

Brief report

Both heterozygous and homozygous α^+ thalassemias protect against severe and fatal *Plasmodium falciparum* malaria on the coast of Kenya

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Although the α^+ thalassemias almost certainly confer protection against death from malaria, this has not been formally documented. We have conducted a study involving 655 case patients with rigorously defined severe malaria and 648 controls, frequency matched on area of residence and ethnic group. The prevalence of both heterozygous and homozygous α^+ thalassemia was reduced in both case patients with severe malaria (ad-

justed odds ratios [ORs], 0.73 and 0.57; 95% confidence intervals [95% CIs], 0.57-0.94 and 0.40-0.81; $P = .013$ and $P = .002$, respectively, compared with controls) and among the subgroup of children who died after admission with severe malaria (OR, 0.60 and 0.37; 95% CI, 0.37-1.00 and 0.16-0.87; $P = .05$ and $P = .02$, respectively, compared with surviving case patients). The lowest ORs were seen for the forms of malaria associated with the highest

mortality—coma and severe anemia complicated by deep, acidotic breathing. Our study supports the conclusion that both heterozygotes and homozygotes enjoy a selective advantage against death from *Plasmodium falciparum* malaria. (Blood. 2005;106:368-371)

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Introduction

The α^+ thalassemias are the commonest known genetic disorder in humans,¹ a situation that probably reflects a selective advantage against death from malaria. However, to date, the protection they afford against severe and fatal falciparum malaria has been investigated in only 2 studies, and these have yielded conflicting results.^{2,3} Here we describe a large case-control study in which we have found that both heterozygous and homozygous α^+ thalassemia are associated with a reduced risk for severe and fatal *Plasmodium falciparum* malaria in children living on the coast of Kenya.

Study design

Case patients with severe malaria and controls

The study was conducted in Kilifi district, where the population is composed predominantly of rural dwellers of the Mijikenda ethnolinguistic group. The epidemiology and clinical characteristics of malaria in the study area have been described in detail previously.^{4,5} Case patients were children with clinical features of severe *P falciparum* malaria who were admitted to the high-dependency unit (HDU) at Kilifi District Hospital (KDH), Kenya, between January 2001 and August 2003. We use 3 clinical features to define severe malaria at our hospital: prostration, coma (Blantyre coma score = 2), and deep breathing. At least 1 of these features is present on admission in approximately 98% of our inpatients who die of malaria.⁶ Children were eligible for the study if they met the following criteria: (1) *P falciparum* malaria with any of the 3 clinical features of severity; (2) residence within the study area of the Kenya Medical Research Institute (KEMRI) Centre for

Geographic Medicine Research, Coast, Kilifi, an area populated by more than 200 000 subjects that has been under continuous demographic surveillance since 2001⁷; and (3) Mijikenda ethnic origin. Children with uncomplicated severe malaria anemia (SMA) (ie, those with *P falciparum* malaria, in association with a hemoglobin level lower than 5 g/dL but with no signs of respiratory distress) are not routinely treated in our HDU because the inpatient mortality rate in this group is less than 1%.⁶ As a result, such children were not included as case patients. All case patients were treated according to standard guidelines, as described in detail previously.⁸ Controls were residents of the same study area who were randomly selected on the basis of ethnic group (Mijikenda) and who were frequency matched to case patients on the basis of location. Controls were younger than 7, but no attempt was made to match on age. The KEMRI National Ethical Review Committee approved the study. Individual written informed consent was provided by all study participants or their parents.

Laboratory methods

Routine hematologic and biochemical data are collected on all children admitted to the HDU at KDH, and malaria parasite densities are determined by standard methods, as described previously.⁸ Case patients and controls were typed for the common African 3.7-kb α -globin deletion by polymerase chain reaction (PCR), as described previously,⁹ using DNA extracted by standard methods (Qiagen DNA Blood Mini Kit; Qiagen, West Sussex, United Kingdom; or Puregene; Gentra Systems, Minneapolis, MN).

Statistical analysis

Categorical data were analyzed using the χ^2 test for trend, and numerical data were compared using analysis of variance (ANOVA). We determined

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Table 1. α^+ thalassemia gene frequencies in case patients and controls

	No. (%)	Genotype frequencies, no. (%)		
		Normal α^- globin genotype, $\alpha\alpha/\alpha\alpha$	Heterozygous α^+ thalassemia, $-\alpha/\alpha\alpha$	Homozygous α^+ thalassemia, $-\alpha/-\alpha$
Controls	648 (100)	212 (32.7)	328 (50.6)	108 (16.7)
Case patients				
with severe malaria	655 (100)	264 (40.3)	308 (47.0)	83 (12.7)

the odds ratios (ORs) for severe malaria, severe malaria subgroup, and inpatient death by α^+ thalassemia genotype using logistic regression, with and without adjustment for Mijikenda ethnic subgroup, age, and sex.

Results and discussion

Our study included 655 case patients and 648 controls. The α^+ thalassemia gene frequencies in case patients and controls and the presenting characteristics of case patients are summarized in Tables 1, 2, and 3. The prevalence of both heterozygous and homozygous α^+ thalassemia was significantly lower in case patients than in controls, and the lowest ORs were seen for homozygosity in children with the syndromes associated with the highest risk for mortality in our population—symptomatic SMA¹⁰ and deep breathing with coma⁶ (Table 4). Although admission hemoglobin concentrations were similar between genotypic groups (Table 3), the trend toward increasing hemoglobin values across the groups is noteworthy, given that heterozygous and homozygous α^+ thalassemia are associated with anemia at steady state.¹¹ We found no effect of α^+ thalassemia on admission parasite density.

Simultaneous with the showing of an association between homozygous and heterozygous α^+ thalassemia and protection from severe *P. falciparum* disease, we found evidence for a protective association against death from malaria. Among children with severe malaria, admitted to our HDU on the basis of strictly defined clinical criteria, the risk for death in hospital was 40% lower in heterozygotes and more than 60% lower in homozygotes than in healthy children (Table 2). Furthermore, among case patients with fatal disease, severe acidosis (base deficit greater than 15) at admission was less common in homozygotes (1 of 7; 14%) than in healthy persons (17 of 33; 51%) or heterozygotes (14 of 32; 44%), though this trend did not reach statistical significance ($\chi^2 = 3.5$; $P = .19$).

The genotypic pattern of protection seen in our study was similar to that seen in Papua, New Guinea, where protection was

greatest for anemia and acidosis²; however, in the latter study, protection did not reach significance in heterozygotes, and the in-hospital mortality rate was too low (15 of 433; 3.5%) to detect a between-genotype difference.² Our observations are not, however, in accord with those from Ghana,³ where protection was seen in heterozygotes (OR, 0.74; 95% confidence interval [95% CI], 0.56-0.98; $P = .03$), but not in homozygotes (OR not reported), and where no protection was seen against in-hospital death despite a mortality rate (11%³) comparable with that seen in our study. These discrepancies may well be attributable to statistical considerations and may be explicable on the basis of the greater power of the current study than previous studies to detect significant differences; however, they may also relate to differences in the design and conduct of these studies. All 3 adopted a case-control design, an approach that is justified on the grounds that severe *P. falciparum* malaria is a relatively rare outcome, affecting, for example, only approximately 10% of 1- to 5-year-olds in the Kilifi study area. Although case-control studies are open to problems of bias and confounding, we believe for several reasons that it is unlikely our results have been affected by such problems. More than 95% of residents of the Kilifi study area are of Mijikenda ancestry. Although this Bantu population can be categorized into 3 main subgroups—Giriama, Chonyi, and Kauma—we have detected no significant differences in the $-\alpha$ allele frequencies between these groups in extensive surveys of more than 4000 children (T.N.W., unpublished data, collected between October 2000 and November 2004). Moreover, we adjusted for these ethnic subgroups in our logistic regression analysis, an exercise that made no material difference to our results. Finally, our controls were frequency-matched on location, through our population database, and our analysis was further adjusted for age. Nevertheless, despite these considerations, we cannot exclude the possibility that unknown biases might have affected our study, which should ideally be replicated using an alternative design such as a family-based or a prospective cohort approach.

Table 2. Clinical characteristics of case patients at time of admission to HDU by α^+ thalassemia genotype

Characteristic	No. (%) [*]			
	All genotypes	$\alpha\alpha/\alpha\alpha$	$-\alpha/\alpha\alpha$	$-\alpha/-\alpha$
Prostration†	275 (42)	106 (40.2)	135 (43.8)	34 (41.0)
Coma†	285 (44)	119 (45.1)	133 (43.2)	33 (39.8)
Deep breathing†	239 (37)	95 (36.0)	111 (36.0)	33 (39.8)
Coma and deep breathing	94 (14)	38 (14.4)	47 (15.3)	9 (10.8)
Symptomatic SMA‡	153 (23)	68 (25.8)	69 (22.4)	15 (18.1)
Metabolic acidosis§	422/598 (71)	175/241 (72.6)	190/283 (67.1)	57/54 (77.0)
Hypoglycemia	100/645 (16)	40/258 (15.5)	43/305 (14.1)	17/82 (20.7)
Fatal outcome¶	72 (11)	33 (12.5)	32 (10.4)	7 (8.4)

^{*}Number and percentage of genotypic group manifesting clinical characteristic. N = 655 case patients in all categories except metabolic acidosis and hypoglycemia, for which denominators are shown.

†Defined as previously described.⁸

‡Hemoglobin less than 5 g/dL, falciparum malaria, acidosis, and deep breathing.

§Base deficit greater than 8 mM.

||Blood glucose less than 2.5 mM.

¶Deaths in hospital after admission.

Table 3. Laboratory findings in case patients by α^+ thalassemia

	Genotype frequencies, mean (SE)		
	Normal α -globin genotype $\alpha\alpha/\alpha\alpha$	Heterozygous α^+ thalassemia $-\alpha/\alpha\alpha$	Homozygous α^+ thalassemia $-\alpha/-\alpha$
Hemoglobin, g/dL	6.95 (0.16)	7.08 (0.16)	7.12 (0.26)
MCV, fL	75.8 (0.6)	72.6 (0.5)	68.2 (1.1)
Platelets, $\times 10^6/L$	162 (10)	159 (9)	187 (20)
Sodium, mM	134 (0)	134 (0)	135 (1)
Potassium, mM	4.4 (0.1)	4.3 (0.1)	4.3 (0.1)
Creatinine, μM	72.2 (2.7)	66.1 (2.3)	71.9 (4.8)
Glucose, mM*	5.8 (0.2)	5.6 (0.2)	5.0 (0.3)
pH*	7.28 (0.01)	7.28 (0.01)	7.27 (0.01)
Bicarbonate, mM*	13.13 (0.33)	13.83 (0.29)	12.85 (0.55)
Base deficit, mM*	12.06 (0.43)	11.24 (0.35)	12.22 (0.67)
Parasitemia, $\log_{10}$ value/ μL	4.8 (4.5-5.0)	4.6 (4.4-4.7)	4.6 (4.5-4.8)

No significant differences were seen with regard to any hematologic or biochemical characteristics with the exception of MCV ($F = 23.21$; $P < .001$).

*Not available for all children; the number of case patients is the same as in Table 1.

What then can such studies tell us about the mechanism by which α^+ thalassemia affords protection against malaria? A number of hypotheses have been proposed, including reduced parasite growth and invasion,¹² increased phagocytosis of infected red blood cells,¹² altered surface antigen expression that potentially augments immunologic clearance,^{13,14} and decreased parasite rosetting¹⁵; nevertheless, for these to be relevant, they must be compatible with observations from epidemiologic studies conducted under conditions of natural malaria transmission. The fact that, in keeping with all previous observations made in vivo,^{2,3,16-19} we found no effect at the level of parasite density makes it unlikely that α^+ thalassemia protects simply by limiting the growth or increasing the clearance of parasites. Instead, it seems more likely that it acts by attenuating the consequences of malaria infection.

Severe and fatal malaria results from a range of interrelated pathophysiologic processes that affect multiple organ systems.⁶ These processes are thought to include the local and systemic release of various cytokines, anemia, decreased red cell deformability and adhesion phenomena such as sequestration and rosetting,²⁰⁻²³ and the clinical features of severe malaria (such as coma, acidosis, and hypovolemic shock), likely reflect the balance of such processes within individual patients.²⁴ That α^+ thalassemia protects against a range of clinical manifestations of severe malaria suggests it does so through a mechanism

Table 4. Odds ratios for malaria-specific syndromes by α^+ thalassemia genotype

Groups	$-\alpha/\alpha\alpha$ (95% CI)*	P^*	$-\alpha/-\alpha$ (95% CI)*	P^*
All severe malaria	0.76 (0.59-0.96)	.02	0.60 (0.44-0.85)	.004
All severe malaria, adjusted†	0.73 (0.57-0.94)	.013	0.57 (0.40-0.81)	.002
Severe malaria syndromes‡				
Prostration	0.81 (0.59-1.10)	.18	0.61 (0.39-0.96)	.03
Coma	0.70 (0.51-0.97)	.03	0.49 (0.31-0.79)	.003
Deep breathing	0.75 (0.54-1.04)	.08	0.64 (0.40-1.03)	.06
Coma and deep breathing	0.81 (0.50-1.30)	.39	0.41 (0.19-0.93)	.03
Symptomatic SMA	0.63 (0.43-0.93)	.02	0.39 (0.21-0.74)	.004
Acidosis	0.68 (0.51-0.90)	.006	0.60 (0.41-0.89)	.01
Hypoglycemia	0.68 (0.42-1.10)	.11	0.75 (0.40-1.40)	.38
Fatal outcome	0.60 (0.37-1.00)	.05	0.37 (0.16-0.87)	.02

Because children may manifest multiple complications of severe malaria, some subjects contributed data to more than 1 clinical subgroup.

*Compared with normal genotype, analyzed by logistic regression.

†Adjusted for minor ethnic group within the Mijikenda tribe, sex, and age.

‡Defined as in Table 1; all ORs were adjusted for minor ethnic groups within the Mijikenda tribe, sex, and age.²

that is central to many of these processes. In this regard, results from a recent study are particularly intriguing. In an extension of the previous study in Papua, New Guinea,² Cockburn and colleagues,²⁵ made 2 related observations: first, that a promoter polymorphism of red cell complement receptor 1 (CR1), an important receptor for rosetting, was significantly associated with protection from severe falciparum malaria; second, that α^+ thalassemia was independently associated with the reduced expression of red cell CR1. These observations support the earlier work of Carlson,¹⁵ who suggested that a number of the hemoglobinopathies might protect against severe malaria by reducing the ability of red cells to form rosettes. Further work will be required to explore these potentially important observations.

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