Karin B. Mirzaev[1] / Darya V. Osipova[2] / Elena J. Kitaeva[3] / Vladimir V. Shprakh[3] / Sherzod P. Abdullaev[1,4] / Denis A. Andreev[2] / Robert B. Mumladze[1] / Dmitriy A. Sychev[1]

Effects of the rs2244613 polymorphism of the *CES1* gene on the antiplatelet effect of the receptor P2Y12 blocker clopidogrel

[1] Russian Medical Academy of Continuous Professional Education, Ministry of Healthcare of the Russian Federation, Moscow, Russian Federation, E-mail: karin05doc@yandex.ru
[2] I.M. Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russian Federation, E-mail: osipova.dasha2013@yandex.ru
[3] Irkutsk State Medical Academy of Postgraduate Education, Irkutsk, Russian Federation
[4] Faculty of Fundamental Medicine, Moscow State University M.V. Lomonosov, Moscow, Russian Federation

Abstract:
Background: The aim of this study was to evaluate the association of the carriage of the rs2244613 polymorphism of the *CES1* gene with clopidogrel resistance as well as to evaluate the effectiveness of antiplatelet therapy in the carriers of this marker who have had acute coronary syndrome (ACS). This study also analyzes the procedure of percutaneous coronary intervention and compares the rs2244613 carrier rate between patients with ACS and healthy participants.
Methods: The study involved 81 patients diagnosed with ACS and 136 conditionally healthy participants. The optical detection of platelet agglutination by VerifyNow was employed to measure residual platelet reactivity in patients with ACS. The rs2244613 polymorphism was determined using real-time polymerase chain reaction.
Results: According to the results, the AA genotype of the rs2244613 polymorphism of the *CES1* gene was detected in 37 patients (45.6%), the CA genotype in 42 patients (51.8%) and the CC genotype in 2 patients (2.6%). The level of residual platelet reactivity in rs2244613 carriers was higher compared with patients who did not have this allelic variant: 183.23 PRU ± 37.24 vs. 154.3 PRU ± 60.36 ($p = 0.01$). The frequencies of the minor allele C were 28.4% and 28.3% in patients with ACS and healthy participants, respectively. The results of the linear statistical model PRU due to *CES1* genotype were as follows: $df = 1$, $F = 6.96$, $p = 0.01$). The standardized beta was 0.285 ($p = 0.01$) and R^2 was 0.081. However, we also added *CYP2C19*2* and **17* into the linear regression model. The results of the model were as follows: $df = 3$, $F = 5.1$, $p = 0.003$) and R^2 was 0.166.
Conclusions: We identified a statistically significant correlation between the carriage of the rs2244613 polymorphism of the *CES1* gene and the level of residual platelet aggregation among patients with ACS and the procedure of percutaneous coronary intervention.

Keywords: acute coronary syndrome (ACS), antiplatelet therapy, CES1, clopidogrel, rs2244613
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Introduction

According to the WHO [http://www.who.int/mediacentre/factsheets/fs310/ru/index1.html], cardiovascular diseases (CVD) are the main cause of mortality in the world. The most common basis for the development of acute coronary syndrome (ACS) is the rupture or erosion of an atherosclerotic plaque, which leads to thrombosis and its further clinical manifestations, such as myocardial infarction (MI), stroke or occlusion of peripheral arteries. Clopidogrel and acetylsalicylic acid are two components of dual antiplatelet therapy, which is the current standard of treatment for the prevention of recurrent cardiovascular outcomes in patients with ACS and undergoing percutaneous coronary intervention (PCI) [1]. Such a therapy aims to prevent the development of thrombotic complications and recurrences of coronary heart disease [2]. However, quite often, patients may suffer from high residual platelet reactivity (HTPR), which is an obstacle in achieving an optimal result of dual antiplatelet therapy [3]. One of the main problems in the effective prevention of thrombotic complications of MI

Karin B. Mirzaev, Darya V. Osipova are the corresponding authors.
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is the problem of the variability of the pharmacological response to antiplatelet drugs, and above all, to clopidogrel. The reasons for this are individual differences in the rate of biotransformation at the level of cytochrome P450 isoenzymes, such as CYP2C19, CYP3A4 and hepatic carboxylesterase-1 (CES1).

Approximately 85% of the original clopidogrel is rapidly hydrolyzed to an inactive carboxylic acid derivative, which is further metabolized by binding to glucuronic acid [4]. This hydrolysis is catalyzed by CES1. In view of the above, we can conclude that existence of polymorphism in the *CES1* gene can significantly affect the metabolic pathway of clopidogrel in the body and, therefore, its activity and antiplatelet effect. In the PAPI study [5], it was found that the carriage of the allelic variant of the c.428G>A (rs71647871) polymorphism of the *CES1* gene is associated with a loss of the carboxylesterase-1 catalytic activity, which may cause an increase in the plasma concentration of the active clopidogrel metabolite and enhance the antiplatelet effect. The carriers of the rs71647871 allelic variant had significantly higher levels of the circulating active metabolite of clopidogrel (30.3 ± 6.1 vs. 19.0 ± 0.4 ng/mL, respectively, p = 0.001) and stronger inhibition of ADP-stimulated platelet aggregation in carriers of the allelic variant A rs71647871 compared with those in non-carriers (43% vs. 29%, respectively, p = 0.003) [5]. In another interesting study by Zhao [6], it was reported that the rs71647871 mutation in the *CES1* gene may be associated with higher residual ADP-induced platelet aggregation. However, the minor allele has a very low incidence (<1%). Furthermore, it was found that rs8192950 polymorphism (T>G) in the *CES1* gene, allele G, was associated with a reduced risk of recurrent ischemic events in patients (logistic regression, OR = 0.53, 95% CI = 0.30–0.94, p = 0.029). Furthermore, it was determined that two polymorphisms rs71647871 (c.428G> A; G143E) and rs71647872 (D260fs) were associated with changes in the activity of the CES1 enzyme and led to a high decrease in its catalytic activity. However, the frequency of the c.428G>A minor allele was 0% in Asian populations and the D260fs allele was found to be an extremely rare mutation. The rs8192950 polymorphism was found in the *CES1* intron and it was demonstrated that patients with it had significantly lower risks of experiencing different clinical events. However, towards an accurate confirmation of this hypothesis, further functional studies of the enzyme must be conducted to determine whether this variant of the *CES1* gene influences the effectiveness of clopidogrel therapy.

So far, many studies have investigated the effect of different polymorphisms of the *CES1* gene on its functional activity and the presence of statistically reliable results. Such studies suggest that the evaluation of its catalytic activity in the body is an important prognostic marker for the development of complications and the effectiveness of therapy. Therefore, the aim of our study was to evaluate the impact of this particular polymorphism rs2244613 of the *CES1* gene on the antiplatelet effect of the clopidogrel receptor blocker P2Y12. This is because such a mechanism has not yet been fully studied in past works.

Materials and methods

The clinical part of the study, including all laboratory and instrumental studies and measurement of residual reactivity of platelets in patients, was conducted at the City Clinical Hospital № 1 named after N.I. Pirogov DZ Moscow. Genotyping for polymorphism c.1168-33A>C (rs2244613) of the *CES1* gene in all study participants was conducted at the Scientific Research Center of the State Budgetary Educational Institution of Higher Professional Education of the Russian Medical Academy of Continuous Professional Education. Permission to conduct the study was obtained from the independent Ethical Committee of the Russian Medical Academy of Continuous Professional Education with the written informed consent from each participant. This study received ethical approval from the IRB.

Study population

The study included 81 patients (64 men and 17 women, average age: 63.9 ± 10.9 years) from the Russian ethnic group (Moscow), who were admitted with a diagnosis of ACS. We clarified the diagnosis of ACS: 50 patients had Q-forming MI, 17 patients had non-Q-forming MI, 10 patients had unstable angina and 4 patients had unspecified MI. ACS with ST segment elevation was observed in 50 patients and in 31 patients, ACS without ST segment elevation was observed. The criteria for inclusion in the clinical part of the study were as follows: the presence of ACS with ST segment elevation/without ST segment elevation, undergoing the PCI procedure (77 patients), using clopidogrel in combination with acetylsalicylic acid in recommended doses and finally submitting informed consent to participate in written form. The exclusion criteria from the clinical part of the study were as follows: hypersensitivity to clopidogrel or other components of the drug; taking ticlopidine, ticagrelor or prasugrel; severe hepatic or renal failure (creatinine clearance <30 mL/min); hemorrhagic syndrome; internal bleeding; intracranial hemorrhage; diseases predisposed to bleeding (e.g. gastric ulcer and duodenal ulcer in the acute stage, ulcerative colitis); has lung tumors, rare hereditary lactose intolerance, lactase deficiency and

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glucose-galactose malabsorption; pregnant or lactating and has children under 18 years of age. The follow-up period lasted for 5 days. During this period, no recurrent MI, stroke, or cardiovascular death were observed in any of the patients. In order to compare the frequency of the prevalence of the rs2244613 polymorphism of the *CES1* gene among patients with CVD and healthy volunteers, 136 conditionally healthy (non-myocardial infraction) participants (26 men and 110 women, mean age: 42.1 ± 11 years) from the Irkutsk Russian ethnic group were included in the study.

Characteristics of healthy study participants

The population part of the study included 136 people. The inclusion criteria for conditionally healthy participants in the study were as follows: self-identification of the subject as a representative of the Russian ethnic group, the absence of CVD, the absence of severe somatic pathologies and informed consent to participate in written form. The exclusion criteria for conditionally healthy study participants were as follows: pregnancy, age of less than 18 years, CVD and the presence of severe somatic pathology.

Laboratory research

The optical detection of platelet agglutination was employed to measure the residual platelets reactivity in patients with ACS. Whole venous blood samples were taken from the cubital vein to measure platelet aggregation for 3–5 days after ACS and PCI. As part of a complex drug therapy for MI, all patients had treatment with clopidogrel and ASA in the following recommended doses: ASA - 300 mg initial dose and 100 mg maintenance dose and clopidogrel - 300 mg initial dose, 75 mg maintenance (11 patients received an initial dose of 600 mg and 2 patients received a maintenance dose of 150 mg). For blood sampling, Greiner Bio-One Vacuette vacuum tubes (Greiner Bio-One, Austria) were used for 2 mL with 3.2% sodium citrate. The study was conducted on the VerifyNow P2Y12 test system (”Accumetrics”, USA) for 1 hour after taking a sample of whole venous blood. This study was observational, so additional interventions and changes in the dosages of the drugs were not conducted. A PRU value of 208 is considered the clopidogrel resistance threshold [7], [8]. Thus, we determined that the PRU value of more than 208 was associated with an insufficient response to clopidogrel and, therefore, with an increased risk of developing thrombotic complications and their clinical outcomes, such as stroke, recurrent heart attack and stent thrombosis. A PRU value of less than 208 corresponds to a normal response to drug therapy.

Genotyping

For genotyping, venous blood collected 2–5 days after PCI in VACUETTE® vacuum tubes (Greiner Bio-One, Austria) with EDTA was used. The carriage of the polymorphic marker *CES1* gene was detected by real-time polymerase chain reaction (PCR) on CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., USA). To determine of the polymorphism of the CES1 gene, the ”GenTest CES1” kit was used (OOO ”Nomotek”, Moscow, Russia). The amplification program included an incubation step at 95 °C for 3 minutes, then denaturation at 95 °C for 10 seconds and annealing at 60 °C for 30 seconds for 50 cycles. The fluorescence signals were developed in the appropriate channels: FAM, HEX or FAM. The DNA extraction was carried out using reagents for the isolation of genomic DNA from whole blood (DNA-EKSTRAN-1) (ZAO Syntol, Russia). Genotyping for *CYP2C19* was performed with the use of an oligonucleotide ligation assay after initial specific amplification by means of a PCR involving three primers for the major variant alleles *CYP2C19*2* (c.G681A; rs4244285), *CYP2C19*3* (c.G636A; rs4986893) and *CYP2C19*17* (c.C806T; rs12248560). Primers: 5′-GATATGCAATAATTTTCCCACTATCATTG-3′ and 5′-GGTGTTCTTTTACTTTCTCCAAAATATCAC-3′ were used to amplify sequence of the *CYP2C19*2* 681G.A (rs4244285) in exon 5; for *CYP2C19*3* (c.636G>; rs4986893), we used 5′-CACCCTGTGATCCCACTTTC-3′ and 5′-ACTTCAGGGCTTGGTCAATA-3′; for *CYP2C19*17* (c.C806T;rs12248560),we used 5′-AAATTTGTGTCTTCTGTTCTCAAA-3′ and 5′-AATCCCAGTTCTGCCAGCTA-3′. The sequence of the G allele-specific probe was 5′-FAMTTATTTCCCGGGAACC-3′ and the sequence of the A allele-specific probe was 5′-VICATTATTTCCCAGGAACC-3′. For rs2244613 gene *CES1*: 5′-AGATTGCCTTTTGCAAAGTT-3′ and 5′-AAGTGCAGTGAGGAGAGTCC-3′ [9].

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Statistical analysis

For the statistical processing of the results, SPSS Statistics 20.0 was used. Average values were presented as M ± SD (M = mean, SD = standard deviation). The normality of the distribution was verified using the Shapiro-Wilk's test. Non-normal distribution for all continuous variables was observed; thus, only non-parametric tests were used. Mann-Whitney test was applied for the comparison of the two independent groups comparison, particularly, the *CES1* genotypes (AA, AC+CC). The Pearson chi-square test (χ^2) was employed to compare the categorical variables. A Hardy-Weinberg test was performed using an online calculator to confirm the independent distribution of alleles in the studied polymorphisms [10]. To assess the differences in the frequency of occurrence of alleles by rs2244163 between patients with ACS and a healthy group, Fisher's exact test was used. In order to demonstrate the *CES1* potential to predict PRU value, linear regression model was developed (described below). Differences were considered significant at $p < 0.05$.

Results

A total of 81 patients (64 men and 17 women) with ACS were included. The average age of patients was 63.9 ± 10.9 years. However, there were no significant differences that could affect platelet aggregation activity, including the size of the initial dose, between the group with a normal genotype and that with polymorphic allele carriers. It was also found that 5 (6.1%) patients had a history of ischemic stroke, 71 (87.6%) patients were diagnosed with acute MI and 10 (12.3%) patients had unstable angina. The distribution of genotypes in the sample did not match the Hardy-Weinberg equilibrium. This result is most likely associated with a small sample size. The frequencies of the AA, CA and CC genotypes were 45.6% (n = 37), 51.8% (n = 42) and 2.6% (n = 2), respectively. The frequencies of alleles A and C were 71.6% and 28.4%, respectively (Table 1).

Table 1: The frequencies of the genotypes and alleles rs2244613 of the *CES1* gene in groups of patients with ACS and in healthy participants.

Groups	n	Genotype			Allele frequency, %		95% CI	Compliance with Hardy-Weinberg distribution		Comparison of allele carrier frequency	
		AA	AC	CC	A	C		χ^2	p-Value	p-Value	OR (95% CI)
Patients with ACS	81	37	42	2	71.6	28.4	0.208–0.345	6.131	0.046	1.000	1.004 (0.6522–1.546)
Healthy participants	136	70	55	11	71.7	28.3	0.233–0.339	0.0018	0.965		

However, we determined that there are significant differences between carriers of the AA genotype and the AC + CC genotype in terms of PRU: 154.3 ± 60.36 (95% CI: 134.17–174.42) vs. 183.23 ± 37.24 (95% CI: 171.9–194.55); range of 86.0–244.0 and $p = 0.01$, respectively. This means that the carriage of the polymorphic marker rs2244613 is likely to be associated with the impaired pharmacological response to clopidogrel therapy.

Moreover, the average percentage of platelet inhibition was lower in patients who were carriers of the allelic variant C (AC + CC) rs2244613 of the *CES1* gene compared with the AA homozygotes: 24.87 ± 2.1 in the AC + CC group vs. 32.81 ± 4.08 in group AA. However, these data were not statistically significant. Then a linear statistical model to predict PRU level was developed. At the first stage, only the *CES1* genotype was included as the predictor. The results of the model PRU due to the *CES1* genotype were as follows: df = 1, F = 6.96, p = 0.01). The standardized beta was 0.285 ($p = 0.01$) and $R^2 = 0.081$ (Table 2). According to the Kruskal-Wallis and Mann-Whitney tests results, the *CYP2C19*2* and **17* genotypes had no influence on the PRU level [9]. However, we also added *CYP2C19*2* and **17* into the linear regression model. With that, the results of the model were as follows: df = 3, F = 5.1, $p = 0.003$) and $R^2 = 0.166$. The results of regression analysis are presented in Table 3. According to the regression results, *CES1* and *CYP2C19*2* are risk factors of increased PRU level. Thus, the model that involves both these genes can better predict the PRU level. Other factors were not included into the regression model, because pairwise comparisons confirmed null hypothesis; thus, there were no correlations of PRU with demographic and clinical parameters.

Table 2: The regression model to predict the PRU level by using the *CES1* genotype only.

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	Beta	B	B Standard error	p-Value
CES1	0.285	28.93	10.96	0.01

Table 3: The linear regression model to predict the PRU level by using the *CES1* and the *CYP2C19*2* and **17* genotypes.

	Beta	B	B Standard error	p-Value
CES1	0.285	28.974	10.82	0.009
CYP2C19*2	0.259	35.77	14.754	0.018
CYP2C19*17	−0.196	−20.351	11.178	0.073

As can be seen from above, the proportion of patients with laboratory resistance to clopidogrel (PRU > 208) was higher among carriers of the allelic variant C (CA + CC) compared with patients without this allelic variant: 27.2% among patients with the CA + CC genotype and 18.9% among homozygotes for the wild type AA allele (OR = 1.607; 95% CI:0.559–4.624; p = 0.01). No significant differences were found after studying the differences in quantitative variables, such as LDL level, percentage of platelet inhibition, triglyceride level, glucose level, etc. between carriers of the normal AA genotype and the CA + CC genotype using the Mann-Whitney test (Table 4 and Table 5).

Table 4: The clinical and demographic data of patients with ACS.

Dimension	Patients (n = 81)	Genotype AA (n = 37)	Genotype AC+CC (n = 44)	p-Value
Age	63.9 ± 10.9	64.3 ± 12.2	63.7 ± 9.8	0.803
Unstable angina, n (%)	10 (12.3)	6 (7.4)	4 (4.9)	0.290
Q-wave myocardial infarction, n (%)	50 (61.7)	21 (25.9)	29 (35.8)	
Not-Q-wave myocardial infarction, n (%)	17 (21.0)	8 (9.8)	9 (11.1)	
Myocardial infraction unspecified, n (%)	4 (4.9)	2 (2.45)	2 (2.45)	
Diabetes II type, n (%)	16 (19.8)	6 (7.4)	10 (12.3)	0.465
Arterial hypertension, n (%)	75 (92.5)	35 (43.2%)	40 (49.3)	0.637
Anemia, n (%)	4 (4.9)	2 (2.45)	2 (2.45)	0.862
Active smoking, n (%)	17 (20.9)	7 (8.6)	10 (12.3)	0.678
Ischemic stroke, n (%)	5 (6.2)	1 (1.2)	4 (4.9)	0.228

Table 5: Laboratory and instrumental measure and concomitant drug therapy 2–5 days after PCI.

Dimension	Patients (n=81)	Genotype AA (n=37)	Genotype AC+CC (n=44)	p-Value
Platelets, 10^9 cells/L	226.42 ± 64.62	230.16 ± 66.15	223.29 ± 63.92	0.638
Total cholesterol, mmol/L	5.1 ± 1.2	5.2 ± 1.07	5.1 ± 1.32	0.614
LDL, mmol/L	2.86 ± 1.15	3.06 ± 1.26	2.7 ± 1.04	0.180
Triglycerides, mmol/L	1.69 ± 0.6	1.57 ± 0.48	1.8 ± 0.79	0.120
Creatinine, mcmol/L	105.1 ± 20.4	106.04 ± 19.48	104.35 ± 21.3	0.711
Glucose,mmol/L	6.8 ± 5.7	7.37 ± 8.24	6.3 ± 1.73	0.455
Hematocrit, %	42.16 ± 6.8	41.8 ± 7.8	42.45 ± 5.88	0.679
Calcium channel blockers, n (%)	5 (6.2)	3 (3.7)	2 (2.5)	0.523
Proton pump inhibitors, n (%)	80 (98.8)	37 (45.6)	43 (53.2)	0.323
Beta blockers, n (%)	71 (89.9)	33 (40.7)	37 (45.6)	0.114
ACE Inhibitors, n (%)	53 (65.4)	25(30.8)	28 (34.6)	0.355

To assess the possibility of extrapolating the findings according to the results of genotyping patients with ACS, conditionally healthy participants were included in the study. When genotyping the healthy participants, 70 (51.5%) had the AA genotype, 55 (40.4%) had the AC genotype and 11 (8.1%) had the CC genotype. The frequency of the minor allele C was 28.3% and that of the allele A was 71.7%. In the group, compliance with the equilibrium frequency distribution of genotypes was observed according to the Hardy-Weinberg equilib-

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rium. A comparison of the carrier frequency of the allelic variant C rs2244613 between healthy participants and patients with ACS revealed no statistically significant differences (Table 1).

Discussion

The study aimed to determine the effect of the carrier of the rs2244613 polymorphism of the *CES1* gene on the effectiveness of antiplatelet therapy and included 81 patients admitted with a diagnosis of ACS. To compare the rs2244613 carrier frequency among patients with ACS and healthy people, 136 conditionally healthy participants were included in the study. The results of the study showed that the carriage of the rs2244613 polymorphism of the *CES1* gene is associated with a higher residual platelet reactivity. This may be associated with a decrease in the concentration of the active metabolite of clopidogrel in the body, which in turn, is related to an increase in the catalytic activity of the CES1 enzyme. We hypothesized that there were no differences in the rs2244613 carrier frequency between healthy people and patients with CVD. A comparison of genotyping data of healthy participants and patients with ACS from the same ethnic group showed no statically significant differences in the carrier frequency rs2244613 ($p > 0.05$).

Clopidogrel is thienopyridine, which is converted into a pharmacologically active thiol metabolite through an inactive intermediate 2-oxo-clopidogrel [4]. The reaction is catalyzed by cytochrome P450 enzymes, including CYP2C19, CYP3A, CYP2B6, CYP1A2 and CYP2C9 [11]. However, only 15% of clopidogrel becomes bioavailable [12]. The active metabolite 5-thiol clopidogrel inhibits ADP-induced platelet activation and aggregation by irreversible binding to the P2Y12 receptor on the platelet surface [13]. Approximately 85% of the starting clopidogrel is rapidly hydrolyzed to an inactive carboxylic acid derivative, which is further metabolized by binding to glucuronic acid [4]. This hydrolysis is catalyzed by CES1, which is mainly expressed in the liver [14]. In addition, recent studies have shown that the hydrolysis of clopidogrel with CES1 results in a more than 1000-fold increase in plasma carboxylic acid inactive compared with the concentration of active clopidogrel derivative in blood [7]. From this, it follows that the production of an inactive metabolite of a drug occurs much more efficiently than the formation of an active metabolite [8]. This suggests the enormous influence of the catalytic activity of the CES1 enzyme on the concentration of clopidogrel in the body.

According to the latest publications on Pharmgkb.org, the leading resource on Pharmacogenetics [https://www.pharmgkb.org/chemical/PA449053/clinicalAnnotation/982030805], the rs71647871 polymorphism of the *CES1* gene was assigned a level of evidence of 2B, as well as another polymorphism of the cytochrome P450 isoenzyme CYP2C19, according to which more studies were conducted. The rs71647871 polymorphism of the *CES1* gene is associated with a decrease in the catalytic activity of the CES1 enzyme and, therefore, with an increase in the concentration of the active metabolite of clopidogrel in the body. Increased evidence suggests that more and more studies on the *CES1* gene reliably confirm the association of a carrier of a particular polymorphism with a change in the catalytic activity of the enzyme and its further effect on clopidogrel pharmacotherapy. Thus, further studies can confirm the feasibility of using genotyping for polymorphisms of the *CES1* gene as a genetic marker in clinical practice.

In our study we obtained interesting results. According to the Kruskal-Wallis and Mann-Whitney tests, the *CYP2C19*2* and **17* genotypes had no influence on the PRU level. However, according to the regression results, *CES1* and *CYP2C19*2* are risk factors of increased PRU level. These results are contradictory and such contradiction cannot be ignored. We believe that for accurate justification the sample must be increased further so that the pairwise comparison test can achieve reliable results. Moreover, we also need to note that even non-significant *CYP2C19*17* shows an association in the opposite direction of that of *CYP2C19*2*, but it did not reach statistical significance potentially due to the low sample size.

In a study by Xie et al. [15], the *CES1P1* rs3785161 polymorphism was associated with a weakened antiplatelet effect of clopidogrel in 162 patients with ischemic heart disease and the rs8192950 polymorphism was associated with a decrease in the risk of dangerous clinical complications, such as recurrent transient polymorphism and shear heart disease. Stroke MI and many CVDs in patients with extracranial or intracranial stenosis of blood vessels were treated with clopidogrel. Another unique study by Neuvonen et al. [16] found that variants of the rs12443580 and rs8192935 polymorphisms of the *CES1* gene had a significant effect on CES1 expression in whole blood, but not in the liver, which indicates the tissue-specific effect of these polymorphisms on CES1 expression. These polymorphisms did not affect clopidogrel pharmacokinetics; in comparison, the *CES1* c.428G> A allelic variant was associated with a significant decrease in clopidogrel hydrolysis. As the average percentage of inhibition of the P2Y12-mediated platelet aggregation with clopidogrel was 21% higher with the *CES1* c.428G> allelic variant ($p = 0.009$) and 12% lower with the *CYP2C19* c.681G> allelic variant ($p = 0.01$), these results demonstrate the need to continue studying the *CES1* gene and its polymorphism, the associated

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functional changes and the effect on clopidogrel pharmacokinetics. This is because it has the potential to play a crucial in antiplatelet therapy adjustment in patients undergoing ACS and PCI.

To the best of our knowledge, our study is the first to study the effect of the rs2244613 polymorphism of the *CES1* gene on antiplatelet therapy with clopidogrel. It was determined that a change in *CES1* at the position c.1168-33A>C can hypothetically affect protein phosphorylation and influence the catalytic function of CES1. This polymorphism can change the secondary structure of the CES1 protein and affect the interaction between the CES1 protein and the ligand. Forexample, in the study of Jian Shi [17], it was determined that the rate of activation of DABE and its intermediate metabolites M1 and M2 were closely related to the activity of CES1. The R^2 values of the correlations between the activation rates of DABE, M1 and M2 to DAB and the CES1 activity were 0.81, 0.62 and 0.78, respectively. It was also reported that the allelic variant G143E was related with the loss-of-function variant for the activation of DABE, M1 and M2 [17]. This means that this allelic variant may affect the structure of the CES1 enzyme, thus changing the ligand-protein binding. However, this hypothesis requires further detailed study.

The results of our study are related to those of the PAPI study, which reported that the rs71647871 polymorphism of the *CES1* gene is associated with a loss of the catalytic activity of carboxylesterase-1, which may cause an increase in the plasma concentration of the active metabolite of clopidogrel and an increase in the antiaggregant effect. In our study, we found that the rs2244613 polymorphism of the *CES1* gene is associated with an increase in the residual reactivity of platelets and, therefore, with a possible increase in the catalytic activity of CES1 and a decrease in the antiplatelet effect of the drug, which may lead to the recurrence development of thrombotic complications in the future. However, studying the effect of the rs2244613 polymorphism on the functional properties of the CES1 enzyme requires further research in order to more accurately assess the antiplatelet effect of clopidogrel and its correlation with various clinical manifestations.

We also need to mention the results of other studies that did not have such significant effects on the association of the *CES1* polymorphism carrier and clinical outcomes in treatment with clopidogrel. Fathy conducted a simple logistic regression association analysis between major adverse cardiovascular event and *CES1* polymorphism and found that genetic factor does not detect statistically significant association [18]. In another study on the *CES1* variant, only four participants in the studied population carried that variant allele, which prevented the authors from testing this association [19]. As demonstrated above, the diversity of polymorphisms and their effects on the catalytic activity of the CES1 enzyme determines the relevance of further studying this gene. Moreover, it is necessary to further assess the feasibility of introducing pharmacogenetic testing in modern clinical practice.

Conclusions

A statistically significant association of the rs2244613 polymorphism with clopidogrel resistance was detected. We have confirmed that this polymorphism is equally common in both healthy and ACS patients. The obtained results confirm the feasibility of using this genetic marker for predicting the pharmacological response to clopidogrel and developing approaches to optimizing the pharmacotherapy of ACS based on *CES1* genotyping. The fact that there is no difference in the carrier frequency between healthy people and patients with CVD from one ethnic group indicates the possibility of extrapolating such results and conclusions between groups and the lack of connection between the polymorphism and the disease. However, further prospective pharmacogenetic studies are needed for a more complete understanding of the relationship between the polymorphic rs2244613 marker carrier and the clinical outcomes of pharmacotherapy and the personalization of dosing regimens for patients taking clopidogrel. It is also necessary to evaluate the combined effect of CES1 enzymes and cytochrome P450 isoenzymes on the pharmacokinetics and pharmacodynamics of clopidogrel and its impact on clinical efficacy and safety.

As for the limitations of this study, first, it is a relatively small sample size. Second, the current study cannot assess the effects of the polymorphisms of other enzymes involved in the metabolism of drugs and their impacts on the final outcome. Third, due to the low level of scientific knowledge on the polymorphism rs2244613 of the *CES1* gene, we cannot fully evaluate its effect on the functional properties of the enzyme.

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