of *VKORC1* and *CYP2C9* allele polymorphisms, affecting the metabolism of warfarin, in acute ischemic stroke Asian indigenous and Caucasian patients in Yakutia.

MATERIAL AND METHODS

The study group includes 15 patients with acute ischemic attacks and stroke, which were hospitalized in the Regional Vascular Center (Yakutsk) in 2013 and received warfarin therapy for secondary stroke prevention. The diagnosis was confirmed by the data of neurological examination, medical history, neuroimaging. Computed tomography (CT) of the brain was performed on 64-slice CT scanner multispiral Siemens SOMATOM Definition AS, the data were

interpreted by radiologist. Ultrasound of the heart and brachiocephalic vessels carried on the unit AcusonS 2000 (SiemensAG). The blood coagulation (international normalized ratio (INR), activated partial thromboplastin time (aPTT), PTI (prothrombin index)), complete blood count, electrocardiogram were performed. All patients provided informed consent. Genetic study was conducted in "Genomic Medicine" laboratory of Medical Clinic of NEFU named after M.K. Ammosov. DNA extraction was performed using a set ExtraGene (Germany) and phenol-chloroform method. Genetic typing of the *CYP2C9*2* (rs1799853), *CYP2C9*3* (rs1057910) and *VKORC1* C1173T (rs9934438) held with PCR kits "Литех" (Moscow).

RESULTS AND DISCUSSION

There were 7 male patients (46.7 %) in the study group (n = 15). The average age was 65 years (min – 41, max – 81). There were 9 patients (60 %) of the indigenous Asian ethnicity, 5 patients (33.3 %) of Caucasian race, and others – 1 (6.7 %). 13 ischemic stroke cases (86.7 %) and 2 transient ischemic attack cases (13.3 %) were diagnosed. The following comorbid diseases were diagnosed: atrial fibrillation – 11 cases (73.3 %), heart defects – 4 (26.7%), including operated heart valves – 2 (13.3%), chronic rheumatic disease – 5 (33.3%), ischemic heart disease (IHD) – 9 (60%), acute myocardial infarction – 1 (6.7%), hypertension – 13 (86.7 %), diabetes mellitus – 3 (20 %), dilatation of the heart – 9 (60 %), osteoarthritis – 1 (6,7 %). Patients had no contraindications to anticoagulation therapy, including hematologic diseases, peptic ulcer disease, liver or kidney failure, hemorrhagic stroke in history, severe degree of hypertension at the time of the survey.

The genotyping of *CYP2C9* identified homozygous of allele *CYP2C9*1* in most cases (n = 12, 80%). Heterozygous carriers of *CYP2C9*2* detected in 3 cases (20%), all of them had heterozygotes with *CYP2C9*1/*2*. The allelic variant *CYP2C9*3* have not been identified in this group.

Genotyping C1173T *VKORC1* found 11 cases of homozygous with allele C (73.3 %). Heterozygotes of this polymorphism C/T were found in 4 cases (26.7 %). Homozygous carriers of the T allele *VKORC1* were not found in this group. The genotypes frequencies of *CYP2C9* and *VKORC1* in the target group are presented in Table 1.

Table 1

CYP2C9 and *VKORC1* genotypes frequencies depending on ethnicity and gender

Ethnicity	Gender	Genotype			
		*CYP2C9 *1/*1*	*CYP2C9 *1/*2*	*VKORC1 1173 C/C*	*VKORC1 1173 C/T*
indigenous of Asian race (n=9) (abs.)	male	3	1	3	1
	female	4	1	4	1
Caucasian race (n=5) (abs.)	male	3	0	3	0
	female	1	1	0	2
others (n=1) (abs.)	female	1	0	1	0
Total (abs, %)	15 (100)	12 (80)	3 (20)	11 (73,3)	4 (26,7)

Thus, the proportion of carriers of increased warfarin sensitivity alleles was 40 % (n = 6), including 4 heterozygous for T allele C1173T polymorphism *VKORC1* (26.7 %) and 3 carrier of *CYP2C9*2* genotype (20 %), while 1 patient was both "defect" alleles carrier. Among the carriers of "defect" alleles there were 4 Asian indigenous patients (genotype CYP2C9 *1/*2 (n = 2 , 22.2 % of this ethnic group) and genotype C/T of polymorphism C1173T *VKORC1* gene (n = 2 , 22.2 %); and there were 2 Caucasian patients, one of which was with two "defect" allele (genotype *CYP2C9* *1/*2 (n = 2 , 40% of this ethnicity) and genotype C/T of C1173T polymorphism *VKORC1* gene (n = 1, 20%)).

It was found that there are ethnic differences in the frequencies of *CYP2C9* gene variants. Thus, according to [5], in the Korean population allelic variant *CYP2C9*3* is less common compared to the European one. Allelic variant *CYP2C9*2* is absent in East Asian populations, including Koreans, or it is present in a small proportion of cases and is more rare than allelic variant *CYP2C9*3*. Accordingly, it was concluded that routine *CYP2C9*2* genotyping no need in this population [5]. Our data, on the contrary, confirm the need for allele *CYP2C9*2* typing for the study of individual sensitivity to warfarin among Asian indigenous in Yakutia.

We present two clinical reports of acute ischemic stroke cases with the mapping *CYP2C9* and *VKORC1* genotyping data on the dynamics of blood coagulation due the warfarin therapy. The demographic and laboratory data, including concomitant diseases, addictions, medications, initial warfarin dose are presented.

Clinical report 1. Patient R., 68 years old, Russian, male gender, diagnosis: Ischemic stroke in the left posterior cerebral artery (cardioembolic variant). Patient also had ischemic stroke in 2012; IHD, miocardial infarction in hystory, exertional angina FC II; permanent atrial fibrillation, tachysystolic variant; heart defect, mitral valve and tricuspidal valve failure 1 degree; secondary dilatation of both atria of the heart; chronic heart failure II A; hypertension stage III; hypertrophy of the left ventricle; multifocal atherosclerosis; osteoarthritis of ankle joints.

Height 175 cm, weight 95 kg. No smoker. Medications: amiodarone, antifungals, sulfonamides at the time of the study – no; statins – yes (atoris 20 mg drug); nonsteroidal anti-inflammatory drugs (NSAIDs) – yes (75 mg per day). Initial INR – 1.14; Platelets – 242 10^9/ L; PTI – 82.1 %; aPTT – 29.7; initial warfarin dose – 2.5 mg. Genotype: combination *CYP2C9*1/*1* allele and C/C of C1173T polymorphism *VKORC1* gene.

INR value has not reached the "therapeutic range" on the initial dose of warfarin 2.5 mg per day (INR less than 2.0). The anticoagulant low-dose was appointed in connection with the need for NSAIDs due to the joints disease. On the 8th day INR value remained low and amounted to 1.21, therefore the dose correction was performed.

Clinical report 2. Patient M., 72 years old, indigenous ethnicity, female gender, diagnosis: Transient ischemic attack in vertebral-basilar system. Patient also had IHD, exertional angina FC II; permanent atrial fibrillation, normosistolic variant; нypertension stage III; hypertensive heart disease III; secondary dilatation of the heart; hypertrophy of the left ventricle; multifocal atherosclerosis.

Height 164 cm, weight 67 kg. No smoker. Medications: amiodarone, antifungals, sulfonamides at the time of the study - no; statins – yes (atoris 20 mg). Initial INR – 1.13; Platelets – 159X10^9/ L; PTI – 81.1 %; aPTT – 25; initial warfarin dose – 2.5 mg. Genotype: combination *CYP2C9*1/*2* and C/C of C1173T polymorphism *VKORC1* gene.

The initial warfarin dose was 2.5 mg per day. The INR value achieved the "therapeutic range" to 7th day (INR 2.26). Subsequently, the warfarin dose 2.5 mg daily was prescribed after discharge from the hospital under the supervision of laboratory INR. During 6 months the patient's INR value was in the range from 2.21 to 2.76 with 2.5 mg warfarin daily.

These case reports demonstrate the various blood coagulation dynamics in response to an identical dose during the first few days of warfarin therapy. Patient M. (case report 2) had genotype *CYP2C9*1/*2* with functionally "defect" allele and thus had «slow» warfarin metabolism, and so there was a more rapid achievement of INR «therapeutic range». Warfarin «slow» metabolism causes the hypocoagulation state for a long time. Carriage of this genotype

suggests a tendency to haemorrhagic complications [6], so recommendation of warfarin low dose need to patients. In case report 2 the therapeutic effect (INR value of 2.0 to 3.0) could be achieved at a low dose warfarin (2.5 mg) during a long time (6 months). Subsequently this patient should be monitored INR carefully to avoid haemorrhagic complications.

Patient R. (case 1) had not "defect" alleles of studied polymorphisms (genotype *CYP2C9 *1/*1*; 1173CC *VKORC1*). During the first days of warfarin therapy the INR value remained low at 2.5 mg and did not reach the "therapeutic threshold". The carriage of this genotype is associated with a "fast" warfarin metabolism and thus to a more rapid elimination of the drug, respectively, the patient needs to the higher dose.

Recently the genetic testing is recommended for optimization of the selection of the warfarin start dose. For example, according to [14], algorithm including genetic typing of genes *CYP2C9* and *VKORC1*, improves the prognosis of the optimal dose in comparison with algorithms based on clinical and demographic factors solely.

CONCLUSION

Preliminary data of the frequency of genetic variants *VKORC1* and *CYP2C9*, affecting sensitivity to anticoagulant therapy, in acute ischemic stroke indigenous Asian and Caucasian patients in Yakutia is presented in this article. The proportion of carriers of *VKORC1* and *CYP2C9* genotypes, determining high sensitivity to warfarin, accounted 40 % in the observed group. There were 22.2% carrier with allele *CYP2C9*2* and 22.2% with T allele of C1173T *VKORC1* polymorphism among Asian indigenous patients.

Blood coagulation control taking into data of *CYP2C9* and *VKORC1* polymorphisms genotyping contributes to the safety and effectiveness of warfarin therapy in secondary prevention of cardioembolic stroke. It is necessary to note the presence of confounding factors affecting metabolism, including the medication, comorbid diseases, gender, ethnicity. Genotyping of *VKORC1* and *CYP2C9*, including *CYP2C9*2* allele, is recommended for personified prevention of cardioembolic stroke in Yakutia.

REFERENCES

1. A polymorphism in the VKORC1 gene is associated with an interindividual variability in dose-anticoagulant effect of warfarin / G. D`Andrea, RL. D`Ambrosio, P. Di Perna [et al.] // Blood. – 2005. - V.105 (2). – P.645 – 649.
2. Antithrombotic therapy in atrial fibrillation: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition) / D.E. Singer, G.W. Albers, J.E. Dalen et al. // Chest. – 2008. - V.133. Suppl. 6. – P.546 – 592.

3. Detection of CYP2C9 polymorphism based on the polymerase chain reaction in Chinese / S.L. Wang, J. Huang, M.D. Lai, J.J. Tsai // Pharmacogenetics. – 1995. - V.5. – P.37 – 42.
4. Effect of VKORC1 haplotypes on transcriptional regulation and warfarin dose / M.J. Rieder, A.P. Reiner, B.F. Gage [et al.] // N. Engl. J. Med. – 2005. - V.352 (22). – P. 2285 – 2293.
5. Frequency of cytochrome P450 2C9 mutant alleles in a Korean population / Y.R. Yoon, J.H. Shon, M.K. Kim et al. // British Journal of Clinical Pharmacology. – 2001.-V. 51(3). – P. 277 – 280.
6. Genetic determinants of response to warfarin during initial anticoagulation. / U. I. Schwarz, M. Ritchie., Y. Bradford [et al.] // N. Engl. J. Med. – 2008.- V.358 (10). – P. 999 – 1008.
7. Guidelines for the Prevention of Stroke in Patients With Stroke or Transient Ischemic Attack / K.L. Furie, S.E. Kasner, R.J. Adams et al. // Stroke. – 2011. - V. 42. – P.227– 276.
8. Hart, R.G. Meta–analysis: Antithrombotic therapy to prevent stroke in patients who have nonvalvular atrial fibrillation / R.G. Hart, L.A. Pearce, M.I. Aguilar // Ann. Intern Med. – 2007.- V.146 (12). – P.857 – 867.
9. Meschia, J.F. Advances in genetics 2010 / J.F. Meschia // Stroke. – 2011. - V.42. – P. 285 – 287.
10. Mutations in VKORC1 cause warfarin resistance and multiple coagulation factor deficiency type / Rost S., Fregin A., Ivaskevicius V. [et al.] // Nature. – 2004. - V. 427. – P.537 – 541.
11. Nasu, K. Genetic analysis of CYP2C9 polymorphism in a Japanese population / K. Nasu, T. Kubota, T. Ishizaki // Pharmacogenetics. – 1997. - V.7 (5). – P.405 – 409.
12. Population variation in VKORC1 haplotype structure / S. Marsh, C.R. King, R.M. Porche-Sorbet. Et al. // Journal of Thrombosis and Haemostasis. – 2006. - V.4 (2). – P.473 – 474.
13. Sanderson, S. CYP2C9 gene variants, drug dose, and bleeding risk in warfarin-treated patients: a HuGEnet systematic review and meta-analysis / S. Sanderson, J. Emery, J. Higgins // Genet Med. – 2005. -7(2) – P.97 –104.
14. Warfarin pharmacogenetics: a single VKORC1 polymorphism is predictive of dose across 3 racial groups / N.A. Limdi, M. Wadelius, L. Cavallari [et al.] // Blood. – 2010. - V. 115 (18). – P.3827 – 3834.

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