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Positively Selected G6PD-Mahidol Mutation Reduces Plasmodium vivax Density in Southeast Asians

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Glucose-6-phosphate dehydrogenase (G6PD) deficiency—the most common known enzymopathy—is associated with neonatal jaundice and hemolytic anemia usually after exposure to certain infections, foods, or medications. Although G6PD-deficient alleles appear to confer a protective effect against malaria, the link with clinical protection from Plasmodium infection remains unclear. We investigated the effect of a common G6PD deficiency variant in Southeast Asia—the G6PD-Mahidol487A variant—on human survival related to vivax and falciparum malaria. Our results show that strong and recent positive selection has targeted the Mahidol variant over the past 1500 years. We found that the G6PD-Mahidol487A variant reduces vivax, but not falciparum, parasite density in humans, which indicates that Plasmodium vivax has been a driving force behind the strong selective advantage conferred by this mutation.

Malaria is a major cause of human mortality worldwide and is considered to be one of the strongest known forces of evolutionary selection in the recent history of the human genome (1). Host genetic defense mechanisms are likely to have evolved to resist malaria infection in regions where the parasites have been historically prevalent. Among malaria-causing parasites, Plasmodium falciparum and Plasmodium vivax seem to have exerted strong selective pressure on the cellular phenotype of human erythrocytes, causing increased prevalence of hemoglobinopathies and other inherited blood disorders (1).

Glucose-6-phosphate dehydrogenase (G6PD) is an X-linked essential enzyme that plays a key role in protecting cells from oxidative stress and is particularly important in red blood cells. G6PD deficiency, affecting more than 400 million people worldwide, is associated with several clinical disorders including neonatal jaundice, hemolytic anemia following infection by certain pathogens, and favaism (2). The high overall frequency of G6PD-deficient alleles in the population is thought to result from their protective effect against malaria. Evolutionary studies of the G6PD loci suggest that local and recent positive selection has targeted the G6PD-deficient allele G6PD4 in Africa (3, 4) and that this process started 2500 to 3800 years before present (YBP) (5). These observations are consistent with signs of recent expansion of P. falciparum in Africa (6). However, the clinical link between G6PD deficiency and malaria is less clear. Although a clinical protective effect of G6PD deficiency against human lethal malaria, P. falciparum, has been shown in Africa (7), several other reports have not found an association (8, 9).

Most clinical, epidemiological and evolutionary studies of the relation between G6PD deficiency and malaria protection have focused on falciparum malaria, particularly in Africa. The role of G6PD-deficient alleles in the susceptibility, or resistance, to vivax malaria has not been accurately tested and remains anecdotal. Nevertheless, P. vivax imposes a considerable burden of disease on the human population and, historically, has been associated with considerable mortality and decreased fertility in human populations (10). Both P. falciparum and P. vivax coexist in Southeast Asia, with P. vivax accounting for over half of malaria cases. Moreover, there is increasing evidence for an ancient origin of P. vivax in Asia (11), where its presence apparently predates that of P. falciparum (12).

We investigated whether G6PD deficiency increases human survival in Southeast Asia, through its effects on vivax and falciparum malaria, in an evolutionary and epidemiological study of the Mahidol487A mutation (MIM no. 305900.005). This G6PD-deficient variant occurs throughout greater Southeast Asia, including mainland China, and is most common in Myanmar [Burma] (13). The average allele frequency in Thailand is 12%, but there is distinct local heterogeneity with increased frequency on the western border, particularly in the Mon, Burmese, and Karen populations (13, 14). This mutation is classified as a moderate-to-mild G6PD variant with a reduction of 5 to 32% of wild-type activity levels in healthy individuals (13, 15).

We first used an evolutionary approach to detect the potential molecular signature of positive selection at the G6PD-Mahidol487A mutation. We genotyped 30 single-nucleotide polymorphisms (SNPs) (including the Mahidol487A variant) (table S1) dispersed along a 2.4-Mb region encompassing G6PD (Fig. 1A) in a panel of 384 unrelated individuals, the majority of whom are Karen, living in the Suan Phung district of Thailand (16). After reconstructing the phase of extended haplotypes (16), we implemented the long-range haplotype (LRH) test, which identifies alleles that have undergone recent positive selection, i.e., alleles associated with high levels of extended homozygosity at nearby markers and present at high population frequencies (3). The frequency of the Mahidol487A mutation in our population sample was 24% and showed very high levels of extended homozygosity: 63% of Mahidol487A-bearing haplotypes showed complete haplotype conservation over the entire 2.4-Mb region (Fig. 1, A and B). The high level of homozygosity surrounding Mahidol487A was highly significant given its frequency (P < 10−5), when compared with the empirical distribution of allelic homozygosity versus frequency for all X-linked HapMap Phase II SNPs in Han Chinese (17), after matching for SNP density (Fig. 1C). However, the power of the LRH test can be challenged by uncertainties related to phase reconstruction and specific population histories (18). To circumvent this, we compared the observed allelic homozygosity associated with Mahidol487A in males only (whose haplotype phase is known) with simulations of a ~1-Mb recombining X-linked region for a population experiencing different demographic regimes (16). Consistently, we found a highly significant signal of positive selection at Mahidol487A, independently of the demographic scenario considered (P < 10−4 for the constant population size, the bottleneck and the expansion models) (Fig. 1D). The low microsatellite diversity associated with Mahidol487A (Fig. 1E) confirmed the results based on the LRH test. Together, our evolutionary analyses show that the Mahidol487A mutation is under recent and strong positive selection in the Karen population, indicating that this mutation has conferred a strong selective advantage in human survival.

We estimated the age of G6PD-Mahidol487A and the selection coefficient that would be consistent with such a strong positive selection. We obtained similar estimates using two different methods (19, 20), which showed that the fre-
frequency of the Mahidol$^{487A}$ mutation started to increase at ~1500 YBP, with a selection intensity of ~0.23 (Table 1). The selection coefficients of Mahidol$^{487A}$ are among the strongest detected so far in the human genome, including human immunodeficiency virus (HIV)-protective haplotypes (~0.30) (21), malaria-protective $G6PD^{4}$ (~0.2) (5, 20) and $\beta$-globin variants (~0.26 and ~0.08) (22, 23), as well as lactase persistence (~0.1) (24).

We investigated the nature of the selective advantage conferred by the $G6PD$-Mahidol$^{487A}$ mutation by testing its influence on the outcome of infection with either $P$. falciparum or $P$. vivax. To this end, we conducted a community-based longitudinal study in the Suan Phung district of Thailand, which has a total population of 5368. From the 3484 participants of the malaria epidemiology study (25), we obtained genotypes at the $G6PD$-Mahidol position from 925 individuals (16). Between 1998 and 2005, there were 1090 $P$. falciparum clinical episodes in 460 of these individuals, and 524 $P$. vivax clinical episodes in 262 of these individuals. Reliable parasite density data was available for 823 observations of $P$. falciparum parasite density in 400 individuals and for 417 observations of $P$. vivax parasite density in 227 individuals. Details on the sample selection procedures are provided in (16) and summarized in fig. S1.

To test for a genetic association between $G6PD$-Mahidol$^{487A}$ and the number of $Plasmodium$ species clinical cases or parasite density, we performed a family-based association test (FBAT), which corrects for spurious association due to population stratification (16). Seventy-seven families were informative for the association with the number of $Plasmodium$ species clinical cases, 44 of which were informative for the association with $P$. falciparum density (i.e., some families were infected by different $Plasmodium$ species at different times) (16). We found that Mahidol$^{487A}$ had no effect on the number of cases of clinical malaria due to either $P$. vivax or $P$. falciparum reported for each individual during the 7-year observation period (16). This is likely due to the high heterogeneity of exposure to infection in this area of low transmission intensity, where virtually all infections by either parasite species lead to symptomatic episodes (25). Our analyses also revealed that Mahidol$^{487A}$ was not significantly associated with $P$. falciparum density, using both annual values of parasite density (16) or mean density values across all years under any genetic model (table S2). By contrast, Mahidol$^{487A}$ was significantly associated with reduced $P$. vivax density using both annual values of parasite density ($\chi^2$ test, $P = 0.029$) and mean density values across years ($\chi^2$ test; dominant model $P = 0.011$, additive model $P = 0.016$, recessive model $P = 0.048$ (table S2)) taking into account age and environmental covariates that affect parasite density (16, 26). A permutation test with 100,000 iterations confirmed that these results were significant ($\chi^2$ test, $P = 0.017$).

To verify this association, we performed a population-based association study between Mahidol$^{487A}$ and $Plasmodium$ parasite density in the whole population, excluding all individuals used in the FBAT analyses (16). Mahidol$^{487A}$ significantly reduced $P$. vivax parasite density (the most significant $P$ value was obtained for the dominant model, $\chi^2$ test, $P = 0.006$), whereas no association was observed with $P$. falciparum parasite density for any genetic model (table S2). When considering both the family-based and the population-based data sets, $P$. vivax parasite density decreased with age ($\chi^2$ test, $P < 0.001$), indicative of the acquisition of antiparasite immunity (Fig. 2A). Mean $P$. vivax density was reduced by 30% in females heterozygous for the Mahidol$^{487}$ mutation (non-Mah/Mah) and 61% in females homozygous for Mahidol$^{487}$ (Mah/Mah) compared with non-Mah/non-Mah females; parasite density was reduced by 40% in hemizygous males for Mahidol$^{487}$ (Mah/Mah) compared with non-Mah/Mah males (Fig. 2B). Mahidol$^{487}$ accounted for 3.3% of the observed variation in $P$. vivax density. Although increasing age was again associated with decreasing $P$. falciparum density ($\chi^2$ test, $P < 0.001$) (Fig. 2C), there was
no significant effect of Mahidol487A on falciparum density (Fig. 2D).

A Mahidol487A gene dose effect on P. vivax density was observed, whereby parasite density tends to be lower in Mah/Mah females with respect to both non-Mah/Mah females and Mah/Y males (Fig. 2B). Two main factors can explain the observed tendency. First, G6PD activity in heterozygous non-Mah/Mah females depends on the random inactivation of the X-chromosome: Each heterozygous female will contain two populations of red blood cells (expressing Mahidol487A or Mahidol487G), and their relative abundance is unpredictable. Indeed, when measuring G6PD activity in Karen individuals using the fluorescence spot test (16), we observed that the non-Mah/Mah genotype can express either normal or deficient phenotypes (table S3). Second, the Mahidol487A mutation markedly increases protein thermal instability with consequences for enzyme activity (15). Hence, in P. vivax infection, which induces high fever, G6PD deficiency may be more pronounced than in noninfected individuals, which contributes to the reduction in parasite density. Because genotype-phenotype correlations for G6PD can be complex, we analyzed our data using G6PD phenotypes rather than genotypes. We confirmed that both age and G6PD-deficient phenotypes significantly decreased P. vivax density ($\chi^2$ test, $P < 0.001$ and $P = 0.039$, respectively) (16).

Although the precise mechanism underlying this protective effect is unknown, it is likely to be related to the effects of G6PD deficiency on red cell physiology, particularly by increasing oxidative stress. Young red cells (i.e., reticulocytes) contain more antioxidant enzymes than mature red cell populations (27). The preference shown by P. vivax for reticulocytes suggests that P. vivax is more sensitive to oxidative stress than P. falciparum, which is known to have no red-cell preference (28). Under these conditions, reduced G6PD activity would have a greater effect on P. vivax.

The historical expansion of malaria has been linked to that of agriculture, which generated breeding grounds for mosquitoes and increased human population density (29), thereby facilitating human-mosquito contact and the conditions for stable malaria transmission. In East Asia, farming is mainly associated with the development of rice culture in China ~8000 YBP. Although there is evidence of rice cultivation in Southeast Asia dating back to 4200 YBP, it developed mainly over the last 2000 years (30). The Karen people, belonging to the Sino-Tibetan language group, are thought to descend from Tibetan people who entered Myanmar ~1500 YBP (31). It is noteworthy that the estimated age of Mahidol487A, at ~1500 YBP, coincides with the proposed arrival of the Karens into the region and with the time at which rice started to be extensively cultivated. This supports a link between the selective advantage conferred by the G6PD-Mahidol487A mutation and its protective effect against vivax malaria.

In conclusion, we showed that the G6PD-Mahidol487A mutation has been under strong positive selection for the last 1500 years and that it reduces P. vivax parasite density in humans. These findings provide evidence that vivax malaria has been a driving force behind the selective advantage conferred by the Mahidol487A mutation and supports the notion that P. vivax historically had a considerable impact on human health (10), at least in Southeast Asia. Indeed, P. vivax infection is not only responsible for clinical malarial attacks, but has also been implicated in causing low birth weight (32) and malnutrition in children (33), both of which have strong impacts on childhood survival. The significant health burden imposed by P. vivax has been seriously underestimated, and increased global efforts to combat malaria should encompass P. vivax as well as P. falciparum malaria.

References and Notes
MicroRNA-206 Delays ALS Progression and Promotes Regeneration of Neuromuscular Synapses in Mice

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by loss of motor neurons, denervation of target muscles, muscle atrophy, and paralysis. Understanding ALS pathogenesis may require a fuller understanding of the bidirectional signaling between motor neurons and skeletal muscle fibers at neuromuscular synapses. Here, we show that a key regulator of this signaling is miR-206, a skeletal muscle-specific microRNA that is dramatically induced in a mouse model of ALS. Mice that are genetically deficient in miR-206 form normal neuromuscular junctions but are highly susceptible to ALS, which suggests that this microRNA delays disease progression. Knockdown of miR-206 in mice expressing a toxic superoxide dismutase 1 (SOD1) mutation exacerbates disease, whereas ectopic expression of miR-206 can significantly delay SOD1-mediated neurodegeneration. This study provides insights into the pathogenesis of ALS and identifies microRNA-206 as a potential therapeutic target for this disease.

16. Materials and methods are available as supporting material on Science Online.
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References
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