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Applications of Genomics to Malaria Treatment

Malaria, a tropical disease characterized primarily by fevers and headaches, is estimated to have killed half of all humanity that ever lived (Durham, 2012). The disease has long been considered both preventable and treatable, but historically malaria has been less emphasized than other epidemics such as the Black Death, yellow fever or polio because of its lower fatality rate and less obviously recognizable symptoms. However, malaria remains a looming problem for the 3.3 billion people at risk in the world today; in 2010, there were 216 million malaria episodes resulting in 655,000 deaths, 91% of which were in Africa and 86% of which were children under the age of 5 (World Malaria Report 2011).

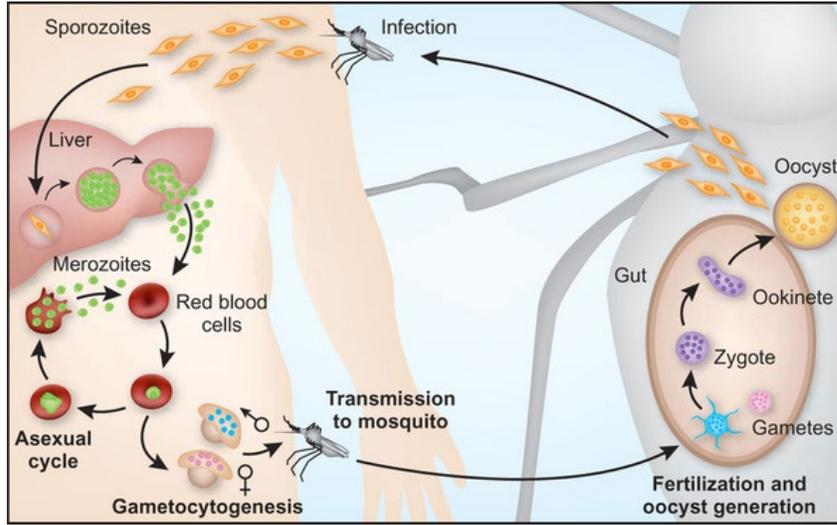
Beyond the misery and mortality caused by the disease itself, there is a well-established link between malaria endemicity and social and economic burden. Malaria's effects on population structure, investment, worker productivity, tourism, and trade impede economic growth (Sachs and Malaney, 2002). Higher fertility rates are observed in malaria-endemic countries, as parents face their children's decreased odds of survival, resulting in less investment in education per child, reduced human capital for women, and the skewing of the population towards young dependents (Sachs and Malaney, 2002). There is clear evidence for this trend linking economic development and national malaria burden – in 1995, the average GDP in malaria-endemic countries was five times smaller than that of non-malarious countries (Sachs and Malaney, 2002). Poverty certainly

contributes to the frequency of malaria transmission, but the disease plainly contributes to poverty in turn.

Worlds away from this (largely sub-Saharan African) public health crisis, the field of genomics is progressing on an equally incredible scale. DNA sequencing technology has even surpassed the trajectory postulated by Moore's Law, meaning that the technology has better than doubled in speed every two years since 2003, opening up possibilities for innovations quite recently thought to be impossible (Burke, 2012). There are several ways that genomics can contribute to the study and treatment of infectious diseases such as malaria. After briefly outlining the traditional understanding malaria, I will explore the ways in which knowledge of the *Plasmodium falciparum* genome, the human genome, and a combination of the two can expand new avenues of malaria treatment.

Pathophysiology and Treatment of Malaria

The deadliest form of malaria, considered here, is caused by the protozoan parasite *Plasmodium falciparum*. It is transmitted through the bite of infected female *Anopheles spp.* mosquitoes. The bite initially injects sporozoites into the bloodstream, where they travel to and reside asymptotically in the liver for one to two weeks. Merozoites are then released from the liver into the blood, where they attack host erythrocytes and cause the episodes recognizable as malaria (Fig. 1). When another mosquito bites the host human, gametocytes are transmitted from the human back to the mosquito, which becomes infected and able to transmit the disease to another human (Durham, 2012).



In 2010, the public received 181 million treatment

courses of the current first-line drug, artemisinin-combination therapy, or ACT (World Malaria Report 2011). However, just as *Plasmodium falciparum* has developed resistance to each of the drugs used to treat malaria over past decades, resistance to ACT is now rapidly developing along the Thailand/Myanmar border (Friedrich, 2012). There are over 30 vaccines in some state of development in the WHO's Rainbow Table for Malaria, targeting all three stages of the *P. falciparum* life cycle, and one vaccine RTS,S/AS01 is currently in Phase 3 trials in Africa (WHO Malaria Vaccine Rainbow Tables). Different drug targets have different implications for treatment: blood-stage intervention controls symptoms but not parasitemia, sexual-stage targets prevent transmission but not illness, and pre-erythrocytic targets prevent symptoms, parasitemia, and transmission (Regules, Cummings & Ockenhouse).

The RTS,S vaccine, which targets the pre-erythrocytic, sporozoite and liver-stage antigens, was engineered from fragments of the *P. falciparum* circumsporozoite protein fused to the hepatitis B surface antigen and an AS01 adjuvant (Opar, 2011). The RTS,S

vaccine has been found to be 30.1% effective in infants 6-12 weeks old and about 50% effective in children aged 5-17 months (“A Phase 3 Trial,” 2012), but the efficacy of the vaccine appears to diminish after about six months (Opar, 2011). The lower levels of anti-circumsporozoite antibody titers in the younger cohort’s blood might be a result of maternally-derived antibodies’ interference with infants’ immune responses, but since a vast majority of malaria’s victims are young children, the relatively low vaccine success rate in infants is not promising. The other vaccines in the WHO’s Rainbow Table are several years out from widespread trials. The rapidly developing tools of genomics, therefore, have much to offer the field of malaria treatment.

Treatment Insights from the *Plasmodium falciparum* Genome

The genome of *P. falciparum* was sequenced in 2002 with the whole-chromosome shotgun sequencing method. At 22.8 million bases long, its genome contains 14 chromosomes and is composed of 80.6% A-T base pairs. Almost two-thirds of the proteins discovered were thought to be unique to *P. falciparum* (Gardner et al, 2002). The parasite belongs to the phylum Apicomplexa, and thus houses plastids called apicoplasts that are requisite for the protozoan’s survival. These organelles have their own genomes that encode approximately 30 proteins, which are potential drug targets as well as *P. falciparum*’s own genome (Gardner et al). The authors who originally published the genome sequence suggested that vaccine development could be furthered by “the identification of hundreds of potential antigens that could be scanned for desired properties such as surface expression or limited antigenic diversity. This could be combined with data on stage-specific expression...to identify potential antigens that are

expressed in one or more stages of the life cycle” (Gardner et al, 2002). They immediately suggested investigation of certain proteins found in the parasite but absent in mammals, such as certain proton translocating pyrophosphatases.

Since then, several genome-wide association studies of *P. falciparum* have identified linkage disequilibrium patterns and selection signatures, looking for regions of positive selection (Carlton, 2007). Mu et al (2007) found regions of the genome with consecutive polymorphic genes or peaks of polymorphism, indications of stabilizing selection. One study partially sequenced the genome of *Plasmodium reichenowi*, *P. falciparum*'s parallel parasite in chimpanzees. Since *P. reichenowi* cannot parasitize humans, and chimpanzees don't develop malaria symptoms upon infection, it is a useful resource for comparison (Carlton, 2007).

Mechanisms of drug resistance were determined from *P. falciparum*'s genome; for example, mutations in the *MAL7P1.27* and *pfdhfr* genes led to resistance in chloroquine, and resistance to sulfadoxine-pyrimethamine developed from mutations in the *pfdhps* gene. Copy-number variations and point mutations at *pfmdr1*, a homolog of human P-glycoprotein, were associated with resistance to mefloquine, quinine, and ART (Mu et al, 2010). The illumination of the parasite's genome sequence has given momentum to the multiplicity of drugs and vaccines currently in development, but *P. falciparum*, on its own, is far from the whole story of new insights into malaria.

The Human Genome and Resistance to Malaria

Research into human genetic resistance to malaria is not a recent development. In fact, malaria was the first known case of a disease for which genetic innate immunity was

recognized as an evolutionary force in humans, going back to J.B.S. Haldane's 1949 work on the sickle-cell trait. There are several hemoglobinopathies that confer malaria resistance, including HbS, HbC, β -thalassemia, and α -thalassemia. G6PD deficiency and pyruvate kinase deficiency have similar effects (Duraisingh and Lodish). HbS and HbC have pleiotropic effects that interact to combat *P. falciparum* parasitemia, including the formation of tachtoids that prevent the erythrocyte from being a viable host and elevated levels of certain micro RNAs, described below (Durham, 2012).

Genome-wide association studies of humans have identified loci associated with malaria resistance, and like the conditions mentioned above, most of the protein products linked to resistance affect erythrocytic surfaces that make the host cell unable to maintain an environment for the parasite. Five different SNPs located within the *ATP2B4* gene at 1q32 have odds ratios of 0.61-0.62 (Timmann et al, 2012). The *ATP2B4* gene encodes erythrocytes' main calcium pump, Ca^{2+} -ATPase type 4. In people with these SNPs, decreased Ca^{2+} concentrations in the compartment separating the parasite from the host erythrocyte may impede the parasites' reproduction and maturation. A malaria-protective intergenic SNP located at 16q22.2 is thought to be linked to the *MARVELD3* gene 6.4 kb downstream, which encodes a protein that is "expressed on endothelial cells and might therefore have a role in microvascular damage caused by endothelial adherence of parasitized erythrocytes" (Timmann et al, 2012).

Toll-like receptors are involved in immune responses to several pathogens, and the adaptor protein Mal, encoded by *TIRAP* at 11q24.2, takes part in the pathway's signaling. Heterozygosity for a SNP encoding a leucine substitution on Mal was implicated in protecting against invasive pneumococcal disease, bacteremia, malaria, and

tuberculosis across eight study populations (Khor et al, 2007), although there has been some debate about the duplicability of these results. Like studies of the *P. falciparum* genome, knowledge of the interactions between the human genome and the processes of malaria have contributed to our understanding of specific mechanisms toward which malaria control measures could be directed.

MicroRNAs and Malaria

An exciting finding intertwining the roles of the human and *P. falciparum* genomes in disease prevention, published in September 2012, concerns the function of microRNA in malaria resistance. MiRNAs appear in red blood cells during erythroblast proliferation. Erythrocytes with the HbS-derived sickle trait have elevated levels of miR-451, miR-233, and let-7i compared with normal erythrocytes, although the reasons for this differential expression are not known (LaMonte et al, 2012). The miRNAs' effect on the parasitic mRNA is to reduce the translation activity of the regulatory PKA subunit, thereby inhibiting erythrocyte invasion and survival, sporozoite motility and hepatocyte invasion, and induction of gametocytogenesis. This particular miRNA effect takes advantage of a powerful opportunity to disrupt *P. falciparum* activity (LaMonte et al, 2012). That the miRNAs themselves confer malaria resistance is supported by the observation that when miR-451, miR-233, and let-7i are inhibited in HbAS and HbSS genotypes, parasite growth increases (LaMonte et al, 2012).

The truly unusual aspect of this resistance mechanism is the way miR-451 operates in the parasite: the miR-451 from the HbS erythrocyte is translocated into the *P. falciparum* parasite and covalently binds to the *Plasmodium* PKA-R mRNA, forming a

chimeric RNA that inhibits translation (LaMonte et al, 2012). This is a departure from all previously observed miRNA-mRNA interactions, in which miRNA binds to RNA-induced silencing complexes of proteins and then to regions of mRNA with complementary base pairs. However, there are no Dicer or Argonaute homologs in *Plasmodium*, ruling out the normal functioning of miRNA (LaMonte et al, 2012). The chimeric bonding was confirmed by real-time PCR and northern blots.

Unlike some of the resistance loci and drug targets suggested from the human and *P. falciparum* genomes, this miRNA effect immediately suggests an application to the treatment of malaria in populations lacking the recessive hemoglobinopathy. When these specific miRNAs are *overexpressed* in the HbAA genotype, malarial resistance increases; in fact, the infection rate of these HbAA erythrocytes was found to be reduced by 46% when miR-451 and miR-223 were overexpressed (LaMonte et al, 2012). The mechanism by which the miRNA crosses from the erythrocyte cytosol, through the parasitophorous vacuolar membrane, and then through the parasite's plasma membrane is unknown, but it will be useful to decipher so that this effect can be manipulated (Duraisingh and Lodish, 2012).

The incidence of malaria has been declining slightly since 2000. Access to insecticide-treated bed nets has vastly improved, thanks to growing donor support (World Malaria Report 2011). As global attention focuses on the disease, research into treatments accelerates. There have been recent setbacks, such as the limited success of the RTS,S vaccine, even as promising research surfaces. Given the wide variety of currently proposed therapies, it is good news that funding from such powerful entities as the United

States government and the Bill and Melinda Gates Foundation is not likely to dry up soon. There is still a great amount of work to be done; the properties of malaria vary in differing regions around the world, and there are more *Plasmodium* species than just *falciparum* that cause malaria. Even when new treatments are developed, the arms race driven by drug resistance will eventually become a problem. But the progress to date in the effort to stop malaria is an example of how the tools of genomics can be used to combat infectious disease. Hopefully, the rapid advances in biotechnology will soon lead to the control and eventual eradication of the disease that has plagued humanity for so long.

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