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Chapter 1

Circumsporozoite Protein from Plasmodium vivax and Its Relationship to Human Malaria

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Abstract

The circumsporozoite surface protein (CSP) is the most abundant polypeptide in the sporozoite covering. This protein is involved in the motility and invasion of the sporozoite during its entrance in the hepatocyte.

Plasmodium vivax CSP sequences analyses revealed that parasites have repeats belonging to three types of peptide repeat units, named VK210, VK247 or *P. vivax*-like, this last differ from the two previously described variants. All *P. vivax* CS genotypes have a worldwide distribution by genetic and serological evaluation. Studies have also reported differences in the infectivity of anophelines to the variant genotypes, indicating that different malaria vectors were more susceptible to the infection by VK210. These findings could be a consequence of differences in the emergence of this genotype in specific regions around the world. These polymorphisms are associated to the increase of nonregulated inflammatory immune responses, which in turn may be associated with the outcome of infection. Geographic coexistence of these variants increase drug resistance and also recurrent parasite behavior. Knowledge of the *P. vivax* genome contributed to several discoveries, however, new studies are still needed to evaluate its potential as a promising vaccine target.

Keywords: epidemiology, treatment response, vaccine, plasmodium vivax

1. Introduction

Malaria remains an important public health problem in several countries of tropical and subtropical regions of the world. In 2019, the disease caused an estimated 229 million clinical cases and around 409,000 deaths worldwide [1].

Five *Plasmodium* species are more frequently associated with human infection: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium knowlesi*. The last species was recently related in a zoonotic transmission in Asia [2]. Although, cases from an outbreak in the Atlantic Forest
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[Image 99x193 to 524x282]

of Rio de Janeiro state, initially diagnosed as *P. vivax* infection, were in fact caused by *Plasmodium simium*, a neotropical primate parasite [3], other simian malaria parasites, morphologically, indistinguishable from *P. vivax*, the *Plasmodium cynomolgi*, have also been shown to have the potential of zoonotic transmission to humans through the bites of infected mosquitoes under natural and experimental conditions [4, 5]. Among the natural human *Plasmodium* species causing malaria in humans, *P. vivax* is the most widely distributed and prevalent outside of Africa [6], producing more than 80 million cases per year in these regions. Once considered clinically mild when compared with *P. falciparum* infection, *P. vivax* malaria causes debilitating effects that affect social and economic indices of the endemic regions and has been associated with the occurrence of severe cases around the world.

The circumsporozoite protein (CSP) of the infective sporozoite of all *Plasmodium* species can be evidenced in the process of maturation and salivary invasion in the vector as well as in human liver cells [7, 8]. Although it has been a major target in the development of recombinant malaria vaccines, this approach had to be re-evaluated because of the discovery of sequence variation in the repetitive sequence of its central portion gene [9, 10]. All CSPs present a central repeat region (CRR) and two conserved domains RI (region I - located in the amino terminal) and RII (region II – located in the carboxyl terminal). Sequence analyses of the *P. vivax* CRR CSP showed two repeats GDRA(A/D)GQPA or ANGA(G/D)(N/D)QPG belonging to one of the nonapeptide repeat units named VK210 or VK247, respectively [11, 12]. Lately, the *P. vivax*-like was named by Qari et al. [10] to describe an 11-mer repeat sequence, APGANQ(E/G)GGAA containing variant, distinct from the two previously described, isolated from an infected individual in Papua New Guinea (Figure 1, [10, 13]). However, phylogenetic analyses of the SSU RNAr and Cyt B markers positioned both *P. vivax* CS genotypes in the same clade after revealing high similarity and diversity equal to zero between VK210 and *P. vivax*-like [14].

Finally, a high frequency of IgG antibodies against the N- and C-terminal regions of the *P. vivax* CSP was detected in comparison to the immune response to the VK210- and VK247-repetitive regions. Such difference was even more pronounced in *P. vivax*-like variant-caused infection cases. So, it appears that differences among the *P. vivax* CS variants are restricted to the central repeated region of the protein, mostly generated by nucleotide variation, with important serological consequences. These are information of great importance since such genetic diversity can be the product of intra-specific biological signatures, with major implications for the *P. vivax* CSP malaria vaccine trials [14].

Figure 1. Schematic representation of the circumsporozoite protein (CSP) of *P. vivax*, comprising the central repeat region (CRR) flanked by the N- and C-terminal domains, including the conserved regions I and II. The CRR can have three forms denoted VK210, VK247, and *P. vivax*-like.
Epidemiological aspects of the *Plasmodium vivax* CSP variants in endemic areas around the world

*P. vivax* is responsible for approximately 7 million malaria cases worldwide [1]. In 2017, 49 countries reported locally acquired cases of *P. vivax*, where it is estimated that more than 2.5 billion people are at risk of infection [15, 16]. The control of *P. vivax* infections is more challenging compared with *P. falciparum* due to some unique biological features, such as the existence of dormant liver forms (hypnozoites) and early sexual parasite development. Thus, treatment of blood-stage infection is less effective to reduce *P. vivax* transmission, and probably the vaccine development against this species will be indispensable for elimination and eradication of disease.

Most efforts to develop a vaccine against *P. vivax* have focused on the CSP. For this reason, it is important to evaluate polymorphisms in *pvcsp* gene and how its variants are widespread in endemic areas [17].

Seroepidemiological and genetic studies performed in malaria endemic areas could provide valuable information on parasite transmission and dispersion. The genetic diversity of the CSP gene has been useful in molecular epidemiological studies, understanding the transmission, dynamics, and evolutionary relationships [14]. Furthermore, eco-epidemiological conditions, biological and genetic characteristics of the parasite, the host immunity, and local vectors may influence the different patterns of demographic expansion. On the other hand, the human intervention can become a factor to reduce the risk of malaria, without necessarily modifying the environment [18]. Serologic surveys in *P. vivax* isolates from Thailand [19, 20] and Myanmar [21] demonstrated the presence of antibodies that recognize recombinant and synthetic polypeptides that represent the VK210 and VK247 (alone or mixed). *PvCSP* antibodies in high-risk malaria areas in Korea [22] and sub-Saharan Africa [23] were detected. The Korean findings suggest that antibody levels for the CSP antigen could become deficient or reduced over the long-term incubation period. Mexican [24, 25] and Peruvian [24] sera reacted with either VK210 or VK247 repeat domains.

It has been shown that the prevalent phenotype of the *P. vivax* parasite in the study sites of Colombia is VK247, whereas VK210 accounts for one-third of the cases, and few *P. vivax* malaria cases correspond to mixed infection [26]. Antibodies to these three variants have been circulating in the Brazilian Amazon population [27–30]. In addition, Oliveira-Ferreira et al. [31] have observed the profile of IgG responders to these *PvCSP* variants, where more responders to VK210 were found, followed by *P. vivax*-like and VK247 in Rondônia state. Cross-sectional cohort study included individuals from three different communities with malaria transmission in Acre state, demonstrating that VK210 presented the highest prevalence of responders to IgG antibodies, followed by *P. vivax*-like and VK247 [32]. Besides, the higher frequency of antibodies to VK210 is according to studies that described this variant as the most common in Amazon, while VK247 was rarely reported as a single infection [14, 27]. However, unlike previous reports from different areas of the Brazilian Amazon, the VK247 variant was found in higher frequencies than VK210 in Goianésia do Pará, Para State [29]. The higher frequencies of VK247 in current infections compared with higher antibody responses to VK210 may suggest that VK210 protein is more immunogenic than VK247 [33, 34], and also that the VK210 variant could have been more prevalent in endemic area from Amazon region in the recent past, as the majority of the patients had a previous history of malaria infections. Nevertheless, nucleic-acid-based diagnosis evaluates the genotypes of the current infection while antibody-based techniques are able to signalize positive results several months and even years after...
Further, there is a possibility that hypnozoites/relapses do influence the antibody response to sporozoites, since the presence of a blood-stage infection may not necessarily indicate a new infection [29, 34].

Using DNA techniques, the VK210 and VK247 sequences were found in P. vivax isolates from Philippines [35] and Cambodia/Thailand [36]. The cosmopolitan variant VK210 also predominate in Sri Lanka [37], Myanmar [38], Iran [39], Vanuatu Island [40], and China [41]. VK247 seems to be more common in South-eastern Asia [42, 43]. In fact, VK210 and VK247 genotypes have a worldwide distribution; however, the P. vivax-like genotype has been only detected in Papua New Guinea, Brazil, Indonesia, and Madagascar [13, 42, 44]. Sequence analysis of the C-terminal nonrepeat region of Myanmar VK210 variants revealed 27 distinct haplotypes. The sequence of haplotype 22 was identical with Sal I (GU339059) and accounted for 9.8% of all the VK210 sequences. The C-terminal nonrepeat region of VK247 variants showed a lower level of genetic diversity than that of VK210 variants [45]. Moreover, pvcsps sequencing for samples collected in Oman displayed the VK210 type with a single haplotype (six repeats of GDRADGQPA and nine repeats of GDRAAGQPA), and the malaria vector in Oman is Anopheles culicifacies [46]. VK210 and VK247 ratio in China was extensively investigated in several provinces, and the coexistence was verified only in Yunnan, Hainan, and Liaoning provinces, whereas in Anhui, Hubei, Guangxi, Guangdong, Guizhou, Sichuan, Jiangsu, also endemic, only the VK210 type was detected.

P. vivax was transmitted by distinct Anopheline species in China, in Henan by An. Sinensis, and in Hainan by Anopheles dirus and Anopheles minimus, but these two provinces had the same genotypes [47].

Malaria transmission currently exists in 21 countries and territories of the American continent, where 132 million people are at risk of infection [48]. In southern Mexico, VK210 and VK247 CSP phenotypes were detected and associated to the parasite infectivity to the local mosquito vectors Anopheles pseudopuntipennis and Anopheles albimanus [49, 50]. In samples of P. vivax from Mexico, Nicaragua, and Peru, it was observed that the genetic diversity of the CSP gene is restricted mainly to the central repeat domain and 3′-terminal portion [18]. This variation occurs due to changes in the type of nucleotides and number of repeats of the repeat region, and it is possible that the repeat region of VK247 is more stable than VK210. The predominance of VK247 parasites was documented on the Pacific Ocean coast of Western Colombia and in Piura, Peru [51]. An. nuneztovari infected with the VK210 and VK247 genotypes in Colombia, showing its importance for malaria transmission in areas with anthropic intervention [52].

P. vivax samples in the North, North-West, and South of Guatemala [53] and in municipalities of Honduras [54] were all of the VK210 genotype. In Brazil, the detection of three P. vivax CSP genotypes has been observed in different areas from Amazon [44, 55–58], pre-Amazon [59], as single and mixed infections, and extra-Amazon region [60]. The VK210 genotype remains the most prevalent, most likely because of the great susceptibility of the An. darlingi vector, which is the most abundant in the country, to this variant [61]. The P. vivax-like genotype had a low frequency of the genotyped samples; this frequency could be due to its recent introduction into the region or due to differences in the development of this genotype in the vectors present in the area [27, 55]. Furthermore, the VK210 and VK247 were detected in An. aquasalis and An. darlingi in endemic areas of Pará State [61]. Although the VK210 genotype remains the most prevalent in Brazil, new evidence reveals a strong adaptation of the VK247 variant in southeastern Pará state, as well as the association of this genotype with high parasitemia.
Anopheles oswaldoi in Acre State [62, 63]. Despite the association between high parasitemia and the VK247 genotype, the introduction of the VK247 and VK210 genotypes may have occurred at different times according to the endemic area [57].

3. CSP variants in Plasmodium vivax isolates from malaria-endemic region and to profile these variants based on sensitivity to antimalarial drugs. As described above, *P. vivax* has a complex life cycle distinct with different stages between vector and host with a dormant liver stage. To achieve optimal results for *P. vivax* infections, effective clearance of both blood-stage parasites to treat the acute infection and liver-stage parasites (radical cure) to prevent relapse is required; however, the continual rise and propagation of resistance against antimalarial drugs are of great concern to successfully [64]. Despite increasing reports of resistance, chloroquine (CQ) remains highly effective for treatment of strains from temperate South American countries, some parts of Eastern Mediterranean, and parts of Southeast Asia [65]. The first evidence that *P. vivax* is developing resistance to CQ was reported in Papua New Guinea by Rieckmann et al. [66]. It is difficult to ascertain how common CQ resistance is in *P. vivax* infection, particularly as resistance does not appear to be absolute [67]. On the other hand, reduction in susceptibility to CQ was reported from Solomon Island [68], Papua New Guinea [69, 70], and India [71]. Brazilian studies also assessed the efficiency of standard supervised therapy or the *in vitro* profile of mefloquine (MQ) and CQ resistance showing failure of the treatment [72–74].

Another important issue is that the response to the treatment might possibly differ depending on the genotype of the parasite. However, the influence of these variants on drug response remains unclear. Studies conducted by Kain et al. [75] showed that the response to CQ may vary depending on the type of *P. vivax* variant, as both single VK210 and VK210/VK247 mixed infections took longer to clear when compared with single VK247 infection in Thailand. Later, two studies conducted in Brazil showed contradictory results. One demonstrates a correlation between the *P. vivax* variant and the response to CQ [56], and the other does not observe any difference in the frequency of the resistant isolates and in the IC50 mean for CQ or mefloquine, according to VK210 subtypes [74]. Additional studies will be necessary to enable a better understanding of whether individuals in endemic areas acquire *P. vivax* CSP variants that have preferential ability to malarial drug resistance.

4. Vaccine containing the three allelic variants of the Plasmodium vivax circumsporozoite antigen. Immunologically, the course of infection by *Plasmodium* depends on the production of pro- and anti-inflammatory cytokines. In cases where an inflammatory pattern is prevalent, the disease tends to be more severe. Nonetheless, the upregulation of anti-inflammatory cytokine appears to occur after the increase in inflammatory cytokines, due to a regulatory mechanism, to prevent the exacerbation of inflammatory response and its deleterious effects [76, 77]. Moreover, *P. vivax* sporozoites are covered with CSP, a highly immunogenic protein, recognized mainly by B lymphocytes.
The good-responder immune profile against CS repeats of VK247 in individuals carrying the typically Amerindian HLA specificity DRB1*16 and the non-responder profile against CS repeats of VK210 in individuals carrying HLA DRB1*07 was previously determined [31]. Patients with the VK210 variant showed a regulatory cytokine profile in plasma, while those infected with the VK247 variant have a predominantly inflammatory cytokine profile and higher parasite loads, which altogether may result in more complications in infection. In other Brazilian Amazon areas (Maranhão and Pará state), the CSP polymorphism is associated with the increase of nonregulated inflammatory immune responses, which in turn may be associated with the outcome of infection [59]. In addition, individuals with the rs16944 CC genotype in the IL1β gene have higher antibody levels to the CSP of P. vivax of VK247 and P. vivax-like variants [29].

The development of a vaccine with satisfactory efficacy for malaria would be an important strategy for the control of the disease, mainly because it provides a tool for the prevention of this parasitosis, with relevant aspects of the cost–benefit relation and would bring a solution that overlays the adaptive strategies of parasites and vectors. Despite the general obstacles that need to be overcome in the development of vaccines against parasitic diseases, researchers who develop vaccines against P. vivax face other adversities. One of them is the fact that these protozoa have the formation of hypnozoites, which can cause relapses months and even years after the primary infection [78]. In endemic areas of malaria transmission, individuals with repeated exposure to the parasite tend to develop clinical immunity to both P. falciparum and P. vivax malaria. Since the central portion of CSP is highly immunogenic and the induction of a protective response in animals immunized with sporozoites and in humans has been observed in experimental models, CSPs are being investigated as candidates for a human vaccine against malaria. However, despite decades of research, a highly effective vaccine still remains elusive. To date, only one vaccine formulation against P. falciparum has been licensed, RTS, S, manufactured by GlaxoSmithKline (GSK), which showed limited protective efficacy in young children (approximately 36%). RTS, S is a recombinant vaccine produced in Saccharomyces cerevisiae, which comprises the C-terminal portion of PfCSP and repeat regions fused to the surface antigen (S) of hepatitis B virus. This vaccine may contribute substantially to the control of malaria when used in combination with other control measures, especially in high-risk areas [79].

This vaccine is based on CSP, the major surface antigen present in sporozoites that is critical in liver-stage development during the pre-erythrocytic life cycle [29]. CSP has a central immunodominant region of tandem repeats flanked by two highly conserved regions that encode the amino terminal and carboxy terminal regions. The most advanced vaccine candidate (phase II clinical trial) to prevent malaria by P. vivax also targets CSP. Nonetheless, differently from P. falciparum, the PvCSP exhibits diversity in its central repetitive domain, defining the variants VK210, VK247, and P. vivax-like. The P. vivax CSP vaccine was also combined into multivalent formulations or chimeric synthetic molecules. Peptides based on the N-terminal, central repeat, and C-terminal regions of PvCSP were immunogenic in individual administrations of experimental models (mice and monkeys) as well as in healthy human volunteers [80]. Another major difficulty that must be overcome is the absence of P. vivax infection in rodents, and in this way, preclinical evaluations on the vaccine protective efficacy are mainly restricted to nonhuman primates. In addition to the ethical issues, these
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Animals must undergo a surgery to remove the spleen in order to promote adequate parasitemia. This procedure may add significant result interpretation bias since organ removal causes immunological changes [81]. Thus, vaccine formulations against *P. vivax* malaria are based on chimeric parasites expressing parasite proteins [82].

The use of transgenic parasites of CSP-based vaccine formulations for the pre-erythrocytic phase of infection has allowed the analysis of functional inhibition of the exogenous CSP, expressed in replacement of the endogenous protein. Likewise, chimeric *P. berghei* parasites expressing the VK210 and VK247 were used to determine the protective efficacy of vaccine formulations consisting of viral vectors carrying *P. vivax* CSP alleles [82]. The results showed that this vaccine could induce protective and effective immune responses against *P. vivax* and that these findings could contribute to the development of a potential vaccine against malaria.

Another subunit vaccine against *P. vivax* malaria is also undergoing clinical trials. Named VMP001, this vaccine was expressed in *Escherichia coli* and encodes a chimeric CSP containing repeated sequences of the two alleles VK210 and VK247. The first Phase 1 trial with VMP001 showed that the vaccine was well tolerated and immunogenic, generating robust humoral and cellular responses to the vaccine antigen. The results did not demonstrate a protective sterilizing immunity; however, a delay to significant parasitemia was observed in more than 50% of the vaccinated individuals, compared with that seen in the control group [83].

Another study for *P. vivax* vaccine [79], which performed the expression in *Pichia pastoris* two chimeric proteins by merging the three central repeat regions of different CSP alleles (VK210, VK247, and *P. vivax*-like), after challenge with *P. berghei ANKA* transgenic parasites expressing Pb/PvVK210 or Pb/PvVK247 sporozoites. Significant time delays to parasitaemia were observed in all vaccinated mice. Thus, these formulations have potential for clinical evaluation due to their potential as protective vaccines against *P. vivax* malaria. Then, a group in Brazil describes the immunogenicity analysis of the vaccine formulations composed only by the *PvCSP-AllFL* chimeric and the influence of the *PvAMA-1* combination [80]. The *PvAMA-1* recombinant protein has also been previously described. The recombinant protein *PvCSP-AllFL* contains both N- and C-terminal and also the central repeats sequence of the *P. vivax* allelic variants while the central region contains six copies of the VK210 sequence (GDRA[A/D]GQPA), followed by six copies of *P. vivax*-like repeats (APGANQEGGAA) and five copies of the VK247 sequence (ANGAGNQPG). Laboratorial analyses using sporozoites from the *P. vivax* strain VK210 and blood-stage isolates demonstrated that these vaccine-elicited antibodies can recognize the native proteins. Immunization using this vaccine induced approximately a five fold decrease in parasitemia as assessed at day 5 post challenge, however, was not enough to neutralize the VK210 sporozoite infection.

5. Concluding remarks

*Plasmodium vivax* is the most widespread and the second most prevalent malaria-causing species in the world. CSP, although highly informative, is not a perfect measure of this parasite genetic diversity. However, this could provide interesting baseline data that allow identifying potential new cases infected by parasites diverse from those currently circulating in a determined area. Individuals residing in malaria-endemic areas may be infected with different parasite genotypes, resulting from multiple bites from infectious mosquitoes or bites from mosquitoes infected with *P. vivax*. Please use Adobe Acrobat Reader to read this book chapter for free. Just open this same document with Adobe Reader. If you do not have it, you can download it here. You can freely access the chapter at the Web Viewer here.
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