Human Genetics and Malaria: Relevance for the Design of Clinical Trials

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(See the article by Crompton et al., on pages 1265–75.)

Perhaps no disease in history has exerted as strong a selective pressure on the human genome as falciparum malaria. Evidence suggests that *Plasmodium falciparum* has infected humans for at least 5000–10,000 years, and human haplotype studies have shown that alleles that may offer protection against malaria have undergone selection during that same time frame [1–3]. Today, that evolutionary pressure continues, with an estimated half-billion episodes of falciparum malaria yearly, which are associated with 1–3 million deaths [4]. Falciparum malaria appears to be the selective force behind the most common hemoglobinopathies and enzymopathies in the world, including sickle cell anemia, various types of thalassemia, and glucose-6-phosphate dehydrogenase (G6PD) deficiency. In 1949, J. B. S. Haldane suggested that the distribution of thalassemia may have been influenced by the selective pressure exerted by malaria [5]. In 1954, A. C. Allison showed an association between the prevalence of sickle cell disease and malaria [6]. Subsequent studies showed decreased prevalence of the sickle hemoglobin gene in those with severe malaria, compared with healthy control subjects [7].

Thus, in this balanced polymorphism, sickle hemoglobin (HbAS), which is caused by a single-nucleotide polymorphism (SNP) in the β-globin gene leading to a Glu6Val substitution, affords protection against mortality from falciparum malaria in heterozygous individuals, balancing the severe consequences of sickle cell disease in homozygous individuals. Subsequent studies have demonstrated a 60%–90% level of protection for sickle cell–heterozygous individuals against the most severe forms of malaria and a lower level of protection against mild malaria [7–10]. Although the mechanisms of protection afforded by sickle heterozygosity are uncertain, important contributors likely include the reduced ability of parasites to grow and multiply in HbAS erythrocytes and the altered display of parasite ligands on the erythrocyte surface, leading to reduced binding of infected erythrocytes to host cells [11–13].

In this issue of the *Journal*, Crompton et al. demonstrate protection in sickle cell–heterozygous individuals, measured by using a surrogate for malaria incidence, the time to the first episode of malaria after enrollment of a healthy cohort [14]. This surrogate is not as precise a measure as true incidence, but it is much easier to assess and is recommended by the World Health Organization as the primary endpoint in Phase III malaria vaccine studies [15]. To ascertain the impact of human genetics on this primary endpoint, Crompton et al. [14] followed a cohort of 225 Malian volunteers for 8 months, performing multiple blood smears during and after the malaria transmission season. In children aged 2–10 years, HbAS was associated with a median 34-day delay in time to first episode of symptomatic malaria. Sickle cell trait led to a larger reduction in time to malaria than any other covariate included in a Cox proportional hazards analysis. Notably, G6PD status, α-thalassemia, and bed net use were not associated with protection from malaria in this study. The authors argue that hemoglobin S status should be evaluated in all vaccine trials to reduce the potential for imbalanced distribution in intervention groups and to increase the power for detection of intervention effects.

The results reported by Crompton et al. [14] add to the expanding knowledge base regarding the impact of human genetics on malaria. The most intensively studied phenotype with respect to human susceptibility to malaria has been severe disease. Multiple polymorphisms that are predicted to affect parasite interactions with
the erythrocyte have been shown to protect against severe malaria. These variants include those in globin (HbS, HbC, and α-thalassemia), erythrocyte surface proteins (band 3 and glycophorin C), and oxidative enzymes (G6PD). Intriguingly, some of these variants may interact with one another to modulate protection against malaria, as evidenced in recent studies suggesting negative epistasis between HbS and α-thalassemia [16].

In addition, the impact of human genetic variation on malaria extends beyond disease expression in the general population. Genetics may particularly affect outcomes in pregnant women, the group at greatest risk, after young children, for severe malaria morbidity. Recent studies have associated variations in band 3, haptoglobin, and Toll-like receptors in the manifestations of pregnancy-associated malaria [37–39]. Genetic differences in drug-metabolizing enzymes may affect the efficacy or toxicity of antimalarial therapy. CYP2C19 genotypes have been associated with the metabolism of proguanil in vitro, but CYP2C19 status did not clearly correlate with clinical efficacy of the drug [40–42]. Variants of CYP2C8 that are prevalent in African populations show decreased metabolism of amodiaquine; the clinical significance of this finding is unknown [43]. Immune responses to malarial infection and to vaccines are also under genetic control; particular HLA haplotypes have been associated with protection from malaria [7, 44]. In searching for genetic factors that affect malaria, animal studies may prove informative, as was true for recent studies implicating pyruvate kinase deficiency in protection [45, 46].

Crompton et al. found that the polymorphism best associated with incidence of malaria, HbAS, also offered protection when assessed on basis of the time to first episode of malaria [14]. The authors argue for the inclusion of sickle cell trait as a covariate in studies of vaccine efficacy. The recommendation is well founded and supports that of Sokhna et al., who earlier found that HbAS was associated with a delay in the rate of parasite reappearance after radical treatment [47]. However, heritability studies have suggested that although about 25% of total variation in malaria incidence and hospitalization can be accounted for by host genetic variation, sickle cell status accounts for only 2% of this variation [48]. Thus, many other genetic factors likely affect the outcome studied by Crompton et al. [14]. Moreover, specific genetic factors will likely affect different populations differently, and certain polymorphisms (e.g., absence of the Duffy antigen, which protects against Plasmodium vivax infection primarily in Africans, and the band 3 mutation that causes ovalocytosis, primarily in Asian populations) have strong geographic determinants. Therefore, it seems prudent to heed the advice of these authors and assess sickle cell status in vaccine study subjects, but it would also be prudent to consider the identification of additional genetic mediators of malaria outcomes a high priority. Further, concern about genetic factors and study outcomes should extend beyond vaccine studies. Genetics can affect treatment outcomes, as was demonstrated in a recent study that showed sulfadoxine-pyrimethamine treatment to be approximately half as likely to fail in children with sickle cell trait, compared with children who had normal hemoglobin [49]. Adding extensive genetic assessments to all malaria treatment efficacy studies may be impractical. Nonetheless, recent characterizations of the impact of human genetics on malaria outcomes, including the report in this issue, remind us of the powerful impact of falciparum malaria on human evolution and therefore of the need to consider human genetics as one assesses malaria outcomes.

References