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学位論文要旨
Dissertation Summary

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論 文 名：抗原多型の少ないマラリアワクチン候補抗原 PfRipr の同定
(Dissertation Title): Identification of Plasmodium falciparum reticulocyte binding protein homologue 5-interacting protein, PfRipr, as a highly conserved blood-stage malaria vaccine candidate

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BACKGROUND.

Malaria is a life threatening disease caused by five parasite species within the genus Plasmodia including, *P.falciparum, P.vivax, P.malariae, P. ovale,* and *P. knowlesi*. Of these, *P.falciparum* causes the most severe infections and number of deaths. An infected female Anopheles Mosquito vector transmits malaria. According to the latest World Health Organization (WHO), there were an estimated 212 million new cases of malaria, and 429,000 deaths, 90% of which occurred in sub-Saharan Africa in 2016. Global efforts to control malaria burden rely heavily on the availability and proper use of insecticides to kill the vector by using Insecticide Treated Nets (ITNs) and In-door Residual Spraying (IRS) and the effective antimalarial drug, artemisinin. In the period between 2000 and 2015 increased funding scaled up the use of these effective malaria control intervention especially in the Africa region. This resulted in the reduction of incidence rates by 37% globally, and 42% in Africa, and mortality rates by 60% globally, and 66% in Africa. However, there is a worrisome concern of development and spread of parasite resistance to the artemisinin, and mosquito resistance to the insecticides, that could threaten the progress. There is therefore, a global demand for development of especially effective malaria vaccines that could complement current effective control measures.

The malaria parasite has a complex life cycle that alternates in both the mosquito vector and the human host. In the human host, different stages of the parasite can be found in different organs of the body of during infection. This exposes different sets of parasite proteins (antigens) to the host immune system. The exposure renders the development of an effective malaria vaccine an uphill task. An effective malaria vaccine would require a polyvalent multicomponent vaccine with a combination of candidate antigens from different stages of the life cycle. Hence the approach of targeting vaccine development by differentiating between stages including, Pre-erythrocytic stage vaccine that targets

prevention of sporozoites entry and development in the liver, Asexual blood-stage vaccine that targets disease prevention through blocking of merozoite invasion and intra-erythrocytic parasite development, and Transmission-blocking vaccine that targets sexual and sporogonic stages to prevent parasite development in the mosquito.

PROBLEM STATEMENT

The RTS,S vaccine, a leading pre-erythrocytic subunit vaccine and only vaccine that has completed phase 3 trial, showed moderate level efficacy of modest duration. Moreover, as analysed, determinants of RTS,S induced immunogenicity in the final results of the phase 3 trial. The analysis revealed anti-CSP antibody titers, a surrogate marker of protection for the magnitude and duration of the vaccine efficacy, waned more rapidly during participant follow-up at especially higher transmission intensity because of reduced titers levels and lesser blood-stage immunity. The finding is a significant limitation that clearly highlights the importance of blood-stage immunity in preventing malaria.

Targeting vaccines against blood-stage merozoite antigens would improve vaccine efficacy. However, the most advanced blood-stage vaccine candidates like FMP2.1/AS02A, a subunit vaccine based on P. *falciparum* 3D7 apical membrane antigen 1 (AMA1) sequence, have suffered poor efficacy in human trials due to high genetic polymorphisms of AMA1 that induce not only allele-specific immune responses but also suboptimal concentrations of functional antibodies against malaria parasites.

THE STUDY RATIONALE

The extensive genetic diversity and polymorphisms in several *P.falciparum* malaria antigen-coding genes arise as a result of selection by the human immune system. Novel, relatively conserved antigens that induce broadly cross-reactive antibody and cell-mediated immune response may provide longer lasting and more efficacious protection. There is therefore, need to prioritize candidate peptides that comprise of conserved epitope targets of immunity in the design of next generation vaccines. The approach of population genetic and structural studies, followed by molecular epidemiological surveys or *in vitro* functional studies has been instrumental in identifying immunologically relevant diversity in pathogens prior to development and testing of vaccines.

SIGNIFICANCE AND OBJECTIVES OF THE THESIS

The thesis attempted to contribute to the 2030 WHO Global Technical Strategic goals of reducing the burden of malaria by identifying of highly conserved *P.falciparum* antigen targets of robust natural immunity across multiple *P. falciparum* strains, for designing an efficacious next-generation malaria vaccine.

Specifically the thesis' aims were,

1. To explore extent of polymorphism and genetic diversity in PfRipr, PfGAMA, PfRALP1 and PfMSPDBL1 by utilizing current genetic population analysis tools. .
2. To evaluate antibodies against WGCFS expressed recombinant PfRipr, PfGAMA, PfRALP1 and PfMSPDBL1 proteins based on *P. falciparum* 3D7 DNA sequence in inhibiting growth (GIA) of strains 3D7 and FVO.

MATERIALS AND METHODS

The study utilized a total of 102 *P. falciparum* clinical isolates from a region of high malaria transmission in Uganda, and *P. falciparum* laboratory strains 3D7 and FVO. We also searched and selected Single Nucleotide Polymorphisms (SNPs) among 164 to 203 *P. falciparum* isolates from the online PlasmoDB. We used tools of population genetic analysis to assess extent of polymorphism and genetic diversity in the four above mentioned merozoite

proteins; PfRipr, PfGAMA、PfRALP1, and PfMSPDBL1. These proteins were recently reported as well characterized potential blood-stage vaccine candidates that are immunogenic with minimal genetic variability in a few field isolates and laboratory strains. *P.falciparum* AMA1 and the housekeeping protein, adenylosuccinate lyase (ADSL) were positive and negative controls respectively. Furthermore, we employed the principle of reverse vaccinology by utilizing WGCFS to express recombinant proteins for the four vaccine candidates based on *P. falciparum* strain 3D7 sequences, immunized rabbits to obtain specific antibodies and performed growth inhibition assays (GIA). The GIA activity of the raised antibodies was demonstrated using both homologous 3D7 and heterologous FVO strains *in vitro*.

RESULTS

We demonstrated that approximately 50% of the selected PlasmoDB SNPs were unique to the Uganda isolates, suggesting a finding of new variants in this population. Genetic analyses showed that *pfgama* and *pfmspdbl*1 are polymorphic and genetically diverse, but both *pfripr* and *pfralp*1 are less polymorphic. *Pfralp1* is however, comparatively more diverse than *pfripr*, due to existence of insertion-deletion (INDELs), asparagine and 6-mer repeat regions in the sequences (Figure1).

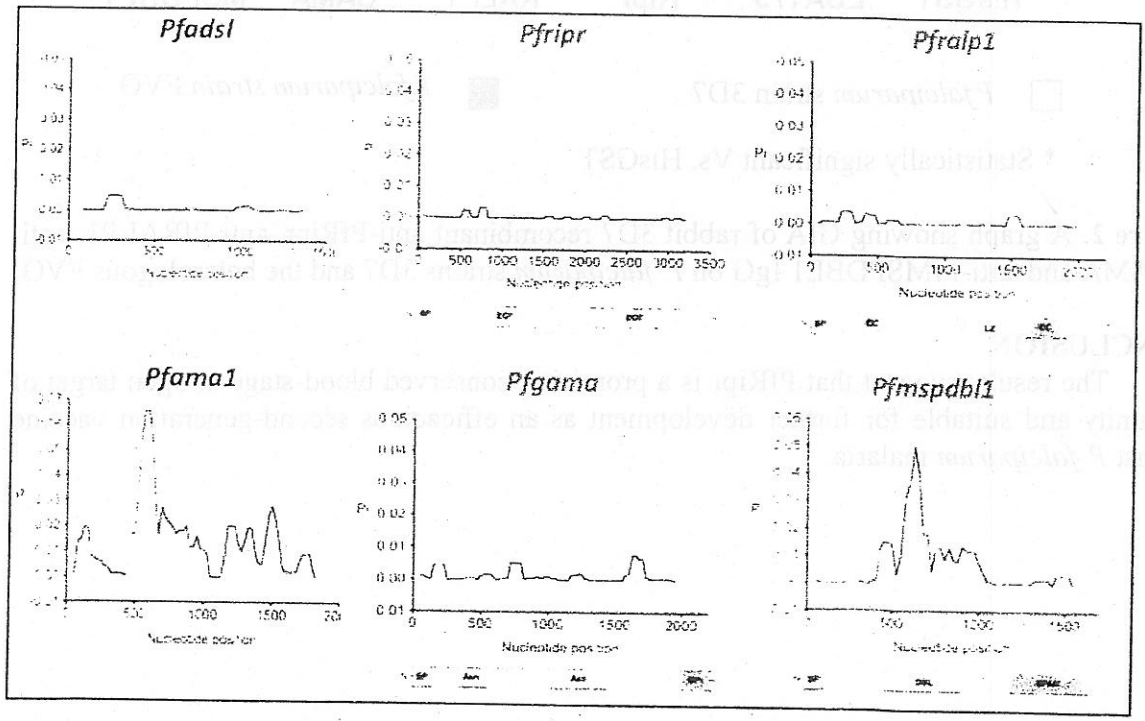

Figure 1. Sliding window analysis of nucleotide diversity to compare levels of diversity across each gene in Ugandan *P.falciparum* isolates.

In addition, with the WGCFS, we successfully expressed a large fragment of amino acids 717 residues recombinant PfRipr protein. The WGCFS expressed recombinant PfRipr was immunogenic in rabbit, and generated quality specific polyclonal antibodies (IgG). Antibodies against 3D7 recombinant proteins; PfGAMA and PfMSPDBL1 inhibited merozoite invasion of the homologous strain 3D7 but not the strain FVO. The antibodies against strain 3D7 recombinant proteins; PfRipr and PfRALP1, potently inhibited merozoite invasion of homologous 3D7 and heterologous strain FVO. However, the GIA of anti-PfRipr IgG was much higher than that of anti-PfRALP1 (Figure 2).

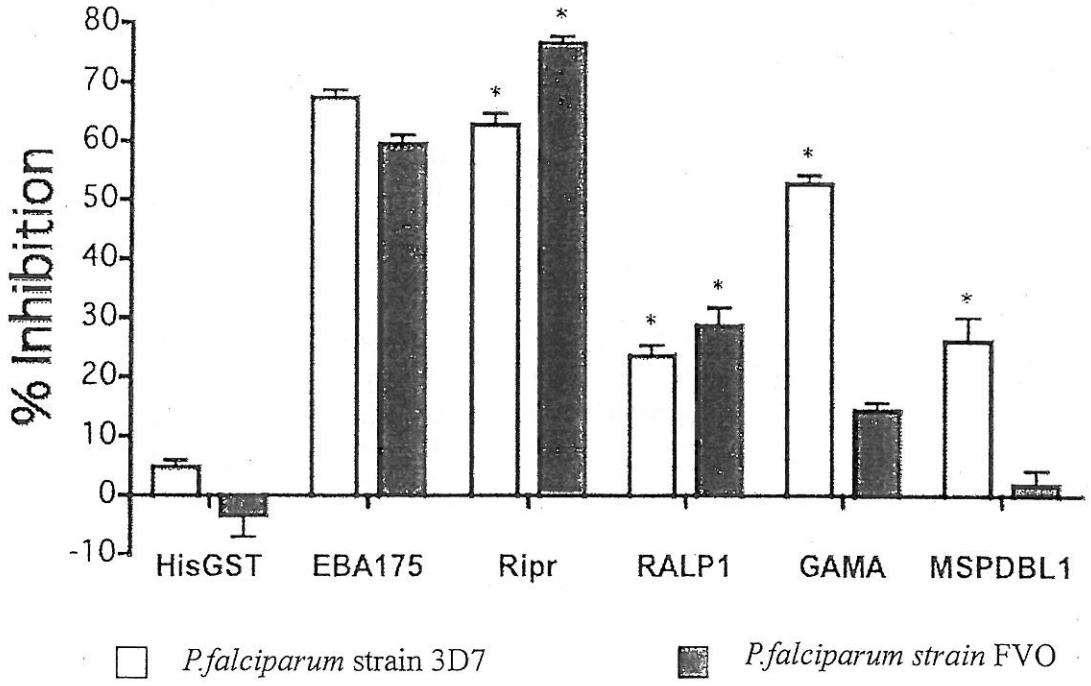

Figure 2. A graph showing GIA of rabbit 3D7 recombinant anti-PfRipr, anti-PfRALP1, anti-PfGAMA and anti-PfMSPDBL1 IgG on *P. falciparum* strains 3D7 and the heterologous FVO.

CONCLUSION

The results suggest that PfRipr is a promising conserved blood-stage antigen target of immunity and suitable for further development as an efficacious second-generation vaccine against *P. falciparum* malaria.