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The impact of gene polymorphism and high on-treatment platelet reactivity on clinical follow-up: outcomes in patients with acute coronary syndrome after drug-eluting stent implantation

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KEYWORDS

- acute coronary syndrome
- clopidogrel
- gene polymorphism
- outcome
- platelet reactivity

Abstract

Aims: The current study sought to evaluate the clinical impact of newly reported genetic variations and their association with clopidogrel high on-treatment platelet reactivity (HTPR) in acute coronary syndrome (ACS) patients after drug-eluting stent (DES) implantation.

Methods and results: The study enrolled 1,016 consecutive patients with ACS undergoing DES implantation. A total of 19 tag single nucleotide polymorphisms (SNPs) were selected from CYP3A4/5, CYP2C19, P2Y12 and ABCB1 genes. ADP-induced light transmittance aggregometry (LTA) was performed to test the post-procedure maximum platelet agglutination (MPA). The primary endpoint was a composite of cardiovascular death, non-fatal myocardial infarction (MI), stent thrombosis, and ischaemic stroke at one-year follow-up after DES placement. The secondary endpoint was the incidence of bleeding events. The post-procedure MPA was calculated and the cut-off point was determined for the HTPR. Using multivariate logistic regression analysis, the carriage of two CYP2C19 LOF alleles was an independent predictor of the post-procedure HTPR (OR: 2.8, 95% CI: 1.70-7.23, p<0.001). Through multivariate Cox regression analysis, the carriage of two CYP2C19 LOF alleles and the post-procedure HTPR were independent predictors of the primary endpoint (HR: 2.3, 95% CI: 1.40-4.97, p<0.001; HR: 2.9, 95% CI: 1.52-5.57, p<0.001, respectively). However, post-procedure MPA did not predict a bleeding event (HR: 0.9, 95% CI: 0.44-1.59, p=0.532).

Conclusions: In patients with ACS, the CYP2C19 LOF allele was associated with post-procedure HTPR and a subsequently increased risk of adverse clinical events at one-year follow-up following DES implantation and clopidogrel administration.

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Introduction

Clopidogrel and aspirin inhibit platelet function, prevent ischaemic events and therefore improve clinical outcomes in patients with acute coronary syndrome (ACS) following percutaneous coronary intervention (PCI) in the drug-eluting stent (DES) era[1-3]. Both the American College of Cardiology and American Heart Association guidelines recommend dual antiplatelet therapy with aspirin and clopidogrel as the standard treatment strategy to avoid thrombotic events after implantation of a DES[4,5]. Despite the introduction of dual antiplatelet therapy treatment, it should be noted that a patient treated by DES remains at risk of an ischaemic event at clinical follow-up. Moreover, persistent high platelet reactivity following PCI is reported to occur, even with adequate pre-treatment of clopidogrel, and is related to an increased risk of adverse cardiovascular events. Indeed, platelet activity in response to clopidogrel treatment is highly variable, with clinical, cellular, and genetic factors considered as important reasons for the variability[6].

Clopidogrel, an inactive prodrug, is metabolised to its active thiol metabolite form by the hepatic cytochrome P450 (CYP) family of isoenzymes, including CYP3A4/5, 2C19, 1A2, 2B6, and 2C9[7-9]. Accumulating evidence now indicates that the polymorphically expressed isoenzymes CYP3A4/5 and CYP2C19 play an integral role in the metabolism of clopidogrel[8,10,11]. The active clopidogrel metabolite directly blocks adenosine diphosphate (ADP) binding to platelet P2Y12 receptors, subsequently inhibiting ADP-mediated activation of the glycoprotein IIb/IIIa complex. Exploration on this issue has demonstrated that several P2Y12 polymorphisms (e.g., C34T, G52T and i-T744C) potentially influence ADP-induced platelet activation[12]. Similarly, *in vitro* and clinical studies have shown that ABCB1 polymorphisms, particularly C3435T, might alter clopidogrel metabolism and its efficacy[13,14]. However, the majority of these studies were primarily carried out in Caucasian populations, with a paucity of large sample size and clinical follow-up results in patients with ACS receiving DES treatment. Therefore, the present study sought to assess the impact of gene polymorphisms (CYP3A4/5, CYP2C19, P2Y12 and ABCB1) on ADP-induced platelet reactivity, and subsequent clinical follow-up outcomes in Chinese high-risk ACS patients receiving clopidogrel after DES implantation.

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Methods

PATIENTS

Between March 2009 and April 2010, 1,127 consecutive patients with ACS following coronary DES implantation were prospectively enrolled in the present study **(Figure 1)**. Patients were considered eligible regardless of the clinical presentation, including unstable angina pectoris (UAP), ST-elevation myocardial infarction (STEMI), and non-ST-elevation myocardial infarction (NSTEMI). Patients were excluded from the study if they had known allergies to antiplatelet therapy, a history of taking clopidogrel within seven days, a left ventricular ejection fraction <30% or New York Heart Association functional Class III or IV, active bleeding and bleeding diatheses, oral anticoagulation therapy with Coumadin, a leukocyte count <3,000/mm^3 and/or a platelet count <100,000/mm^3, an aspartate aminotransferase level or an alanine aminotransferase level ≥3 times the upper normal limit, a serum creatinine concentration ≥2.5 mg/dl, a stroke within six months, non-cardiac disease with a life expectancy of <1 year, or if they were unable to undergo a DES implantation.

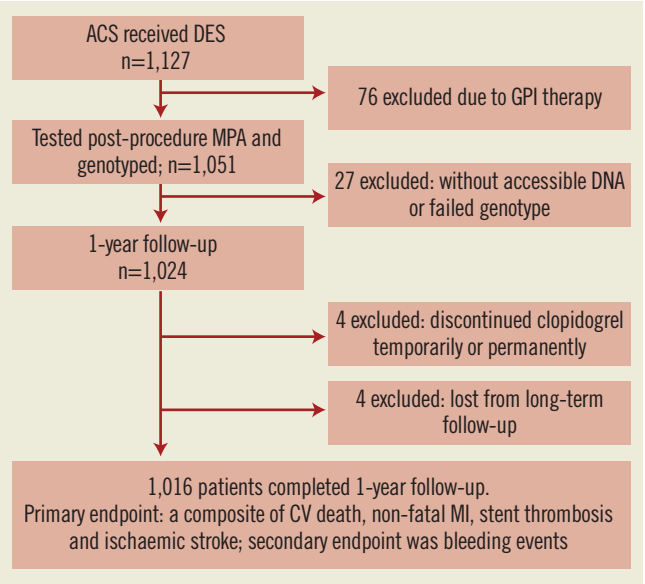

Figure 1. *Flowchart of the study. ACS: acute coronary syndrome; CV: cardiovascular; DES: drug-eluting stent; GPI: glycoprotein IIb/IIIa receptor inhibitor; MI: myocardial infarction; MPA: maximum platelet agglutination*

INTERVENTIONAL PROCEDURES AND MEDICATIONS

All enrolled patients were pre-treated with aspirin (300 mg) and a loading dose of 600 mg clopidogrel before PCI. Stents were deployed according to the standard techniques. Maintenance doses of antiplatelet agents taken for at least 12 months included aspirin (100 mg per day) and clopidogrel (75 mg per day). During the PCI procedure, almost all patients received intravenous unfractionated heparin; however, patients who received a glycoprotein IIb/IIIa receptor inhibitor (GPI) intravenously were excluded (n=76). Compliance with clopidogrel and aspirin therapy was verified by nursing staff in the hospital and ensured through structured or telephone interview during follow-up. Hypertension was defined as a blood pressure of ≥140/90 mmHg taken from a mean of three independent blood pressure measurements, or the use of antihypertensive drugs[15]. Dyslipidaemia and diabetes were defined according to the Third Report of the National Cholesterol Education Program, and the American Diabetes Association, respectively[16,17].

SELECT tagSNPs

Using the pairwise tagging approach, tag single nucleotide polymorphisms (SNPs) were selected from the HapMap CHB databank

(public data release #2/phase III, Feb 2009) with the aid of tagSNPs' online software (http://hapmap.ncbi.nlm.nih.gov/cgi-perl/gbrowse/hapmap 3r2_B36/, Broad Institute, Cambridge, MA, USA). TagSNPs covered the complete CYP3A4 region, from 3,000 bp upstream to 1,000 bp downstream. Common variants were defined as those with a minor allele frequency cut-off of 0.05, with a linkage disequilibrium (LD) measure r2 threshold of 0.8. All selected tagSNPs were ensured from different haplotype regions using this protocol. In total, four tagSNPs (rs2242480C>T, rs2404955G>A, rs2246709A>G and rs4646437C>T) were identified over the entire CYP3A4 gene. In addition, two tagSNPs (rs3800959T>C and rs15524T>C) from the CYP3A5 gene and seven haplotype tagging SNPs (CYP2C19*2-rs4244285G>A, rs7916649G>A, rs3758581G>A, rs11528090T>G, rs4304697G>A, rs3814637C>T and rs10786172A>G) from the entire CYP2C19 gene were selected. On this basis, we also enrolled CYP2C19*3-rs4986893G>A, CYP2C19*17 -rs12248560C>T, P2Y12 (C34T -rs6785930, G52T -rs6809699 and i-T744C -rs2046934), and ABCB1 (rs1045642-3435C>T) **(Online Table 1)**. No deviation from Hardy-Weinberg equilibrium was detected in any enrolled tagSNPs **(Online Table 2)**.

BLOOD SAMPLING AND GENOTYPING

The study protocol was approved by the Institutional Ethics Committee of Shenyang Northern Hospital (No.2011C02), China. Written informed consent was obtained from each participant. DNA was extracted from peripheral blood using TIANamp Blood DNA kits (Tiangen Biotech Co., Ltd., Beijing, China). DNA concentration and quality were assessed by absorbance spectrophotometry and agarose gel electrophoresis. All 19 selected SNPs were genotyped using standard polymerase chain reaction (PCR) techniques. PCR cycling conditions are shown in detail in **Online Table 1**. Each PCR amplification was performed using 0.1 µg DNA, 15 pmol of each primer, 0.4 mmol/L dNTPs, 5 µl of 10× reaction buffer with mgCl2, and 4U rTaq DNA polymerase (total volume 50 µl). DNA sequencing was performed on an ABI Prism 3730 genetic analyser (Applied Biosystems, Foster City, CA, USA) using an ABI dye terminator cycle sequencing kit (Applied Biosystems). There were 27 patients without accessible DNA or failed genotyping.

PLATELET FUNCTION TESTING

Venous blood samples were obtained at least 12 hours after DES implantation, then added to tubes containing 3.2% trisodium citrate. All blood samples were processed within 90 minutes after collection. Platelet aggregation was analysed in platelet-rich plasma (PRP) by optical turbidimetric aggregometry using a four-channel Platelet Aggregation Chromogenic Kinetic System (Helena Laboratories, Beaumont City, TX, USA). PRP was prepared by centrifugation at 200 g for 10 minutes. After adjustment from baseline, 20 µmol/L ADP was added, and aggregation was recorded for five minutes. Results are expressed as a percentage of maximal light transmission from platelet-poor plasma obtained from the same patient. The coefficient of variation of this optical aggregometry assay was 5.2%.

STUDY ENDPOINTS AND DEFINITIONS

The primary endpoint of the present study was a composite of cardiovascular (CV) death, non-fatal myocardial infarction (MI), stent thrombosis, and ischaemic stroke during the initial year after admission. The diagnosis of myocardial infarction was defined as ischaemic symptoms with electrocardiogram (ECG) abnormalities and above the normal limits of elevated troponin[18]. The estimated stent thrombosis (definite) was defined according to the Academic Research Consortium[19]. The secondary endpoint was bleeding events. Bleeding was quantified according to Thrombolysis in Myocardial Infarction (TIMI) criteria[20]. All events were reported to and judged by an independent committee blinded to the genotype and platelet function of the patients.

CLINICAL FOLLOW-UP

Patients were interviewed by telephone every 30 days. Patients exhibiting cardiac symptoms underwent a complete clinical, ECG, and laboratory check-up in an outpatient clinic. All clinical information, from referring physicians, relatives and hospital readmissions, was collated. Source documentation was checked to ensure high-quality data. During the initial year of follow-up, four patients were lost to follow-up study. There were four patients excluded from the study due to discontinued aspirin and/or clopidogrel temporarily or permanently. The reasons for the discontinuation were as follows: two patients received non-cardiac surgery, one patient experienced a severe gastrointestinal reaction, and one patient required dental treatment.

STATISTICAL METHODS

Under three models (codominant, dominant and recessive), the relationships between all enrolled SNPs and the post-procedure high on-treatment platelet reactivity (HTPR) were analysed. A combined receiver operator curve (ROC) analysis was used to determine the cut-off point of post-procedure maximum platelet agglutination (MPA) for the HTPR (MedCalc software, Mariakerke, Belgium). Patients above the cut-off value were considered to exhibit HTPR. Comparisons were performed with the Kaplan-Meier method and the log-rank test. The odds ratio (OR) was estimated by logistic regression analysis. Categorical variables are expressed as n (%) and continuous variables as mean±SD. The Fisher's exact test and Mann-Whitney rank sum test were used for comparison of categorical and continuous variables.

Multivariate logistic regression analysis was used to evaluate the following risk factors on the occurrence of post-procedure HTPR: gene polymorphism, age, gender, body mass index, diabetes mellitus, left ventricular ejection fraction (LVEF), hypercholesterolaemia, hypertension, active smoker, previous MI, previous PCI and proton pump inhibitors. Multivariate Cox regression analysis was used to evaluate the prognostic significance of the following risk factors on the occurrence of ischaemic events or bleeding events within one year of discharge: post-procedure HTPR, gene polymorphism, LVEF, diabetes mellitus, hypercholesterolaemia, hypertension, active smoker, previous MI and previous PCI. The measure of effect was the hazard

ratio (HR). A probability value of <0.05 was considered statistically significant. All abovementioned analyses were performed using SigmaStat software (Systat Software Inc., Point Richmond, CA, USA).

Results

STUDY POPULATION

Within the present study, 1,016 patients with ACS treated by DES were assessed. All patients were from the Chinese Han population. Clinical and procedural characteristics according to the cut-off point of post-procedure HTPR are shown in **Table 1**. The proportion of diabetes mellitus in the HTPR group was higher than that in the non-HTPR group (45.1% vs. 40.7%, p=0.029).

At one-year follow-up, a total of 78 ischaemic events occurred (7.68%): 15 (1.48%) CV deaths, 38 (3.74%) non-fatal MIs, 16 (1.57%) stent thromboses and 9 (0.89%) ischaemic strokes. Sixty-five bleeding events (6.40%) occurred, which included four (0.39%) cases of major bleeding and 61 (6.01%) cases of minor bleeding.

ROC ANALYSES

We calculated the cut-off value (namely, MPA >60.7, as the HTPR) using the ROC curve analysis in accordance with or without the composite primary endpoints **(Figure 2A)**. Thus, 288 patients (28.3%) above the HTPR cut-off point were identified. The area under the ROC curve is equal to 0.783 (95% CI: 0.757-0.808, p<0.001). We also calculated the cut-off point (MPA <50.5) according to the bleeding event; however, the area under the curve is only 0.498 (95% CI: 0.515-1.273, p=0.059, **Figure 2B**).

GENOTYPING AND HTPR

In the HTPR group, the proportion of LOF alleles of CYP2C19*2 and *3 is higher than that in the non-HTPR group. No correlation was found between other individual tagSNPs and HTPR **(Table 2)**. The post-procedure platelet aggregation was calculated according to three models **(Table 3)**.

GENOTYPING, HTPR AND OUTCOMES

Patients with post-procedure HTPR had a significantly high risk of ischaemic events compared to the patients without HTPR **(Figure 3)**. Meanwhile, patients carrying two CYP2C19 LOF alleles were associated with a higher risk of ischaemic events compared to those carrying no or one CYP2C19 LOF allele at one-year follow-up **(Figure 4)**. There was no significant difference in the bleeding risk between the groups **(Table 4)**.

The carrying of two CYP2C19 LOF alleles was an independent predictor for the risk of post-procedure HTPR (OR: 2.8, 95% CI: 1.70-7.23, p<0.001). Diabetes mellitus was associated with a risk trend of post-procedure HTPR (OR: 1.3, 95% CI: 0.97-2.48, p=0.057, **Table 5**).

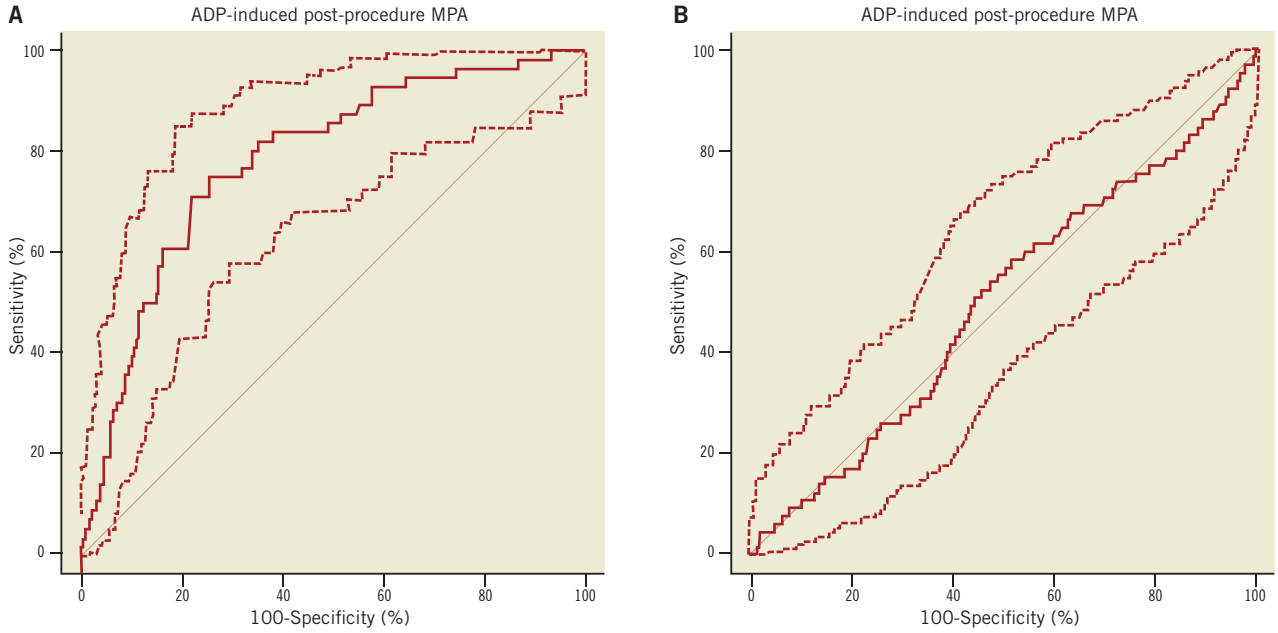

Figure 2. *ROC curve of MPA for the enrolled patients. A) Combined receiver operator curve for 20 mM ADP-induced post-procedural platelet aggregation measured by LTA. A criterion value of >60.7% aggregation provided the HPR-ADP cut-point. Dashed lines represent 95% confidence intervals. Sensitivity: 75.0 (95% CI: 61.6-85.6), specificity: 74.4 (95% CI: 61.6-85.6), area under the ROC curve (AUC): 0.783 (95% CI: 0.757-0.808, p<0.001). B) Combined receiver operator curve for 20 mM ADP-induced post-procedural platelet aggregation measured by LTA. A criterion value of <50.5% aggregation provided the bleeding cut-point. Dashed lines represent 95% confidence intervals. Sensitivity: 58.46 (95% CI: 45.6-70.6), specificity: 48.26 (95% CI: 45.0-51.5), area under the ROC curve (AUC): 0.498 (95% CI: 0.515-1.273, p=0.059). ADP: adenosine diphosphate; CAD: coronary artery disease; HPR: high platelet reactivity; LTA: light transmittance aggregometry; MPA: maximum platelet agglutination; ROC: receiver operator curve*

Table 1. Baseline characteristics of the study population.

Variable		Overall (n=1,016)	According to post-procedure MPA cut-off		p*
			MPA <60.7 (n=728)	MPA >60.7 (n=288)	
Male, n (%)		599 (59.0)	425 (58.4)	174 (60.4)	0.548
Age, yrs		61.2±12.6	60.8±11.5	61.7±10.3	0.363
Body mass index, kg/m^2		25.4±3.1	25.2±3.3	26.2±2.9	0.332
LVEF, %		56.8±9.4	55.9±10.2	57.0±9.9	0.274
Active smoker, n (%)		517 (50.9)	374 (51.4)	143 (49.7)	0.191
Previous MI, n (%)		211 (20.8)	151 (20.7)	60 (20.8)	0.928
Previous PCI, n (%)		266 (26.2)	187 (25.7)	79 (27.4)	0.109
Hypertension (%)		643 (63.3)	457 (62.8)	186 (64.6)	0.243
Hypercholesterolaemia (%)		131 (12.9)	90 (12.4)	41 (14.2)	0.358
Diabetes mellitus, n (%)		426 (41.9)	296 (40.7)	130 (45.1)	0.029
Family history of CAD, n (%)		60 (5.9)	43 (5.9)	17 (5.9)	0.926
Clinical presentation	UAP	552 (54.3)	392 (53.8)	160 (55.6)	0.322
	STEMI	96 (9.5)	68 (9.3)	28 (9.7)	0.431
	NSTEMI	368 (36.2)	268 (36.8)	100 (34.7)	0.633
Laboratory examination	Platelet count, ×109/L	195.8±51.4	194.9±51.7	196.8±50.4	0.274
	Fasting blood glucose, mmol/L	7.5±2.9	7.4±3.1	7.7±2.5	0.451
	Serum creatinine, mmol/L	90.4±22.5	91.1±28.2	89.3±20.5	0.103
Medication at admission, n (%)	Statins	909 (89.5)	650 (89.3)	259 (89.9)	0.733
	β-blocker	710 (69.9)	508 (69.8)	202 (70.1)	0.317
	ACE inhibitor/AT1 inhibitor	802 (78.9)	575 (79.0)	227 (78.8)	0.906
	Proton pump inhibitors	59 (5.8)	43 (5.9)	16 (5.6)	0.272
	Urgent PCI**	315 (31.0)	223 (30.6)	92 (31.9)	0.110
	Selective PCI**	701 (69.0)	505 (69.4)	196 (68.1)	0.534
Stented vessel, n (%)	Left anterior descending coronary artery	729 (71.8)	517 (71.0)	212 (73.6)	0.209
	Left circumflex coronary artery	432 (42.5)	312 (42.9)	120 (41.7)	0.325
	Right coronary artery	701 (69.0)	498 (68.4)	203 (70.5)	0.732
	Left main coronary artery	83 (8.17)	54 (7.4)	29 (10.1)	0.102

* All p-values were adjusted by multivariate logistic analysis including age, male gender, diabetes mellitus, active smoker, body mass index, LVEF, hypertension, platelet count and proton pump inhibitors. Data presented are means + SDs or numbers of patients (percentages). **Urgent PCI: PCI within 24 hours after admission; Selective PCI: PCI more than 24 hours after admission. ACE: angiotensin-converting enzyme; AT1: angiotensin 1; CAD: coronary artery disease; LVEF: left ventricular ejection fraction; MI: myocardial infarction; MPA: maximum platelet agglutination; NSTEMI: non-ST-elevation myocardial infarction; PCI: percutaneous coronary intervention; STEMI: ST-elevation myocardial infarction; UAP: unstable angina pectoris

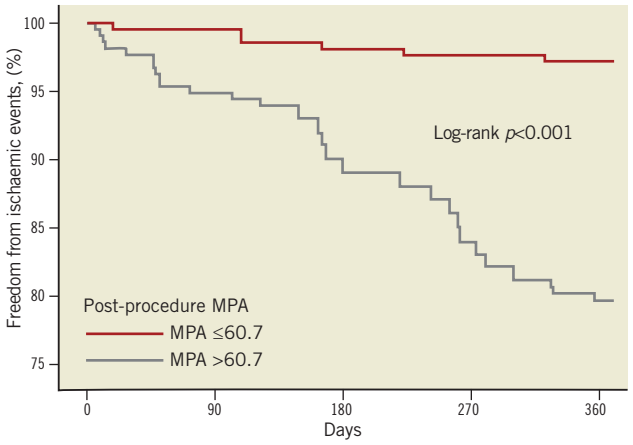

Figure 3. *Kaplan-Meier analysis of the clinical endpoint between the HTPR group and non-HTPR group.*

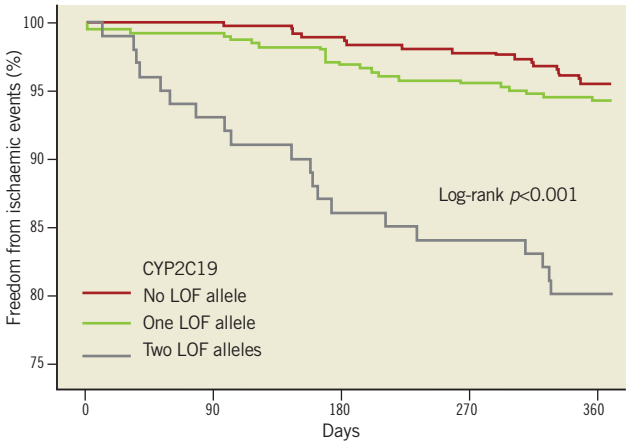

Figure 4. *Kaplan-Meier analysis of the clinical ischaemic endpoint for the presence of the CYP2C19 LOF alleles polymorphism.*

Table 2. Genotyping according to post-procedure HTPR or non-HTPR.

SNP		Model	Overall (n=1,016)	HTPR group MPA <60.7 (n=728)	Non-HTPR group MPA >60.7 (n=288)	p*
CYP2C19	*2-rs4244285 G>A	CO, GG/GA/AA	445/458/113	312/352/64	133/106/49	<0.001
		DO, GG/GA+AA	445/571	312/416	133/155	0.023
		RE, GG+GA/AA	910/114	664/64	239/49	<0.001
	rs7916649 G>A	CO, GG/GA/AA	298/530/188	197/401/130	101/129/58	0.174
		DO, GG/GA+AA	298/718	197/531	101/187	0.234
		RE, GG+GA/AA	828/188	598/130	230/58	0.379
	rs3758581 G>A	CO, GG/GA/AA	749/241/26	541/169/18	208/72/8	0.782
		DO, GG/GA+AA	749/267	541/187	208/90	0.362
		RE, GG+GA/AA	990/26	710/18	280/8	0.463
	rs11528090 T>G	CO, TT/ TG/ GG	377/498/141	286/343/99	91/155/42	0.648
		DO, TT/ TG+GG	377/639	286/442	91/197	0.378
		RE, TT+TG/ GG	875/141	629/99	246/42	0.721
	rs4304697 G>A	CO, GG/GA/AA	724/260/32	523/183/22	201/77/10	0.117
		DO, GG/GA+AA	724/292	523/205	201/87	0.231
		RE, GG+GA/AA	984/32	706/22	278/10	0.192
	rs3814637 C>T	CO, CC/CT/TT	647/318/51	435/254/35	208/64/16	0.432
		DO, CC/CT+TT	647/369	435/289	208/80	0.592
		RE, CC+CT/TT	965/51	693/35	272/16	0.614
	rs10786172 A>G	CO, AA/AG/GG	695/287/34	506/200/22	189/87/12	0.101
		DO, AA/AG+GG	695/321	506/222	189/99	0.297
		RE, AA+AG/GG	982/34	706/22	276/12	0.115
	*3-rs4986893 G>A	CO, GG/GA/AA	959/55/2	696/31/1	261/26/1	0.022
		DO, GG/GA+AA	959/57	696/32	261/27	0.004
		RE, GG+GA/AA	1,014/2	727/1	287/1	<0.001
	*17-rs12248560 C>T	CO, CC/CT/TT	998/18/0	715/13/0	283/5/0	NA
		DO, CC/CT+TT	998/18	715/13	283/5	0.372
		RE, CC+CT/TT	1,016/0	728/0	288/0	NA
CYP3A4	rs2242480 C>T	CO, CC/CT/TT	568/386/62	416/269/43	152/117/19	0.493
		DO, CC/CT+TT	568/448	416/312	152/136	0.821
		RE, CC+CT/TT	954/62	685/43	269/19	0.645
	rs2404955 G>A	CO, GG/GA/AA	461/457/98	344/320/64	117/137/34	0.456
		DO, GG/GA+AA	461/555	344/384	117/171	0.873
		RE, GG+GA/AA	918/98	664/64	254/34	0.342
	rs2246709 A>G	CO, AA/AG/GG	307/525/184	214/287/127	93/138/57	0.914
		DO, AA/AG+GG	307/709	214/514	93/195	0.533
		RE, AA+AG/GG	832/184	601/127	231/57	0.662
	rs4646437 C>T	CO, CC/CT/TT	770/223/23	551/160/17	219/63/6	0.418
		DO, CC/CT+TT	770/246	551/177	219/69	0.517
		RE, CC+CT/TT	993/23	711/17	282/6	0.218
CYP3A5	rs3800959 T>C	CO, TT/TC/CC	728/262/26	521/189/18	207/73/8	0.322
		DO, TT/TC+CC	728/288	521/207	207/81	0.427
		RE, TT+TC/CC	990/26	710/18	280/8	0.378
	rs15524 T>C	CO, TT/TC/CC	471/454/91	337/326/65	134/128/26	0.235
		DO, TT/TC+CC	471/545	337/391	134/154	0.435
		RE, TT+TC/CC	925/91	663/65	262/26	0.414
P2Y12	rs6785930-34C>T	CO, CC/CT/TT	527/412/77	380/293/55	147/119/22	0.871
		DO, CC/CT+TT	527/489	380/348	147/141	0.534
		RE, CC+CT/TT	939/77	673/55	266/22	0.290
	rs6809699-52G>T	CO, GG/GT/TT	836/176/4	595/130/3	241/46/1	0.116
		DO, GG/GT+TT	836/180	595/133	241/47	0.236
		RE, GG+GT/TT	1,012/4	725/3	287/1	0.094
	rs2046934-i744T>C	CO, TT/TC/CC	659/328/29	474/233/21	185/95/8	0.277
		DO, TT/TC+CC	659/357	474/254	185/103	0.534
		RE, TT+TC/CC	987/29	707/21	280/8	0.107
ABCB1	rs1045642-3435C>T	CO, CC/CT/TT	339/489/188	243/352/133	96/137/55	0.228
		DO, CC/CT+TT	339/677	243/485	96/192	0.374
		RE, CC+CT/TT	828/188	595/133	233/55	0.117

* All p-values were adjusted by multivariate logistic analysis including age, male gender, diabetes mellitus, active smoker, body mass index, left ventricular ejection fraction, hypertension, platelet count and proton pump inhibitors. CO: codominant model; DO: dominant model; HTPR: high on-treatment platelet reactivity; MPA: maximum platelet agglutination; RE: recessive model; SNP: single nucleotide polymorphism

Table 3. The post-procedure MPA according to genotype.

Gene	SNP	Codominant model			p*	Dominant model		p*	Recessive model		p*
		W/W	W/M	M/M		W/W	W/M+ M/M		W/W+ W/M	M/M	
CYP2C19	rs4244285 G>A	45.4±22.1	49.2±19.0	62.4±16.2	<0.001	45.4±22.1	55.1±20.1	0.006	46.6±19.2	62.4±16.2	<0.001
	rs7916649 G>A	45.8±19.3	47.3±24.0	47.5±18.3	0.558	45.8±19.3	47.4±24.2	0.231	46.7±24.3	47.5±18.3	0.738
	rs3758581 G>A	46.7±16.1	47.5±18.3	48.1±19.7	0.233	46.7±16.1	47.6±18.9	0.683	46.9±19.1	48.1±19.7	0.115
	rs11528090 T>G	45.6±27.1	48.2±19.1	47.1±21.4	0.579	45.6±27.1	47.7±20.3	0.674	47.4±19.6	47.1±21.4	0.913
	rs4304697 G>A	46.3±18.3	47.9±19.9	45.5±19.5	0.434	46.3±18.3	47.6±20.7	0.235	47.5±20.5	45.5±19.5	0.258
	rs3814637 C>T	48.1±19.1	46.8±24.6	47.0±17.8	0.792	48.1±19.1	46.9±25.7	0.127	47.6±24.9	47.0±17.8	0.190
	rs10786172 A>G	49.8±24.1	46.8±25.5	44.8±16.7	0.504	49.8±24.1	46.2±25.8	0.329	47.8±25.6	44.8±16.7	0.176
	rs4986893 G>A	41.8±25.5	64.3±21.3	61.7±10.7	0.009	41.8±25.5	64.1±21.9	<0.001	47.2±23.2	61.7±10.7	<0.001
	rs12248560 C>T	47.3±21.3	48.3±19.4	ND	NA	47.3±21.3	48.3±19.4	0.678	47.3±21.0	ND	NA
CYP3A4	rs2242480 C>T	46.9±21.3	46.3±23.6	51.5±24.6	0.214	46.9±21.3	47.9±23.9	0.374	47.2±21.9	51.5±24.6	0.109
	rs2404955 G>A	46.3±23.6	44.6±22.5	47.9±19.0	0.219	46.3±23.6	47.6±22.9	0.314	46.9±23.8	47.9±19.0	0.115
	rs2246709 A>G	47.5±22.4	46.2±21.1	47.2±19.2	0.477	47.5±22.4	46.7±21.4	0.756	47.4±22.7	47.2±19.2	0.844
	rs4646437 C>T	47.1±21.4	46.1±19.3	48.1±21.7	0.576	47.1±21.4	47.6±19.9	0.745	47.2±21.6	48.1±21.7	0.776
CYP3A5	rs3800959 T>C	46.9±19.5	49.8±24.7	43.5±19.5	0.271	46.9±19.5	47.8±25.2	0.154	48.5±20.3	43.5±19.5	0.096
	rs15524 T>C	47.5±17.8	46.8±25.7	45.2±17.8	0.530	47.5±17.8	46.5±25.9	0.616	47.4±18.9	45.2±17.8	0.419
P2Y12	34C/T-rs6785930	46.8±16.7	47.7±21.6	44.8±16.9	0.831	46.8±16.7	47.5±21.9	0.810	47.4±17.7	44.8±16.9	0.533
	52G/T-rs6809699	47.9±23.7	45.3±23.6	45.7±13.7	0.586	47.9±23.7	45.4±23.8	0.283	47.4±23.8	45.7±13.7	0.867
	i-744T/C-rs2046934	46.5±24.6	47.9±22.4	41.5±14.6	0.531	46.5±24.6	47.6±22.7	0.632	47.5±24.9	41.5±14.6	0.102
ABCB1	rs1045642-3435C>T	47.7±21.5	45.9±21.4	46.8±16.6	0.628	47.7±21.5	46.4±21.5	0.453	47.5±23.3	46.8±16.6	0.517

* All p-values were adjusted by multivariate logistic analysis including age, male gender, diabetes mellitus, active smoker, body mass index, left ventricular ejection fraction, hypertension, platelet count and proton pump inhibitors. MPA: maximum platelet agglutination; M/M: mutant-type homozygotes; W/M: mutant heterozygotes; W/W: wild-type homozygotes

The post-procedure HTPR was an independent predictor for the composite of ischaemic events (HR: 2.9, 95% CI: 1.52-5.57, p<0.001). Compared with the carriage of no or one LOF allele, the carriage of two CYP2C19 LOF alleles predicted the composite risk of ischaemic events (HR: 2.3, 95% CI: 1.40-4.97, p<0.001; **Table 6**). There were no variables predicting the risk of a bleeding event **(Table 7)**.

Discussion

To our knowledge, this is the first study in East Asia to investigate the correlation among post-procedure platelet reactivity, SNPs (genes of liver metabolic enzymes, platelet surface receptors and intestinal absorption), and clinical outcomes in ACS patients with clopidogrel administration and coronary DES treatment. The following are the

Table 4. The prevalence of post-procedure HTPR and clinical events according to CYP2C19 LOF alleles.

Variables	Total cohort n=1,016	According to MPA cut-off		p*	According to CYP2C19 LOF alleles			p*
		MPA ≤60.7 (n=728)	MPA >60.7 (n=288)		No LOF *1/*1 n=413	1 LOF *1/*2 or *1/*3 n=463	2 LOF *2/*2 or *2/*3 or *3/*3 n=140	
MPA-ADP	47.3±21.0	42.4±17.8	69.9±9.6	NA	42.2±21.8	47.2±20.1	63.4±27.2	<0.001
HTPR, n (%)	288 (28.34)	NA	NA	NA	98 (23.73)	112 (24.19)	78 (55.71)	<0.001
Primary endpoint								
Composite endpoint	78 (7.68)	20 (2.75)	58 (20.1)	<0.001	21 (5.08)	28 (6.05)	29 (20.71)	<0.001
CV death	15 (1.48)	4 (0.55)	11 (3.82)	0.009	5 (1.21)	6 (1.30)	4 (2.86)	0.004
Non-fatal MI	38 (3.74)	11 (1.51)	27 (9.38)	0.002	10 (2.42)	13 (2.81)	15 (10.71)	0.007
Stent thrombosis	16 (1.57)	2 (0.27)	14 (4.86)	<0.001	3 (0.73)	5 (1.08)	8 (5.71)	<0.001
Ischaemic stroke	9 (0.89)	3 (0.41)	6 (2.08)	0.004	3 (0.73)	4 (0.86)	2 (1.43)	0.018
Secondary endpoint								
Bleeding events	65 (6.40%)	46 (6.32)	19 (6.60)	0.649	27 (6.54)	29 (6.26)	9 (6.43)	0.733

* All p-value were adjusted by multivariate logistic analysis including age, male gender, diabetes mellitus, active smoker, body mass index, left ventricular ejection fraction, hypertension, platelet count and proton pump inhibitors. ADP: adenosine diphosphate; HTPR: high on-treatment platelet reactivity; MPA: maximum platelet agglutination

Table 5. Predictors of post-procedure HTPR by multivariate logistic regression analysis.

Variable		Odds ratio	95% CI	*p*-value
CYP2C19 LOF allele carriage	one LOF allele	1.4	0.75-3.81	0.109
	two LOF alleles	2.8	1.70-7.23	<0.001
Age		1.1	0.72-1.78	0.733
Male		1.2	0.91-1.37	0.832
Body mass index		1.4	0.88-2.37	0.131
LVEF		1.2	1.15-2.78	0.279
Diabetes mellitus		1.3	0.97-2.48	0.057
Hypercholesterolaemia		1.0	0.57-1.94	0.212
Hypertension		1.2	0.69-2.35	0.436
Active smoker		1.1	0.64-1.85	0.276
Previous MI		1.5	0.56-3.24	0.479
Previous PCI		1.3	0.59-2.78	0.356
Proton pump inhibitors		1.3	0.71-2.13	0.437

CI: confidence interval; HTPR: high on-treatment platelet reactivity; LOF: loss of function; LVEF: left ventricular ejection fraction; MI: myocardial infarction; PCI: percutaneous coronary intervention

main findings of the present study: (1) the post-procedure HTPR was associated with a significantly increased risk of adverse clinical events; (2) the carrying of two CYP2C19 LOF alleles was associated with an increased post-procedure platelet reactivity and a significantly high incidence of ischaemic events.

Individual HTPR is an emerging issue in interventional cardiology as several studies have reported widely variable response to the therapeutic actions[21,22]. The absolute level of platelet reactivity during treatment has been proposed as a better measure of thrombotic risk than responsiveness to clopidogrel, and any definition of HTPR will only be meaningful when a cut-off or target value is identified[23]. The ROC curve analysis has been used extensively to define the optimal cut-off point for the definition of HTPR according to the clinical endpoint[23]. In this study, the post-procedure HTPR was

Table 6. Predictors of composite ischaemic events by multivariate Cox regression analysis.

Variable		Hazard ratio	95% CI	*p*-value
Post-procedure HTPR		2.9	1.52-5.57	<0.001
CYP2C19 LOF allele carriage	one LOF allele	1.3	0.75-3.81	0.107
	two LOF alleles	2.3	1.40-4.97	<0.001
Diabetes mellitus		1.3	0.72-3.18	0.083
LVEF		1.2	1.07-2.67	0.234
Hypercholesterolaemia		1.2	0.65-1.85	0.197
Hypertension		1.1	0.59-2.17	0.294
Active smoker		1.2	0.61-2.09	0.211
Previous MI		1.6	0.73-3.78	0.431
Previous PCI		1.2	0.64-2.53	0.401

CI: confidence interval; HTPR: high on-treatment platelet reactivity; LOF: loss of function; LVEF: left ventricular ejection fraction; MI: myocardial infarction; PCI: percutaneous coronary intervention

Table 7. Predictors of bleeding events by multivariate Cox regression analysis.

Variable	Hazard ratio	95% CI	*p*-value
Post-procedure MPA	0.9	0.44-1.59	0.532
Carriage of GOF allele (CYP2C19*17)	1.1	0.67-1.92	0.215
Diabetes mellitus	1.2	0.68-2.12	0.384
LVEF	1.1	1.04-1.98	0.247
Hypercholesterolaemia	1.1	0.59-1.89	0.563
Hypertension	1.3	0.78-2.56	0.194
Active smoker	1.4	0.67-2.88	0.134
Previous MI	1.2	0.45-2.13	0.782
Previous PCI	1.3	0.74-2.33	0.376

CI: confidence interval; GOF: gain of function; HTPR: high on-treatment platelet reactivity; LVEF: left ventricular ejection fraction; MI: myocardial infarction; PCI: percutaneous coronary intervention

defined as the cut-point of 60.7% 20 µmol/l ADP-induced MPA which correlated with the occurrence of ischaemic events, with an area under the curve of 0.783.

Studies have consistently demonstrated that HTPR is an independent predictor for the occurrence of thrombotic/ischaemic events after PCI[24,25]. A previous meta-analysis of 4,564 patients with coronary artery disease undergoing PCI showed that the high residual platelet reactivity was significantly associated with an increased risk of death or recurrent cardiovascular events in patients with a poor response to clopidogrel[22]. Similarly, the present study confirmed that post-procedure HTPR was strongly associated with the incidence of ischaemic events (HR 2.9, 95% CI: 1.52-5.57, p<0.001).

Furthermore, Siller-Matula et al found that personalised antiplatelet treatment resulted in an improved efficacy with an equal safety compared to the standard treatment[26]. Recently, Collet et al randomly assigned 2,440 patients scheduled for coronary stenting at 38 centres to a strategy of platelet-function monitoring, with drug adjustment (double dose of clopidogrel or shift to prasugrel) in patients who had a poor response to antiplatelet therapy, or to a conventional strategy without monitoring and drug adjustment[27]. Collet's result showed no significant improvements in clinical outcomes with platelet-function monitoring and treatment adjustment for coronary stenting. Thus, the association between optimised high platelet reactivity and the prognosis of patients after PCI remains unsettled. In the present study, all enrolled patients were high-risk ACS (UAP: 54.3%, NSTEMI: 36.2%, STEMI: 9.5%) and had a high prevalence of diabetes (41.9%), and 31.0% received urgent DES implantation. These factors were different from Collet's study population, and may be attributed to the different clinical prognostic. It should be noted that the reactivity of prasugrel was potentially influenced by the gene polymorphism in Collet's study[28].

Combining clinical studies and investigations into CYP metabolism *in vitro* suggested that polymorphism was related to reduced CYP function involving the conversion of clopidogrel to its active metabolite, subsequently altering the degree of clopidogrel-induced

platelet inhibition[29,30]. Frere et al identified the CYP2C19*2 allele to be more frequent in patients without an efficient response to clopidogrel[31]. Mega et al demonstrated that tripling the maintenance dose of clopidogrel (225 mg daily) in patients carrying CYP2C19*2 heterozygotes achieved equivalent levels of platelet reactivity compared to those seen with a standard dose (75 mg daily) in non-carriers. Conversely, doses as high as 300 mg daily did not result in comparable degrees of platelet inhibition for patients carrying CYP2C19*2 homozygotes[32]. The findings of the current study are consistent with these previous reports even though there are differences of ethnicity between the subject populations, Chinese and Caucasian.

In a cohort of 1,477 clopidogrel-treated patients with ACS, carriers of at least one CYP2C19 LOF allele had a 53% increased risk of death from CV death, MI or ischaemic stroke, and a threefold increased risk of stent thrombosis when compared with non-carriers[33]. The present study found that patients carrying two CYP2C19 LOF alleles were 2.3 times more at risk of suffering adverse ischaemic events during the first year after DES implantation (HR: 2.3, 95% CI: 1.40-4.97, p<0.001). Of note, a directionally consistent hazard was observed among carriers of two CYP2C19 LOF alleles for clinical ischaemic events when compared with carriers of no or one CYP2C19 LOF allele. These findings indicate that gene polymorphisms are capable of affecting the efficacy of clopidogrel, and subsequently of patient outcomes. However, a recent meta-analysis by Holmes et al concluded that the CYP2C19 genotype is not a significant predictor of clinical outcomes in patients treated with clopidogrel, which was contrary to a previous meta-analysis[34,35]. Although the finding was interesting, several methodological issues were of concern. The analysis by Holme et al included patients in whom there was relatively little or no benefit of clopidogrel, thus curtailing the ability to observe any pharmacokinetic effects, and it included outcomes that occurred in patients who were no longer taking clopidogrel. Also, testing the association of the CYP2C19 genotype with responsiveness to clopidogrel in patients not taking clopidogrel was perhaps problematic, and the meta-analysis included outcomes such as elective target lesion revascularisation and non-CV death, in which clopidogrel had no definite effect. Therefore, we speculate that the CYP2C19 genotype could influence platelet function and clinical events in strict conditions (such as DES, taking clopidogrel, cardiovascular adverse events and so on).

Pharmacological approaches have consistently identified the P2Y12 receptor as being involved in dense granule secretion, fibrinogen receptor activation, P-selectin expression, and thrombus formation[36-39]. Despite this, our cohort exhibited no significant association between ADP-induced platelet reactivity and P2Y12 polymorphisms (C34T, G52T and i-T744C). The ABCB1 gene encodes the intestinal efflux transporter P-glycoprotein, which modulates the absorption of clopidogrel. The ABCB1 C3435T has been extensively studied and some researches have shown that the ABCB1 C3435T genotype influenced the impaired function of P-glycoprotein which could hinder the absorption of clopidogrel[14]. Though a number of investigators have evaluated the relationship between the ABCB1 polymorphism and clopidogrel response, the results were inconclusive[13,40,41]. In the present study, the ABCB1 C3435T genotype did not significantly influence the antiplatelet effect and clinical outcomes of clopidogrel in our patients. Recent evidence suggests that harbouring the CYP2C19 gain of function (GOF) allele (*17) might increase the risk of bleeding[42]. However, the frequency of the CYP2C19 GOF allele (*17) (0.894%) in our population is lower than that in Western populations (18-28%), and the CYP2C19*17 polymorphisms did not significantly influence the clopidogrel pharmacodynamics and long-term clinical outcomes[42,43].

The safety and efficacy of altering therapy in response to genotype is not entirely known. Whereas neither genotyping nor platelet function tests alone adequately describe the global risk profile of an individual patient treated with clopidogrel, point-of-care platelet function testing to identify high-risk patients combined with CYP2C19 genetic testing may be more effective in identifying high-risk individuals for alternative antiplatelet therapies. Ultimately, prospective randomised clinical trials will be needed to test specific personalised antiplatelet algorithms to provide the evidence base necessary for widespread adoption into clinical practice.

Study limitations

The current study contains some limitations. First, single assessment of platelet function combined with only one method of testing platelet function may be regarded as insufficient to diagnose the response to antiplatelet therapy comprehensively. Future studies will incorporate multiple tests to improve determination of platelet function in response to clopidogrel. Second, the results gathered may be specific to the patient cohort and the way their cases were clinically managed. The difference in results from previous studies would not rule out the reason for the selective migration. Even though there is no reason to suspect, *a priori*, that there would be different results in other populations, further studies in diverse populations are required. Finally, the low allele frequency of other known alleles should not alter our conclusions, but may be relevant for individual patients.

Conclusions

In Chinese ACS patients with clopidogrel administration after DES implantation, the post-procedure HTPR and carriage of two CYP2C19 LOF alleles were significantly associated with an increased risk of adverse ischaemic events at one-year follow-up, and the carriage of two CYP2C19 LOF alleles was an independent predictor of post-procedure HTPR.

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Conflict of interest statement

The authors have no conflicts of interest to declare.

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Online data supplement

Online Table 1. The primers, PCR product length and reaction conditions of selected tagSNPs.

Online Table 2. The Hardy-Weinberg equilibrium test in the enrolled patients.

Online data supplement

Online Table 1. The primers, PCR product length and reaction conditions of selected tagSNPs.

Gene	SNP	Position	Primers	PCR product length°Cbp°C	PCR reaction conditions
CYP2C19	rs4244285 G>A	Exon 5	F:5'-CCTATGCTATCATCTCCAAA-3'	576	94°C4m(94°C30s-54°C30s-72°C40s)°C30-72°C7m-4°C∞
			R:5'-TACGCAAGCAGTCACATAAC-3'		
	rs7916649 G>A	Exon 2	F:5'-AGAGTGCTGATAAATTTCTC-3'	637	94°C4m(94°C30s-55°C30s-72°C40s)°C30-72°C7m-4°C∞
			R:5'-GAGAAGCTCTGCTAGTCTG-3'		
	rs3758581 G>A	Exon 7	F:5'-CTTGTTTCTTCATCTAGTCAG-3'	583	94°C4m(94°C30s-55°C30s-72°C40s)°C30-72°C7m-4°C∞
			R:5'-GAGGTAGTTTCTGAATTTAACG-3'		
	rs11528090 T>G	Intro 7	F:5'-GGCATAATCAGGGAATATTG--3'	227	94°C4m(94°C30s-55°C30s-72°C40s)°C30-72°C7m-4°C∞
			R: 5'--TGAAGTGCCATGTGTACAAC--3'		
	rs4304697 G>A	Intro 5	F: 5'--TCCCTGCAATGTGATCTG--3'	903	94°C4m(94°C30s-55°C30s-72°C40s)°C30-72°C7m-4°C∞
			R:5'--GATACTACAAAGATAGTCCCTG--3'		
	rs3814637 C>T	Intro 1	F: 5'--GTCAACTTGGGCTGTAAT--3'	631	94°C4m(94°C30s-55°C30s-72°C40s)°C30-72°C7m-4°C∞
			R: 5'--TAGAGGATGGGAGGTAGG--3'		
	rs10786172 A>G	Intro 7	F: 5'--CACAGTAGAGGAAGATAACTG--3'	298	94°C4m(94°C30s-55°C30s-72°C40s)°C30-72°C7m-4°C∞
			R: 5'--AGCCTCACTACATTTCTAGG--3'		
	rs4986893 G>A	Exon 4	F: 5'--TCCCTGCAATGTGATCTG--3'	903	94°C4m(94°C30s-55°C30s-72°C40s)°C30-72°C7m-4°C∞
			R:5'--GATACTACAAAGATAGTCCCTG--3'		
	rs12248560 C>T	Intro 1	F: 5'--GATGGAGAAGGGAGAACTC--3'	308	94°C4m(94°C30s-55°C30s-72°C40s)°C30-72°C7m-4°C∞
			R: 5'--TGCCACACAGCTCATAGC--3'		
CYP3A4	rs2242480 C>T	Intro 10	F: 5'--TTCTCTTCATCTAAACTGTG--3'	420	94°C4m(94°C30s-56°C30s-72°C40s)°C30-72°C7m-4°C∞
			R: 5'--ATCTTACGCTTCTGCCAGTA--3'		
	rs2404955 G>A	Intro 1	F: 5'--AGGGTATGTTCTTGGTAGC--3'	401	94°C4m(94°C30s-56°C30s-72°C40s)°C30-72°C7m-4°C∞
			R: 5'--GATGAAAGGAATTGAAGAC--3'		
	rs2246709 A>G	Intro 7	F: 5'--AAGATGTGATAGGCCACAATC--3'	469	94°C4m(94°C30s-54°C30s-72°C40s)°C30-72°C7m-4°C∞
			R: 5'--TCACCATGTAATTCATCCAC--3'		
	rs4646437 C>T	Intro 7	F: 5'--AACTTCCACATATGTGTGAG--3'	390	94°C4m(94°C30s-55°C30s-72°C40s)°C30-72°C7m-4°C∞
			R: 5'--CCAACCAGAAGAGTAAAAGA--3'		
CYP3A5	rs3800959 T>C	Intro 5	F: 5'--TTGTCCTTACAACACATACAC--3'	493	94°C4m(94°C30s-58°C30s-72°C40s)°C30-72°C7m-4°C∞
			R: 5'--TGTTATCTTCTAATCACGGAC--3'		
	rs15524 T>C	Exon 14	F: 5'--GATACATGGTGTTAAGAGTCG--3'	481	94°C4m(94°C30s-55°C30s-72°C40s)°C30-72°C7m-4°C∞
			R: 5'--GGTAGTCCTATGAGAAGGCAG-3'		
P2RY12	rs678593034 C>T	Exon 2	F: 5'--CTTTTAGAGGAGGCTGTGTC--3'	298	94°C4m(94°C30s-54°C30s-72°C40s)°C30-72°C7m-4°C∞
			R: 5'--AAACAGGACAGTGTAGAGCA--3'		
	rs680969952 G>T	Intro 2	F: 5'--ATAATTAAGGACAGAAAGG--3'	345	94°C4m(94°C30s-55°C30s-72°C40s)°C30-72°C7m-4°C∞
			R: 5'--TAAGAGTCAGAAATGGCCTG--3'		
	rs2046934 i-744T/C				
ABCB1	rs1045642-3435C>T	Exon 7	F: 5'-- TCAAAGTGTGCTGGTCCTG--3'	452	94°C4m(94°C30s-63°C30s-72°C40s)°C30-72°C7m-4°C∞
			R: 5'-- ACAAGGAGGGTCAGGTGATC --3'		

PCR: polymerase chain reaction; SNP: single nucleotide polymorphism

Online Table 2. The Hardy-Weinberg equilibrium test in the enrolled patients.

Gene	SNP	Genotype			Allele		χ^2	*p*
		WT/WT	WT/MT	MT/MT	Wild	Mutant		
CYP2C19	rs4244285 G>A	445	458	113	1348	684	0.089	0.766
	rs7916649 G>A	298	530	188	1126	906	3.150	0.076
	rs3758581 G>A	749	241	26	1739	293	1.537	0.215
	rs11528090 T>G	377	498	141	1252	780	1.333	0.248
	rs4304697 G>A	724	260	32	1708	324	2.085	0.149
	rs3814637 C>T	647	318	51	1612	420	2.111	0.146
	rs10786172 A>G	695	287	34	1677	355	0.423	0.515
	rs4986893 G>A	959	55	2	1973	59	1.619	0.203
	rs12248560 C>T	998	18	0	2014	18	0.081	0.776
CYP3A4	rs2242480 C>T	568	386	62	1522	510	0.112	0.738
	rs2404955 G>A	461	457	98	1379	653	0.992	0.319
	rs2246709 A>G	307	525	184	1139	893	2.423	0.120
	rs4646437 C>T	770	223	23	1763	269	2.013	0.156
CYP3A5	rs3800959 T>C	728	262	26	1718	314	0.174	0.676
	rs15524 T>C	471	454	91	1396	636	1.549	0.213
P2Y12	rs6785930-34C>T	527	412	77	1466	566	0.081	0.775
	rs6809699-52G>T	836	176	4	1848	184	2.722	0.099
	rs2046934-i-744T>C-	659	328	29	1646	386	2.441	0.118
ABCB1	rs1045642-3435C>T	339	489	188	1167	865	0.249	0.618

MT/MT: mutant-type homozygotes; WT/MT: mutant heterozygotes; WT/WT: wild-type homozygotes