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PEAR1, PON1, CYP2C19, CYP1A2 and F2R Polymorphisms are Associated with MACE in Clopidogrel-Treated Patients with Acute Coronary Syndrome Undergoing Percutaneous Coronary Intervention

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Objective: The objective of this study was to evaluate the impact of clopidogrel-related gene polymorphisms on the occurrence of recurrent thrombotic events and cardiovascular death in patients with acute coronary syndrome (ACS) following percutaneous coronary intervention (PCI).

Methods: We conducted genotype testing for 26 specific loci mapped to 18 clopidogrel-associated genes in ACS patients who had undergone PCI and were receiving dual antiplatelet therapy only involving clopidogrel. We documented major adverse cardiovascular events (MACE) and clinical endpoints, analyzing the effect of genetic polymorphisms on treatment outcomes.

Results: A total of 200 patients were enrolled in the study, with ischemic events occurring in 21 cases. Carriers of the T-allele for rs41273215 (*PEAR1*), rs662 (*PON1*), and the A-allele for rs4244285 (*CYP2C19*), as well as the C-allele for rs762551 (*CYP1A2*), exhibited a significant increase in the risk of MACE (OR = 2.76, 95% CI = 1.46–5.22, P = 0.002; OR = 3.72, 95% CI = 1.82–7.64, P = 0.0003; OR = 3.86, 95% CI = 1.89–7.86, P = 0.0002; OR = 2.40, 95% CI = 1.27–4.55, P = 0.007). Notably, the variant T-allele of rs168753 (*F2R*) was associated with a significant reduction in the risk of such events (OR = 0.29, 95% CI = 0.12–0.67, P = 0.004). No significant associations were found between other single nucleotide polymorphisms (SNPs) and clinical endpoints.

Conclusion: Polymorphisms in rs41273215 (*PEAR1*), rs662 (*PON1*), rs4244285 (*CYP2C19*), and rs762551 (*CYP1A2*) were correlated with an increased risk of MACE in PCI patients. Conversely, the rs168753 (*F2R*) polymorphism was linked to improved cardiovascular outcomes. Genotyping for these polymorphisms could be instrumental in identifying patients at heightened risk for MACE.

Keywords: clopidogrel, percutaneous coronary intervention, acute coronary syndrome, single nucleotide polymorphism, MACE

Introduction

Acute coronary syndrome (ACS) is a form of coronary heart disease predominantly triggered by the rupture of atherosclerotic plaques. The combination of aspirin with a P2Y12 receptor antagonist, as part of dual antiplatelet therapy (DAPT), is recommended to mitigate the risk of major adverse cardiovascular events (MACE)[1,2] and holds a pivotal role in the secondary prevention for patients with ACS undergoing percutaneous coronary intervention (PCI). While current guidelines[3,4] endorse ticagrelor as the preferred option over clopidogrel for DAPT, recent meta-analyses indicate that

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a clopidogrel-based DAPT strategy offers comparable cardiovascular protection with a reduced risk of bleeding and an overall greater benefit.[5,6] In European and American populations, the regimen of aspirin plus ticagrelor is deemed more efficacious and safer compared to the combination with clopidogrel. However, although the Asian demographic also stands to gain from this treatment, they are at an increased risk of bleeding, necessitating a prudent selection of antiplatelet agents.[7] Consequently, clopidogrel maintains a significant role in the antiplatelet management of ACS.

However, the metabolic pathway of clopidogrel was intricate, and its effect is affected by multiple enzymes. After oral administration, only 15% of clopidogrel can be absorbed successfully, others is hydrolyzed to inactive carboxylic acid derivatives by esterase.[8] As a precursor, clopidogrel is converted into its active metabolite by the P450 enzyme system in the liver.[9] The intestinal transport of clopidogrel is modulated by ATP binding box B subfamily member 1 (*ABCB1*), while the cytochrome P450 family 2 subfamily C polypeptide 19 (*CYP2C19*), paraoxonase-1 (*PON1*) and *CES1* play a key role in the process of transformation. The active metabolite of clopidogrel targets the P2RY12 receptor, blocking ADP binding and receptor activation. Research also indicates that various genes, including nitric oxide synthase 3(*NOS3*), beta-1,4-galactosyltransferase 2(*B4GALT2*), platelet endothelial aggregation receptor 1(*PEAR1*), insulin receptor substrate 1(*IRS-1*), coagulation factor II thrombin receptor (*F2R*), thromboxane A2 receptor (*TBXA2R*), integrin subunit beta 3(*ITGB3*), integrin subunit alpha 2(*ITGA2*), solute carrier family 14 member 2(*SLC14A2*), and N-6 adenine-specific DNA methyltransferase 1(*N6AMT1*), influence clopidogrel's pharmacodynamics.[10–15] Mutations at important sites of these genes may help to explain the variation of antiplatelet effect of clopidogrel.[16]

Although some studies have identified the impact of genetic polymorphisms on the reduced effectiveness of clopidogrel, potentially resulting in MACE post-PCI, a comprehensive investigation of these genes is still lacking. Current research primarily concentrates on the *CYP2C19* and CYP450 enzymes, as well as the *ABCB1* gene.[17,18] Furthermore, this study adopts a broader approach to genetic analysis, examining a wider spectrum of genetic influences. Therefore, this study is designed to evaluate the association between clopidogrel-related gene polymorphisms and MACE, and to explore a more comprehensive set of genetic factors, with the aim of providing a deeper scientific basis for personalized treatment.

Materials and Methods

Patient Selection

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 helsinki Declaration and its later amendments or comparable ethical standards, and approved by the Ethics Committee of Fuwai Central China Cardiovascular Hospital, Henan Provincial People's Hospital, Central China Fuwai Hospital of Zhengzhou University (ID: 2022–06).

This retrospective case-control study involved 200 patients with Acute Coronary Syndrome (ACS) who underwent Percutaneous Coronary Intervention (PCI) with stent implantation at Fuwai Central China Cardiovascular Hospital between January 2019 and December 2020. The inclusion and exclusion criteria are as follow:

Inclusion Criteria:

1. Definitive Diagnosis of ACS: The diagnosis of ACS hinges on an extensive evaluation encompassing the patient's clinical presentation, physical signs, electrocardiogram (ECG), laboratory findings, and imaging studies. The diagnoses encompassed by this criterion include ST-segment Elevation Myocardial Infarction (STEMI), Non-ST-segment Elevation Myocardial Infarction (NSTEMI), and Unstable Angina (UA).
2. Dual Antiplatelet Therapy Received: Patients were treated with a combination of aspirin and clopidogrel.
3. Complete Clinical Information Available: Only patients with comprehensive medical records suitable for research analysis were included.

Exclusion Criteria:

1. Contraindications to PCI: Patients who cannot undergo PCI due to various reasons.
2. Allergy or intolerance to clopidogrel: Patients with allergic reactions or unable to tolerate the medication.

3. Severe organ dysfunction: Including severe liver dysfunction, significant valvular disease, or renal impairment.
4. Active bleeding or hemorrhagic disease history: Patients with active bleeding or a history of hemorrhagic disease.
5. Other dual antiplatelet therapy regimens: Patients on ticagrelor antiplatelet therapy.

This study encompassed a total of 200 participants, comprising 21 patients who experienced clinical adverse events and 179 control patients without such events.

Study Design

Patient demographics and clinical characteristics, including age, gender, biochemical indicators, body mass index (BMI), comorbidities, smoking status, alcohol consumption, history of stent placement, and concomitant medication were extracted from the hospital's information system. A loading dose of 300 mg of clopidogrel was administered to patients prior to PCI, followed by a daily maintenance dose of 75mg. Preceding the operation, 5mL of blood was collected intravenously from each patient, placed into heparin-coated tubes, and stored at −80°C for subsequent genotyping. Post-PCI, patients were prescribed a one-year course of dual antiplatelet therapy, consisting of 75 mg/day of clopidogrel and 100 mg/day of aspirin. Discharge education focused on reducing atherosclerosis risk factors, which included controlling blood pressure and glucose levels, lowering LDL cholesterol, ceasing smoking and alcohol consumption, achieving weight loss, and engaging in moderate exercise.

All enrolled patients were monitored by cardiovascular clinic review and readmission records. The primary clinical endpoints for this follow-up were MACE including acute cerebral infarction, acute myocardial infarction, and cardiovascular related death. Based on the incidence of these primary outcomes, patients were divided into "case group" and "control group". The "case group" refers to patients who experienced at least one primary endpoint within one year, either through readmission or revisit. In contrast, the 'control group' consisted of patients who remained free from any primary endpoints over the same duration. A comparative analysis of multiple clopidogrel-associated genotypes was performed between these two cohorts.

Gene Selection

Clopidogrel's pharmacogenetics significantly influence its efficacy. Genes directly involved in clopidogrel metabolism include: *CYP2C19, CYP3A4, ABCB1, CES1, CES1P1, PON1, P2RY12*. Genes selected based on literature reviews that affect clopidogrel pharmacodynamics include: *NOS3, B4GALT2, PEAR1, IRS-1, F2R, TBXA2R, ITGB3, ITGA2, SLC14A2, PTGS1* and *N6AMT1*.[8,19–28] Considering comprehensively, we selected these genes as the object of this study. Mutation frequencies for all loci in different populations are shown in Table S1.

Genotyping

For the analysis of clopidogrel single nucleotide polymorphisms (SNPs), genotypes were ascertained utilizing the MassArray iPLEX assay system (Agena, San Diego, United States). Peripheral blood samples were collected, and approximately 10–20 nanograms of genomic DNA for genotyping. Table 1 presents the list of polymerase chain reaction (PCR) primers employed in this study, which were meticulously designed using the Assay Design 4.0 software (Agena, San Diego, United States). The PCR was conducted to separately amplify the target DNA fragments, with the resulting products then subjected in locus-specific single-base extension reactions. The implementation of rigorous quality control measures in our study refers to several aspects of the MassARRAY process. Firstly, the system's chemistry is robust and reproducible, which is essential for obtaining reliable results. Secondly, the MassARRAY system includes advanced data analysis software that helps in the accurate interpretation of the genotyping data. Thirdly, our study likely involved the use of multiplexed PCR reactions and single base extension reactions, which are designed to be highly specific, further ensuring the accuracy of the genotype calls. Rigorous quality control measures were implemented, ensuring the data's availability and reliability.

Statistics Analysis

All data were subjected to statistical analysis using SPSS 25.0 software from SPSS Inc., Chicago, Illinois, USA, and PLINK v1.90 software. Continuous variables were presented as mean ± standard deviation (SD) and were analyzed employing the

Table 1 Selected Variants for Clopidogrel

Gene	Variant	Allele	SNP position	Primers (5'-3')	MAF in control	MAF in case	HWE P-value
B4GALT2	rs1061781	C>T	Missense	F: ACGTTGGATGAGGTTCGTTATGCTTGTCGC R: ACGTTGGATGACTGGGATGAAGATCTCACG	0.108	0.095	0.707
PEAR1	rs12041331	G>A	Other	F: ACGTTGGATGGAAGTCCCTTCTGCTGTCTC R: ACGTTGGATGTAGAGTTCCTGGTGGACAAG	0.364	0.286	0.263
PEAR1	rs41273215	C>T	Intronic	F: ACGTTGGATGTTCCTGGATGAACTCTCAGC R: ACGTTGGATGTTCAAAGCTTTGGTGTGCCC	0.333	0.571	0.108
PEAR1	rs57731889	C>T	Intronic	F: ACGTTGGATGAGACTAGAGTTTCCTGGCGG R: ACGTTGGATGTTAGCACCAAATGTCCCCAG	0.435	0.309	1.000
IRS1	rs13431554	A>G	3' UTR	F: ACGTTGGATGGTGAATTAAGACCTTGGCGG R: ACGTTGGATGTTGGCTTCCACCCATTCTTC	0.221	0.214	0.831
MED12L, P2RY12	rs6785930	G>A	Other	F: ACGTTGGATGGGACCTGGGTGATTTTGTAG R: ACGTTGGATGCCAACAAGAAATGCAAGCCG	0.245	0.167	0.429
MED12L, P2RY12	rs2046934	A>G	Other	F: ACGTTGGATGTATGGCATCTACATCTTGGG R: ACGTTGGATGCAATTTCACTTATCTCTGG	0.150	0.105	0.568
MED12L, P2RY12	rs6787801	A>G	Other	F: ACGTTGGATGGATGACTCTCATTAATGACC R: ACGTTGGATGGAGATGAGCAAAAAAAAAGTG	0.460	0.548	0.449
ITGA2	rs1126643	C>T	Missense	F: ACGTTGGATGTGGCCTATTAGCACCAAAAC R: ACGTTGGATGCCAGACATCCCAATATGGTG	0.332	0.333	0.498
ITGA2	rs1062535	G>A	Intronic	F: ACGTTGGATGGCTTATTCAGCAGCTTCTGG R: ACGTTGGATGCCATCATGTGATTCACCGTC	0.333	0.333	0.403
F2R	rs168753	A>T	Intronic	F: ACGTTGGATGTGCCTTGTTGATGCGTTCAC R: ACGTTGGATGGGGATCTAAGGTGGCATTTG	0.461	0.222	0.649
ABCB1	rs1045642	G>A	Missense	F: ACGTTGGATGAAGGCATGTATGTTGGCCTC R: ACGTTGGATGTTGCCTATGGAGACAACAGC	0.455	0.381	0.230
ABCB1	rs1128503	A>G	Synonymous	F: ACGTTGGATGTTTCTCACTCGTCCTGGTAG R: ACGTTGGATGCACAGCCACTGTTTCCAACC	0.308	0.333	0.156
PON1	rs662	C>T	Missense	F: ACGTTGGATGCCTGAGCACTTTTATGGCAC R: ACGTTGGATGACATACGACCACGCTAAACC	0.355	0.619	0.865
CYP3A4	rs2242480	C>T	Intronic	F: ACGTTGGATGGCAGGAGGAAATTGATGCAG R: ACGTTGGATGTGCTAAGGTTTCACCTCCTC	0.270	0.190	0.353
NOS3	rs1799983	G>T	Missense	F: ACGTTGGATGACCTCAAGGACCAGCTCGG R: ACGTTGGATGAAACGGTCGCTTCGACGTG	0.134	0.150	1.000
PTGS1	rs1330344	T>C	5' Flanking	F: ACGTTGGATGCACCCATCTGCACTCAAAAC R: ACGTTGGATGTCTGATTCTGAGGTGAAGGC	0.413	0.357	0.540
CYP2C19	rs12248560	C>T	5' Flanking	F: ACGTTGGATGTGAGCTGAGGTCTTCTGATG R: ACGTTGGATGCAAATTTGTGTCTTCTGTTC	0.015	0.000	0.042
CYP2C19	rs4986893	G>A	Stop Codon	F: ACGTTGGATGAACATCAGGATTGTAAGCAC R: ACGTTGGATGGACTGTAAGTGGTTTCTCAG	0.028	0.048	1.000
CYP2C19	rs4244285	G>A	Synonymous	F: ACGTTGGATGCACTTTCCATAAAAGCAAGG R: ACGTTGGATGGCAATAATTTTCCCACTATC	0.255	0.525	0.515
CYP1A2	rs762551	A>C	Intronic	F: ACGTTGGATGCAGCTGGATACCAGAAAGAC R: ACGTTGGATGTCTGTGATGCTCAAAGGGTG	0.387	0.548	0.512
CES1P1	rs3785161	A>C	5' Flanking	F: ACGTTGGATGTGCCCAGAGCACTCTGTATC R: ACGTTGGATGACACATATAGGGTGGAGGAG	0.280	0.262	0.714

(*Continued*)

Table 1 (Continued).

Gene	Variant	Allele	SNP position	Primers (5'-3')	MAF in control	MAF in case	HWE P-value
CES1	rs8192950	T>G	Intronic	F: ACGTTGGATGCTCTTTCTCATTTGGGATGC R: ACGTTGGATGATGTGCTTCTTGTTGGTGGG	0.183	0.190	0.454
ITGB3	rs5918	T>C	Missense	F: ACGTTGGATGTTGCTGGACTTCTCTTTGGG R: ACGTTGGATGCAGATTCTCCTTCAGGTCAC	0.003	0.048	1.000
SLC14A2	rs12456693	C>T	Other	F: ACGTTGGATGAATGTCTCCAGTGCTTCCTC R: ACGTTGGATGTGAGAATTTCCTGTTAACCC	0.138	0.143	0.108
N6AMT1	rs2254638	A>G	Intronic	F: ACGTTGGATGTCCCCATTAAAAAGATCAGC R: ACGTTGGATGAGCTTGTCACTGGGTAGTTG	0.450	0.548	0.762

Student's *t*-test. Categorical variables were expressed in terms of numbers and percentages and were examined using the chi-square ($\chi 2$) test. The chi-square test was also utilized to evaluate Hardy-Weinberg equilibrium (HWE). Linkage disequilibrium (LD) patterns and haplotype structures were assessed with Haploview software version 4.2, developed by Daly Laboratories, USA. The significance of any observed haplotype associations was determined using the chi-square test. OR and 95% CI were applied to evaluate the association of genetic variants with the MACE during the follow-up period using unconditional logistic regression. A P-value of less than 0.05 was considered to indicate statistical significance.

Results

Characteristics of Enrolled Population

Comparative analysis revealed no significant differences between the two groups with respect to age, gender, body mass index (BMI), and medical history—encompassing conditions such as hypertension, diabetes, hyperlipidemia, coronary heart disease, prior coronary artery stent implantation, alcohol consumption, and smoking. Similarly, biochemical indicators, including total cholesterol, triglycerides, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol, were not significantly different. Regarding concurrent medication, the study patients were treated with a range of statins, including atorvastatin, rosuvastatin, pitavastatin, simvastatin, and fluvastatin, alongside beta-blockers such as metoprolol and bisoprolol. The primary clinical endpoints of major adverse cardiovascular events (MACE) comprised stent thrombosis, myocardial infarction, stroke, and death. A detailed overview of the clinical characteristics is presented in Table 2.

Table 2 Clinical Characteristics of Patients with MACE (Case) and Without MACE (Control)

Variable	Case (n=21)	Control (n = 179)	*P*-value
Age, mean±SD	68.48±6.42	64.96±11.20	0.222
Male, n (%)	12(57.1%)	111(62.0%)	0.664
TC (mmol/L)	3.36±0.69	3.43±0.91	0.736
TG (mmol/L)	1.36±0.44	1.61±1.07	0.350
HDL-C (mmol/L)	0.98±0.20	1.02±0.27	0.539
LDL-C (mmol/L)	2.05±0.61	2.02±0.82	0.884
PLT (10^9/L)	205.62±70.85	210.64±59.79	0.721
Systolic pressure	132.43±24.08	135.94±17.93	0.822

(*Continued*)

Table 2 (Continued).

Variable	Case (n=21)	Control (n = 179)	*P*-value
Diastolic pressure	82.10±15.24	83.49±11.81	0.620
Risk factors			
BMI, mean±SD	25.14±2.67	25.99±3.91	0.334
Hypertension, n (%)	14(66.7%)	119(66.5%)	0.986
Diabetes, n (%)	4(19.0%)	64(35.8%)	0.126
Hyperlipidemia, n (%)	1(4.8%)	28(15.6%)	0.311
Coronary heart disease, n (%)	16(76.2%)	133(74.3%)	0.851
Prior coronary stents, n (%)	8(38.1%)	40(22.3%)	0.110
Tobacco use, n(%)			0.120
Never	16(76.2%)	105(58.6%)	–
Current	3(14.3%)	49(27.4%)	–
Ex-tobacco use	2(9.5%)	25(14.0%)	–
Alcohol use, n(%)			0.848
Never	14(66.7%)	123(68.7%)	–
Social alcohol use	2(9.5%)	24(13.4%)	–
Regular alcohol use	5(23.8%)	32(17.9%)	–
Concomitant medication			
Statin drugs			
Atorvastatin, n (%)	8(38.1%)	67(37.4%)	0.971
Rosuvastatin, n (%)	13(61.9%)	102(57.0%)	0.891
Pitavastatin, n (%)	0 (0.0%)	2(1.1%)	0.626
Simvastatin, n (%)	0 (0.0%)	1(0.5%)	0.731
Fluvastatin, n (%)	0 (0.0%)	2(1.1%)	0.626
Beta blockers			
Metoprolol, n (%)	16(76.2%)	134(74.9%)	0.894
Bisoprolol, n (%)	1(4.8%)	11(6.1%)	0.863
Outcome, n (%)			
Stent thrombosis	6(28.6%)	–	–
Myocardial infarction	10(47.6%)	–	–
Acute cerebral infarction	3(14.3%)	–	–
CV-related death	2(9.5%)	–	–

Abbreviations: TC, Total Cholesterol; TG, Triglycerides; HDL-C, High-Density Lipoprotein Cholesterol; LDL-C, Low-Density Lipoprotein Cholesterol; BMI, Body Mass Index; CV, cardiovascular.

Association of Genetic Polymorphisms and Clinical End-Points

The allelic frequencies and HWE test results are shown in Table 1. Three SNPs of rs4986893 (*CYP2C19*3*), rs12248560 (*CYP2C19*17*) and rs5918 (*ITGB3*) had minor allele frequency (MAF) lower than 0.05. Variants with MAF < 0.05 have specific significance in genetic studies, and they may be associated with rare diseases, drug responsiveness, and the genetic structure of populations. None of the SNPs were significantly different from HWE for all patients (p>0.05). The findings from the logistic regression analysis, contrasting cases with controls, are detailed in Table 3. Compared to controls, the cases demonstrated significantly higher mutation frequencies in carriers of the T allele of rs41273215 (*PEAR1*), with an odds ratio (OR) of 2.76 and a 95% confidence interval (CI) of 1.46 to 5.22, achieving statistical significance at P = 0.002. Similarly, T-allele carriers of rs662 (*PON1*) showed an increased risk with an OR of 3.72 and a 95% CI of 1.82 to 7.64, with P = 0.0003.

Table 3 The Associations of Clopidogrel Metabolism-Related SNPs with MACE on Logistic Regression Analysis

Gene	Variant	Logistic regression, OR, 95% CI	P-value
B4GALT2	rs1061781	0.39, 0.09–1.68	0.209
PEAR1	rs12041331	0.65, 0.32–1.35	0.253
PEAR1	rs41273215	2.76, 1.46–5.22	0.002*
PEAR1	rs57731889	0.55, 0.27–1.09	0.088
IRS1	rs13431554	0.96, 0.44–2.10	0.909
MED12L,P2RY12	rs6785930	0.56, 0.23–1.35	0.198
MED12L,P2RY12	rs2046934	0.65, 0.22–1.89	0.431
MED12L,P2RY12	rs6787801	1.53, 0.78–2.97	0.215
ITGA2	rs1126643	1.01, 0.50–2.05	0.980
ITGA2	rs1062535	1.01, 0.49–2.05	0.990
F2R	rs168753	0.29, 0.12–0.67	0.004*
ABCB1	rs1045642	0.70, 0.36–1.38	0.301
ABCB1	rs1128503	1.16, 0.56–2.40	0.684
PON1	rs662	3.72, 1.82–7.64	0.0003*
CYP3A4	rs2242480	0.59, 0.26–1.35	0.208
NOS3	rs1799983	1.16, 0.46–2.87	0.757
PTGS1	rs1330344	0.76, 0.38–1.51	0.428
CYP2C19	rs4986893	1.98, 0.40–9.83	0.405
CYP2C19	rs4244285	3.86, 1.89–7.86	0.0002*
CYP1A2	rs762551	2.40, 1.27–4.55	0.007*
CES1P1	rs3785161	0.90, 0.44–1.86	0.780
CES1	rs8192950	1.06, 0.46–2.46	0.888
SLC14A2	rs12456693	1.05, 0.44–2.46	0.920
N6AMT1	rs2254638	1.56, 0.81–2.99	0.180

Note: *P < 0.05, which is statistically significant.

Table 4 The Frequency of Each Haplotype Within a Block and the Association of Haplotype and Clinical Endpoint

Gene	Block	Haplotype Frequency	Case, Control Frequencies	*P*-value
PEAR1	CT	0.434	0.272, 0.383	0.660
PEAR1	TC	0.332	0.450, 0.423	0.128
PEAR1	CC	0.234	0.278, 0.194	0.023*
ITGA2	TA	0.332	0.361, 0.307	0.346
ITGA2	CG	0.668	0.639, 0.693	0.346

Note: *P < 0.05, which is statistically significant.

A-allele carriers of rs4244285 (*CYP2C19*) also exhibited a higher risk, with an OR of 3.86 and a 95% CI of 1.89 to 7.86, and P = 0.0002. C-allele carriers of rs762551 (*CYP1A2*) had an OR of 2.40 and a 95% CI of 1.27 to 4.55, with P = 0.007. Conversely, the variant T-allele of rs168753 (*F2R*) was associated with a significantly decreased risk of MACE, with an OR of 0.29 and a 95% CI of 0.12 to 0.67, and P = 0.004. No significant associations were observed between other genotypes and clinical events, with all P values exceeding the threshold of 0.05. We analyzed participants in groups according to selected genotypes to see if clinical characteristics differed, as shown in Table S2, and listed the distribution of selected genotypes between cases and controls in Table S3.

Haplotype Analysis and Test of Association

The final LD analysis identified three distinct haplotypes among our patient cohort, specifically mapped to the *PEAR1, P2RY12, ITGA2, ABCB1, CYP2C19*, and *CES1* genes (as illustrated in Figures S1 and S2, which display the linkage disequilibrium plots for the selected SNPs).

The significance of the haplotypic association is presented in Table 4. Despite the relatively low frequency of the CC haplotype (*PEAR1*), which was observed at 0.234, it was associated with an increased risk of recurrent clinical events, with a statistically significant P-value of 0.023. In contrast, the remaining haplotypes did not appear to be significant risk factors for the recurrence of Major Adverse Cardiovascular Events (MACE) among Chinese patients.

Discussion

Our study evaluated the correlation between genetic polymorphisms and the occurrence of MACE in patients who underwent PCI and were subsequently treated with aspirin and clopidogrel. The findings revealed that specific polymorphisms—rs41273215 (*PEAR1*), rs662 (*PON1*), rs4244285 (*CYP2C19*), and rs762551 (*CYP1A2*)—were significantly associated with an elevated risk of experiencing MACE. In contrast, the rs168753 (*F2R*) polymorphism demonstrated a significant inverse relationship, being linked to a reduced risk of such events. The genetic loci identified in this research are notable for their high mutation frequencies across diverse global populations, with mutation rates ranging from 0% to 85.02%. This variation suggests a level of generalizability in our results, implying their potential indirect applicability to other demographic groups.

CYP2C19 is a drug-metabolizing enzyme essential for the biotransformation of clopidogrel. The *CYP2C19*2* and *CYP2C17*3* loss of function (LOF) alleles is found to disrupt bioactivity, diminish the concentration of active clopidogrel metabolites, and compromise the antiplatelet efficacy of clopidogrel. This disruption can subsequently elevate the risk of adverse cardiovascular events.[29] A previous study identified a significantly increased risk of adverse outcomes in carriers unadjusted for treatment (15.6%, P<0.05).[30] Nonetheless, a contrasting study reported that patients possessing LOF alleles, predominantly *CYP2C19*2* did not exhibit an increased risk of of MACE even though there was a notable increase in the risk of MACE.[31]

PEAR1, predominantly expressed in platelets and endothelial cells, facilitates platelet adhesion and aggregation, playing a crucial role in thrombosis as well as in sustaining the homeostatic balance of platelet aggregation. A previous report has indicated

that patients possessing a polymorphism in *PEAR1* are correlated with an elevated risk of acute myocardial infarction and other adverse events subsequent to clopidogrel administration.[32] Another study has documented that single nucleotide polymorphisms (SNPs) within *PEAR1*—specifically rs11264580, rs3737224, and rs41273215—are significantly associated with heightened ADP-induced platelet aggregation in Chinese patients with coronary heart disease (CHD) who are on a dual antiplatelet regimen comprising aspirin and clopidogrel. This finding underscores the significance of these SNPs in platelet activation.[25]

The PON1 enzyme plays a pivotal role in the metabolic pathway of clopidogrel, facilitating the conversion of 2-O-clopidogrel into its active metabolites. Polymorphisms within the *PON1* gene have the potential to diminish the enzymatic activity of PON1, thereby attenuating the antiplatelet effects of clopidogrel and potentially resulting in clopidogrel resistance.[33,34] Relevant reports have suggested that *PON1* gene polymorphisms might influence the therapeutic efficacy of clopidogrel and patient prognosis.[21,35–39] However, the precise connection between CHD and these prognostic factors remains to be fully elucidated. Our investigation has identified that *PON1* gene mutation are significant predictors of patient prognosis following PCI with clopidogrel, aligning with findings from prior research.

The CYP1A2 enzyme is produced following expression of the *CYP1A2* gene. Most of the polymorphic variants of the *CYP1A2* gene detected varied greatly in different populations worldwide. However, four alleles including *CYP1A2*1C, CYP1A2*1F, CYP1A2*1J* and *CYP1A2*1K* were described as common variants in at least one ethnic group.[40,41] A substantial multicenter clinical trial, enrolling 2732 patients, was conducted to genotype and evaluate the link between the *CYP1A2*1C* allele and the enduring clinical efficacy and safety profile of clopidogrel. The findings indicated that patients possessing the *CYP1A2*1C* allele demonstrated a marked increase in responsiveness to clopidogrel. Conversely, these individuals also experienced a notably higher mortality rate attributed to major bleeding incidents.[13]

The presence of the rs168753 *(F2R*) T allele may confer an effect akin to partial inhibition of the PAR-1 receptor, potentially exerting a synergistic antiplatelet influence when combined with clopidogrel's blockade of the P2Y12 receptors.[42] Current evidence is limited and marred by inconsistent findings concerning the link between the *F2R* polymorphism and clinical outcomes in patients. A previous study indicated that individuals carrying the T allele exhibited a diminished risk of ischemic events among a cohort of 503 patients post-PCI who were administered DAPT with aspirin and clopidogrel for a period of 12 months.[43] Additionally, another investigation observed that, among patients with a mild ischemic stroke or transient ischemic attack (TIA) treated with clopidogrel and aspirin, those possessing the rs168753 (*F2R*) T allele experienced a reduced incidence of recurrent strokes compared to non-carriers.[44] In our current study, we have discerned that the rs168753 variant within the *F2R* gene might modulate the therapeutic efficacy of clopidogrel in PCI patients. Notably, the T-allele variant of *F2R* rs168753 was associated with a significant reduction in the likelihood of MACE.

Our study suggests that polymorphisms in the *F2R* gene, in addition to *CYP2C19, CYP1A2, PEAR1*, and *PON1*, may also be associated with the efficacy of clopidogrel in Chinese patients with ACS after PCI. The precise interplay between the *F2R* gene and the incidence of cardiovascular adverse events merits further elucidation in future studies. Physicians have the potential to utilize genetic testing as a means to ascertain which patients are likely to derive clinical benefits from clopidogrel therapy.

This study has several inherent limitations that warrant consideration: Firstly, the sample size was relatively modest, comprising only 200 participants who fulfilled the specified inclusion and exclusion criteria. Secondly, various factors influencing the outcomes were not fully accounted for, including the severity of patients' conditions, the specific type of acute coronary syndrome (ACS), the quantity of stents implanted, the duration of disease, and other relevant variables. Thirdly, the study's population was exclusively Chinese, potentially restricting the generalizability of the results to other ethnicities or populations.

Conclusions

In conclusion, the minor allele of rs41273215 (*PEAR1*), rs662 (*PON1*), rs4244285 (*CYP2C19*), and rs762551 (*CYP1A2*) related to clopidogrel metabolism conferred an elevated risk of MACE in patients with ACS who have undergone PCI. In contrast, the rs168753 (*F2R*) polymorphism has been found to mitigate this risk. However, it is imperative that these findings be substantiated in larger-scale validation studies.

Data Sharing Statement

Data used in this study are available upon reasonable request to the corresponding authors.

Ethical Approval

This study was approved by the Institutional Review Board (Ethics approval number: 2022-06). Our research uses anonymized information data to conduct research that does not cause harm to humans, does not involve sensitive personal information and the ethics committee agrees to waive informed consent.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no conflicts of interest in this work.

References

1. Collet JP, Thiele H, Barbato E, et al. 2020 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. *Eur Heart J*. 2021;42(14):1289–1367. doi:10.1093/eurheartj/ehaa575
2. Nakamura M, Kimura K, Kimura T, et al. JCS 2020 guideline focused update on antithrombotic therapy in patients with coronary artery disease. *Circ J*. 2020;84(5):831–865. doi:10.1253/circj.CJ-19-1109
3. Valgimigli M, Bueno H, Byrne RA, et al. 2017 ESC focused update on dual antiplatelet therapy in coronary artery disease developed in collaboration with EACTS: the Task Force for dual antiplatelet therapy in coronary artery disease of the European Society of Cardiology (ESC) and of the European Association for Cardio-Thoracic Surgery (EACTS). *Eur Heart J*. 2018;39(3):213–260. doi:10.1093/eurheartj/ehx419
4. Ibanez B, James S, Agewall S, et al. 2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: the task force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC). *Eur Heart J*. 2018;39(2):119–177. doi:10.1093/eurheartj/ehx393
5. Patti G, Grisafi L, Spinoni EG, Rognoni A, Mennuni M. Safety and efficacy of selective, clopidogrel-based strategies in acute coronary syndrome: a study-level meta-analysis. *Thromb Haemost*. 2022; 122(10):1732–1743.
6. Sun M, Cui W, Li L. Comparison of clinical outcomes between ticagrelor and clopidogrel in acute coronary syndrome: a comprehensive meta-analysis. *Front Cardiovasc Med*. 2021;8:818215. doi:10.3389/fcvm.2021.818215
7. Wu H, Xiang X, Li D, Shen S, Li X. Efficacy and safety of ticagrelor compared to clopidogrel in patients undergoing percutaneous coronary intervention: a meta-analysis. *Curr Pharm Des*. 2020;26(46):5988–5997. doi:10.2174/1381612826666200614184007
8. Lins R, Broekhuysen J, Necciari J, Deroubaix X. Pharmacokinetic profile of 14C-labeled clopidogrel. *Semin Thromb Hemost*. 1999;25 Suppl 2:29–33.
9. Savi P, Pereillo JM, Uzabiaga MF, et al. Identification and biological activity of the active metabolite of clopidogrel. *Thromb Haemost*. 2000;84 (5):891–896. doi:10.1055/s-0037-1614133
10. Zhang LJ, Zhang YQ, Han X, Zhang ZT, Zhang ZQ. Association of VEGFR-2 gene polymorphisms with clopidogrel resistance in patients with coronary heart disease. *Am J Ther*. 2016;23(6):e1663–e1670. doi:10.1097/MJT.0000000000000231
11. Tatarunas V, Kupstyte-Kristapone N, Norvilaite R, et al. The impact of CYP2C19 and CYP4F2 variants and clinical factors on treatment outcomes during antiplatelet therapy. *Pharmacogenomics*. 2019;20(7):483–492. doi:10.2217/pgs-2018-0178
12. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, et al. 807 C/T Polymorphism of the glycoprotein Ia gene and pharmacogenetic modulation of platelet response to dual antiplatelet treatment. *Blood Coagul Fibrinolysis*. 2004;15(5):427–433. doi:10.1097/01.mbc.0000114439.81125.86
13. Cresci S, Depta JP, Lenzini PA, et al. Cytochrome p450 gene variants, race, and mortality among clopidogrel-treated patients after acute myocardial infarction. *Circ Cardiovasc Genet*. 2014;7(3):277–286. doi:10.1161/CIRCGENETICS.113.000303
14. Tan SSN, Fong AYY, Mejin M, et al. Association of CYP2C19*2 polymorphism with clopidogrel response and 1-year major adverse cardiovascular events in a multiethnic population with drug-eluting stents. *Pharmacogenomics*. 2017;18(13):1225–1239. doi:10.2217/pgs-2017-0078
15. Lewis JP, Horenstein RB, Ryan K, et al. The functional G143E variant of carboxylesterase 1 is associated with increased clopidogrel active metabolite levels and greater clopidogrel response. *Pharmacogenet Genomics*. 2013;23(1):1–8. doi:10.1097/FPC.0b013e32835aa8a2
16. Marchini JF, Pinto MR, Novaes GC, et al. Decreased platelet responsiveness to clopidogrel correlates with CYP2C19 and PON1 polymorphisms in atherosclerotic patients. *Braz J Med Biol Res*. 2017;50(1):e5660. doi:10.1590/1414-431x20165660
17. Wu Y, Liu Y, Chang Y, Liu M. Integration of CRISPR/Cas13a and V-Shape PCR for rapid, sensitive, and specific genotyping of CYP2C19 gene polymorphisms. *Anal Chem*. 2023;95(26):10127–10135. doi:10.1021/acs.analchem.3c01968
18. Mouhrach I, Bouguenouch L, Kamal A, et al. Influence of CYP450 Enzymes and ABCB1 polymorphisms on clopidogrel response in Moroccan patients with acute coronary syndromes. *Pharmgenomics Pers Med*. 2023;16:901–909. doi:10.2147/PGPM.S390092
19. Zhu HJ, Wang X, Gawronski BE, Brinda BJ, Angiolillo DJ, Markowitz JS. Carboxylesterase 1 as a determinant of clopidogrel metabolism and activation. *J Pharmacol Exp Ther*. 2013;344(3):665–672. doi:10.1124/jpet.112.201640

20. Levin LS, Cloyd WH, Beaudry RJ Jr, Yingling TH. Hemangioma of the incisive papilla: a case report. *J Md State Dent Assoc*. 1981;24(1):21–22.
21. Bouman HJ, Schomig E, van Werkum JW, et al. Paraoxonase-1 is a major determinant of clopidogrel efficacy. *Nat Med*. 2011;17(1):110–116. doi:10.1038/nm.2281
22. Pan Y, Chen W, Wang Y, et al. Association between ABCB1 polymorphisms and outcomes of clopidogrel treatment in patients with minor stroke or transient ischemic attack: secondary analysis of a randomized clinical trial. *JAMA Neurol*. 2019;76(5):552–560. doi:10.1001/jamaneurol.2018.4775
23. Scott SA, Collet JP, Baber U, et al. Exome sequencing of extreme clopidogrel response phenotypes identifies B4GALT2 as a determinant of on-treatment platelet reactivity. *Clin Pharmacol Ther*. 2016;100(3):287–294. doi:10.1002/cpt.401
24. Lewis JP, Ryan K, O'Connell JR, et al. Genetic variation in PEAR1 is associated with platelet aggregation and cardiovascular outcomes. *Circ Cardiovasc Genet*. 2013;6(2):184–192. doi:10.1161/CIRCGENETICS.111.964627
25. Yao Y, Tang XF, Zhang JH, et al. Association of PEAR1 genetic variants with platelet reactivity in response to dual antiplatelet therapy with aspirin and clopidogrel in the Chinese patient population after percutaneous coronary intervention. *Thromb Res*. 2016;141:28–34. doi:10.1016/j.thromres.2016.02.031
26. Zhang D, Zhang X, Liu D, et al. Association between insulin receptor substrate-1 polymorphisms and high platelet reactivity with clopidogrel therapy in coronary artery disease patients with type 2 diabetes mellitus. *Cardiovasc Diabetol*. 2016;15(1):50. doi:10.1186/s12933-016-0362-0
27. Grinshtein YI, Kosinova AA, Grinshtein IY, Subbotina TN, Savchenko AA. The prognostic value of combinations of genetic polymorphisms in the ITGB3, ITGA2, and CYP2C19*2 genes in predicting cardiovascular outcomes after coronary bypass grafting. *Genet Test Mol Biomarkers*. 2018;22(4):259–265. doi:10.1089/gtmb.2017.0177
28. Zhong WP, Wu H, Chen JY, et al. Genomewide association study identifies novel genetic loci that modify antiplatelet effects and pharmacokinetics of clopidogrel. *Clin Pharmacol Ther*. 2017;101(6):791–802. doi:10.1002/cpt.589
29. Xi Z, Fang F, Wang J, AlHelal J, Zhou Y, Liu W. CYP2C19 genotype and adverse cardiovascular outcomes after stent implantation in clopidogrel-treated Asian populations: a systematic review and meta-analysis. *Platelets*. 2019;30(2):229–240. doi:10.1080/09537104.2017.1413178
30. Hulot JS, Chevalier B, Belle L, et al. Routine CYP2C19 genotyping to adjust thienopyridine treatment after primary PCI for STEMI: results of the GIANT Study. *JACC: Cardiovasc Interv*. 2020;13(5):621–630. doi:10.1016/j.jcin.2020.01.219
31. Zabalza M, Subirana I, Sala J, et al. Meta-analyses of the association between cytochrome CYP2C19 loss- and gain-of-function polymorphisms and cardiovascular outcomes in patients with coronary artery disease treated with clopidogrel. *Heart*. 2012;98(2):100–108. doi:10.1136/hrt.2011.227652
32. Su J, Zheng N, Li Z, et al. Association of GCK gene DNA methylation with the risk of clopidogrel resistance in acute coronary syndrome patients. *J Clin Lab Anal*. 2020;34(2):e23040. doi:10.1002/jcla.23040
33. Li X, Wang Z, Wang Q, Xu Q, Lv Q. Clopidogrel-associated genetic variants on inhibition of platelet activity and clinical outcome for acute coronary syndrome patients. *Basic Clin Pharmacol Toxicol*. 2019;124(1):84–93. doi:10.1111/bcpt.13110
34. Peng W, Shi X, Xu X, Lin Y. Both CYP2C19 and PON1 Q192R genotypes influence platelet response to clopidogrel by thrombelastography in patients with acute coronary syndrome. *Cardiovasc Ther*. 2019;2019:3470145. doi:10.1155/2019/3470145
35. Saiz-Rodriguez M, Belmonte C, Caniego JL, et al. Influence of CYP450 Enzymes, CES1, PON1, ABCB1, and P2RY12 polymorphisms on clopidogrel response in patients subjected to a percutaneous neurointervention. *Clin Ther*. 2019;41(6):1199–1212e1192. doi:10.1016/j.clinthera.2019.04.037
36. Park KW, Park JJ, Kang J, et al. Paraoxonase 1 gene polymorphism does not affect clopidogrel response variability but is associated with clinical outcome after PCI. *PLoS One*. 2013;8(2):e52779. doi:10.1371/journal.pone.0052779
37. Wu H, Qian J, Xu J, et al. Besides CYP2C19, PON1 genetic variant influences post-clopidogrel platelet reactivity in Chinese patients. *Int J Cardiol*. 2013;165(1):204–206. doi:10.1016/j.ijcard.2012.08.017
38. Campo G, Ferraresi P, Marchesini J, Bernardi F, Valgimigli M. Relationship between paraoxonase Q192R gene polymorphism and on-clopidogrel platelet reactivity over time in patients treated with percutaneous coronary intervention. *J Thromb Haemost*. 2011;9(10):2106–2108. doi:10.1111/j.1538-7836.2011.04457.x
39. Li X, Zhang L, Chen X, et al. PON1 Q192R genotype influences clopidogrel responsiveness by relative platelet inhibition instead of on-treatment platelet reactivity. *Thromb Res*. 2013;132(4):444–449. doi:10.1016/j.thromres.2013.08.004
40. Ito M, Katono Y, Oda A, Hirasawa N, Hiratsuka M. Functional characterization of 20 allelic variants of CYP1A2. *Drug Metab Pharmacokinet*. 2015;30(3):247–252. doi:10.1016/j.dmpk.2015.03.001
41. Thorn CF, Aklillu E, Klein TE, Altman RB. PharmGKB summary: very important pharmacogene information for CYP1A2. *Pharmacogenet Genomics*. 2012;22(1):73–77. doi:10.1097/FPC.0b013e32834c6efd
42. Kreutz RP, Breall JA, Kreutz Y, et al. Protease activated receptor-1 (PAR-1) mediated platelet aggregation is dependent on clopidogrel response. *Thromb Res*. 2012;130(2):198–202. doi:10.1016/j.thromres.2012.02.049
43. Zhang S, Zhu J, Li H, et al. Associations of CYP2C19 and F2R genetic polymorphisms with platelet reactivity in Chinese ischemic stroke patients receiving clopidogrel therapy. *Pharmacogenet Genomics*. 2022;32(4):138–143. doi:10.1097/FPC.0000000000000462
44. Pan Y, Wangqin R, Li H, et al. F2R polymorphisms and clopidogrel efficacy and safety in patients with minor stroke or TIA. *Neurology*. 2021;96(1):e1–e9. doi:10.1212/WNL.0000000000011078