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Naturally acquired antibody responses to recombinant Pfs230 and Pfs48/45 transmission blocking vaccine candidates

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Summary *Objectives:* Pfs48/45 and Pfs230 are *Plasmodium falciparum* sexual stage proteins and promising malaria transmission-blocking vaccine candidates. Antibody responses against these proteins may be naturally acquired and target antigens may be under selective pressure. This has consequences for the future evaluation of vaccine immunogenicity and efficacy in populations naturally exposed to malaria.

Abbreviations: SNP, single nucleotide polymorphism; MTBV, Malaria transmission blocking vaccines; TRA, transmission reducing activity; RDT, rapid diagnostic test; GLURP, glutamate rich protein; SMFA, standard membrane feeding assay; MSP-2, merozoite surface protein-2; MOI, multiplicity of infection.

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Transmission;
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Methods: We determined naturally acquired antibody responses to the recombinant proteins Pfs48/45-10C and Pfs230-230CMB in children from three malaria endemic settings in Ghana, Tanzania and Burkina Faso. We also examined genetic polymorphisms in the *P. falciparum* gene *pfs48/45*.

Results: Antibody prevalence was 1.1−18.2% for 10C and 6.7−18.9% for 230CMB. In Burkina Faso we observed evidence of an age-dependent acquisition pattern for both 10C ($p < 0.001$) and 230CMB ($p = 0.031$). Membrane feeding assays on a separate dataset demonstrated an association between functional transmission reducing activity and antibody prevalence for both 10C ($p = 0.017$) and 230CMB ($p = 0.049$). 17 single nucleotide polymorphisms were found in *pfs48/45* (from 126 samples), with 5 non-synonymous SNPs in the Pfs48/45 10C region.

Conclusions: We conclude there are naturally acquired antibody responses to both vaccine candidates which have functional relevance by reducing the transmissibility of infected individuals. We identified genetic polymorphisms, in *pfs48/45* which exhibited geographical specificity.

1. Introduction

The recent decline in the burden of malaria, particularly in sub-Saharan Africa has re-emphasized elimination as an attainable goal for many malaria endemic countries.[1–3] Novel malaria control strategies that specifically aim to reduce malaria transmission may be required to move from malaria control to elimination.[4] Malaria transmission blocking vaccines (MTBV) are high on the priority list for malaria elimination and eradication strategies.[5–7] The transmission of malaria from man to mosquito depends on the presence of mature sexual stage parasites, gametocytes, in the human peripheral blood. Once ingested by blood feeding mosquitoes, male and female gametocytes activate to become gametes that fuse to form zygotes that penetrate the mosquito midgut wall as ookinetes to form oocysts. These oocysts enlarge over time to release sporozoites that migrate to the mosquito salivary glands and render the mosquito infectious to human beings upon their next feeding. MTBV aim to elicit antibodies that are ingested when a mosquito takes a blood meal which reduce or arrest parasite development, thereby blocking transmission to the next host.[8] Transmission-blocking antigens can be categorized as those that play a role before zygote formation (pre-fertilization) and those that affect the subsequent development of mosquito stages (post-fertilization). Pre-fertilization proteins Pfs48/45 and Pfs230 are both found on the surface of gametocytes and humans harbouring gametocytes in the peripheral blood are therefore exposed to these proteins.[8,9] This exposure allows the acquisition of immune response during natural malaria infections. Antibody responses to both proteins have been detected in naturally exposed populations and have been associated with functional transmission reducing activity (TRA).[10–12] Immune recognition may also result in selective pressure that gives rise to genetic polymorphisms associated with reduced susceptibility of parasites to natural or vaccine-induced immune responses. For *pfs48/45*, 5 main non synonymous genetic polymorphisms have been described previously with clear geographical clustering.[13] Both the presence of naturally acquired antibody responses and genetic polymorphisms in vaccine protein regions are of great importance for the planning and evaluation of vaccine trials in naturally exposed populations.

Recent work indicated that antibody responses to a Pfs230 but not a Pfs25-based vaccine candidate may be recognized by naturally exposed populations.[14] Here we determine naturally acquired antibody responses to MTBV candidates Pfs48/45-10C and Pfs230-230CMB, explore the functionality of naturally acquired antibody responses to these recombinant proteins and describe genetic polymorphisms of Pfs48/45 in local isolates of *Plasmodium falciparum*.

2. Materials and methods

Study areas and populations

Three study sites were selected to reflect different levels of transmission intensity: a site of hyper endemicity in Ouahigouya, Burkina Faso, meso endemicity in Bondo, Tanzania and hypo endemicity in Asutsuare in Ghana. One hundred and eight children were randomly sampled from 1 school in Ghana, 200 children across 2 schools in Burkina Faso and 202 children across 2 schools in Tanzania using sampling strategies described by Brooker et al.[15] Two cross sectional surveys were conducted during the peak transmission season and at the end of the dry season in 2011 and 2012 at each study site.[15] Ethical permission was granted from LSHTM (approval number 5946) and from local ethics committees in Burkina Faso (AEP-007/05/11/CIB/CNRFP), Tanzania (Kilimanjaro Christian Medical Centre IRB 2011-553) and Ghana (Noguchi Memorial Institute for Medical Research-IRB 040/10-11). Written consent was gained from participant's guardians prior to sampling. At each survey, finger prick samples of approximately 300 μL were taken in BD microtainers (Becton Dickinson, Oxford, UK) for microscopy, plasma collection and filter paper storage (Whatman 3MM, Maidstone, UK). Plasma was diluted to 1/20 in 0.05% sodium azide in phosphate buffered saline (PBS), which was stored at −20 °C until use. Blood spot filter papers were air dried overnight, then sealed into individual plastic bags with silica desiccant, and stored at