End of project popular science description

Introduction

Malaria is an infectious disease of serious public health concern and a major cause of morbidity and mortality in sub-Saharan Africa. Children under 5 years old, pregnant women and nonimmune travelers are the most at risk. There are effective drugs for treatment however, emergence of resistant parasites threatens their long term usefulness. Further, the current vaccine approved for use in children has low efficacy. More studies are needed to better understand malaria drug resistance and the immune response to inform the development of better interventions against malaria. It was in this context that this project was conceived. The project was a South-driven capacity building research collaboration involving five partner institutions with the overall aim of building human and Institutional capacity to conduct cutting edge malaria vaccine related research to improve the general well being of populations living in malaria endemic areas. The partners were the Noguchi Memorial Institute for Medical Research (NMIMR), Kintampo Health Research Centre, Navrongo Health Research Centre in Ghana and Statens Serum Institut (SSI), University of Copenhagen (Uni-CP) in Denmark. The significant human resource gap the project sought to fill was the lack of expertise in methodologies for studying malaria-specific antibody functionality while generating important data on malaria immunity and parasite genetics that can inform vaccine design and other interventions. The main approach of the project was to build capacity in the training of PhD students and transfer of research capacity from SSI and Uni-CP to Ghana. The project's specific objectives were organized into five work packages (WP). First, we conducted a longitudinal cohort study in Danfa, a high malaria endemic area in Ghana and using an ongoing blood draw protocol to optimize opsonic phagocytosis and invasion inhibition bioassays (WP1). This WP ensured the availability of optimized bioassays and biological samples with accompanied morbidity data for the execution of the remaining WPs. Next, we assessed antibody functionality in individuals naturally exposed to *Plasmodium falciparum* and the interaction between antibodies and host genetic factors in relation to the risk of clinical malaria (WP2). This work package was the focus of the training of one PhD student. The second PhD student assessed *P. falciparum* genetic variations that underlie malaria infection outcomes and also drug resistance profiles of parasites in the study communities (WP3) while the third focused on statistical modeling of age-specific risk of malaria and relative contributions of host, parasite and environmental associated factors to the risk of malaria (WP4). The fourth PhD student assessed antibody functionality in GMZ2 (a malaria vaccine candidate tested in phase 2b) immunized individuals in relation to vaccine efficacy (WP5). In addition, a postdoc was employed to assist with the capacity building of the PhD students and to help develop approaches for genotyping specific host genes to be incorporated into the antibody data analysis (WP6).

Results

The longitudinal cohort study successfully recruited a total of total of 973 children (aged 0.5 to 13 years old) who met the inclusion criteria. Of these, 848 (87.2%) completed the 50-week longitudinal follow-up. At the time of enrolment, 99 (11.7%) children had asymptomatic *P. falciparum* infection, of which 46 (46.5%) were detected by microscopy (microscopic) while the remaining 53 (53.5%) were only detectable by polymerase chain reaction (PCR) and were considered as sub-microscopic infections. The remaining 749 (88.3%) children had no *P. falciparum* infection at baseline that could be detected by either by microscopy or PCR. Asymptomatic baseline malaria parasite infections were more common in older children (5 to 13 years old) compared to those who were younger (0 to 5 years old). The study found both baseline *P. falciparum* asymptomatic microscopic and more strongly submicroscopic infections to be associated with protection against febrile malaria in the ensuing transmission season. This could have important implications for malaria sero-epidemiological studies and vaccine trials [1]. Further analysis of the parasite genetics data showed majority of samples were polyclonal indicating high within host diversity and thus low inbreeding rates relative to the population. There was an overall low nucleotide diversity observed among the subpopulations (0.0028) an indication of similar evolution events occurring in the population, irrespective of the outcome of the infection. Parasites genotyped in symptomatic and those in asymptomatic infections seemed to cluster together showing relatedness. The main molecular markers associated with chloroqunine resistance *Pfcrt* K76T was significantly low (16-18%). There was an overall 90% parasite population reversion to the wildtype haplotype CMNT. Pfmdr1 (multidrug resistance) molecular markers were generally at low prevalence except for the Y184F marker observed at a prevalence of 70% and singly accounting for the high prevalence of the NFD haplotype associated with lumefantrine and mefloquine resistance. The sulfadoxine-pyrimethamine (SP) molecular markers showed relatively high resistance across the study communities but the surrogate quintuple mutation IRNGE was low (2.5%) justifying the continuous use of SP in pregnancy. The *PfK13* mutation associated with artemisinin resistance was not observed in the study, evidence that resistance to artemisinin is still not established in the population (manuscript in preparation). Capacity for antibody functional studies were successfully acquired and used to analyse both the cohort and GMZ2 study samples. The results of the cohort study indicated that children with higher baseline opsonic phagocytosis (OP) and invasion inhibition activities have a higher probability of remaining free from febrile malaria during the transmission season (manuscript in preparation). We also demonstrated that peripheral merozoite surface proteins are important targets of naturally acquired immunity against malaria [2] and that neutrophils are the dominant phagocytic cells in the OP mechanism [3]. The function of cytophilic antibodies targeting key blood stage malaria antigens in the OP mechanisms and protection against malaria were also described in different populations [4,5] and we showed that higher antibody breadth correlated with both OP and protection

against malaria [6]. In the analysis of the GMZ2 samples, we found the vaccine to be more efficacious in older children, suggesting that GMZ2 antibodies may act in concert with naturally acquired immunity [7]. Further analysis revealed that additional epitopes from the variable regions of GLURP and MSP3 may contribute to better GMZ2 efficacy if incorporated in future designs of the vaccine [8]. We also developed mathematical models to evaluating the predictive performance of malaria antibodies and human *FCGR3B* gene polymorphisms on *P. falciparum* infection outcome using several predictors. We found that *FCGR3B-c.233C>A* (rs5030738) genotype and IgG against the apical membrane antigen 1 were better compared to the other antibodies and studied *FCGR3B* genotypes in classifying or predicting malaria risk among children [9]. Finally, using a severe malaria cohort, we also identified for the first time that polymorphism in the IgG3 hinge region that encodes differences in the hinge length was associated with the risk of cerebral malaria in children [10]. Of the four PhD candidates, three have successfully completed while the last candidate is expected to defend before the end of 2022.

Conclusions

The *P. falciparum* genetics study provides important data on malaria drug resistance parasite profiles in the study population which is vital to the control efforts by the Ghana Health Service and the National Malaria Control Program. The findings suggest that artemisinin based chemotherapy against malaria can still be effective in the communities since the parasites lack the resistance mutations. Also, a policy change to reintroduce chloroquine as a treatment option for malaria may be possible in the near future with the low prevalence of choloroquine resitance mutations observed. The findings from our immunological studies and predictive modeling identified key antigenic targets. These can help inform improved strategies in designing more efficacious next generation malaria vaccines that may benefit from multiple immune mechanisms such as opsonic phagocytosis and invasion inhibition for enhanced efficacy. The human and institutional capacity built through this project contributes directly to the critical workforce needed in conducting cutting edge malaria vaccine and parasite drug resistance studies in Ghana and Africa.

Recommendations

1) The current artemisinin-based combination therapy for the treatment of malaria in Ghana should be continued and genomic surveillance for the emergence of resistance mutations carried out more routinely; 2) blood stage malaria vaccine designs should incorporate antigenic targets such as GLURP R2, AMA1 and MSP3 which were identified as targets of functional antibodies in both the opsonic phagocytosis and invasion inhibition mechanisms and 3) both human and institutional capacity building in malaria research should continue to ensure highly trained workforce for high impact malaria studies.

References

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