X-Linked G6PD Deficiency Protects Hemizygous Males but Not Heterozygous Females against Severe Malaria

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Abbreviations: CI, confidence interval; G6PD, glucose-6-phosphate dehydrogenase; Hb, hemoglobin; OR, odds ratio

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ABSTRACT

Background

Glucose-6-phosphate dehydrogenase (G6PD) is important in the control of oxidant stress in erythrocytes, the host cells for *Plasmodium falciparum*. Mutations in this enzyme produce X-linked deficiency states associated with protection against malaria, notably in Africa where the A– form of G6PD deficiency is widespread. Some reports have proposed that heterozygous females with mosaic populations of normal and deficient erythrocytes (due to random X chromosome inactivation) have malaria resistance similar to or greater than hemizygous males with populations of uniformly deficient erythrocytes. These proposals are paradoxical, and they are not consistent with currently hypothesized mechanisms of protection.

Methods and Findings

We conducted large case-control studies of the A– form of G6PD deficiency in cases of severe or uncomplicated malaria among two ethnic populations of rural Mali, West Africa, where malaria is hyperendemic. Our results indicate that the uniform state of G6PD deficiency in hemizygous male children conferred significant protection against severe, life-threatening malaria, and that it may have likewise protected homozygous female children. No such protection was evident from the mosaic state of G6PD deficiency in heterozygous females. We also found no significant differences in the parasite densities of males and females with differences in G6PD status. Pooled odds ratios from meta-analysis of our data and data from a previous study confirmed highly significant protection against severe malaria in hemizygous males but not in heterozygous females. Among the different forms of severe malaria, protection was principally evident against cerebral malaria, the most frequent form of life-threatening malaria in these studies.

Conclusions

The A– form of G6PD deficiency in Africa is under strong natural selection from the preferential protection it provides to hemizygous males against life-threatening malaria. Little or no such protection is present among heterozygous females. Although these conclusions are consistent with data from at least one previous study, they have not heretofore been realized to our knowledge, and they therefore give fresh perspectives on malaria protection by G6PD deficiency as an X-linked trait.

The Editors' Summary of this article follows the references.

Introduction

Glucose-6-phosphate dehydrogenase (G6PD) is important in the generation of reduced glutathione, a product key in the control of oxidative damage in erythrocytes. Although complete loss of this enzyme is presumably lethal, mutant forms of G6PD with partially deficient activity are common and have been associated with protection against malaria. Geographic distribution of these G6PD variants correlates with historic distributions of the disease [1,2], and a mutant allele *(A–)* encoding G6PD with 10%–50% of normal enzyme activity is widespread in Africa [3–5]. However, conclusions from case-control studies [5–7] and in vitro parasite culture experiments [8–10] have been conflicting and have not been reconciled satisfactorily with the differential expression of *G6PD**A– in males and females. For example, studies using parasite densities but not clinical findings as indicators of disease severity suggested resistance in heterozygous females only, whereas hemizygous males paradoxically showed higher parasitemias despite more severe enzyme deficiency [7]. A report of near-equivalent protection against life-threatening malaria in both males and females [5] has also been difficult to reconcile with the differential expression of G6PD deficiency between the sexes, as hemizygous males would be expected to show an advantage over heterozygous females under at least one mechanism of protection currently advocated [11].

The heavy impact of natural selection from malaria lies in its severe manifestations that kill young children. In rural villages of West Africa where malaria is hyperendemic and adequate medication can be difficult to obtain, virtually all children experience malaria episodes early in life, and approximately 20% die from the disease by age five [12]. A tremendous selective advantage is therefore available to children who can tolerate episodes of *P. falciparum* infection and not have malaria progress from its uncomplicated form to severe and fatal disease.

Following our demonstration of protection against severe relative to uncomplicated malaria by hemoglobin (Hb) C in Dogon children of Mali [13], we asked whether the A– form of G6PD deficiency might also protect against severe malaria, and whether it might do so in a sex-specific manner. Here we report the results of two case-control studies in Malian villages where parasitization is ubiquitous and virtually all children experience malaria in their early years.

Methods

Patient Populations and Malaria Case Definitions

Our case-control studies included two populations of patients presenting to similarly equipped, physician-staffed clinics: (i) 488 control individuals with uncomplicated malaria and 67 patients with severe malaria from the Dogon of Bandiagara, Mali, during two annual transmission seasons (1997–1998); and (ii) 2,277 controls with uncomplicated malaria and 365 patients with severe malaria from the predominantly (82%) Malinké inhabitants of Kangaba and Kela, Mali, during four annual transmission seasons (2001–2004). In accordance with World Health Organization guidelines, uncomplicated (mild) malaria was defined by treatment-seeking behavior for symptoms consistent with malaria (i.e., fever, headache) plus axillary temperature above 37.5 °C plus observed parasite density below 500,000/μl. Severe (life-threatening) malaria was defined as either hyperparasitemia (≥500,000/μl) or the presence of any parasite density in association with one or more of the following clinical criteria: cerebral malaria (Blantyre coma score ≤2, witnessed convulsions), severe anemia (hematocrit <15% or hemoglobin <5 g/dl [equivalent to 50 g/l]), respiratory distress, or prostration (inability to sit unassisted in a child usually able to do so) [14]. Patients with uncomplicated malaria were treated with oral chloroquine (first-line malaria treatment in Mali during the study period) and monitored for clinical and parasitologic treatment failures, which were treated with either oral sulfadoxine–pyrimethamine (second-line malaria treatment during the study period) or parenteral quinine, as appropriate. Patients with severe malaria, or with uncomplicated malaria and parasite densities of 100,000–500,000/μl, were treated parenterally with quinine. Study protocols were approved by Institutional Review Boards of the Faculté de Médecine, de Pharmacie et d'Odontostomatologie, University of Bamako and the National Institute of Allergy and Infectious Diseases. Community permission and individual written informed consent were provided by a parent or guardian of all participating children, as described [15].

Laboratory Procedures and Statistical Analysis

Parasite densities in malaria patients were counted as described [13]. The *(A–)* allele of the gene responsible for G6PD deficiency in Mali was identified by restriction fragment length polymorphism analysis of PCR-amplified DNA samples. Blood was spotted onto strips of filter paper (Schleicher & Schuell 907, http://www.arraying.com), and DNA was extracted using the QIAamp kit (Qiagen, http://www.qiagen.com). Under conditions intended to eliminate risk of cross-contamination and with appropriate water-only negative controls, exon 4 of *G6PD* was amplified using a nested PCR protocol: 1 μg of genomic DNA was first amplified using primers 5′-GTCTTCTGGGTCAGGGAT-3′ (forward) and 5′-GGAGAAAGCTCTCTCTCC-3′ (reverse). Denaturation at 94 °C for 2 min was followed by 45 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, extension at 72 °C for 60 s, and final extension at 72 °C for 4 min. Nested amplification was performed using primers 5′-CCTGTTCCCTCTGCCACA-3′ (forward) and 5′-GGGGGTCTCAAGAAGTAC-3′ (reverse). Denaturation at 94 °C for 2 min was followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 60 s, extension at 72 °C for 30 s, and a final extension at 72 °C for 4 min. Amplification products were recovered with separate pipettors in laboratory areas apart from the PCR-preparation bench and digested with the restriction endonuclease NlaIII (NEB, http://www.neb.com) or its isoschizomer Hsp92II (Promega, http://www.promega.com) to detect the *G6PD**A– mutation at nucleotide position 202. Complete cutting of PCR product identified hemizygous males and homozygous females; cutting of half of the product identified heterozygous females. Hb typing was performed by standard cellulose acetate electrophoresis (Helena Laboratories, http://www.helena.com) and in some cases confirmed by HPLC (D-10 instrument; Bio-Rad, http://www.bio-rad.com).

Odds ratios (ORs), exact 95% confidence intervals (CIs), Fisher's exact tests, and *p*-values were calculated by EpiInfo 2000 software (Centers for Disease Control and Prevention,

Table 1. Distribution of Severe and Uncomplicated Malaria in Recruited Children According to Ethnicity, Sex, and *G6PD* Genotype

Group	Illness	All		Male		Female		
		Deficient	Normal	Hemizygous	Normal	Heterozygous	Homozygous	Normal
Dogon	Severe malaria	5 (7.5)	62 (92.5)	0 (0)	37 (100)	5 (16.7)	0 (0)	25 (83.3)
	Uncomplicated malaria	81 (16.6)	407 (83.4)	34 (13.8)	213 (86.2)	46 (19.1)	1 (0.4)	194 (80.5)
Malinké predominant	Severe malaria	40 (11.0)	325 (89.0)	15 (7.7)	180 (92.3)	22 (12.9)	3 (1.8)	145 (85.3)
	Uncomplicated malaria	340 (14.9)	1,937 (85.1)	152 (14.1)	926 (85.9)	148 (12.4)	40 (3.3)	1,011 (84.3)
Stratified analysis	Pooled OR (95% CI)			0.42 (0.23–0.73)		1.00 (0.62–1.55)	0.51 (0.10–1.63)	
	p-Value			<0.001		>0.999	0.35	

Data shown as number of cases (%) by sex and *G6PD* genotype.
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http://www.cdc.gov) or by GraphPad Instat version 3.01 (GraphPad Software, http://www.graphpad.com). Exact conditional likelihood methods were used to calculate pooled ORs, 95% CIs, and two-sided *p*-values (StatsDirect version 2.5.7, http://www.statsdirect.com). Assumptions of fixed effects meta-analysis were confirmed by tests for homogeneity of the ORs from the different geographic groups. Means of $\log_{10}$-transformed *P. falciparum* densities were compared by ANOVA (Epi Info2000, http://www.cdc.gov/epiinfo).

Results

Among all children presenting to our clinics with fever and other symptoms of illness, our clinical and laboratory determinations identified 3,197 with uncomplicated (n = 2,765) or severe ($n = 432$) forms of malaria. Two observations indicated the populations of children in the clinics were representative of the populations at large. First, in Kela, the one village in our program small enough to realistically include children from all families, all but 112 of 1,288 age-eligible children in the population (~50% of the cases in the Malinké-predominant group of Table 1) were seen for malaria and enrolled in our case-control study. From village census information, we located the 112 children, met with their families, and learned that reasons they had not come to the clinic for malaria treatment were various, including that they received other sources of care for uncomplicated malaria (e.g., family self treatment or seeing a local pharmacist), or that the children might have had malaria without sufficiently intense symptoms to seek care (e.g., reduced symptoms in some cases because of the presence of sickle trait) (unpublished data). These 112 children showed proportions of *G6PD**A– alleles in males and females (7/58 = 12.1% and 8/54 = 14.8%, respectively) that did not differ significantly from those in the uncomplicated malaria groups (152/1,078 = 14.1% and 188/1,199 = 15.7%; Fisher's exact test *p*-values 0.85 and 1.00, respectively). Second, in the larger village of Bandiagara contributing to the Dogon study group, we showed that the prevalence of Hb C in uncomplicated malaria patients was the same as that in more than 7,000 individuals from the overall population [13], a finding consistent with universal experience of Dogon children with parasitization and malaria. Since the malaria cases from that study also provided test samples for our present report, the observed prevalence of G6PD A– in our uncomplicated malaria control group likely reflects the prevalence of G6PD A– in the Dogon population at large.

Table 1 presents the distributions of malaria cases in the Dogon and Malinké-predominant groups by ethnicity, sex, and *G6PD* genotype. These distributions show similar prevalence of the *G6PD**A– allele in controls with uncomplicated malaria among all children in both ethnic groups (16.6% and 14.9%, respectively; Chi^2 $p = 0.39$). Relative to this prevalence, the *G6PD**A– allele is less frequent in the severe malaria cases (7.5% and 11.0%, respectively; Chi^2 $p = 0.01$ for the groups taken together).

The A– form of G6PD deficiency in sub-Saharan Africa is a sex-linked trait characterized by two mutations in the *G6PD* gene-coding sequence on the X chromosome (nucleotide changes A376G, resulting in an Asn → Asp mutation, and G202A, resulting in a Val → Met mutation). In males with G6PD A–, all encoded copies of the enzyme are deficient because only one version of the X chromosome is present (hemizygous state). However, G6PD A– females can have either homozygous deficiency in which all encoded copies of the enzyme are deficient (because both of the two inherited X chromosomes carry the mutant allele) or, more frequently, a heterozygous, mosaic state of G6PD-deficient and G6PD-normal cells (because random inactivation of one of the two inherited X chromosomes results in some cells with normal enzyme and others with mutant enzyme). We therefore examined our data for evidence of differential protection against severe malaria by the uniform (hemizygous, homozygous) states of G6PD deficiency relative to its mosaic state. By stratified OR analysis of the case-control recruitments from the Dogon and Malinké-predominant groups, we found that the protection due to G6PD deficiency against severe malaria occurs entirely in males (Table 1; pooled OR = 0.42; 95% CI 0.23–0.73; $p < 0.001$). No evidence of protection against severe malaria was evident among heterozygous females mosaic for G6PD A– (pooled OR = 1.00; 95% CI 0.62–1.55; $p > 0.999$). As a test for any evidence that ethnicity might act as a confounder in these results, we repeated the analysis with only malaria cases of identifiable Malinké ethnicity (82%) from the Malinké-predominant group. Calculations confirmed reduced odds of severe malaria in male children with G6PD A– (OR = 0.37; 95% CI 0.18–0.70; $p = 0.001$) but not in the heterozygous female children with severe malaria (OR = 1.00; 95% CI 0.59–1.64; $p = 0.99$).

Children who survive repeated *P. falciparum* infections in hyperendemic areas of sub-Saharan Africa eventually acquire immunity against malaria that protects them against its severe, life-threatening forms. This immunity is evident in

Table 2. Distribution of Severe and Uncomplicated Malaria in HbAA Children ≤5 y According to Ethnicity, Sex, and *G6PD* Genotype

Group	Illness	Male		Female		
		Hemizygous	Normal	Heterozygous	Homozygous	Normal
Dogon	Severe malaria	0 (0)	27 (100)	4 (17.4)	0 (0)	19 (82.6)
	Uncomplicated malaria	15 (14.6)	88 (85.4)	15 (15.5)	0 (0)	82 (84.5)
Malinké predominant	Severe malaria	7 (5.9)	111 (94.1)	13 (11.1)	1 (0.9)	103 (88.0)
	Uncomplicated malaria	71 (15.5)	387 (84.5)	55 (12.3)	15 (3.4)	377 (84.3)
Stratified analysis	Pooled OR (95% CI)	0.28 (0.11–0.62)		0.92 (0.49–1.65)	0.24[a] (0.01–1.63)	
	p-Value	<0.001		0.89	0.21	

Data shown as number of cases (%) by sex and *G6PD* genotype.
[a]OR with Fisher's exact CI for Malinké-predominant group only, as no homozygotes with malaria were recruited in the Dogon group.
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much-reduced morbidity and mortality rates in children after the first few years of life [16,17]. Mutant hemoglobins also contribute to protection against severe malaria in West Africa [13,18,19]. To minimize the influence of the effects of these forms of protection as potential confounding variables, we determined the ORs for children both 5 y old and younger and of normal HbAA type. Results from these calculations (Table 2) provided even stronger evidence that G6PD A– protects males against severe malaria (pooled OR = 0.28; 95% CI 0.11–0.62; $p < 0.001$), whereas mosaic females again showed no evidence of significant protection (pooled OR = 0.92; 95% CI 0.49–1.65; $p = 0.89$). For homozygous females 5 y old and younger in the Malinké-predominant group the OR = 0.24; 95% CI 0.01–1.63; $p = 0.21$. G6PD-deficient males among children 5 y old or younger with uncomplicated malaria were not significantly older than their female counterparts (aged 3.17 ± 1.29 y versus 2.95 ± 1.23 y; $p = 0.22$), so their differential protection relative to heterozygous females could not be explained by greater levels of age-dependent acquired immunity.

In concert with analyses of the clinical presentations, we compared the parasite densities of male and female children 5 y old or younger with different G6PD status. Among children with malaria and HbAA type, no significant differences were evident in males and females with or without G6PD A– (Table 3). For uncomplicated cases the interaction of sickle- or HbC-trait and G6PD deficiency and parasite densities in male children was also not significant ($p = 0.08$).

Discussion

In rural villages of Mali, where virtually all young children experience episodes of malaria, protective hemoglobinopathies and erythrocyte polymorphisms offer a tremendous survival benefit when they prevent progression of uncomplicated malaria to severe, life-threatening disease. Our study was designed to test for protection by the A– form of G6PD deficiency and determine whether the effect of this sex-linked polymorphism would be more evident in male or female children. Since children who never contract malaria are rare at our study sites, we used case-control comparisons of severe malaria patient "cases" against uncomplicated malaria patient "controls" as described by Hayes et al. [20] for epidemiological assessments of protection.

Our results from the Malinké and Dogon children in two regions 600 km apart show that G6PD A– provides substantial protection against severe malaria in hemizygous males but little or no protection in heterozygous females. After correcting for the confounding variables of age-dependent acquired immunity and potential effects of hemoglobins C and S, we calculate a pooled OR of 0.28 (95% CI 0.11–0.62) for the males 5 y old and below in these two groups. While our case-control design does not allow the calculation of relative risk that would be available from a prospective cohort study, the nearly complete recruitment of children with malaria in the Kela region of our study suggests roughly two-thirds less risk of severe malaria in young G6PD-deficient relative to G6PD-normal males. This level of protection from G6PD A– in hemizygous males 5 y old and younger is comparable to levels reported for hemoglobins C and S in certain West African populations [13,18,19].

Because homozygous G6PD-deficient females are relatively rare, the number of these individuals that could be recruited to our studies was low. Nevertheless, cohort sizes from the Kela region were sufficiently large to detect a possible trend to protection against severe malaria in these individuals. For female children 5 y old and younger, the age range benefiting most from this effect, the OR of this trend was calculated to be 0.24 (95% CI 0.01–1.63), a value comparable to that for young hemizygous males. Pooled OR calculations including homozygous females 5 y old and younger from additional regions were not possible as sufficient recruitment numbers were not available. Additional studies with larger groups will be necessary to confirm the significance of this trend in young homozygous females.

Two other studies have reported clinical investigations of severe malaria in relation to G6PD A–. In Nigeria, Gilles et al. [6] observed a protective effect of G6PD A– against convulsions or coma in male children 6 mo to 4 y of age; numbers from the female cases were not considered statistically significant for conclusions. In a later and much larger study from The Gambia and Kenya, Ruwende et al. [5] concluded that G6PD A– is associated with a 46%–58% reduction in the risk of severe malaria for both hemizygous males and heterozygous females. Those conclusions are different from ours and led us to look more deeply into the details of the authors' data and analysis.

Our first comment on the different conclusions from our study and that of Ruwende et al. [5] concerns the control groups. Ruwende et al. [5] compared cases of uncomplicated or severe malaria in children up to 10 y of age against age-

Table 3. Parasite Densities in Cases of Severe or Uncomplicated Malaria in Children ≤5 y According to Hb Type, Sex, and *G6PD* Genotype

Illness	Hb Type	Males		Females		
		Hemizygous	Normal	Heterozygous	Homozygous	Normal
Severe malaria	AA	4.38 (3.91–5.17) $n = 7$, $p = 0.26$	4.66 (4.27–5.06) $n = 138$	4.80 (4.78–5.11) $n = 17$, $p = 0.12$	4.77 $n = 1$, $p = 0.70$	4.51 (4.14–4.94), $n = 122$
	AS or AC	4.16 (3.59–4.73), $n = 2$, $p = 0.47$	4.63 (4.14–5.06), $n = 26$	4.35 (3.54–4.76) $n = 3$, $p = 0.80$	0	4.21 (3.47–5.06) $n = 10$
Uncomplicated malaria	AA	4.30 (3.81–4.88) $n = 86$, $p = 0.79$	4.28 (3.88–4.77) $n = 475$	4.21 (3.88–4.80) $n = 70$, $p = 0.30$	4.12 (3.84–4.49) $n = 15$, $p = 0.29$	4.30 (3.93–4.78) $n = 459$
	AS or AC	4.04 (3.47–4.58) $n = 22$, $p = 0.08$	4.32 (3.96–4.79), $n = 98$	4.04 (3.33–4.68) $n = 11$, $p = 0.80$	0	4.24 (3.86–4.77), $n = 94$

Data shown are mean (interquartile range) of $\log_{10}$-transformed parasitemias/μl of whole blood by sex and *G6PD* genotype. *p*-Values reflect the probability that the difference between two groups was observed by chance.

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and location-matched control children who did not have malaria. Controls in Kenya included healthy community children with or without asymptomatic parasitemia; controls in The Gambia included inpatient and outpatient children with nonmalarial illnesses and no *P. falciparum* parasitemia. Comparative assessments of G6PD deficiency in cases of severe versus uncomplicated malaria were not reported. In contrast, our study was performed over two or four transmission seasons in Malian villages where nearly every child experienced malaria. Malaria-free "control" groups in this context would have been highly skewed with confounding variables if not impossible. Additionally, subjective thresholds of treatment-seeking behavior for uncomplicated malaria can vary among different village settings. We therefore focused on the natural selection of G6PD deficiency that arises from its ability to protect children against progression from uncomplicated to severe, life-threatening malaria.

Ruwende et al. [5] provided data from The Gambia and Kenya on the prevalence of G6PD A– in children with severe or uncomplicated malaria. Using these data in stratified analysis, we calculate a pooled OR of 0.68 (95% CI 0.33–1.39; $p = 0.32$) for the protective effect of G6PD A– in the hemizygous males, whereas for the heterozygous females the pooled OR is 0.91 (95% CI 0.55–1.53; $p = 0.71$) (Table S1). Inclusion in these data of children older than 5 y and children with Hb types other than AA could have masked a greater trend to protection in the hemizygous males. The numbers of recruited homozygous female cases were few and showed no suggestion of protection.

Taken together, what do the different clinical studies of severe malaria in relation to the G6PD A– form of deficiency indicate for an overall estimate of relative protection? In the investigations producing the data from the Dogon, Malinké, Kenyan, and Gambian populations, the study designs, definitions of severe and uncomplicated malaria, and methodological details were similar in their significant aspects and are, therefore, informative when analyzed together. Earlier data from the Nigerian study [6] have to be excluded because of that study's highly restrictive definition of severe malaria (only cases of convulsions or coma in the presence of high fever and parasitemia ≥100,000/μl). We therefore performed fixed effects meta-analysis on the data from Ruwende et al. [5] and from all children in our study (data from Table 1, not Table 2, to avoid selective exclusion of cases in this combined treatment). Pooled ORs from all four sets of data confirmed highly significant protection for hemizygous males (OR = 0.51; 95% CI 0.33–0.77; $p < 0.001$), but not for heterozygous females (OR = 0.96; 95% CI 0.68–1.34; $p = 0.87$). This overall indication of protection is probably underestimated for hemizygous HbAA males who are 5 y old and younger, in light of our findings that G6PD deficiency reduces the risk of severe malaria more in this population.

We also performed meta-analysis of our data and those of Ruwende et al. [5] for evidence of protection against two leading forms of severe malaria highly fatal to African children, cerebral malaria and severe anemia. Greater numbers of cases of cerebral malaria were present in the data than cases of severe anemia (Table S2). Results from this analysis showed statistically significant protection against cerebral malaria for hemizygous males (pooled OR = 0.44; 95% CI 0.24–0.77; $p = 0.002$) but not for heterozygous females (pooled OR = 0.91; 95% CI 0.59–1.39; $p = 0.76$). No significant OR for protection could be demonstrated for the recruitment groups with severe anemia (Table S2).

G6PD deficiency provides an important example of a sex-linked locus that can be maintained in the absence of heterozygote advantage [21]. Haplotype diversity and linkage disequilibrium analysis has indicated that the *G6PD**A– allele arose within the past 3,840–11,760 years and spread with agriculture and malaria in Africa [22]. It is a balanced polymorphism, as the protection provided by this G6PD deficiency against severe malaria in hemizygous males (and perhaps homozygous females) is also associated with risks of life-threatening complications, e.g., neonatal jaundice and devastating hemolytic crises precipitated by viral infections or ingestion of medicinal or dietary oxidants [23]. In hemizygous males and homozygous females, the risk of hemolysis and protection against severe malaria would both reflect the presence of uniformly G6PD-deficient populations of erythrocytes, whereas in heterozygous females the relative reduction of these risks and lack of protection are attributable to mosaic populations of G6PD-normal and G6PD-deficient erythrocytes circulating in the bloodstream. Our evidence that the A– form of G6PD deficiency protects against severe malaria in its uniform (hemizygous, homozy-

gous) but not mosaic (heterozygous) state appears more consistent than do previous proposals with currently hypothesized mechanisms of protection. Enhanced phagocytosis of parasitized erythrocytes [11] or effects on the pathogenic consequences of parasitized erythrocytes in the microcirculation [24] would be expected to operate preferentially in individuals whose erythrocytes are uniformly deficient in G6PD.

Supporting Information

Table S1. Distribution of Uncomplicated and Severe Malaria Cases in a Previously Reported Case-Control Study from The Gambia and Kenya

Data from Ruwende et al. [5] were used in stratified analysis to calculate pooled ORs for the protective effect of the A– form of G6PD deficiency in hemizygous males and heterozygous females against severe malaria.

Found at doi:10.1371/journal.pmed.0040066.st001 (50 KB DOC).

Table S2. Distribution of Uncomplicated, Cerebral, and Severe Anemia Malaria Cases in the Present Study and in a Previously Reported Case-Control Study from The Gambia and Kenya

Data from the present study (Table 1) and from Ruwende et al. [5] were used in fixed effects meta-analysis to calculate pooled ORs for the relative protection of the A– form of G6PD deficient males and females against cerebral malaria and severe anemia.

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Editors' Summary

Background. "Favism" is a condition that results from a deficiency in an enzyme called glucose-6-phosphate dehydrogenase (G6PD), and this disorder is thought to be the commonest enzyme-deficiency disease worldwide. The disease is named favism after the Italian word for broad beans (fava), which cause a classic reaction when eaten by people with G6PD deficiency. The G6PD enzyme is particularly important in red blood cells, where it protects against damage that can be caused by certain drugs or other stresses. There are a number of normal variants of G6PD, with G6PD A and G6PD B being common in Africa. However, abnormal mutations in the gene can lead to anemia as a result of the red blood cells breaking down in response to certain drugs or types of food, or in other situations. G6PD deficiency is not spread evenly around the world; it is particularly common in Africa and the Mediterranean, and up to 20%–25% of people in certain African regions can have the condition. Although there can be serious clinical outcomes from G6PD deficiency that result from the red blood cells being broken down, G6PD deficiency may protect against malaria.

Why Was This Study Done? The researchers here wanted to find out whether, in regions where virtually all children get malaria during early childhood, a mutation in G6PD A (written as G6PD A–) that leads to G6PD deficiency protects children from having their malaria episodes worsen into severe, life-threatening disease. They also wanted to look at whether the degree of protection from G6PD A– deficiency differed between boys and girls. The reason for looking at this had to do with the genetics of G6PD deficiency. The gene coding for G6PD is carried on the X chromosome, of which males have only one copy, whereas females have two. Therefore, males will be G6PD deficient when they inherit only one mutant gene, but females need to inherit two abnormal genes (or have the normal gene turned off, which happens by a process known as X-inactivation in a proportion of female cells).

What Did the Researchers Do and Find? This study was carried out in two regions of Mali, West Africa, where malaria affects virtually all children under the age of 5 years. The children recruited at each study site came from two distinct cultural groups, the Dogon people of Bandiagara and the Malinké people of Kangaba and Kela. The researchers studied children coming to the medical clinics in each area with either uncomplicated malaria (i.e., mild symptoms) or severe malaria. All patients were treated normally according to the standard practices for this region. Blood samples were collected from each child in order to see how many malaria parasites were in the blood, as well as to analyze each patient's DNA to work out whether they had a mutant form of the *G6PD**A gene that causes the G6PD A– form of deficiency.

Overall, 3,197 children were recruited into the study, 2,765 of whom had uncomplicated malaria and 432 had severe malaria. In both ethnic groups, children with severe malaria were much less likely to have the mutant form of the *G6PD**A gene than children with uncomplicated malaria, showing that the gene mutation protected children from having their malaria progress to severe malaria. The researchers then looked at whether the protection given by the mutant forms of G6PD affected boys and girls differently. The researchers found that protection against severe malaria occurred in boys but not girls who had inherited one mutant *G6PD**A– gene.

What Do These Findings Mean? These data show that the mutation in *G6PD**A that leads to the G6PD A– deficiency gives children substantial protection against severe malaria, but this protection seems to be the case for boys and not girls who have only one mutant copy of the gene. There seems to be around a two-thirds drop in risk of severe malaria in boys with G6PD deficiency as compared to normal boys. At present the reason for this difference in protection is not clear, because it is not known how malaria parasites are affected by red blood cells that lack G6PD activity.

Additional Information. Please access these Web sites via the online version of this summary at http://dx.doi.org/10.1371/journal.pmed.0040066.

- Medline Plus has an article on glucose-6-phosphate dehydrogenase deficiency
- Wikipedia has an entry on glucose-6-phosphate dehydrogenase deficiency (Wikipedia is a free internet encyclopedia that anyone can edit)
- Information on malaria is available from the US Centers for Disease Control