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ORIGINAL RESEARCH

The Diversity of *CYP2C19* Polymorphisms in the Thai Population: Implications for Precision Medicine

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Introduction: *CYP2C19* plays a major role in the metabolism of various drugs. The most common genetic variants were the *CYP2C19*2* and **3* alleles (*rs4244285* and *rs4986893*, non-functional variants). In previous studies, we found that genetic polymorphisms in *CYP2C19* variants influenced the active metabolites of clopidogrel and caused major adverse cardiovascular and cerebrovascular effects. However, the distribution of *CYP2C19* varies among ethnic groups and according to adverse drug reactions. This study aimed to investigate the frequency of *CYP2C19* genetic polymorphisms in the Thai population and analyze the differences in the frequency of *CYP2C19* genetic polymorphisms between Thai and other populations.

Methods: This study enrolled 211 unrelated healthy Thai individuals in total. We performed a real-time polymerase chain reaction to genotype *CYP2C19*2* (681G > A) and *CYP2C19*3* (636G > A).

Results: In the Thai population, the *CYP2C19*1* allele was the most prevalent at 70.14%, while the *CYP2C19*2* and **3* alleles were found at frequencies of 25.36% and 4.50%, respectively. Conversely, the *CYP2C19*3* allele was not detected in Caucasian, Hispanic, African, Italian, Macedonian, Tanzanian, or North Indian populations. The phenotypic profile of this gene revealed that the frequency of intermediate metabolizers (IMs) is nearly equal to that of extensive metabolizers (EMs), at 42.65% and 48.82% respectively, with genotypes **1/*2* (36.02%) and **1/*3* (6.63%). Likewise, poor metabolizers (PMs) with genotypes **2/*2* (6.16%), **2/*3* (2.37%), and **3/*3* (<1%) are more prevalent in our population as well.

Conclusion: The distribution of *CYP2C19* genotype and phenotype influenced by non-functional alleles has potential as a pharmacogenomics biomarker for precision medicine and is dependent on an ethnic-specific genetic variation database.

Keywords: *CYP2C19* gene, genetic diversity, Thai population, interethnic differences

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Introduction

Cytochrome P450 (CYP) proteins form a superfamily of enzymes that are involved in the metabolism of drugs, fatty acids, steroids, and xenobiotics.[1,2] Research has demonstrated that the metabolic activity of CYP enzymes is influenced by genetic polymorphisms.[3,4] These polymorphisms in drug-metabolizing enzyme genes contribute to variations in pharmacological responses and the risk of adverse drug events among individuals and across different ethnic groups.[5] The human *CYP2C* subfamily includes four members: *CYP2C8, CYP2C9, CYP2C18*, and *CYP2C19*.[6]

The *CYP2C19* gene is located on chromosome 10 (10q24.1-q24.3) and is responsible for approximately 10% of drug metabolism in clinical practice.[7,8] *CYP2C19* plays a crucial role in metabolizing various therapeutic drugs, including clopidogrel, phenytoin, omeprazole, proguanil, diazepam, citalopram, imipramine, amitriptyline, and clomipramine.[9] The most common genetic variant of *CYP2C19* is the *CYP2C19*2* allele (*rs4244285*, c. G681A), a single base pair mutation in exon 5 that results in a splicing defect, impairing enzyme function.[10] Another significant variant, *CYP2C19*3* (*rs4986893*), is a G636A mutation in exon 4, producing a premature stop codon.[11] *CYP2C19* genotypes and phenotypes are categorized as follows: **1/*1* (extensive metabolizers, EMs, two functional alleles); **1/*2* and **1/*3* (intermediate metabolizers, IM, one null allele and one functional allele); and **2/*2, *2/*3*, and **3/*3* (poor metabolizers, PM, two non-functional alleles).[12–14] Numerous studies have shown a correlation between *CYP2C19* polymorphisms and enzyme activity in patients treated with relevant drugs.[15–17]

Clopidogrel is commonly used to treat myocardial infarction (MI), stroke, acute coronary syndrome (ACS), and atherosclerotic vascular disease.[18] Notably, CYP2C19 plays a crucial role in converting clopidogrel into its active metabolite, clopi-H4.[19] This active metabolite inhibits the adenosine diphosphate P2Y12 receptor, thereby reducing platelet activation. According to the Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for clopidogrel, alleles associated with the *CYP2C19* poor metabolizers phenotype (**2/*2, *2/*3*, and **3/*3*) are significantly linked to lower levels of active clopidogrel metabolites and an increased risk of major adverse cardiovascular and cerebrovascular events.[20] Furthermore, the *CYP2C19* intermediate metabolizers (IMs) phenotype also reduces the effectiveness of clopidogrel.

The distribution of poor metabolizers (PMs) with genotypes **2/*2, *2/*3*, and **3/*3* vary among different populations. The highest prevalence is observed in East Asians (12.97%) and Asians (8.15%), followed by African Americans (4.05%) and Europeans (2.38%). A similar pattern is seen with intermediate metabolizers (IMs), with East Asians having the highest frequency (45.92%), followed by Asians (40.80%), African-Americans (31.39%), and Europeans (26.10%).[21] These genetic variations in *CYP2C19* across different ethnicities are crucial for understanding individual differences in treatment effectiveness and safety, especially in the context of pharmacogenomics-based dosing guidelines. Consequently, this study aimed to investigate the prevalence of *CYP2C19* genetic variations in the Thai population and compare them with those in other populations.

Materials and Methods

Thai Subjects

This cross-sectional study recruited 211 unrelated, healthy individuals of Thai ethnicity, with clinical data obtained from the Excellence Pharmacogenomics and Precision Medicine Centre, College of Pharmacy, Rangsit University, between November 2021 and February 2023 (Supplement 1). All participants were native Thais who were lived in the location of Thailand and had no history of adverse drug reactions (ADRs). We used self-identified race/ethnicity (SIRE) method to confirm the ethnicity, specifically three generations were confirmed as Thais. Thailand is centrally located in Mainland Southeast Asia, bordered by Myanmar to the west, Laos to the northeast, Cambodia to the east, and Malaysia to the south. The study received approval from the Ethics Review Board of Rangsit University (COA. No. RSUERB2021-104), and according to the Declaration of Helsinki with a written informed consent was obtained from all participants.

CYP2C19 Genotyping

Genomic DNA was extracted from EDTA whole blood using a Geneaid DNA Isolation Kit (Geneaid Biotech Ltd., New Taipei City, Taiwan). The quality and quantity of the genomic DNA were measured using an ultraviolet-visible

spectrophotometer (Zhengzhou, Henan, China). Genotyping of *CYP2C19*2* (681G > A, *rs4244285*, non-functional variant) and *CYP2C19*3* (636G > A, *rs4986893*, non-functional variant) was performed using TaqMan assays (C__30634128_10 and C__27861809_10 respectively; ABI, Foster City, CA, USA). The PCR cycling conditions included 50 cycles of denaturation at 92°C for 15 seconds, and annealing and extension at 60°C for 1.30 minutes. *CYP2C19* variants were identified using a QIAGEN QIAquant 96 real-time PCR system (Qiagen, Hilden, Germany).

CYP2C19 Phenotype

According to the Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline, *CYP2C19*1/*1* is categorized as an extensive metabolizers (EMs) with two normal-function alleles. Intermediate metabolizers (IMs) have one normal and one non-functional allele (*CYP2C19*1/*2* and *CYP2C19*1/*3*). Poor metabolizers (PMs) carry two non-functional alleles (*CYP2C19*2/*2, CYP2C19*2/*3*, and *CYP2C19*3/*3*).

Statistical Analysis

The frequencies of the two common *CYP2C19* variants were analyzed using the Arlequin program version 3.1 for Hardy–Weinberg equilibrium testing. Allele and phenotype frequencies of *CYP2C19* in the Thai population were compared with those in other populations using R Statistical Software (v4.1.2; R Core Team 2021). Odds ratios for all comparisons were illustrated in a forest plot using the Meta for R package (v4.2.0; Viechtbauer 2010). A significant difference was identified for each population compared with the Thai population if the p-value was less than 0.05.

Results

The Frequency of *CYP2C19* Alleles in Thai Population

A total of 211 unrelated healthy Thai participants were included in this study, comprising fifty-five males (26.07%) and one hundred fifty-six females (73.93%). The mean age of the participants was 38.3 years, ranging from 19 to 75 years. We determined the allele frequency distribution of *CYP2C19* in the Thai population (Table 1). The *CYP2C19*1* allele was the most common, with a frequency of 70.14%. The frequencies of the *CYP2C19*2* and *CYP2C19*3* alleles were 25.36% (n = 107) and 4.50% (n = 19), respectively. Among the healthy Thai participants, the *CYP2C19* genotypes were distributed as follows: 103 (48.82%) were extensive metabolizers (EMs, *CYP2C19*1/*1*), 76 (36.02%) were intermediate metabolizers (IMs, **1/*2* and **1/*3*), and 14 (6.63%), 13 (6.16%), and 5 (2.37%) were poor metabolizers (PMs, **2/*2* and **2/*3*). The frequency of the *CYP2C19*3/*3* (PMs) genotype was less than 1.00% of the total Thai population. The allele frequencies of *CYP2C19*2* and **3* were tested for Hardy–Weinberg equilibrium (p-value < 0.05), showing no significant deviation in the Thai population.

Table 1 Alleles and Genotyping Frequencies for *CYP2C19* Genes in the Thai Population (n = 211)

***CYP2C19* Alleles (n = 422)**		**n**	**Allele Frequencies, %**
*CYP2C19*1*		296	70.14
*CYP2C19*2*, c.*G681A*		107	25.36
*CYP2C19*3* c.*C806T*		19	4.50
***CYP2C19* genotyping (n = 211)**	**Predicted phenotype**	**n**	**Genotype frequencies, %**
*CYP2C19*1/*1*	EMs	103	48.82
*CYP2C19*1/*2*	IMs	76	36.02
*CYP2C19*1/*3*	IMs	14	6.63
*CYP2C19*2/*2*	PMs	13	6.16
*CYP2C19*2/*3*	PMs	5	2.37
*CYP2C19*3/*3*	PMs	0	0.00

Abbreviations: EMs, Extensive metabolizers; IMs, intermediate metabolizers; PMs, poor metabolizers.

Comparative of *CYP2C19* Genotype and Phenotype Frequency Between the Thai and Other Populations

The frequencies of the *CYP2C19*2* and **3* alleles were evaluated in Thai and various other populations, as shown in Tables 2 and 3 and Figure 1A and B. This study found that the *CYP2C19*2* allele was particularly frequent. The highest frequencies of the *CYP2C19*2* allele were observed in Indians (37.90%),[30] Native Japanese (34.50%),[33] and Bhutanese (30.14%),[34] followed by North Indians (29.75%),[29] Koreans (28.40%),[33] Thais (25.36%), Han Chinese (24.67%),[31]

Table 2 The Allele Frequencies of *CYP2C19*2* in Many Populations

Ethnicity	Allele Frequency, %	OR (95% CI)	p-value	References
Thai (n = 211)	25.36	Present study		
Italian (n = 360)	11.10	**0.36 (0.23–0.58)**	**1.21×10^{-5}**	[22]
Macedonia (n = 184)	14.40	**0.47 (0.27–0.82)**	**0.0056**	[23]
Caucasian (n = 216)	16.20	**0.56 (0.34–0.93)**	**0.0176**	[24]
Hispanic (n =500)	12.80	**0.43 (0.28–0.66)**	**6.04×10^{-5}**	[25]
African (n = 99)	20.20	0.74 (0.39–1.35)	0.3208	[26]
African-American (n = 500)	19.40	0.70 (0.47–1.05)	0.0711	[25]
Venezuelan (n = 281)	19.70	0.71 (0.45–1.11)	0.1249	[27]
Tanzanian (n = 212)	17.90	0.64 (0.39–1.04)	0.0600	[28]
North Indians (n = 121)	29.75	1.23 (0.72–2.08)	0.4426	[29]
Indian (n = 112)	37.90	**1.74 (1.03–2.93)**	**0.0298**	[30]
Han Chinese (n = 96)	24.67	0.97 (0.53–1.740)	1.0000	[31]
Native Japanese (n = 200)	34.50	1.53 (0.98–2.39)	0.0529	[32]
Korean (n = 271)	28.40	1.15 (0.75–1.77)	0.5362	[33]
Bhutanese (n = 443)	30.14	1.26 (0.86–1.86)	0.2307	[34]
Vietnamese (n = 275)	20.50	0.74 (0.47–1.17)	0.1899	[35]
Malaysian (n = 62)	5.70	**0.20 (0.05–0.58)**	**0.0007**	[36]

Notes: Bold font indicates significantly different (p-value < 0.05), allele frequency compared between different ethnicities and Thais.
Abbreviations: OR, odds ratio; 95% CI, 95% Confidence Interval.

Table 3 The Allele Frequencies of *CYP2C19*3* in Many Populations

Ethnicity	Allele Frequency, %	OR (95% CI)	p-value	References
Thai (n = 211)	4.50	Present study		
Italian (n = 360)	0	**0 (0.00–0.29)**	**0.0001**	[22]
Macedonia (n = 184)	0	**0 (0.00–0.57)**	**0.0042**	[23]
Caucasian (n = 216)	0	**0 (0.00–0.48)**	**0.0016**	[24]
Hispanic (n =500)	0	**0 (0.00–0.21)**	**1.58×10^{-5}**	[25]
African (n = 99)	0	**0 (0.00–1.06)**	**0.0620**	[26]
African-American (n = 500)	0.40	**0.09 (0.01–0.44)**	**0.0005**	[25]
Venezuelan (n = 281)	1.26	0.32 (0.07–1.18)	0.0847	[27]
Tanzanian (n = 212)	0	**0 (0.00–0.49)**	**0.0018**	[28]
North Indians (n = 121)	0	**0 (0.00–0.87)**	**0.0291**	[29]
Indian (n = 112)	2.20	0.41 (0.04–2.03)	0.3412	[30]
Han Chinese (n = 96)	3.27	0.72 (0.12–2.99)	0.7595	[31]
Native Japanese (n = 200)	9.00	2.22 (0.92–5.75)	0.0718	[32]
Korean (n = 271)	10.10	**2.48 (1.10–6.13)**	**0.0224**	[33]
Bhutanese (n = 443)	15.69	**4.20 (2.04–9.78)**	**8.94×10^{-6}**	[34]
Vietnamese (n = 275)	2.50	0.59 (0.18–1.80)	0.3151	[35]
Malaysian (n = 62)	6.50	1.55 (0.34–5.79)	0.5002	[36]

Notes: Bold font indicates significantly different (p-value < 0.05), allele frequency compared between different groups ethnicities and Thais.
Abbreviations: OR, odds ratio; 95% CI, 95% Confidence Interval.

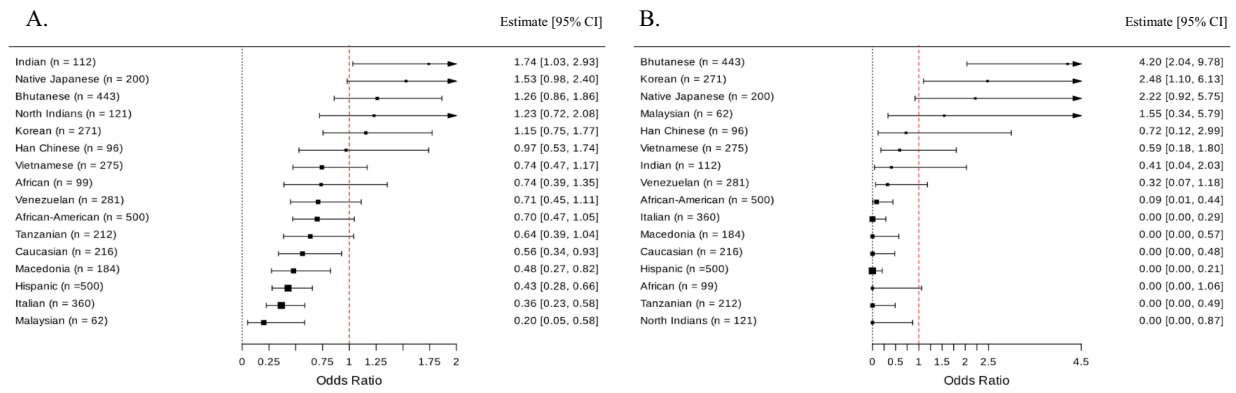

Figure 1 Forest plot displaying the odds ratios for the frequencies of *CYP2C19*2* (**A**) and *CYP2C19*3* (**B**) alleles across multiple populations in comparison to the Thai population allele frequency.

Vietnamese (20.50%),[35] and Africans (20.20%)[26] (Table 2 and Figure 1A). Conversely, the Bhutanese population had the highest observed frequency of the *CYP2C19*3* allele (15.69%)[34] (Table 3 and Figure 1B). High distributions of the *CYP2C19*3* allele were also observed in Koreans (10.10%),[33] Native Japanese (9.00%),[32] Malaysians (6.50%),[36] and Thais (4.50%), while the *CYP2C19*3* allele was not found in Caucasian, Hispanic, African, Italian, Macedonian, Tanzanian, and North Indian populations.[22–26,28,29]

A comparison of *CYP2C19*2* and **3* frequencies between the Thai population and other populations is presented in Tables 2 and 3 and Figure 1A and B. The distribution of *CYP2C19* variations was similar among the Thai, Han Chinese, Vietnamese, and Venezuelan populations (p-value > 0.05). However, the *CYP2C19*2* allele showed significant differences between the Thai population and others, such as Italian (p-value = 1.21×10^{-5}), Macedonian (p-value = 0.0056), Caucasian (p-value = 0.0176), Hispanic (p = 6.04×10^{-5}), Indian (p-value = 0.0298), and Malaysian populations (p-value = 0.0007). A strong significant difference was also observed for *CYP2C19*3* frequencies when comparing Thai with Bhutanese and Hispanic populations (p-value = 8.94×10^{-6} and 1.58 x 10^{-5}, respectively).

Phenotyping of *CYP2C19*1/*1* (extensive metabolizers, EMs) was highly prevalent in several populations (Table 4 and Figures 2 and 3). The highest frequencies of *CYP2C19*1/*1* were observed in the Italian population (79.44%) compared to the Thai population (p-value = 8.22×10^{-14}).[22] Intermediate metabolizers (*CYP2C19 *1/*2* and **1/*3*) showed the following phenotype distribution: Italian (18.89%), Macedonian (19.02%), Caucasian (18.52%), Hispanic (18.00%), African (19.19%), African-American (23.60%), and Malaysian (14.52%) populations.[22–26,36] Significant differences in IMs prevalence were observed when compared with Thais (p-value < 0.05), with the highest frequency detected in the Bhutanese population (79.01%; OR = 5.05, 95% CI = 3.49–7.34, p-value = 7.83×10^{-20}).[34]

The distribution of poor metabolizers (PMs) of *CYP2C19 (*2/*2, *2/*3*, and **3/*3*) was 19.00% in Native Japanese,[32] 14.02% in Koreans,[33] 10.42% in Han Chinese,[31] 9.82% in Indians,[30] 8.53% in Thais, 7.44% in North Indians,[29] 6.32% in Bhutanese,[34] 6.06% in Africans,[26] 4.84% in Malaysians,[36] 4.63% in Venezuelans,[27] 4.40% in African-Americans,[25] 4.17% in Caucasians,[24] 4.00% in Vietnamese,[35] 3.77% in Tanzanians,[28] 2.72% in Macedonians,[23] 1.67% in Italians,[22] and 1.60% in Hispanics.[25] The PMs prevalence was significantly higher in Thai patients compared to Italian (p-value = 0.0001), Macedonian (p-value = 0.0168), and Hispanic (p-value = 0.0006) patients. Conversely, the PMs prevalence in the Thai population was lower than in the Native Japanese population (OR = 2.51, 95% CI = 1.34–4.86, p-value = 0.0024). The odds ratios for these comparisons are illustrated in Figure 2A and B using forest plots.

Discussion

According to the Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines, the *CYP2C19* gene is a significant pharmacogenomic polymorphism in various populations.[20,37] In clinical settings, *CYP2C19* plays a pivotal role in metabolizing approximately 8–10% of commonly used medications, including proton pump inhibitors, anticonvulsants, antiplatelet agents, and antidepressants.[14,24] Notably, patients carrying homozygous alleles (PMs) and

Table 4 Phenotyping Frequencies of *CYP2C19**2* and **3* Polymorphism Among Different Ethnicities Compared with Thai Population

Ethnicity	Phenotyping Frequencies, n (%)	OR (95% CI)	p-value	References
EMs (1/*1*)**				
Thai (n = 211)	103 (48.82)	Present study		
Italian (n = 360)	286 (79.44)	**4.04 (2.75–5.98)**	**8.22×10^{-14}**	[22]
Macedonia (n = 184)	77 (41.85)	0.76 (0.49–1.15)	0.1882	[23]
Caucasian (n = 216)	89 (41.20)	0.74 (0.49–1.09)	0.1204	[24]
Hispanic (n = 250)	124 (49.60)	1.03 (0.70–1.51)	0.9255	[25]
African (n = 99)	33 (33.33)	**0.53 (0.31–0.89)**	**0.0139**	[26]
African-American (n = 250)	88 (35.20)	**0.57 (0.38–0.84)**	**0.0033**	[25]
Venezuelan (n = 281)	177 (62.99)	**1.78 (1.22–2.61)**	**0.0018**	[27]
Tanzanian (n = 106)	72 (67.92)	**2.21 (1.3 3–3.75)**	**0.0013**	[28]
North Indians (n = 121)	58 (47.93)	0.97 (0.60–1.55)	0.9094	[29]
Indian (n = 112)	33 (29.46)	**0.44 (0.26–0.73)**	**0.0009**	[30]
Han Chinese (n = 96)	50 (52.08)	1.14 (0.68–1.90)	0.6238	[31]
Native Japanese (n = 200)	62 (31.00)	**0.47 (0.31–0.72)**	**0.0003**	[32]
Korean (n = 271)	97 (35.79)	**0.59 (0.39–0.86)**	**0.0051**	[33]
Bhutanese (n = 443)	254 (57.34)	1.41 (1.00–1.99)	0.0439	[34]
Vietnamese (n = 275)	160 (58.18)	1.46 (1.00–2.13)	0.0436	[35]
Malaysian (n = 62)	41 (66.13)	**2.04 (1.09–3.89)**	**0.0203**	[36]
IMs (1/*2* and **1/*3*)**				
Thai (n = 211)	90 (42.65)	Present study		
Italian (n = 360)	68 (18.89)	**0.31 (0.21–0.47)**	**2.17×10^{-9}**	[22]
Macedonia (n = 184)	35 (19.02)	**0.32 (0.19–0.51)**	**4.96×10^{-7}**	[23]
Caucasian (n = 216)	40 (18.52)	**0.31 (0.19–0.48)**	**6.37×10^{-8}**	[24]
Hispanic (n =250)	45 (18.00)	**0.29 (0.19–0.46)**	**7.04×10^{-9}**	[25]
African (n = 99)	19 (19.19)	**0.32 (0.17–0.58)**	**4.25×10^{-5}**	[26]
African-American (n = 250)	59 (23.60)	**0.42 (0.27–0.63)**	**1.61×10^{-5}**	[25]
Venezuelan (n = 281)	90 (32.03)	**0.63 (0.43–0.93)**	**0.0180**	[27]
Tanzanian (n = 106)	30 (28.30)	**0.53 (0.31–0.90)**	**0.0142**	[28]
North Indians (n = 121)	54 (44.63)	1.08 (0.67–1.74)	0.7315	[29]
Indian (n = 112)	68 (60.71)	**2.07 (1.27–3.41)**	**0.0023**	[30]
Han Chinese (n = 96)	32 (33.33)	0.67 (0.39–1.14)	0.1325	[31]
Native Japanese (n = 200)	98 (49.00)	1.29 (0.86–1.94)	0.1998	[32]
Korean (n = 271)	128 (47.23)	1.20 (0.82–1.76)	0.3564	[33]
Bhutanese (n = 443)	350 (79.01)	**5.05 (3.49–7.34)**	**7.83×10^{-20}**	[34]
Vietnamese (n = 275)	99 (36.00)	0.76 (0.52–1.11)	0.1591	[35]
Malaysian (n = 62)	9 (14.52)	**0.23 (0.09–0.50)**	**3.81×10^{-5}**	[36]
PMs (2/*2*, **2/*3* and **3/*3*)**				
Thai (n = 211)	18 (8.53)	Present study		
Italian (n = 360)	**6 (1.67)**	**0.18 (0.06–0.49)**	**0.0001**	[22]
Macedonia (n = 184)	**5 (2.72)**	**0.30 (0.08–0.86)**	**0.0168**	[23]
Caucasian (n = 216)	9 (4.17)	0.47 (0.18–1.13)	0.0745	[24]
Hispanic (n =250)	**4 (1.60)**	**0.17 (0.04–0.54)**	**0.0006**	[25]
African (n = 99)	6 (6.06)	0.69 (0.22–1.89)	0.5038	[26]
African-American (n = 250)	11 (4.40)	0.49 (0.21–1.14)	0.0833	[25]
Venezuelan (n = 281)	13 (4.63)	0.52 (0.23–1.15)1	0.0920	[27]

(*Continued*)

Table 4 (Continued).

Ethnicity	Phenotyping Frequencies, n (%)	OR (95% CI)	p-value	References
Tanzanian (n = 106)	4 (3.77)	0.42 (0.10–1.33)	0.1595	[28]
North Indians (n = 121)	9 (7.44)	0.86 (0.33–2.10)	0.8361	[29]
Indian (n = 112)	11 (9.82)	1.17 (0.48–2.73)	0.6876	[30]
Han Chinese (n = 96)	10 (10.42)	1.25 (0.49–2.99)	0.6695	[31]
Native Japanese (n = 200)	**38 (19.00)**	**2.51 (1.34–4.86)**	**0.0024**	[32]
Korean (n = 271)	38 (14.02)	1.75 (0.94–3.36)	0.0642	[33]
Bhutanese (n = 443)	28 (6.32)	0.72 (0.38–1.43)	0.3274	[34]
Vietnamese (n = 275)	11 (4.00)	0.45 (0.19–1.03)	0.0518	[35]
Malaysian (n = 62)	3 (4.84)	0.55 (0.09–1.97)	0.4260	[36]

Notes: Bold font indicates significantly different (p-value < 0.05), phenotyping frequencies compared between different ethnicities and Thais.
Abbreviations: OR, odds ratio; 95% CI, 95% Confidence Interval.

heterozygous alleles (IMs) of *CYP2C19*2* and **3* variants are reported to have approximately 1.8-fold and 1.5-fold increased risk, respectively, of developing severe adverse cardiovascular events (e.g.death, myocardial infarction, stroke) following clopidogrel treatment.[38,39] Thus, non-functional *CYP2C19* variants significantly impact the dosage requirements for clopidogrel therapy in individual patients.

In our study, the most prevalent non-functional variant was the *CYP2C19*2* allele, found in approximately 25.36% of our sample, consistent with previous reports in various populations, including Asian populations (Han Chinese, Native Japanese, Korean, Bhutanese, Vietnamese, and North Indians), Africans, African-Americans, Venezuelans, and Tanzanians.[25–28,31–36] In contrast, Caucasian, Hispanic, Macedonian, and Italian populations exhibited lower frequencies of the *CYP2C19*2* allele compared to the Thai population.[22–25] However, these variations were attributed to the distribution of non-functional *CYP2C19* alleles in each population and their impact on clopidogrel efficacy. Further investigations are necessary to confirm the influence of *CYP2C19*2* distribution across ethnicities and the effects on clinically relevant medications.

The allele frequency of *CYP2C19*3* (c C806T) in our study was approximately 4.50%, similar to earlier reports in the Thai population.[40] Additionally, the frequency of *CYP2C19*3* in Han Chinese was 3.27%, consistent with other Asian populations, as shown in Table 3 and Figure 1B. Interestingly, the distribution of *CYP2C19*3* in these Asian populations, including Thai, closely resembled each other, except for Bhutanese, Korean, and North Indian populations. Specifically, we observed a higher distribution of *CYP2C19*3* in the Bhutanese population compared to the Thai population (p-value = 8.94×10^{-6}). However, the

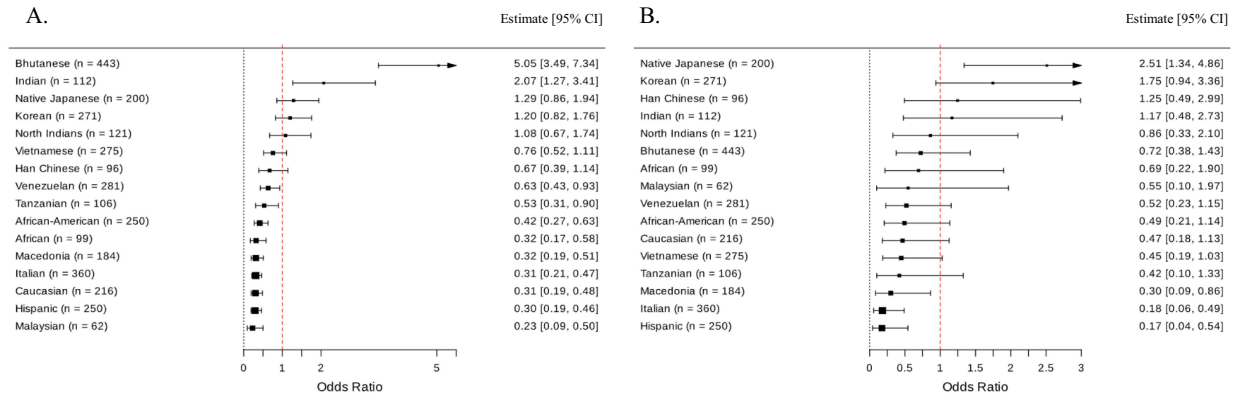

Figure 2 Forest plot displaying the odds ratios for the frequencies of *CYP2C19* intermediate metabolizers (IMs; **1/2 + *1/3*) diplotypes (**A**) and *CYP2C19* poor metabolizers (PMs; **2/*2 + *2/*3 + *3/*3*) diplotypes (**B**) across multiple populations in comparison to the Thai population diplotype frequency.

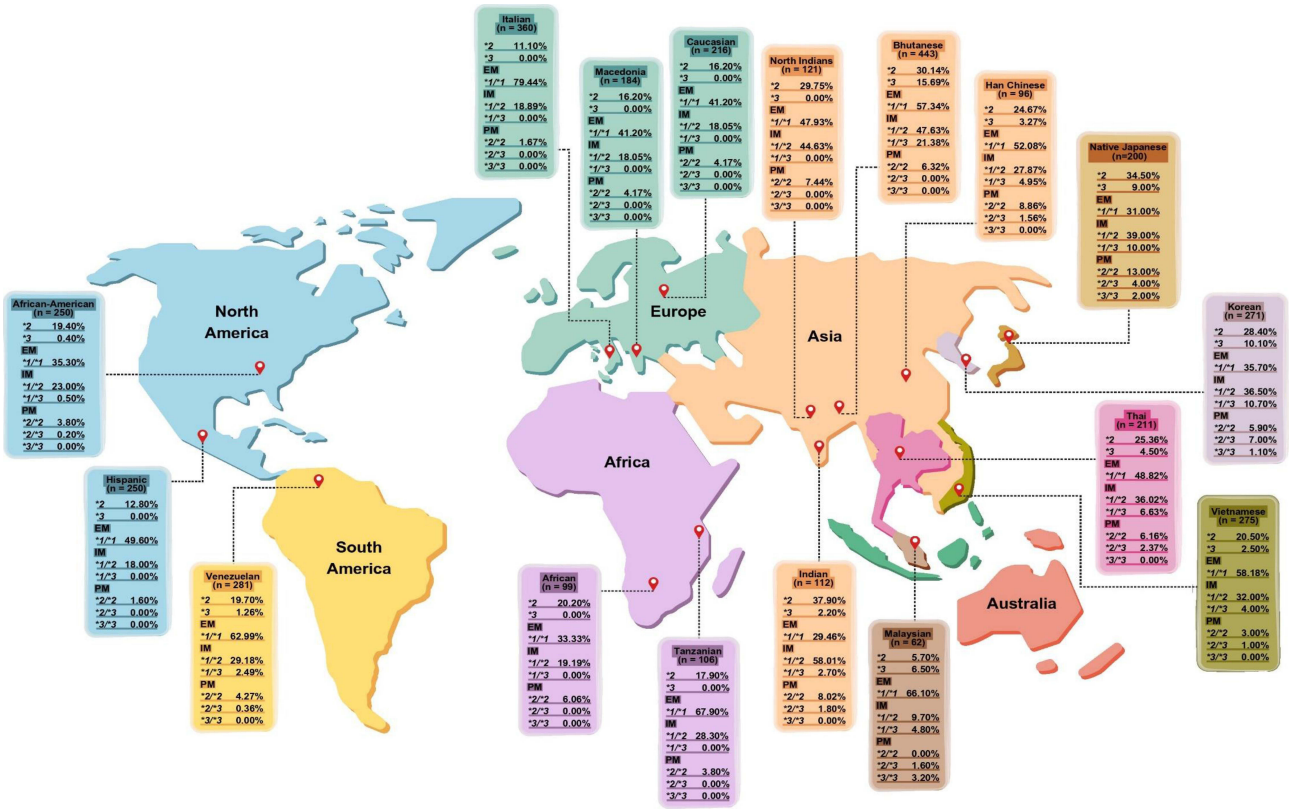

Figure 3 Distribution of *CYP2C19* polymorphisms in Thais and ethnic groups.

prevalence of the *CYP2C19*3* allele was absent in Italian, Macedonian, Caucasian, Hispanic, African, Tanzanian, and North Indian populations.[22–26,28,29] This genetic variability across racial and ethnic groups underscores the importance of *CYP2C19*3* allele frequency as a pharmacogenomics marker for screening different ethnicities to prevent therapeutic failures in individual patients, particularly in the Asian population.

None of the Thai participants in our study carried the homozygous *CYP2C19*3/*3* allele. However, PMs phenotypes based on *CYP2C19*2/*2* and **2/*3* genotypes were present in approximately 6.16% and 2.37% of the sample, respectively. Our findings align with previous research indicating a PMs distribution of approximately 19% in Asian populations compared to around 2% in Caucasians.[41] Notably, substantial variation in the distribution of these *CYP2C19* genotypes was observed across populations, with less than 1% occurrence of these variants (**1/*3, *2/*3*, and **3/*3*) in European and African American populations, contrasting with frequencies of **1/*3* (7.34%), **2/*3* (3.33%), and **3/*3* (0.44%) in East Asian populations.[42] Given the genetic diversity of *CYP2C19* variants across ethnicities, determining the *CYP2C19*3* genotype and phenotype before treatment initiation is particularly advantageous for Asian populations. Moreover, the understanding of *CYP2C19* polymorphisms can significantly impact patient prognosis and outcomes, particularly in the context of drugs like clopidogrel, where genetic variations affect drug metabolism and efficacy. However, the relationship between *CYP2C19* phenotype frequency and drug efficacy or adverse drug reactions (ADRs) warrants further investigation in other populations.

Conversely, the *CYP2C19*17* variant (c. −806C>T) demonstrates ultra-rapid *CYP2C19* activity and holds a significant role in the metabolic pathways of therapeutic drugs, which could heighten the risk of treatment failure with this substrate. From a clinical standpoint, the *CYP2C19*17* variant strongly correlates with an increased risk of clopidogrel-induced bleeding in patients with cardiovascular and cerebrovascular diseases (OR = 1.89, 95% CI: 1.09–3.25, p-value = 0.02). This association is attributed to the heightened transcriptional activity of the CYP2C19 enzyme and its exaggerated response to clopidogrel's prodrug.[43] Conversely, this variant exhibits a notable association with a reduced risk of major adverse cardiovascular and cerebrovascular events in patients with coronary artery disease (OR = 0.76, 95%

CI: 0.60–0.98, p = 0.03).[43] Furthermore, the *CYP2C19*17* genotype has been observed at higher frequencies among European and African populations, approximately 15.0–25.0%, whereas the Asian population exhibits a much lower frequency, approximately 4%.[44] Given these trends, further exploration of the frequency of *CYP2C19*17* variants and their association with adverse drug reactions is warranted across various populations.

One limitation of our study stems from the genotyping method employed for *CYP2C19*. We utilized real-time PCR to detect two single nucleotide polymorphisms (SNPs), *rs4244285* (**2*) and *rs4986893* (**3*). In cases where individuals did not carry these two variants, we presumed the genotype to be **1*. However, this assumption may not accurately represent the true **1* status, as other untested variants could be present. This methodological approach has the potential to affect the precision of our genotyping Results and subsequent interpretations of *CYP2C19* metabolic phenotypes. While real-time PCR is a highly reliable technique, the importance of validation cannot be overstated. To mitigate this concern, future studies should consider validating genotyping results using DNA sequencing methods, which offer a more comprehensive analysis of *CYP2C19* variants and minimize potential errors. By integrating such validation steps, we can enhance the accuracy of genotyping and ensure a more dependable identification of metabolic phenotypes, thereby enhancing the relevance of our findings in clinical practice. In future research, we plan to include clinical data to investigate how these genetic variations impact drug response and adverse reactions in real-world scenarios.

In conclusion, the distribution of the *CYP2C19* gene via non-functional alleles holds promise for implementation in clinical practice and relies on an ethnic-specific genetic variation database. Specifically, the genotype and phenotype of *CYP2C19*3* could serve as a pharmacogenomics biomarker in Thai and Asian populations for screening, mitigating genetic associations with adverse drug reactions induced by certain medications.

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Disclosure

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