

Proof-of-concept development of a cost-effective PACE-based assay for *CYP2C19* genotyping

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ABSTRACT

Background: Pharmacogenomic testing of the *CYP2C19* gene is a fundamental component of precision medicine, enabling personalized dose adjustments for critical medications, including antiplatelet agents and antidepressants. However, the high cost of currently available commercial genotyping kits creates a financial burden that limits patient accessibility to these essential tests.

Objectives: This study aimed to preliminarily develop and analytically evaluate a novel PCR Allelic Competitive Extension (PACE) assay for genotyping *CYP2C19**2, *3, and *17.

Materials and methods: Thirty DNA samples extracted from buffy coat were analyzed using specifically designed allele-specific primers for *CYP2C19**2, *3, and *17. The genotyping results were reported as zygosity status and evaluated for concordance with the TaqMan™ Drug Metabolism Genotyping Assay.

Results: The analysis identified *1/*1 (53.3%), *1/*2 (30.0%), *1/*3 (10.0%), and *2/*2 (6.7%) genotypes, while the allele frequencies were 73.3% for *1, 21.7% for *2, 5.0% for *3, and 0% for *17. The results from the PACE technique demonstrated 100% concordance with those obtained from the commercial TaqMan™ assay and produced clear, well-separated data points in the allelic discrimination plot analysis.

Conclusion: The novel PACE technique provides high accuracy, equivalent to commercially available test kits, while offering a more cost-effective approach, with a reagent cost of only USD 4.34 (135 Thai baht) per sample for *CYP2C19**2, *3, and *17 genotyping. It represents an alternative to *CYP2C19* genotyping, supporting the advancement of personalized medicine and reducing the long-term financial burden on healthcare.

Introduction

Pharmacogenomic testing has emerged as a fundamental component of precision medicine, enabling the integration of individual genetic variability into predictions of drug response, therapeutic efficacy, and the risk of adverse drug reactions. Genetic polymorphisms, particularly in the cytochrome P450 (CYP450) gene family, which is involved in drug metabolism, transport, and pharmacological targets, substantially affect drug pharmacokinetics and significantly influence plasma concentrations and clinical outcomes. The implementation of

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pharmacogenomics into clinical practice supports the selection of appropriate medications and individualized dose adjustments tailored to each patient's genetic profile. This personalized therapy enhances treatment effectiveness, reduces the incidence of adverse drug reactions and drug-related toxicity. Moreover, integrating pharmacogenomics-guided strategies into healthcare systems has the potential to reduce long-term healthcare costs by reducing inappropriate medication use and preventing avoidable drug-related complications. Therefore, pharmacogenomics testing is considered a critical tool for supporting clinical decision-making, enhancing medication safety, and advancing the delivery of personalized patient care.

Cytochrome P450 (CYP450) enzymes are encoded by *CYP* genes and are responsible for drug metabolism. These enzymes play a critical role in catalyzing chemical modifications of pharmaceutical compounds, thereby facilitating their elimination from the body. Genetic variability within *CYP* genes significantly influences enzyme activity and contributes to individual differences in drug response.¹ Based on variations in the *CYP* gene, patients can be categorized into four major phenotypes. There are poor metabolizers (PM), intermediate metabolizers (IM), extensive metabolizers (EM), and ultra-rapid metabolizers (UM), each associated with distinct drug-response risks. PM usually contains homozygous null-functional or deletion of *CYP* alleles, exhibiting elevated plasma drug concentrations, leading to an increased risk of drug toxicity and adverse drug reactions. In contrast, UM with two or more functional *CYP* alleles may experience subtherapeutic drug levels, resulting in diminished treatment efficacy and potential therapeutic failure.²

CYP2C19 is a major enzyme in the cytochrome P450 family encoded by the *CYP2C19* gene and is essential for the metabolic activation of prodrug antiplatelet agents, such as clopidogrel, antidepressant drugs, and proton pump inhibitors.^{3,4} The distribution of *CYP2C19* polymorphisms varies among ancestral and geographic regions, resulting in inter-ethnic differences in drug metabolism phenotypes and pharmacological response. Loss-of-function alleles, especially *CYP2C19**2 and *3, are more frequent in Asian populations than in European and African populations, leading to higher frequencies of intermediate- and poor-metabolizer phenotypes.^{5,6} In contrast, the gain-of-function *CYP2C19**17 allele is common among European and Middle Eastern populations and associated with rapid and ultrarapid metabolizer phenotypes.^{5,6} These genetic differences specific to ethnicity have important implications in pharmacogenomics-guided therapy, especially for drugs metabolized through the *CYP2C19* pathway.

Among the Thai population, the *CYP2C19* allele frequencies were 25.60%, 2.50%, and 1.80% for the *CYP2C19**2, *CYP2C19**3, and *CYP2C19**17 alleles, respectively.^{4,7} The importance of *CYP2C19* genotyping

lies in its ability to predict varied drug responses and optimize therapeutic outcomes. The *CYP2C19**1/*1 genotype is the most prevalent, corresponding to normal metabolizers (NM) that show a normal drug response. Two *CYP2C19**2 or *CYP2C19**3 alleles result in the PM phenotype, which significantly reduces or abolishes *CYP2C19* activity, diminishing drug response and possibly leading to drug toxicity. While the *CYP2C19**17 allele is associated with ultra-rapid metabolizer (UM) status due to increased enzyme activity, the drug dose may need to be increased to achieve adequate therapeutic efficacy.^{8,9} Therefore, genotyping of clinically relevant *CYP2C19* alleles is essential for tailoring personalized dosing, which prevents drug toxicity in poor metabolizers and therapeutic failure in ultra-rapid metabolizers. PCR-based techniques, such as real-time PCR assays, have been widely used to provide rapid and accurate detection of star alleles (*2, *3, *17) for clinical pharmacogenomic applications. Validation studies have demonstrated concordance with DNA sequencing, the analytical gold standard.^{10,11} However, currently available commercial *CYP2C19* genotyping kits remain relatively expensive, limiting access for patients who benefit from pharmacogenomic testing due to the associated financial burden. To address this limitation, this study develops an alternative genotyping approach that would reduce testing costs and improve patient access to personalized treatment. This was achieved through the design and implementation of a *CYP2C19* genotyping assay using the PCR Allelic Competitive Extension (PACE) technique.

PACE is an advanced genotyping technology designed for the highly accurate detection of DNA sequence variations. This method utilizes unlabeled allele-specific primers in combination with a universal fluorescent reporting cassette, and the result indicates zygosity status; this technique enables cost-effective, high-throughput genotyping. The PACE platform has been widely used in genetic research, molecular breeding, and pathogen detection due to its high sensitivity, specificity, scalability, and operational efficiency.¹²⁻¹⁴ Although this technique has not been reported for use in human gene variation analysis, particularly for single-nucleotide polymorphism (SNP) detection, previous studies have demonstrated successful development of SNP assay designs for plant disease detection, with consistent experimental performance.

Materials and methods

Study design and sample recruitment

This study aims to develop a *CYP2C19* genotyping assay based on the PCR Allelic Competitive Extension (PACE) technique and to compare the results with the commercial *CYP2C19* genotyping kits (TaqMan Drug Metabolism Genotyping Assays, Applied Biosystems™, USA). Thirty DNA samples were obtained from residual clinical buffy coat specimens from patients

archived in the Molecular Biology Laboratory, Division of Transfusion Science, Department of Medical Technology, Faculty of Associated Medical Sciences, Chiang Mai University. These residual specimens were originally collected for pharmacogenomics routine testing in this laboratory and were subsequently used for research under institutional ethical approval.

DNA extraction and preparation

DNA was extracted from the buffy coat using a QIAamp® Blood Mini Kit (QIAGEN, Germany). Purity and dsDNA concentration were measured by a Qubit fluorometer (Invitrogen, USA). Then, DNA concentration was adjusted to 4 ng/μL and stored at -20 °C until further analysis.

Primer and control template design for PACE and modification

The primer sequences were designed as described in Table 1. Two allele-specific forward primers, each terminating with a nucleotide specific to a given allele, and one common reverse primer were designed based on the rs identifiers rs4244285, rs4986893, and rs12248560, corresponding to the *CYP2C19*2*, *CYP2C19*3*, and *CYP2C19*17* alleles, respectively. In addition, approximately 500 bp DNA templates representing wild-type and mutant alleles were

synthesized based on the gene *CYP2C19* sequence (Homo sapiens, chromosome 10, GRCh38.p14, NCBI Reference Sequence: NC_000010.11). The genomic regions spanning positions 94,781,609-94,782,109 for *CYP2C19*2*, 94,780,403-94,780,903 for *CYP2C19*3*, and 94,761,650-94,762,150 for *CYP2C19*17* were selected. These synthetic templates were used as reference controls for zygosity determination.

Analysis of *CYP2C19* alleles using TaqMan™ Drug Metabolism Genotyping Assays

PCR reaction consisted of 5 μL of TaqPath™ ProAmp™ Master Mix, 0.5 μL of each TaqMan™ Drug Metabolism Genotyping Assay (rs4244285, rs4986893, and rs12248560), and 5 μL of genomic DNA (4 ng/μL). Nuclease-free water was added to adjust the total reaction volume to 10 μL.

PCR amplification was performed using a real-time PCR system (QuantStudio™ 5, Applied Biosystems) with the following thermal cycling conditions: a pre-read step at 60 °C for 30 seconds, followed by enzyme activation at 95 °C for 5 minutes. This was followed by 40 amplification cycles consisting of DNA denaturation at 95 °C for 15 seconds and extension at 60 °C for 90 seconds. A final post-read step was performed at 60 °C for 30 seconds. Fluorescence signals were collected during the pre-read, extension, and post-read steps.

Table 1. Allele-specific primers designed for PACE-PCR genotyping of *CYP2C19*2*, *CYP2C19*3*, and *CYP2C19*17*.

ID		Sequence (5'→3')
rs12248560	Allele-specific primer 1	GAAGGTGACCAAGTTCATGCTCAAATTTGTGTCTTCTGTTCTCAAAGC
rs12248560	Allele-specific primer 2	GAAGGTCTGGAGTCAACGGATTTCAAATTTGTGTCTTCTGTTCTCAAAGT
rs12248560	Common, Reverse primer	CCATCGTGGCGCATTATCTCTTACAT
rs4986893	Allele-specific primer 1	GAAGGTGACCAAGTTCATGCTCAAAAACTGGCCTTACCTGGATC
rs4986893	Allele-specific primer 2	GAAGGTCTGGAGTCAACGGATTGCAAAAACTGGCCTTACCTGGATT
rs4986893	Common, Reverse primer	AACATCAGGATTGTAAGCACCCCT
rs4244285	Allele-specific primer 1	GAAGGTGACCAAGTTCATGCTTTCCCACTATCATTGATTATTTCCCG
rs4244285	Allele-specific primer 2	GAAGGTCTGGAGTCAACGGATTAATTTCCCACTATCATTGATTATTTCCCA
rs4244285	Common, Reverse primer	GCAAGGTTTTTAAGTAATTTGTTATGGGT

Analysis of *CYP2C19* alleles using PCR Allelic Competitive Extension assay

For *CYP2C19* allele detection using PACE, the assay was prepared as follows. First, a PACE assay primer mix was prepared, consisting of two allele-specific forward primers, each at a final concentration of 12 μM, and one common reverse primer at a final concentration of 30 μM. Next, the PACE genotyping reaction master mix was prepared by combining 5 μL of 2×PACE Genotyping Master Mix (GMM) with 0.138 μL of the PACE assay primer mix. Finally, 5 μL of genomic DNA (4 ng/μL) was added to each reaction, resulting in a total reaction volume of 10 μL.

PCR amplification was conducted using a real-time PCR system (CFX Opus 96, Bio-Rad) with the following cycling parameters: DNA denaturation at 94 °C for 15 minutes, followed by 9 cycles of 94 °C for 20 seconds and 65 °C for 1 minute. followed by 30 cycles of 94 °C for 20 seconds and 57 °C for 1 minute, and a final hold at 37 °C for 1 minute. The fluorescent signal was measured at the endpoint.

Statistical analysis

Genotyping results obtained from the PACE assay were compared with those generated by the commercial *CYP2C19* genotyping kit. The overall

concordance rate between the two methods was calculated as the proportion of identical genotype calls across all samples. The 95% confidence interval (CI) for concordance was estimated using the Wilson score method.¹⁵ Agreement between the two genotyping approaches was evaluated using Cohen's kappa coefficient (κ). The strength of agreement was interpreted as follows: values <0.20 indicate poor agreement; 0.20-0.40, fair agreement; 0.41-0.60, moderate agreement; 0.61-0.80, substantial agreement; and >0.80, almost perfect agreement.¹⁶ Allele and genotype frequencies of *CYP2C19* variants were calculated by direct counting from the genotyping results of the 30 DNA samples and expressed as percentages.

Results

CYP2C19 genotyping using the PACE technique was performed, and allelic discrimination plots for *CYP2C19*2*, *CYP2C19*3*, and *CYP2C19*17* alleles are shown in Figures 1-3, respectively. The genotyping results from all three alleles were subsequently analyzed together. A total of 30 DNA samples were examined, of which 16 samples were identified as

*CYP2C19*1/*1*, 9 samples as *CYP2C19*1/*2*, 3 samples as *CYP2C19*1/*3*, and 2 samples as *CYP2C19*2/*2* (Table 2). The genotyping results obtained using the PACE method demonstrated complete agreement with those generated by the TaqManTM Drug Metabolism Genotyping Assay, yielding an overall concordance rate of 100% (95%) (CI: 88.7-100.0%) using the Wilson score method. Agreement between the two genotyping approaches showed an almost perfect level of agreement, with a Cohen's kappa coefficient (κ) of 1.00. Among the samples analyzed, *CYP2C19*1/*1* was the most prevalent genotype (53.3%), followed by *CYP2C19*1/*2* (30.0%), *CYP2C19*1/*3* (10.0%), and *CYP2C19*2/*2* (6.7%). Notably, no *CYP2C19*17* variant was detected in this study population. Based on direct counting, the allele frequencies were 73.3% for *CYP2C19*1*, 21.7% for *CYP2C19*2*, and 5.0% for *CYP2C19*3*, while *CYP2C19*17* was not observed. No *CYP2C19*17*-positive clinical samples were identified in this cohort. Therefore, evaluation of *CYP2C19*17* detection was limited to synthetic homozygous mutant, heterozygous mutant, and wild-type control templates.

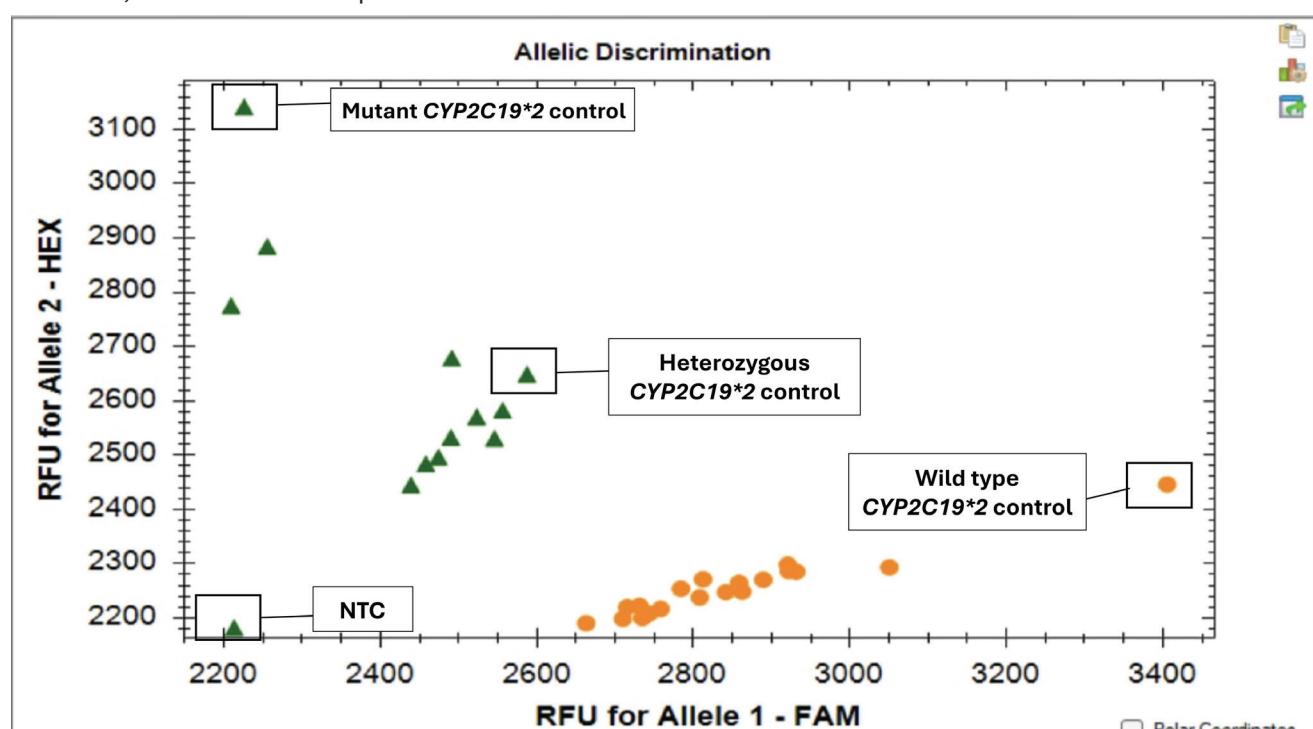


Figure 1. PACE-PCR genotyping of *CYP2C19*2*. Allelic discrimination plots show genotype distributions. Green dots represent samples carrying mutant alleles, including homozygous and heterozygous genotypes, while orange dots represent wild-type samples.

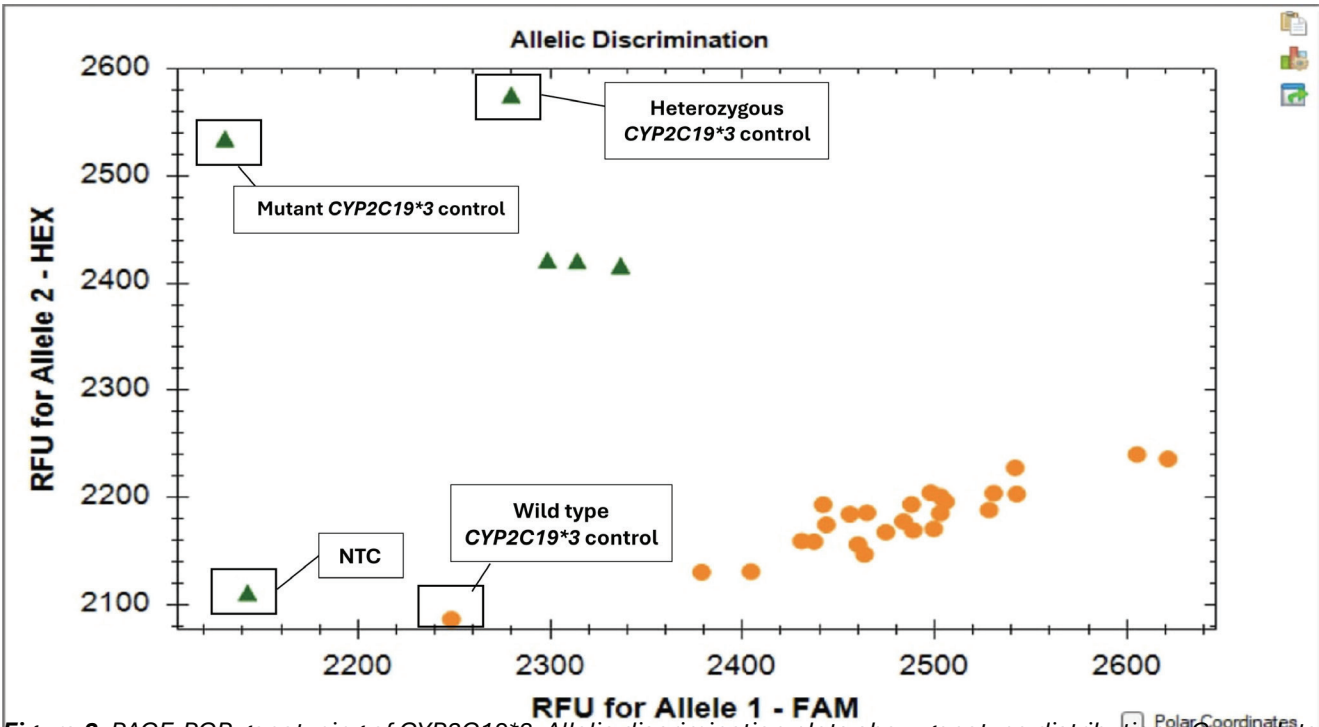


Figure 2. PACE-PCR genotyping of CYP2C19*3. Allelic discrimination plots show genotype distributions. Green dots represent samples carrying mutant alleles, including homozygous and heterozygous genotypes, while orange dots represent wild-type samples.

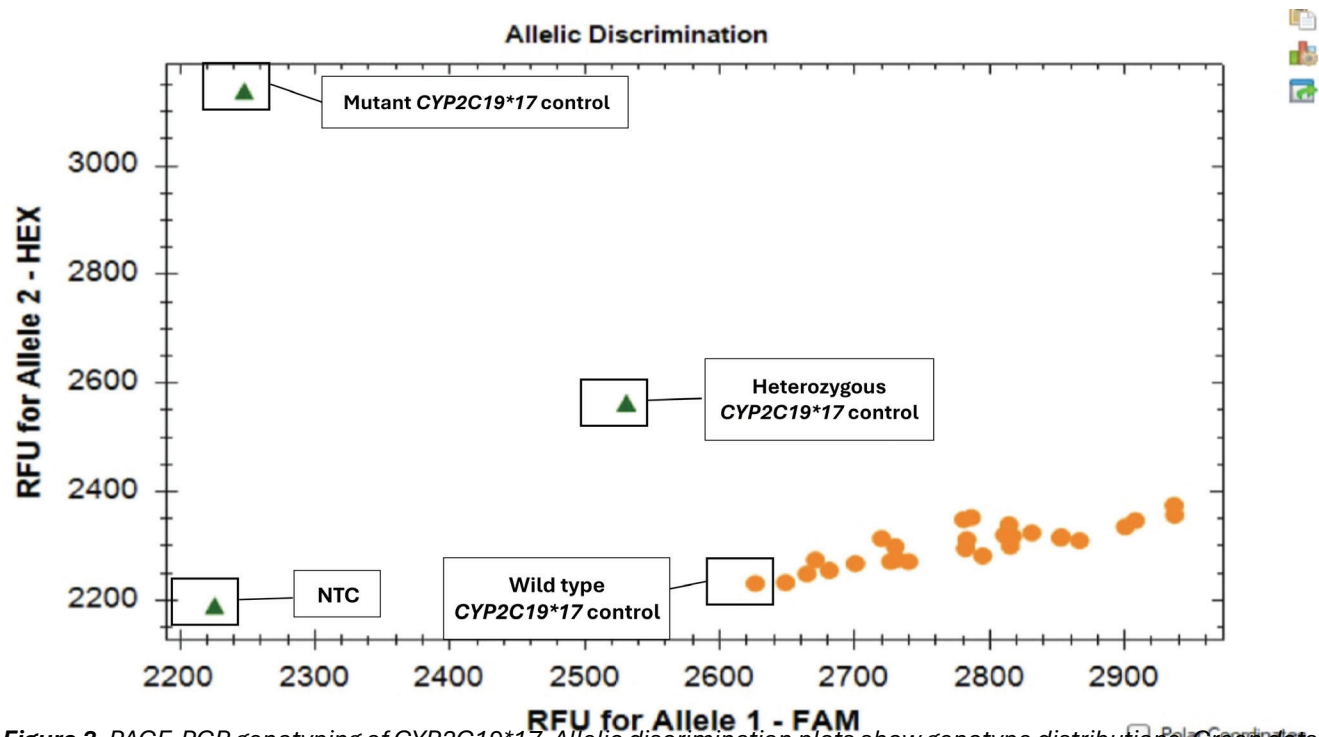


Figure 3. PACE-PCR genotyping of CYP2C19*17. Allelic discrimination plots show genotype distributions. Green dots represent samples carrying mutant alleles, including homozygous and heterozygous genotypes, while orange dots represent wild-type samples.

Table 2. Summary of *CYP2C19* genotyping results from *CYP2C19*2*, *CYP2C19*3* and *CYP2C19*17* allelic discrimination plots.

Sample ID	<i>CYP2C19</i> Diplotype	<i>CYP2C19*2</i> (G>A)	<i>CYP2C19*3</i> (G>A)	<i>CYP2C19*17</i> (C>T)
S001	*1/*2	A/G	G/G	C/C
S002	*2/*2	A/A	G/G	C/C
S003	*1/*2	A/G	G/G	C/C
S004	*1/*1	G/G	G/G	C/C
S65056	*1/*3	G/G	G/A	C/C
S65073	*1/*1	G/G	G/G	C/C
S65145	*1/*1	G/G	G/G	C/C
S65157	*1/*1	G/G	G/G	C/C
S65160	*1/*1	G/G	G/G	C/C
S66027	*1/*2	A/G	G/G	C/C
S66087	*1/*2	A/G	G/G	C/C
S66101	*1/*2	A/G	G/G	C/C
S66116	*1/*1	G/G	G/G	C/C
S66117	*1/*1	G/G	G/G	C/C
S66118	*1/*1	G/G	G/G	C/C
S66124	*1/*1	G/G	G/G	C/C
S66138	*1/*2	A/G	G/G	C/C
S66155	*2/*2	A/A	G/G	C/C
S66166	*1/*3	G/G	G/A	C/C
S67018	*1/*1	G/G	G/G	C/C
S67046	*1/*2	A/G	G/G	C/C
S67066	*1/*1	G/G	G/G	C/C
S67109	*1/*1	G/G	G/G	C/C
S67178	*1/*1	G/G	G/G	C/C
S67179	*1/*3	G/G	G/A	C/C
S67196	*1/*3	G/G	G/A	C/C
S68011	*1/*1	G/G	G/G	C/C
S68083	*1/*2	A/G	G/G	C/C
S68087	*1/*2	A/G	G/G	C/C
S68094	*1/*2	A/G	G/G	C/C

Discussion

This study successfully developed a *CYP2C19* genotyping assay based on the PACE technique and compared it against a commercially available TaqMan™ Drug Metabolism Genotyping Assay. The PACE assay presented perfect concordance (100%) with the commercial method, with a Cohen's kappa coefficient of 1.00. These findings confirm that the PACE assay provides highly accurate, reliable genotype calls for the *CYP2C19* variants and clear allelic discrimination. However, the current study presents only an initial proof-of-concept evaluation. Comprehensive analytical characteristics, including limit of detection, specificity, intra- and inter-assay precision, reproducibility across operators and instruments, and robustness under varying experimental conditions, were not fully investigated and should be addressed in future studies prior to routine clinical implementation. Together, without confirmation by sequencing-based methods, the current study should be considered as a comparative analytical evaluation rather than a complete method validation.

Compare PACE to other commercial real-time methods in terms of time-consuming and cost per assay sample. PCR hands-on time is the same, but reagent costs are USD 4.34 (135 Thai baht) per sample for PACE, which may be lower than those of other commercial real-time genotyping assays. This estimated assay cost represents only direct reagent consumable expenses. The calculation did not include labor costs, equipment acquisition or depreciation, maintenance, infrastructure, or other operational expenses. Therefore, the present analysis should not be interpreted as a formal cost-effectiveness evaluation. Nevertheless, the lower reagent cost observed for the PACE assay suggests potential economic feasibility for pharmacogenomics screening applications, particularly in resource-limited settings.

Using this new approach, the genotype and allele frequency distributions observed in this study are consistent with those reported in larger Thai populations. Previous studies have consistently shown that the wild-type allele *CYP2C19*1* has the highest frequency, typically ranging from approximately 70-73%, while *CYP2C19*2* is the most prevalent loss-of-function variant, with approximately 21-25% of the population carrying it, representing the major genetic determinant of the poor metabolizer phenotype.^{17,18} The predominance of the *CYP2C19*1/*1* genotype observed in this study (53.3%) is highly comparable to that reported by Nakhonsri et al. (48.82%), suggesting that approximately half of the Thai population exhibits extensive metabolizer (EM) phenotype.¹⁸ For intermediate metabolizers (IM) phenotype, *CYP2C19*1/*2* and *CYP2C19*1/*3* genotypes have been previously reported as 36.02% and 6.63%, respectively.¹⁸ Similarly, those were observed in our study as 30.0% and 10.0%,

respectively. For the poor metabolizer (PM) phenotype, although the allele frequency of *CYP2C19*3* in this study (5%) was slightly higher than that reported by Areesinpitak et al. (3%),¹⁷ it was comparable to the findings of Nakhonsri et al. (4.5%).¹⁸ Regional variation among study populations, such as differences between northeastern and northern Thai cohorts, may partly explain the observed variation in *CYP2C19*3* allele frequency across studies. However, we observed only *CYP2C19*2/*2* genotype (6.7%), while Nakhonsri et al. showed both *CYP2C19*2/*2* (6.16%) and *CYP2C19*2/*3* (2.37%).^{17,18} Since *CYP2C19*17*, the gain-of-function variant, is known to occur at a lower frequency in Asian populations, approximately 1.8-2% of Thai individuals, the absence of this allele in this study is unexpected. This discrepancy is likely attributable to the relatively small sample size (N=30), which reduces the probability of detecting this allele in smaller cohorts.^{17,18} Although complete concordance (100%) and $\kappa=1.00$ were observed between the PACE assay and the commercial TaqMan™ assay, these findings should be interpreted with caution due to the relatively small sample size and limited genotype diversity.

Therefore, this study should be considered as a preliminary analytical validation rather than a complete assessment of assay performance.

The observed genotype distribution in this study also highlights the potential clinical relevance of *CYP2C19* pharmacogenomics in the Thai population. The clinical relevance in translating *CYP2C19* genotype into predicted metabolizer phenotype for pharmacogenomics-guided therapy was shown in Table 3. Loss-of-function alleles (*2 and *3) of *CYP2C19* are associated with reduced enzymatic activity and impaired conversion of clopidogrel to its active metabolite, which may increase the risk of adverse cardiovascular events during antiplatelet therapy.²⁰ Additionally, these variants could affect the metabolism of proton pump inhibitors and some antidepressants.²¹ In contrast, the *CYP2C19*17* gain-of-function allele has been associated with increased enzyme activity, which may contribute to altered therapeutic response and reduced plasma drug concentrations. Thus, accurate *CYP2C19* genotyping may enable the implementation of personalized pharmacotherapy and precision medicine.

Future studies should include a larger sample size and a more genetically diverse population to further validate the analytical performance of this newly developed PACE assay, along with confirmation by a sequencing-based method. Expanding the panel to include additional clinically relevant or rare *CYP2C19* variants and evaluating reproducibility and inter-laboratory robustness are recommended to support the clinical implementation of this assay.

Table 3. *CYP2C19* genotype–phenotype associations and potential pharmacogenomic implications.¹⁹

<i>CYP2C19</i> genotype	Predicted phenotype	Expected enzyme activity	Potential clinical implication
*1/*1	Normal metabolizer (NM)	Normal	Expected standard drug response
*1/*2, *1/*3	Intermediate metabolizer (IM)	Reduced	Reduced clopidogrel activation; altered response to PPIs/ antidepressants
*2/*2, *2/*3, *3/*3	Poor metabolizer (PM)	Markedly reduced or absent	Increased risk of clopidogrel therapeutic failure and drug toxicity
*1/*17	Rapid metabolizer (RM)	Increased	Potential reduced drug exposure
*17/*17	Ultrarapid metabolizer (UM)	Significantly increased	Increased metabolism and potential therapeutic failure for some drugs

Note: Source: PharmGKB (<https://www.clinpgx.org/page/cyp2c19RefMaterials> (accessed on 29 May 2026))

Limitations

Although this study showed 100% concordance between the newly developed PACE and the commercial TaqMan™ genotyping assay, the PACE still has some limitations. Firstly, the sample size was small (N=30), which could limit the statistical robustness of concordance analysis and allele frequency estimation. Consequently, the observed 100% concordance and $\kappa=1.00$ may overestimate assay performance due to limited genetic variability within the study cohort. Secondly, although complete concordance between the PACE assay and the commercial TaqMan™ genotyping assay was observed, the comparator method was limited to real-time PCR genotyping without confirmation by a gold-standard sequencing-based approach, such as Sanger sequencing. Therefore, potential shared systematic errors between the two PCR-based methods cannot be completely excluded. Thirdly, the study evaluated only three *CYP2C19* variants (*2, *3, and *17), and other clinically relevant alleles reported in the PharmVar database (URL <https://www.pharmvar.org/>) were not assessed. Finally, no *CYP2C19**17-positive clinical samples were included in this sample set, preventing direct evaluation of assay sensitivity for this allele in patient specimens.

Therefore, the current study demonstrated the technical feasibility of detecting *CYP2C19**17 using synthetic control templates and is considered a preliminary study.

Conclusion

As an affordable alternative to commercial genotyping platforms, this study successfully developed a *CYP2C19* genotyping assay based on the PACE technique. Complete concordance with the TaqMan™ Drug Metabolism Genotyping Assay was observed with nearly perfect agreement ($\kappa=1.00$). The newly developed PACE assay showed promising preliminary analytical performance and identified the *CYP2C19**2, *3, and *17 variants. This assay demonstrated lower direct reagent costs and may represent a potentially affordable alternative for *CYP2C19* genotyping.

However, further comprehensive analytical and clinical validation studies, together with a comprehensive economic evaluation, are required before conclusions regarding cost-effectiveness can be established and implemented in routine pharmacogenomic testing.

Ethical approval

This research was granted exemption from ethical review by the Research Ethics Committee of the Faculty of Associated Medical Sciences, Chiang Mai University (Research Project Code AMSEC-67EM-014; date of approval: 19 March 2024).

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Conflict of interest

The authors declare no conflict of interest.

CRediT authorship contribution statement

Chonticha Sirikul: conceptualization, methodology, data curation, formal analysis, writing: original draft, funding acquisition; **Auttachai Saejia:** methodology, data analysis guidance, sample preparation; **Nampeung Anukul:** conceptualization, supervision, validation, writing: original draft, review and editing.

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