Genetic Diversity of *Plasmodium falciparum* Reticulo-cyte-Binding Protein Homolog 5 (RH5) in Brazilian Amazon Isolates

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Background: Malaria caused by *Plasmodium falciparum* continues to pose a serious health challenge in the Brazilian Amazon, where residual transmission sustains a significant burden despite intensified control strategies. Among parasite invasion ligands, the reticulocyte-binding protein homolog 5 (RH5) has emerged as a leading vaccine candidate due to its essential, non-redundant role in merozoite entry and its unusually restricted genetic variability. **Methods:** To investigate the regional landscape of genetic variation, we analyzed the *pfrh5* gene in 131 isolates collected between 2016 and 2024 across five Amazonian municipalities. Diversity and structure were profiled through nucleotide/haplotype diversity, linkage disequilibrium and population differentiation, with temporal-stability analysis, haplotype-network inference, and structural mapping with in-silico B-/T-cell epitope prediction. **Results:** Diversity was minimal (six haplotypes from four nonsynonymous sites). C203**Y** predominated; Y147**H**/H148**D** was scarcely observed and restricted to Boa Vista (perfect linkage); I410**M** was infrequent, sometimes co-occurring with C203**Y**. Despite sparse variation, clear geographic structure separated Pacaraima from Cruzeiro do Sul/Mâncio Lima/Guajará, which were otherwise undifferentiated. Allele frequencies were stable across 2016–2024, neutrality analyses showed no strong departures, and pooled data indicated excess identical haplotypes consistent with Y203 dominance. Structural/epitope mapping placed C203**Y** at the basigin-binding interface and Y147**H**/H148**D/**I410**M** within predicted T CD4 epitopes. **Conclusions:** In the studied malaria-endemic municipalities, *pfrh5* is highly conserved and regionally structured, with no evidence of strong selection, reinforcing RH5 as a promising vaccine target and underscoring the need for continued genomic surveillance.

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