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**STUDYING THE POLYMORPHISM OF *CYP2C19*, *P2RY12*, *ITGB3*
GENES AND CLOPIDOGREL RESISTANCE IN PATIENTS
UNDERGOING PERCUTANEOUS CORONARY INTERVENTION**

Major: Internal medicine

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SUMMARY OF MEDICAL DOCTORAL THESIS

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INTRODUCTION

Even with clopidogrel therapy, cardiovascular events (CVEs) can still happen, and genetic variants linked to clopidogrel resistance can affect both short-term and long-term treatment outcomes. The gene encoding the enzyme involved in clopidogrel metabolism (*CYP2C19*) has single nucleotide polymorphisms (SNPs) that have been linked to clopidogrel resistance and poor clinical outcomes following percutaneous coronary intervention (PCI). However, only around 12% of clopidogrel resistance instances can be explained by the *CYP2C19* gene polymorphism. There has been some recent evidence that suggests SNPs of genes encoding platelet membrane receptors such as *P2Y12* (*P2RY12*) - the direct target receptor of clopidogrel, or Glycoprotein IIb/IIIa (*ITGB3*) - an essential receptor for thrombus development - are linked to both clopidogrel resistance and CVEs.

In Vietnam, several studies have investigated the association between SNPs of *CYP2C19* gene and clopidogrel resistance, as well as outcomes in certain groups of patients with coronary artery disease. However, there is still lack of data available on patients who have undergone coronary artery stenting. Furthermore, no studies have been found that investigate this relationship for SNPs of genes such as *P2RY12* and *ITGB3* in Vietnamese subjects. Additionally, assessing how this combination of SNPs is related to clinical characteristics and treatment outcomes in patients undergoing coronary artery intervention is of immense importance and requires research to contribute to personalized treatment.

Research Objectives:

1. *Investigating the genetic polymorphisms of CYP2C19, P2RY12, ITGB3, and clopidogrel resistance in patients undergoing percutaneous*

coronary intervention..

2. Evaluating the association between CYP2C19, P2RY12, and ITGB3 gene polymorphisms and clopidogrel resistance, clinical and paraclinical characteristics, and treatment outcomes in 12 months of follow-up in patients undergoing percutaneous coronary intervention.

NEW CONTRIBUTIONS OF THE THESIS

- Identifying the polymorphic characteristics of the *CYP2C19*, *P2RY12*, and *ITGB3* genes in patients undergoing percutaneous coronary intervention.
- Determining the relationship between *CYP2C19*, *P2RY12*, and *ITGB3* gene polymorphisms and clopidogrel resistance, as well as some clinical characteristics.
- Determining the association between *CYP2C19*, *P2RY12*, *ITGB3* gene polymorphisms, clopidogrel resistance, and treatment outcomes followed-up in 12 months.

THESIS STRUCTURE

The thesis consists of 133 pages (excluding appendices and references). Introduction: 2 pages; Literature Review: 32 pages; Subjects and Methods: 30 pages; Results: 31 pages. Discussion: 34 pages. Limitations: 1 page. Conclusion: 2 pages. Recommendation: 1 page. With 52 tables, 5 charts, and 12 figures. There are 155 references, including 23 Vietnamese and 132 English documents.

CHAPTER 1

LITERATURE REVIEW

1.1. Overview of Coronary Artery Disease

1.2. Effect of clopidogrel resistance in the treatment of coronary artery disease

This inadequate antiplatelet aggregation response to clopidogrel

is called "clopidogrel resistance". The prevalence of clopidogrel resistance in the Asian population is reported to be between 17.2% and 81.6%. In Vietnam, some studies showed this rate ranges from 29.3% to 34.68%. The mechanism of this condition is not fully understood and depends on many factors, including both genetic and non-genetic factors. Numerous studies have shown that the occurrence of clopidogrel resistance is a significant risk factor for cardiovascular events in patients after coronary intervention.

1.3. Genetic polymorphisms of *CYP2C19*, *P2RY12*, *ITGB3* and their association with clopidogrel resistance

1.3.2. Influence of *CYP2C19* gene polymorphism on clopidogrel resistance

The *CYP2C19* gene is located on chromosome 10q23.33, contains 9 exons and 8 introns. The *CYP2C19**2 and *CYP2C19**3 alleles produce premature termination codons that result in a loss of function of the *CYP2C19* enzyme. Since *CYP2C19**2 and *CYP2C19**3 are non-functional alleles associated with clopidogrel resistance and are prevalent in Asians.

1.3.3. The influence of the *P2RY12* gene polymorphism on clopidogrel resistance

The *P2RY12* gene is located on chromosome 3q25.1 and consists of 3 exons and 2 introns. A meta-analysis by Cui G. of 15 studies (5,769 patients) showed that the *G52T* polymorphism is associated with clopidogrel resistance in dominant genetic model ($p < 0.05$). At the same time, there was a clear correlation between the *C34T* polymorphism and clopidogrel resistance under dominant, recessive, and additive genetic models ($p < 0.05$).

1.3.4. Influence of *ITGB3* gene polymorphism on clopidogrel resistance

The gene encoding *ITGB3* is located on chromosome 17q21.32, consists of 15 exons. The common polymorphism of the *ITGB3* gene is *T1565C* on exon 2 (rs5918). This SNP has been shown in some studies to be a risk factor for cardiovascular disease and clopidogrel resistance.

1.4. Domestical and international research

1.4.1. Domestical Research

- A study by Dao Van Don (2020) on 116 patients with acute coronary syndrome (ACS) showed a clopidogrel resistance rate of 29.3%. Patients with an intermediate/poor *CYP2C19* metabolizer phenotype had a higher rate of clopidogrel resistance compared to patients with rapid *CYP2C19* metabolizer phenotype.
- A study by Tran Hoa (2020) on over 650 patients who underwent PCI with clopidogrel treatment showed 5 genotypes with the following distribution percentages: *CYP2C19* *1/*1 (40%), *CYP2C19* *1/*2 (42.2%), *CYP2C19* *1/*3 (6%), *CYP2C19* *2/*2 (3.2%), *CYP2C19* *2/*3 (8.6%).

1.4.2. International research

- A study by Grinshtein Y. et al. (2018) on 130 patients who underwent coronary artery bypass grafting showed that patients with the gene combination of *ITGB3*+*CYP2C19**2 or with the *2 allele of *CYP2C19* had more major adverse cardiac events (MACE) with HR of 4; 95% CI: 2.19 - 7.29, $p = 0.008$.
- A study by Pandey C. et al. (2019) on 207 patients with acute myocardial infarction using dual antiplatelet therapy (aspirin and

clopidogrel) showed that there was an independent and statistically significant association between non-response to the medication and the *CYP2C19*2* gene (OR = 3.33; 95% CI: 1.04 - 10.69; $p = 0.044$) after adjusting for demographic and clinical factors.

- A meta-analysis conducted by Zhao K. et al. (2019), including 14 studies with a total of 8,698 patients, identified an association between the *P2RY12* gene polymorphism and adverse events in patients treated with clopidogrel, showing that ischemic events were associated with the *C34T* variant (OR = 1.70; 95% CI: 1.22 - 2.36; $p < 0.05$).

CHAPTER 2

SUBJECTS AND METHODS

2.1. Research Subjects

The study was conducted on patients with coronary artery disease (CAD) who underwent successful coronary stent placement and were treated with clopidogrel, meeting the following criteria:

*** Inclusion criteria**

- Patient over 18 years old.
- The patient was diagnosed with ACS or CCS, had an indication for intervention, and successfully underwent coronary stenting.
- The patient was treated with clopidogrel (Plavix) at a dose of 75 mg/day for at least 7 consecutive days.
- The patients or their legal representative agrees to participate in the study.

*** Exclusion criteria**

- Allergic to clopidogrel or using a lower or higher dose than the antiplatelet treatment regimen.
- Using of P2Y12 receptor inhibitors other than clopidogrel (ticagrelor or prasugrel) or nonsteroidal anti-inflammatory drugs (NSAIDs)

within 7 days prior to the study.

- Using oral anticoagulants or omeprazole or esomeprazole in your treatment.
- With a history of: previous coronary artery intervention or coronary artery bypass graft surgery or stroke (cerebral hemorrhage, cerebral infarction).
- Major surgery within 7 days prior to the study.
- Hemoglobin below 80 g/l or above 170 g/l.
- Platelets below 100 G/l or above 450 G/l.
- Patients or families with hematological disorders.
- Hepatitis, cirrhosis, liver cancer.
- Severe infection at the time of the study.
- Has a glomerular filtration rate below 30 ml/min or is undergoing dialysis.

2.2. Methods

2.2.1. Study design

- Prospective, cross-sectional descriptive study and 12-month follow-up.

2.2.1.1. Location and time of study

- Department of Cardiovascular Intervention, 103 Military Hospital.
- Department of Cardiovascular Intervention, 175 Military Hospital.
- Platelet aggregation tests were conducted at the Department of Hematology, Military Hospital 103 and the Department of Hematology, Military Hospital 175.
- Genetic polymorphism testing of SNPs: *CYP2C19 G681A*, *CYP2C19 G636A*, *P2RY12 C34T*, *P2RY12 G52T*, and *ITGB3 T1565C* was conducted at the Institute of Military Medical and Pharmaceutical Research, Vietnam Military Medical University.
- The research period is from 09/2021 to 12/2023.

2.2.1.2. Study sample size

- Objective 1: Use the sample size formula for descriptive estimation studies for a proportion.

$$n = Z_{1-\alpha/2}^2 \frac{p(1-p)}{d^2}$$

According to research by Guha S. et al. (2009), the rate of clopidogrel resistance is $p = 12.5\% \rightarrow n = 168.07$.

$$n = \frac{\left\{ Z_{1-\frac{\alpha}{2}} \sqrt{2P_2(1-P_2)} + Z_{1-\beta} \sqrt{P_1(1-P_1) + P_2(1-P_2)} \right\}^2}{(P_1 - P_2)^2}$$

- Objective 2: Use the sample size calculation formula for a study on the association between clopidogrel resistance based on genotype.

Table 2.1. Calculate the sample size based on studies of each gene polymorphism.

Study	Genotype	P ₁	P ₂	n
Alhazzani A. et al	<i>CYP2C19 G681A: GA+AA</i>	60%	16%	13
	<i>CYP2C19 G636A: GA+AA</i>	48%	20%	35
Hidayat R. et al	<i>P2RY12 C34T: CT+TT</i>	16.2%	83.8%	5
	<i>P2RY12 G52T: GT+TT</i>	15.8%	84.2%	5
Arya V. et al	<i>ITGB3 T1565C: TC+CC</i>	37.8%	14.3%	40

So the minimum sample size for each group is 40. At the same time, we chose a sample overage rate of 20% for 12-month longitudinal follow-up. So $n \geq (2 \times 40) \times 1.2 = 96$. In summary, the minimum sample size required to ensure both research objectives is 169 patients.

2.2.2. Patient admission and data collection process

- **Step 1:** Patients with CAD who underwent successful coronary stent placement and were treated with clopidogrel were selected, explained

the study, and invited to participate. In which, clopidogrel is treated according to the "Diagnosis and Treatment of Coronary Artery Disease" protocol of the Vietnamese Ministry of Health.

- **Step 2:** The doctor examines and records clinical and paraclinical information, lesion characteristics, and coronary artery interventions according to the procedure of the Vietnamese Ministry of Health.

- **Step 3:** Collect venous blood samples for platelet aggregation testing using the optical transmission method (LTA) at a time when clopidogrel 75 mg/day has been used for at least 7 days according to a standardized protocol.

- **Step 4:** Collect venous blood samples for genetic testing of the following SNPs: *CYP2C19* (*G681A* and *G636A*), *P2RY12* (*C34T* and *G52T*), and *ITGB3* (*T1565C*) using the Sanger sequencing method according to a standardized procedure.

- **Step 5:** Monitor the patient and record treatment outcomes.

+ Post-PCI treatment method: The patient was counselled and explained the importance of regular follow-up visits. Based on individual characteristics, the patient was treated according to recommendations and maintained consistent medical treatment following the guidelines of the Vietnamese Ministry of Health.

+ Monitoring method: The patient continued to receive a daily dose of 75 mg clopidogrel until discharge, as well as maintaining this dose of clopidogrel periodically every month (with a prescription included) according to the DAPT treatment recommendations after intervention from the Vietnamese Ministry of Health. Simultaneously, the patient was followed up periodically at 1, 3, 6, 9, and 12 months after coronary artery intervention at the cardiology clinic of 103 Military Hospital and the internal cardiology clinic of 175 Military Hospital, starting

from the time of discharge. The follow-up data were collected from periodic treatment documents, hospital admission records for cardiovascular events (if any), and clinical examination information at the clinic using the research patient follow-up form, including: assessing treatment adherence, checking medication prescriptions, and directly interviewing patients to determine if they maintained the correct clopidogrel dosage; Clinical signs of myocardial ischemia; Record any cardiovascular events if they occur.

Immediately after obtaining the results of the genetic test, especially for the *CYP2C19* gene polymorphism, if loss-of-function alleles are present or if the patient experiences ischemic events during clopidogrel use, the patient is immediately tested for the gene polymorphism. At the same time, the patient is counselled and recommended to switch antiplatelet therapy from clopidogrel to ticagrelor. If the patient does not agree to adjust their medication as recommended, continue clopidogrel and monitor and record any adverse events until the end of the study period.

2.2.3. Diagnostic criteria used in the study

2.2.3.1. *Hypertension (According to the recommendations of the Vietnam Society of Cardiology - 2018)*

2.2.3.2. *Body mass index (According to WHO - 2004)*

2.2.3.3. *Diabetes (According to ADA - 2020)*

2.2.3.4. *Dyslipidemia (According to NCEP-ATP III - 2020)*

2.2.3.5. *Smoking (According to Mohammadi S. - 2021)*

2.2.3.6. *Family history of early coronary artery disease (According to Tran Hoa - 2020)*

2.2.3.7. *Diagnosis of acute coronary syndrome (According to the Vietnamese Ministry of Health - 2019)*

2.2.3.8. *Diagnosis of chronic coronary artery syndrome (According to the Vietnamese Ministry of Health - 2020)*

2.2.3.9. *Diagnosis of chronic heart failure (According to the Vietnamese Ministry of Health - 2020)*

2.2.3.10. *Clopidogrel resistance*

Clopidogrel resistance in patients after PCI with stent placement is defined as platelet aggregation > 50% after taking clopidogrel 75 mg/day for ≥ 7 days, with testing performed using the LTA method with ADP 5 $\mu\text{mol/l}$ (Blinden - 2007, Hwang – 2011 và Lee – 2014).

2.2.3.11. *Assessing the characteristics of coronary artery lesion and intervention*

2.2.3.12. *Cardiovascular events monitored over 12 months*

- Primary endpoints: Major adverse cardiovascular events (MACE) include all-cause mortality, non-fatal myocardial infarction, stroke, and target vessel revascularization, defined according to Khan U. (2017).

- Secondary endpoints: stent thrombosis, rehospitalization for heart failure or recurrent angina.

2.5. Data analysis

Processed using SPSS 26.0 and Excel 2013 software. The result is considered statistically significant when $p < 0.05$.

CHAPTER 3

RESULTS

3.1. General characteristics

Age, $\bar{X} \pm SD$	Male, n (%)	Female, n (%)	p (T-test)
	128 (74.85)	43 (25.15)	
61.99 $\pm$ 11.26	59.91 $\pm$ 11.11	68.21 $\pm$ 9.3	<0.05

Comment: Males are predominant at 74.85% and have a lower average age than females with $p < 0.05$.

3.2. Polymorphic characteristics of *CYP2C19*, *P2RY12*, *ITGB3* genes, clopidogrel resistance

Table 3.7. Characteristics of *CYP2C19* gene polymorphism (n=171)

Gene	Genotype, n (%)		Allele, n (%)		p
<i>CYP2C19</i> G681A	GG	94 (54.97)	G 253 (73.98)	A 89 (26.02)	>0.05
	GA	65 (38.01)			
	AA	12 (7.02)			
<i>CYP2C19</i> G636A	GG	149 (87.13)	G 318 (92.98)	A 24 (7.02)	>0.05
	GA	20 (11.70)			
	AA	2 (1.17)			

p: Hardy-Weinberg equilibrium test

Comment: The allele frequencies of G and A for the *CYP2C19* G681A and G636A were 73.98%; 92.98% and 26.02%; 7.02%, respectively.

Table 3.8. Phenotype characteristics of the *CYP2C19* gene (n=171)

<i>CYP2C19</i> phenotype	n (%)
Normal metabolism (NM)	81 (47,37)
Intermediate metabolism (IM)	69 (40,35)
Poor metabolism (PM)	21 (12,28)

Comment: The moderate and poor metabolizer phenotypes of the *CYP2C19* gene accounted for 40.35% and 12.28%, respectively.

Table 3.9. *P2RY12* gene polymorphism characteristics (n=171)

Gene	Genotype, n (%)		Allele, n (%)		p
<i>P2RY12</i> <i>C34T</i>	CC	98 (57.31)	C 255 (74.56)	T 87 (25.44)	>0.05
	CT	59 (34.50)			
	TT	14 (8.19)			
<i>P2RY12</i> <i>G52T</i>	GG	135 (78.95)	G 302 (88.30)	T 40 (11.70)	>0.05
	GT	32 (18.71)			
	TT	4 (2.34)			

p: Hardy-Weinberg equilibrium test

Comment: The C and T allele frequencies of *P2RY12 C34T* were 74.56% and 25.44%. The G and T allele frequencies of *P2RY12 G52T* were 88.30% and 11.70%.

Table 3.10. Characteristics of the *ITGB3* gene polymorphism (n=171)

Gene	Genotype, n (%)		Allele, n (%)		p
<i>ITGB3</i> <i>T1565C</i>	TT	156 (91.23)	T 327 (95.61)	C 15 (4.39)	>0.05
	TC	15 (8.77)			
	CC	0 (0)			

p: Hardy-Weinberg equilibrium test

Comment: The T and C allele frequencies of *ITGB3 T1565C* were 95.61% and 4.39%.

Table 3.14. The relationship between clinical factors and clopidogrel resistance

Features		Clopidogrel resistance (n=75)	Non clopidogrel resistance (n=96)	OR [95%CI]	p
BMI (kg/m ²)	≥ 23, n (%)	44 (25.73)	41 (23.98)	1.94 [1.03-3.51]	<0.05 ^a
	< 23, n (%)	31 (18.13)	55 (32.16)		
	$\bar{X} \pm SD$	23.62±2.89	22.74±2.58		<0.05 ^b
Smoking, n (%)	Yes	41 (23.98)	21 (12.28)	4.30 [2.21-8.36]	<0.05 ^a
	No	34 (19.88)	75 (43.86)		

p^a : Mantel-Haenszel test; p^b : T-test

Comment: Patients with BMI ≥23 kg/m² or smoking, the rate of clopidogrel resistance was higher than the others group with OR were 1.94 and 4.30, respectively ($p < 0.05$).

Table 3.15. Relationship between some paraclinical factors and clopidogrel resistance

Features, n (%)		Clopidogrel resistance (n=75)	Non clopidogrel resistance (n=96)	OR [95%CI]	p
eGFR (ml/min)	≥ 60	21 (12.28)	13 (7.60)	2.48 [1.14-5.37]	<0.05
	< 60	54 (31.58)	83 (48.54)		
NT-proBNP (pg/ml)	<300	32 (18.71)	62 (36.26)	2,45 [1.31-4.55]	<0.05
	≥300	43 (25.15)	34 (19.88)		

p : Mantel-Haenszel test

Comment: Patients with eGFR <60 ml/min or NT-proBNP >300 pg/ml, the rate of clopidogrel resistance was 2.48 and 2.45 times higher than in the other groups with $p < 0.05$.

Table 3.17. The relationship between medications used in treatment and clopidogrel resistance

Medications, n (%)		Clopidogrel resistance (n=75)	Non clopidogrel resistance (n=96)	OR [95 %CI]	p
Aldosteron antagonist	Yes	38 (22.22)	29 (16.96)	2.37 [1.26-4.44]	<0.05
	No	37 (21.64)	67 (39.18)		
PPI	Yes	70 (40.94)	76 (44.44)	3.68 [1.31-10.34]	<0.05
	No	5 (2.92)	20 (10.70)		

p: Mantel-Haenszel test

Comment: Patients using PPI or aldosteron antagonist had a 3.68 and 2.37 times higher risk of clopidogrel resistance with $p < 0.05$.

3.3. The relationship between *CYP2C19*, *P2RY12*, *ITGB3* gene polymorphisms and clopidogrel resistance, clinical and paraclinical characteristics, and treatment outcomes in 12 months.

Table 3.19. The relationship between the *CYP2C19* genotype and clopidogrel resistance

Gene	Clopidogrel resistance (n=75)	Non clopidogrel resistance (n=96)	OR [95%CI]	p
<i>CYP2C19</i> G681A, n (%)				
GG	31 (18.13)	63 (36.84)		<0.05 ^a
GA	35 (20.47)	30 (17.54)		
AA	9 (5.26)	3 (1.75)		
(GA+AA) vs GG			2.83 [1.51-5.30]	<0.05 ^b
<i>CYP2C19</i> G636A, n (%)				
GG	55 (32.16)	94 (54.97)		<0.05 ^a
GA	18 (10.53)	2 (1.17)		
AA	2 (1.17)	0 (0)		
(GA+AA) vs GG			17.09 [3.84-75.92]	<0.05 ^b

p^a : χ^2 test; p^b : Mantel-Haenszel test

Comment: Patients carrying allele A of *CYP2C19* G681A or *CYP2C19* G636A had a 2.83 and 17.09 times higher rate of clopidogrel resistance with $p < 0.05$.

Table 3.19. The relationship between the *CYP2C19* phenonotype and clopidogrel resistance

Phenotype	Clopidogrel resistance (n=75)	Non clopidogrel resistance (n=96)	OR [95%CI]	p
NM, n (%)	19 (11.11)	62 (36.26)		<0.05 ^a
IM, n (%)	39 (22.81)	30 (17.54)		
PM, n (%)	17 (9.94)	4 (2.34)		
NM vs (IM+PM)	56 (32.75)	34 (19.88)	5.37 [2.76-10.48]	<0.05 ^b

p^a : χ^2 test; p^b : Mantel-Haenszel test

Comment: Patients with IM+PM phenotype had a 5.37 times higher rate of clopidogrel resistance than NM phenotype with $p < 0.05$.

Table 3.23. The relationship between the *P2RY12* genotype and clopidogrel resistance

Genotype	Clopidogrel resistance (n=75)	Non clopidogrel resistance (n=96)	OR [95%CI]	p
<i>P2RY12</i> C34T, n (%)				
CC	36 (21.05)	62 (36.26)		>0.05 ^a
CT	30 (17.54)	29 (16.96)		
TT	9 (5.26)	5 (2.92)		
(CT+TT) vs CC			1.97 [1.06-3.66]	<0.05 ^b

p^a : χ^2 test; p^b : Mantel-Haenszel test

Comment: The rate of clopidogrel resistance in patients with the CT+TT genotype of *P2RY12* C34T was 1.97 times higher than the CC

genotype with $p < 0.05$.

Table 3.26. The UAC value evaluates the clopidogrel resistance prediction of gene polymorphisms

SNP	UAC	p
Allele A of <i>CYP2C19</i> G681A	0,627	<0,05
Allele A of <i>CYP2C19</i> G636A	0,623	<0,05

Comments: Allele A of *CYP2C19* G681A and G636A had predictive values of clopidogrel resistance with UAC were 0.627 and 0.623 ($p < 0.05$).

Table 3.27. Multivariate regression analysis of the association with clopidogrel resistance

Variables	Adjusted OR [95% CI]	p
Age > 60	0.76 [0.29 – 1.95]	>0.05
Male	0.40 [0.12 – 1.27]	>0.05
Smoking	7.90 [2.67 – 23.38]	<0.05
Hypertension	0.99 [0.34 – 2.90]	>0.05
Diabetes	1.03 [0.38 – 2.75]	>0.05
Lipidemia disorder	1.46 [0.53 – 4.00]	>0.05
Overweight-Obesity	1.51 [0.60 – 3.82]	>0.05
eGFR < 60 ml/min	3.78 [1.16 – 12.23]	<0.05
NT-proBNP > 300 pg/ml	1.47 [0.60 – 3.59]	>0.05
PPI intake	4.87 [1.07 – 22.20]	<0.05
Aldosteron antagonist intake	2.20 [0.90 – 5.34]	>0.05
Allele A of <i>CYP2C19</i> G681A	6.05 [2.44 – 15.00]	<0.05
Allele A of <i>CYP2C19</i> G636A	16.04 [2.90 – 88.67]	<0.05
Allele T of <i>P2RY12</i> C34T	2.02 [0.85 – 4.82]	>0.05
Allele T of <i>P2RY12</i> G52T	2.77 [0.95 – 8.06]	>0.05
Allele C of <i>ITGB3</i> T1565C	1.20 [0.25 – 5.80]	>0.05

p: Multivariate logistic regression analysis

Comments: Smoking status, eGFR < 60 ml/min, use of PPI in treatment, allele A of *CYP2C19* G681A and G636A gene polymorphisms were independent risk factors for clopidogrel resistance with adjusted OR of 7.90; 3.78; 4.87; 6.05 and 16.04, respectively ($p < 0.05$).

3.3.2. The relationship between *CYP2C19*, *P2RY12*, and *ITGB3* gene polymorphisms and certain clinical and paraclinical characteristics

Table 3.30. Association between *P2RY12* G52T gene polymorphism and some clinical characteristics

Feature	GG (n=135)	GT+TT (n=36)	p
Overweight-Obesity	61 (35.67)	24 (14.04)	<0.05

p : χ^2 test

Comment: There is relationship between the GT+TT genotype of the *P2RY12* G52T and overweight and obesity with $p < 0.05$.

Table 3.32. Association between *ITGB3* gene polymorphism and some clinical characteristics

Number of coronary artery branches damaged	<i>ITGB3</i> T1565C		p
	TT (n=156)	TC+CC (n=15)	
1 branch	76 (44.44)	3 (1.75)	<0.05
2 branches	50 (29.24)	4 (2.34)	
3 branches	30 (17.54)	8 (4.68)	

p : χ^2 test

Comment: In patients carrying the T allele of the *ITGB3* T1565C, the number of damaged coronary artery branches increased gradually with $p < 0.05$.

3.3.3. Association between *CYP2C19*, *P2RY12*, *ITGB3* gene polymorphisms, clopidogrel resistance, and treatment outcomes during 12-month follow-up.

Table 3.34. Characteristics of cardiovascular events during longitudinal follow-up

Features (n=171)	n (%)
MACE	
Cardiovascular death	3 (1.75)
All-cause mortality	4 (2.34)
Non-fatal myocardial infarction	0 (0)
Intra stent restenosis	1 (0.58)
Revascularization of target coronary artery lesions	1 (0.58)
Stroke	2 (1.17)
Secondary endpoints	
Stent thrombosis	0 (0)
Rehospitalization for heart failure	7 (4.09)
Rehospitalization for recurrent angina	17 (9.94)

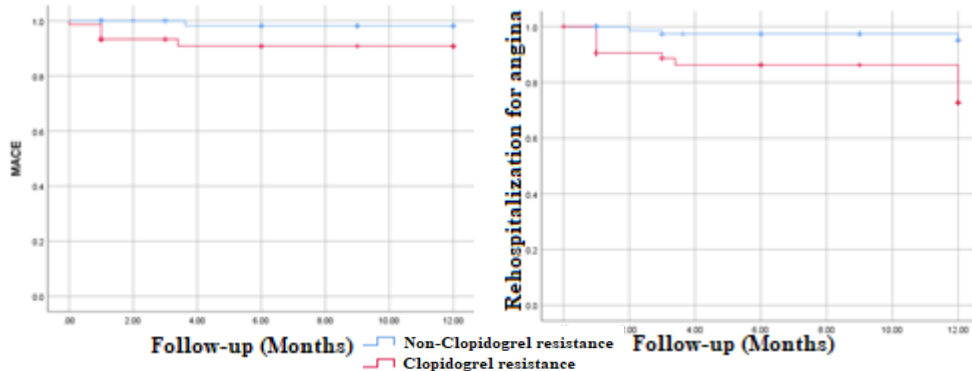
Comments: The rate of MACE was 4.09%, rehospitalization for recurrent angina was 9.94% and rehospitalization for heart failure was 4.09%.

Table 3.35. The association between clopidogrel resistance and cardiovascular events

Features, n(%)		Clopidogrel resistance (n=75)	Non clopidogrel resistance (n=96)	OR [95%CI]	p
MACE	Yes	6 (3.51)	1 (0.58)	8.26	>0.05
	No	69 (40.35)	95 (55.56)	[0.97-70.18]	
Rehospitalization for heart failure	Yes	5 (2.92)	2 (1.17)	3.35	>0.05
	No	70 (40.94)	94 (54.97)	[0.63-17.81]	
Rehospitalization for recurrent angina	Yes	14 (8.19)	3 (1.75)	7.11	<0.05
	No	61 (35.67)	93 (54.39)	[1.96-25.79]	

p: Mantel-Haenszel test

Comments: Patients with clopidogrel resistance had a 7.11-fold higher risk of rehospitalization for angina with $p < 0.05$.



Figures 3.4 and 3.5. The Kaplan-Meier curve shows the association between clopidogrel resistance and MACE and rehospitalization for recurrent angina. Comments: Clopidogrel resistance increased MACE and recurrent chest pain hospitalization events, with p log rank (Mantel-Cox) values of 0.02 and 0.001, respectively, when analyzed using Kaplan-Meier curves.

Table 3.36. The association between CYP2C19 gene polymorphism and cardiovascular events

Features, n (%)		CYP2C19 G636A		p
		GG (n=149)	GA+AA (n=22)	
MACE	Yes	6 (3.51)	1 (0.58)	>0.05 ^a
	No	143 (83.63)	21 (12.28)	
Rehospitalization for heart failure	Yes	4 (2.34)	3 (1.75)	<0.05 ^a
	No	145 (84.80)	19 (11.11)	
Rehospitalization for recurrent angina	Yes	11 (6.43)	6 (3.51)	<0.05 ^b
	No	138 (80.70)	16 (9.36)	

p^a : Fisher's exact test; p^b : χ^2 test

Comment: Allele A of *CYP2C19* G636A was associated with rehospitalization for heart failure and recurrent angina with $p < 0.05$.

Table 3.41. Multivariate regression analysis of factors influencing rehospitalization for recurrent angina

Variables	Adjusted OR [95% CI]	p
Age > 60	1.02 [0.27-3.77]	>0.05
Male	25.26 [1.85-344.89]	<0.05
Smoking	0.43 [0.10-1.92]	>0.05
Hypertension	0.81 [0.16-4.01]	>0.05
Diabetes	4.84 [1.22-19.09]	<0.05
Overweight-Obesity	0.74 [0.20-2.65]	>0.05
Lipidemia disorder	4.42 [0.76-25.68]	>0.05
Clopidogrel resistance	15.72 [2.76-89.23]	<0.05
Allele A of <i>CYP2C19</i> G681A	0.73 [0.19-2.81]	>0.05
Allele A of <i>CYP2C19</i> G636A	2.57 [0.59-11.18]	>0.05
Allele T of <i>P2RY12</i> C34T	1.00 [0.29-3.41]	>0.05
Allele T of <i>P2RY12</i> G52T	0.06 [0.006-1.83]	>0.05
Allele C of <i>ITGB3</i> T1565C	1.10 [0.09-3.63]	>0.05

p: Multivariate logistic regression analysis

Comments: Clopidogrel resistance, along with male gender and diabetes, were independent risk factors for rehospitalization for recurrent angina with OR of 15.72; 25.26 and 4.84 with $p < 0.05$.

CHAPTER 4

DISCUSION

4.2. Polymorphic characteristics of *CYP2C19*, *P2RY12*, *ITGB3* genes and clopidogrel resistance

4.2.1. Polymorphic characteristics of the *CYP2C19* gene

The results of the *CYP2C19* gene polymorphism genotypes and allele frequencies are consistent with the studies by Sugimoto K. (2008), Kim (2010), or the studies by Đào Văn Đôn (2020) and Vu Ngọc Trung (2021) on the Vietnamese population. *CYP2C19* gene polymorphism with studies on Vietnamese patients by Vu Thi Thom (2018), Tran Hoa (2021), and Vu Ngọc Trung (2021).

4.2.2. Polymorphic characteristics of the *P2RY12* gene

We observed that the allele frequency of these variants is similar to the frequency reported in Li M.'s study on ACS patients or Hidayat R.'s study on Indonesian patients.

4.2.3. Polymorphic characteristics of the *ITGB3* gene

This result is consistent with the studies by Nguyen Thi Minh Ngoc (2017) and Zhang Y. (2013).

4.2.4. Clopidogrel resistance characteristics

The rate of clopidogrel resistance in our study was quite high at 43.86%, which is higher than Dao Van Don's study at 29.3%. However, this result is quite similar to the study by Park K. (2011), which was 46%; or the study by Amin A. (2017), which was 38%.

Our study found that overweight-obesity was positively correlated with platelet aggregation, which in turn leads to an increased risk of clopidogrel resistance. This result is also consistent with the study by Dogan A. (2016), which showed that leptin levels are elevated in obese individuals, and platelets have leptin receptors on their surface. Additionally, our study also shows that smoking is an independent risk factor for clopidogrel resistance. This can be explained by the fact that tobacco smoke contains substances that affect the activity of the CYP1A2 enzyme involved in the metabolism of clopidogrel into its active metabolite.

Our study found an association between increased NT-proBNP levels and clopidogrel resistance. During the acute phase of CAD, platelet adhesion and aggregation are strong, which can lead to clopidogrel resistance.

On the other hand, we found that impaired renal was an independent risk factor for clopidogrel resistance. Therefore, patients with low eGFR should be closely monitored and evaluated periodically to reduce clopidogrel resistance.

Our study found that in the group of patients PPIs, the rate of clopidogrel resistance was 3.68 times higher compared to the other group, with $p < 0.05$, and was also an independent risk factor for this condition. It has been shown that PPIs have different levels of CYP2C19 inhibition; for example, omeprazole is a stronger inhibitor than other PPIs in the group. Therefore, the FDA has included omeprazole in the clopidogrel interaction warning.

4.3. The relationship between *CYP2C19*, *P2RY12*, *ITGB3* gene polymorphisms, clopidogrel resistance, clinical and paraclinical characteristics, and cardiovascular events

4.3.1. The relationship between *CYP2C19*, *P2RY12*, and *ITGB3* gene polymorphisms and clopidogrel resistance

Our study shows that patients carrying the *CYP2C19* G681A gene polymorphism A allele have a 2.83-fold increased rate of clopidogrel resistance with $p < 0.05$, and it is also an independent risk factor for this condition. Our results are consistent with some studies on Vietnamese patients. Ying S.'s (2020) meta-analysis showed that the *CYP2C19* G681A gene polymorphism is closely associated with clopidogrel resistance, with the A allele and GA, AA genotypes potentially increasing clopidogrel resistance, particularly in Asians.

For the *CYP2C19* G636A, we also observed similar results. This result is consistent with the study by Alhazzani A. (2017), which showed that the A allele of the *CYP2C19* G636A SNP increases poor response to clopidogrel with an OR of 3.45; 95% CI: 1.57 - 7.70, $p < 0.05$.

Patients with intermediate and poor metabolizer phenotypes (IM+PM) had a higher rate of clopidogrel resistance compared to the normal metabolizer (NM) group (32.75% vs. 19.88%) with an OR of 5.37 and $p < 0.05$. On the Vietnamese population, this result is consistent with the findings of Dao Van Don (2020) and Hwang J (2011).

When analyzing the ROC curve according to the dominant genetic model, we found that patients carrying the A allele of the *CYP2C19* G681A and G636A gene polymorphisms significantly predicted clopidogrel resistance. Collet J.'s study also yielded similar results, showing that *CYP2C19* loss-of-function variants are a significant determinant of prognosis for patients treated with clopidogrel after acute MI, including MACE (HR = 1.55; 95% CI: 1.11 - 2.17) and stent thrombosis (HR = 2.67; 95% CI = 1.69 - 4.22). This suggests that testing for the *CYP2C19* G681A and G636A gene polymorphisms is a useful tool for clinicians to predict clopidogrel resistance.

Our study shows that the T allele of *P2RY12* G52T significantly affects platelet aggregation, particularly in the *TT* genotype. At the same time, the T allele of *P2RY12* C34T is significantly associated with a higher risk of clopidogrel resistance. The mechanism that could explain this result is that these gene polymorphisms affect the binding of the drug to the P2Y12 receptor and the signaling pathway within the

cell after being activated by clopidogrel. Additionally, another potential mechanism is that gene polymorphisms can lead to variable receptor expression, altering drug efficacy by affecting drug-receptor binding affinity.

Our study found that platelet aggregation and clopidogrel resistance were higher in the group of patients with the *TC+CC* genotype compared to the group with the *TT* genotype, although this was not statistically significant. This result is quite similar to the studies by Grinshtein Y and Morawski W, who did not find a statistically significant difference in platelet aggregation.

4.3.2. The relationship between CYP2C19, P2RY12, ITGB3 gene polymorphisms and certain clinical and paraclinical characteristics

We also observed a statistically significant association ($p < 0.05$) between the *GT+TT* genotype of the *P2RY12 G52T* and overweight-obesity. However, Li M.'s (2017) study showed no similar difference.

On the other hand, we also found that the C allele of the *ITGB3 T1565C* was associated with an increased number of damaged coronary artery branches with $p < 0.05$. This result is consistent with the studies by Conkbayir C. et al. (2021), which showed that the C allele was higher in the CAD group compared to the control group with $p = 0.001$.

4.3.3. The association between CYP2C19, P2RY12, ITGB3 gene polymorphisms, clopidogrel resistance, and cardiovascular events

We observed that MACE accounted for 4.09%, and the rate of rehospitalization for recurrent angina was 9.94%. This result is consistent with reports from several studies showing that the rate of MACE is trending downward due to the development of the PCI

specialty.

Our research results show that clopidogrel resistance increased MACE and was an independent risk factor for readmission due to recurrent angina. This could be explained by clopidogrel resistance leading to reduced inhibition of platelet aggregation, resulting in a high thrombotic state for the patient, which is a significant part of the important pathophysiological process of MACE.

When considering the relationship between *CYP2C19* gene polymorphisms and MACEs, our results differ from some domestic and international publications. Another reason that can be explained is the "East Asian anomaly." East Asian patients have a lower rate of ischemic events and experience more bleeding events compared to white patients. However, when considering the event of rehospitalization due to heart failure, our study showed that the A allele of the *CYP2C19* G636A gene polymorphism was associated with the event of rehospitalization due to heart failure with $p < 0.05$ in univariate analysis. This can be explained by the fact that the *CYP2C19* G636A gene polymorphism is closely associated with clopidogrel resistance during treatment, which can lead to reduced effectiveness of coronary revascularization and further promote ischemia, resulting in heart failure.

LIMITATIONS

1. The degree of platelet aggregation at different treatment follow-up time points has not been assessed, therefore a complete evaluation of the drug's response during treatment is not available. Monitoring platelet aggregation with clopidogrel at multiple time points will provide more accurate data on treatment response to the drug.
2. The number of patients in the ACS and CCS groups is significantly

different. Although due to objective conditions at the time of the study, the main patients admitted for treatment were ACS patients, this significantly affected the assessment of the relationship between clopidogrel resistance and gene polymorphisms for the surveyed cardiovascular events.

3. The expression levels of these genes have not yet been evaluated. If additional data on gene expression levels can be analyzed, it would provide a more solid basis for evaluating the relationship between genotype and phenotype with regard to clopidogrel resistance and coronary artery disease characteristics.

CONCLUSIONS

Through a study of 171 patients who underwent coronary artery stenting with clopidogrel treatment and genetic polymorphism testing of *CYP2C19*, *P2RY12*, and *ITGB3* from September 2021 to December 2023 at Military Hospital 103 and Military Hospital 175, we draw the following conclusions:

1. Polymorphisms of the *CYP2C19*, *P2RY12*, *ITGB3* genes and clopidogrel resistance.

- The minor allele frequencies of the *CYP2C19* *G681A*, *CYP2C19* *G636A*, *P2RY12* *C34T*, *P2RY12* *G52T*, and *ITGB3* *T1565C* were 26.02%, 7.02%, 25.44%, 11.70%, and 4.39%, respectively. The intermediate metabolic phenotype of the *CYP2C19* gene was 40.35%, and the poor phenotype was 12.28%.

- The rate of clopidogrel resistance was 43.86%.

- The conditions of BMI ≥ 23 kg/m², NT-proBNP concentration ≥ 300 pg/ml, and the use of aldosterone antagonists increased the risk of clopidogrel resistance with OR of 1.94, 2.45, and 2.37 with $p < 0.05$ in univariate analysis. Smoking, eGFR < 60 ml/min, and PPI use for

treatment are independent risk factors for clopidogrel resistance in multivariate regression analysis, with adjusted OR of 7.90, 3.78, and 4.87 ($p < 0.05$).

2. The relationship between *CYP2C19*, *P2RY12*, *ITGB3* gene polymorphisms and clopidogrel resistance, as well as clinical, paraclinical characteristics, and cardiovascular events monitored over 12 months

**** The relationship between *CYP2C19*, *P2RY12*, and *ITGB3* gene polymorphisms and clopidogrel resistance***

- The *CYP2C19* G681A and G636A gene polymorphisms' A alleles, and the *P2RY12* C34T gene polymorphism's T allele, increased the risk of clopidogrel resistance with OR of 2.83, 17.09, and 1.97 with $p < 0.05$ in univariate analysis.

- Intermediate and poor metabolizer phenotypes of the *CYP2C19* gene have a 5.37-fold higher rate of clopidogrel resistance compared to the normal phenotype group, with $p < 0.05$.

- The *CYP2C19* gene variants G681A and G636A are independent risk factors (adjusted OR of 6.05 and 16.04) and are significant in predicting clopidogrel resistance (UAC of 0.627 and 0.623) with $p < 0.05$.

- There was no statistically significant relationship found between the *P2RY12* G52T and *ITGB3* T1565C gene polymorphisms and clopidogrel resistance.

**** The relationship between *CYP2C19*, *P2RY12*, *ITGB3* gene polymorphisms and clinical, paraclinical characteristics***

- There is a statistically significant association ($p < 0.05$) between the GT+TT genotype of the *P2RY12* G52T gene polymorphism and overweight-obesity.

- The C allele of the *ITGB3* T1565C gene polymorphism is associated

with an increased number of damaged coronary artery branches with $p < 0.05$.

*** *The association between CYP2C19, P2RY12, ITGB3 gene polymorphisms, clopidogrel resistance and cardiovascular events***

- Clopidogrel resistance had a 7.11 times higher risk of rehospitalization for angina compared to the non-resistant group, with $p < 0.05$. Clopidogrel resistance increased MACE and rehospitalization for recurrent angina during the 12-month follow-up period, with p log rank (Mantel-Cox) values of 0.02 and 0.001, respectively, when analyzed using Kaplan-Meier curves.

- Clopidogrel resistance, along with male gender and diabetes, were independent risk factors for readmission due to recurrent angina, with adjusted OR of 15.72, 25.26, and 4.84, respectively, with $p < 0.05$ in multivariate logistic regression analysis.

- The *CYP2C19* G636A polymorphism's A allele was associated with rehospitalization for heart failure and recurrent angina with $p < 0.05$ in univariate analysis.

RECOMMENDATIONS

- In patients indicated for clopidogrel treatment with a high risk of resistance, such as $\text{BMI} \geq 23 \text{ kg/m}^2$, smoking, $\text{eGFR} < 60 \text{ ml/min}$, NT-proBNP level $\geq 300 \text{ pg/ml}$, or those using PPIs or aldosterone receptor antagonists, platelet aggregation testing should be performed during treatment to determine clopidogrel resistance and optimize the antiplatelet drug regimen.

- It is necessary to consider conducting genetic polymorphism testing for *CYP2C19* (G681A, G636A) and *P2RY12* C34T to provide additional tools for predicting and diagnosing clopidogrel resistance, thereby enabling a switch to an appropriate antiplatelet regimen to personalize treatment.

PUBLICATION RELATED TO THE THESIS

1. Ta Anh Hoang, Nguyen Duy Toan, Truong Dinh Cam (2024), “Investigating the features of platelet aggregation and clopidogrel resistance in patients undergone percutaneous coronary intervention”, Journal of 108 - Clinical Medicine and Phamarcy, Volume 19, issue 2/2024: 44-50.
2. Ta Anh Hoang, Nguyen Duy Toan, Hoang Van Tong, Truong Dinh Cam (2024), “Study on characteristics of *CYP2C19* gene polymorphism and its relationship with clopidogrel resistance in patients undergoing percutaneous coronary intervention”, Journal of Military Pharmaco-medicine, issue 8/2024:113-123.
3. Ta Anh Hoang, Nguyen Duy Toan, Hoang Van Tong, Truong Dinh Cam (2024), “Investigating the *ITGB3* genetic polymorphism characteristics in patients undergoing coronary stenting intervention”, Vietnam Medical Journal, Volume 542, issue 3: 156-160.
4. Ta Anh Hoang, Nguyen Duy Toan, Bui Duc Thanh, Hoang Van Tong, Nguyen Oanh Oanh, Luong Cong Thuc, Truong Dinh Cam, “Association of *P2RY12* Gene Variants and Non-Genetic Factors With Clopidogrel Responsiveness in Vietnamese Patients After Percutaneous Coronary Intervention: A Cross-Sectional Study”, J Clin Lab Anal. 2025 Feb 10:e70003. doi: 10.1002/jcla.70003. Epub ahead of print. PMID: 39927599.