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The impact of CYP4F2, ABCB1, and GGCX polymorphisms on bleeding episodes associated with acenocoumarol in Russian patients with atrial fibrillation

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Abstract

Background: Oral anticoagulants are commonly used to treat patients with thromboembolic pathology. Genetic variations could influence personal response to anticoagulant drugs. Acenocoumarol (AC) is a vitamin K antagonist used in anticoagulant therapy and as a prophylaxis measure in Europe. In this study, we assessed the effect of CYP4F2 rs2108622, ABCB1, and GGCX polymorphisms on the safety profile and regime dosing of AC in patients with nonvalvular atrial fibrillation.

Methods: Fifty patients aged 40–70 years were included. All patients received AC in the dose of 1–6 mg daily with a target international normalized ratio of 2.0–3.0. Genotyping for polymorphism markers *C3435T* for the *ABCB1* gene, rs2108622 for the *CYP4F2* gene, and rs11676382 for the *GGCX* gene were designed using polymerase chain reaction and restriction fragment length polymorphism. Statistical analysis was performed using the Fisher exact test and the Mann-Whitney U test.

Results: We found that *CYP4F2* rs2108622 *CT* carriers required a higher AC dose than *CC* ($p = 0.0366$), and *CT* and *TT* carriers required a higher AC dose than *CC* ($p = 0.0314$).

Conclusions: We found that *ABCB1 CT* and *TT* genotypes are associated with a higher risk of bleeding. No influence of ABCB1 and GGCX polymorphisms on the doses of AC was established. CYP4F2 could still be a genetic factor responsible for the personal variability of AC metabolism.

Keywords: ABCB1; acenocoumarol; CYP4F2; GGCX; pharmacogenetics.

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Introduction

Atrial fibrillation (AF) is one of the most common and clinically important variations of cardiac arrhythmia. Its prevalence worldwide is currently increasing [1]. AF leads to a variety of severe complications such as heart failure and systemic embolism. Although AF-related embolism may occur in any artery, occlusion most typically happens in cerebral arteries. Stroke is the most frequent and menacing complication of AF. This condition causes a significant change in the quality of life and requires lifelong oral anticoagulant (OA) therapy. The most widely used OAs are coumarins such as warfarin and acenocoumarol (AC), which have been proven effective in preventing thromboembolic complications [2]. Arterial, venous, and systemic embolism are the leading causes of working incapacity and mortality in industrialized countries [3]. One of the main roles in thrombosis prevention belongs to a coumarin group OA also known as vitamin K antagonists. Their efficacy has been proven in multicentered clinical trials and meta-analysis studies [4]. For a long time, AC had the third largest sales volume among OAs in Russia, surpassed only by warfarin and phenylins, and is now widely available in our country. OAs exert their effect by reducing the synthesis of the prothrombin complex coagulation factors. However, the mechanism of the principal adverse reaction they cause – bleeding – is the same. It occurs in 25% of the patients, including life-threatening events that 1.5% of patients develop [5]. Genetic characteristics of a patient can play a significant role in shaping an individual reaction to OAs. That has been proven for another OA – warfarin [6]. Algorithms of

personalized warfarin dose prediction have been implemented in the clinic, which has had a statistically significant improvement on the effectiveness and safety of the treatment. The therapeutic dose of warfarin is related to *VKORC1* and *CYP2C9* genes that influence drug pharmacokinetics and vitamin K metabolism [7]. It is notable that cytochrome P-450 4F2 is thought to take part in vitamin K metabolism, particularly its hydroxylation, because a similar reaction with vitamin E is catalyzed by cytochrome P-450 4F2 (CYP4F2) [8, 9]. Another hypothesis states that CYP4F2 is involved in AC metabolism because rs2108622 polymorphism is associated with the difference in levels of clotting factors II, VII, IX, and X after using AC. There is a significant locus related to the coumarin mechanism of action – *ABCB1* gene – that encodes P-glycoprotein. P-glycoprotein is a transporter that plays the key role in eliminating most medicinal agents. This transporter is expressed in intestinal, liver, proximal kidney tubular, and blood-brain barrier cells [10]. Some studies have shown the influence of P-glycoprotein on the sensitivity to warfarin [11]. The polymorphism marker C3435T of the *ABCB1* gene is associated with the P-glycoprotein expression level. People with *CC* genotype have a higher transporter expression level than *TT* homozygotes. This peculiarity may influence drug pharmacokinetics [12]. Our pilot study shows that *ABCB1 CT* and *TT* genotypes were found to be significantly associated with a higher risk of bleeding [13]. Another key enzyme that may influence the pharmacological reaction is γ-glutamyl carboxylase (encoded by the *GGCX* gene), which catalyzes carboxylation. In the process of carboxylation, γ-glutamyl carboxylase oxidizes vitamin K hydroquinone to vitamin K 2,3-epoxide. It then goes on to become the active form K1H2 as the result of vitamin K epoxide reductase (VKORC1) action [14].

Goals

The goal of the following research was to study associations between rs2108622 *CYP4F2*, *ABCB1*, and *GGCX* gene polymorphisms and both bleeding events and thrombosis episodes in patients on AC with a high risk of thromboembolic complications.

Materials and methods

The retrospective cohort study included 50 participants, age 59 ± 9 years (mean ± SD), 34 men (68%) and 16 women (32%), all of Russian nationality, and all taking AC, mean dose 2.9 ± 1 mg. Included in the study were patients experiencing nonvalvular AF, permanently receiving AC (Syncumar, ICN Hungary Co. Ltd., Hungary) as a measure of thrombosis prophylaxis (Table 1). All subjects signed an informed consent. The inclusion criteria were as follows: patients with permanent AF and same dose of AC (1–6 mg/day), with an international normalized ratio (INR) target value of 2–3 for at least 1 month. Patients were excluded from the study if they had any of the counterindications to receiving AC (according to the registry instruction manual of the National Pharmaceutical) or had a medical condition that could influence AC distribution, metabolism, or elimination (oncological diseases, diabetes mellitus, and kidney and liver diseases). Another exclusion criterion was incompliance with the monthly INR measurements in accordance with clinical guidelines [15]. We registered episodes of hypocoagulation (INR >3) and bleeding throughout the observation period, which was 1–6 months long. We registered episodes of bleeding according to questionnaires and patient charts. We differentiated between minor and major bleedings as is outlined in the ROCET-AF study of Rivaroxaban. Therefore, the bleeding was considered major if associated with a fatal outcome, or with a fall in hemoglobin concentration of ≥2 g/dL, or leading to transfusion of ≥2 U of packed red blood cells or whole blood. All intracerebral bleeding was interpreted as hemorrhagic strokes. If the bleeding did not meet any of the aforementioned criteria, but required any medical intervention (unscheduled consultation and interruption of medication), it was considered clinically relevant, but nonmajor. All other episodes are classified as minor bleeding [16].

INR measurement was performed with thromboplastin produced by the "Technological Standard" company (Russia) with an international sensitivity index of 1.3.

CYP4F2 rs2108622, *ABCB1*, and *GGCX* genes were screened in all patients. Genotyping was performed using the polymerase chain reaction (PCR)-restriction fragment length polymorphism method. PCR primers were designed using "PrimerSelect" 4.05©1993–2000 DNASTAR, Inc., software and synthesized by "Syntol" company (Russia). All of the enzymes were produced by SybEnzyme (Russia). The division of restriction fragments was performed using electrophoresis in 10% acrylamide gel [17].

Statistical analysis

The statistical significance of the differences in the results was assessed using the Fisher exact test, the Mann-Whitney U test, and the Bonferroni correction for multiple comparisons. Statistical analysis was performed using Instat software.

Table 1: Demographic characteristics of patients.

Demographic characteristic	Patients (n = 50)
Age, mean ± SD, years	59 ± 8
Gender, n (%)	
Male	34 (68%)
Female	16 (32%)
Weight, mean ± SD, kg	85.7 ± 11.3
Height, mean ± SD, cm	171.44 ± 7.5
Stable AC dose, mean ± SD, mg/day	2.93 ± 1.07
Target INR	2.0–3.0

Ethics

The Ethics Committee of the Russian Medical Academy of Postgraduate Education, Moscow, Russia, approved the study. Written informed consent was obtained from all subjects.

Results

The results of genotyping in all 50 patients are presented in Table 2.

The allele frequencies by loci for the *CYP4F2* rs2108622 gene were as follows: CC – 30 patients (60%), CT – 17 patients (34%), and TT – 3 patients (6%). The *ABCB1* gene allele frequencies of the polymorphism marker C3435T were as follows: CC genotype – 10 patients (20%), CT genotype – 25 patients (50%), and TT genotype – 15 patients (30%). With the *GGCX* gene, 44 patients (88%) had CC genotype and 6 patients (12%) had CG genotype (Table 3).

Bleeding is the most common adverse effect of all coumarine anticoagulants. The data were analyzed to find an association between adjusted AC doses and the development of bleeding events.

Patients who had episodes of bleeding and abnormal hypocoagulation were divided in two groups for comparison: CYP4F2 (CC), ABCB1 (CC), and GGCX(CC) genotypes and all of the other patients [not CYP4F2 (CC), ABCB1 (CC), and GGCX (CC)].

In the case of the *CYP4F2* rs2108622 gene, 14 (47%) of 30 patients with CC genotype and 6 (30%) of 20 patients with CT and TT genotypes had bleeding episodes ($p = 0.2576$). An episode of INR >3 was registered in 17 (57%) of 30 CC genotype patients and in 12 (60%) of 20 CT and TT genotype patients ($p = 1.0$). In both cases, the difference between the groups was not statistically significant.

In the case of *ABCB1* gene, 1 (10%) of 10 patients with CC genotype and 19 (47.5%) of 40 patients with CT and TT

Table 2: Allelic frequency and genotype frequency distribution of *CYP4F2*, *ABCB1*, and *GGCX* in Russian patients.

Gene	Single-nucleotide polymorphism	Genotype	Frequency number (%)	Allele	Frequency number (%) (95% CI)	Hardy–Weinberg equilibrium, p-value
CYP4F2	Rs2108622	*CC*	30 (60%)	C	77 (77%) (68.8–85.25)	0.77
		CT	17 (34%)		23 (23%) (14.8–31.25)	
		TT	3 (6%)	T		
ABCB1	C3435T	*CC*	10 (20%)	C	45 (45%) (32.25–54.75)	0.94
		CT	25 (50%)	T		
		TT	15 (30%)		55 (55%) (45.25–64.75)	
GGCX	Rs11676382	*CC*	44 (88%)	C	94 (94%) (89.35–98.65)	0.65
		CG	6 (12%)	G	6 (6%) (1.35–10.65)	

Table 3: Effects of the *CYP4F2* Rs2108622, *ABCB1*, and *GGCX* genotypes on bleeding and hypocoagulation episodes frequencies in Russian patients.

Genotype	No. patients (%)	No. bleedings (%)	INR > 3 (%)	Comparison among different genotypes	No. bleedings	INR > 3
						p-Value
CYP4F2						
	50	**20**	**29**	CC vs. TT	0.3438	0.5794
CC	30 (60)	14 (70)	17 (58.6)	CC vs. CT	0.6522	0.7588
CT	17 (34)	6 (30)	11 (38)	CC vs. CT TT	0.2576	1.0
TT	3 (6)	0	1 (3.4)	CT vs. CC TT	0.8549	0.5565
ABCB1						
	50	**20**	**29**	CC vs. TT	0.1794	0.2290
CC	10 (20)	1 (5)	8 (27.6)	CC vs. CT	0.0280	0.2516
CT	25 (50)	13 (65)	13 (44.8)	CC vs. CT TT	0.0366	0.1674
TT	15 (30)	6 (30)	8 (27.6)	CT vs. CC TT	0.1482	0.5672
GGCX						
	50	**20**	**29**	CC vs. CG	0.3811	0.3803
CC	44 (88)	19 (95)	24 (82.8)			
CG	6 (12)	1 (5)	5 (17.2)			

genotypes had bleeding episodes, the difference being statistically significant (p = 0.0366). An episode of INR >3 was registered in 8 (80%) of 10 CC genotype patients and in 21 (52%) of 40 CT and TT genotype patients, the difference being not statistically significant (p = 0.1674).

Polymorphism markers Rs2108622 for the *CYP4F2* gene and Rs11676382 for the *GGCX* gene association with bleeding and hypocoagulation episodes were also studied.

In the case of the *GGCX* gene, 19 (43%) of 44 patients with CC genotype and 1 (17%) of 6 patients with CT and TT genotypes had bleeding episodes (p = 0.3811). An episode of INR >3 was registered in 24 (54%) of 44 CC genotype patients and in 5 (83%) of 6 CT and TT genotype patients (p = 0.3803). In both cases, the difference between the groups was not statistically significant.

Thus, no association between polymorphism markers Rs2108622 for the *CYP4F2* gene and Rs11676382 for the *GGCX* gene and bleeding or hypocoagulation episodes has been established in our study.

A statistically significant difference has, however, been found in the process of individually adjusted dose analysis between different CYP4F2 rs2108622 genotype groups. It has been established that patients with CC genotype have a proven lower adjusted dose of AC than the CT (p = 0.0366) and the CT/TT genotype groups (p = 0.0314). The dose analysis for other genes has shown no statistically significant pattern. The results are presented in Table 4.

Discussion

We studied the association between *CYP4F2*, *ABCB1*, and *GGCX* genes and both risk of bleeding and therapeutic dose of AC. The allele frequencies of CYP4F2 rs2108622, C3435T ABCB1, and Rs11676382 GGCX between Russian and other ethnic groups were also compared. To our knowledge, this is the first research to study the effect of CYP4F2, ABCB1, and GGCX variations on AC requirements in Russian population. We found no statistically significant difference in the allele frequency of CYP4F2 rs2108622 of Russian (23%), Hispanic (23%), and Caucasians (34%) patients [18]. We have found no difference in the frequency of the *ABCB1* gene C3435 polymorphism between Russian patients (55%) and Caucasians (50%) (German; n = 188) [12]. We have also found relatable results for Rs11676382 GGCX with 5% occurrence in Russian patients and 8% in Caucasians [19]. Our study shows that rs2108622 CYP4F2 may influence AC dose. However, it is notable that the Bonferroni correction method shows the differences that we found are not statistically significant.

Danese et al. [20] studied the influence of rs2108622 *CYP4F2* gene polymorphism on coumarine anticoagulant doses in a meta-analysis. They compared homozygotes for the wild-type C allele with T-allele carriers and found that patients with T-allele required an 8.3% higher dose of coumarins. In 100 white men from Spain, the initial response to 3 mg of AC over three consecutive days was influenced by CYP4F2 genotype: c.1297A carriers needed approximately 4 mg/week more than c.1297GG subjects to achieve a steady INR [21]. Wypasek et al. [22] in their study showed that CYP4F2 may explain 18% of the acenocoumarol dose variability in Slavic patients. According to our results, ABCB1 and GGCX polymorphisms have no influence on acenocoumarol dose. However, Saraeva et al. [23] were the first ones to show that the C3435T polymorphism of the *ABCB1* gene has a role in sensitivity to acenocoumarol. According to their data, CC homozygotes require lesser dose than TT homozygotes.

According to literature, a significant number of patients with a T-allele of the *ABCB1* gene have decreased P-glycoprotein expression compared with C allele homozygotes. Decreased P-glycoprotein expression may lead to slowed acenocoumarol elimination that causes drug retention and adverse effect development [24]. Our data serve to prove that statement.

In 2013, 548 patients participated in a multicentered randomized trial by Verhoef et al. It showed that the "genotype-guided dosing of acenocoumarol or phenprocoumon did not improve the percentage of time in the therapeutic INR range during the 12 weeks after the initiation of therapy" [25]. However, genotyping was conducted only for *CYP2C9* and *VKORC1* genes, which might have influenced

Table 4: Effects of the *CYP4F2* Rs2108622, *ABCB1*, and *GGCX* genotypes on mean daily AC dose in Russians patients.

Gene	Single-nucleotide polymorphism	Data			p-Value			Mean dose		
		CC	*CT* (*CG*)	*TT*	*CC* vs. *CT*	*CC* vs. *TT*	*CC* vs. *CT*+*TT*	*CC*	*CT* (*CG*)	*TT*
CYP4F2	rs2108622	30	17	3	0.0366	0.4177	0.0314	2.7 ± 1.02	3.35 ± 1.1	3.17 ± 0.76
ABCB1	rs1045642	10	25	15	0.0592	0.1796	0.0855	3.45 ± 1.34	2.64 ± 1.01	3.1 ± 0.86
GGCX	rs11676382	44	6	0	0.7121			2.91 ± 1.0	3.08 ± 1.69	

the result of the study. It is also important to remember that acenocoumarol and warfarin metabolism differ significantly; therefore, *CYP2C9* and *VKORC1* gene polymorphisms cannot fully explain the acenocoumarol dosing variability. Many individual dose adjustment algorithms are currently being designed for coumarin anticoagulants such as warfarin, acenocoumarol, and phenprocoumon. Their metabolism is regulated by *CYP2C9*, *VKOR1*, and *CYP4F2* genes, with CYP2C9 and VKOR1 playing the key role [26]. Coumarin anticoagulants continue to be the most widely used group of anticoagulants worldwide [27]. However, coumarins have a narrow therapeutic range, and therefore selecting the precise maintenance dose is of outmost importance [28]. The advantages of introducing a pharmacogenetic algorithm for acenocoumarol are currently being studied [26, 29]. Genetically significant for acenocoumarol are VKOR1, APOE, CYP2C9*3, and ABCB1. ABCB1 in particular should be considered for hypocoagulation and bleeding episode prevention in patients taking acenocoumarol [30]. Such an algorithm would make it possible to predict acenocoumarol dose requirement to minimize adverse events and to enhance efficacy individually for every patient [31]. A dose dependence from the *UGT1A1* gene, encoding uridine diphosphate glucuronosyltransferase enzyme, has been shown for warfarin [32, 33]. No similar studies have been conducted for acenocoumarol.

Limitations

The main limitation of our study is the low number of patients included. This can lead to false-positive or false-negative results. The number of patients included in the study should be increased so that more accurate statistical results can be received.

Conclusions

We found that patients with CC genotype of rs2108622 gene CYP4F2 have a statistically significant lower adjusted dose of acenocoumarol than the CT and CT/TT genotype group. Patients with CC genotype for the *ABCB1* gene have a lower bleeding risk compared with CT and TT genotype patients. No association between polymorphism markers Rs2108622 for the *CYP4F2* gene and Rs11676382 for the *GGCX* gene with bleeding events and hypocoagulation episodes has been established in our study.

Further research of acenocoumarol pharmacogenetics is required to design an algorithm for personal dose requirement prediction because the data available at the moment are insufficient.

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