Genetic Diversity of the PvCSP Across Nine Municipalities in the Brazilian Amazon Region

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Introduction: The circumsporozoite protein of *Plasmodium vivax* (*Pv*CSP) is the main surface antigen of the sporozoite stage and plays a critical role in hepatocyte invasion. Three variants VK210, VK247 and *P. vivax*-like circulate in endemic regions and are key targets for vaccine development. However, PvCSP exhibits significant genetic polymorphism, particularly in its central repeat region (CRR), which may affect antigenicity and immune recognition. Characterizing PvCSP diversity is essential to support vaccine design and understand parasite population dynamics. **Methodology:** A total of 114 dried blood spot samples collected from multiple municipalities in the Brazilian Amazon were analyzed. Genomic DNA was extracted from filter paper and subjected to nested PCR targeting the PvCSP gene, using primer pairs designed to amplify the region spanning from conserved region I (RI) to conserved region II (RII). Amplified fragments were visualized by agarose gel electrophoresis. PCR products were directly purified; when multiple bands were observed, higher molecular weight bands were excised and purified prior to Sanger sequencing. Sequences were edited and aligned for CRR pattern analysis. **Results:** Sixty-two samples were positive for PvCSP amplification, yielding 63 high-quality sequences. All isolates were identified as the VK210 variant. Comparison with the Sal-1 reference sequence showed that three isolates were identical to Sal-1, whereas the remaining sequences generated 20 distinct CRR patterns. These patterns differed in the number and arrangement of repeat units and included both synonymous and non-synonymous polymorphisms, demonstrating substantial intra-variant structural diversity across municipalities. **Conclusion:** Despite the exclusive detection of VK210, considerable structural diversity was observed within the CRR, an immunodominant region of PvCSP, reinforcing the importance of detailed molecular characterization in endemic settings to PvCSP-based vaccine design.

Keywords: Gene polymorphism, Molecular epidemiology, Genetic diversity