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Genetic diversity of *pvcsp* and *pvs25* in *Plasmodium vivax* isolates in malaria-endemic areas in Asia, Africa, and America: A systematic review

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Analysis of genetic diversity is an important tool for evaluating *Plasmodium vivax* drug resistance, relapse patterns, and vaccine development. *P. vivax* circumsporozoite protein (*pvcsp*) is involved in sporozoite binding to liver cells. The three *pvcsp* patterns, *i.e.*, VK210, VK247, or *P. vivax*-like types, are indentified in *P. vivax* isolates, which are characterized by repeated sequences. The VK210 pattern is a major *pvcsp* genetic diversity distributed worldwide, demonstrating *P. vivax* variation. Moreover, the circumsporozoite proteins play an essential role in parasite transformation in mosquito development. Among various *P. vivax* sexual stage antigens, *pvs25* is an important antigen for transmission-blocking vaccine (TBV) development. This systemic review focuses on the analysis of current information on the genetic diversity of *pvcsp* and *pvs25* in *P. vivax* isolates from different malaria-endemic areas. The prevalence and patterns of *pvcsp* and *pvs25* reported from various studies depend on geographical distribution and the time of sample collection. The information might facilitate further vaccine development and *P. vivax* control and elimination.

Key words: *Plasmodium vivax,* genetic diversity, *Pvcsp, Pvs25,* transmission-blocking vaccine (TBV).

INTRODUCTION

Malaria is one of the leading worldwide public health problems that affect many tropical regions (Howes et al., 2016). It is caused by intercellular protozoa belonging to the genus *Plasmodium* which is transmitted to humans by infected female *Anopheles* mosquitoes. Five *Plasmodium* species that infect humans include *Plasmodium falciparum*, *Plasmodium vivax, Plasmodium ovale, Plasmodium malariae*, and *Plasmodium knowlesi*. Falciparum malaria is the most virulent species, while *P. vivax* is the most widespread species, with about 80 to 300 million clinical cases annually worldwide (WHO, 2022). *P. vivax* infection may lead to relapse due to the

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release of hypnozoites from livers.

Circumsporozoite proteins are significant molecular markers for understanding *P. vivax* diversity. They constitute the most critical targets for vaccines and have extensively been studied concerning antigenicity and polymorphisms (Kaur et al., 2019). There are three main alleles of *P. vivax* circumsporozoite proteins (*pvcsp*), that is, VK210, VK247, and *P. vivax*-like. VK210 and VK247 distribute worldwide, while *P. vivax*-like subtype is rare. VK247, particularly VK210, are high antigenicity (Cheng et al., 2013). *Pvs25* is well-known as transmission-blocking vaccine (TBV) candidate antigen of *P. vivax*. This antigen is highly expressed on the surface membrane of zygotes and ookinetes of *P. vivax* malaria. This antigen is involved in ookinete survival and oocyst development in the mosquito (Yu et al., 2022). Considering the genetic diversity of *P. vivax*, it is necessary to evaluate the transmission dynamics of vivax malaria accurately (Kang et al., 2013). This systemic review focuses on analyzing current information on the genetic diversity of *pvcsp* and *pvs25* in *P. vivax* isolates from different malaria-endemic areas.

MATERIALS AND METHODS

Data extraction and collection

The systematic review was performed between 2004 and 2020 using PubMed, ScienceDirect, and Scopus databases. The search terms applied were: "*P. vivax*," AND/OR "*P. vivax*," AND "genetic diversity," AND "*P. vivax* circumsporozoite protein," AND/OR "Pvcsp," AND/OR "*P. vivax* sexual stage antigen" AND/OR "Pvs25". The articles published from various journals were retrieved and saved in EndNote X7 reference management software (Clarivate™, Boston, MA, USA) for further analysis. The inclusion criteria were (i) articles related to *P. vivax* gene diversity, (ii) articles related to *P. vivax* circumsporozoite protein (*pvcsp*) and/or *P. vivax* sexual stage antigen (*pvs25*), and (iii) articles available as full-text in English language. The articles were excluded if they were: (i) articles with unclear methodology; (ii) articles related to other *P. vivax* genes; (iii) duplicate articles; (iv) review articles including systematic analysis and meta-analysis; or (v) letters to the editor or editorials.

Two reviewers extracted data independently, and the disparity was resolved by discussion and suggestions from the third reviewer. The following study characteristics were extracted from each article that fulfilled the eligibility criteria: study's objective(s), laboratory technique(s) applied, sample size, type of sample, the country where the study was conducted, and key findings and conclusion. Qualitative data are presented as percentages.

RESULTS

A total of 828 related research articles published between 2004 and 2020 were retrieved from PubMed, Science Direct, and Scopus databases. Two hundred and seventy duplicate articles, 34 review articles, and 79 non-full-text articles were excluded. The remaining 445 articles were available as full texts for eligibility screening. Finally, 43 articles out of 445 articles fulfilling the eligibility criteria were included in the analysis (Figure 1). The analysis of articles related to the genetic diversity of *pvcsp* and *pvs25* are summarized in Tables 1 and 2, respectively.

DISCUSSION

Genetic diversity of *pvcsp*

The circumsporozoite protein (CSP) of the infective sporozoite is the main focus of research and development of recombinant malaria vaccine. A total of 35 research articles reported during 1991-2019 were related to *pvcsp* diversity in *P. vivax* isolates mainly from Asia [Bangladesh (Kibria et al., 2015), China (Huang et al., 2014; Liu et al., 2014), India (Kim et al., 2006; Kaur et al., 2019), Iran (Gholizadeh et al., 2013), Korea (Kho et al., 1999; Kim et al., 2002; Choi et al., 2011; Cheng et al., 2013; Cho et al., 2013)] and South/Central America[Brazil (Kremsner et al., 1992; González et al., 2001; Arruda et al., 2007; Gomes et al., 2016; Pratt-Riccio et al., 2019; Santos et al., 2019), Honduras (Lopez et al., 2012), Mexico (González-Cerón et al., 2013; González-Cerón et al., 2019), Nicaragua (González-Cerón et al., 2013), and Peru (González-Cerón et al., 2013)]. The remaining articles were reported from Europe [Azerbaijan (Kain et al., 1991), Greece (Ioannidis et al., 2013)], Oceania (Gopinath et al., 1994), and West Africa (Chenet et al., 2012)]. The articles involved 31 studies in humans and 4 studies in mosquitoes.

Analysis of genetic diversity is an important tool for determining parasites' populations and origins, whether import or autochthonous cases (Liu et al., 2014). Several molecular techniques were used for the analysis of *pvcsp* variants. These included DNA sequencing, real-time PCR (RT-PCR), PCR-restriction fragment length polymorphism (PCR-RFLP), and nested PCR. Those which detected antibodies against PvCSP included the enzyme-linked immunosorbent assay (ELISA) and indirect fluorescent antibody technique (IFAT). The application of different analysis techniques could influence the analysis results and diversity of frequency. Studies conducted in South America in 1991, India in 2019, Republic Korea in 1991, and Bangladesh in 2015 using different molecular techniques, that is, PCR-RFLP, and nested PCR revealed the diversity of frequency of the *P. vivax* variants (Kain et al., 1991; Kho et al., 1999; Kibria et al., 2015; Kaur et al., 2019). The CSP variation by using PCR-RFLP is particular to each *P. vivax* variant with high sensitivity to detect low parasite levels and also provides information on the prevalence and distribution of the infection of *pvcsp* genotypes in endemic and non-endemic regions, enabling understanding of their phylogeny. Serological methods help identify areas that need malaria to manage and estimate the surveillance system in certain areas (Cho et al., 2013). Antibody exposure via CSP-ELISA could give helpful information

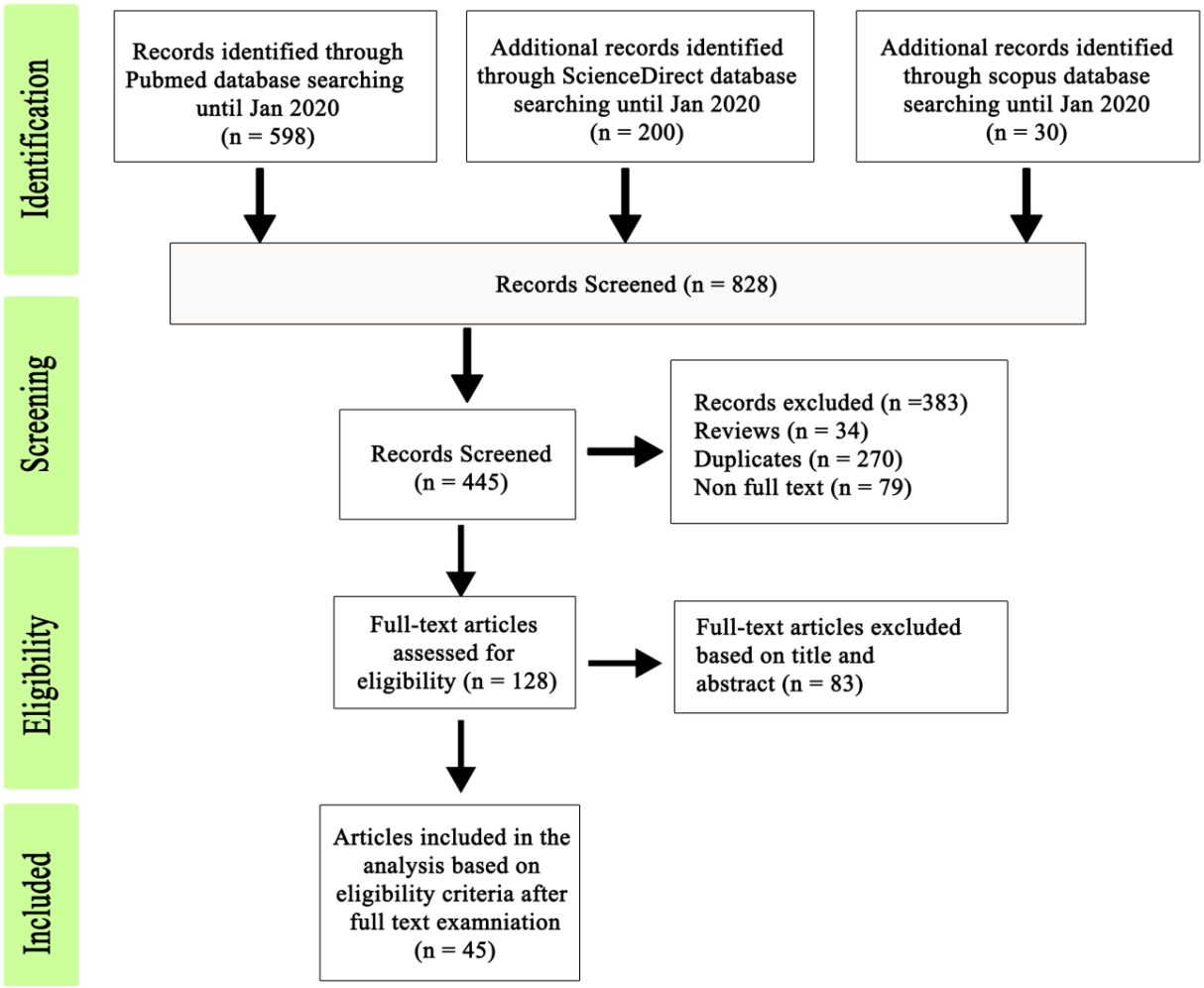

Figure 1. Flow chart summarizing the inclusion and exclusion of the articles for the study.
Source: Authors 2023

about malaria prevalence in certain areas and individuals.

P. vivax global genetic diversity

The knowledges of *P. vivax* diversity help to understand the parasites' populations, evolution and origins. Most human studies focused on genetic diversity of the two main subtypes of *pvcsp,* that is, VK210 and VK247 alone or together with genetic diversity of other *P. vivax* genes (*pvmsp-1, pvmsp-3□, pvtrap, pvdbp, pvama-1,* and *pvs25*) (Brown et al., 1992; Kremsner et al., 1992; Gopinath et al., 1994; Kho et al., 1999; Kim et al., 2002, 2006, 2010; Choi et al., 2011; Chenet et al., 2012; Lopez et al., 2012; González-Cerón et al., 2013; Ioannidis et al., 2013; Huang et al., 2014; Liu et al., 2014; Gomes et al., 2016; Goryacheva et al., 2018; Kaur et al., 2019; Pratt-Riccio et al., 2019). Other human studies involved the investigation of antibodies against both *pvcsp* variants using ELISA, protein assay, and immune blotting (Kremsner et al., 1992; González et al., 2001; Arruda et al., 2007; Cheng et al., 2013; Cho et al., 2013).

Pvcsp VK210 infection is more prevalent in most countries, particularly in the regions of the New World, such as Mexico and Guatemala. *Pvcsp* VK210 was reported as the main variant in Azerbaijan (Leclerc et al., 2004), Bangladesh (Kibria et al., 2015), China (Liu et al., 2014), India (Kaur et al., 2019), Iran (Gholizadeh et al., 2013), Pakistan (Raza et al., 2013), Papua New Guinea (Gopinath et al., 1994), Peru (González-Cerón et al., 2013), and Thailand (Brown et al., 1992; Imwong et al., 2005). VK247 infection is more often recognized in countries such as Thailand (Brown et al., 1992; Imwong et al., 2005) and Papua New Guinea (Gopinath et al., 1994). In Southeast Asia, high genetic diversity and multiplicity of infection of *pvcsp* was reported observed. Increasing *P. vivax* genetic diversity is observed in endemic regions with interstate migration, which requires considerable awareness of malaria control (Gopinath et al., 1994; González et al., 2001; Huang et al., 2014;

Table 1. Genetic diversity of *pvcsp* of *P. vivax*.

Reference	Country	Year	Type of sample	Sample size	Study objective	Method	Genotype and sequence	Key findings/Conclusion
Leclerc et al. (2004)	Azerbaijan	2004	Human blood	36	To investigate the extent of polymorphism of *pvcsp* and *pvmsp-1* genes in *P. vivax* isolates from five localities of central Azerbaijan	RT-PCR, DNA sequencing	VK210 (70%)	Results did not support the use of *pvcsp* sequence analysis for tracking the geographic origin of Azeri isolates probably due to small sample size.
Kibria et al. (2015)	Bangladesh	2015	Human blood	102	To investigate the genetic diversity of *P. vivax* using three well-established markers, *pvcsp, pvmsp-1*, and *pvmsp-3α,* in selected endemic areas of Bangladesh	PCR-RFLP	VK210 (64.7%), VK247 (35.3%)	VK210 and VK247 repeat types of pvcsp: found in field isolates (higher prevalence of VK210) High genetic diversity of *P. vivax* isolates in Bangladesh, suggesting transmission dynamics Essential information to support and monitor malaria control measures, including the design and evaluation of new drugs and vaccines
Kremsner et al. (1992)	Brazil	1992	Human blood	160	To establish antibodies of *P. vivax*, the prevalence and titers of anti-sporozoite antibodies to *P. falciparum* , *P. vivax* and *P. malariae*	ELIZA	VK 247 (25%)	VK 247: widely distributed in the Amazon region of Brazil, but unknown variants may also exist
Alves et al. (2007)	Brazil	2007	Human blood	55	To standardize new PCR-RFLP method for identification of the three described *pvcsp* gene variants	PCR-RFLP	VK247 (58%)	Developed method: adequate sensitivity and accuracy for *P. vivax* genotyping, enabling a better understanding of their phylogeny
Arruda et al. (2007)	Brazil	2007	Human blood	1,206	To investigate the prevalence of malaria infection and antibodies against the repetitive epitopes of the CS proteins of *P. falciparum* , *P. malariae, P. vivax* VK210, *P. vivax* VK247, and *P. vivax*-like in the states of Rondônia, Pará, Mato Grosso, Amazonas, and Acre	ELISA	VK210 (28%), VK247 (66%)	Sero-epidemiological stratification of malaria: valuable tool to recognize how variations inhabit and years of contact with vectors contribute to the level of antibodies and intensity of transmission in malaria-endemic areas Transmission of *P. vivax* VK247 and *P. vivax*-like: common in this region, with variation from state to state
Cassiano et al. (2011)	Brazil	2011	Human blood, Mosquitoes	90	To describe a novel PCR assay using primers for specific regions in the sequences of the CS gene to identify human *Plasmodium* species, and the use of RFLP to discriminate *P. vivax* variants in mosquitoes	PCR-RFLP	*Pvcsp* (84.2%)	The method: highly specific to each *Plasmodium* species and *P. vivax* variants, with comparable efficiency to the gold standard (nested PCR)
Chenet et al. (2012)	Peru	2012	Human blood	106	To investigate the frequencies of *Pvcsp, Pvtrap, Pvdbp, Pvmsp-1* and *Pvama-1* genotypes in Peruvian *P. vivax* isolates collected during 2006-2007	PCR, DNA sequencing	VK210 (97%), VK247 (28%)	Information on the frequency and distribution of haplotypes in different *P. vivax* endemic areas: important for vaccine development strategy
Gomes et al. (2016)	Brazil	2016	Human	91	To investigate the distribution and pattern of *P. vivax* in the town of Oiapoque, Amapá State using the *pvcsp* gene as a marker	PCR-RFLP	VK210 (62.64%), *P. vivax*-like (2.2%), VK247 (1.1%)	Unclear whether *P. vivax* commonly spread from one area to another by migrants or emerge mainly from local endemic populations
Santos et al. (2019)	Brazil	2019	Mosquitoes	895	To describe and compare the distribution of *pvcsp* genotypes in Anopheline mosquitoes from four states of the Brazilian Amazon	ELISA	VK247 (42.20%), VK210 (29.10%)	VK247: more frequent than VK210 in Roraima state (*An. albitarsis s.l* = main vector) Genotyping: adapting in *Anopheles* species

Table 1. Cont'd

Pratt-Riccio et al. (2019)	Brazil	2019	Human blood	95	To characterize *Pvcsp* variants in malaria-endemic region in Brazil and to profile these variants based on parasite sensitivity to chloroquine and mefloquine	PCR, ELISA	VK210 (40%)	VK210: most frequently observed, significant genetic variability *Pvcsp* variants: appeared to influence symptom severity, humoral response patterns, parasite burden, and cytokine balance but unclear influence of these variants on drug response
Huang et al. (2014)	China	2014	Human blood	45	To better understand transmission dynamics of *P. vivax* in China, the extent of genetic diversity of *P. vivax* populations in Bozhou of Anhui province, using three polymorphic genetic markers *pvmsp-1, pvmsp-3α, pvcsp*	PCR-RFLP	VK210 (41.5%)	Some degree of *P. vivax* genetic diversity in these three polymorphic markers (16 unique haplotypes observed among 45 samples) Relatively uniformed genotypes: *pvmsp-3α* type A (100%), *pvmsp-1* R2 (75.6%), *pvcsp* VK210 (41.5%)
Liu et al. (2014)	China	2014	Human blood	1	To describe genetic variants of *pvcsp* in an imported *P. vivax* case from Southeast Asia, who relapsed three months after leaving the area	Nested PCR	VK210 (75%)	Genotyping: useful tool to determine the origin of *P. vivax* and discriminate imported cases from autochthonous cases
González et al. (2001)	Colombia	2001	Human, Mosquitoes	70	To investigate frequencies of the two *pvcsp* variants (VK210, VK247) in wild *P. vivax* isolates from Colombia	IFAT	VK210 (32.8%), VK247 (59.7%)	Useful information on the behavior of *pvcsp* and the complex relationship between parasites, vectors and human immune response Sporozoites carrying the VK247 sequence: more frequently produced in *Anopheles albimanus* than sporozoites with the VK210
Ioannidis et al. (2013)	Greece	2013	Human	1	To perform a phylogenetic analysis of the genes encoding *pvmsp-1* and *pvcsp* in an *P. vivax* isolate from an autochthonous clinical case	PCR	CSP (75%)	Emerging malaria infection in Greece (the doorstep to Europe for thousands of immigrants) Based on *pvcsp* genetic marker: close proximity of the strain in both regions with strains from South America in both trees (Brazil, Colombia), denoting a possible origin of the *Plasmodium* strain from South America
Lopez et al. (2012)	Honduras	2012	Human blood	84	To characterize the genetic variation of *P. vivax* and *P. falciparum* in Honduras using the three polymorphic markers *pvama-1, pvcsp, pvmsp-1, pfmsp-1, pfmsp-2*	PCR	VK210 (100%)	Genetic diversity of both *Plasmodium* spp. despite Honduras being considered a low malaria transmission area Pv VK210: only variant of *pvcsp* Valuable tools to distinguish relapses recurrences and reinfections, and to establish eventual changes in parasite population dynamic
Kaur et al. (2019)	India	2019	Human blood	143	To investigate the genetic diversity of *pvcsp* and *pvs25* among severe and non-severe *P. vivax* isolates from a tertiary hospital (PGIMER, Chandigarh)	NestedPCR	VK210 (99%)	*P. vivax* VK210: predominant in both complicated and uncomplicated groups Population genetic studies: required for understanding the population for identification of signatures of balancing

Table 1. Cont'd.

								selection within *P. vivax* surface antigens Better understanding of genetic variability in different geographical regions: enlightening the role of these genetic variants in severe *P. vivax* malaria
(Kim et al. (2006)	India	2006	Human blood	151	To investigate the genetic diversity of *P. vivax* using the highly polymorphic, single-copy, unlinked genes *pvcsp, pvmsp-1, pvmsp- 3α* in Kollakata	Nested PCR	CSP (65%)	Low rate of multiple genotypes infections despite a high degree of genetic diversity
Gholizadeh et al. (2013)	Iran	2013	Mosquitoes	137	To examine the susceptibility of *A. stephensi* (Asian malaria vector) to *P. vivax* VK210 and/or VK247 in southeastern Iran	PCR-RFLP	VK210 (70.5%), VK247 (17.5%)	Different population structures according to geographical origin Transmission of VK210 especially VK210B haplotypes by *A. stephensi* mysorensis, a good vector in southeastern Iran
Cheng et al. (2013)	Korea	2013	Human blood	194	To investigate the reactivity of human sera obtained from patients infected with VK210 and VK247 on the conserved chimeric rPvCSP and the relationship between clinical response and patients' age and parasitemia	Protein array, Immunoblot assay	VK210 (70.8%), VK247 (100%)	Soluble recombinant chimeric *pvcsp* protein: successfully expressed and purified, which included conserved regions and variant regions of *pvcsp* VK210 and VK247 Specific serological reaction of rPvCSP-c to both VK210 and VK247, suggesting the chimeric protein a potential global serologic marker
(Cho et al. (2013)	Korea	2013	Human blood	1,825	To measure anti-CSP antibodies and compare malaria prevalence	PCR, ELIZA, Western blot	CSP (18.14%)	Antibody detection using CSP-ELISA: useful information on malaria prevalence to identify areas that require malaria control and surveillance
Kho et al. (1999)	Korea (south)	1999	Human blood	30	To investigate polymorphism in *pvcsp* gene of *P. vivax* isolates in Korea	PCR-RFLP	VK210 (75%)	The two genotypes (SK-A, SK-B) coexisting in the epidemic area of South Korea & East Asian isolates RFLP: useful tool for studying *pvcsp* polymorphism in South Korea
Kim et al. (2002)	Korea (south)	2002	Human blood	21	To elucidate the origin of *P. vivax* in Korea	PCR	CSP (99.2%)	Two main subtypes: VK210 & VK247 and another subtype (18 amino acid repeats and different amino acid repeat patterns compared with previous resurgent Korean isolates) Difference in *pvcsp* gene sequences with other geographically dispersed strains
Choi et al. (2011)	Korea (south)	2011	Human Blood	3	To characterize genetic sequences of *pvmsp-1* and *pvcsp* in three imported cases To discriminate an imported *P. vivax* case that was misdiagnosed as indigenous by genetic analysis	PCR and DNA sequencing	CSP (94%)	Genetic monitoring of imported and autochthonous malaria: essential in addition to systemic and continuous monitoring of indigenous malaria to eradicate malaria worldwide

Table 1. Cont'd

Goryacheva et al. (2018)	Kyrgyz Republic	2018	Human Blood	50	To investigate polymorphisms and distribution of *pvmsp-1* haplotypes, *pvmsp-3α, pvcsp*, and *pvdbpII* in parasite populations in Kyrgyz Republic and compared to those in Tajikistan and other countries of Central Asia	PCR	VK210 (13%)	*P. vivax* populations: formed by different isolates from several different population genetic processes South-western area: high level of interstate migration, requiring considerable attention to malaria control
González-Cerón et al. (2013)	Mexico, Nicaragua, and Peru	2013	Human blood	470	To investigate *pvcsp* diversity and gene polymorphism in isolates from the Pacific Ocean coast of Mexico, Nicaragua, and Peru	PCR-RFLP	VK210 (53%), VK247 (25%)	Genome sequences and analysis of haplotype structure: help to reveal complex distribution in Latin America, as well as the relationships with global genetic diversity
Gonzalez-Ceron et al. (2007)	Mexico	2007	Mosquitoes	9	To investigate development of *pvcsp* VK210 in *An. pseudopunctipennis* and *An. albimanus*	PCR, IFA, Western blot	VK210 (16%)	VK210 in *An. pseudopunctipennis:* not observed at the hemocelic midgut surface, and any developing oocysts or undeveloped or damaged parasites occurred on this location VK210 ookinetes: could not escape from and are destroyed within the midgut lumen of *An. pseudopunctipennis*
González-Cerón et al. (2019)	Mexico.	2019	Human blood, Mosquitoes	120	To explore susceptibility of *An. albimanus* and *An. pseudopunctipennis* to *pvcsp* VK/*pvs25*-130 haplotypes from southern Mexico	PCR	VK210 (12.5%), VK247 (20%)	Distribution and dispersion of *P. vivax* haplotypes: might depend on the competence of vector species, which is important for defining control measures Higher frequency of VK247/*pvs25*- infections compared to other haplotypes: in agreement with its higher parasitemia and gametocytaemia
Kim et al. (2010)	Myanmar	2010	Human blood	100	To investigate the prevalence of the *pvcsp* VK247 variant in Myanmar	PCR, IFAT, ELISA	VK210 (47%), VK247 (1%)	Geographic location of Myanmar: large diversity of *Pvcsp* genotypes and serotypes Population migration: may introduce *P. vivax* population with different alleles and complexity
Kim et al. (2011b)	Myanmar	2011	Human blood	100	To clone *pvcsp* VK247 gene expressed in *Escherichia coli* and characterize the antigenicity to determine its usefulness for diagnosis of patients infected with the VK247 variant subtype of *P. vivax*	PCR, Western blot analysis	VK247 (72.4%)	Malaria prevalence in Myanmar: complicated situation. Pure VK247 genotype: rare
Raza et al. (2013)	Pakistan	2013	Human blood	250	To bridge the existing knowledge gap on population structure of *P. vivax* from Pakistan using these two polymorphic genes *pvcsp* and *pvmsp*	Nested PCR/RFLP	VK 210 (85.5%), VK 247 (14.5%)	Increased sequence variation in *P.vivax*: high transmission rates and difficulty in malaria control Extensive polymorphism and diversity of *pvcsp* and *pvmsp*: natural selective pressure on the parasite for its survival and transmission
Gopinath et al. (1994)	Papua New Guinea	1994	Human blood	126	To confirm the global distribution of the *P. vivax*-like parasite in Papua New Guinea	PCR and hybridized with probes specific for the VK210,	VK210 (87.3%), VK247 (38.1 %)	Not confirmed for the existence of the third *pvcsp* or new *P. vivax*-like infection

Table 1. Cont'd

						VK247, and *P. vivax*-like CS variants		
Kain et al. (1991)	South America, West Africa, and The Indian subcontinent	1991	Human blood	6	To develop the method to release and amplify *P. vivax* DNA from whole blood blotted onto filter paper and applied for analysis of the distribution of *pvcsp* in *P. vivax* isolates from South America, Asia, and Africa	PCR	VK210 & VK247	VK247: globally distributed Possibility of simultaneous infection with both VK210 and VK247 Possibility of ineffective single epitope vaccine based on the predominant VK210 form
Maneerattanasak et al. (2016)	Thailand	2016	Human blood	254	To analyze changes in the genetic diversity of *P. vivax* genes from field isolates collected at different times along the Thai-Myanmar border	Nested-PCR, PCR–RFLP	CSP (16.9 %)	High genetic diversity and multiplicity of infection levels in *P. vivax* isolates along the Thai-Myanmar border despite the low level of malaria transmission
Brown et al. (1992)	Thailand	1992	Human blood	137	To investigate the prevalence of *pvcsp* VK210 and VK247 variants	PCR	VK210 (70%), VK247 (7%)	PCR: not practical for diagnosis of mixed infections of both variants in the clinical setting
Imwong et al. (2005)	Thailand	2005	Human blood	100	To develop the field applicably methods of genotyping, which could also complement clinical trials in *P. vivax.*	PCR	VK210 (90%), VK247 (9%)	Genotyping protocols: useful for assessment of *in vivo* drug efficacy in clinical trials conducted in endemic areas and for epidemiological studies of *P. vivax* infections Distinct allelic variants: randomly distributed without evidence of linkage disequilibrium between the two loci

Direct immunofluorescent test (IFAT).
Source: Authors 2023

Goryacheva et al., 2018).

Pvcsp VK247 was reported as the main variant in Colombia (González et al., 2001). On the other hand, the predominant *pvcsp* subtypes reported in some countries (Brazil, Korea, Mexico, and Myanmar) varied according to the study areas and years of investigation. Some studies reported a higher prevalence of *pvcsp* VK210 (Kho et al., 1999; Alves et al., 2007; Gonzalez-Ceron et al., 2007; Cheng et al., 2013; Gomes et al., 2016), while others reported a higher prevalence of *pvcsp* VK247 (Arruda et al., 2007; Kim et al., 2011b; Cheng et al., 2013; González-Cerón et al., 2019). Studies conducted in Brazil in 2007, 2011, and 2012 using PCR-RFLP showed variations in the genetic diversity of *pvcsp* (Alves et al., 2007; Cassiano et al., 2011; Chenet et al., 2012). In 2007, the prevalence of VK247 was 58%. In 2012, the prevalence of VK210 and VK247 were 97 and 28%, respectively (Brown et al., 1992; Alves et al., 2007; Chaurio et al., 2016). The naturally acquired antibodies that identified the VK247 sequence are less prevalent than those recognizing VK210 in some countries like Brazil (Kremsner et al., 1992; Arruda et al., 2007; Pratt-Riccio et al., 2019). The diversity of *P. vivax* suggests the dynamics of parasite transmission which might be contributed to pathogenesis and disease symptoms (Pratt-Riccio et al., 2019). A low rate of multiple genotype infections was observed in some countries, such as India, despite a high degree of genetic diversity (Kim et al., 2006). Population migration may introduce *P. vivax* population with different alleles and complexity (Kim et al., 2010). The difference in diversity patterns of *pvcsp* may depend on natural selective pressure (Raza et al., 2013) and competence of vector species (González-Cerón et al., 2019).

The competence of vector species, susceptibility of *Anopheles* vector to *pvcsp* VK210 and VK247 variants, and the antigenic diversity of these two variants were investigated (Gonzalez-Ceron et al., 2007; Gholizadeh et al., 2013; González-Cerón et al., 2019; Santos et al., 2019). The results provide helpful information on the behavior of *pvcsp* and the complex relationship between parasites, vectors, and human immune

Table 2. Genetic diversity of *pvs25* of *P. vivax*.

Reference	Country	Year	Type of sample	Sample size	Objective	Method of detection	Gene frequency	Findings
Feng et al. (2011)	China	2011	Human blood	30	To analyze the genetic diversity of the two candidate transmission-blocking vaccines (TBVs) genes – *pvs25* and *pvs28* in *P. vivax* isolates from Yunnan Province, China	PCR	*Pvs25*= 100%	Limited genetic diversity of *pvs25* & *pvs28*, suggesting antigenic diversity may not be a particular problem for Sal I based TBVs in most *P. vivax*-endemic areas of China
Kaur et al. (2019)	India	2019	Human blood	143	To study the genetic diversity of *pvcsp* and *pvs25* among complicated and uncomplicated *P. vivax* isolates	Nested PCR	*Pvs25*= 100%	Analysis of genetic variability: understanding of the role of genetic variants in severe *P. vivax* malaria The presence of 100% of double mutants carrying combination of E97Q/I130T in both groups of patients *Pvs25*: a promising vaccine candidate gene
Kang et al. (2013)	Korea	2013	Human blood	86	To investigate genetic variations of the four TBV candidate antigens (*pvs25, pvs28, pvs48/45, pvwrap*) in *P. vivax* Korean isolates	PCR	*Pvs25*= 69.8%	Limited genetic diversity of sexual stage antigens: most likely attributed to the expression of these proteins across mosquito stages, which might avoid immune pressure in humans
González-Cerón et al. (2019)	Mexico	2019	mosquitoes	120	To investigate the susceptibility of *A. albimanus* and *A. pseudopunctipennis* from various geographical sites of southern Mexico to *pvcsp/pvs25* haplotypes	PCR	*Pvs25*= 67.5%	Higher frequency of *Vk247/pvs25* infections compared to other haplotypes: in agreement with its higher parasitemia and gametocytaemia Distribution and dispersion of *P. vivax* haplotypes: might depend on the competence of vector species (important for defining control measures)
Kim et al. (2011a)	Myanmar	2011	Human blood	112	To investigate the profile of antibodies against several antigens of *P. vivax* and *P. falciparum* in Mandalay, Myanmar	IFAT, ELISA	*Pvs25*= 36.7%	Geographic location of Myanmar: contribute to the large diversity in serology of *P. vivax* and *P. falciparum* and thus, low possibility of antibody detection to support the results of microscopic examination
Zhao et al. (2017)	Myanmar	2017	Human blood	1,005	To compare three molecular methods for detecting asymptomatic and submicroscopic *Plasmodium* infections in healthy residents of malaria hypoendemic region in Southeast Asia To assess the prevalence of *P. vivax* gametocyte using RT-PCR and qRT-PCR targeting the *pvs25*	Nested PCR, Nested RT-PCR, CLIP-PCR	*Pvs25*= 54.76%	Nested PCR targeting rRNA: most sensitive *P. vivax:* predominant species 115/210 cases: *pvs25*-positive by qRT-PCR, indicating that a large proportion of asymptomatic individuals were gametocyte carriers
Kuamsab et al. (2012)	Thailand	2012	Human blood	235	To develop a multiplex-nested RT-PCR targeting *pfs25* and *pvs25* mRNA to detect mature gametocytes of *P. falciparum* and *P. vivax*	Nested PCR	*Pvs25*= 91.1%	Patterns and dynamics of gametocyte carriage in malaria-infected individuals: important contributions to the capability of malaria transmission and persistence in each endemic area

Table 2. Cont'd

								Simultaneous detection of both *P. falciparum and P. vivax* gametocytemia by multiplex-nested RT-PCR: useful in areas where both malaria species co-circulate
Chaurio et al. (2016)	USA	2016	Human blood	325	To study the evolution of two major transmission-blocking vaccine antigens, *pvs25* and *pvs28*	PCR	-	The geographic pattern of genetic differentiation and the evidence for positive selection: strongly suggest that the functional consequences of the observed polymorphism should be evaluated during the development of TBVs that include *pvs25* and *pvs28*
Kongkasuriya chai et al. (2004)	USA	2004	Mice blood	25	To investigate the potential of DNA vaccine as the delivery method for *P. vivax* homologs of *pvs25* and *pvs28*	PCR, ELISA, Western blot analysis	*Pvs25* =64%	DNA vaccine based on the *P. vivax* antigens *pvs25 and pvs28*: high titers of specific and effective antibodies to block parasite transmission

Source: Authors 2023

response. Sporozoites carrying the VK247 sequence were shown to be more frequently produced in *Anopheles albimanus* than those carrying VK210 (González et al., 2001). *Anopheles stephensi* mysorensis was shown to be a good vector in southeastern Iran for the transmission of VK210, especially VK210B haplotypes (Gholizadeh et al., 2013). Altogether, the data support the role of *pvcsp* analysis as a tool for parasite control and drug and vaccine development for *P. vivax.* In addition, they also provide useful information on the behavior of *pvcsp* and the complex relationship between parasites, vectors and human immune response (González et al., 2001). Sero-epidemiological stratification of malaria provides a valuable tool to recognize how variations and years of contact with vectors contribute to the level of antibodies and intensity of transmission in malaria-endemic areas (Arruda et al., 2007). Moreover, information on the genetic diversity of *P. vivax* would be helpful for understanding disease severity and thus, malaria control; Choi et al., 2011).

Genetic diversity of pvs25

P. vivax sexual stage antigen provides a strong immunogenicity and potential transmission-inhibiting activities (Kaur et al., 2019). This sexual stage antigen is therefore, considered a transmission-blocking vaccine (TBV). To develop TBV, several antigens have been explored. The PVS25 is a promising sexual stage antigen for vaccine candidate (Kongkasuriyachai et al., 2004; Kim et al., 2011a; Kang et al., 2013; Chaurio et al., 2016).

A total of 9 research articles reported during 2004-2019 were related to *pvs25* diversity in *P. vivax* isolates, mainly from Asia [China (Feng et al., 2011), India (Kaur et al., 2019), Korea (Kang et al., 2013), Myanmar (Kongkasuriyachai et al., 2004; Kim et al., 2011a), and Thailand (Kuamsab et al., 2012)] and South/Central America [Mexico (González-Cerón et al., 2019) and USA (Kongkasuriyachai et al., 2004; Chaurio et al., 2016)]. These involved 7 studies in humans, and 1 study each in mosquitoes (González-Cerón et al., 2019) and mice (Kongkasuriyachai et al., 2004). The objectives of the studies were (i) to investigate the genetic diversity/evolution of *pvs25* in clinical samples collected from various malaria-endemic areas (Feng et al., 2011; Kim et al., 2011a; Kuamsab et al., 2012; Kang et al., 2013; Chaurio et al., 2016; Zhao et al., 2017; Kaur et al., 2019), (ii) to investigate the susceptibility of *Anopheles* mosquitoes to *pvs25* (González-Cerón et al., 2019), (iii) to evaluate the potential of DNA vaccine as the delivery method for *pvs25* (Kongkasuriyachai et al., 2004), and (iv) to develop sensitive and specific molecular methods for *pvs25* detection (Kuamsab et al., 2012; Zhao et al., 2017). The molecular analysis techniques for *pvs25* detection included PCR (Kongkasuriyachai et al., 2004; Feng et al., 2011; Kang et al., 2013; Chaurio et al., 2016; González-Cerón et al., 2019), nested PCR (Kuamsab et al., 2012; Zhao et al., 2017; Kaur et al., 2019), nested RT-PCR (Zhao et al., 2017), capture and ligation probe-PCR (CLIP-PCR) (Zhao et al., 2017), and Western blot (Kongkasuriyachai et al., 2004).

Those which detected antibodies against *pvs25* included ELISA (Kongkasuriyachai et al., 2004; Kim et al., 2011a) and IFAT (Kim et al., 2011a).

The diversity of *pvs25* reported in these studies ranged from 36.7 to 100%. In Indian complicated and uncomplicated *P. vivax* patients, 100% double mutants of *pvs25* were observed (Kaur et al., 2019); all patients had high parasite density and gametocytotemia. Studies conducted in Korea, Myanmar and the USA suggested variations in the genetic diversity of *pvs25* (Kongkasuriyachai et al., 2004; Kim et al., 2011a; Kang et al., 2013; Chaurio et al., 2016). The patterns of *pvs25* polymorphisms were similar among endemic areas, which provide low dynamic changes. *pvs25* might therefore, be a good candidate for TBVs development.

PVS25 protein is expressed before fertilization, achieving peak synthesis in the first hours quickly later, and then most abundantly revealed on the surface of the developing zygotes and ookinetes. The polymorphisms may confer an adaptive advantage to the parasite. The genetic variation observed in that gene and their geographic distribution should be considered by vaccine developers. The geographic pattern of genetic differentiation and the evidence for positive selection strongly suggest that the functional consequences of the observed polymorphisms should be evaluated during the development of TBVs (Kongkasuriyachai et al., 2004). The obligation of several vaccination doses remains the main barrier to DNA and protein immunization. The DNA vaccine based on *pvs25* was shown to increase the titers of specific antibodies that efficiently blocked transmission of the parasite.

Another concern of TBVs, is that genetic polymorphisms might be altered the antigen structure, which reflects to reduce immunogenicity and lead to vaccine ineffectiveness. Although the systematic review showed a similar pattern of *pvs25* worldwide, the genetic change should be further monitored.

Conclusion

The overall prevalence and patterns of *pvcsp* and *pvs25* reported from various studies depend on geographical distribution and the time of sample collection. This systemic review showed different patterns of *pvcsp* among endemic areas, but the sub-genotypes were also reported, which serve as the molecular tool for investigating parasite population, parasite evolution and parasite transmission pattern. While the *pvs25* showed a similar pattern in several countries, this low genetic diversity has been proposed as a promising antigen for vaccine development (TBV). The results of this study will be used as a baseline for future studies.

CONFLICT OF INTERESTS

The authors have not declared any conflicts of interests.

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