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Genetic polymorphisms of *Plasmodium vivax* transmission-blocking vaccine candidates *Pvs*48/45 and *Pvs*47 in Thailand

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Abstract

Background The genetic diversity of malaria parasites varies between regions in the world. The genetic polymorphisms of the genes *Pvs*48/45 and *Pvs*47 which encode gametocyte/gamete proteins of *Plasmodium vivax*, were studied because of their potential as transmission-blocking vaccine (TBV) targets. The aim of the present study was to investigate the genetic diversity of *Pvs*48/45 and *Pvs*47 in clinical isolates from endemic areas of Thailand.

Methods *Plasmodium vivax* samples collected from four provinces neighbouring either Myanmar or Malaysia were analysed using polymerase chain reaction and nucleotide sequencing.

Results Fifteen and 18 amino acid substitutions were observed in 36 Pvs48/45 and 62 Pvs47 deduced amino acid sequences, respectively. Eleven haplotypes were identified in Pvs48/45 and 26 in Pvs47. Overall, low nucleotide diversities were observed for *Pvs*48/45 ($\pi = 0.00104$) and *Pvs*47 ($\pi = 0.00321$). Tajima's D, and Fu and Li's D* and F* values were negative for both genes, *Pvs*48/45 and *Pvs*47 while a significant difference was found in *Pvs48/45* ($P < 0.05$).

Conclusion The limited polymorphism of the two investigated TBV candidate antigens observed in this study is consistent with findings in worldwide isolates. The collected genetic diversity data could be helpful for developing effective TBVs in malaria-endemic areas.

Keywords *Plasmodium vivax*, Transmission-blocking vaccine, Pvs48/45, Pvs47, Genetic polymorphism

Background

Malaria is an infectious disease caused by parasites of the genus *Plasmodium*. Malaria is transmitted to humans through the bite of infected female *Anopheles* mosquitoes. It is a significant global health concern, particularly in tropical and subtropical regions, with 249 million malaria cases in 85 malaria-endemic countries in 2022 [1]. Among five species of human malaria parasites, *Plasmodium vivax* is responsible for most malaria cases throughout Asia–Pacific and Central and South America [1]. In Thailand, *P. vivax* causes more than 93% of all malaria cases [2]. Despite a decrease in the global incidence of malaria during the last decade and the fact that *P. vivax* strains are still sensitive to the current antimalarial treatments, a significant challenge remains due to the emergence of drug-resistant *P. vivax* malaria and the absence of a vaccine to prevent it.

Malaria vaccines have been developed using antigens from different stages of the malaria parasite. The transmission-blocking vaccines (TBVs) target the sexual stages of *Plasmodium* to reduce or interrupt the transmission of the parasite [3, 4]. Target of the vaccines are antigens expressed on the surface of gametocyte, gamete, zygote and ookinete. Recently, many potential TBV candidates have been tested and the effective

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target antigens of TBVs have been demonstrated [5–7]. *Plasmodium vivax* 48/45 (*Pvs*48/45) and *Plasmodium falciparum* 48/45 (*Pfs*48/45), are specifically expressed in gametocytes circulating within the vertebrate host [8]. Others, such as *Pfs*25 in *P. falciparum* and *Pvs*25 and *Pvs*28 in *P. vivax*, are exclusively expressed by the zygote and ookinete within the midgut of anopheline vectors [9–11].

Pvs48/45 and Pvs47 of *P. vivax* belong to the six-cysteine (6-Cys) domain protein family which is characterized by a cysteine-rich domain and is encoded by 10 genes in the *Plasmodium* genome. [12, 13]. The open reading frame (ORF) of *Pvs*48/45 has a length of 1,353 bp. The encoded protein of 450 amino acids (aa) length contains two copies of the s48-45 six-Cys domain at positions 48–162 aa and 298–418 aa, respectively. Pvs48/45 protein is expressed on the surface of gametocytes/gametes and plays a key role in gamete fusion during fertilization. Recently, the genetic diversity of *Pvs*48/45 and *Pvs*47 genes and polymorphisms of their amino acid sequences from *P. vivax* isolates from different geographical areas have been investigated [6, 14–18]. In the present study, the genetic diversity of these two TBV candidate antigens (Pvs48/45 and Pvs47) was analysed in *P. vivax* clinical isolates from Thailand.

Methods

Collection of study samples

Between 2006 and 2019, 100 *P. vivax* samples were collected from malaria patients in two regions of Thailand, in the west along the Thai-Myanmar border from Tak Province (45 samples) and Kanchanaburi Province (10 samples) and in the south at the Thai-Myanmar border from Ranong Province (20 samples) and at the Thai-Malaysian border from Yala Province (25 samples). *Plasmodium vivax* infection was diagnosed by microscopic examination of Giemsa-stained thick and thin blood smears and confirmed by polymerase chain reaction (PCR). All participants provided their informed consent for inclusion before participating in the study. The study was reviewed and approved by the Ethics Committee of Thammasat University (COA No. 042/2564).

Extraction of *Plasmodium* genomic DNA

The parasite genomic DNA was extracted from ethylene diamine tetra-acetic acid (EDTA) blood samples using a QIAamp DNA extraction mini-kit (Qiagen, California, USA) according to the manufacturer's instruction. The DNA was stored at − 20 °C until used as template for gene amplification by polymerase chain reaction (PCR).

Amplification and detection of *Pvs*48/45 and *Pvs*47 gene polymorphisms

The *Pvs48/45* and *Pvs47 P. vivax* genes were amplified by PCR using the previously described specific primers and methods with some modifications [18]. For amplification, the PCR was carried out with the reaction mixture including 0.1 µM of each primer, 1.5 mM $MgCl_2$ (Thermo Scientific, Massachusetts, USA), 1 × Taq buffered with KCl (Thermo Scientific, Massachusetts, USA), 0.2 mM deoxynucleotides (dNTPs) (Bioline, London, UK), 1 µl of genomic DNA and 2 units of Taq DNA polymerase (Thermo Scientific, Massachusetts, USA). The amplification conditions were denaturation at 95 °C for 3 min, followed by 35 cycles of 95 °C for 1 min, 58 °C for 1 min, and 67 °C for 90 s, with a final extension at 72 °C for 5 min. The PCR products were then analysed on a 1.2% agarose gel and visualized and photographed under a UV illuminator.

Purification of PCR products and DNA sequencing

The PCR products of the *Pvs*48/45 and *Pvs*47 genes were purified using a QIAquick PCR purification kit (Qiagen, Germany) and sequenced using an ABI 3730XL DNA Analyzer (Thermo Scientific, Massachusetts, USA) via bidirectional sequencing, covering both forward and reverse strands (Bionics Co., Ltd., Seoul, South Korea). The nucleotide sequences were submitted to GenBank. (GenBank accession numbers: PQ441910–PQ441920 for *Pvs*48/45 and PQ441921–PQ441946 for *Pvs*47).

Genetic diversity and neutrality of *Pvs*48/45 and *Pvs*47 genes

The nucleotide and deduced amino acid sequences were separately aligned and analysed using MEGA X (https://www.megasoftware.net/index.html). All *Pvs*48/45 and *Pvs*47 sequences were compared with the reference sequences of the *Pvs*48/45 and *Pvs*47 genes from the Salvador I (Sal-I) strain, PVX_083235 and PVX_083240, respectively, that are available in GenBank. The haplotypes of Pvs48/45 and Pvs47 were analysed based on the amino acid substitutions of the obtained sequences. Segregating sites (S), nucleotide diversity (π), the average number of nucleotide differences (k), number of haplotypes (H), and haplotype diversity (Hd) were determined using DnaSP version 6.0 (http://www.ub.edu/dnasp/). Likewise, deviation from the neutral theory of evolution of the *Pvs*48/45 and *Pvs*47 genes, Tajima's D neutrality test, and the Fu and Li's D* and F* were determined using DnaSP. Under neutrality, Tajima's D is anticipated to be 0. Significantly positive Tajima's D values indicate recent balancing selection or a population bottleneck, whereas negative values suggest population expansion or

directional selection [19]. The synonymous (dS) and non-synonymous (dN) substitutions were computed and compared with the *Z*-test ($P < 0.05$) in MEGA X using Nei and Gojobori's method [20] with the Jukes and Cantor (JC) correction and standard error by 1000 bootstrap replications. The recombination parameter (R), which included the effective population size and probability of recombination between adjacent nucleotides per generation (Ra, Rb), and the minimum number of recombination events (Rm) were analysed using DnaSP.

Haplotype networks and worldwide genetic diversity of *Pvs*48/45 and *Pvs*47

The worldwide relationship of diversity in *Pvs*48/45 and *Pvs*47 genes was analysed by haplotype networks. Nucleic acid sequences were obtained from GenBank (Table S1) including the sequences from Colombia, Vanuatu, Iran, India, South Korea, China, Indonesia, and Thailand for *Pvs*48/45 and sequences from Colombia, Vanuatu, India, South Korea, Indonesia, and Thailand for *Pvs*47. The haplotype network was constructed by Popart (http:// popart.otago.ac.nz) using the Median-Joining method [21]. Furthermore, the genetic diversity between populations was estimated by evaluating the fixation index (F_{ST}) using DnaSP. High genetic diversity is indicated by $F_{ST} > 0.15$ [22].

Results

Genetic diversity of *Pvs*48/45

Thirty-six *P. vivax* samples were successfully amplified with a product size of 1,605 bp. Seventeen single nucleotide polymorphisms (SNPs) were observed as compared with the Sal-I strain sequences. Among these, 100% mutation was detected at position 631 (A), 750 (C) and 1,253 (G). In the deduced amino acid sequences, 15 amino acids substitutions (non-synonymous) (R4P, K26R, E35K, V41L, N109T, E127Q, E136Q, K185R, H211N, K212T, D214N, K250N, D335Y, A376T and K418R) and two synonymous substitutions (Q5Q and E228E) were detected. Of these, three amino acid substitutions (H211N, K250N and K418R) were fixed. Therefore, 11 haplotypes (H1-H11) were classified from amino acids substitutions as presented in Table 1. H2 was the most prevalent haplotype (55.6%) in the present study with 5 amino acid substitutions (211N, 250N, 335Y, 376 T and 418R).

Genetic diversity of *Pvs*47

Sixty-two *P. vivax* samples were successfully amplified with a product size of 1,507 bp. In comparison with the Sal-I strain sequences, the *Pvs*47 gene revealed higher polymorphism than the *Pvs*48/45 gene with 24 SNPs. Among these, 100% mutation was detected at position 22 (A), 24 (C), 26 (A) and 27 (G). In the deduced amino acid sequences, 18 non-synonymous substitutions (L3P, F6V, F22L, F24L, K27E, S57I/T, S62N, L82V, K164T, V230I, M233I, F237I, E240D, F247L, I262K/T, E263D, I273V and A373V) and five synonymous substitutions (T26T, G298G, I322I, S352S and F369F) were detected. Among these, three amino acid substitutions (F22L, F24L and K27E) were fixed. Therefore, 26 haplotypes (H1-H26) were classified from amino acids substitutions as presented in Table 2. H1 was the most prevalent haplotype (16.1%) in the present study with 8 amino acids

Table 1 Pvs48/45 amino acid haplotypes in *P. vivax* clinical isolates from Thailand

Haplotypes	Frequency (%)	Amino acid position														
		4	26	35	41	109	127	136	185	211	212	214	250	335	376	418
Sal-1 strain	–	R	K	E	V	N	E	E	K	H	K	D	K	D	A	K
Haplotype 1 (H1)	5 (13.9)	.	.	.	.	.	.	.	.	**N**	.	.	**N**	.	**T**	**R**
Haplotype 2 (H2)	20 (55.6)	.	.	.	.	.	.	.	.	**N**	.	.	**N**	**Y**	**T**	**R**
Haplotype 3 (H3)	1 (2.8)	.	.	.	.	.	.	.	**R**	**N**	.	**N**	**N**	.	.	**R**
Haplotype 4 (H4)	1 (2.8)	.	**R**	.	.	.	.	.	.	**N**	.	.	**N**	**Y**	**T**	**R**
Haplotype 5 (H5)	2 (5.6)	.	.	**K**	.	.	.	.	.	**N**	.	.	**N**	**Y**	**T**	**R**
Haplotype 6 (H6)	1 (2.8)	.	.	**K**	.	.	.	.	.	**N**	.	.	**N**	.	**T**	**R**
Haplotype 7 (H7)	1 (2.8)	.	.	.	.	.	.	.	.	**N**	**T**	.	**N**	**Y**	**T**	**R**
Haplotype 8 (H8)	1 (2.8)	.	.	.	.	**T**	.	.	.	**N**	.	.	**N**	**Y**	**T**	**R**
Haplotype 9 (H9)	2 (5.6)	.	.	.	.	.	.	.	.	**N**	.	.	**N**	.	.	**R**
Haplotype 10 (H10)	1 (2.8)	.	.	.	.	.	.	.	.	**N**	.	.	**N**	**Y**	.	**R**
Haplotype 11 (H11)	1 (2.8)	**P**	.	**K**	**L**	.	**Q**	**Q**	.	**N**	.	.	**N**	**Y**	**T**	**R**
Total	36 (100)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Dots (.) represent identical amino acid residues compared to the reference Sal-I strain. Bold letters represent amino acid substitutions compared to the Sal-I strain

Table 2 Pvs47 amino acid haplotypes in *P. vivax* clinical isolates from Thailand

Haplotypes	Frequency (%)				Amino acid position														
		3	**6**	**22**	**24**	**27**	**57**	**62**	**82**	**164**	**230**	**233**	**237**	**240**	**247**	**262**	**263**	**273**	**373**
Sal-1 strain	–	L	F	F	F	K	S	S	L	K	V	M	F	E	F	I	E	I	A
Haplotype 1 (H1)	10 (16.1)	.	.	**L**	**L**	**E**	.	**N**	.	.	**I**	**I**	.	.	.	.	.	**V**	**V**
Haplotype 2 (H2)	2 (3.2)	.	.	**L**	**L**	**E**	.	.	.	.	**I**	**I**	.	.	.	.	.	.	.
Haplotype 3 (H3)	3 (4.8)	.	.	**L**	**L**	**E**	**T**	.	.	.	.	**I**	.	.	.	**K**	.	.	.
Haplotype 4 (H4)	1 (1.6)	.	.	**L**	**L**	**E**	.	**N**	.	**T**	**I**	**I**	.	.	.	.	.	**V**	.
Haplotype 5 (H5)	7 (11.3)	.	.	**L**	**L**	**E**	.	.	.	.	.	**I**	.	.	.	**K**	.	.	.
Haplotype 6 (H6)	1 (1.6)	.	.	**L**	**L**	**E**	**I**	.	**V**	.	.	**I**	.	.	.	**K**	.	.	.
Haplotype 7 (H7)	2 (3.2)	.	.	**L**	**L**	**E**	.	.	.	.	**I**	**I**	.	.	.	**T**	.	.	.
Haplotype 8 (H8)	2 (3.2)	.	.	**L**	**L**	**E**	.	.	.	.	**I**	**I**	**I**	.	.	**K**	.	.	.
Haplotype 9 (H9)	2 (3.2)	.	.	**L**	**L**	**E**	.	.	.	.	**I**	**I**	.	.	.	.	.	**V**	**V**
Haplotype 10 (H10)	1 (1.6)	.	.	**L**	**L**	**E**	.	.	.	.	**I**	**I**	.	.	.	.	.	**V**	.
Haplotype 11 (H11)	4 (6.5)	.	.	**L**	**L**	**E**	.	.	.	.	**I**	**I**	.	.	.	**T**	.	.	**V**
Haplotype 12 (H12)	1 (1.6)	.	.	**L**	**L**	**E**	**T**	.	.	.	**I**	**I**	.	.	.	**T**	.	.	.
Haplotype 13 (H13)	1 (1.6)	.	**V**	**L**	**L**	**E**	.	.	.	.	**I**	**I**	.	.	.	**K**	.	.	**V**
Haplotype 14 (H14)	2 (3.2)	.	.	**L**	**L**	**E**	.	.	.	.	.	**I**	.	.	.	**K**	.	.	**V**
Haplotype 15 (H15)	1 (1.6)	.	.	**L**	**L**	**E**	.	**N**	.	.	**I**	**I**	.	.	.	**K**	.	.	**V**
Haplotype 16 (H16)	7 (11.3)	.	.	**L**	**L**	**E**	.	.	.	.	.	**I**	.	**D**	.	**K**	.	.	.
Haplotype 17 (H17)	2 (3.2)	.	.	**L**	**L**	**E**	.	.	.	.	.	**I**	**I**	.	.	**K**	.	.	.
Haplotype 18 (H18)	1 (1.6)	.	.	**L**	**L**	**E**	.	**N**	.	.	.	**I**	.	.	.	**K**	.	.	.
Haplotype 19 (H19)	1 (1.6)	.	.	**L**	**L**	**E**	.	.	.	.	.	.	.	.	.	**K**	.	.	.
Haplotype 20 (H20)	1 (1.6)	**P**	.	**L**	**L**	**E**	.	.	.	.	.	**I**	.	.	.	**K**	.	.	**V**
Haplotype 21 (H21)	1 (1.6)	.	.	**L**	**L**	**E**	.	.	.	.	.	**I**	.	.	.	.	.	.	.
Haplotype 22 (H22)	1 (1.6)	.	.	**L**	**L**	**E**	.	.	.	.	**I**	**I**	.	.	.	.	.	.	**V**
Haplotype 23 (H23)	5 (8.1)	.	.	**L**	**L**	**E**	.	**N**	.	.	**I**	**I**	.	.	.	.	.	**V**	.
Haplotype 24 (H24)	1 (1.6)	.	.	**L**	**L**	**E**	**T**	.	.	.	**I**	**I**	.	.	.	.	**D**	.	**V**
Haplotype 25 (H25)	1 (1.6)	.	.	**L**	**L**	**E**	.	.	.	.	.	**I**	.	.	**L**	.	.	.	**V**
Haplotype 26 (H26)	1 (1.6)	**P**	.	**L**	**L**	**E**	.	.	.	.	**I**	**I**	.	.	.	**T**	.	.	.
Total	62 (100)	–	–	–	–	–	–	–	–	.	–	–	–	–	–	–	–	–	–

Dots (.) represent identical amino acid residues compared to the reference Sal-I strain. Bold letters represent amino acid substitutions compared to the Sal-I strain

substitutions (22L, 24L, 27E, 62N, 230I, 233I, 273 V and 373 V) followed by H5 and H16 (11.3%). There was no sample haplotype that was identical to the Sal-I strain (wildtype).

Genetic diversity and natural selection of *Pvs*48/45 and *Pvs*47

Analysis of genetic diversity and natural selection of *Pvs*48/45 and *Pvs*47 of Thai isolates revealed that the average number of nucleotide differences of *Pvs*48/45 (K = 1.40317) was 2.6-fold lower than that of *Pvs*47 (K = 3.67319) (Tables 3, 4). The haplotype diversity (Hd) of *Pvs*48/45 and *Pvs*47 of all samples was 0.67930 and 0.94183, respectively, whereas the nucleotide diversity (π) was 0.00104 and 0.00321, respectively. Tajima's D values for *Pvs*48/45 and *Pvs*47 were negative (−0.88082 and −0.67448), with a significant difference observed in *Pvs*48/45 ($P < 0.05$), indicating evidence of purifying selection, particularly in isolates from Tak Province ($P < 0.05$). The Fu and Li's D* and F* values of both genes were consistent with Tajima's D values. A significant difference of Fu and Li's D* and F* values was found in *Pvs*48/45 with the value of − 3.31623 ($P < 0.02$) and − 3.35817 ($P < 0.02$), respectively. One haplotype (H) was found in isolates from Yala for both genes, *Pvs*48/45 and *Pvs*47, this might have been caused by clonal transmission in the same area. The dN-dS value of *Pvs*48/45 was positive (0.00088 ± 0.00055) whereas the one for *Pvs*47 was negative (−0.00172 ± 0.00160), no significant difference was found ($P > 0.10$) in both genes. High recombination parameters were found in *Pvs*47 (Ra: 0.0638, Rb: 82.2, Rm: 3) compared to *Pvs*48/45 (Ra: 0, Rb: 0.001, Rm: 2). These high parameter values indicated high meiotic recombination that generated genetic diversity of the gene.

Table 3 Nucleotide diversity and neutrality test of *Pvs*48/45 in *P. vivax* clinical isolates from Thailand

Sample areas	Frequency (%)	S	H	K	Hd	π	Tajima's D (*P* value)	Fu and Li's D* (*P* value)	Fu and Li's F* (*P* value)	dN-dS ± SE
Tak	24 (66.7)	14	10	1.79710	0.79710	0.00133	− 1.82397 ($P<0.05$)	− 2.73505 ($P<0.05$)	− 2.87079 ($P<0.05$)	0.00102 ± 0.00069
Kanchanaburi	5 (13.9)	2	2	0.80000	0.40000	0.00059	− 0.97256 ($P>0.10$)	− 0.97256 ($P>0.10$)	− 0.95440 ($P>0.10$)	0.00077 ± 0.00053
Ranong	2 (5.6)	2	2	2.00000	1.00000	0.00148	0	0	0	0.00192 ± 0.00131
Yala	5 (13.9)	0	1	0	0	0	0	0	0	0
Total	36 (100)	14	11	1.40317	0.67930	0.00104	− 0.88082 ($P<0.05$)	− 3.31623 ($P<0.02$)	− 3.35817 ($P<0.02$)	0.00088 ± 0.00055

Population genetic structure and worldwide Pvs48/45 and Pvs47 haplotype networks

Haplotype networks of worldwide *Pvs*48/45 and *Pvs*47 sequences are presented in Figs. 1 and 2, respectively. Thirty-seven haplotypes were identified in 344 *Pvs*48/45 sequences from 8 countries. Haplotype prevalence of global *Pvs*48/45 ranged from 0.3% to 27.9% with 2 haplotypes at high frequency, H1 (89/344, 25.9%) and H4 (96/344, 27.9%) (Fig. 1). Singletons were found for 16 haplotypes (16/37, 43.2%). The worldwide haplotype network of *Pvs*47 showed a more complicated relationship than *Pvs*48/45 (Fig. 2). Forty-five haplotypes were identified in 138 *Pvs*47 sequences. The haplotype prevalence of *Pvs*47 ranged from 0.7% to 24.6% with the predominant haplotype H1 (34/138, 24.6%) (Fig. 2). Singletons were found in 29 haplotypes (29/45, 64.4%). Interestingly, the frequency of singletons was high in Thai sequences for both *Pvs*48/45 (previous study 4/16, 25.0% [6] and present study 4/16, 25.0%) and *Pvs47* (previous study 5/29, 17.2% and present study 15/27, 51.7%), which indicates a high level of genetic diversity in the Thai population.

The genetic diversity between global populations was investigated, excluding populations with fewer than 20 individuals from the analysis [23, 24]. The diversity of F_{ST} values between *P. vivax Pvs*48/45 and *Pvs*47 from different regions is presented in Tables 5 and 6. Pairwise comparisons of *Pvs*48/45 from the present study in Thailand with those from South Korea and Iran showed high F_{ST} values of 0.22297 and 0.20186, respectively (Table 5). Similarly, comparisons between Iran and South Korea (0.38383) and between Iran and China (0.26870) also revealed high differentiation. Most pairwise comparisons were highly significant, except those involving China. The pairwise comparison of the *Pvs*47 gene between the present study in Thailand and South Korea showed a highly significant F_{ST} value of 0.30849 (Table 6). The high value of F_{ST} fixation index among different regions indicated a high diversity of *Pvs*48/45 and *Pvs*47 between populations.

Discussion

Malaria is an ancient disease that continues to be a significant public health concern in the present era. *Plasmodium vivax* is the most widespread species of human malaria parasites throughout Asia–Pacific and Central and South America [1]. Although most symptoms of *P. vivax* infection are relatively benign recurrent relapse infections, anti-malarial drug resistance and lack of preventive vaccine pose significant challenges to controlling *P. vivax*.

Genetic variations of genes encoding potential vaccine candidate antigens in natural parasite populations represent one of the major obstacles in developing an effective malaria vaccine. The vaccines tend to elicit variant-specific immunity that allows immune escape of other variants. TBVs are considered as a crucial tool for malaria control and elimination, potentially playing an important role in preventing the spread of *P. vivax* parasites. Several studies demonstrated that TBV candidates exhibit limited polymorphism [6, 14, 16, 18, 25–27].

Presently, genetic diversity of *Pvs*48/45 and *Pvs*47 has been reported from some malaria-endemic countries, including Colombia [6], Vanuatu [6], Iran [15], India [16], South Korea [14, 16], China [14], Indonesia [18], and Thailand [6]. In the present study from Thailand, the results demonstrated low genetic variation in the studied population (*Pvs*48/45, $\pi = 0.00104$; *Pvs*47, $\pi = 0.00321$). Fifteen and 18 amino acid substitutions were observed in Pvs48/45 and Pvs47, respectively. High values of recombination parameters were observed in *Pvs*47 in the studied population (Rb = 82.2), suggesting an increased probability of inter- or intra-allelic recombination of *Pvs*47.

The comparison of amino acid substitutions of Pvs48/45 in Thailand with results of previous studies in other countries indicated that the majority of the substitutions observed in Thai isolates (H211N and K250N, 100%) [present study, 6] were similar to the amino acid substitutions reported in isolates from China [14] and Iran [17]. Six amino acid substitutions

Table 4 Nucleotide diversity and neutrality test of *Pvs*47 in *P. vivax* clinical isolates from Thailand

Sample areas	Frequency (%)	S	H	K	Hd	π	Tajima's D (*P* value)	Fu and Li's D* (*P* value)	Fu and Li's F* (*P* value)	dN-dS ± SE
Tak	45 (72.6)	17	24	3.55657	0.93535	0.00311	− 0.58493 ($P > 0.10$)	− 0.95297 ($P > 0.10$)	− 0.98027 ($P > 0.10$)	0.00239 ± 0.00145
Kanchanaburi	6 (9.7)	6	5	2.26667	0.93333	0.00198	− 0.78648 ($P > 0.10$)	− 0.90815 ($P > 0.10$)	− 0.94350 ($P > 0.10$)	0.00079 ± 0.00164
Ranong	4 (6.5)	4	2	2.00000	0.50000	0.00175	− 0.78012 ($P > 0.10$)	− 0.78012 ($P > 0.10$)	− 0.72052 ($P > 0.10$)	0.00223 ± 0.00111
Yala	7 (11.3)	0	1	0	0	0	0	0	0	0
Total	62 (100)	20	28	3.67319	0.94183	0.00321	− 0.67448 ($P > 0.10$)	− 1.18822 ($P > 0.10$)	− 1.19484 ($P > 0.10$)	− 0.00172 ± 0.00160

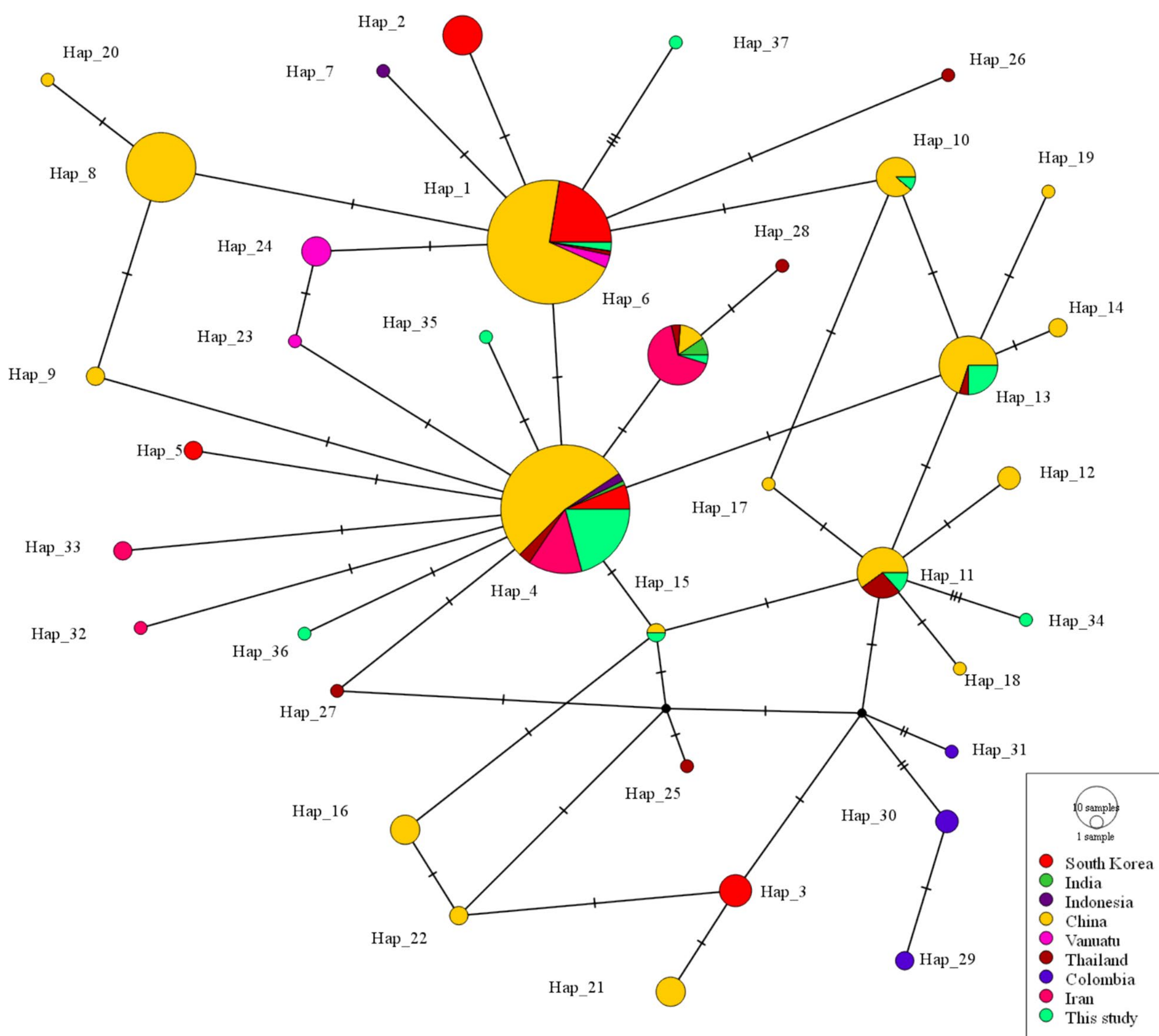

Fig. 1 Haplotype network of global *P. vivax Pvs*48/45. Branch lengths are proportional to divergence; node sizes are proportional to the total haplotype frequencies. The network presents 37 haplotypes found in 344 sequences. Each colour corresponds to a different geographical origin. Lines separating haplotypes represent mutational steps

detected in a previous study in Thailand (E35K, H211N, K250N, D335Y, A376T, and K418R) were found in the present study while 9 amino acid substitutions (R4P, K26R, V41L, N109T, E127Q, E136Q, K185R, K212T, and D214N) were only detected in the present study. Substitution K250N was fixed (100%) and D335Y was highly conserved in most of the studied populations [16, 18, 6,] with exception of Latin American sequences [6, 15]. Substitution E353Q was only found in Latin American sequences and I380T was only found in Korean sequences [16, 18]. The position and prevalence of amino acid substitutions varied in different populations. This suggested that some parasite genotypes may have different geographic distributions [22].

Amino acid substitutions in Pvs47 detected in all samples of the present study were F22L, F24L and K27E which is similar to South Korea [16, 18], India [18], Indonesia [18], Vanuatu [6], and a previous study in Thailand [6]. Other amino acid substitutions that were often detected in the present study, V230I (56.5) and M233I (98.4%), were not found in Colombia and Vanuatu sequences. The amino acid substitutions S62N, K164T and I273V were only found in Thai sequences [present study, 6] while E240D was only found in Vanuatu

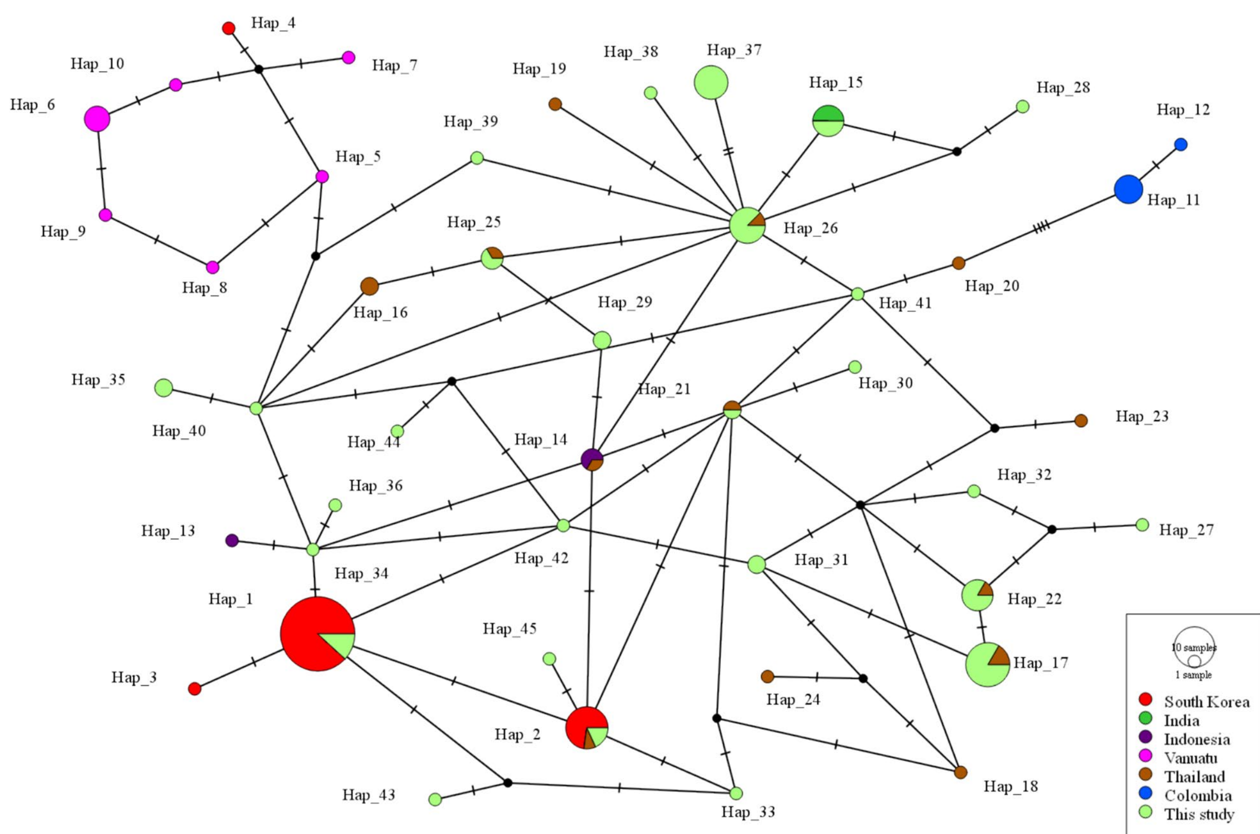

Fig. 2 Haplotype network of global *P. vivax Pvs*47. Branch lengths are proportional to divergence; node sizes are proportional to the total haplotype frequencies. The network presents 45 haplotypes found in 138 sequences. Each colour corresponds to a different geographical origin. Lines separating haplotypes represent mutational steps

Table 5 Genetic diversity of *Pvs*48/45 from four populations by pairwise F_{ST} values (*p* value)

	Thailand, present study	China	South Korea	Iran
Thailand, present study				
China	0.10544 (0.48)			
South Korea	0.22297 (< 0.01)	0.07038 (0.08)		
Iran	0.20186 (< 0.01)	0.26870 (0.17)	0.38383 (< 0.01)	

Table 6 Genetic diversity of *Pvs*47 from two population by pairwise F_{ST} values (*p* value)

	Thailand, present study	South Korea
Thailand, present study		
South Korea	0.30849 (< 0.01)	

sequences [6]. The five amino acid substitutions newly found in the present study were L3P, F6V, K164T, F247L, and E263D. Interestingly, the amino acid changes found in Pvs48/45 and Pvs47 in Thailand changed with time when comparing a previous study with the present study [6, present study]. Therefore, an increase in genetic diversity of *Pvs*48/45 and *Pvs*47 might hinder the efficacy of a vaccine.

The haplotype networks of *Pvs*48/45 and *Pvs*47 from worldwide populations indicate geographical differentiation of these genes. Most of the high frequency haplotypes were shared between populations, especially between the Asian and Latin American populations. The results support a substantial geographic differentiation between *P. vivax* populations supported by a high F_{ST} value.

Studies of various vaccine candidates have highlighted the challenge of genetic polymorphisms in natural parasites which poses a significant hurdle to vaccine development. The results of the present study show low nucleotide diversity of *Pvs*48/45 ($\pi = 0.00104$) and *Pvs*47 ($\pi = 0.00321$). The Tajima's D values of both *Pvs*48/45 and *Pvs*47 were negative with significant difference ($P < 0.005$) in *Pvs*48/45 that were consistent with Fu and Li's D* and F* values. Therefore, the Pvs48/45 and Pvs47 could be useful in TBVs development.

In conclusion, the present study revealed genetic diversity and amino acid substitutions of Pvs48/45 and Pvs47 in Thai isolates. The results are consistent with previous findings and indicate that *P. vivax* TBV candidate antigens exhibit limited sequence polymorphism. However, *P. vivax* isolates from different regions exhibit varying genetic diversity, which might be affected by factors such as anti-malarial drug resistance, transmission intensity, recombination rate, genetic drift, and natural selection. These genetic data could be valuable for malaria control, offering crucial insights for developing effective anti-malarial TBVs and serving as genetic markers to describe malaria parasite diversity in different endemic regions.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12936-025-05305-w.

Additional file 1.

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Author contributions
J.K. Conceptualization, Methodology, Formal analysis, Resources, Data curation, Writing—original draft, Writing—review and editing, Supervision, Funding acquisition. N.S. Formal analysis, Data curation, Writing—review and editing. B.E. and S.H. Formal analysis and Methodology. All authors read and approved the final manuscript.

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Availability of data and materials
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The study was reviewed and approved by the Ethics Committee of Thammasat University (COA No. 042/2564).

Competing interests
The authors declare no competing interests.

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