

## Brief report

 $\alpha^+$ -thalassemia protects African children from severe malaria

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The high frequency of  $\alpha^+$ -thalassemia in malaria-endemic regions may reflect natural selection due to protection from potentially fatal severe malaria. In Africa, bearing 90% of global malaria morbidity and mortality, this has not yet been observed. We tested this hypothesis in an unmatched case-control study among 301 Ghanaian children with severe malaria and 2107 controls (62% parasitemic). In

control children,  $\alpha^+$ -thalassemia affected neither prevalence nor density of *Plasmodium falciparum*. However, heterozygous  $\alpha^+$ -thalassemia was observed in 32.6% of controls but in only 26.2% of cases (odds ratio [OR], 0.74; 95% confidence interval [CI], 0.56-0.98). Protection against severe malaria was found to be pronounced comparing severe malaria patients with parasitemic controls (adjusted OR in children

< 5 years of age, 0.52; 95% CI, 0.34-0.78) and to wane with age. No protective effect was discernible for homozygous children. Our findings provide evidence for natural selection of  $\alpha^+$ -thalassemia in Africa due to protection from severe malaria. (Blood. 2004;104:2003-2006)

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## Introduction

Severe *Plasmodium falciparum* malaria is a major cause of death in sub-Saharan Africa, particularly in children younger than 5 years of age.<sup>1</sup> Interindividual variation in susceptibility and manifestation can be attributed partially to innate host factors such as the sickle cell trait.<sup>2-4</sup> According to Haldane's "malaria hypothesis,"<sup>5</sup> these factors are subject to selection in endemic regions because of the resistance they confer against malaria. Another hemoglobin disorder,  $\alpha^+$ -thalassemia, reaches a prevalence of more than 80% in parts of Southeast Asia and Melanesia.<sup>6-8</sup> Heterozygosity is characterized by the commonly deletional loss of one of the duplicated  $\alpha$ -globin genes ( $-\alpha/\alpha\alpha$ ) and slight hematologic changes. Homozygous individuals ( $-\alpha/-\alpha$ ) have mild microcytic anemia.<sup>9</sup>

In Melanesia, the geographic correlation between the prevalence of  $\alpha^+$ -thalassemia and malarial endemicity suggests natural selection.<sup>6</sup> In one case-control study in Papua New Guinea, the risk of severe malaria was reduced by 60% and 34% in homozygous and heterozygous children, respectively.<sup>7</sup> In sub-Saharan Africa,  $\alpha^+$ -thalassemia affects up to 50% of the population, but protection against uncomplicated or severe malaria could not be demonstrated so far.<sup>3,9,10</sup>

We examined whether  $\alpha^+$ -thalassemia confers protection from severe falciparum malaria in Ghana.

## Study design

## Patients and controls

We conducted an unmatched case-control study in the city of Tamale and its vicinity, northern Ghana, during the rainy season 2002 (August to

November). In the study area, malaria is hyperendemic (F.P.M., unpublished observations, December 1, 2003). Ethical clearance was obtained from the Ethics Committee, University for Development Studies, Tamale, and informed consent, from the participants' parents. We recruited 290 children with severe malaria according to WHO definition at Tamale Teaching Hospital.<sup>11</sup> An additional 11 children with severe malaria were detected during the survey mentioned later in this paragraph and classified as cases. In the 301 index patients, symptoms defining severe malaria were as follows: severe anemia, 57%; prostration, 32%; respiratory distress, 22%; multiple convulsions, 20%; impaired consciousness, 19%; jaundice, 11%; circulatory collapse, 3%; and hemoglobinuria, 3%. Controls were 2107 children from Tamale and the surrounding districts who were recruited following a 2-stage cluster sampling strategy with probability proportional to population size. There were 30 census units included, and within each 70 or more children aged 6 months to 9 years were randomly selected. Due to ethnic conflicts, ethnicity could not be dependably assessed, but the vast majority of cases and controls belonged to the Dagomba ethnic group. Severe malaria patients received artesunate (Plasmodium; Mepha, Basel, Switzerland) for 5 days (5 mg/kg body weight, double dose on first day) and supportive care. Uncomplicated malaria or parasitemia higher than 5000/ $\mu$ L was treated with sulfadoxine-pyrimethamine.

## Microscopy and PCR assays

Venous blood was taken and DNA extracted (QIAmp; Qiagen, Hilden, Germany). Malaria parasites were counted per 200 or more white blood cells on Giemsa-stained thick blood films. Polymerase chain reaction (PCR) assays were applied to ascertain *P. falciparum* and to determine the common West African  $-\alpha^{3,7}$  deletion type of  $\alpha^+$ -thalassemia.<sup>12,13</sup>

## Statistical analysis

Geometric mean parasite densities (GMPDs) and 95% confidence intervals (95% CIs) were calculated. Continuous variables were compared by *t* test,

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Submitted December 1, 2003; accepted May 1, 2004. Prepublished online as Blood First Edition Paper, June 22, 2004; DOI 10.1182/blood-2003-11-4090.

Supported by Charité grant 2003-676.

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**Table 1. Characteristics of control group children and severe malaria patients**

	Control group			Severe malaria patients
	All	Aparasitemic children	Parasitemic children	
No.	2107	811	1296	301
Age, mo, median (range)	50 (6-108)*	48 (6-108)*	60 (6-108)*†	24 (6-102)
Proportion younger than 5 y of age, no. (%)	1093 (51.9)*	479 (59.1)*	614 (47.4)*†	261 (86.7)
Female, no. (%)	1050 (49.8)	400 (49.3)	650 (50.2)	159 (52.8)
Rural residence, no. (%)	1353 (64.2)*	326 (40.2)	1027 (79.2)*†	104 (34.6)
Geometric mean parasite density, per $\mu\text{L}$ (95% CI)	1419 (1274-1581)*	0	1419 (1274-1581)*	27 164 (20 246-36 447)
Heterozygous $\alpha^+$ -thalassemia, no. (%)	686 (32.6)	256 (31.6)	430 (33.2)	79 (26.2)‡
Homozygous $\alpha^+$ -thalassemia, no. (%)	86 (4.1)	35 (4.3)	51 (3.9)	15 (5.0)

\*Difference to severe malaria patients,  $P < .0001$ .

†Difference between aparasitemic and parasitemic control children,  $P < .0001$ .

‡Heterozygous  $\alpha^+$ -thalassemia occurs less frequently in severe malaria patients than in all controls ( $P = .03$ ) and than in parasitemic control children ( $P = .02$ ).

analysis of variance, Mann-Whitney  $U$  test, and Kruskal-Wallis test, and proportions were compared by  $\chi^2$ -tests. The odds of severe malaria in children with  $\alpha^+$ -thalassemia were estimated in logistic regression models adjusting for sex, age (months), and residence (urban, Tamale district; rural, surrounding districts).

## Results and discussion

In control children, *P falciparum* occurred in 62% and  $\alpha^+$ -thalassemia occurred in 37% (Table 1). The  $\alpha$ -globin genotype did not influence *P falciparum* prevalence ( $\alpha\alpha/\alpha\alpha$ , 815 [61%] of 1335 children;  $-\alpha/\alpha\alpha$ , 430 [63%] of 686 children;  $-\alpha/-\alpha$ , 51 [59%] of 86 children), febrile parasitemia ( $\alpha\alpha/\alpha\alpha$ , 58 [4.4%] of 1332 children;  $-\alpha/\alpha\alpha$ , 38 [5.6%] of 684 children;  $-\alpha/-\alpha$ , 5 [5.8%] of 86 children), or parasite density (GMPDs [95% CIs]: 1500/ $\mu\text{L}$  [1313-1712]; 1282/ $\mu\text{L}$  [1053-1562]; 1409/ $\mu\text{L}$  [807-2462]).

We compared the frequencies of  $\alpha^+$ -thalassemia in cases and controls (Table 1). Cases and controls differed with respect to residence, but heterozygous and homozygous  $\alpha^+$ -thalassemia occurred at similar prevalence in rural (477 [32.7%] of 1457 children and 62 [4.3%] of 145 children, respectively) and urban (288 [30.3%] of 951 children and 39 [4.1%] of 951 children, respectively;  $P = .4$ ) areas. Children with heterozygous  $\alpha^+$ -thalassemia had reduced odds of severe malaria when compared with all controls (odds ratio [OR], 0.74; 95% CI, 0.56-0.98;  $P = .03$ ) or with parasitemic controls (OR, 0.72; 95% CI, 0.54-0.97;  $P = .03$ ). These associations were stronger in children younger than 5 years, and strongest when parasitemic children were chosen as the control group (Table 2). In these, logistic regression analysis revealed that heterozygous  $\alpha^+$ -thalassemia reduced the odds of severe malaria by 48% (Table 3). Further age

stratification showed a waning protective effect with increasing age (ORs [95% CIs]: 0.5-2 years, 0.48 [0.28-0.81]; 3-4 years, 0.60 [0.31-1.18]; 5-6 years, 1.55 [0.65-3.72];  $\geq 7$  years, 2.01 [0.60-6.75]). In the logistic regression model, the interaction between age and heterozygous  $\alpha$ -thalassemia, (ie, the change of effect with age) was significant ( $P = .0005$ ). Irrespective of age, homozygous  $\alpha^+$ -thalassemia did not reduce the odds of severe malaria.

In severe malaria patients, GMPDs were virtually identical in  $\alpha$ -globin normal, heterozygous, and homozygous children (GMPD [95% CI]: 27 797/ $\mu\text{L}$  [19 532-39 560], 25 763/ $\mu\text{L}$  [14 485-45 821], and 26 182/ $\mu\text{L}$  [6194-110 665], respectively). Also, heterozygous and homozygous  $\alpha^+$ -thalassemia occurred at similar frequencies in the 2 predominant syndromes, that is, severe anemia without cerebral involvement ( $n = 99$ ;  $-\alpha/\alpha\alpha$ , 25.3%;  $-\alpha/-\alpha$ , 3.0%) and cerebral involvement (prostration, convulsions, or impaired consciousness,  $n = 186$ ; 27.4%, 6.5%,  $P = .4$ ). In 285 hospitalized severe malaria patients with available follow-up data, fatality rates did not differ significantly between children with a normal  $\alpha$ -globin genotype (21 [10.9%] of 193 children), heterozygous  $\alpha^+$ -thalassemia (10 [12.8%] of 78 children), and homozygous  $\alpha^+$ -thalassemia (1 [7.1%] of 14 children).

We show for the first time that heterozygous  $\alpha^+$ -thalassemia protects from severe malaria in African children. Our findings are different from those in New Guinea where protection against severe malaria was stronger in homozygous than in heterozygous children.<sup>7</sup> We could not detect protection in homozygotes, which is possibly due to their small proportion and the statistical limitations associated. Considering the high malaria burden in sub-Saharan Africa, the comparatively lower frequency of  $\alpha^+$ -thalassemia in Ghana could indicate a balanced polymorphism.<sup>14</sup> Yet, there is no evidence for a reduced biologic fitness of homozygous individuals,

**Table 2. Distribution of  $\alpha$ -globin genotypes in Ghanaian children with severe malaria and in control groups**

Stratum	No.	Genotype frequencies (no., %)		
		Normal $\alpha$ -globin genotype	Heterozygous $\alpha^+$ -thalassemia	Homozygous $\alpha^+$ -thalassemia
<b>Younger than 5 years of age</b>				
Severe malaria	261	186 (71.3)	62 (23.7)	13 (5.0)
All controls	1093	699 (63.9)	357 (32.7)	37 (3.4)
Aparasitemic controls	479	309 (64.5)	153 (31.9)	17 (3.6)
Parasitemic controls	614	390 (63.5)	204 (33.2)	20 (3.3)
<b>5 years of age or older</b>				
Severe malaria cases	40	21 (52.5)	17 (42.5)	2 (5.0)
All controls	1014	636 (62.7)	329 (32.5)	49 (4.8)
Aparasitemic controls	332	211 (63.6)	103 (31.0)	18 (5.4)
Parasitemic controls	682	425 (62.3)	226 (33.1)	31 (4.6)

**Table 3. Associations with severe malaria in  $\alpha$ -thalassemic children younger than 5 years of age**

	Heterozygous $\alpha^+$ -thalassemia	Homozygous $\alpha^+$ -thalassemia
<b>Univariate analysis (OR, 95% CI)</b>		
Severe malaria vs all controls	0.65 (0.47-0.90); <i>P</i> = .008	1.32 (0.63-2.60); <i>P</i> = .40
Severe malaria vs aparasitemic controls	0.67 (0.47-0.96); <i>P</i> = .02	1.27 (0.55-2.85); <i>P</i> = .53
Severe malaria vs parasitemic controls	0.64 (0.45-0.90); <i>P</i> = .008	1.36 (0.61-2.95); <i>P</i> = .40
<b>Multivariate analysis (OR, 95% CI)</b>		
Severe malaria vs all controls	0.71 (0.51-0.99); <i>P</i> = .04	1.47 (0.73-2.96); <i>P</i> = .28
Severe malaria vs aparasitemic controls	0.75 (0.52-1.07); <i>P</i> = .11	1.22 (0.57-2.63); <i>P</i> = .60
Severe malaria vs parasitemic controls	0.52 (0.34-0.78); <i>P</i> = .002	1.87 (0.76-4.62); <i>P</i> = .17

Children with a normal  $\alpha$ -globin genotype serve as reference groups. Multivariate ORs are derived from logistic regression models with severe malaria as outcome variable and sex, age (months), and residence (urban/rural) as independent variables. In children 5 years of age or older,  $\alpha$ -globin genotypes did not differ significantly between cases and controls.

or for such to be present particularly in Africa. Close-to-fixation frequencies of  $\alpha^+$ -thalassemia in Asia and Melanesia suggest additional selecting factors (eg, an increased susceptibility to potentially beneficial *Plasmodium vivax* infections).<sup>15</sup> Also,  $\alpha^+$ -thalassemia in New Guinea conferred protection against common nonmalaria diseases (eg, respiratory infections).<sup>7</sup> In West Africa, *P vivax* is virtually absent and  $\alpha^+$ -thalassemic resistance to nonmalaria illness has not been reported.

Regarding possible confounding due to other protective traits or ethnicity, no correlation of  $\alpha$ -globin genotypes with hemoglobin C, hemoglobin S, or glucose-6-phosphate dehydrogenase deficiency was observed in a subset of children (F.P.M., unpublished observations, December 1, 2003). Gene frequencies in children of rural or urban origin were very similar. Considering that ethnicity may determine residence, and vice versa, this renders ethnic bias unlikely. Moreover, in contrast to HbS,  $\alpha$ -globin genotype frequencies in Ghana are remarkably stable in geographic terms, that is, they are almost identical in northern and southern parts of the country.<sup>16</sup>

The mechanism of protection in  $\alpha^+$ -thalassemia remains obscure. Consistent with previous findings,<sup>7,9,10</sup>  $\alpha^+$ -thalassemia did not influence susceptibility to infection per se. Rather, the heterozygous trait ameliorated disease manifestation, that is, it influenced progression from parasitemia to severe malaria. However, fatality was unchanged, indicating an abrogation of protection once a critical stage of disease is reached. *P falciparum*-infected  $\alpha$ -thalassemic red cells show a decreased capacity to form rosettes, a pathogenic marker of severe malaria,<sup>17</sup> but in vitro results on inhibited parasite multiplication are conflicting.<sup>18-21</sup> Increased antibody binding to parasitized thalassemic red cells suggests raised neoantigen expression, which would result in enhanced immune recognition and increased parasite clearance.<sup>21</sup> The protective effect of heterozygous  $\alpha^+$ -thalassemia was strongest in the youngest children, that is, during a time when malaria-associated mortality is highest. Similarly, protective effects of the sickle cell trait against severe and

uncomplicated malaria in Kenyan and Nigerian children, respectively, have been reported to be restricted to young age groups.<sup>2,4</sup> These observations agree with the notion that innate resistance acts mainly before specific immunity has developed.<sup>14</sup> The restriction of heterozygous protection to young children could thus indicate a predominance of nonimmune mechanisms, or, alternatively, an accelerated acquisition of immunity. Development of immunity with age in  $\alpha$ -globin normal children could explain the decline in heterozygous advantage, but the reasons for the indication of its reversal remain unclear. Older children were rare among severe malaria patients and, thus, this observation requires verification. Irrespective of its underlying causes, protection in young heterozygous children improves the chance of survival and, thus, of reaching an age at which the physical capacity to cope with the infection is higher. At any rate, the number of severe malaria cases prevented among young heterozygous children clearly outweigh a yet-to-be-confirmed risk increase in older children.

In Melanesia, increased susceptibility to *P vivax* in young  $\alpha^+$ -thalassemic children has been suggested to induce cross-reactive immune protection against subsequent falciparum malaria.<sup>15</sup> Preliminary data indicate no likewise excess of *Plasmodium ovale* or *Plasmodium malariae* in  $\alpha^+$ -thalassemic children in Ghana (F.P.M., unpublished observations, December 1, 2003).

Our results demonstrate that heterozygous  $\alpha^+$ -thalassemia confers protection against severe malaria. This increased biologic fitness may have contributed to its high frequency in sub-Saharan Africa.

## Acknowledgments

We thank the children and their parents who participated in this study. We are grateful to the members of the Northern Region Malaria Project (NORMAP) for patient recruitment and to Baerbel Jakob and Susanne Roewer for technical assistance.

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