BMC Infectious Diseases

RESEARCH Open Access

Genetic polymorphism of merozoite surface protein 1 and merozoite surface protein 2 in the Vietnam *Plasmodium falciparum* population

Tuấn Cường Võ[1,2], Hương Giang Lê[1,2], Jung-Mi Kang[1,2], Nguyen Thi Minh Trinh[3], Huynh Hong Quang[3*] and Byoung-Kuk Na[1,2*]

Abstract

Background *Plasmodium falciparum* merozoite surface proteins 1 (PfMSP1) and 2 (PfMSP2) are potential candidates for malaria vaccine development. However, the genetic diversity of these genes in the global *P. falciparum* population presents a significant challenge in developing an effective vaccine. Hence, understanding the genetic diversity and evolutionary trends in the global *P. falciparum* population is crucial.

Methods This study analyzed the genetic variations and evolutionary changes of *pfmsp1* and *pfmsp2* in *P. falciparum* isolates from the Central Highland and South-Central regions of Vietnam. DNASTAR and MEGA7 programs were utilized for analyses. The polymorphic nature of global *pfmsp1* and *pfmsp2* was also investigated.

Results A total of 337 sequences of *pfmsp1* and 289 sequences of *pfmsp2* were obtained. The *pfmsp1* and *pfmsp2* from Vietnam revealed a higher degree of genetic homogeneity compared to those from other malaria-endemic countries. Remarkably, the allele diversity patterns of Vietnam *pfmsp1* and *pfmsp2* differed significantly from those of neighboring countries in the Greater Mekong Subregion. Declines in allele diversity and polymorphic patterns of Vietnam *pfmsp1* and *pfmsp2* were observed.

Conclusions The Vietnam *P. falciparum* population might be genetically isolated from the parasite populations in other neighboring GMS countries, likely due to geographical barriers and distinct evolutionary pressures. Furthermore, bottleneck effects or selective sweeps may have contributed to the genetic homogeneity of Vietnam *pfmsp1* and *pfmsp2*.

Keywords *Plasmodium falciparum*, Merozoite surface proteins, Genetic diversity, Vietnam

*Correspondence:
Huynh Hong Quang
huynhquangimpe@yahoo.com
Byoung-Kuk Na
bkna@gnu.ac.kr
[1]Department of Parasitology and Tropical Medicine, Institute of Medical Science, Gyeongsang National University College of Medicine, Jinju 52727, Korea
[2]Department of Convergence Medical Science, Gyeongsang National University, Jinju 52727, Korea
[3]Tropical Diseases Clinical and Treatment Research Department, Institute of Malariology, Parasitology, and Entomology Quy Nhon, Quy Nhon, Vietnam

Introduction

Malaria, an acute febrile disease caused by infections with *Plasmodium* parasites, continues to be a significant global health challenge. According to the World Malaria Report 2023 from the World Health Organization (WHO), there were 249 million global malaria cases, making an increase of 5 million from the previous year, and an estimated 608,000 deaths were reported in 2022 [1]. Despite achievements in malaria control within the Greater Mekong Subregion (GMS) over recent decades, a surge in indigenous malaria cases from 90,082 to 170,527 was observed between 2021 and 2022. Specifically, the number of *P. falciparum* cases almost doubled during this period, with instances rising from 16,490 to 30,789 [1]. The rapid spread of antimalarial drug-resistant *P. falciparum* strains in the GMS poses a significant challenge to the region's efforts to eliminate falciparum malaria [2, 3]. Although notable reductions in malaria morbidity and mortality have occurred in Vietnam over the last two decades [4], the disease remains endemic in the central and southern provinces, particularly in the Central Highland region where *P. falciparum* is the predominant species [5]. A total of 3,200 malaria cases were reported in Vietnam in 2019, among which 3,110 were *P. falciparum* cases [1]. The malaria incidences in Vietnam have decreased sharply since 2019, but the epidemic has continued with fluctuating annual cases thereafter. Understanding the genetic makeup and evolutionary trends of the *P. falciparum* population in certain endemic areas is crucial as it provides valuable insights to the epidemiological patterns and genetic characteristics of the parasites.

P. falciparum merozoite surface proteins (PfMSPs) are multigene family proteins that are expressed on the surfaces of merozoites and sporozoites of the parasite and play critical roles in the invasion of the parasite into host cells [6]. Over 10 distinct genes encoding PfMSPs have been identified in *P. falciparum*, among which *pfmsp1* and *pfmsp2* are the most intensively investigated due to their reliability as polymorphic markers. PfMSP1 is a transmembrane protein divided into 17 distinct blocks, with block II displaying the most significant polymorphic character [7, 8]. PfMSP1 is typically classified into three allelic variants, K1, MAD20, and RO33, based on the polymorphisms observed in block II [9, 10]. The K1 and MAD20 types contain different tripeptide repeat sequences, and the variations in the repeat sequences and different numbers of repeats generate genetic polymorphisms within each of these types. While, RO33 has quite different sequences lacking typical repeat sequences. PfMSP2 consists of a central repeat region (CRR) flanked by conserved N-terminal and C-terminal regions and is classified into two distinct variants, 3D7 and FC27, depending on the sequence variations in the CRR [11, 12]. In particular, high levels of size polymorphisms are identified in the two regions; R1 corresponding to the GSA-rich repeat units and R2 flanking the poly-threonine stretch. Due to these polymorphisms in *pfmsp1* and *pfmsp2*, they are recognized as reliable polymorphic markers for studying genetic heterogenicity and evolutionary aspects of the *P. falciparum* population [13, 14].

Comprehensive studies analyzing the genetic nature of *pfmsp* genes in *P. falciparum* isolates from countries in the GMS have been conducted [15–19]. These studies indicated substantial genetic heterogeneities of *pfmsp* genes in the *P. falciparum* population in the GMS. The genetic characteristics of *pfmsp* genes in Vietnam *P. falciparum* have been partially characterized [20–22]. Similar to the *pfmsp* genes from other GMS countries, those in Vietnam *P. falciparum* also appear to exhibit considerable genetic diversity. However, these prior studies were limited by their reliance on simple typing instead of sequencing analysis and by being outdated, suggesting they may not accurately reflect the current genetic composition of the genes in Vietnam *P. falciparum* population. In this study, we explored the genetic diversities of *pfmsp1* and *pfmsp2* in Vietnam *P. falciparum* isolates collected in the Central Highland and South-Central regions of Vietnam between 2018 and 2022. We also comparatively analyzed the genetic differences among *pfmsp1* and *pfmsp2* populations from Vietnam and other malaria-endemic countries, including GMS countries.

Materials and methods

Parasite samples and study area

A total of 382 blood samples were collected from *P. falciparum*-infected individuals residing in three provinces in the Central Highlands (Dak Lak, Dak Nong, and Gia Lai) and two provinces in the South-Central regions (Phu Yen and Khanh Hoa), Vietnam, during 2018–2022 (Fig. 1). *P. falcipar*um infection was diagnosed by microscopic analysis of thin and thick blood smears. The dried blood filter (DBF) was prepared from each patient by finger-prick method and *P. falciparum* infection was verified by a species-specific polymerase chain reaction (PCR) targeting the 18S ribosomal RNA (rRNA) gene as reported previously [5, 23]. The study protocol was reviewed and approved by the Ethics Committee of the Ministry of Health, Institute of Malariology, Parasitology and Entomology Quy Nhon, Vietnam (IRB Approval numbers: 386/VSR-LSDT, 45/VSR-NCDT, and 637/VSR-NCDT).

Amplifications and sequence analyses of Vietnam *pfmsp* genes

Parasite DNA was isolated from each DBF using the QIAamp DNA Blood Kit (Qiagen, Hilden, Germany). Nested PCRs to amplify the *pfmsp1* block II and *pfmsp2* block III were performed using the primer sets and

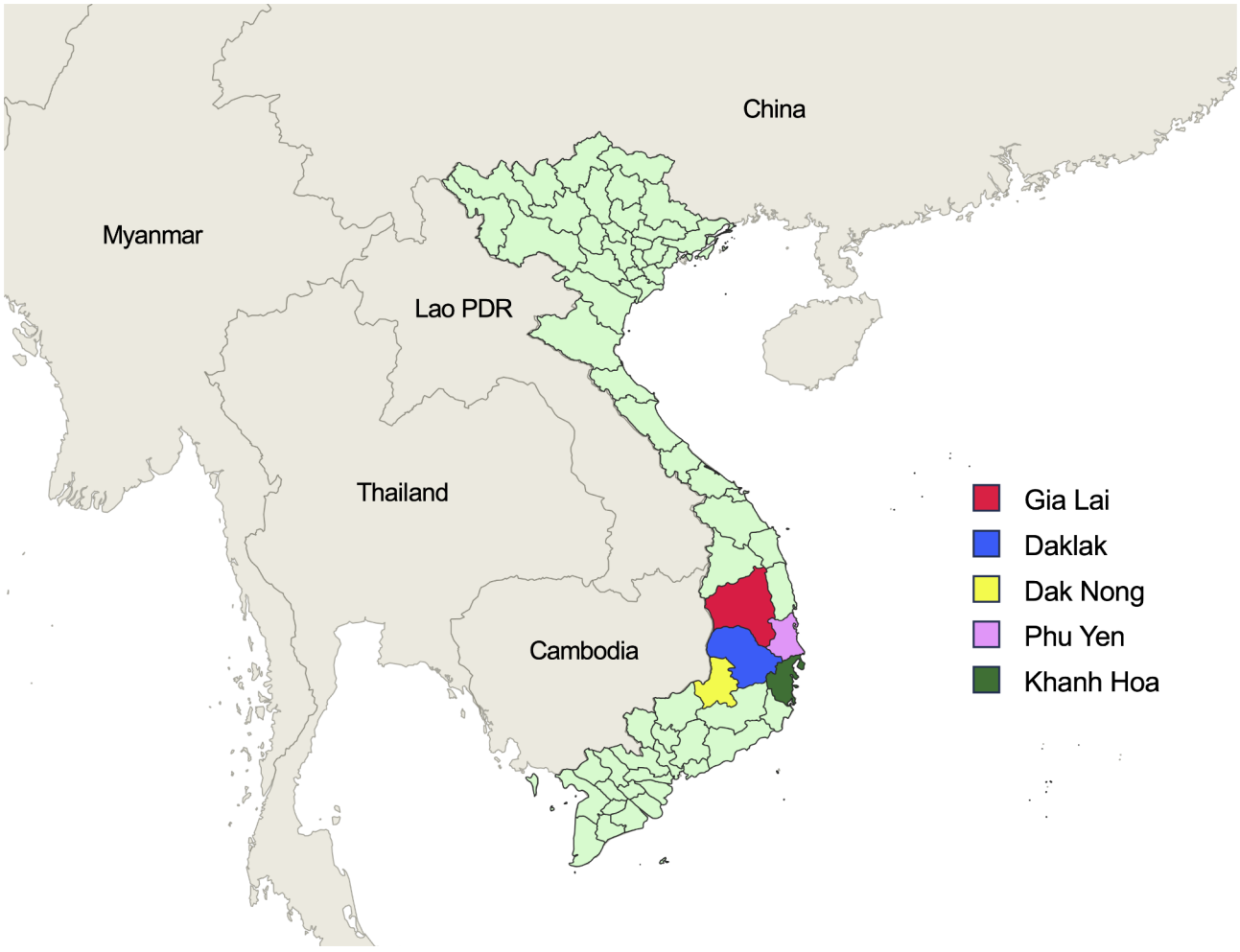

Fig. 1 Map of blood collection areas. The blood samples used in this study were collected from *P. falciparum*-infected patients residing in five provinces of Vietnam from 2018 to 2022

protocols described in previous studies [15, 17, 24]. Each PCR product was cloned into a T&A cloning vector (Real Biotech Corporation, Banqiao City, Taiwan) and transformed into *Escherichia coli* DH5α competent cells. The nucleotide sequences of the cloned gene were analyzed using the automated Sanger method with M13 forward and reverse primers. The nucleotide sequences of Vietnam *pfmsp* genes have been deposited in the GenBank with the accession numbers: *pfmsp1* (OR425413–OR425789) and *pfmsp2* (OR425790–OR426078).

Genetic diversity analysis

The nucleotide and inferred amino acid sequences of Vietnam *pfmsp* genes were analyzed using EditSeq and SeqMan in the DNASTAR package (DNASTAR, Madison, WI, USA). Sequences were analyzed based on the reference sequences: MAD20 (GenBank No.: X05624.2), K1 (GenBank No.: NC_004330.2), and RO33 (GenBank No.: M55001.1) for *pfmsp1*; 3D7 (GenBank No.: X53832) and FC27 (GenBank No.: J03828) for *pfmsp2*. The *pfmsp* sequences obtained in this study were also comparatively analyzed with the previously reported *pfmsp* sequences from Vietnam and other countries including the GMS countries (Supplemental File 1: Table S1).

Results

Genetic diversity of Vietnam *pfmsp1* block II

A total of 377 *pfmsp1* block II sequences were successfully obtained from Vietnam *P. falciparum*, with MAD20 alleles being overwhelmingly predominant (375/377, 99.47%). Each of the K1 and RO33 alleles was identified only once, respectively (Fig. 2). Block II of the MAD20 alleles comprised various combinations and arrangements of seven distinct peptide repeat motifs (PRMs) of SGG, SVT, SVA, SKG, SSG, PVA, and TVA, yielding a total of 25 unique alleles of MAD20 (A1–A25). None of the allele shared the same PRM configuration with the reference sequence (X05624.2). The construction of each MAD20 allele varied, containing PRMs ranging from 1 to 14. The two alleles, A14 and A20, were the

MAD20 X05624.2	PRMs 213331131	*n*
A1	1	1
A2	121	3
A3	431	9
A4	12121	3
A5	43131	1
A6	43531	1
A7	121331	2
A8	121211	1
A9	431131	2
A10	52113311	14
A11	43113131	2
A12	42113311	1
A13	4311313131	5

MAD20 X05624.2	PRMs 213331131	*n*
A14	12121133331	180
A15	13121133331	1
A16	12121132331	1
A17	12121173331	1
A18	12121133361	1
A19	121212333331	2
A20	431131313131	139
A21	413111361331	1
A22	431131316131	1
A23	431131313531	1
A24	431171313131	1
A25	43113131313131	1

PRMs	
SGG	1
SVT	2
SVA	3
SKG	4
SSG	5
PVA	6
TVA	7

K1 NC_004330.2	PRMs 141414141412332	*n*
A1	1222332	1

PRMs	
SAQ	1
SGT	2
SGP	3
SGA	4

RO33 M55001.1	LEALEDAVLTGYSLFQKEKMVLKDGANTQV//DLKHRVQNYLFTIKELKYPELFDLTNHM	*n*
A1	..............................//..............................	1

Fig. 2 Polymorphic patterns in Vietnam *pfmsp1* block II. A total of 27 different alleles were found in Vietnam *pfmsp1* block II, including 25 alleles for MAD20 type, 1 allele for K1 type, and 1 allele for RO33 type. Vietnam MAD20 block II consisted of seven different PRMs, including SGG, SVT, SVA, SKG, SSG, PVA, and TVA. Meanwhile, Vietnam K1 block II included three distinct PRMs: SAQ, SGT, and SGP. Vietnam RO33 block II had sequences identical to the reference sequence. The total number of isolates for each allele is indicated

most prevalent, accounting for 180 and 139 sequences, respectively. Block II of the K1 allele also demonstrated genetic variation relative to the reference sequence (NC_004330.2) and contained only three PRMs: SAQ, SGT, and SGP. In contrast, block II of the RO33 allele was identical in sequence to the reference (M55001.1).

Genetic differences of *pfmsp1* block II in the global population

The allelic diversity of Vietnam *pfmsp1* block II was compared with those of other countries and previously reported Vietnam *pfmsp1* (Fig. 3). The MAD20 alleles were predominant across all Vietnam *pfmsp1* populations, a trend similarly observed in *pfmsp1* populations of GMS countries, including Thailand and Myanmar, as well as Pacific countries such as the Philippines, PNG, and SI. Conversely, K1 alleles were primarily found in *pfmsp1* populations from India, Africa, and South American countries. Notably, the RO33 allele was predominant in the Vanuatu *pfmsp1* population. The global *pfmsp1* population displayed significant genetic heterogeneity in MAD20 and K1 alleles, attributed to various compositions and arrangements of PRMs. For MAD20 alleles, 10 different PRMs including SGG, SVT, SVA, SKG, SSG, PVA, TVA, SGT, SGA, and SVG were identified globally (Fig. 4). These PRMs were unequally distributed, with SGG, SVT, and SVA universally present across all populations. Additionally, SKG and SSG appeared in Asia, the Pacific, and Africa, but were absent in South American populations. The five PRMs, namely PVA, TVA, SGT, SGA, and SVG, were uniquely identified in either Vietnam or Indian populations, with PVA and TVA being novel PRMs first detected in the Vietnam MAD20 alleles analyzed in this study. A similar variation in PRM distribution was found in global K1 alleles (Fig. 4). A total of 17 distinct PRMs were detected in global K1 alleles. Among these, SGT and SGP were consistently observed across all populations, while SAG was present in K1 alleles of all countries analyzed except Brazil. SGA occurred in some countries across Asia, Africa, and South America, but

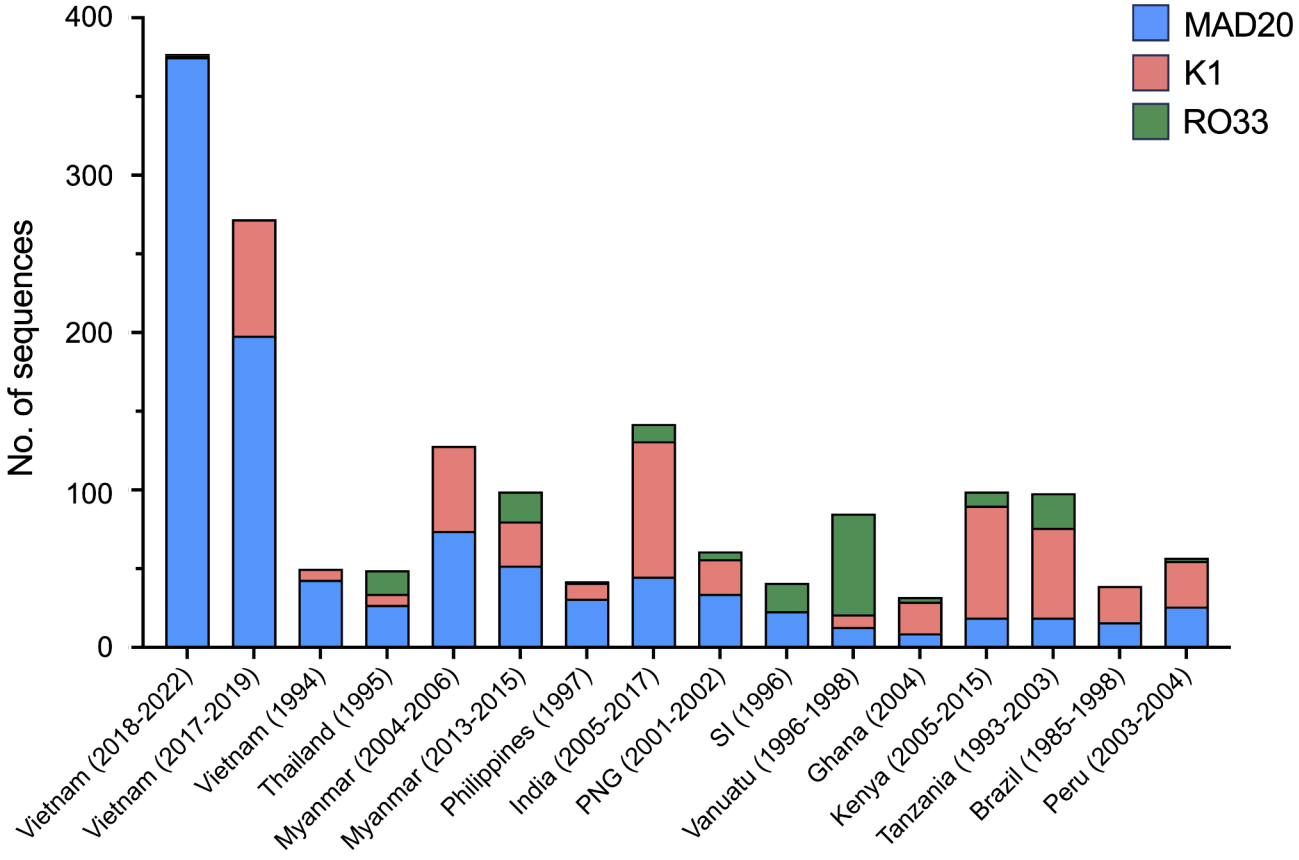

Fig. 3 Allelic diversity of the global *pfmsp1* block II. Different proportions of three *pfmsp1* types including MAD20, K1, and RO33 were observed in the global *pfmsp1* populations

not in the Pacific region. The remaining 13 PRMs showed unique occurrence in specific countries such as India, Kenya, or Tanzania. These PRMs contributed to various combinations and arrangements within global MAD20 and K1 alleles, resulting in significant size variations and genetic heterogeneity of *pfmsp1* by country (Fig. 5). Worldwide, MAD20 alleles varied immensely, containing 1 to 19 PRMs, with prevalent sizes ranging from 9 to 15 PRMs in the global MAD20 population. In comparison, global K1 alleles exhibited even greater size diversity due to different compositions of PRM compositions, ranging from 4 to 25. Each country displayed variability in the number of PRMs present, with pronounce size variations of PRMs in both MAD20 and K1 alleles notably in *pfmsp1* from India and African countries.

Genetic diversity of Vietnam *pfmsp2* block III

A total of 289 Vietnam *pfmsp2* block III sequences were successfully obtained from the samples analyzed in this study. These sequences were categorized into 3D7 types. They displayed polymorphic characters, forming 7 distinct alleles (A1–A7) distinguished by sequence polymorphisms (Fig. 6). The A4 and A2 alleles were predominant, accounting for 52.2% (151/289) and 41.9% (121/289), respectively. In the E1 region, only four amino acid changes (T44E, N47K, P48T, and P49S) were observed, categorized into two groups of paired amino acid substitutions. Specifically, the T44E/P49S pair was present in 280 sequences (96.9%), while the N47K/P48T pair was found in 9 sequences (3.1%). Different numbers, types, and arrangements of PRMs were observed in the R1 region, contributing to the size polymorphisms of Vietnam *pfmsp2* block III. GAGGSGSA, GGSGSA, and GAGASGSA served the fundamental units of PRMs. In the R2 region, all sequences exhibited poly-threonine (poly-T) signature characteristic of the 3D7 type. Alleles A1–A6 contained 8 threonines (T8), while A7 featured 14 threonines (T14). Compared to the 3D7 reference sequence, a notable characteristic in the E3 region of all Vietnam *pfmsp2* was the insertion of 11 amino acids at position 156: PKGKGEVQKPN for A1–A6 alleles and PKGNGGVQEPN for the A7 allele. Additionally, an amino acid change of E154K was identified in the A7 allele.

Fig. 4 PRM profiles of the global *pfmsp1* block II. Differences in PRM types in the global MAD20 and K1 populations. MAD20 block II comprised 10 different PRMs, while K1 block II included 17 distinct PRMs

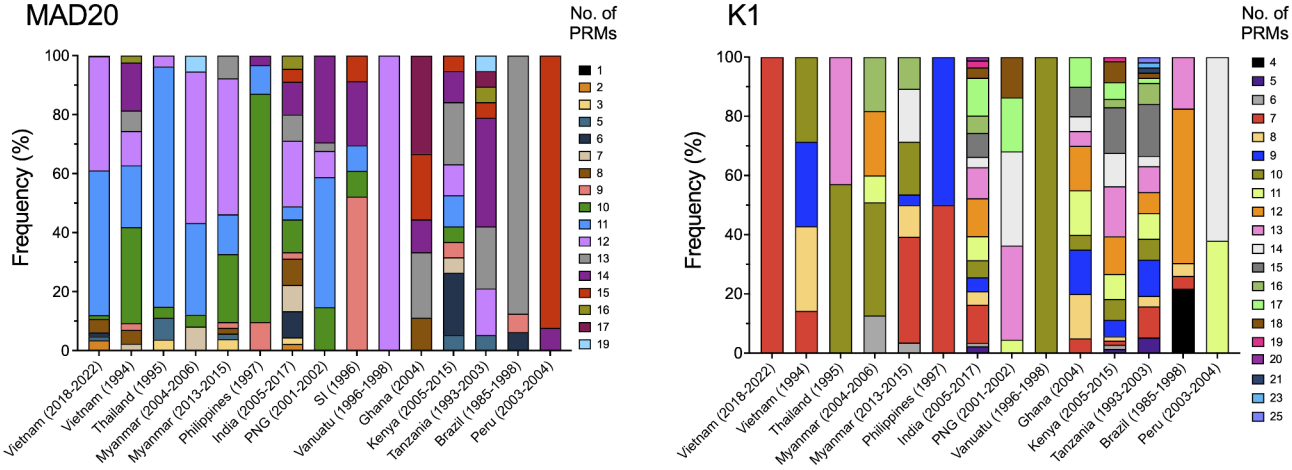

Fig. 5 Size polymorphism patterns of the global *pfmsp1* block II. Size differences in the global *pfmsp1* block II were caused by varying numbers of PRMs. The global MAD20 block II exhibited 17 different sizes with PRM counts ranging from 1 to 19. The global K1 block II displayed 20 different size polymorphisms resulting from distinct PRMs counts that ranged from 4 to 25

Genetic differences of *pfmsp2* block III in the global population

The distribution of alleles in the current Vietnam *pfmsp2* was compared to *pfmsp2* from Vietnam (1994 and 2017–2019) and other countries including Myanmar, Thailand, India, PNG, and Gambia (Fig. 7). All populations exhibited the presence of both allelic types of 3D7 and FC27 in a substantial proportion except the current

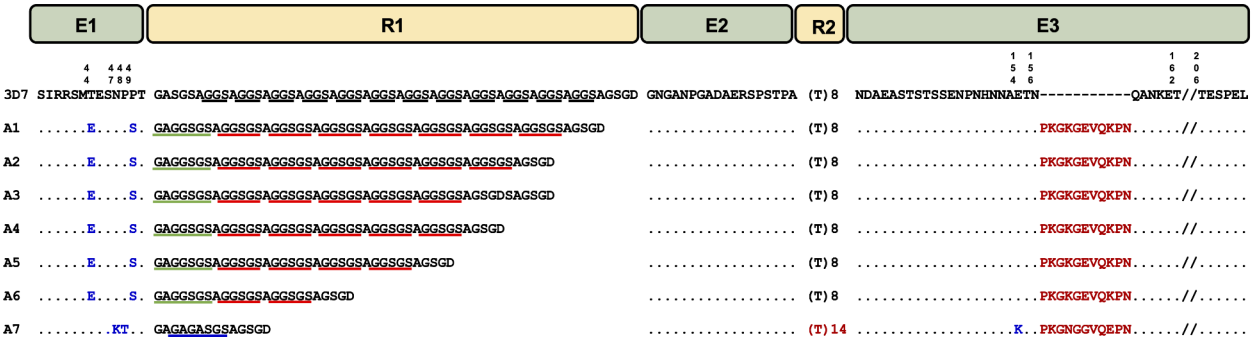

Fig. 6 Polymorphic patterns in Vietnam *pfmsp2* block III. In Vietnam, seven distinct alleles of the 3D7 types (A1–A7) were identified in the *pfmsp2* population

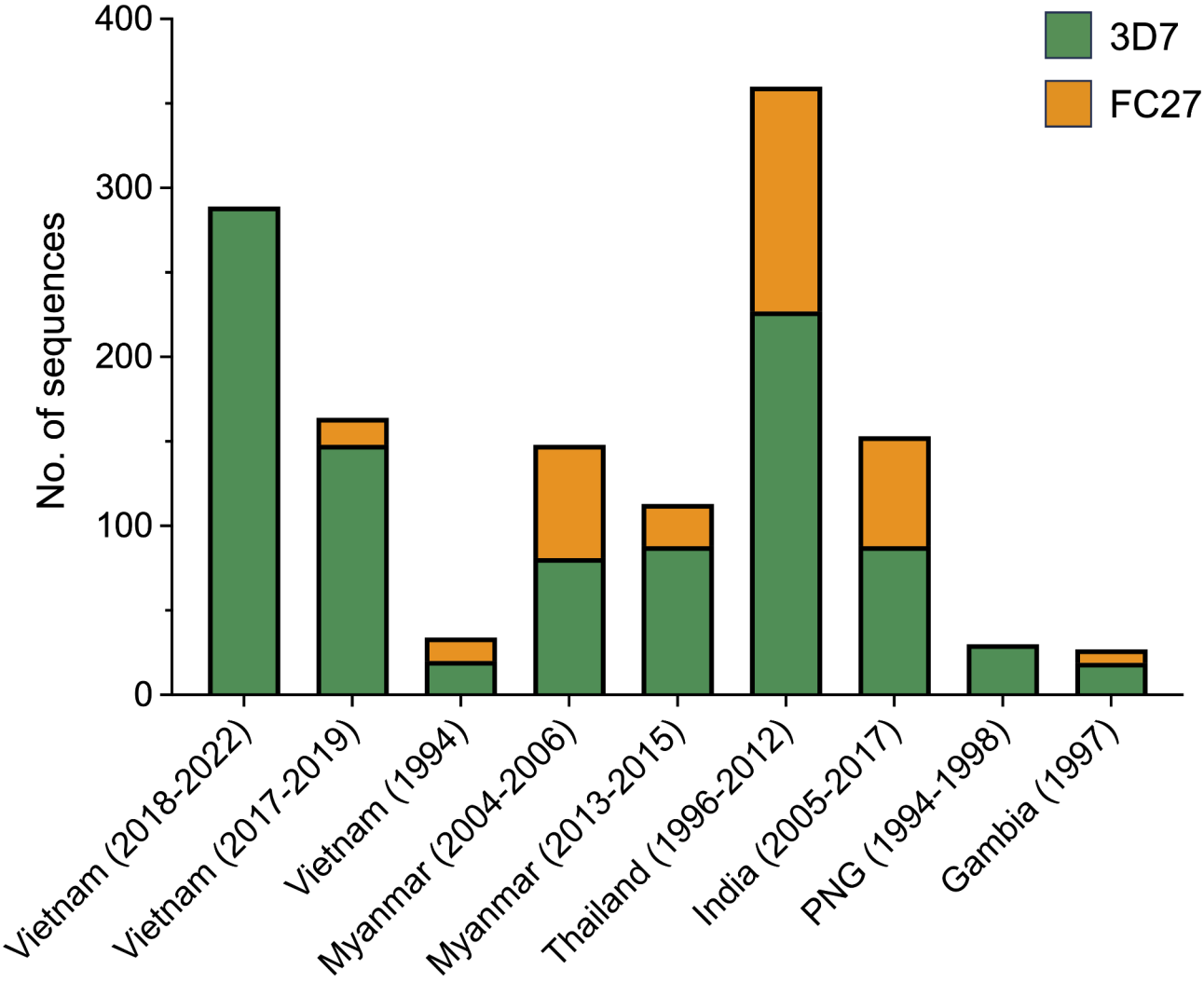

Fig. 7 Allelic diversity of the global *pfmsp2* block III. In global populations, different proportions of two *pfmsp2* subtypes, namely 3D7 and FC27, were evident

Vietnam and PNG. The previously reported Vietnam *pfmsp2* (Vietnam 1994 and 2017–2019) harbored both 3D7 and FC27, though they shared overlapping collection sites with the present study. Significant genetic diversity was observed in the global *pfmsp2* block III. The regions of R1, R2, and E3 were the primary contributors to genetic diversity among and between *pfmsp2* populations (Fig. 8). The R1 region exhibited exceptionally

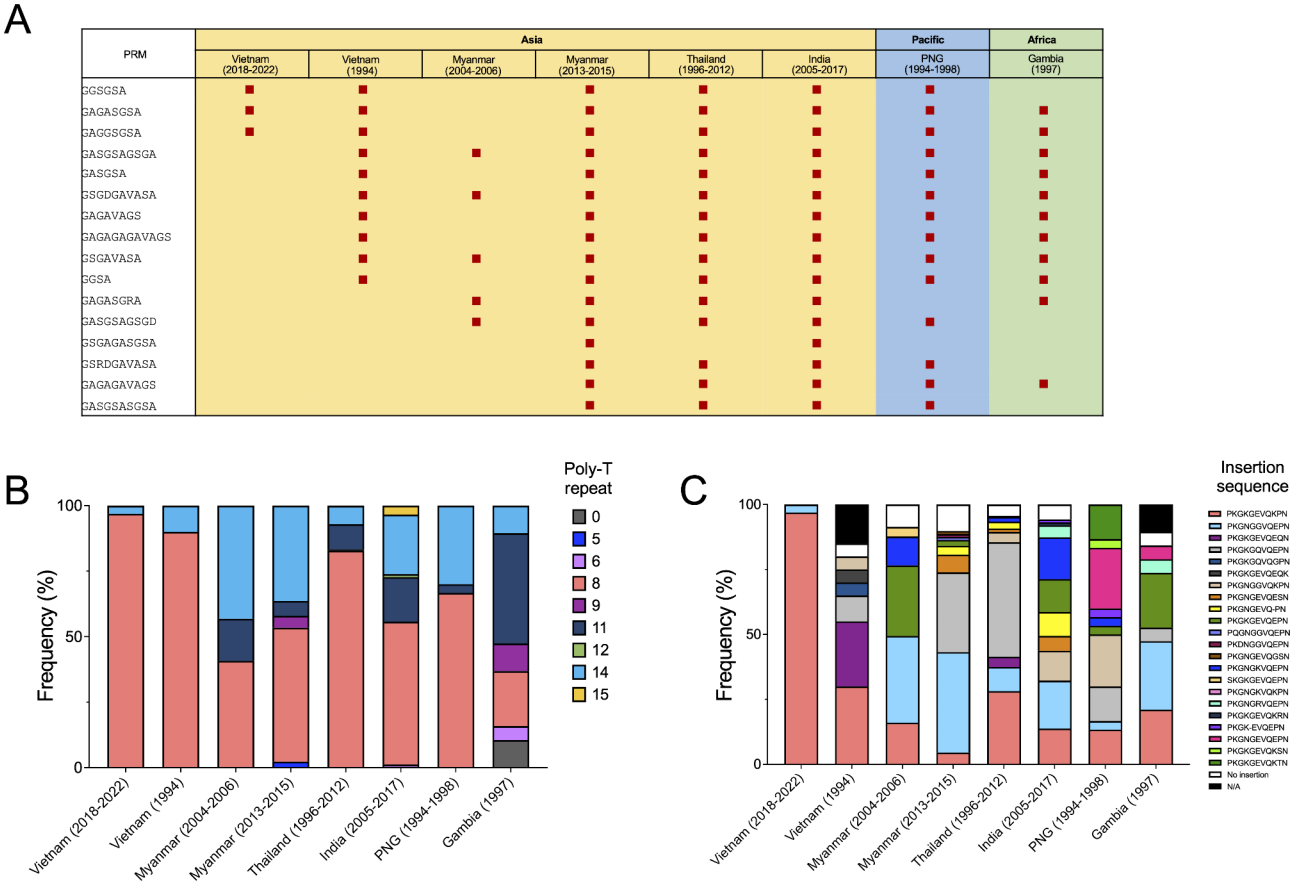

Fig. 8 Comparison of polymorphism patterns in the R1, R2, and E3 regions of the global *pfmsp2* 3D7 type. (**A**) Distinct PRM profiles in the R1 region. (**B**) Varied poly-T patterns in the R2 region. (**C**) Diverse insertion sequences in the E3 region. N/A, not available for analysis due to the sequence missing corresponding to the region

diverse polymorphisms, characterized by 16 different types of PRM with unequal arrangements and repetitions (Fig. 8A). Three Asian *pfmsp2* populations, Myanmar (2013–2015), Thailand, and India, possessed all PRM types. The PNG and Gambia populations also included 14 and 11 different PRM types, respectively. Notably, two Vietnam *pfmsp2* populations exhibited distinct patterns. While the Vietnam *pfmsp2* analyzed in this study displayed only 3 PRM types, Vietnam (1994) *pfmsp2* comprised 10 different PRM types. In the R2 region, only two types of poly-T (T8 and T14) were identified in the Vietnam *pfmsp2* population, whereas a variety of poly-T types were observed in the global population (Fig. 8B). The prevalence of poly-T types varied globally, with T8 and T14 being the dominant species. Intriguingly, T8 was markedly prevalent in the Vietnam *pfmsp2* population. The E3 region of *pfmsp2* also demonstrated substantial genetic diversity in the global population. A range of insertion types with diverse sequences was evident in the global *pfmsp2* population (Fig. 8C). The PKGKGEVQKPN insertion appeared in all populations, albeit at varying frequencies by country. Additionally, *pfmsp2* sequences lacking insertion were present in the global population, excluding PNG.

Discussion

PfMSPs are key contenders in the development of vaccines against falciparum malaria; however, the extensive genetic diversity of these genes within the global *P. falciparum* population poses significant challenges to vaccine development. In our study, we examined the genetic polymorphisms of two *pfmsp* genes, *pfmsp1* and *pfmsp2*, in *P. falciparum* isolates from Vietnam and compared their genetic diversities with those from other countries, including those in the GMS.

It has been previously suggested that *pfmsp1* block II and *pfmsp2* block III exhibit high levels of polymorphism [13, 14]. In contrast, our analysis revealed a marked genetic uniformity in the Vietnam *pfmsp1* and *pfmsp2* genes, with the dominant presence of MAD20 alleles in *pfmsp1* and 3D7 alleles in *pfmsp2*. This uniformity sets the Vietnamese isolates apart from their counterparts in neighboring GMS countries like Myanmar and Thailand. Notably, when compared with earlier analyses of the

Vietnam *pfmsp1* and *pfmsp2* populations (2017–2019) [22], a significant reduction in allelic variation was noted in recent populations, despite their shared geographical origins. No mixed infections of *pfmsp1* and *pfmsp2* were identified in the *P. falciparum* population analyzed in this study. However, in our previous study on the genetic makeup analysis of Vietnam *pfmsp1*, mixed infections with different alleles were detected [5], albeit at low frequencies. This discrepancy may be attributed to the preferred amplification and cloning of the major alleles in mixed infection samples, if present. Nevertheless, our study revealed that Vietnam *pfmsp1* and *pfmsp2* exhibited a higher degree of genetic homogeneity compared to those from other malaria-endemic countries. PRMs are significant factors in inducing genetic complexity in Vietnam *pfmsp1* and *pfmsp2*. Common PRMs identified in other global *pfmsp1* and *pfmsp2* populations were also found in Vietnam *pfmsp1* and *pfmsp2* as major PRMs. However, two novel PRMs (PVA, and TVA) that not been reported in global *pfmsp1* were also discovered in Vietnam *pfmsp1*, though their prevalence was low. The most notable features in Vietnam *pfmsp2* included the low diversity of PRM types in the R1 region and the exceedingly high frequency of a single type of insertion (PKGKGEVQKPN, 96.9%) in the E3 region. Compared to previous Vietnam sequences (1994) that exhibited more than five different insertion in the E3 region, only two types of insertion sequence (PKGKGEVQKPN and PKGNGGVQEPN) were identified in the Vietnam *pfmsp2* analyzed in this study. Malaria prevalence and transmission intensity, particularly *P. falciparum*, have been sharply declined in the endemic areas of Vietnam in recent few years [1, 4], The recent decrease of overall genetic diversity in Vietnam *pfmsp1* and *pfmsp2* populations could be attributed to declined transmission intensity in the country, limiting genetic exchange and recombination in the population.

Differences in genetic diversity of other vaccine candidates between Vietnam and other GMS countries have previously been reported. The pattern of restricted genetic diversity in Vietnam's *P. falciparum* is not confined solely to MSP family genes but also extends to other genes. In Vietnam *P. falciparum*, *pfama1* and *pfeba-175* demonstrated notably lower genetic diversity and a unique genetic profile compared to other GMS countries, such as Myanmar and Thailand [25, 26]. This phenomenon was not exclusive to *P. falciparum* genes. *P. vivax* circumsporozoite surface protein (*pvcsp*) of Vietnam also exhibited a distinct allelic pattern relative to *pvcsp* from other GMS countries [27]. The genetic distinctiveness of Vietnam's *Plasmodium* populations compared to those of other GMS countries could be attributed to geographic feature. The Truong Son Range (Annamite Range), stretching approximately 1,100 km across the north and south of western Vietnam, may act as a topographical barrier that impedes the transmission of parasites and mosquito vectors between western Vietnam and eastern Laos and Cambodia [26]. Foehn effect across the Range also renders dissimilar environmental conditions in the western and the eastern sides of the Range, resulting in different transmission settings that can influence transmission patterns and genetic dynamics of the parasites. Furthermore, this barrier could also restrict the migratory patterns of the human population, limiting transmission. Due to these environmental factors, the *Plasmodium* population in Central Vietnam could be undergoing unique evolutionary changes, independent from those in the neighboring GMS countries, resulting in the distinctive genetic lineage. However, this study still presents limitations. The samples from GMS countries were collected at varying time points, which challenges the distinction of precise genetic variations in contemporary *P. falciparum* populations. To better understand the genetic dynamics of *Plasmodium* species in this region, comprehensive examinations with larger-scale samples and more systematic methodologies are required, especially in Laos and Cambodia, which border Vietnam.

The results of this study also indicated a decrease the sequence diversity of Vietnam's *pfmsp1* and *pfmsp2* compared to earlier periods, potentially due to population bottlenecks in the parasite population. The recent decrease in malaria cases in this endemic region, attributed to sustained control efforts, may lead to a bottleneck effect, significantly reducing genetic polymorphism [28]. Conversely, the reduction in polymorphism at these sites could result from a selective sweep, where newly developed directional selection favors certain alleles over others [29].

Conclusion

Vietnam *pfmsp1* and *pfmsp2* exhibit less genetic diversity than those from other malaria-endemic countries, including the GMS countries. Vietnam *P. falciparum* population is likely genetically isolated from the parasite populations in other neighboring GMS countries, possibly due to geographical barriers and unique evolutionary pressures. The bottleneck effect or selective sweep may contribute to the genetic homogeneity of Vietnam *pfmsp1* and *pfmsp2*, which warrants further investigation. This study provides a comprehensive understanding of the genetic characteristics and population structure of the Vietnam *P. falciparum* population, but a more comprehensive analysis of the genetic diversity and evolutionary aspects of the population is also necessary.

Abbreviations

CRR	Central repeat region
DBF	Dried blood filter
GMS	Greater Mekong Subregion

MSP	Merozoite surface protein
P. falciparum	*Plasmodiumfalciparum*
PfMSP1	*P. falciparum* merozoite surface protein 1
PfMSP2	*P. falciparum* merozoite surface protein 2
PCR	Polymerase chain reaction
PRM	Peptide repeat motif
poly-T	Poly-threonine
rRNA	ribosomal RNA
WHO	World Health Organization

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-024-10116-6.

Supplementary Material 1

Acknowledgements
The authors thank the staff in the Tropical Diseases Clinical and Treatment ResearchDepartment, Institute of Malariology, Parasitology, and Entomology, Quy Nhon and the healthprofessionals for their contribution and technical support in field study. This research was supported by a National Research Foundation of Korea (NRF) grants from the Government of Korea (NRF-2023M3A9H506175721 and NRF-2024M3A9H5043141).

Author contributions
TCV, HHQ, and BKN conceptualize the study. TCV, HGL, JMK, TMTN, and HHQ performed experiment or contributed in sample collection. TCV, HGL, JMK, HHQ, and BKN analyzed and interpreted the data. HHQ and BKN designed and supervised the experiments. TCV and BKN wrote the draft of the manuscript. HGL, JMK, TMTN, and HHQ reviewed the manuscript. All authors have agreed to the published version of the manuscript. All authors read and approved the final manuscript.

Funding
This research was supported by a National Research Foundation of Korea (NRF) grants from.
the Government of Korea (NRF-2023M3A9H506175721 and NRF-2024M3A9H5043141).

Data availability
Sequence data that support the findings of this study have been deposited in the GenBank with the accession numbers of pfmsp1 (OR425413–OR425789) and pfmsp2 (OR425790–OR426078).

Declarations

Ethics approval and consent to participate
The study protocol was reviewed and approved by the Ethics Committee of the Ministry of Health, Institute of Malariology, Parasitology and Entomology Quy Nhon, Vietnam (IRB Approval numbers: 386/VSR-LSDT, 45/VSR-NCDT, and 637/VSR-NCDT). Verbal informed consent was obtained from all the participants in this study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 6 August 2024 / Accepted: 24 October 2024
Published online: 28 October 2024

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