Multiple Mutations of CYP2C19, PON1 and ABCB1 Influence Platelet Response, Bleeding and Thrombosis Risk to Clopidogrel after Percutaneous Coronary Intervention: A Retrospective Study

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Abstract

Background: Different combinations of multiple mutations of CYP2C19, PON1 and ABCB1 for the efficiency and safety of Clopidogrel in patients undergoing percutaneous coronary intervention (PCI) remain unclear.

Methods: 263 Chinese Han patients receiving 75mg Clopidogrel and 100mg Aspirin every day for 12 months after PCI were enrolled in this study. ADP-induced platelet aggregation rates, thrombosis and bleeding risk are used to compare the Clopidogrel response among the combined genetic mutation.

Results: Our study demonstrated that multiple genetic mutations are very common in patients receiving Clopidogrel and Aspirin after PCI. Patients who have high ADP-induced platelet aggregation rate with more mutations of CYP2C19, PON1 and ABCB1. Patients with full mutations of CYP2C19, PON1 and ABCB1 have highest risk of thrombosis and lowest risk of bleeding in patients receiving clopidogrel and aspirin with one-year follow-up duration.

Conclusions: We summarized multiple genetic polymorphism influences platelet reactivity, bleeding and thrombosis risk to Clopidogrel after percutaneous coronary intervention.

Background

Percutaneous coronary intervention (PCI) is a common revascularization strategy to Coronary atherosclerotic heart disease. Post-procedural thrombotic events often happen in the implanted vascular stent. Dual-antiplatelet therapy strategies after PCI, with aspirin and Clopidogrel, is firstly recommended to reduce the thrombosis risk in European and American Guidelines [1]. However, clopidogrel's active metabolite generation is unpredictable, which was influenced by the function of CYP2C19, PON1 and ABCB1. Cytochrome 4502C19 (CYP2C19) catalyzes the prodrug clopidogrel into 2-oxo-clopidogrel [2]. The paraoxonase (PON-1) enzyme transforms 2-oxo-clopidogrel into its active form [3, 4]. The gene ABCB1 encoding the P Glycol-protein (P-gp) regulates the clopidogrel absorption in systemic circulation [5, 6]. Patients carried CYP2C19*2, CYP2C19*3, PON-1Q192R or ABCB1 C3435T homozygous mutant genotypes experienced low clopidogrel response and a high risk of adverse thrombotic events when receiving aspirin and Clopidogrel after PCI. While the evidence for effects of alleles heterozygous mutations of CYP2C19, PON1 and ABCB1 in patients with acute coronary syndrome (ACS) or coronary artery disease (CAD) undergoing PCI receiving Clopidogrel is still controversial and inconclusive [3, 5, 7–10]. The efficiency and safety of Clopidogrel on carriers of multiple heterozygous mutations of CYP2C19, PON1 and ABCB1 also remains unclear. This study aims to demonstrate the influence of Clopidogrel on Platelet response, Bleeding and Thrombosis Risk in patients undergoing PCI with multiple heterozygous mutation of CYP2C19, PON1 and ABCB1 gene.

Methods

Study Participants

263 Chinese Han patients receiving 75mg Clopidogrel and 100mg Aspirin every day for 12 months after PCI from The Fifth People's Hospital of Shanghai were enrolled in this retrospective study between June 2016 and March 2020. The inclusion criteria were: (1) patients with genotyping of the CYP2C19*2, CYP2C19*3, PON-1Q192R and ABCB1 C3435T with polymerase chain reaction (PCR); (2) patients with regular following up for 12 months; (3)

patients with continuous drug prescription records; (4) patients who calculated the ADP dependent Platelet aggregation rate when clopidogrel treated for at least 7 days and stayed at a stable plasma concentration. The exclusion criteria: (1) patients who experienced the major bleeding and thrombotic events in the last 6 months before PCI; (2) Patients who received anticoagulation drugs in the last 6 months before PCI; (3) patients received CYP2C19 or ABCB1 inhibitors (such as Omeprazole or Clarithromycin) during 12 months after PCI; (3) patients who stopped or adjusted the dose of clopidogrel and aspirin during dual-antiplatelet therapy; (4) patients who switched to ticagrelor or prasugrel; (5) patients with severe renal and hepatic function failure. (6) patients with large surgery during the observation period.

The study was approved by the ethics committee of The Fifth People's Hospital of Shanghai.

Platelet aggregation assays

Blood samples for platelet aggregation ratio assays were done after 7 days with clopidogrel and aspirin in patients undergoing PCI. Blood samples (3 ml) were collected in the tube containing 10 μ mol/L sodium citrate. The platelet-rich plasma (PRP) and the platelet-poor plasma (PPP) were separated by centrifuged. The PPP and PRP samples were heated to 37°C for 3 min, then 20 μ mol/L of ADP was added to the samples to measure platelet aggregation rate (PAR).

Genotyping

Details on Genotyping technology has been published previously [3, 11]. All patients enrolled in this study have undergone genetic testing for CYP2C19, ABCB1 and PON1 genotypes. The DNA were extracted and the single nucleotide polymorphisms (SNPs) of each allele (CYP2C19*2, CYP2C19*3, ABCB1 and PON-1) were determined by the DNA assay method on a Real-Time PCR System according to the manufacturer's instructions. Carrier status for all alleles was available and reported as wild type, heterozygous or homozygous for the minor allele.

Outcomes and follow-up

The thrombotic and bleeding events were extracted from out-patients follow-ups for 12 months after PCI. Thrombotic events are composed of stent thrombus, Stroke, TIA and target vessel-related MI. The bleeding events is consisting of major bleeding (Cerebral hemorrhage, Gastrointestinal bleeding, Fundus bleeding) and minor bleeding (Subcutaneous bleeding, Gums bleeding and so on)

Statistical analysis

Variables are presented as the mean $\pm$ standard deviation (SD) or frequencies (percentage). Continuous variables were compared using Student's t-test or one-way ANOVA, and categorical variables were compared using the chi-squared test or Fisher's exact test. To examine the association between the risk factors and the ADP induced platelet aggregation rate, we performed univariate and multivariate Cox regression analyses. To avoid the potential bias of covariates between high and low ADP induced platelet aggregation rate, a case-control matching method was used to match variables that included age, gender, hemoglobin and hepatic function. Matching tolerance was 0.02. To compare the predictability for high ADP induced platelet aggregation rate among the genotyping mutation, logistic regression analysis was performed. Thrombosis and bleeding event rates were estimated by the Kaplan Meier method according to the multiple genetic mutation groups and compared by log-rank tests.

Results

Identification of Multiple mutations of CYP2C19, ABCB1 and PON-1 in Han Chinese Patients.

CYP2C19, PON-1 and ABCB1 genotypes were assayed in 263 patients undergoing PCI. The distribution of CYP2C19*2, CYP2C19*3, PON Q192R and ABCB1 3435 C allelic and genotype frequency are shown in Table 1. Approximately 46% of the population had one or two heterozygous mutation of CYP2C19*2 or CYP2C19*3 (CYP2C19 Intermediate metabolite, IM) and 14% had homozygous alleles (CYP2C19 poor metabolite, PM). In terms of the prevalence of ABCB1 3435 C > T polymorphism, 40% of the population had one T-allele (CT, heterozygous mutation) and 17% had two T-alleles (TT, homozygous mutation). In terms of the prevalence of PON1 polymorphism, 46% of the population had one or more A-allele (GA or AA) and 18% had two A-alleles (AA). Only 19 patients with no mutation of CYP2C19, PON1 and ABCB1 gene; 24 patients with three heterozygous mutation of CYP2C19, PON1 and ABCB1 gene; 25 patients with single homozygous mutation alone in CYP2C19, PON1 and ABCB1 gene (Table 1).

Table 1
The distribution of multiple mutations of CYP2C19, ABCB1 and PON-1

	Total	CYP2C19 metabolic phenotype		
		EM (*1/*1)	IM (*1/*2, *3)	PM (*2/*2)
CYP2C19 metabolic phenotype	263	97(0.37)	126(0.48)	40(0.15)
ABCB1 3435C > T genotype				
CC	107(0.41)	31(0.32)	60(0.48)	16(0.4)
TC	109(0.41)	41(0.42)	47(0.37)	21(0.53)
TT	47(0.18)	25(0.26)	19(0.15)	3(0.08)
PON1 575A > G genotype				
GG	92(0.35)	29(0.3)	46(0.37)	17(0.43)
GA	127(0.48)	46(0.47)	62(0.49)	19(0.48)
AA	44(0.17)	22(0.23)	18(0.14)	4(0.10)
Combined genetic status(ABCB1 + PON1)				
0 mutation	36(0.14)	9(0.09)	21(0.17)	6(0.15)
ABCB1(mu)/PON1(mu) (ABCB1 CT or PON1 GA)	127(0.48)	42(0.43)	64(0.51)	21(0.53)
ABCB1(mu) + PON1(mu) (ABCB1 CT + PON1 GA)	100(0.38)	46(0.47)	41(0.33)	13(0.33)
The values are expressed as n (%) of patients.				
EM , extensive metabolizer, CYP2C19 *1*1; IM , intermediate metabolizer, CYP2C19 *1*2 or *1*3; PM , poor metabolizer, CYP2C19 *2*2 or *3*3 or *2*3. CC , ABCB1 3435C > T wild allele; CT , ABCB1 3435C > T heterozygotic allele; TT , ABCB1 3435C > T homozygotic allele. GG , PON1 575A > G wild allele; GA , PON1 575A > G heterozygotic allele; AA , PON1 575A > G homozygotic allele. 0 mutation : ABCB1 CC + PON1GG; ABCB1(mu)/PON1(mu) : ABCB1CT/TT + PON1GG or ABCB1 CC + PON1GA/AA; ABCB1(mu) + PON1(mu) : ABCB1CT/TT + PON1GA/AA.				

Risk factors of high platelet aggregation rate on patients with dual antiplatelet therapy.

A total of 263 patients were enrolled in our study. Table 2 shows the baseline characteristics of the overall population according to the ADP dependent Platelet aggregation rate. The patients with high ADP-Platelet aggregation were older and had lower hemoglobin. They also exhibited a higher prevalence of acute MI as an index diagnosis, chronic liver disease, CYP2C19 variants, as well as a greater incidence of higher ADP dependent Platelet aggregation compared with those who achieved a successful anti-platelet therapy (Table 2).

Table 2
Patient characteristics of high and low ADP-induced Platelet aggregation

ADP-dependent Platelet Aggregation Rate			
Covariste	< 50% (n = 157)	≥ 50% (n = 60)	P value
Age, y	66.49 ± 11.07	71.53 ± 9.55	0.002
Male/Female	130/27	38/22	0.002
BMI	24.85 ± 2.87	24.26 ± 2.86	0.220
Medical history			
Hypertension	103(0.66)	37(0.62)	0.558
Diabetes	50(0.32)	23(0.38)	0.366
AMI	62(0.39)	14(0.23)	0.026
ACS	24(0.15)	11(0.18)	0.585
Ischemic stroke	11(0.07)	3(0.05)	0.591
DBP (mmHg)	76.98 ± 13.81	74.48 ± 11.56	0.215
SBP (mmHg)	132.87 ± 22.64	130.58 ± 22.27	0.504
mmHg)			
HR (/min)	74.54 ± 15.95	73.78 ± 12.52	0.742
PLT(×10 ⁹ /L)	205.05 ± 62.35	198.92 ± 69.45	0.386
HGB(g/L)	138.15 ± 16.38	131.46 ± 15.51	0.007
HbA _{1c} (%)	6.54 ± 1.48	6.74 ± 1.74	0.384
Viscosity	1.38 ± 0.11	1.37 ± 0.12	0.681
ALT(U/L)	31.16 ± 28.04	26.77 ± 31.60	0.321
AST(U/L)	83.82 ± 120.44	30.74 ± 40.49	0.001
SCr(μmol/L)	83.01 ± 37.28	84.20 ± 25.48	0.820

The values are expressed as the mean ± SD or n (%) of patients.

BMI, body mass index; **AMI**, acute myocardial Infarction; **ACS**, acute coronary syndromes; **DBP**, Diastolic blood pressure; **SBP**, Systolic blood pressure; **HR**: heart rate; **HGB**, hemoglobin : **PLT**, platelet count; **HbA_{1c}**, hemoglobin A1c; **Viscosity**, blood viscosity; **ALT**, alanine aminotransferase; **AST**, Aspartate aminotransferase; **SCr**, Serum creatinine; **TC**, total cholesterol; **TG**, Triglycerides; **eGFR**, estimated glomerular filtration rate; **CYP2C19 *1*1**, CYP2C19 wild allele; **CYP2C19 *1*2 or *1*3**, CYP2C19 heterozygotic allele; **CYP2C19 *2*2 or *3*3 or *2*3**, CYP2C19 homozygotic allele. **CC**, ABCB1 3435C > T wild allele; **CT**, ABCB1 3435C > T heterozygotic allele; **TT**, ABCB1 3435C > T homozygotic allele. **GG**, PON1 575A > G wild allele; **GA**: PON1 575A > G heterozygotic allele; **AA**, PON1 575A > G homozygotic allele.

ADP-dependent Platelet Aggregation Rate			
TC (mmol/L)	3.83 ± 1.07	3.66 ± 0.98	0.281
TG (mmol/L)	1.66 ± 1.21	1.48 ± 0.80	0.290
eGFR(ml/min/1.73m ²)	82.27 ± 20.29	77.90 ± 16.68	0.175
CYP2C19			0.004
*1*1	59(0.38)	15(0.25)	0.08
*1*2/*1*3	75(0.48)	33(0.55)	0.341
*2*2/*3*3	23(0.15)	12(0.2)	0.338
PON1			
GG	54(0.34)	20(0.33)	0.883
GA	80(0.51)	27(0.45)	0.433
AA	23(0.15)	13(0.22)	0.214
ABCB1			
CC	67(0.43)	25(0.42)	0.302
CT	63(0.4)	21(0.35)	0.448
TT	27(0.17)	14(0.23)	0.707
The values are expressed as the mean ± SD or n (%) of patients.			
BMI , body mass index; AMI , acute myocardial Infarction; ACS , acute coronary syndromes; DBP , Diastolic blood pressure; SBP ,Systolic blood pressure; HR : heart rate; HGB , hemoglobin : PLT , platelet count; HbA_{1c} , hemoglobin A1c; Viscosity , blood viscosity; ALT , alanine aminotransferase; AST , Aspartate aminotransferase; SCr , Serum creatinine; TC , total cholesterol; TG , Triglycerides; eGFR , estimated glomerular filtration rate; CYP2C19 *1*1 , CYP2C19 wild allele; CYP2C19 *1*2 or *1*3 , CYP2C19 heterozygotic allele; CYP2C19 *2*2 or *3*3 or *2*3 , CYP2C19 homozygotic allele. CC , ABCB1 3435C > T wild allele; CT , ABCB1 3435C > T heterozygotic allele; TT , ABCB1 3435C > T homozygotic allele. GG , PON1 575A > G wild allele; GA : PON1 575A > G heterozygotic allele; AA , PON1 575A > G homozygotic allele.			

Genetic polymorphism is associated with high platelet aggregation rate.

To order to compare the difference distribution of genotyping mutation between high and low ADP-induced platelet aggregation rates, a 1:1 case-control matching procedure was performed to avoid the potential bias of covariates. After matching for age gender, AMI, hemoglobin and hepatic function, there were no differences in age gender, AMI, hemoglobin and hepatic functionafter matching between high and low ADP-induced platelet aggregation rates. There remained a significantly higher mutation of CYP2C19, PON1 and ABCB1 gene in high ADP-induced platelet aggregation rate (Table 3).

Table 3

Relationship between genotype mutations and ADP-induced platelet aggregation rate after 1:1 propensity score matching.

ADP-dependent Platelet aggregation				
Covariste	< 50% (n = 47)	≥ 50% (n = 60)	P value	Multivariate analysis HR (95% CI)
Age, y	68.42 ± 7.46	71.53 ± 9.55	0.478	0.970(0.890–1.506)
Male/Female	30/17	38/22	0.625	1.440(0.334–6.209)
Medical history				
Hypertension	30(0.64)	37(0.62)	0.864	0.903(0.281–2.899)
Diabetes	12(0.26)	23(0.38)	0.944	0.944(0.192–4.647)
AMI	7(0.15)	14(0.23)	0.099	0.214(0.034–1.338)
HGB(g/L)	132.28 ± 14.04	131.46 ± 15.51	0.459	1.019(0.969–1.073)
HbA _{1C} (%)	6.54 ± 1.61	6.74 ± 1.74	0.670	1.089(0.735–1.613)
ALT (U/L)	20.95 ± 12.49	26.77 ± 31.60	0.100	1.045(0.992–1.101)
AST (U/L)	32.01 ± 39.60	30.74 ± 40.49	0.365	0.991(0.971–1.011)
SCr (μmol/L)	78.36 ± 28.08	84.20 ± 25.48	0.665	1.006(0.980–1.032)
TC (mmol/L)	3.89 ± 1.06	3.66 ± 0.98	0.133	0.571(0.274–1.187)
TG (mmol/L)	1.62 ± 1.36	1.48 ± 0.80	0.929	0.979(0.608–1.574)
CYP2C19			0.001	
*1*1	24(0.51)	15(0.25)	1	
*1*2/*1*3	22(0.47)	33(0.55)	< 0.001	0.005(0.000-0.079)
*2*2/*3*3	1(0.02)	12(0.20)	0.015	0.023(0.002–0.309)
PON1			0.003	
GG	24(0.51)	20(0.33)	1	
GA	21(0.45)	27(0.45)	0.001	0.015(0.001–0.182)
AA	2(0.04)	13(0.22)	0.015	0.055(0.005–0.565)
ABCB1			0.025	
CC	27(0.57)	25(0.42)	1	

The values are expressed as the mean ± SD or n (%) of patients.

AMI, acute myocardial Infarction; **HGB**, hemoglobin; **HbA_{1C}**, hemoglobin A_{1C}; **ALT**, alanine aminotransferase; **AST**, Aspartate aminotransferase; **SCr**, Serum creatinine; **TC**, total cholesterol; **TG**, Triglycerides.

ADP-dependent Platelet aggregation				
CT	17(0.36)	21(0.35)	0.007	0.74(0.011–0.493)
TT	3(0.06)	14(0.23)	0.080	0.183(0.027–1.222)
The values are expressed as the mean ± SD or n (%) of patients.				
AMI , acute myocardial Infarction; HGB , hemoglobin; HbA_{1C} , hemoglobin A _{1C} ; ALT , alanine aminotransferase; AST , Aspartate aminotransferase; SCr , Serum creatinine; TC , total cholesterol; TG , Triglycerides.				

Multiple genetic mutations influenced the adverse outcomes of dual antiplatelet therapy.

Subsequently, the bleeding and thrombotic events were analyzed to explore the correlation between multiple mutations and adverse outcomes. The rate of any bleeding and major thrombosis is significantly different in individual CYP2C19, PON1 and ABCB1 loss-of-function allele carriers compared with noncarriers. The lower risk of bleeding and higher risk of thrombosis was observed on interaction among carriers with different number of variants. Besides, regardless of carrying or not carrying CYP2C19 variants, PON1 or ABCB1 variants increased the rate of thrombotic events (Table 4).

Table 4
The correlation between multiple mutations and adverse outcomes

	Total adverse outcomes						
	(263)	Bleeding events			Thrombotic events		
	NO	Yes	P value		NO	Yes	P value
Total	(215)	(48)			(234)	(29)	
CYP2C19 metabolic phenotype				0.169			0.546
*1*1	97	73(0.34)	24(0.5)	0.037	89(0.38)	8(0.28)	0.271
*1*2/*1*3	126	106(0.49)	20(0.42)	0.338	110(0.47)	16(0.55)	0.406
*2*2/*3*3	40	36(0.17)	4(0.08)	0.142	35(0.15)	5(0.17)	0.747
ABCB1 3435C > T genotype				0.083			0.006
CC	107	94(0.44)	13(0.27)	0.034	99(0.42)	8(0.28)	0.128
TC	109	83(0.39)	26(0.54)	0.048	99(0.42)	10(0.34)	0.420
TT	47	38(0.18)	9(0.19)	0.86	36(0.15)	11(0.38)	0.003
PON1 575A > G genotype				0.665			0.006
GG	92	77(0.36)	15(0.31)	0.549	88(0.38)	4(0.14)	0.011
GA	127	101(0.47)	26(0.54)	0.367	112(0.48)	15(0.52)	0.695
AA	44	37(0.17)	7(0.15)	0.659	34(0.15)	10(0.34)	0.007
Combined genetic status(CYP2C19 + ABCB1 + PON1)				0.021			0.036
CYP2C19(No) + ABCB1(No) + PON1(No)	9	5(0.02)	4(0.08)		9(0.04)	0(0)	
CYP2C19(No) + ABCB1(Mu)/PON1(Mu)	42	35(0.16)	7(0.15)		41(0.18)	1(0.03)	
CYP2C19(No) + ABCB1(Mu) + PON1(Mu)	46	33(0.15)	13(0.27)		39(0.17)	7(0.24)	
CYP2C19(Mu) + ABCB1(No) + PON1(No)	27	26(0.12)	1(0.02)		27(0.12)	0(0)	

The values are expressed as n (%) of patients.

CC, ABCB1 3435C > T wild allele; **CT**, ABCB1 3435C > T heterozygotic allele; **TT**, ABCB1 3435C > T homozygotic allele. **GG**, PON1 575A > G wild allele; **GA**, PON1 575A > G heterozygotic allele; **AA**, PON1 575A > G homozygotic allele. **CYP2C19(No)**, CYP2C19*1*1; **CYP2C19(Mu)**, CYP2C19*1*2 or *1*3 or *2*2 or *3*3; **ABCB1(No)**, ABCB1 CC, ABCB1 3435C > T wild allele; **ABCB1(Mu)**, ABCB1CT/TT, ABCB1 3435C > T heterozygotic or homozygotic allele; **PON1(No)**, PON1GG, PON1 575A > G wild allele; **PON1(Mu)**, PON1GA/AA, PON1 575A > G heterozygotic or homozygotic allele.

	Total (263)	adverse outcomes			
		Bleeding events		Thrombotic events	
CYP2C19(Mu) + ABCB1(Mu)/PON1(Mu)	85	74(0.34)	11(0.23)	74(0.32)	11(0.38)
CYP2C19(Mu) + ABCB1 (Mu) + PON1(Mu)	54	42(0.2)	12(0.25)	44(0.19)	10(0.34)
The values are expressed as n (%) of patients.					
CC , ABCB1 3435C > T wild allele; CT , ABCB1 3435C > T heterozygotic allele; TT , ABCB1 3435C > T homozygotic allele. GG , PON1 575A > G wild allele; GA , PON1 575A > G heterozygotic allele; AA , PON1 575A > G homozygotic allele. CYP2C19(No) , CYP2C19*1*1; CYP2C19(Mu) , CYP2C19*1*2 or *1*3 or *2*2 or *3*3; ABCB1(No) , ABCB1 CC, ABCB1 3435C > T wild allele; ABCB1(Mu) , ABCB1CT/TT, ABCB1 3435C > T heterozygotic or homozygotic allele; PON1(No) , PON1GG, PON1 575A > G wild allele; PON1(Mu) , PON1GA/AA, PON1 575A > G heterozygotic or homozygotic allele.					

Difference between multiple heterozygous mutation and single homozygous mutation

In order to compare the difference between multiple heterozygous mutation and single homozygous mutation, we extracted the information of patients with three heterozygous and single homozygous mutation of CYP2C19, PON1 and ABCB1 polymorphism. Patients with multiple heterozygous genotype in the clopidogrel plus aspirin group had a higher risk of bleeding (33% vs 8%; $P = 0.028$ for Chi-square test) and lower risk of thrombosis (0% vs 24%; $p = 0.01$ for Chi-square test) (Table 5). While, the thrombosis risk are not different in the two groups.

Table 5

Baseline Characteristics Between Single Homozygous mutation and multiple Heterozygous mutation in Patients with Clopidogrel Treatment After PCI

Genotyping mutation			
Covariste	3 Heterozygous 0 Homozygous (n = 24)	1 Homozygous 0 Heterozygous (n = 25)	P value
Age, y	68.96 ± 14.08	66.44 ± 11.14	0.490
Male/Female	17/7	18/7	0.928
BMI	26.02 ± 3.17	24.82 ± 2.6	0.178
Medical history			
Hypertension	15(0.63)	15(0.6)	0.858
Diabetes	11(0.46)	7(0.28)	0.196
AMI	10(0.42)	8(0.32)	0.483
ACS	4(0.17)	5(0.2)	0.763
Stroke	1(0.04)	3(0.12)	0.317
SBP (mmHg)	132 ± 23.18	137.04 ± 22.54	0.444
PLT (×10 ⁹ /L)	213.88 ± 70.13	197.75 ± 54.05	0.377
HGB (g/L)	131.5 ± 24	134.75 ± 14.27	0.571
ADP-PAR (%)	37.83 ± 20.38	39 ± 21.88	0.868
HbA _{1c} (%)	6.86 ± 1.95	6.99 ± 1.93	0.817
Viscosity	1.39 ± 0.12	1.38 ± 0.11	0.846
ALT (U/L)	30.07 ± 15.27	34.28 ± 49.21	0.690
AST (U/L)	89.95 ± 100.62	59.44 ± 91.33	0.272
SCr (μmol/L)	78.96 ± 18.59	83.44 ± 26.52	0.505
TC (mmol/L)	3.93 ± 1.01	4.13 ± 1.11	0.521
TG (mmol/L)	1.61 ± 0.99	1.79 ± 1.05	0.557
eGFR(ml/min/1.73m ²)	82.9 ± 17.37	80.52 ± 19.66	0.680

The values are expressed as the mean ± SD or n (%) of patients.

BMI, body mass index; **AMI**, acute myocardial Infarction; **ACS**, acute coronary syndromes; **SBP**, Systolic blood pressure; **HR**: heart rate; **HGB**, hemoglobin : **PLT**, platelet count; **ADP-PAR**: ADP induced platelet aggregation rate; **HbA_{1c}**, hemoglobin A1c; **Viscosity**, blood viscosity; **ALT**, alanine aminotransferase; **AST**, Aspartate aminotransferase; **SCr**, Serum creatinine; **TC**, total cholesterol; **TG**, Triglycerides; **eGFR**, estimated glomerular filtration rate.

Genotyping mutation			
Bleeding events	8(0.33)	2(0.08)	0.028
Thrombotic events	0(0)	6(0.24)	0.010
The values are expressed as the mean $\pm$ SD or n (%) of patients.			
BMI , body mass index; AMI , acute myocardial Infarction; ACS , acute coronary syndromes; SBP , Systolic blood pressure; HR : heart rate; HGB , hemoglobin : PLT , platelet count; ADP-PAR : ADP induced platelet aggregation rate; HbA_{1c} , hemoglobin A1c; Viscosity , blood viscosity; ALT , alanine aminotransferase; AST , Aspartate aminotransferase; SCr , Serum creatinine; TC , total cholesterol; TG , Triglycerides; eGFR , estimated glomerular filtration rate.			

Discussion

Our study evaluated the ADP-induced Platelet aggregation rate and safety outcomes in patients with the combined mutations of the CYP2C19, PON1 and ABCB1 undergoing PCI receiving clopidogrel and aspirin. Our study demonstrated that patients with multiple mutations of CYP2C19, PON1 and ABCB1 have higher ADP-induced Platelet aggregation rate and more bleeding and thrombotic events. Patients with all mutations of CYP2C19, PON1 and ABCB1 have highest risk of thrombosis and lowest risk of bleeding in patients receiving clopidogrel and aspirin with one-year follow-up.

Multiple studies have consistently shown that PCI-treated patients with impaired clopidogrel-induced platelet inhibition increased the risk for stent thrombosis and ischemic events. Low platelet reactivity is associated with an increased risk of bleeding events, while high platelet reactivity (HPR) is correlated with a high incidence of thrombotic events[12]. Different platelet function testing, such as VerifyNow, Multiplate, vasodilator-stimulated phosphoprotein, thromboelastography (TEG) with platelet mapping and light transmission aggregometry are available for the assessment of on-treatment platelet reactivity to adenosine diphosphate (ADP) [13, 14]. However, based on the results of platelet function testing, a series of studies were conducted to escalate the appropriate P2Y₁₂ inhibitors on clinical therapy and failed to improve the safety outcomes[15, 16]. Peng and his collaborators[3] explored the relationships of the effects of CYP2C19 and PON1 Q192R polymorphism and ADP-induced platelet inhibition by thromboelastography (TEG) in ACS patients with clopidogrel. They found individual CYP2C19 and PON1 variants influenced platelet response on clopidogrel and both LOF CYP2C19 and PON1 192R variants are independent risk factors of high platelet reactivity. However, they didn't evaluate the influence of the multiple variants on safety outcomes. Some researchers measured P2Y₁₂ platelet reaction units using the VerifyNow P2Y₁₂ test to evaluate the effect of clopidogrel on platelet aggregation. Treatment with prasugrel significantly reduced HPR compared with clopidogrel by P2Y₁₂ reaction unit thresholds[17]. For patients with long-term DAPT (aspirin + clopidogrel) after PCI with coronary stent implantation, switching from clopidogrel to prasugrel resulted in a stable reduction in PRU, regardless of CYP2C19 polymorphism[18]. In our study, we used ADP-induced platelet aggregation rate to evaluate the platelet response on clopidogrel. After PCI, several established risk factors, such as age, gender, hepatic function, Hemoglobin and genetic polymorphism have been incorporated into a statistical model for clopidogrel response prediction. While these factors could be used to fully explain future bleeding and thrombosis events. In order to exclude the potential bias of other factors, we performed case-control matching procedure and compared the consistency between the genetic polymorphism

and ADP-induced platelet aggregation rate, we found patients with allelic mutations of CYP2C19, PON1 and ABCB1 have high platelet aggregation rate on clopidogrel.

Besides, we also found patients with combined mutations of CYP2C19, PON1 and ABCB1 have higher risk of thrombosis and lower risk of bleeding in patients receiving clopidogrel and aspirin with one-year follow-up. In a PCI population taking clopidogrel, the interest in genetic testing for clopidogrel metabolism has grown in recent years to augment standard risk evaluation. The allelic mutant of CYP2C19 which play a significant role in the first oxidation step will lead to lower ADP-induced platelet aggregation rate and higher risk of thrombosis. But the influence on individual PON1 and ABCB1 variants is contradictory and controversial. Chen et al[10] and Hulot et al[19] found the allelic mutant of PON1 was not a major determinant on the clopidogrel pharmacokinetics, pharmacodynamics, and clinical efficacy. While Nishio et al[20], Chen et al[8] and Li et al[21] drew the inconsistent conclusions that PON1 Q192R genotype influenced clopidogrel responsiveness by relative platelet inhibition, especially in CYP2C19 loss-of-function carriers. Similar disputes also occurred in ABCB1 genotype[22–25]. Most studies revealed the individual CYP2C19, PON1 and ABCB1 variants on the platelet reactivity and clinical outcomes on patients receiving long-term DAPT (aspirin + clopidogrel) after PCI. A multicomponent guiding approach to personalize clopidogrel treatment is needed[26]. Some studies focus on the combined status of two at-risk variants in PCI patients on clopidogrel[3, 8, 9, 20]. In the prior studies, both the CYP2C19 poor metabolism status and ABCB1 3435 TT genotype was considered to influence the clopidogrel metabolization and transportation and continuously elicited clopidogrel response[5]. The individuals carrying both at-risk variants showed the highest event rate compared to those with other combined genetic subsets. They could not find any association between adverse events and PON-1 genotypes. However, Peng et al found both mutations of CYP2C19 and PON1 192R variants are independent risk factors of high platelet reactivity except ABCB1 variants[3]. There is a paucity of data regarding the combined effect of three at-risk variants, and no data are available to evaluate the incremental prognostic value of their combined effect over conventional clinical risk factors. In our study, we evaluated the combined effect of three at-risk variants on clinical outcomes of patients receiving clopidogrel and aspirin. The lower risk of bleeding and higher risk of thrombosis was observed on interaction among carriers with different number of variants. Besides, regardless of carrying or not carrying CYP2C19 variants, both of PON1 and ABCB1 variants increased the rate of thrombotic events.

There were still some limitations in our study. Firstly, the sample size was relatively small. Secondly, we didn't use another clinically validated and standardized platelet function testing, such as P2Y₁₂ reaction unit (PRU), vasodilator-stimulated phosphoprotein or TEG with platelet mapping, to define the platelet reactivity in the follow up duration 12 months. Lastly, in this retrospective study, we only included clinical cases with genetic test and platelet aggregation rate and complete follow-up data within one year. This may lead to bias in the inclusion of cases for ignoring patients who transferred to other hospitals and lost to follow-up after adverse events. Finally, we did not exclude the effects of other metabolism enzymes on clopidogrel resistance. Despite we concluded that patients with all mutations of CYP2C19, PON1 and ABCB1 have highest risk of thrombosis and lowest risk of bleeding in patients receiving clopidogrel and aspirin with one-year follow-up duration. Further large-scale and prospective multi-center collaborative research is still essential to draw definite conclusions on the current issues.

Conclusion

Multiple genetic polymorphism influence platelet reactivity, bleeding and thrombosis risk to Clopidogrel after percutaneous coronary intervention.

Abbreviations

PCI, Percutaneous Coronary Intervention; **ADP**, adenosine diphosphate; **BMI**, body mass index; **AMI**, acute myocardial Infarction; **ACS**, acute coronary syndromes; **SBP**, Systolic bloodpressure; **HR** heart rate; **HGB**, hemoglobin; **PLT**, platelet count; **ADP-PAR**: ADP induced platelet aggregation rate; **HbA_{1c}**, hemoglobin A1c; **Viscosity**, blood viscosity; **ALT**, alanineaminotransferase; **AST**, Aspartate aminotransferase; **SCr**, Serum creatinine; **TC**, total cholesterol; **TG**, Triglycerides; **eGFR**, estimated glomerular filtration rate; **CC**, ABCB1 3435C > T wild allele; **CT**, ABCB1 3435C > T heterozygotic allele; **TT**, ABCB1 3435C > T homozygotic allele. **GG**, PON1 575A > G wild allele; **GA**: PON1 575A > G heterozygotic allele; **AA**, PON1 575A > G homozygotic allele. **CYP2C19(No)**, CYP2C19*1*1; **CYP2C19(Mu)**, CYP2C19*1*2 or *1*3 or *2*2 or *3*3; **ABCB1(No)**, ABCB1 CC, ABCB1 3435C > T wild allele; **ABCB1(Mu)**, ABCB1 CT/TT, ABCB1 3435C > T heterozygotic or homozygotic allele; **PON1(No)**, PON1 GG, PON1 575A > G wild allele; **PON1(Mu)**, PON1 GA/AA, PON1 575A > G heterozygotic or homozygotic allele.

Declarations

Ethics approval and consent to participate

The protocol used was approved by the Ethics Committee of The Fifth people's Hospital of Shanghai, Fudan University (No. 2018-192). Due to the retrospective nature of the study, informed consent was waived by the Ethics Committee of The Fifth people's Hospital of Shanghai. All the human data have been performed in accordance with the declaration of Helsinki. All methods were carried out in accordance with the declaration of Helsinki and relevant guidelines and regulations.

Consent for publication

All the authors are consent for publication.

Availability of data and material

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Conflicts of interests

All the authors have no conflict of interest to declare.

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Author contributions

Yan Yu and Hui Jin conceived and designed this study. Patients follow-up and Data extraction was carried out by Xiaoying Shen and Hui Jin. All statistical analysis was performed by Jinfei Song and was double checked by Hui Jin. This manuscript was drafted by Yu Yan, Qing Liang and Guangchun Sun are critically reviewed for improving overall quality.

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