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The *ABCB1*, *CYP2C19*, *CYP3A5* and *CYP4F2* genetic polymorphisms and platelet reactivity in the early phases of acute coronary syndromes

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Abstract

Background: The aim was to study seven polymorphic markers of genes encoding proteins involved in the absorption, metabolism and pharmacokinetics of clopidogrel among patients with an acute coronary syndrome (ACS), who have undergone percutaneous coronary intervention (PCI).

Patients and methods: Eighty-one ACS and PCI patients older than 18 years and treated with dual antiplatelet therapy were enrolled in the study. Platelet function testing and *ABCB1*, *CYP2C19*, *CYP3A5* and *CYP4F2* genotyping were performed. The predictive role of categorical variables, such as genotypes (carriers and non-carriers of polymorphism), on platelet reactivity (platelet reactivity units [PRU] platelet inhibition [PI]) was assessed by logistic regression (for categorical outcomes) and linear regression (for continuous outcomes) analysis. A p-value < 0.05 was considered significant. The allele frequencies were estimated by gene counting, and Hardy-Weinberg equilibrium was tested using the chi-square test.

Results: Regarding clopidogrel response, 62 patients (76.5%) were clopidogrel responders and 19 were non-responders (23.5%). Mean PRU value and the percentage of platelet inhibition were 170.0 ± 50.9 PRU and $28.6 \pm 19.9\%$, respectively. The effects of the *CYP2C19*2* polymorphisms on PRU (166.0 ± 50.8 vs. 190.7 ± 48.2, $p < 0.038$) and PI (30.6 ± 20.0 vs. 18.1 ± 16.3, $p < 0.013$) were observed, and the rates of high platelet reactivity (HPR) were lower in *CYP2C19*1/*1* than those in *CYP2C19*1/*2* + *CYP2C19*2/*2* (16.2% vs. 53.8% $p < 0.0067$). In comparison, no significant difference in PRU value and PI was observed at <5 days between the rest of polymorphisms ($p > 0.05$). Based on the logistic regression analysis, *CYP2C19*2* (OR: 4.365, CI: 1.25–17.67, $p = 0.022$) was an independent predictor of HPR at <5 days, as was the stent diameter (OR: 0.219, CI: 0.002–0.229, $p = 0.049$). The remaining polymorphisms had no influence.

Conclusions: The reactivity of the on-clopidogrel platelet in the early phase of ACS is influenced primarily by the *CYP2C19* polymorphisms. We believe that the findings of the present study could supply additional evidence regarding the clinical appropriateness of the *CYP2C19* genetic testing for designing suitable antiplatelet therapy in the early phase of ACS.

Keywords: acute coronary syndrome; clopidogrel; cytochrome P450; percutaneous coronary intervention; pharmacogenetics; polymorphism.

Introduction

As a standard of care for patients with acute coronary syndrome (ACS) undergoing percutaneous coronary intervention (PCI), dual antiplatelet therapy is routinely used [1]. The P2Y12 inhibitor clopidogrel is a prodrug; therefore, it needs to be metabolized in the liver, which makes its pharmacokinetics dependent on the activity of the enzymes involved. For clopidogrel, these enzymes are CYP2C19, CYP2B6, CYP1A2 for the transformation of the prodrug into 2-oxo-clopidogrel as stage one, and CYP2C19, CYP2B6, CYP3A4, CYP3A5, CYP2C9 to transform that intermediate product into an active metabolite as stage two [2]. The metabolism of clopidogrel involves

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a two-stage transformation and tiny transporter P-glycoproteins to facilitate prodrug absorption at the apical membranes of the intestinal cells. This P-glycoprotein is encoded by the *ABCB1* gene. As a result, there are numerous sites for a possible breakdown, which can lead to an altered response to clopidogrel. These causes for altered clopidogrel response could be divided into three groups: (1) pharmacokinetic markers like intestinal absorption, (2) metabolic activation in the liver and (3) pharmacodynamic markers. These causes have a clear link to variants of the genes coding the enzymes involved, which are called polymorphisms. These polymorphisms, which are either suspected or proven causes, are mentioned in this article as follows: *ABCB1 C3435T*, *ABCB1 C>T*, *rs4148738* for P-glycoprotein; *CYP2C19*17 C-806T*, *CYP2C19*2 681G>A*, *CYP2C19*3 636G>A*, *CYP3A5*3 A6986G* for genes *CYP2C19* and *CYP3A5* of the cytochrome P450 enzymes family; and *CYP4F2 C(Val433Met)T* as a pharmacodynamic marker for the CYP4F2 enzyme, which is also responsible for the epoxyeicosatrienoic acid metabolism [3].

This altered response to clopidogrel poses a challenge to physicians and could potentially lead towards complications, such as stent thrombosis or bleeding [4, 5]. Although studies suggest that the presence of polymorphisms does affect laboratory clopidogrel resistance, the clinical implications for routine genetic testing are not stated according to the current guidelines [6]. The reason for this is that the 'critical mass' of the studies, which show that altered response to clopidogrel might seriously jeopardize the safety of percutaneous coronary intervention and cause stent thrombosis or bleeding, has not yet been achieved.

One way to assess the response to clopidogrel is to measure platelet reactivity with the VerifyNow P2Y12 assay. The therapeutic range is over 85, but less than 208 platelet reactivity units (PRUs) [7]. While a PRU level under 85 may be a predictor of a bleeding event, a PRU level over 208 is a sign that a higher clopidogrel reactivity is present and a physician might consider a need for further action. This is the time for a personalized approach. Past trials have assessed doubling and tripling the dose of clopidogrel for low responders [8, 9]. However, the current strategy for low responders is switching to another P2Y12 inhibitor like ticagrelor or prasugrel [10].

Polymorphisms in these genes occur differently among populations: while *CYP2C19*2* and *CYP2C19*3* are seen among Asians, *CYP2C19*17* is more often present among Caucasians [11]. Therefore, a physician might consider measuring platelet reactivity for such a patient or switch to another P2Y12 inhibitor.

Patients and methods

Study population

The study protocol was approved by the Ethics Committee of the Russian Medical Academy of Continuous Professional Education and the Ministry of Health of the Russian Federation, Moscow. All patients gave written informed consent prior to their participation. The study was conducted in accordance with the Declaration of Helsinki and consistent with applicable guidelines for good clinical practice. The patients included in this study were recruited from October 2014 to September 2015 in the Moscow City Clinical Hospital №1, Moscow, Russian Federation. The study population consisted of 81 ACS and PCI patients older than 18 years; they were treated with dual antiplatelet therapy, 100 mg of aspirin daily and either a 300 or 600 mg loading dose following 75 mg maintenance dose daily of clopidogrel. The major exclusion criteria included high risk of bleeding, thrombocytopenia, contraindications to aspirin or clopidogrel, malignancies, pregnancy, and cerebrovascular events within the past 3 months. These factors included age, sex, smoking, diabetes mellitus, hypertension (BP ≥ 140/90 mmHg), hyperlipidemia, body mass index, family history of coronary artery disease, previous myocardial infarction, and coronary stents, which were obtained from patient's files or interview.

Blood sampling

A total of two blood samples from each of the 81 patients were collected on the 3rd and 5th day for genotyping and platelet reactivity. Blood (2 mL each) for DNA analysis was sampled from the peripheral vein using ethylene diamine tetra acetate (EDTA) tubes (VACUETTE®, Greiner Bio-One, Austria) and stored at −80 °C. Blood samples (2 mL each) for the measurement of platelet activity in response to ADP were taken in tubes with 3.2% sodium citrate at 4 h after the dose of clopidogrel was taken [12].

Extraction of the peripheral blood DNA

Genomic DNA was extracted from the peripheral blood by using 'DNA – EKSTRAN-1' (ZAO Syntol, Russia) according to the manufacturer's protocol. The quantitative concentration of DNA was measured by the Nanodrop Spectrophotometer (ND-2000, USA).

ABCB1, *CYP2C19*, *CYP3A5* and *CYP4F2* genotyping

A panel of seven SNPs of *ABCB1 (C3435T, rs1045642)*, *ABCB1 (C>T, rs4148738)*, *CYP2C19*2 (681G>A, rs4244285)*, *CYP2C19*3 (636G>A, rs4986893)*, *CYP2C19*17 (C-806T, rs1224856)*, *CYP3A5*3 (A6986G, rs776746)* and *CYP4F2 (C>T, Val433Met, rs2108622)* was selected based on previous investigations [2, 12, 13]. Base numbering and allele definitions were defined by the nomenclature of the Human Cytochrome P450 (CYP) Allele Nomenclature Committee (www.pharmvar.org). The kit that was used according to the manufacturer's instructions for genotype determination was a TaqMan® Single

Nucleotide Polymorphism Genotyping Assay kit and a TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA) with an ABI PRISM® Sequence Detector 7000 (Applied Biosystems). The PCR reactions were carried out in a 10-μL reaction volume containing genomic DNA 15 ng, oligonucleotide primers 0.5 pM, 1 μL 10 PCR buffer, deoxynucleotides (dNTPs) 250 μM, magnesium chloride 3 mM and DNA polymerase 0.25 U. The cycling program consisted of preliminary denaturation at 95 °C for 10 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 60 s, and elongation at 72 °C for 60 s, followed by a final elongation step at 72 °C for 7 min [14].

Platelet function assay

Platelet function tests were performed within 1 h of sample acquisition by utilizing VerifyNow P2Y12 assay (Accumetrics, San Diego, CA, USA) within 5 days after clopidogrel loading (early phase). Glycoprotein IIb/IIIa inhibitors and intravenous anticoagulants, except the unfractionated heparin, were not used in this study. The level of platelet aggregation is expressed in P2Y12 reactivity unit (PRU) and in platelet percentage inhibition (PI). The VerifyNow system is a point-of-care platelet function testing system. The VerifyNow P2Y12 assay measures the ADP-induced platelet aggregation as PRU. The device activates platelets through the thrombin receptor pathway, which is P2Y12-receptor-independent, and provides a total platelet function baseline, which is reported as 'Base PRU'. The percent of ADP-induced platelet aggregation inhibition is calculated from the PRU and Base PRU values. A value for higher platelet reactivity (HPR) was defined as PRU > 208 in the present study, despite the lack of consensus on the cutoff value associated with clinical outcomes [15]. The reference values were PRU values < 208 (responders to clopidogrel) or PRU values > 208 (high OTR to clopidogrel or clopidogrel non-responders). Moreover, a cutoff for lower platelet reactivity (LPR) was defined as PRU < 85 in the current study.

Statistical analysis

The qualitative variables were expressed as relative and absolute frequencies and the quantitative variables as the mean (M) ± standard deviation (SD). The Kolmogorov Smirnov test was used to assess normality. Continuous variable comparisons were performed using the Student's t-test and analysis of variance. The χ^2-test and analysis of variance (ANOVA) were used to compare the variables between the subgroups, and the Fisher exact test was used when any expected cell count was <5 for a contingency table. Differences in data between subgroups were tested by either ANOVA (when values were normally distributed) and the non-parametric Mann-Whitney U-test or Kruskal-Wallis test (when the distribution normality test failed). The predictive role of categorical variables, such as genotypes (carriers and non-carriers of polymorphism) on platelet reactivity (PRU, PI) was assessed by logistic regression (for categorical outcomes) and linear regression (for continuous outcomes) analysis. A p-value < 0.05 was considered significant. The allele frequencies were estimated by gene counting, and the Hardy-Weinberg equilibrium was tested using the χ^2-test. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) 20.0 (IBM Corporation, USA).

Results

Characteristics of the study population and genotyping

A total of 81 Caucasian patients were included in the study. Of the 81 ASC patients, 77 received PCI, and four received standard medical treatment without PCI. No significant differences were observed in the demographic, clinical, and laboratory findings between the study subgroups according to platelet reactivity levels (Table 1).

All patients were successfully genotyped for seven genetic variants, *ABCB1 (C3435T, rs1045642)*, *ABCB1 (C > T, rs4148738)*, *CYP2C19*2 (681G > A, rs4244285)*, *CYP2C19*3 (636G > A, rs4986893)*, *CYP2C19*17 (C-806T, rs1224856)*, *CYP3A5*3 (A6986G, rs776746)* and *CYP4F2 (C > T, Val433Met, rs2108622)* (Table 2). Apart from *ABCB1 (C3435T, rs1045642)* and *CYP3A5*3 (A6986G, rs776746)*, all the genetic variants showed no significant deviations from the Hardy-Weinberg equilibrium (Table 2).

Effects of the *ABCB1*, *CYP2C19*, *CYP3A5* and *CYP4F2* genotypes on the P2Y12-antiplatelet effect of clopidogrel

Regarding clopidogrel response, 62 patients (76.5%) were clopidogrel responders and 19 were non-responders (23.5%). The mean PRU value and the percentage of platelet inhibition were 170.0 ± 50.9 PRU and 28.6 ± 19.9%, respectively. No significant differences in Base PRU levels were observed among any genotypes. At <5 days (an average of 3.4 days), the effects of the *ABCB1*, *CYP2C19*, *CYP3A5* and *CYP4F2* genotypes on the P2Y12-reactivity unit (PRU) values and the percentage of platelet inhibition (PI) after ACS with PCI (ANOVA, U-test) are shown in Table 3.

The effects of the *CYP2C19*2* polymorphisms on PRU (166.0 ± 50.8 vs. 190.7 ± 48.2, $p < 0.038$) and PI (30.6 ± 20.0 vs. 18.1 ± 16.3, $p < 0.013$) (Table 3) were observed, and the rates of HPR were lower in *CYP2C19*1/*1* than those in *CYP2C19*1/*2 + CYP2C19*2/*2* (16.2% vs. 53.8% $p < 0.0067$). In comparison, no significant difference in PRU value and PI was observed at <5 days between the remaining polymorphisms ($p > 0.05$).

By logistic regression analysis, *CYP2C19*2* (OR: 4.365, CI: 1.25–17.67, $p = 0.022$) was found to be an independent predictor of HPR at <5 days, as was also the stent diameter (OR: 0.219, CI: 0.002–0.229, $p = 0.049$). The remaining polymorphisms had no influence.

Table 1: Baseline demographics and clinical characteristics of the study cohort with resistance to clopidogrel (PRU > 208) and a normal response to clopidogrel (PRU < 208).

Characteristics	Total (n = 81)	Patients with PRU < 208 (n = 62)	Patients with PRU > 208 (n = 19)	p-Value
Age, years mean ± SD	63.9 ± 10.9	63.2 ± 11.0	66.2 ± 10.4	0.265
Men, n (%)	64 (79.0)	50 (80.6)	14 (73.7)	0.360
BMI, mean ± SD	27.8 ± 3.1	28.0 ± 3.2	27.8 ± 3.1	0.361
Sub-type ACS, n (%)				
UA, n	10 (12.3)	9 (14.5)	1 (5.3)	0.290
STEMI, n	50 (61.7)	35 (56.5)	15 (78.9)	
NSTEMI, n	17 (21.0)	14 (22.6)	3 (15.8)	
Indeterminate, n	4 (4.9)	4 (6.5)	0 (0.0)	
Risk factors, n (%)				
DM, n	16 (19.8)	12 (19.4)	4 (21.1)	0.552
HBP, n	75 (92.5)	58 (95.1)	17 (89.5)	0.340
Current smoking status, n	17 (21.5)	12 (19.3)	5 (26.3)	0.385
Prior MI, n	14 (17.2)	8 (12.9)	6 (31.5)	0.497
Prior stroke, n	5 (6.2)	4 (6.5)	1 (5.6)	0.686
Prior PCI, n	12 (14.8)	8 (12.9)	4 (21.1)	0.295
CHF II-III FC, n	3 (3.7)	1 (1.6)	2 (10.5)	0.136
Arrhythmia, n	8 (9.9)	4 (6.5)	4 (21.1)	0.083
Stent thrombosis, n	2 (2.5)	1 (1.7)	1 (5.3)	0.416
Drug-eluting stent, n	30 (38.9)	25 (43.1)	5 (26.3)	0.331
Involved vessel				
LM, n	4 (4.9)	4 (6.5)	0 (0.0)	0.335
LAD, n	39 (49.4)	32 (53.3)	7 (36.8)	0.161
LCX, n	27 (34.2)	22 (36.7)	5 (26.3)	0.295
LMA, n	4 (4.9)	3 (4.8)	1 (5.3)	0.665
RCA, n	23 (28.3)	15 (24.6)	8 (42.1)	0.115
Stent diameter, mm	2.75 ± 6.8	2.6 ± 0.8	3.0 ± 0.3	0.049
Stent length, mm	25.5 ± 5.1	26.3 (10.9)	22.7 (6.4)	0.178
Medication, n (%)				
Aspirin, n	81 (100)	62 (100)	19 (100)	–
Statin, n	81 (100)	62 (100)	19 (100)	–
CCB, n	5 (6.2)	2 (3.2)	3 (15.8)	0.081
Diuretics, n	22 (27.2)	17 (27.4)	5 (26.3)	0.588
PPI, n	80 (98.8)	62 (100)	18 (94.7)	0.235
β-Blockers, n	71 (89.9)	54 (90.0)	17 (89.5)	0.621
ACE-inhibitors, n	62 (76.5)	49 (79.0)	13 (68.4)	0.254

ACS, acute coronary syndrome; STEMI, ST-segment elevation myocardial infarction; NSTEMI, non-ST-segment elevation myocardial infarction; UA, unstable angina; CHF, chronic heart failure; FC, functional class; ACE, angiotensin converting enzyme inhibitor; BMI, body mass index; CCB, calcium channel blocker; DM, diabetes mellitus; HBP, hypertension; IM, intermediate metabolizer; LAD, left anterior descending artery; LCX, left circumflex artery; LM, left main coronary artery; LMA, left marginal artery; RCA, right coronary artery; MI, myocardial infarction; PCI, percutaneous coronary intervention; PM, poor metabolizer; PPI, proton pump inhibitor. Student's t-test for continuous variables and χ^2-test for categorical variables, $p < 0.05$ for all.

Linear regression was used to calculate the odds ratio (OR) of changes in on-clopidogrel platelet reactivity (PRU and PI) between the carriers and non-carriers of the minor allelic variant: for PRU – *CYPC19*2*: 190.7 ± 48.2 vs. 166.0 ± 50.8 (OR = 0.27; p = 0.026) and *CYPC19*17*: 153.8 ± 62.3 vs. 176.8 ± 44.1 (OR = 0.3; p = 0.01); for PI: 18.1 ± 16.3 vs. 30.6 ± 20.8 (OR = 0.33; p = 0.005) and *CYPC19*17*: 34.9 ± 25.0 vs. 25.9 ± 16.9 (OR = 0.32; p = 0.006), the remaining polymorphisms had no influence.

Linkage disequilibrium analysis and haplotype analysis

We used 'SNPStats' for the analysis of linkage disequilibrium and haplotype analysis (http://bioinfo.iconcologia.net/en/SNPStats_web) [16]. The polymorphisms are considered to be in 'strong LD' if the one-sided upper 95% confidence bound on D′ is ≥0.98 and the lower bound is ≥0.70. On the one hand, the *ABCB1 rs1045642* and *ABCB1*

Table 2: Genotype frequencies and Hardy-Weinberg equilibrium test.

Allele	Genotype, n (%)			Minorallele, %	HWE[a]	
	Homozygous noncarriers	Heterozygous carriers	Homozygous carriers		χ^2	p-Value
ABCB1 (rs1045642)						
Obs.	CC 17 (21.0%)	CT 51 (63.0%)	TT 13 (16.0%)	47.5	5.57	0.018
Exp.	22.3	40.4	18.3			
ABCB1 (rs4148738)						
Obs.	CC 15 (18.5%)	CT 45 (55.6%)	TT 21 (25.9%)	53.7	1.11	0.291
Exp.	17.4	40.3	23.4			
*CYP2C19*2 (rs4244285)*						
Obs.	GG 68 (84.0%)	AG 13 (16.0%)	AA 0 (0.0%)	8.0	0.62	0.432
Exp.	68.5	12.0%	0.5			
*CYP2C19*3 (rs4986893)*						
Obs.	GG 81 (100%)	AG 0 (0.0%)	AA 0 (0.0%)	0.0	–	–
Exp.	–	–	–			
*CYP2C19*17 (rs1224856)*						
Obs.	CC 57 (70.4%)	CT 23 (28.4%)	TT 1 (1.2%)	25.4	0.62	0.428
Exp.	57.9%	21.1	1.9			
*CYP3A5*3 (rs776746)*						
Obs.	GG 78 (96.3%)	AG 3 (3.7%)	AA 0 (0.0%)	1.35	11.9	0.001
Exp.	77.1	4.8	0.1			
*CYP4F2*3 (rs2108622)*						
Obs.	CC 40 (49.4%)	CT 38 (46.9%)	AA 3 (3.7%)	27.1	2.79	0.095
Exp.	43.0	32.0	6.0			

[a]HWE, Hardy-Weinberg equilibrium test. obs., observed genotype; Exp., expected genotype.

Table 3: The effects of the *ABCB1*, *CYP2C19* and *CYP4F2* genotypes on the P2Y12-reactivity unit (PRU) values and the percentage of platelet inhibition (PI) after ACS with PCI (ANOVA, U-test).[a]

Genotype	PRU value, mean ± SD	p-Value	Percentageinhibition (PI), %	p-Value
ABCB1				
rs1045642				
CC	187.0 ± 26.6	0.123	20.0 ± 13.1	0.077
CT + TT	165.5 ± 54.9		30.9 ± 20.8	
rs4148738				
CC	169.2 ± 52.8	0.946	29.2 ± 17.7	0.657
CT + TT	170.1 ± 50.9		28.6 ± 19.9	
CYP2C19				
rs4244285				
GG	166.0 ± 50.8	0.038	30.6 ± 20.0	0.013
GA + AA	190.7 ± 48.2		18.1 ± 16.3	
rs4986893				
GG	170.0 ± 50.9	–	28.6 ± 19.9	–
GA + AA	–		–	
rs1224856				
CC	176.8 ± 44.1	0.064	25.9 ± 16.9	0.076
CT + TT	153.8 ± 62.3		34.9 ± 25.0	
CYP4F2				
rs2108622				
CC	174.8 ± 51.0	0.407	27.7 ± 18.3	0.902
CT + TT	165.3 ± 51.0		29.5 ± 21.5	

[a]Analysis of variance (ANOVA) was used to calculate the difference in PRU value, and Mann-Whitney U-test was used to calculate the difference in percentage inhibition.

rs4148738 polymorphisms showed a strong linkage disequilibrium (D′ = 0.72 and D′ = 0.99, relatively). On the other hand, the polymorphisms in the *CYP2C19* gene *(*2 (rs4244285)* and **17 (rs1224856))* were in low linkage disequilibrium (D′ = 0.36) ($p < 0.05$, Figure 1). The distribution and association with the platelet reactivity of these haplotypes are shown in Table 4. If some haplotype has a significant association ($p < 0.05$) with higher mean difference of PRU or PI than reference haplotype, it is considered characteristic of poor clopidogrel response (Table 4).

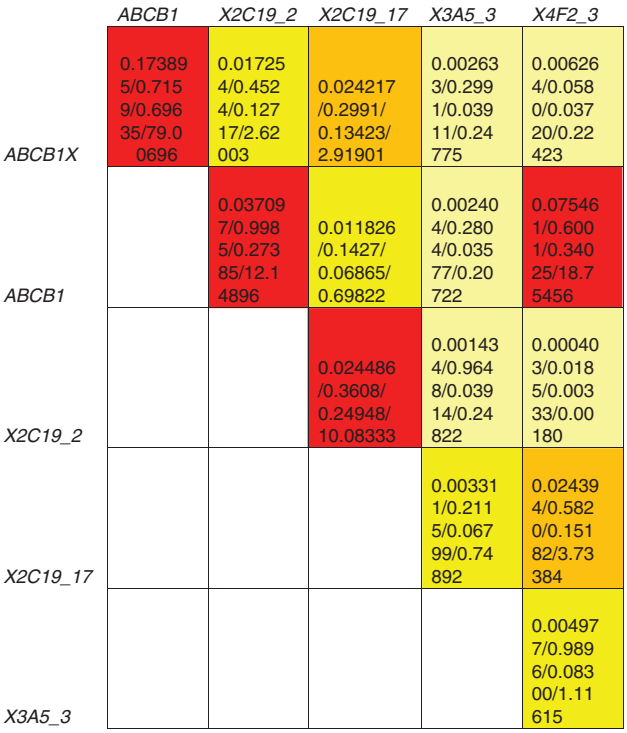

Figure 1: The linkage disequilibrium analysis of the *ABCB1*, *CYP2C19*, *CYP3A5* and *CYP4F2* polymorphisms.

Discussion

Eighty-one ACS patients undergoing PCI were included in this study at <5 days. This time frame was chosen because most cases of stent thrombosis occur within the first 30 days (especially within the first 7 days) after stent implantation [17]. This research was designed to explore the effects of the *ABCB1*, *CYP2C19*, *CYP3A5* and *CYP4F2* genetic polymorphisms on clopidogrel response among patients with ACS and PCI. Previous studies have reported that *ABCB1 (C3435T, rs1045642)*, *ABCB1 (C > T, rs4148738)*, *CYP2C19*2 (681G > A, rs4244285)*, *CYP2C19*3 (636G > A, rs4986893)*, *CYP2C19*17 (C-806T, rs1224856)* and *CYP3A5*3 (A6986G, rs776746)* can affect clopidogrel pharmacokinetics and pharmacodynamics [18] in both healthy volunteers and ACS patients. The polymorphisms of the *CYP4F2 (C > T, Val433Met, rs2108622)* genes selected in this study have no impact on clopidogrel pharmacokinetics, but this gene coding cytochrome P450 4F2 subfamily – an enzyme that takes part in the biosynthesis of epoxyeicosatrienoic acids – is an important vasoactive product of arachidonic acid metabolism with a wide range of biological actions in the cardiovascular system, including influence on platelet reactivity. Simultaneously, there was a paucity of data regarding the combined effect of several *ABCB1*, *CYP2C19*, *CYP3A5* and *CYP4F2* at-risk alleles, and insufficient data to measure the linkage disequilibrium analysis and haplotype analysis. We found that *CYP2C19*2* increases the on-clopidogrel platelet reactivity, which is associated with a higher risk of ischemic cardiovascular events [19]. Furthermore, we observed that *CYP2C19*17* was associated with lower platelet reactivity than in non-carriers. Previous studies that enrolled Russian patients [20–25] investigated the role of the *CYP2C19* polymorphism, *ABCB1* genes and other risk factors on the poor clopidogrel antiplatelet effect. For example, Komarov et al. showed that male sex,

Table 4: Haplotype association with P2Y12-reactivity unit (PRU) and percentage of platelet inhibition (PI) after ACS with PCI for all SNPs (n = 81, crude analysis).

ABCB1		CYP				Haplotype analysis results		
(rs1045642)	*(rs4148738)*	*CYP 2C19*2 (rs4244285)*	*2C19*17 (rs1224856)*	*3A5*3 (rs776746)*	*4F2*3 (rs2108622)*	Haplotype frequency	PRU mean difference (95% CI)	p-Value
PRU								
T	C	G	C	G	C	0.3011	0.00	–
T	C	G	C	G	T	0.0336	−60.65 (−92.21 to 29.08)	0.00023
PI, %								
T	C	G	C	G	C	0.3146	0.00	–
C	T	A	T	G	C	0.0313	−20.91 (−37.14 to 4.67)	0.013
T	T	G	T	G	T	0.0122	46.29 (23.1–69.48)	0.00014
T	C	G	C	G	T	0.0062	68.2 (35.59–100.81)	<0.0001

low ejection fraction, multivascular lesion of coronary arteries, the *ABCB1 C3435T* polymorphism and proton pump inhibitor intake significantly increase the risk of clopidogrel non-responsiveness, and that *CYP2C19*2* carriers has a 2.4-times increased risk of thrombotic cardiovascular complications (95% CI = 1.2–4.9; p = 0.01) [20]. The frequency of both alleles *CYP2C19*2* and *CYP2C19*17* among the current ACS patients was close to that observed among Russian patients from the Moscow region and from Northern, Central, and Eastern Siberia in other previously published studies with ACS patients.

None of the other polymorphisms was associated with differences in the results of the 5-day platelet reactivity analysis by the VerifyNow P2Y12 assay.

According to the linkage disequilibrium analysis, the *ABCB1 rs1045642* and *ABCB1 rs4148738* polymorphisms showed a strong linkage disequilibrium (D′ = 0.72 and D′ = 0.99, relatively). In contrast, the polymorphisms in the *CYP2C19* gene *[*2 (rs4244285)* and **17 (rs1224856)]* were in low linkage disequilibrium (D′ = 0.36) (p < 0.05).

There are about 34 *CYP2C19* alleles, including *CYP2C19*2*, *CYP2C19*3* and *CYP2C19*17*. The most common *CYP2C19* polymorphisms are *CYP2C19*2*, *CYP2C19*3*, and *CYP2C19*17* [26]. The carriers of the *CYP2C19*2* and *CYP2C19*3* polymorphisms are poor metabolizers (PM), i.e. they have decreased activity of liver enzymes and reduced clopidogrel biotransformation. Carriers of the *CYP2C19*17* polymorphism are ultrarapid metabolizers (UM). About 50% of the Mongoloid, 34% of Negroid and 18% of Caucasians are *CYP2C19*2* carriers [3, 27]. The *CYP2C19*3* allele frequency is less than 1% in Caucasians and Negroid and less than 7% in Mongoloids [28]. *CYP2C19*17* has been found in 25.7% of Germans [29], 22% of Norwegians [30] and less than 4% of the Asian population (Korean, Japanese and Chinese) [31]. *CYP2C19*2* has been shown to be a strong determinant of ischemic cardiovascular events in Asian patients, whereas the relation between the *CYP2C19*2* allele and the magnitude of risk has been inconsistent in Caucasians [3, 28, 32, 33]. The evaluation of the differences in the prevalence of the *CYP2C19* gene polymorphisms is of utmost importance in multiethnic countries, such as Russia. According to the results obtained in previous studies, 16%–27.5% of ACS patients from different counties in Russia have at least one *CYP2C19*2* allele variant, which changes the clopidogrel metabolism. For instance, the results of previous studies in both healthy volunteers and ACS patients showed that 23.0% of Russians, 21% of Buryats, 38.0% of Kalmyks, 20.0% of Tatars, 12.0% of Ingushes, 18.0% of Chechens, 14.0% of Circassian, 18.8% of Carachay, 12.7% of Avar, 5.0% of Dargin, 14.5% of Lak, 23% of Yakuts, 15% of Altayans and 15% of Tuvinians carried at least one *CYP2C19*2* allele variant [34–40].

Based on the association between *CYP2C19* and clopidogrel pharmacokinetics, numerous studies have confirmed an important role of the *CYP2C19* loss-of-function alleles in HPR, as determined by ADP-induced platelet reactivity in CAD patients [3, 9, 41–47]. In addition, some studies have also revealed that *CYP2C19*17* (increased activity allele) results in enhanced platelet inhibition and, possibly, an increased bleeding risk [3, 48, 49]. Several clinical studies have detected a significant association between the *CYP2C19*2* allele and adverse cardiovascular events, including an increased risk for stent thrombosis [2, 3, 12, 28, 41, 43, 50, 51]. Furthermore, studies revealed that the usage of *CYP2C19*-genotyping among patients treated with clopidogrel leads to a larger overall benefit compared with lower risk indications, such as medical management of ACS and stable CAD [52, 53]. Therefore, the influence of *CYP2C19* on clinical outcomes has been most evident among ACS with PCI patients. The reported meta-analyses suggest that the clopidogrel-treated ACS/PCI patients who are *CYP2C19*2* carriers have an increased risk of main adverse cardiovascular events compared with *CYP2C19*1/*1* patients [54].

In fact, it is very difficult to perform large randomized studies guided by genotype-directed treatment. In previous studies, the *CYP2C19* genotype-directed antiplatelet therapy trials have been reported (CLOVIS-2, ELEVATE TIMI-56, RAPID-GENE, RAPID STEMI, ACCEL-AMI-2C19, ACCEL-2C19, GeCCO, PAPI-2, GIANT, TAILOR-PCI, NCT01097343). For example, in RAPID GENE trials, it was determined that the *CYP2C19* genetic testing after ACS with PCI can be done effectively for genotype-directed treatment [55], which subsequently was expanded in the RAPID STEMI trial [56]. Although the reported 'pharmacodynamic' randomized studies support the *CYP2C19* genotype-directed antiplatelet therapy, clinical trials powered for actual clinical outcomes are ultimately more likely to definitively establish or refute the clinical utility of the *CYP2C19* genotyping.

The Society for Cardiovascular Angiography and Interventions, the American Heart Association (AHA), the American College of Cardiology Foundation (ACCF), the Society of Thoracic Surgeons and European Society of Cardiology (ESC) do not recommend routine genetic testing prior to clopidogrel administration, because they lack sufficient evidence. However, they did suggest consideration of genetic testing for patients at high risk of suffering from adverse cardiovascular events (high-risk PCI procedures, previous stent thrombosis, DM, etc.) and that CYP2C19 poor metabolizers should be prescribed an

alternative antiplatelet regimen [57]. Similarly, the Royal Dutch Association for the Advancement of Pharmacy Pharmacogenetics Working Group (KNMPPWG), and the Clinical Pharmacogenetics Implementation Consortium (CPIC) recommend genetic testing and genotype-directed treatment for high-risk patients [58]. According to the CPIC guidelines, the switch to an alternative P2Y12 inhibitor (e.g. prasugrel, ticagrelor) is supported [59].

There is a need to mention some limitations to this study. First, it includes a relatively small number of patients. Second, the current study does not evaluate the association of the clinical outcome and the genetic polymorphism.

Conclusions

We found that on-clopidogrel platelet reactivity in the early phase of ACS is influenced primarily by the *CYP2C19* polymorphisms. We believe that the findings of the present study could provide additional evidence regarding the clinical appropriateness of the *CYP2C19* genetic testing for tailoring antiplatelet therapy in the early phase of ACS. However, in order to draw definite conclusions, further large-scale research studies are necessary.

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