

Role of *Plasmodium falciparum* genetic backgrounds in tolerance to antimalarial-resistance



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This dissertation is submitted for the degree of
Master of Philosophy

Hughes Hall College

February 2021

Declaration

I declare that this thesis is the result of my own work and includes nothing which is the outcome of work done in collaboration except where specifically stated in the text. It does not exceed the prescribed 20,000-word limit for the School of Biological Sciences Degree Committee.

Acknowledgement

I would like to thank my supervisor Dr Marcus Lee for accepting me as his first MPhil student and been such an amazing supervisor. He has been a supervisor with an extraordinary level of patience in and out of the laboratories, and has endlessly been supportive and encouraging throughout my MPhil. Working with him has exposed me to extensive perspectives of malaria research and particularly advanced perceptions of *Plasmodium* and drug resistance. I would like to thank members of team 226 for their great support and always readily answering my endless questions that I have learnt a lot from. My time with this team has been an incredible life changing experience and greatly inspirational for my career prospects of a malaria scientist.

I am grateful to Wellcome Sanger Institute for this studentship that has given me the opportunity to be enlightened on pioneering scientific research areas and methods in an environment where every conversation has been a learning experience. I want to thank Christina Hedberg-Delouka and Annabel Smith of the Sanger graduate office for their enormous administrative support and ensuring student welfare especially during the initial Covid-19 lockdown that left many students overwhelmed.

I am thankful to MRC Unit The Gambia at LSHTM who's partnership with Sanger has provided me with this opportunity, and for their developmental training that has equipped me with foundational skills to successfully take up and complete this MPhil.

I would like to thank my family and friends for their continuous support and encouraging words throughout this period, especially my husband for been very supportive of my career development. My deepest gratitude goes to my mother who has tried in every way possible way to make this journey a pleasant one beyond her comfort.

Lastly, I am enormously thanking my son Sulayman who's understanding, kind and positive words have been my strength. Despite the stressful interruptions of plans to see each other due to the pandemic that kept him away from me for a year, he was continuously supportive and considerate, patiently waiting for my calls even when I left the lab at late nights and constantly checking if I have eaten or taking breaks to rest. I dedicate every word of this thesis to him for his endless and unconditional solace.

Abstract

Malaria remains a global public health burden with the highest mortality rate amongst vector-borne diseases, affecting mostly children under five years of age making it the leading cause of child deaths. There has been significant reduction of the malaria burden as a result of global efforts in implementing control interventions to address malaria morbidity and mortality especially since the introduction of artemisinin. However, the burden of malaria remains high in many endemic areas despite the substantial decline in global spatial distribution and burden since 2000, with over 90% of people within sub-Saharan Africa residing in endemic areas. There has been increasing reports of *Plasmodium falciparum* tolerance to several partner drugs currently in use in artemisinin-based combination therapies in certain endemic countries which has been associated with certain genetic backgrounds.

In this study, the impact of resistance-associated mutations on parasite resistance and fitness, and how the genetic background of the parasite affects these phenotypes was investigated. In the first research chapter, the growth phenotypes of a panel of barcoded *P. falciparum* parasites were measured in parallel using barcode sequencing (BarSeq). These barcoded parasites cover different *P. falciparum* strains from different geographic locations, and are grown *in vitro* in competition with each other in the presence of antimalarial compounds. BarSeq was then used to measure the different phenotypes to antimalarials based on the genetic background. Another chapter in the study established the impact of potential antimalarial compounds on genetically modified *P. falciparum* parasites harbouring *Pfkelch13* mutations. These experimental compounds were chosen because of their known activity on human *Kelch*-like ECH-associated protein1 (Keap1), and were tested in drug response assays and established to have activity against parasites. Another element in this

chapter established the generation of CRISPR plasmids for editing the *Pfkelch13* gene. These individual donors were deconvoluted from a complex pool of plasmids with a common pDC2 backbone that encoded all 64 possible codons at the critical position 580 of *Pfkelch13* that is the site of the most prevalent artemisinin-resistance variant. The isolation of each possible individual donor was carried out and sub-pools of plasmids were generated. This was done to facilitate future work to examine which amino acid may be more efficient in replacing cysteine at position 580 and the impact of parasite genetic background on the outcome of the allele.

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