Mutability studies of sexual-stage specific antigens, *PFS230* and *PFS48/45* of *Plasmodium falciparum* population in Africa and their potential implications on transmission blocking vaccines

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Overview

BACKGROUND:

- Malaria caused by *P. falciparum* remains the deadliest form of malaria in sub-Saharan Africa, affecting millions of individuals especially children under the age of five.
- Currently, artemisinin-based combination therapy (ACTs) are serving as a major frontline treatment in the control of malaria .
- The emergence of resistance to ACTs and other control interventions has spurred the need for new treatment options and prophylaxis with keen interest in vaccine development. However, this has proven to be challenging.
- The most advanced malaria vaccine, RTS'S/AS01 has showed modest efficacy of about 39% against clinical malaria, which studies have attributed to the high genetic variation within its targeted antigen, the circumsporozoite protein (CSP).
- There exist two bottlenecks with reduced parasite population in the host that constitute two potentially weak points in the life cycle of the parasite; the pre-erythrocytic stage and the stage of gametocytes uptake into the midgut of the mosquito (**Figure 1**).
- Two antigens, *pfs230* (**Figure 2**) and *pfs48/45* (**Figure 3**) that are expressed on the surface of gametocytes have been shown to elicit strong antibody responses and are good targets for transmission blocking vaccines (the later of the two bottlenecks).
- However, the genetic variation within these antigens have not been fully explored in the African parasite population.
- Using NGS data for 310 *P. falciparum* sequenced samples from five countries: Congo DR, Gambia, Ghana, Tanzania and Thailand, the per-nucleotide genomic mutability was estimated for the pfs230 and pfs48/45 antigens
- This will provide the needed insight for further analysis of *pfs230* and *pfs48/45* for an effective vaccine design

OBJECTIVES:

- Map P. falciparum sequences to the 3D7 reference genome
- Estimate the per-nucleotide genomic mutability
- Evaluate the population structures and differentiation within Africa

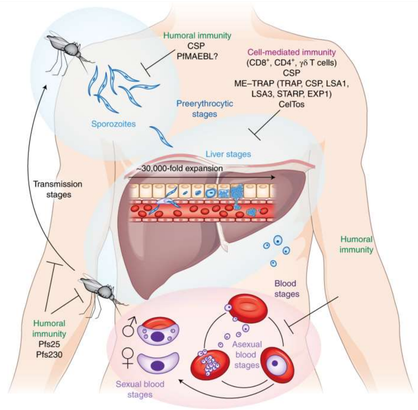

Figure 1. Life cycle of *Plasmodium falciparum*.

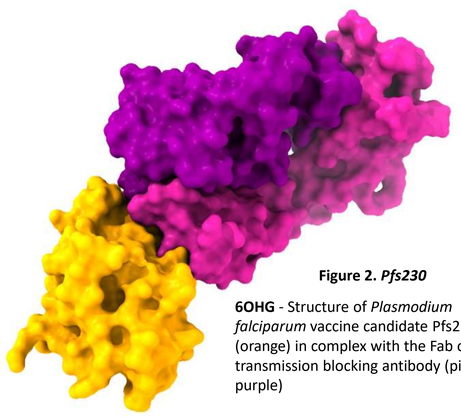

Figure 2. *Pfs230*

6OHG - Structure of *Plasmodium falciparum* vaccine candidate Pfs230D1M (orange) in complex with the Fab of a transmission blocking antibody (pink & purple)

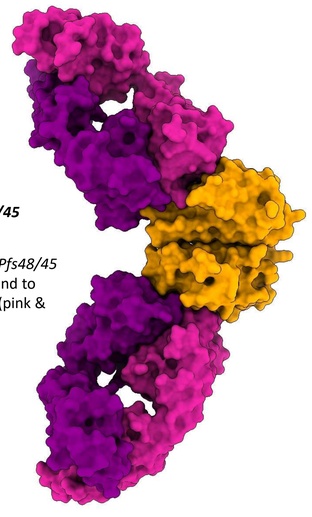

Figure 3. *Pfs48/45*

6H5N - *Plasmodium falciparum Pfs48/45* C-terminal domain (orange) bound to monoclonal antibody 85RF45.1 (pink & purple)

Approach

Results

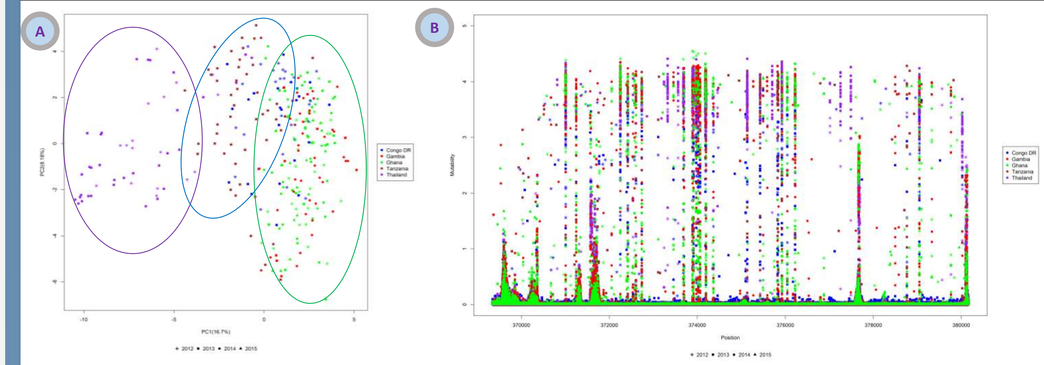

Figure 4.
A) First and Second principal components plot for *pfs230* antigen with three clusters: i) purple circle represent Thailand population, ii) blue circle encapsulates Congo DR and Tanzania samples , and iii) green circle represent Ghana and Gambia cluster.
B) Plot of per-nucleotide mutability of *pfs230* antigen for each of the 310 samples from the five countries. There were a combine total of 49 loci with mutability score above 2.0 for at least 5 samples from each country.

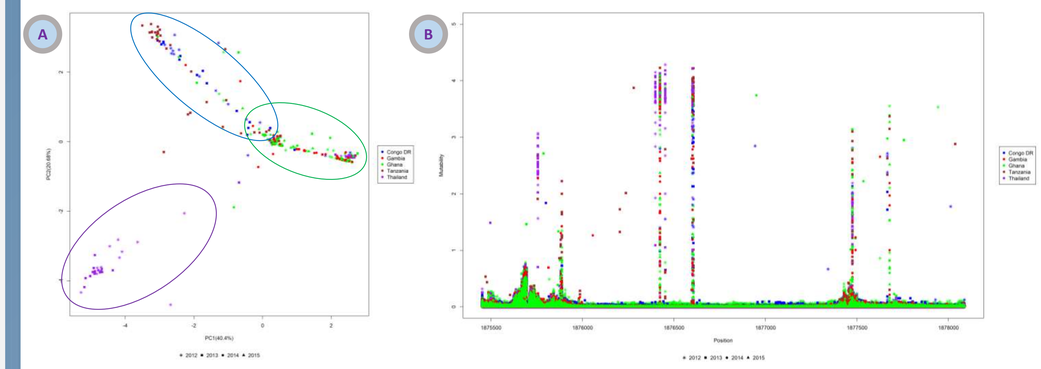

Figure 5.
A) First and Second principal components plot for *pfs48/45* antigen with three clusters: i) purple circle represent Thailand population, ii) blue circle encapsulates Congo DR and Tanzania samples , and iii) green circle represent Ghana and Gambia cluster.
B) Plot of per-nucleotide mutability of *pfs48/45* antigen for each of the 310 samples from the five countries. There was a combined total of 8 loci with mutability score above 2.0 for at least 5 samples from each country.

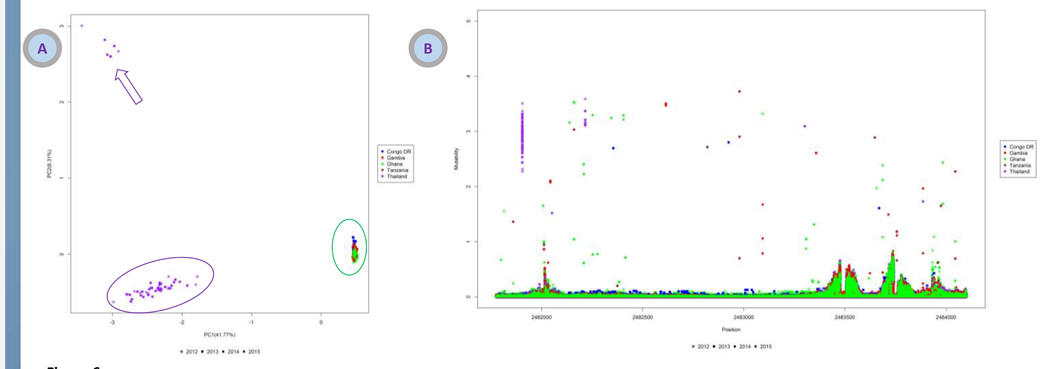

Figure 6.
A) First and Second principal components plot for the housekeeping antigen (PF3D7_1361900) with two clusters: i) purple circle represent Thailand population, and ii) green circle represent the African countries cluster. Also, some Thailand samples were outliers.
B) Plot of per-nucleotide mutability for the housekeeping antigen (PF3D7_1361900) for each of the 310 samples from the five countries. There were two loci with mutability score above 2.0 for at least 5 samples from the Thailand population but none from the other countries.

Summary and Conclusion

- The population structure for the two antigen *pfs230* and *pfs48/45* revealed three clusters (**Figures 4A & 5A**);
  - One for the Thailand population
  - Another for the West African countries – Ghana and Gambia
  - And the third for Congo DR and Tanzania.
- As a control a housekeeping antigen was added to the analysis. The population structure for this antigen revealed two clusters **(Figure 6A):**
  - One for the African countries' populations
  - And the other for the Thailand population
  - The Thailand cluster was due to two loci with higher mutability values and some outliers.
- There were a combine total of 49 loci with mutability score above 2.0 for at least five samples from each country for the *pfs230* antigen (**Figure 4B**).
- Out of these 49 loci,
  - 12 loci were shared among all countries,
  - 4 loci were shared only among African countries
  - 7 loci were found only in the Ghanaian population
  - And 9 loci were only found in the Thailand population
- The *pfs48/45* antigen contained a combine total of 8 loci with mutability score above 2.0 for at least five samples from each country (**Figure 5B**).
  - Of which 3 loci were found only in the Thailand population
  - And the rest shared by at least two countries.
- These shows that the *pfs230* antigen is relatively more mutable than the *pfs48/45* antigen just as suggested by other research studies
- The housekeeping antigen contained only two loci with mutability score above 2.0 for at least five samples and all these loci were from the Thailand population (**Figure 6B**).
- These results gives a general overview of how mutable these antigens are,.
- Thus further population genetics analysis into the nucleotides at these highly mutable loci will throw more light on how effectively *pfs230* and *pfs48/45 may work as vaccines.*

Future work

- Call single nucleotide polymorphisms (SNPs)
- Analyse the functional effects of SNPs
- Curate and predict epitopes
- Analyse SNP effects on epitopes

Reference

- J. Heide, K. C. Vaughan, A. Sette, T. Jacobs, and J. S. Zur Wiesch, "Comprehensive review of human plasmodium falciparum-specific CD8+ T cell epitopes," *Front. Immunol.*, vol. 10, no. MAR, p. 397, 2019, doi: 10.3389/fimmu.2019.00397.
- A. M. Dondorp, F. Nosten, P. Yi, D. Das, A. P. Phyo, J. Tarning, K. M. Lwin, F. Ariey, W. Hanpithakpong, S. J. Lee, P. Ringwald, K. Silamut, M. Imwong, K. Chotivanich, P. Lim, T. Herdman, S. S. An, S. Yeung, P. Singhasivanon, N. P. J. Day, N. Lindegardh, D. Socheat, and N. J. White, "Artemisinin resistance in Plasmodium falciparum malaria," *N. Engl. J. Med.*, vol. 361, no. 5, pp. 455–467, Jul. 2009, doi: 10.1056/NEJMoa0808859.
- WHO, "World malaria report 2019," 2019. https://www.who.int/news-room/feature-stories/detail/world-malaria-report-2019 (accessed Jan. 21, 2020).