Evaluation of genetic variability and its potential impact on the antigenicity of B-cell epitopes of the Pv41 and PvGAMA protein in Brazilian isolates of *Plasmodium vivax*

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Malaria parasites exhibit extensive genetic polymorphisms, many of which have evolved to evade host immune responses, posing a significant challenge to developing an effective malaria vaccine. In this context, the protein Pv41 (Plasmodium vivax Protein 41), a conserved surface protein expressed during the asexual (merozoite) stage, and PvGAMA (P. vivax GPI-anchored micronemal antigen), a GPI-anchored protein involved in parasite cell adhesion, has been identified as both antigenic and immunogenic. To investigate the genetic variability of these proteins and their potential impact on immune recognition, the study followed these steps: (1) amplification of the genes encoding Pv41 and PvGAMA from isolates obtained from 72 isolates of Pv41 and 24 isolates of PvGAMA different endemic regions of the Amazon - Belém (2), Mâncio Lima (10), Cruzeiro do Sul (10), São Gabriel da Cachoeira (21), Guajará (2), Rio Branco (5), Manaus (8), Oiapoque (7), Porto Velho (7) using polymerase chain reaction (PCR), followed by DNA sequencing via the Sanger method; (2) identification of B-cell epitopes using multiple immunoinformatics tools, including Bepipred, BCPREDS, and Vaxijen; and (3) analysis of the effect of antigenic variation in the most promising epitopes on the observed immune response. Analysis of the pv41 gene revealed both synonymous and non-synonymous mutations. Notably, one non-synonymous mutation occurred within the PEKQIDEHL epitope at position 170 (H170L) and A509T, affecting the antigenicity of this predicted epitope. In the N-terminal region of PvGAMA, two non-synonymous mutations were identified. Sequencing of this region from samples collected in Porto Velho (9) and Manaus (15) revealed a single nucleotide polymorphism (SNP) common to both locations (95% prevalence). This SNP (T269C) represents a non-synonymous mutation that distinguishes the reference strain Sal-1 from the VCG-I strain (Colombia) and P01 strain (Papua Indonesia). In summary, this study indicates that Pv41 and PvGAMA exhibit moderate sequence variation, which may influence potential B-cell epitopes and antibody recognition. Despite these observed polymorphisms, further research is necessary to assess the functional impact of specific antibodies on both conserved and mutated regions.

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