Scientific Background

My scientific career began when I completed my bachelor's in biology at Kingston University London. I selected modules relating to infectious diseases and studied several major human pathogens including malaria, leishmaniasis and dengue. I selected a parasitology project which explored the advantages of using molecular techniques for the accurate identification of nematode species. After completing my bachelors, I went travelling to Indonesia and witnessed how infectious diseases such as malaria and dengue impacted individuals. This experience inspired me to pursue a master's degree in infectious diseases at the University of Kent, where I not only gained laboratory skills but also developed valuable communication skills, through presenting data, poster creation, and scientific blog writing. I achieved the highest grade in my class, and as a result of my accomplishments, I was awarded 'School of Biosciences Postgraduate Student Prize' for best performing student in November 2016 with the grade distinction/très bien.

In 2018, I began working as a Research Assistant/Engineer at the University of Cambridge, where I worked on various research projects related to bacterial pathogens, specifically *Staphylococcus spp.* One of my key achievements in this role was developing a method for simultaneously extraction of clinically relevant bacterial organisms. This accomplishment resulted in a joint-first author publication in *Scientific Reports*, 2021.

During my time in Cambridge, I attended various talks on malaria and pursued my passion for the disease by joining the Wellcome Sanger Institute/Genomic Surveillance Unit. Sequencing the genome of *Plasmodium falciparum* presents significant challenges, including high levels of human DNA contamination and genome complexity due to highly repetitive A-T rich regions. To address these issues, my research focused on troubleshooting the latest long-read sequencing platforms such as Oxford Nanopore. By optimising the use of nanopore on malaria, I aimed to improve the quality of *P. falciparum* genome sequencing data and implement this technology on field isolates in Ghana. Working alongside Dr William Hamilton (Wellcome Sanger Institute, UK) and collaborating with Dr Lucas Amenga-Etego (West African Centre for Cell Biology of Infectious Pathogens, Ghana), I conducted fieldwork and provided training in a resource-limited setting in Ghana for five weeks. Despite the difficulty of working with field isolates where the parasitaemia is <1%, coupled with high levels of human DNA contamination, we were successful in sequencing *P. falciparum.*

This experience was immensely gratifying as we were able to not only introduce this technology to young Ghanaian scientists but also promote scientific collaboration and innovation in developing countries. I achieved several personal milestones through this project, such as a job promotion, publishing a first-author paper (available on bioRxiv), and gaining valuable insights from skilled scientists in Ghana. Additionally, I collaborated with Dr Antoine Claessens (University of Montpellier) to develop methods for long-read sequencing using PacBio technology. The aim was to sequence full-length genomes of *P. falciparum* isolates from Africa, and these genomes have laid the foundation of my PhD project 'Single-cell transcriptomics to discover how *P. falciparum* evades the immune system', supervised by Dr Claessens. This PhD is part of a longitudinal study 'Malaria Integrative Analysis to Discover Biomarkers of Infection Outcome' (MADBIO). My passion for pursuing this PhD stems from my belief that a comprehensive understanding of how *P. falciparum* can evade detection in the human host for prolonged periods will be instrumental in the eradication of malaria. By employing single-cell transcriptomics, we can gain valuable insights into the complex mechanisms that enable this parasite to remain undetected with hopes of paving the way for the more effective therapies and preventative measures.

Malaria Integrative Analysis to Discover Biomarkers of Infection Outcome (MADBIO)

Summary

A *P. falciparum* infection can result in a wide range of outcomes, from asymptomatic to uncomplicated or severe malaria and death. The vast majority of all *P. falciparum* infected carriers worldwide are asymptomatic. The discrepancy in infection outcomes suggests a large diversity of parasite virulence and/or host susceptibility. The host-pathogen interaction is mediated at least partly by the parasite antigenic variation and the host immune response.

To disentangle these two variables, the main objective of the MADBIO is to define the natural progression of *P. falciparum* infection, based on host and parasite transcriptomes. With the sequencing of unicellular RNA at several stages of an infection, we will identify biomarkers predictive of its outcome and discover the mechanisms that can shift host-pathogen interactions towards virulence or parasite elimination. The long-term goal is to target the most virulent parasites and protect the most vulnerable humans, to help eradicate malaria.

To achieve this, we will draw blood (fingerprick or venous blood) at regular intervals from each participant who has agreed to participate in the study, and for which we have received signed informed consent. Plasma, peripheral blood mononuclear cells (PBMC), and infected red blood cells will be frozen separately. Plasma and PBMCs will be used to characterize the host immune response by flow cytometry and RNA-seq, respectively. The antigenic variation of the parasites, involving the regular change of the type of *var* gene expressed, will be studied by RNA-seq and qRT-PCR.

The longitudinal approach of the MADBIO project will make it possible to decipher the mechanisms of the host-pathogen interaction of *P. falciparum* in its natural host. Most importantly, the discovery of host and parasitic biomarkers of infection outcome will enable us to identify and protect those most at risk, but also to lead malaria elimination campaigns against long-lasting infections.

Introduction

Most of the half a million annual deaths by malaria are due *to P. falciparum*. This unicellular eukaryote parasite is transmitted by female Anopheles mosquitoes. A *P. falciparum* infection results in a wide range of outcomes, from asymptomatic to uncomplicated or severe malaria and death. On any given day, the vast majority of all *P. falciparum* infected carriers worldwide are asymptomatic[1]. In cohorts from The Gambia and Mozambique, the proportion of infections needing anti-malarial treatment was in the order of 10%, while roughly half of the asymptomatic infections naturally cleared within a few weeks and the rest remained chronic for at least 6 months [2,3].These long-lasting chronic infections are key to the parasite survival in the dry season when there is no transmission. This reservoir is arguably the biggest challenge for malaria eradication, as clearing all infections includes treating carriers without clinical symptoms who are unlikely to seek treatment. Of the parasite Variant Surface Antigens (VSA), *P.f.* Erythrocyte Membrane Protein 1 (PfEMP1) is the major ligand binding to human endothelial cell protein receptors[4]. PfEMP1 is encoded by a family of ~60 var genes that undergo mutually exclusive expression (Fig 1). Each *P. falciparum* isolate typically contains a unique repertoire of 60 *var* sequences, making the worldwide total number of *var* gene sequences virtually infinite. The sequence polymorphism and the regular switching of surface PfEMP1 antigens would mediate antigenic variation to evade the immune response[5].

In asymptomatic infections, var gene expression has hardly been investigated. One study in Kenya points to a homogeneous var expression pattern[6]., with an overall lower abundance of PfEMP1 at the surface of the RBC, as an additional immune evasion strategy. Importantly, the antigenic variation hypothesis has never been tested at multiple timepoints from human chronic infections.

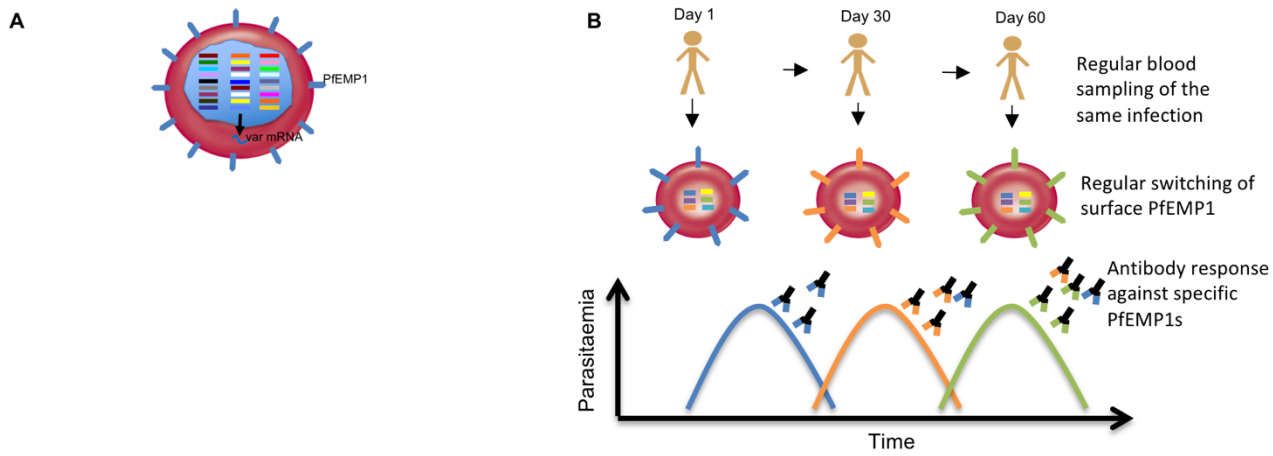

Fig 1. Mutually exclusive expression of the *var* gene family and antigenic variation. (A) Mutually exclusive expression of *var* genes, resulting in a single type of PfEMP1 at the surface of the iRBC (B) The antigenic variation hypothesis. Regular switching of surface-exposed PfEMP1 would lead to a burst of parasitemia immediately followed by sequential acquisition of specific antibodies.

To the best of our knowledge, at the early stage of a natural infection (before the onset of symptoms) parasite and host transcriptomes have never been recorded so far. This is the challenge we wish to address through the recruitment of a cohort of children aged 6-10 years old over the wet season and the following dry season, and collect venous blood samples before during and after a *P. falciparum* infection (Fig 2). Ultimately, through the use of transcriptome sequencing from both host and parasites cells, we will identify genes specifically expressed in symptomatic and asymptomatic infections.

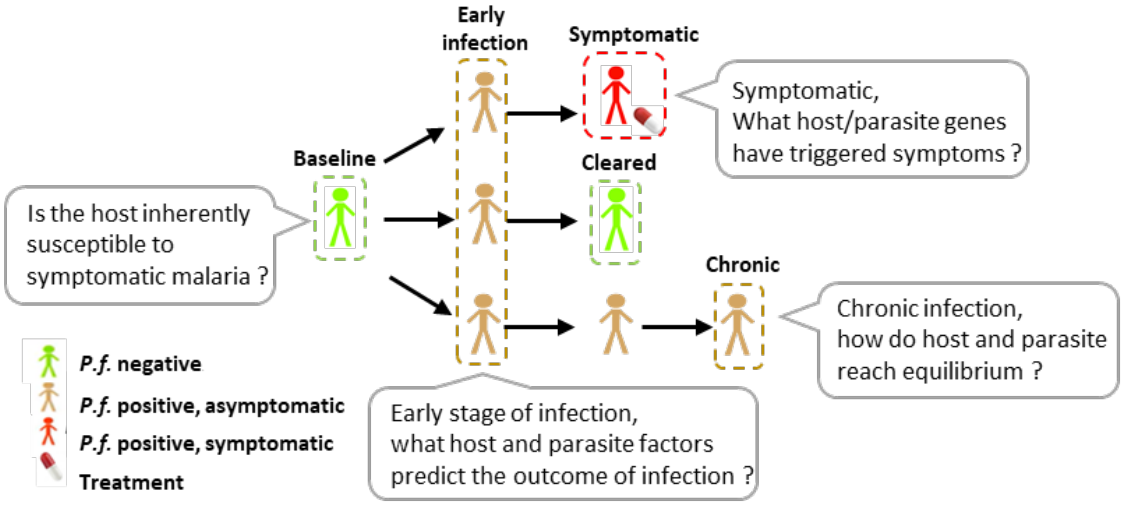

Figure 2. Design of our longitudinal cohort that includes three categories of children, those who develop symptoms (symptomatic), those who naturally clear the parasite (cleared) and those with persistent infections (Chronic).

Study Design

Overview

The goal is to recruit 100 children, all treated with anti-malarial and anti-parasitic drugs on the first day. Fifteen days later, a first 'baseline level' venous sampling is performed on each child, then a fingerprick is taken every week for up to 3 months. A venous blood sample is collected as soon as the child is positive, and at the day the child develops symptoms OR after one month if the child remains asymptomatic.
At the end of the 3 months of follow-up, 25 asymptomatic positive children will be selected to be sampled at month 6 and month 9.

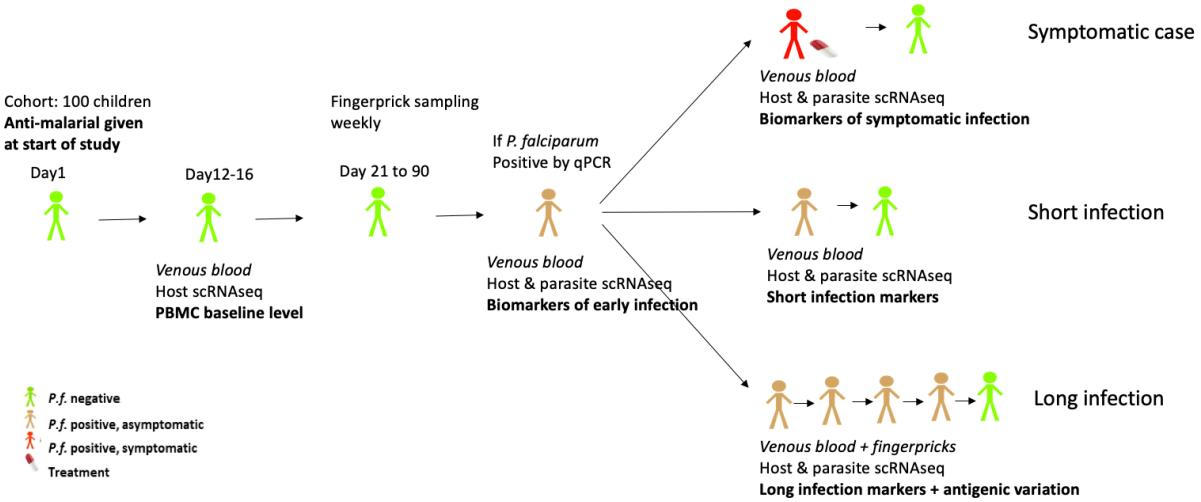

Fig 3. MADBIO Cohort design. All recruited children will be treated at the start of the study, hence *P. falciparum* negative. Once a child becomes infected with *P. falciparum*, three outcomes are possible: the infection resolves itself after a few days/weeks, the infection remains asymptomatic chronic, or the infection becomes symptomatic, in which case it will be treated with antimalarials immediately.

<u>Working Plan (working alongside a postdoc from Ghana)</u>

Dr Antoine Claessens will travel to Ghana in June to visit the lab and meet WACCBIP/NHRC staff. Note: I have previously conducted research in the lab.

See application form for a breakdown of lab work that will be conducted in phase 1, 2, 3 and 4.

Phase 1: Recruitment

Phase 2: PBMC Baseline level on all children of the cohort.

Phase 3: Weekly fingerprick, plus follow-up of positive cases
Phase 4: Long-term follow-up of 12 asymptomatic chronic infections

Scientific Impact of MADIO

The global battle against malaria started in 1955 when the WHO aimed to eradicate it within a few years. The initial progress was quickly curbed by the parasite and the vector acquiring drug resistance. Nowadays resistance against Artemisinin Combination Therapy has spread throughout South East Asia, and the only vaccine available (RTS,S) is of very limited efficacy. We argue that our failure to eliminate this deadly disease is at least partly due to our lack of understanding of the host-pathogen

interaction, and that the most physiologically relevant approach is a longitudinal study in an endemic country. The MADBIO was formed to tackle novel biological questions that can only be addressed with the original cohort design and state-of-the-art single-cell technology. In some malaria endemic areas, *Plasmodium* prevalence is so high that it is difficult to distinguish *Plasmodium*-related symptoms from other viral or bacterial co-infections. Indeed, people with 'flu-like symptoms' generally assume to have malaria and take antimalarials even without diagnostics. Gene expression data could provide a signature to distinguish malaria from other febrile illnesses. Here, the short-term goal is to identify host and parasite biomarkers, and the long-term goal is to develop devices predicting the outcome of an infection. In the context of a malaria elimination program, defining the natural progression of asymptomatic *P. falciparum* infection will inform policy decision on treatments that cure all infections versus only symptomatic ones.

References

1. Barry, A. *et al.* Higher gametocyte production and mosquito infectivity in chronic compared to incident Plasmodium falciparum infections. *Nat. Commun.* (2021). doi:10.1038/s41467-021-22573-7
2. Galatas, B. *et al.* Dynamics of Afebrile Plasmodium falciparum Infections in Mozambican Men. 1–8 (2018). doi:10.1093/cid/ciy219
3. Collins, K. A. *et al.* MAJOR ARTICLE A Cohort Study on the Duration of Plasmodium falciparum Infections During the Dry Season in The Gambia. 1–10 (2022).
4. Bull, P. C. & Abdi, A. I. The Role of PfEMP1 as targets of naturally acquired immunity to childhood malaria. *Parasitology* **143**, 171–186 (2016).
5. Nyarko, P. B. & Claessens, A. Understanding Host–Pathogen–Vector Interactions with Chronic Asymptomatic Malaria Infections. *Trends Parasitol.* 1–10 (2020). doi:10.1016/j.pt.2020.09.017
6. Warimwe, G. M. *et al.* Plasmodium falciparum var Gene Expression Homogeneity as a Marker of the Host-Parasite Relationship under Different Levels of Naturally Acquired Immunity to Malaria. *PLoS One* **8**, (2013).