Archives of the Balkan Medical Union
vol. 59, no. 4, pp. 343-351
December 2024
Copyright © 2024 Balkan Medical Union

ORIGINAL PAPER

OPTIMIZED T-ARMS PCR FOR DETECTING CYP2C19*2 POLYMORPHISM IN THE MEKONG DELTA KHMER POPULATION

Thi P. A. NGUYEN[1], Anh N. B. TRAN[2], Trang P. T. NGUYEN[1], Nguyen T. T. CAO[3], Khang T. DO[1], Khuong T. T. TRAN[2]✉

[1] Department of Molecular Biology, Institute of Food and Biotechnology – Can Tho University, Vietnam
[2] Stem Cell Laboratory, Institue of Food and Biotechnology – Can Tho University, Vietnam
[3] Department of Biology – Genetics, Faculty of Basic Sciences – Can Tho University of Medicine and Pharmacy, Vietnam

Received 14th Sept 2024, Accepted 21st Oct 2024
https://doi.org/10.31688/ABMU.2024.59.4.03

Abstract

Introduction. The CYP2C19*2 single nucleotide polymorphism (SNP) significantly alters the metabolism of clopidogrel, a key antiplatelet drug prescribed to reduce stroke risk, thereby increasing the risk of cardiac complications in affected individuals.
The objective of the study was to identify this polymorphism, for minimizing adverse drug reactions and enabling timely interventions. While various methods for rapid SNP detection are available, choosing the most appropriate technique can be challenging. Tetra ARMS PCR is a widely used method for SNP detection that we aimed to optimize, specifically to detect CYP2C19*2 polymorphism.
Material and methods. The PCR mixture and thermal cycling time points were examined and modified to improve PCR performance on detecting CYP2C19. Isopropanol was used to extract the DNA from the oral mucosal cell samples taken from 35 randomly selected Khmer volunteers, regardless of their age, gender, or health status.

Résumé

PCR T-ARMS optimisée pour la détection du polymorphisme CYP2C19*2 dans la population Khmère du Delta du Mékong

Introduction. Le polymorphisme nucléotidique simple (SNP) CYP2C19*2 modifie considérablement le métabolisme du clopidogrel, un antiplaquettaire clé prescrit pour réduire le risque d'accident vasculaire cérébral, augmentant ainsi le risque de complications cardiaques chez les personnes concernées.
L'objectif de l'étude était d'identifier ce polymorphisme, pour minimiser les réactions indésirables et permettre des interventions rapides. Bien que diverses méthodes de détection rapide des SNP soient disponibles, le choix de la technique la plus appropriée peut être difficile. La PCR Tetra ARMS est une méthode largement utilisée pour la détection des SNP que nous avons cherché à optimiser, en particulier pour détecter le polymorphisme CYP2C19*2 dans cette étude.
Matériel et méthodes. Le mélange de PCR et les points temporels du cycle thermique ont été examinés

✉ Address for correspondence: Thi Thanh Khuong TRAN
Stem Cell Laboratory, Institue of Food and Biotechnology – Can Tho University, Vietnam
Email: tttkhuong@ctu.edu.vn; Phone +84-901002285

Results. The outcomes demonstrated that, under modified circumstances, optimal amplification happened after 35 cycles with an annealing temperature of 56°C and 5% dimethyl sulfoxide (DMSO). The detected genotypes were *1/*1 (21 out of 35) and *1/*2 (14 out of 35), with no *2/2 identified.
Conclusions. Our research aligns with previous studies, showing T-ARMS PCR as an effective, cost-efficient, and rapid way for screening CYP2C19*2 polymorphisms. These findings lay the foundation for future studies on stroke intervention among Mekong Delta Khmer populations.

Keywords: clopidogrel, CYP2C19*2, Khmer, polymorphism, T-ARMS PCR.

List of abbreviations:
CYP2C19 – cytochrome P450 2C19
SNP – single nucleotide polymorphism
PCR – polymerase chain reaction
T-ARMS – Tetra-primer Amplification Refractory Mutation System
DNA – deoxyribonucleic acid
dNTP – deoxynucleotide triphosphates
UV – ultraviolet

Les résultats ont démontré que, dans des circonstances modifiées, l'amplification optimale s'est produite après 35 cycles avec une température de recuit de 56°C et 5% de diméthyl sulfoxyde. Les génotypes détectés étaient *1/*1 (21 sur 35) et *1/*2 (14 sur 35), sans *2/2 identifié. Notre recherche s'aligne sur des études précédentes, montrant que la PCR T-ARMS est un moyen efficace, rentable et rapide de dépister les polymorphismes du CYP2C19*2.
Conclusions. Ces résultats jettent les bases de futures études sur l'intervention contre les accidents vasculaires cérébraux parmi les populations khmères du delta du Mékong.

Mots-clés: clopidogrel, CYP2C19*2, Khmer, polymorphisme, PCR T-ARMS.

Introduction

Under the development of technical and information technology, the selection and design of a polymorphic recognition method customized to user needs can be done easily. Genetic variations in the drug-metabolizing enzyme gene groups have been one of the key targets in most research for more than six decades, specifically the variations resulting from point mutations of a gene/gene cluster. For certain genes, these associations have been verified and have made great contributions to medicine. The cytochrome P450 enzyme family, comprising CYP1A2, CYP3A4/5, and notably, CYP2C19, is the major groups of enzymes that contribute to the metabolism of clopidogrel[1]. Within these gene clusters, single nucleotide polymorphisms (SNPs) are prevalent, exhibiting various mutations such as nucleotide additions, deletions, or repeats[2,3,4]. These mutations give rise to phenotypic manifestations ranging from loss of function to enhanced gene activity, categorized into poor drug metabolism, intermediate drug metabolism, normal drug metabolism, and ultrarapid drug metabolism[5]. The enzymatic activity of CYP450 enzymes is influenced by both endogenous and exogenous factors, impacting the body's drug metabolism and absorption rates, as evidenced by SNP polymorphisms[6].

Among these enzymes, CYP2C19 is particularly noteworthy, catalyzing the conversion of 15% of clopidogrel in the liver and small intestine into its active form[7]. Notably, CYP2C19*2 represents a significant variant within the CYP2C19 gene. The polymorphism of CYP2C19 variant*2 (rs4244285) caused by the substitution point mutation of G681A has a significant impact on the patient's ability to tolerate and potentially compromise the drug's efficacy. The impact of CYP2C19*2 polymorphism is contingent upon the specific variants harbored by individual patients[8-10]. Recent research conducted among the Vietnamese population indicated that the prevalence of the CYP2C192 genotype was 20.5% and 26.4%[11,12]. Despite significant similarities in the proportion of phenotypes containing SNPs between these studies, research on the CYP2C192 variant in Vietnam has predominantly focused on the Kinh ethnic group, with limited data available on ethnic minorities such as the Khmer population. Although various methods exist for surveying these genetic variations, many are associated with significant time and cost requirements.

The Tetra-primer ARMS-PCR technique emerges as a promising method for detecting SNP polymorphisms, offering simplicity and high accuracy. However, its adoption is still constrained in Vietnam, necessitating further exploration and optimization for efficient screening of CYP2C19*2 polymorphism.

Table 1. Primers sequences used in T-ARMS PCR in this research [14]

Primer	*5'-3'*	*Expected band size (bp)*
Outer Forward	TACAACCAGAGCTTGGCATATT	311 bp
Outer Reverse	TCAATGAATCACAAATACGCAA	
Inner Forward (allele G)	CCCACTATCATTGATTATTTCACG	219 bp
Inner Reverse (allele A)	GGTTTTTAAGTAATTTGTTATGGGTTACT	144 bp

Table 2. Parameters changes that were made in this research to optimize the reaction

Name	*Parameters*	*Degrees*			
Heat cycle	Numbers of cycle	30 cycles	32 cycles	35 cycles	40 cycles
	Annealing time	20 seconds	30 seconds	45 seconds	1 minute
Reaction components	$MgCl_2$ concentration	2.0 mM	3.0 mM	3.5 mM	4.0 mM
	Use of DMSO	Yes		No	

The primary objective of the study was to optimize and evaluate the T-ARMS-PCR technique for the detection of CYP2C19*2 polymorphism in the Khmer ethnic population of the Mekong Delta region, Vietnam. Additionally, the study aimed to determine the prevalence of the CYP2C19*2 genotype in this population, addressing the current gap in genetic data of this group. By optimizing the T-ARMS-PCR method, the study aimed to provide a reliable, cost-effective approach for SNP detection, which can be used to improve pharmacogenetic testing and contribute to personalized medicine in the region.

Material and methods

Chemicals

Agarose powder (Sigma); DNA 100bp HyperLadder™ (Meridian Bioscience); EDTA 0.1M solution; Isopropanol; Loading buffer 6X; Master Mix 10X (ThermoFisher); Platinium Taq polymerase (Invitrogen); Proteinase K solution; Safeview dye (Meridian Bioscience); Saline solution 0.9%; SDS 0.5%; Tris-EDTA solution (0.1X); T-ARMS PCR primers for rs4244285 gene region; TBE 10X (Sigma).

Materials

A cross-sectional descriptive study was conducted on 35 Khmer students who voluntarily enrolled from May 2023 to April 2024 in Can Tho University, Vietnam. Samples (buccal cells) were collected from volunteers who are physically healthy at the samples-collecting period. The volunteers were all introduced to the research purposes and had given their signed consents. The study was approved by the Ethics Committee (approval number BQ2023-02/VCNSHTP on April 6th, 2023), confirming compliance with ethical principles, including the protection of participants' rights, privacy, and confidentiality.

Sample Collection and DNA Extraction

Oral mucosa samples were collected from 35 volunteers following the method described by Aidar & Liner[13] with modifications. The samples were resuspended in 0.9% saline solution, iced and transported to the Molecular Biology Laboratory at Can Tho University. Upon arrival, the samples were centrifuged, and the resulting pellets were subjected to DNA extraction using Cell Lysis Buffer (Tris-HCl, SDS, EDTA, and NaCl) and Proteinase K activated enzyme solution (ABT, Vietnam). DNA purity and concentration were assessed with NanoDrop One meter (Thermo Scientific, USA) and visualized on a 0.8% agarose gel.

T-ARMS PCR Optimization

Optimization of the Tetra-primers ARMS PCR protocol was performed to enhance its efficiency. Modifications to the thermal cycling conditions and mixture components were based on the method reported[14] (Table 1). The original PCR settings involved an initiation at 95°C for 5 minutes, followed by 32 cycles of denaturation at 95°C (30 seconds), annealing at 56°C (30 seconds), extension at 72°C for 20 seconds, and lastly storage at 4°C.

Various parameters were assessed to determine optimal conditions for each step of the PCR protocol. Denaturing, annealing, and extension times were evaluated at different intervals, ranging from 20 seconds to a minute. Additionally, the concentrations of $MgCl_2$ and the presence of dimethyl sulfoxide (DMSO) were varied to optimize amplification efficiency. $MgCl_2$ concentrations were analyzed at 2 mM, 3 mM, and 4 mM, while DMSO was evaluated

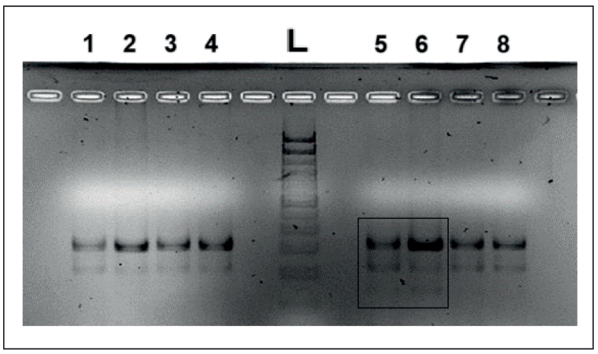


Figure 1. CYP2C19*2 thermal cycles optimization results on 2% agarose gel. The order of the wells are as follows: (1 – 2) PCR results of 30 thermal cycles; (3 – 4) PCR results of 32 thermal cycles; (L) DNA ladder 1000 bp; (5 – 6) PCR results of 35 thermal cycles; (7 – 8) PCR results of 40 thermal cycles. All amplifications were performed with a singular DNA extracted sample.

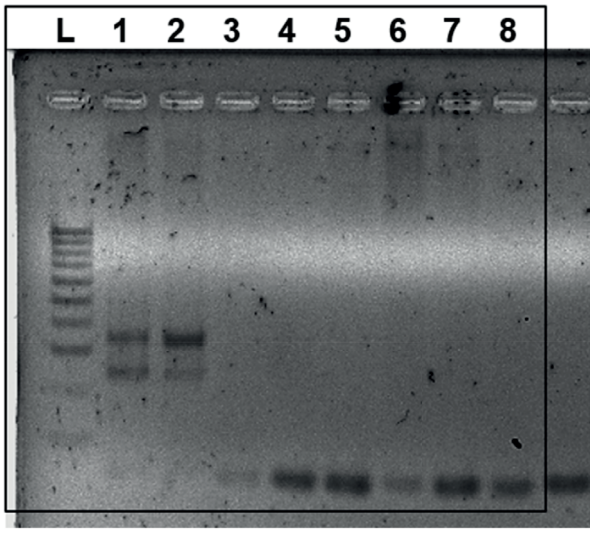


Figure 2. CYP2C19*2 $MgCl_2$ concentration PCR optimization results on 2% agarose gel. The order of the wells are as follows: (L) DNA ladder 1000 bp; (1 – 2) PCR results of 2.0 mM concentration; (3 – 4) PCR results of 3.0 mM concentration; (5 – 6) PCR results of 3.5 mM concentration; (7 – 8) PCR results of 4.0 mM concentration. All amplifications were performed with a singular DNA extracted sample for 35 cycles, with DMSO.

at a concentration of 5%. The resulting amplification products were visualized on a 2% gel containing Safeview dye and visualized under UV light.

Statistical analysis

All statistical probabilities calculations used in this study were conducted on Minitab software (version 18.1) with a confidence level of 95% (95% CI) to evaluate the genotype frequency of Vietnam Khmer ethnicity population.

Results

Result of T-ARMS PCR amplification with modified conditions

The extracted DNA was evaluated for its purity and its concentration to select qualified samples to perform PCR reactions. The closer the purity of the sample was to the DNA extraction standard (A_{260}/A_{280} = 1.8 – 2.0; concentration ≥ 30 ng/µl), the clearer the bands. In total, we had collected 35 qualified DNA samples with A260/A280 index from 1.4 – 2.0 and with concentration ≥16 ng/µl.

Achieving the highest efficiency requires the researcher to re-evaluate all factors in the mixture and its thermal cycle, making the optimization phase laborious. The thermal cycle and reaction composition proposed in the study of Rupoma[14] were repeated before conducting the necessary investigation. The amplification reaction based on the original study gave blurry results, which were difficult to observe even on agarose gels containing a higher percentage than suggested. After investigation, the thermal cycle for the rs4244285 region was modified to ensure clear and accurate amplification of CYP2C19 polymorphisms. More specifically, the optimal annealing temperature for all three primer pairs was achieved at 56°C with a total of 35 cycles. This change can occur because the input samples were not purified, so the reaction needs more time to completely break down the bonds between the DNA strands and the binding proteins to release the two separate DNA strands. These changes need to happen at the same time, changing only one element will not give the desired amplification results; if the coupling time is too long, it may inhibit the Taq polymerase binding to the template strand; leading to amplification failure. In addition, T-ARMS PCR reaction was best visualized at a final 2 mM $MgCl_2$ concentration in the mixture along with DMSO 5% present. The final reaction mixture (25 µl) included: 2.5 µl Taq mix PCR 10X buffer (Invitrogen, USA), T-ARMS primers (10 pmol/µl) in equal volume, 3 µl sample DNA, 1U Taq polymerase, 10 mM dNTP mix (Invitrogen, USA), BiH_2O with DMSO 5%. The PCR product of the outer primer pair was used as an internal control for each reaction. This meant that we only report results showing the 311-bp fragment on the 2% agarose gel. This helps to eliminate false positive bands.

Based on the results of the 2% agarose gel electrophoresis (Figure 1), representative DNA samples after performing the T-ARMS-PCR reaction all yielded bands as reported[14]. The expected sizes of the products were 311 bp, 219 bp and 144 bp, respectively. Among them, the 2-band samples, with product

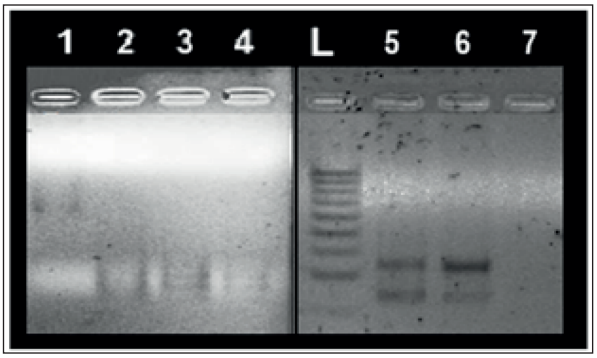


Figure 3. Assessment of DMSO 5% role in aiding CYP2C19*2 PCR amplifications results on 2% agarose gel.
The orders are as follows: (1 – 2 – 3) are amplification without presence of DMSO; (L) DNA ladder 1000 bp; (5 – 6) are amplification with presence of DMSO. Well 7 is the negative control – no DNA presence.
All amplifications were performed with a singular DNA extracted sample for 35 cycles.

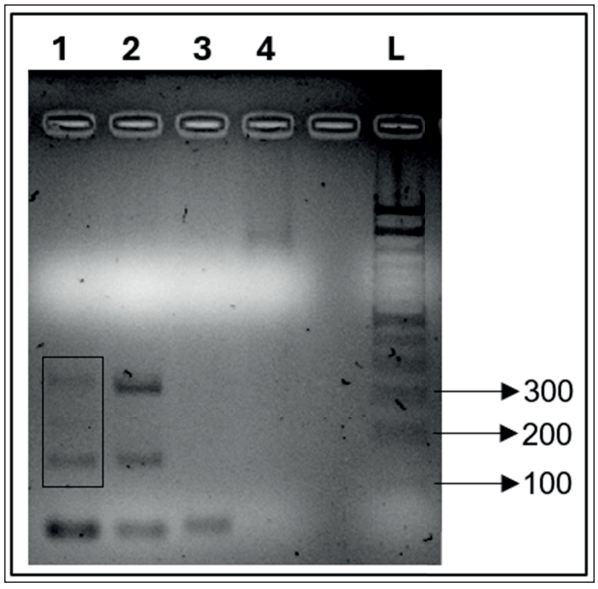


Figure 4. CYP2C19*2 PCR annealing temperature optimization results on 2% agarose gel. The orders are as follows: (1) is amplification achieved at 45 seconds annealing time; (2) is amplification achieved at 1 minute annealing time; (3) is amplification achieved at 30 seconds annealing time; (4) is amplification achieved at 20 seconds annealing time; (L) DNA ladder 1000 bp. All amplifications were performed with a singular DNA extracted sample for 35 cycles, with DMSO.

sizes roughly of 311 bp and 219 bp, were samples carrying the wild genotype (*1/*1). The 3-band samples were samples with heterozygous genotype (*1/*2). The 2-band samples with expected product sizes of 311 bp and 144 bp were homozygous samples carrying the mutated genotype (*2/*2) (Figure 2).

Result of detecting allele frequency and genotype frequency on CYP2C19*2

Among the 35 Khmer samples analyzed, 21 samples had the wildtype genotype (*1/*1) and 14 samples had the genotype (*1/*2). The genotype (*2/*2) did not appear among the 35 samples we had gathered. The presence of CYP2C19*2/*2 genotype is proportionately correlated to the sample numbers, owed to its rarity among the CYP2C19*2 genotypes. The most recent multi-nationality study conducted in Vietnam (2019) focusing on the northern region of the country with five different nationalities reported the presence of *2/*2 genotype as such: Kinh people (3 out of 100), Tay people (13 out of 103), Muong people (11 out of 99). Our finding is also consistent with similar research among other Asian populations, i.e. the genotype frequency of CYP2C19*2/2* in 6,686 volunteers of the Hakka ethnic population, southern China was 9.75% (652 out of 6686). CYP2C19*2/*2 genotype was also reported at a similarly low rate 9.9% (93/939, p = 0.016) in an upscale hospital-based co-hort study in Meizhou, China (2023)[15].

The prevalence of mutant CYP2C19 alleles shows ethnic differences, with significant differences existing between Caucasian and Asian groups. The frequency of CYP2C19*2 was recorded at a range from 2% to 7% in Caucasians and 14% to 25% in Asians[16-18]. This shows that in this study, the percentage of CYP2C19*2 allele is compatible with previous studies. Based on Table 3, the result suggested that the percentage of the CYP2C19*2 allele in the Khmer ethnic group in this study is 20%.

Discussion

Extracting DNA from oral mucosa is a quick, convenient, controllable, and mostly painless method for sample-givers. Currently, this is the most popular form of sample collection, both in research and clinical examination[19]. DNA degradation during the extraction process is inevitable to some extent due to the nature of the buffers, chemical and physical effect throughout the extraction. In this research, we use DNA extraction via isopropanol. This method limits exposure to toxic chemicals (i.e., chloroform) but has certain disadvantages in incubating time and sample quality when compared to other methods[13,20]. To tackle this problem, we suggest using the presence of a successfully amplified housekeeping gene on the same chromosome as a control.

The specificity of a PCR reaction depends on factors such as the ratio between outer and inner primer pairs, $MgCl_2$ concentration, as well as other factors to limit unwanted by-products. More specifically,

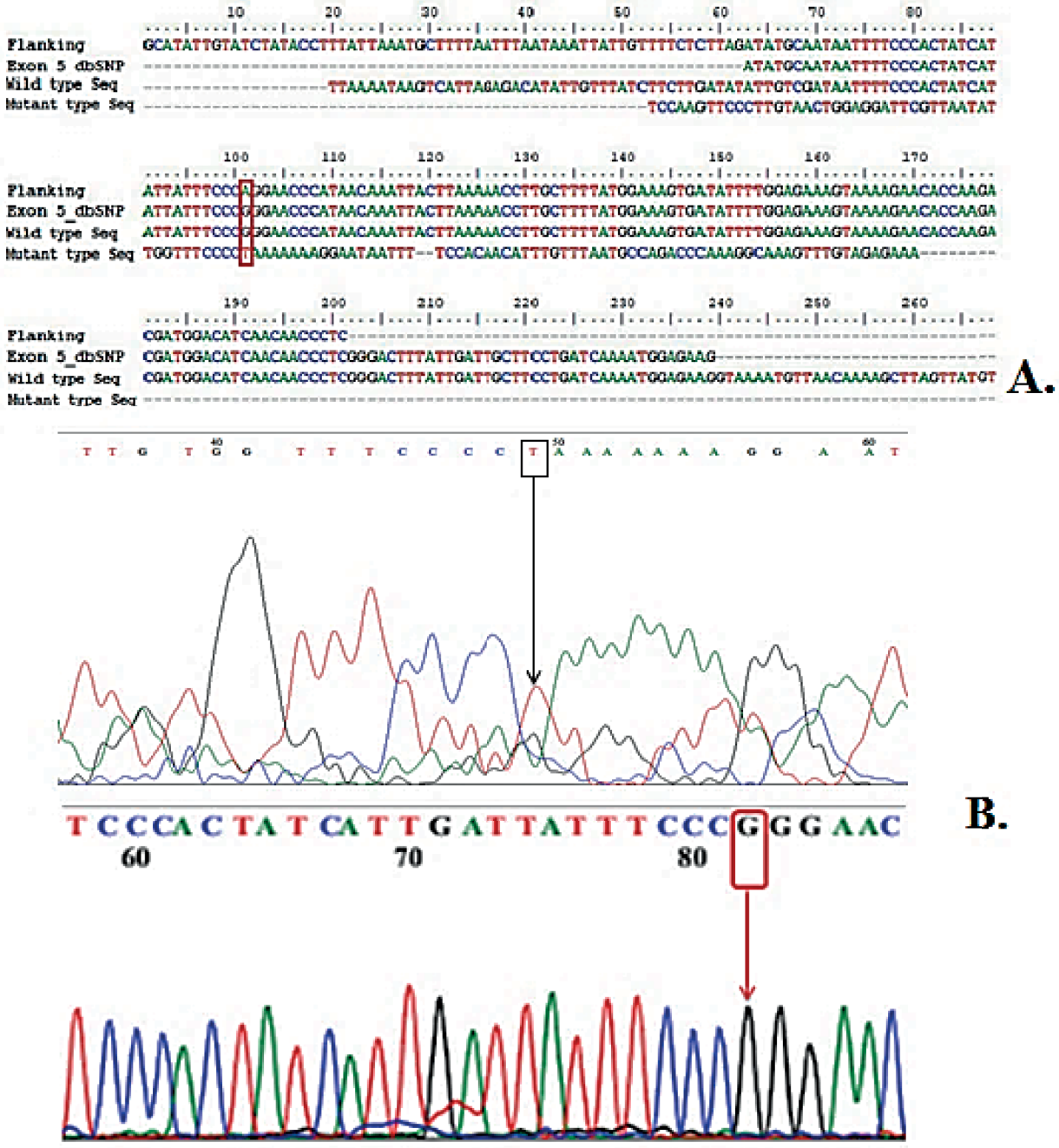


Figure 5. (A) CYP2C19*2 polymorphism Sequence Alignment of amplified T-ARMS PCR Sanger sequencing results. The alignment was as follows: "Flanking" sequence represented the CYP2C19*2 SNP-carrier gene region on exon 5, chromosome 10. "Exon 5_dbSNP" represented the no-mutation CYP2C19*2 gene region on exon 5, chromosome 10. "Outer primer" represented the sequence amplified by T-ARMS PCR outer forward primer. "IR primer" represented the sequence amplified by T-ARMS PCR inner reverse primer, which is the SNP (G>A) detecting strand. The flanking and Exon 5 sequences were obtained through NCBI database (last accessed on 22/06/2024). (B) Sequencing results showing SNP positions in amplified product of a heterozygote sample. The upper sequence showed the G mutation whereas, in the same codon, no mutation was presented (under part).

they are key factors that play a role in determining the correct pairing and formation of heterozygous bands (GA – *1/*2) or homozygous recessive bands (AA – *2/*2). In this study, the ratio of 2:1 and 1:1 for the outer – inner pair has no significant effect on the amplification results. At ratio levels less than 1:1, primer pairs that amplify 219 bp and 144 bp bands cannot be observed on agarose gels. Changing the $MgCl_2$ concentration will enhance or limit the ability of Taq polymerase enzyme depending on the types of

Table 3. Allele frequency and genotype frequency on CYP2C19*2 in Khmer people

	*Sample size (n^1/n) **	*Frequency (%)*	
Genotype	*1/*1 (21/35)	60%	95% CI** [42,9 – 77,1]
	*1/*2 (14/35)	40%	95% CI** [22,9 – 57,1]
	*2/*2 (no data)	(no data)	(no data)
Allele type	*1	80%	95% CI** [70 – 88,6]
	*2	20%	95% CI** [11,4 – 30]

Note: *The number of times each genotype (n1) appears over the sum (n).
** The data in the problem are calculated within the confidence range of 95%

Table 4. Allele frequency of CYP2C19*2 on Khmer people compared with previous results on multiple ethnicities

	*Allele CYP2C19*2 (%)*	*Sample size*	*References*
Khmer	20%	35	Our report
Kinh	26.4%	165	[16]
Kinh	24%	90	[27]
Kinh*	30.4%	140	[29]
Kinh	20.5%	100	[28]
Tay	38.3%	103	[28]
Muong	33.8%	99	[28]
H'Mong	53.75%	40	[28]
Nung	43.9%	33	[28]

amplification that is being used[21-23] At $MgCl_2$ concentrations higher than 2 mM, the CYP2C19 T-ARMS amplification band becomes lighter.

DMSO is a compound with high polarity, high dielectric constant and has been utilized extensively in chemical and biological research. It has been demonstrated to have the ability to influence numerous molecular processes, notably replication, regulation of gene expression, recombination, and PCR in vitro. DMSO, glycerol or betaine are additives that have been shown to support amplification reactions of gene regions with high GC% as well as the ability to reduce the final temperature for the annealing stage (reducing 0.75°C for every 2% DMSO of the total reaction volume)[12,18,23,24]. Each additive has its role in different PCR products. Some additives work better in specific PCR reactions which fail for others. When used in PCR, DMSO binds to the genetic material at the cytosine site and changes its form, thereby making the DNA more vulnerable to heat denaturation. Since most primers are rich in G-C content, DMSO helps primers anneal to the template indirectly, which improves amplification. This is particularly beneficial for templates that include a considerable amount of GC since the stronger hydrogen bonds make it harder to denaturize the template and encourage the formation of intermolecular secondary structures, which might interfere with primer annealing. The percentage of DMSO investigated in this study showed the best result at 5% concentration. Interestingly, higher DMSO concentrations have been shown to be effective in amplifying genomic regions rich in GC% content (higher than 60-70%)[25].

T-ARMS-PCR is considered a method with immense potential for investigating polymorphisms in different gene regions. The variable distribution of CYP2C19*2 alleles may contribute to pharmaceutical improvement, as well as clinical trials on related diseases. In patients treated with clopidogrel, it has been shown that carriers of the CYP2C19*2 mutation may have risks of myocardial infarction, ischemic stroke, etc. compared with people who do not carry the gene[5,26,27]. For that reason, performing a screening for CYP2C19*2 allele is necessary. Not only can it aid the treatment-providers to carefully consider the underlying genetic factors, evaluate the proper drug dosage, but also can help improve treatment efficiency and shorten the spectrum while limiting drug side effects. Table 4 shows that the result of CYP2C19*2 SNP-carrier's percentages in Khmer people presented similarity to earlier reports[28]. For instance, it is the same as the allelic percentage surveyed on Kinh people, 26.4%, 24% and 20.5%[27-30]. Interestingly, when compared with the proportion of Kinh people in the

group with underlying heart diseases, the percentages of gene-carrier went up (30.4%)[29]. On a broader scale, research had been conducted on many Asian countries and correlation was found[11,18,19,30]. Thereby, it can be suggested that the ratio of CYP2C19*2 allele-carrier reflects the relationship between mutant alleles and patients.

With 86.2% of the total population, the Kinh ethnic group is the largest in Vietnam and is regarded to be representative of the country's population. Although Kinh people are found in all provinces, they tend to reside in the Delta and big cities. Our research mainly focused on the minority present in the far southern part of Vietnam, more specifically the Khmer people. Among the other 53 ethnic minorities, the Khmer people make up a total of 0.0137% of the whole population (based on Vietnam's population report in 2019, with 1.3 million per 95.78 million people). Khmer people lead a quiet but colorful, heavily tradition-influenced life which leads to their resistance to accepting modern medical screenings and testing. Obtaining consents were, no doubt, that our team had to overcome. When comparing the frequency percentage of CYP2C19*2 allele in the Khmer ethnic group and other ethnic minorities such as Tay people, Muong people, Hmong people, and Nung people, the numbers representing the Khmer ethnic group are significantly lower than that of other ethnic minorities. It can be concluded that the allelic frequency of CYP2C19*2 could have differed depending on the patient's ethnicity[19, 26-30].

Nevertheless, the study data have certainly provided new insight into the allelic frequency of CYP2C19*2 polymorphisms in Vietnamese Khmer community. However, this research had several limitations in terms of methodologies and sample size. The number of volunteers is quite small (35 volunteers) with no detection of the (*2/*2) genotype, therefore research needs further explorations with broader sample size. We recommend expanding the research with a larger sample size to increase accuracy (sample size ≥ 1000) for future aspects of CYP2C19*2 polymorphism. Furthermore, it is essential that the correlation between CYP2C19*2 polymorphisms and clinical manifestations of related diseases, such as cardiovascular disease, be studied thoroughly.

Conclusions

The allelic frequency of CYP2C19 polymorphism *2 in Vietnamese Khmer people was 20%, which is similar to other previous studies on the subject. Overall, the data of this research had provided new information on polymorphisms of the CYP2C19*2 gene region in the Vietnamese Khmer ethnic group. The research partially contributes to improving drug therapies in Vietnam and can be beneficial in terms of reducing drug side effects, as well as increasing treatment outcomes for clopidogrel-treated patients.

Authors' Contributions

Conceptualization: K.T.T.T. and N.T.T.C.; methodology: T.P.A.N.; software: A.N.B.T. and T.P.T.N.; validation: K.T.D.; formal analysis: K.T.T.T.; investigation: T.P.A.N.; resources: T.P.T.N.; data curation: N.T.T.C.; writing-original draft preparation: A.N.B.T.; writing-reviewing and editing: A.N.B.T. and N.T.T.C.; visualization: K.T.D.; project administration: K.T.T.T. and T.P.T.N. All authors have read and agreed with the final version of the article.

Compliance with Ethics Requirements:

"The authors declare no conflict of interest regarding this article"

"The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study."

Acknowledgments

"We thank the staff and facilities at the Institute of Food and Biotechnology, Can Tho University, for their crucial support in conducting this study. Funding for this study was provided by the Can Tho University, Code: T2023-181."

References

1. Dorji P W, Tshering G, Na-Bangchang K. CYP2C9, CYP2C19, CYP2D6 and CYP3A5 polymorphisms in South-East and East Asian populations: A systematic review. *Journal of Clinical Pharmacy and Therapeutics*. 2019;44(4):508-524.
2. Sienkiewicz-Oleszkiewicz B, Wiela-Hojeska A. CYP2C19 polymorphism in relation to the pharmacotherapy optimization of commonly used drugs. *Die Pharmazie-An International Journal of Pharmaceutical Sciences* 2018;73(11):619-624.
3. Yusmiati Y, Muliaty D. The pharmacogenetics of cytochrome P450 2C19 enzymes-effects on clopidogrel and proton pump inhibitors. *The Indonesian Biomedical Journal*. 2014;6(1):33-44
4. Biswas M. Global distribution of CYP2C19 risk phenotypes affecting safety and effectiveness of medications. *The Pharmacogenomics Journal* 2021;21(2):190-199.
5. Giantini A, Timan IS, Listiyaningsih E, et al. Comparison of light transmission aggregometry and in detecting clopidogrel resistance and factors affecting clopidogrel resistance in AMI-EST patients undergoing percutaneous coronary intervention: a cross-sectional study. *The Indonesian Biomedical Journal* 2021;13(2):163-9.
6. McDermott J H, Leach M, Sen D, et al. The role of CYP2C19 genotyping to guide antiplatelet therapy following ischemic

stroke or transient ischemic attack. *Expert Review of Clinical Pharmacology* 2022;15(7):811-825.
7. Yuan XW, Yuan SY, Wu GX, Wu ZX, Guan ZY. Genetic polymorphism of clopidogrel metabolism related gene CYP2C19 gene in Chinese from Foshan area of Guangdong Province. *Hematology* 2022;27(1):1056–61.
8. Mao L, Jian C, Changzhi L, et al. Cytochrome CYP2C19 polymorphism and risk of adverse clinical events in clopidogrel-treated patients: A meta-analysis based on 23,035 subjects. *Archives of Cardiovascular Diseases* 2013;106(10):517–27.
9. Montazid MS, Sajib AA, Hasan KN, et al. Multiplex allele-specific detection of clinically important CYP2C19 variants associated with clopidogrel metabolism in a Bangladeshi population sample. *Meta Gene* 2021;27(100830):100830.
10. Xie J, Pan T, Luo W, Zhang S, Fang Y, Xu Z. CYP2C19 *2/*2 genotype is a risk factor for multi-site arteriosclerosis: A hospital-based cohort study. *International Journal of General Medicine* 2023;16:5139–46.
11. Alrajeh KY, Roman YM. The frequency of major CYP2C19 genetic polymorphisms in women of Asian, native Hawaiian and pacific islander subgroups. *Personalized Medicine* 2022;19(4):327–39.
12. Wang Z, Ma R, Li X, et al. Clinical efficacy of clopidogrel and ticagrelor in patients undergoing off-pump coronary artery bypass grafting: a retrospective cohort study. *International Journal of Surgery* 2024;110(6):3450–60.
13. Aidar M, Line SRP. A simple and cost-effective protocol for DNA isolation from buccal epithelial cells. *Brazilian Dental Journal* 2007;18(2):148–52.
14. Rupoma AA. The SNP CYP2C19* 2 (rs4244285) genotyping using tetra primer arms PCR method [Dr. dissertation]. Bangladesh: Brac University; 2020.
15. Medrano RFV, de Oliveira CA. Guidelines for the tetra-primer ARMS–PCR technique development. *Molecular Biotechnology* 2014;56(7): 599-608.
16. Mesrian TH, Mojtabavi NM, Rahgozar S, Rasa SMM, Vallian S. Modified tetra-primer ARMS PCR as a single-nucleotide polymorphism genotyping tool. *Genetic Testing and Molecular Biomarkers* 2015;19(3):156–61.
17. Luo H, Poland R, Lin K, Wan Y. Genetic polymorphism of cytochrome P450 2C19 in Mexican Americans: A cross-ethnic comparative study. *Clinical Pharmacology & Therapeutics* 2006;80(1):33–40.
18. Chen L, Qin S, Xie J, et al. Genetic polymorphism analysis of CYP2C19 in Chinese Han populations from different geographic areas of mainland China. *Pharmacogenomics.* 2008;9(6): 691-702.
19. Kurose K, Sugiyama E, Saito Y. Population differences in major functional polymorphisms of pharmacokinetics/pharmacodynamics-related genes in eastern Asians and Europeans: Implications in the clinical trials for novel drug development. *Drug Metabolism and Pharmacokinetics* 2012;27(1):9–54.
20. Adamowicz MS, Stasulli DM, Sobestanovich EM, Bille TW. Evaluation of methods to improve the extraction and recovery of DNA from cotton swabs for forensic analysis. *PLoS One* 2014;9(12): e116351.
21. Dewi AL, Paramita DK, Fachiroh J. Development of tetra-primer amplification refractory mutation system (ARMS) PCR for detection of CHRNA3 rs8040868. *The Indonesian Biomedical Journal* 2021;13(2):192-200.
22. Suhda S, Paramita D K, and Fachiroh J. Tetra primer ARMS PCR optimization to detect single nucleotide polymorphisms of the CYP2E1 gene. *Asian Pacific Journal of Cancer Prevention* 2016;17(7): 3065-3069.
23. Jurišić V, Obradović J, Tošić N, Pavlović S, Kulić M, Djordjević N. Effects of DMSO, glycerol, betaine and their combinations in detecting single nucleotide polymorphisms of epidermal growth factor receptor (EGFR) gene promoter sequence in non-small-cell lung cancer (NSCLC) patients. *Journal of Pharmaceutical and Biomedical Analysis* 2016;128:275–9.
24. Varadharajan B, Parani M. DMSO and betaine significantly enhance the PCR amplification of ITS2 DNA barcodes from plants. *Genome* 2021;64(3):165–71.
25. Sidhu MK, Liao MY, Rashidbaigi A. Dimethyl sulfoxide improves RNA amplification. *Biotechniques* 1996;21(1):44–47.
26. Hoang DT, Van Hiep T, Thi Phuong Nguyen T, et al. Exploring the Kinh Vietnamese genomic database for the polymorphisms of the P450 genes towards precision public health. *Annals of Human Biology* 2022;49(2):152–5.
27. Yamada S, Onda M, Kato S, et al. Genetic differences in CYP2C19 single nucleotide polymorphisms among four Asian populations. *Journal of Gastroenterology* 2001;36(10):669–72.
28. Vu NP, Nguyen HTT, Tran NTB, et al. CYP2C19 genetic polymorphism in the Vietnamese population. *Annals of Human Biology* 2019;46(6):491–7.
29. Vu NT, Nguyen TTM, Trinh HH, et al. The effects of CYP2C19 genotype and other factors on platelet aggregation in Vietnamese patients with acute coronary syndrome. *Academia Journal of Scientific Research* 2018;6(4): 172-179.
30. Zhong Z, Hou J, Li B, et al. Analysis of CYP2C19 genetic polymorphism in a large ethnic Hakka population in southern China. *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research* 2017;(23):6186–92.