Hemoglobin Variants and Disease Manifestations in Severe Falciparum Malaria

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SICKLE CELL DISEASE, THALASSEMIAS, and other hemoglobinopathies are among the most common genetic disorders of humans. Their high prevalences in malaria-endemic areas are considered to result from balancing selection, in that reduced fitness of affected individuals is counterbalanced by some mode of protection against malaria.1

Malaria may present as a mild febrile illness or, in cases of Plasmodium falciparum infections, as a severe, life-threatening syndrome.2 While severe falciparum malaria does not affect more than 1% to 2% of those infected, it causes more than 1 million cases of childhood mortality annually3 and therefore may be considered most relevant to natural selection. The severe falciparum malaria syndrome comprises a number of distinct but overlapping clinical signs.2 The prominent ones are cerebral malaria and severe anemia, but the syndrome has been found to be complex and to include additional signs such as respiratory distress, hyperlactatemia, and acidosis, which are important predictors of mortality.4,5 The pathogenesis and interdependence of the various signs are not completely understood, but the complex and varied clinical presentation suggests more than a single pathophysiological process.

Sickle cell hemoglobin S (HbS) and hemoglobin C (HbC) are structural variants of B-globin that differ from each other by a single amino acid residue.6 α-Thalassemias result from an impaired production of α-globin, whereby α+ and α0-forms are caused by deletions that leave 1 functional copy of the duplicated α-globin genes and abolish both of them, respectively. In Africa, the clinically silent −α 3.7 deletion prevails, and α2-thalassemias are rare.1

Sickle cell hemoglobin S has long been recognized as protective against mild and severe malaria, and the high degree of protection found in various, often heterogeneous, study groups sug-

Context The geographical distributions of hemoglobin S (HbS), hemoglobin C (HbC), and α-thalassemia (−α) strongly suggest suggest balancing selection with malaria. However, whereas several studies indicate that the HbS carrier state protects against all major forms of clinical malaria, malaria protection on clinical grounds has been more difficult to confirm for HbC and −α, and questions remain as to whether it applies to all forms of the disease.

Objective To assess the association between major clinical forms of severe falciparum malaria and HbS, HbC, and −α.

Design, Setting, and Participants Case-control study of 2591 children with severe falciparum malaria enrolled at a tertiary referral center in Ghana, West Africa, and 2048 age-, sex-, and ethnicity-matched control participants recruited by community surveys.

Main Outcome Measures Frequencies of HbS, HbC, and −α in patients and controls, including stratifications of patients for signs of disease.

Results Patients presented with partly overlapping signs of disease, including severe anemia (64%), cerebral malaria (22%), respiratory distress (30%), hyperparasitemia (32%), prostration (52%), acidosis (59%), and hyperlactatemia (56%). Carrier states of HbC and −α were found in 1.4%, 9.4%, and 25.2% of the patients, respectively, and 14.8%, 8.7%, and 27.3% of controls. The HbS carrier state was negatively associated with all forms of the disease studied (overall odds ratio [OR], 0.82; 95% CI, 0.69-0.96). The HbC carrier state showed a negative association selectively with cerebral malaria (OR, 0.82; 95% CI, 0.69-0.96). The −α carrier state showed a negative association selectively with severe anemia (OR, 0.82; 95% CI, 0.69-0.96).

Conclusion Whereas the HbS carrier state was found to be negatively associated with all major forms of severe falciparum malaria, the negative associations of the carrier states of HbC and −α appeared to be limited to cerebral malaria and severe anemia, respectively.

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suggests that it has an effect on most if not all forms of clinical malaria.6-10

In contrast, malaria protection afforded by HbC and –α has been more difficult to confirm.6,7 More recently, several studies have indicated that both confer significant protection against the severe form of the disease.8-14 However, certain lines of evidence raise the question of whether HbC and –α provide equal protection from all forms of severe malaria.10-12 Data obtained in a small patient group suggested that –α might be negatively associated with severe anemia only.13,14 Hemoglobin C was recently found to inhibit the adherence of P falciparum-infected erythrocytes to vascular endothelial cells in vitro.17 Because postmortem findings of parasitized cells seques-
tered in small blood vessels of the brain are considered the hallmark of cere-
bral malaria,18 the in vitro finding may suggest that HbC may have a particu-
lar influence on this form of the disease.

After studying more than 2500 children with severe falciparum malaria, we determined the associations of HbS, HbC, and –α with the syndrome as a whole and with cerebral malaria and severe anemia specifically.

METHODS
Study Participants
Ethical approval was obtained from the Committee for Research, Publications and Ethics of the School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. All procedures were explained to parents or guardians of the participating children in the local language, and written or thumb-printed in-
formed consent was obtained.

Patients were recruited in Komfo Anokye Teaching Hospital, a tertiary re-
ferral center in Kumasi, between 2001 and 2005 in parallel with the Severe Ma-
laria in African Children study.19 All pa-
tients aged 6 months to 10 years were screened for malaria parasitemia using Giemsa-stained blood films. In pa-
tients with malaria, the consciousness level according to the Blantyre Coma

Score (BCS)2 was determined at the time of admission, as were acid-base status and blood concentrations of glucose, lactate, and hemoglobin. Patients positive for asexual P falciparum parasit-

emia with either a BCS less than 3, hemoglobin concentration less than 5
g/dL, or lactate concentration greater than 5 mmol/L were enrolled after con-
sent had been obtained from accom-
panying parents or guardians.3 Para-
site densities were determined per 200 leukocytes and calculated assuming a leukocyte count of 8000 cells/µL of blood.20

Prostration (age-dependent incapacity of the child to suck, sit, stand, or walk) and respiratory distress (irregu-
lar or deep, acidotic breathing) were as-
sessed in addition to the BCS, which was repeated 1 hour after admission. Cerebral malaria was defined by a BCS of less than 3 for at least 1 hour with or without convulsions; patients with convulsions and a higher BCS were not included because of uncertainties in ex-
cluding febrile seizures. Lumbar puncture was performed on unconscious pa-
tients, and those with cerebrospinal fluid findings indicative of meningitis were excluded. Blood culture was per-
fomed on a minority of patients at the discretion of the admitting physician, but the results were not recorded. Pa-
tients with parasite densities greater than 200 000 parasites/µL were classi-

fied as hyperparasitemic; those with a base deficit greater than 3.0 mEq/L as acidotic; and those with a blood glu-
cose level less than 39.6 mg/dL (2.2
mmol/L) as hypoglycemic. Jaundice, hemoglobinuria, and abnormal bleeding are rare signs of severe malaria in this setting and were not recorded. Pa-
tients were treated according to local guidelines.

Control participants were identi-

fied by community surveys designed to search for children, frequency-
matching the patients for age, sex, and ethnicity. Ethnicity was determined by the participants using investigator-
defined categories. Children were re-
cruited who appeared healthy by physi-

cal examination and did not have serious illness according to informa-
tion provided by parents or guardians.

Genotyping
From patients and controls, 0.5 to 1
mL of venous blood was collected into
citrate and subjected to density-

gradient centrifugation. The granulo-
cyte fraction was stored in 4-M urea and used for DNA extraction (Nucleo-
Mag 96 Blood; Macherey-Nagel, Düren, Germany). Genotyping for HbS, HbC, and the –α 3.7 deletion was performed11,22 using a LightTyper
(Roche Diagnostics, Basel, Switzer-
land) for the analysis of HbS and HbC.

Statistical Analysis
Odds ratios (ORs) and 95% confi-
dence intervals (CIs) were calculated using STATA version 9.2 (StatCorp, College Station, Tex). All statistical analyses were 2-sided; P<.05 was considered significant. For multivari-
ate analyses, multiple logistic regres-
sions were performed to evaluate the effects of genotypes on phenotypes, in-
cluding adjustments for age, sex, and ethnicity and for mutual influences of α- and β-globin variants. For all com-
parisons, the control group (n=2048) was used as the reference group. Co-
efficients were log-transformed to cal-
culate the ORs and CIs. Proportions of
categorical variables were compared by χ2 tests. In continuous exposures, Wil-
coxon or Kruskall-Wallis tests were per-
fomed. The assumption of a Hardy-
Weinberg equilibrium was tested using a z test based on a χ statistic. Odds ra-
tios obtained in subgroups were com-
pared using tests of interaction and ex-
pressed as OR ratios.23 Epistasis was assessed by performing the Wald test on interactions between α- and β-glo-
bin genotypes in a logistic regression including age, sex, and ethnicity as co-

TABLE 1

<table>
<thead>
<tr>
<th>Table 1: ORs and CIs for Serious Illnesses According to Various Hb Variants</th>
<th>HbS</th>
<th>HbC</th>
<th>–α</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCS</td>
<td>2.62 (1.21–5.67)</td>
<td>1.94 (0.93–4.04)</td>
<td>1.93 (0.92–4.02)</td>
</tr>
<tr>
<td>Cerebral Malaria</td>
<td>6.34 (2.40–16.6)</td>
<td>4.51 (1.67–12.0)</td>
<td>4.44 (1.58–12.0)</td>
</tr>
<tr>
<td>Cerebral Palsy</td>
<td>3.04 (1.17–7.80)</td>
<td>2.52 (1.01–6.21)</td>
<td>2.49 (1.00–6.41)</td>
</tr>
</tbody>
</table>

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typing for HbS, HbC, and −α showed marked differences between patients and controls in the frequencies of heterozygous HbS (HbAS) and, accordingly, of homozygous wild-type genotype for β-globin (normal β-globin genotype). In the control group, all genotype distributions were in Hardy-Weinberg equilibrium (HbS and HbC [P > .61] and −α [P > .23]). In the patient group, HbS and HbC genotype frequencies deviated from Hardy-Weinberg equilibrium (P < .001), but −α genotype frequencies did not (P > .41). When patients were grouped according to genotypes, no significant differences were observed in laboratory findings except that patients with HbAS had lower parasite densities than children with normal β-globin genotype (Table 2).

Patients and controls were compared regarding α- and β-globin genotype frequencies by multiple logistic regression including adjustments for age, sex, and ethnicity and for mutual influences of α- and β-globin variants. Evaluating the entire case group collectively, HbAS was found much less frequently among patients than among controls, indicating a strong negative association (Table 3). In contrast, no significant negative association was found with heterozygous HbC (HbAC). Likewise, associations of homozygous HbC and hemoglobin C disease (HbSC) were not significant (OR, 0.38; 95% CI, 0.13-1.06; P = .07; and OR, 0.44; 95% CI, 0.18-1.04; P = .06, respectively), whereby low genotype frequencies limited accuracy and statistical power of these assessments. Heterozygous −α (−α/αα) showed a significant negative association with disease (Table 3), whereas a negative association of the homozygous form (−α/−α) was not statistically significant (OR, 0.67; 95% CI, 0.38-1.16; P = .11). In the control group, all patients and controls showed Hardy-Weinberg genotype distributions were in Hardy-Weinberg equilibrium (HbS and HbC [P > .61] and −α [P > .23]). In the patient group, HbS and HbC genotype frequencies deviated from Hardy-Weinberg equilibrium (P < .001), but −α genotype frequencies did not (P > .41). When patients were grouped according to genotypes, no significant differences were observed in laboratory findings except that patients with HbAS had lower parasite densities than children with normal β-globin genotype (Table 2).

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Table 1. Demographic Data of the Study Group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Controls (n = 2048)</th>
<th>Severe Malaria (n = 2591)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male-female ratio</td>
<td>53.47</td>
<td>54.46</td>
</tr>
<tr>
<td>Age, median (10th-90th percentile), mo</td>
<td>30 (11-73)</td>
<td>20 (8-60)</td>
</tr>
<tr>
<td>Ethnic group, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Akan</td>
<td>62.9</td>
<td>67.5</td>
</tr>
<tr>
<td>Northem*</td>
<td>29.3</td>
<td>29.6</td>
</tr>
<tr>
<td>Ewe</td>
<td>1.5</td>
<td>2.9</td>
</tr>
</tbody>
</table>

*Immigrants from the Sahel zone in Northern Ghana, comprising Dagomba, Sissala, Gonja, and Kusasi.

Table 2. Clinical and Laboratory Findings in Genetic Subgroups of Children With Severe Falciparum Malaria (N = 2591)*

<table>
<thead>
<tr>
<th>Findings</th>
<th>β-Globin Variants</th>
<th>α-Globin Variants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (10th-90th percentile), mo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male-female ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parasite density, median (10th-90th percentile), 1000 parasites/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe anemia, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebral malaria, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prostration, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory distress, %‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactate, median (10th-90th percentile), mmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperlactatemia, %§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base excess, median (10th-90th percentile), mEq/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acidity, %</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations: −α/−α, homozygous α-thalassemia; −α/αα, heterozygous α-thalassemia; αα/αα, homozygous wild-type genotype for α-globin; HbAA, homozygous wild-type genotype for β-globin; HbAC, heterozygous hemoglobin C; HbAS, heterozygous sickle cell hemoglobin; HbCC, homozygous hemoglobin C; HbSC, hemoglobin C disease.

†Children with sickle cell disease were excluded because, often being severely anemic per se, they could not be classified with regard to severe malaria anemia. Values of subgroups were compared using Kruskal-Wallis or Mann-Whitney tests; no significant differences were obtained except when noted, whereby P values were corrected for multiple comparisons of the 10 independent parameters of age, sex, parasite density, hemoglobin concentration, cerebral malaria, prostration, respiratory distress, lactate concentration, and acidosis.

‡Different from HbAA and HbAC (Mann-Whitney test, corrected P = .02 and P = .03, respectively), which is in accordance with previous findings.‡

§n = 2381.

¶n = 2582.

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A significant antagonistic epistasis was observed between $\alpha^{+}/\alpha^{+}$ and $\alpha^{-}/\alpha^{-}$, as indicated by an increase in the OR for $\alpha^{-}/\alpha^{-}$ from 0.06 to 0.11 ($P = .04$). Significant epistasis was not found between $\alpha^{+}/\alpha^{+}$ and $\alpha^{-}/\alpha^{-}$. Possible epistasis involving $\alpha^{-}/\alpha^{-}$ was not assessed because of the low frequency of $\alpha^{-}/\alpha^{-}$.

Because the low genotype frequencies of homozygotes and compound heterozygotes limited the statistical power substantially, we restricted further analyses to heterozygotes. Based on evidence obtained in previous studies, the patient group was stratified for severe anemia and cerebral malaria. Heterozygous HbS showed clear negative associations in both subgroups (Table 3). A significant negative association of $\alpha^{-}/\alpha^{-}$ was found in the subgroup of patients with severe anemia and cerebral malaria. HbAC showed a uniform pattern of negative associations in all subgroups studied (Figure). Negative associations of HbAC were exclusively seen in subgroups containing patients with cerebral malaria, thereby confirming the selectivity of association with this sign of the disease.

The stratification resulted in a subgroup of patients with cerebral malaria alone, ie, cerebral malaria in the absence of additional signs of disease, and this subgroup showed a negative association with $\alpha^{-}/\alpha^{-}$. This subgroup was of considerable size (n = 281), and the OR differed significantly from that in the main severe-anemia subgroup (OR, 1.08 vs 0.76 [Figure]; OR ratio, 1.42; 95% CI, 1.02-1.99; uncorrected $P = .04$). However, the statistical significance is questionable, because the data result from multiple comparisons.

**COMMENT**

Our results indicate that, whereas HbAS was negatively associated with all forms of severe falciparum malaria studied, the negative associations of HbAC and $\alpha^{-}/\alpha^{-}$ were restricted to cerebral malaria and severe anemia, respectively.

**Table 3.** Distributions of $\beta$-Globin and $\alpha$-Globin Genotypes in Children With Severe Falciparum Malaria and in Subgroups of Children With Severe Anemia and Cerebral Malaria

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls, % (n = 2048)</th>
<th>Total (n = 2591)</th>
<th>Severe Anemia (n = 1649)</th>
<th>Cerebral Malaria (n = 581)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>OR (95% CI)†</td>
<td>$P$ Value</td>
<td>%</td>
</tr>
<tr>
<td>$\beta$-Globin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HbAA</td>
<td>75.3</td>
<td>88.6</td>
<td>1 [Reference]</td>
<td>88.5</td>
</tr>
<tr>
<td>HbAS</td>
<td>14.8</td>
<td>1.4</td>
<td>0.08 (0.06-0.12)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>HbAC</td>
<td>8.7</td>
<td>9.4</td>
<td>0.90 (0.73-1.11)</td>
<td>.30</td>
</tr>
<tr>
<td>$\alpha$-Globin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\alpha^{-}/\alpha^{-}$</td>
<td>69.5 72.8 1 [Reference]</td>
<td>73.5 1 [Reference]</td>
<td>.03</td>
<td>24.7 0.82 (0.69-0.96)</td>
</tr>
<tr>
<td>$\alpha^{+}/\alpha^{+}$</td>
<td>27.3 25.2 0.85 (0.74-0.96)</td>
<td>73.5 1 [Reference]</td>
<td>.03</td>
<td>24.7 0.82 (0.69-0.96)</td>
</tr>
</tbody>
</table>

Abbreviations: $\alpha^{-}/\alpha^{-}$, heterozygous $\alpha^{-}$-thalassemia; $\alpha^{+}/\alpha^{+}$, homozygous wild-type genotype for $\alpha$-globin; CI, confidence interval; HbAA, homozygous wild-type genotype for $\beta$-globin; HbAC, heterozygous hemoglobin C; HbAS, heterozygous sickle cell hemoglobin; OR, odds ratio.

* Assessed by logistic regression compared with all controls adjusting for age, sex, and ethnicity, and mutual influences of $\alpha$- and $\beta$-globin genotypes.

† $P$ values corrected for 2 comparisons (severe anemia and cerebral malaria; the analyses of negative associations with severe malaria collectively [total] were hypothesis-driven).
Associations of HbAS, HbAC, and −α/αα are shown with severe falciparum malaria stratified for signs of disease as indicated at left; plots were further stratified for the presence or absence of cerebral malaria and for the presence or absence of severe anemia. Error bars indicate 95% confidence intervals (CIs). Axes in blue indicate odds ratio range from 0.2 to 2.0. The selectivity of negative associations found with HbAC and −α/αα is not caused by confounding effects. Fatality indicates the percent fatality during hospitalization, whereby 2539 (98.0%) of 2591 patients underwent follow-up until discharge from the hospital or death.
subgroup analyses indicated that the selectivity of the \(\alpha\)/\(\alpha\) association was not caused by confounding effects of other signs of the disease. Therefore, it may be concluded that HbAC selectively protects against cerebral malaria and that –\(\alpha\)/\(\alpha\) selectively protects against severe anemia.

Protection by HbAS appeared to exceed 90% in the study group as a whole and 80% in all subgroups. Although the latter had previously not been shown formally, it could have been predicted from the consistently high degrees of protection that had been found in heterogeneous patient groups.6-10

A significant epistatic effect between –\(\alpha\) and HbAS has previously been reported26 that was limited to the homozygous form –\(\alpha\)/–\(\alpha\). While we could not assess the interaction between –\(\alpha\)/–\(\alpha\) and HbAS in our study population because of low genotype frequency of –\(\alpha\)/–\(\alpha\), we did find marginal but significant epistasis between heterozygous –\(\alpha\)/\(\alpha\) and HbAS and thereby extend the previous observation. No significant epistasis was found between –\(\alpha\)/\(\alpha\) and HbAC. Epistasis was not addressed in any of the subgroup analyses because it influenced the HbAS effect only, which was not the focus of the present study, and because it was marginal and not significant in any of the subgroups studied.

In comparison, to the findings obtained with HbAS, the selectivity of the HbAC association for cerebral malaria is remarkable, given the close structural similarity between HbS and HbC.1 That no significant negative association of HbAC with severe malaria was seen collectively may be the result of the relatively small proportion of patients with cerebral malaria enrolled in our study group (22% [581/2591]), which in part may be due to stringent inclusion criteria. In previous studies describing such an association, 51% (34/67) and 39% (115/290) of the study groups comprised patients with cerebral malaria,10,11 which may have contributed an HbAC effect sufficiently strong to infer protection against severe malaria collectively. The selectivity for cerebral malaria may have been missed because the study groups were too small to allow for appropriate stratifications required to analyze any single one of the largely overlapping disease signs10,11 or because no stratifications were made.9

Cerebral malaria is considered to result from impairments in cerebral perfusion and local alterations of the blood-brain barrier caused by adherence of parasitized erythrocytes to microvascular endothelial cells,18,27 which is mediated by Plasmodium falciparum-erythrocyte-membrane-protein 1 (PIEMP-1) expressed on the surface of parasitized erythrocytes.28 Recent laboratory studies have shown that HbAC alters the display of PIEMP-1 at the erythrocyte surface, causing an approximately 30% reduction in endothelial adhesion.17 The selectivity of the HbAC effect may indicate a critical role of PIEMP-1 display in the pathogenesis of cerebral malaria. Conversely, it may also indicate that PIEMP-1 display is less critical for other signs of severe malaria.

Whereas HbAC appeared to be selectively associated with cerebral malaria, the negative association of –\(\alpha\)/\(\alpha\) appeared to be limited to severe anemia. This is in agreement with previous findings. A failure of –\(\alpha\)/\(\alpha\) (and –\(\alpha\)/–\(\alpha\)) to protect 56 patients from coma has also been noted by Allen et al.12 in Papua New Guinea but was interpreted as resulting from a poor definition of the cerebral phenotype, which has been found to comprise a variety of etiologies.29 In our setting, we have also observed heterogeneity in the etiology of syndromes commonly classified as severe malaria.30 However, the clear negative associations observed with HbS provide strong circumstantial evidence for a predominant role of malaria as the cause of disease in our patient group, given that HbS has never been reported to be associated with protection against diseases other than malaria.24 More recently, Wambua et al15 and Pasvol16 speculated about a possible selectivity of –\(\alpha\)/\(\alpha\) to protect against severe anemia based on an inability to find protection among 19 cases of cerebral malaria.

Our stratifications resulted in the identification of a subgroup of 281 patients with severe anemia without additional signs of disease, who showed no negative association with –\(\alpha\)/\(\alpha\). This finding became apparent on multiple comparisons and therefore was statistically not significant. We believe, however, that it merits some discussion because, with 281 patients, the subgroup was of considerable size and, furthermore, the hypothesis that the association of –\(\alpha\)/\(\alpha\) would be specific for a complicated form of severe anemia may give rise to an interesting idea about the mode of malaria protection conferred by –\(\alpha\)/\(\alpha\).

The pathogenesis of malaria anemia is considered to include intravascular hemolysis, extravascular clearance of parasitized and nonparasitized erythrocytes, and bone-marrow dysfunction, with no understanding yet as to the relative contributions of these factors in mild and severe anemia.31 Similarly, the mechanism of malaria protection by \(\alpha\)-thalassemia remains a matter of discussion. Experimental data may support control of parasitism22 or selectivity for anemia in general33 but not selectivity for a complicated form of severe anemia. This, in fact, supports statistical concerns that this part of our –\(\alpha\)/\(\alpha\) findings may be spurious.

On the other hand, 2 lines of evidence may contribute to explain the selectivity of an association of –\(\alpha\)/\(\alpha\) with a complicated form of severe anemia. First, experience with anemia of other etiologies shows that a rapid decrease in hemoglobin concentrations generally causes tissue hypoxia with symptoms resembling those seen as additional complications in complicated forms of severe malaria anemia, whereas very low hemoglobin concentrations may well be tolerated if they develop at a rate sufficiently slow to allow compensatory mechanisms to become effective.34 Observations of drug-resistant malaria suggest that severe malaria anemia without additional...
complications may develop over a prolonged period.\textsuperscript{15,16}

Second, evidence has been presented indicating that $\alpha$/$\alpha$ may cause an increased erythrocyte turnover.\textsuperscript{37} Thus, it may be hypothesized that the 2 forms of severe malaria anemia differ in that they result from a slow and a rapid decrease in hemoglobin concentrations, respectively. The protective effect of $\alpha$/$\alpha$ might be restricted to the latter because a constitutively accelerated erythrocyte production in $\alpha$/$\alpha$ may dampen a rapid decrease in hemoglobin concentration, whereas it may have little effect on a slow decrease, which leaves time for maximum stimulation of erythropoiesis irrespective of the constitutive level. Further studies are needed to support these speculations.

The data presented in this study suggest that the specific malaria-protective effects of HbAC and $\alpha$/$\alpha$ result from interferences of these hemoglobin variants with distinct pathophysiological events. The genetic associations presented provide circumstantial evidence only and do not monitor biological processes directly. They may, however, stimulate attempts to design clinical studies and experimental models to confirm the genetic results at the functional level.

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