Associations between erythrocyte polymorphisms and risks of uncomplicated and severe malaria in Ugandan children: A case control study

Arthur Mpimbaza1, *, Andrew Walakira2, Grace Ndeezi3, Anne Katahoire1, Charles Karamagi3,4, Samuel L. Nsobya2, Stephen Tukwasibwe2, Victor Asua2, Philip J. Rosenthal5

1 Child Health and Development Centre, Makerere University-College of Health Sciences, Kampala, Uganda, 2 Infectious Diseases Research Collaboration, Kampala, Uganda, 3 Department of Pediatrics and Child Health, Makerere University-College of Health Sciences, Kampala, Uganda, 4 Clinical Epidemiology Unit, Department of Medicine, Makerere University-College of Health Sciences, Kampala, Uganda, 5 Department of Medicine, University of California, San Francisco, CA, United States of America

* arthurwakg@yahoo.com

Abstract

Background
Evidence for association between sickle cell and alpha thalassemia trait and severe malaria is compelling. However, for these polymorphisms associations with uncomplicated malaria, and for G6PD deficiency associations with uncomplicated and severe malaria, findings have been inconsistent. We studied samples from a three-arm case-control study with the objective of determining associations between common host erythrocyte polymorphisms and both uncomplicated and severe malaria, including different severe malaria phenotypes.

Method
We assessed hemoglobin abnormalities, α-thalassemia, and G6PD deficiency by molecular methods in 325 children with severe malaria age-matched to 325 children with uncomplicated malaria and 325 healthy community controls. Conditional logistic regression was used to measure associations between specified genotypes and malaria outcomes.

Results
No tested polymorphisms offered significant protection against uncomplicated malaria. α-thalassemia homozygotes (_α/α) had increased risk of uncomplicated malaria (OR 2.40; 95%CI 1.15, 5.03, p = 0.020). HbAS and α-thalassemia heterozygous (_α/αα) genotypes protected against severe malaria compared to uncomplicated malaria (HbAS OR 0.46; 0.23, 0.95, p = 0.036; _α/αα OR 0.51; 0.24, 0.77; p = 0.001) or community (HbAS OR 0.23; 0.11, 0.50; p<0.001; _α/αα OR 0.49; 0.32, 0.76; p = 0.002) controls. The α-thalassemia homozygous (_α/α) genotype protected against severe malaria when compared to uncomplicated malaria controls (OR 0.34; 95%CI 0.156, 0.73, p = 0.005), but not community
controls (OR 1.03; 0.46, 2.27, p = 0.935). Stratifying by the severe malaria phenotype, compared to community controls, the protective effect of HbAS was limited to children with severe anemia (OR 0.17; 95%CI 0.04, 0.65; p = 0.009) and that of _α/αα to those with altered consciousness (OR 0.24; 0.09, 0.59; p = 0.002). A negative epistatic effect was seen between HbAS and _α/αα; protection compared to uncomplicated malaria controls was not seen in individuals with both polymorphisms (OR 0.45; 0.11, 1.84; p = 0.269). G6PD deficiency was not protective against severe malaria.

Conclusion
Associations were complex, with HbAS principally protective against severe anemia, _α/αα against altered consciousness, and negative epistasis between the two polymorphisms.

Introduction
Several human genetic polymorphisms appear to protect against lethal falciparum malaria[1]. As first proposed for thalassemias by Haldane in 1949 [2], some polymorphisms that alter erythrocyte function persist in human populations as balanced polymorphisms by protecting against lethal malaria in heterozygotes, offsetting the negative consequences of the homozygous state[2]. The best characterized of these polymorphisms are common disorders of hemoglobin, with single amino acid substitutions in β-globin (hemoglobin S, C, and E) or reduced production of α- (α-thalassemia) or β- (β-thalassemia) globin [3], and deficiency in glucose-6-phosphate dehydrogenase (G6PD), which protects erythrocytes from oxidative stress[1].

Multiple studies have shown that hemoglobin S heterozygotes (HbAS) are protected against uncomplicated[4–9] and severe[4–6, 10–14] malaria due to Plasmodium falciparum. The mechanism of protection conferred by HbAS is incompletely understood [15, 16]. Potential mechanisms include increased splenic phagocytosis [2], premature hemolysis and parasite death[17], impaired hemoglobin digestion[18], altered cytoadherence [19], translocation of HbS-specific microRNAs [20], and growth arrest due to hemoglobin polymerization under low oxygen conditions[21]. HbC heterozygotes (HbAC) are also protected against severe malaria [12]. α-thalassemia heterozygosity (_α/αα) [12, 22–24] and homozygosity (_α/_α) [22, 23, 25] are reported to protect against severe malaria; protection against uncomplicated malaria is less certain, with studies yielding contradictory results [26].

The common G6PD deficiency genotype in African populations is G6PD A- (V68M and N126D), with 8–20% of wild type enzyme activity. G6PD deficiency may protect against malaria by increasing oxidative stress in P. falciparum-infected erythrocytes, causing premature lysis [27]. A case-control study showed significant association between G6PD A- and risk of severe malaria, with protection against cerebral malaria, but increased risk of severe anemia [28]. However, compared to HbAS or α-thalassemia, associations between G6PD A- deficiency and risk of severe malaria have been less straightforward, with studies yielding inconsistent results [29, 30].

A recent case-control study of determinants of severe malaria in Uganda enabled comparison of genotypes in children with severe malaria with those in two control groups: children with uncomplicated malaria and healthy community controls [31]. The objective of this study was to determine the association between common host erythrocyte polymorphisms and malaria outcomes including uncomplicated malaria and different manifestations of severe malaria.
Methods

Study design

A matched-case control study was conducted to identify determinants of severe malaria in Ugandan children [31]. Cases were children, aged 6 months to less than 10 years of age with severe malaria, defined as children hospitalized at Jinja Hospital with a positive malaria blood smear (>2500 parasites/ul) and any of the following severe malaria criteria: 1) severe anemia (Hb<5g/dl), 2) impaired consciousness (Blantyre Coma Score < 4)[32], or 3) respiratory distress (inter-costal or sub-costal recession without crackles or other evidence of pneumonia upon auscultation). Two types of controls were selected for each case. First, uncomplicated malaria controls were defined as children with fever and a blood smear positive for *P. falciparum* parasites (>2500 parasites/ul), but without clinical evidence of severe malaria, danger signs [32], or other known causes of febrile illness. Second, community controls, included to allow study of risk factors for uncomplicated malaria, were defined as children without a history of fever in the 2 weeks prior to enrollment recruited from the same village as a matched case. We sought a representative community sample, and in this high-transmission setting some community controls had asymptomatic parasitemia. Such controls were not excluded as this might have introduced bias [33, 34]. Target age groupings for matching were 6 to <12 months, 1 to <3 years, 3 to <5 years, and ≥5 years.

Malaria microscopy

Thick blood smears were stained with 10% Giemsa for 10 minutes and were initially read as positive or negative for malaria parasites by health facility staff as part of their routine practice. Enrollment of severe malaria cases and uncomplicated malaria controls was confirmed upon ascertainment of a positive malaria smear and adequacy of parasite density by a study laboratory technician. Thick blood smears reported as positive were sent to a central laboratory to be read by two expert microscopists for determination of parasite densities. Results were considered final if the first and second expert readings agreed on parasites densities (<25% level of disagreement). If the first and second expert readings were discordant, a third expert, reader broke the tie.

Characterization of human genotypes

For each child, a blood sample was collected into an EDTA tube, and DNA was purified as previously described [35]. Characterization of genotypes for hemoglobin (HbS and HbC), the α-thalassemia 3.7 kb deletion, and the common African G6PD deficiency genotype (G6PD A-) was performed with PCR-based assays, as described previously[35]. Outcomes for each tested genotype were categorized as wild type, heterozygous, or homozygous. For α-thalassemia, the αα/αα genotype represented wild type, _α/αα the heterozygote (silent carrier), and _α/α the homozygote (trait). For G6PD A-, females were considered wild type (A/A), heterozygous (A/A-) or homozygous (A-/A-), whereas males were considered wild type (A) or hemizygous (A-).

Sample size estimation

Sample size was powered to detect a difference in the odds of α-thalassemia heterozygosity (_α/αα) between severe malaria cases and either uncomplicated malaria or healthy community controls. The population prevalence of _α/αα was estimated at 44%, and we projected exclusion of 5% of enrolled patients due to false positive malaria tests. Based on these assumptions, we required a sample size of 325 children in each arm to detect a minimum odds ratio of 0.5
for risk of severe malaria among children with α-thalassemia heterozygosity, assuming power of 80%, and alpha of 0.05.

Analysis
Data were analyzed using STATA (version 14; STATA Corp.). Age of participants was summarized using medians. Gender, ethnicity (Nilotic vs. Bantu, based on the mother’s tribe as reported by the caregiver), and different manifestations of severe malaria were reported as proportions. Differences in distribution of baseline characteristics between cases and controls were compared using the Wilcoxon signed-rank test and the chi-square test for continuous and categorical data, respectively. Prevalence of genotypes and allele frequencies were calculated for different groups. Adherence to Hardy–Weinberg Equilibrium (HWE) was assessed in community controls based on observed versus expected genotypes; an exact test (p < 0.05) was considered a violation of HWE (S1 Table). Conditional logistic regression with adjustment for age (to account for residual confounding) and caretaker’s ethnicity (Nilotic vs. Bantu), a potential confounder [35], was used to measure associations between each polymorphism and risks of uncomplicated malaria (compared to community controls) and severe malaria. For association with severe malaria (including the sub-groups of severe anemia and impaired consciousness), comparisons were with uncomplicated malaria and community control groups. To assess if α/αα interacted with HbAS, we used two different approaches. First, we presented the individual effects of each polymorphism and the joint effects relative to wild type as the reference category in a conditional logistic regression model, determining the relative excess risk due to interaction, attributable proportion, and synergy index on an additive scale. As these measures were developed for risk rather than preventive factors [36], we recoded outcomes such that severe malaria was the reference and uncomplicated malaria associated with increased risk, resulting in transformation of the exposure variables as risk and not protective factors. Second, the significance of the product terms between HbAS and α/αα in the regression model was assessed using the Wald statistic.

Ethics approval
Institutional approval was obtained from the Uganda National Council of Science and Technology and the Institutional Review Boards of the College of Health Sciences, Makerere University, and the University of California San Francisco. Written informed consent was obtained from each caregiver. Interviews were conducted in accordance with good clinical practice, applicable patient privacy requirements, and the guiding principles of the Declaration of Helsinki.

Results
Study participants
A total of 2054 children were screened, including 1009 severe malaria cases, 528 with uncomplicated malaria, and 517 community controls (Fig 1). Among cases, the most common reason for exclusion was lack of specified severe malaria criteria (427, 67.0%). We excluded 196 (37%) uncomplicated malaria controls; low parasitemia, evidence of another illness, and evidence of severe malaria were the most common reasons for exclusion. We excluded 185 (35.7%) community controls; history of fever in the past two weeks was the most common reason for exclusion. Of 332 sets of cases and controls initially enrolled, 7 sets were excluded; 3 had a case with a negative confirmatory malaria test, 3 a missing record, and 1 a severe malaria case
accidentally enrolled twice, but considered for analysis only once, leaving 325 children in each group eligible for analysis.

**Characteristics of the study groups**

The median ages for children in the 3 study groups were similar, but severe malaria cases were younger than both community (median 1.98 vs. 2.15 years, p < 0.001) and uncomplicated malaria (2.13 years, p < 0.001) controls (Table 1). The distribution of age groups based on matching criteria among cases and controls was similar, but the severe malaria case group had a larger proportion of children less than one year of age (20.0%) compared to community (13.4%) and uncomplicated malaria (14.1%) controls. Distributions of females and Nilotics

| Variable                      | Community controls N = 325 | Uncomplicated malaria controls N = 325 | Severe malaria cases N = 325 | p-value<sup>a</sup> | p-value<sup>b</sup> | p-value<sup>c</sup> |
|-------------------------------|---------------------------|---------------------------------------|----------------------------|---------------------|---------------------|---------------------|
| Age in years (IQR)            | 2.15 (1.35–3.17)          | 2.13 (1.34–3.15)                      | 1.98 (1.11–2.95)           | 0.449               | <0.001              | <0.001              |
| Age category                  |                           |                                       |                            |                     |                     |                     |
| < 1 year                      | 44 (13.5%)                | 46 (14.1%)                            | 65 (20.0%)                 | 0.926               | <0.001              | <0.001              |
| 1 to < 3 years                | 183 (56.3%)               | 184 (56.6%)                           | 181 (55.6%)                |                     |                     |                     |
| 3 to < 5 years                | 78 (24.0%)                | 70 (21.5%)                            | 57 (17.5%)                 |                     |                     |                     |
| 5 years or more               | 20 (6.1%)                 | 25 (7.6%)                             | 22 (6.7%)                  |                     |                     |                     |
| Females, n (%)                | 174 (53.7%)               | 157 (48.4%)                           | 152 (46.7%)                | 0.172               | 0.07                | 0.624               |
| Ethnicity (Nilotic versus Bantu) | 15 (4.6%)                 | 17 (5.2%)                             | 11 (3.4%)                  | 0.723               | 0.393               | 0.200               |

<sup>a</sup> Community controls vs. uncomplicated malaria controls

<sup>b</sup> community controls vs. severe malaria

<sup>c</sup> uncomplicated malaria controls vs. severe malaria.
were similar across cases and controls (Table 1). Matching by age group was successful for 73.4% of severe malaria and uncomplicated malaria, 72.7% of severe malaria and community, and 71.0% of uncomplicated malaria and community case-control pairs.

Characteristics of children with severe malaria (cases)
Among the 325 children with severe malaria, 446 severe malaria criteria were observed, most commonly severe anemia (Hb < 5g/dl; 49.1%), impaired consciousness (32.3%), and respiratory distress (18.6%). Of the 325 children with severe malaria, 222 (68.3%) of them presented with a single severe malaria-defining syndrome, including severe anemia (64.9%), impaired consciousness (26.6%) and respiratory distress (8.5%). In the 103 children presenting with more than one defining criterion, severe anemia with unconsciousness was the most common (37.9%), followed by respiratory distress with unconsciousness (24.3%).

Prevalence of erythrocyte polymorphisms
Mutant genotypes were common for all polymorphisms studied, except HbC, with many more heterozygous than homozygous alleles identified (Table 2). Among community controls, allele frequencies were 6.2% for HbS, 0% for HbC, 22.3% for α-thalassemia, and 10.2% for G6PDA; none of the allele distributions violated Hardy Weinberg equilibrium (S1 Table).

Association between host polymorphisms and risks of uncomplicated malaria compared to community controls
Hemoglobin S heterozygotes had marginal protection against uncomplicated malaria (OR 0.60; 95%CI 0.34, 1.03, p = 0.065) (Table 3). α-thalassemia heterozygotes (_α/αα) did not demonstrate a significant association with uncomplicated malaria (OR 1.11; 95% CI 0.74, 1.56, 2007).
Table 3. Association between specific host polymorphisms and risk of malaria infection and severe malaria.

| Host polymorphism | Prevalence n/N (%) | Adjusted analysis |
|-------------------|--------------------|-------------------|
|                   | Community controls | Uncomplicated malaria | Severe malaria cases | Severe malaria cases vs. Community controls | Severe malaria cases vs. Uncomplicated malaria controls | Severe malaria cases vs. Community Controls |
|                   |                    |                   |                   | OR (95%CI); p-value | OR (95%CI); p-value | OR (95%CI); p-value |
| All children      |                    |                   |                   |                   |                   |                   |
| AS                | 42/317 (13.2%)     | 27/309 (8.7%)     | 14/311 (4.5%)     | 0.60 (0.34, 1.03); 0.065 | 0.46 (0.23, 0.95); 0.036 | 0.23 (0.11, 0.50); <0.001 |
| _α/αα             | 104/304 (34.2%)    | 102/300 (34.0%)   | 64/302 (21.1%)    | 1.11 (0.74, 1.56); 0.697 | 0.51 (0.24, 0.77); 0.001 | 0.49 (0.32, 0.76); 0.002 |
| _α/ _α            | 16/304 (5.2%)      | 31/300 (10.3%)    | 15/302 (4.9%)     | 2.40 (1.15, 5.03); 0.020 | 0.34 (0.16, 0.73); 0.005 | 1.03 (0.46, 2.27); 0.935 |
| G6PD-Females Heterozygous A/A- | 20/172 (11.6%) | 26/151 (17.2%) | 30/148 (20.2%) | 1.43 (0.65, 3.14); 0.370 | 1.98 (0.78, 5.03); 0.150 | 1.94 (0.75, 4.99); 0.169 |
| G6PD-Females Homozygous A-/A- | 5/172 (2.9%) | 7/151 (4.6%) | 5/148 (3.3%) | 3.21 (0.28, 35.7); 0.341 | 0.42 (0.07, 2.64); 0.350 | 1.16 (0.25, 5.40); 0.847 |
| G6PD Males Hemizygote A- | 20/142 (14.0%) | 30/162 (18.5%) | 26/166 (15.6%) | 1.52 (0.67, 3.41); 0.307 | 0.99 (0.40, 2.42); 0.986 | 0.86 (0.35, 2.08); 0.745 |
| Children with severe malaria attributed to severe anemia only   | 18/135 (13.3%) | 11/132 (8.3%) | 6/130 (4.6%) | NA | 0.47 (0.13, 1.65); 0.245 | 0.17 (0.04, 0.65); 0.009 |
| _α/αα             | 36/129 (27.9%)    | 45/124 (36.2%)   | 33/128 (25.7%)   | NA | 0.66 (0.35, 1.23); 0.193 | 0.78 (0.38, 1.57); 0.487 |
| _α/ _α            | 6/129 (4.6%)      | 13/124 (10.4%)   | 10/128 (7.8%)    | NA | 0.55(0.21, 1.44); 0.226 | 1.60 (0.51, 5.03); 0.415 |
| G6PD-Females Heterozygous A/A- | 7/72 (9.7%) | 12/64 (18.7%) | 11/67 (16.4%) | NA | 1.91 (0.45, 7.99); 0.372 | 0.83 (0.15, 4.40); 0.835 |
| G6PD-Females Homozygous A-/A- | 3/72 (4.1%) | 5/64 (7.8%) | 2/67 (2.9%) | NA | 0.22 (0.02, 2.39); 0.218 | 0.29 (0.001, 96.1); 0.678 |
| G6PD Males Hemizygote A- | 9/61 (14.7%) | 10/68 (14.7%) | 10/66 (15.1%) | NA | 3.24 (0.56, 21.2); 0.179 | 0.25 (0.02, 2.42); 0.233 |
| Children with severe malaria attributed to impaired consciousness only   | 7/84 (8.3%) | 4/83 (4.8%) | 6/84 (7.1%) | NA | 1.34 (0.37, 4.83); 0.647 | 0.80 (0.24, 2.66); 0.724 |
| _α/αα             | 34/80 (42.5%)     | 26/80 (32.5%)    | 13/79 (16.4%)    | NA | 0.31 (0.12, 0.80); 0.016 | 0.24 (0.09, 0.59); 0.002 |
| _α/ _α            | 7/80 (8.7%)       | 10/80 (12.5%)    | 2/79 (2.5%)      | NA | 0.08 (0.009, 0.72); 0.025 | 0.47 (0.08, 2.63); 0.396 |
| G6PD-Females Heterozygous A/A- | 2/44 (4.5%) | 7/39 (17.9%) | 6/35 (17.1%) | NA | * | * |
| G6PD-Females Homozygous A-/A- | 1/44 (2.2%) | 1/39 (2.5%) | 0 | NA | * | * |
| G6PD Males Hemizygote A- | 5/40 (12.5%) | 11/45 (24.4%) | 9/48 (18.7%) | NA | 0.37 (0.05, 2.41); 0.305 | 1.21 (0.29, 5.02); 0.784 |

NA: Not applicable

* Adjusted analysis: uncomplicated malaria cases vs. community controls

b* Adjusted analysis: severe malaria cases vs. uncomplicated malaria controls

* Adjusted analysis: severe malaria cases vs. community controls

d* Analysis limited to case control pairs; cases are defined by the listed severe malaria phenotype

* Insufficient numbers resulting in unintelligible effect estimates and confidence intervals

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p = 0.697), and α-thalassemia homozygotes (_α/α) had increased risk of uncomplicated malaria (OR 2.40; 95% CI 1.15, 5.03, p = 0.020). G6PD A- was not associated with risk of uncomplicated malaria in female heterozygotes (OR 1.43; 95% CI 0.65, 3.14, p = 0.370), female homozygotes (OR 3.21; 95% CI 0.28, 35.7, p = 0.341), or male hemizygotes (OR 1.52; 95% CI 0.67, 3.41, p = 0.307).

Association between host polymorphisms and risks of severe malaria compared to uncomplicated malaria controls

Hemoglobin S heterozygous (OR 0.46; 95% CI 0.23, 0.95, p = 0.036), _α/αα (OR 0.51; 95% CI 0.24, 0.77, p = 0.001) and _α/_α (OR 0.34; 95% CI 0.16, 0.73, p = 0.005) genotypes demonstrated protection against severe malaria when compared to uncomplicated malaria controls (Table 3). Upon sub-group analysis by type of severe malaria, the protective effect of HbAS was not demonstrated for severe anemia or impaired consciousness. In contrast, the protective effects of _α/αα and _α/_α were significant for children with impaired consciousness, a surrogate for cerebral malaria (OR 0.31; 95% CI 0.12, 0.80, p = 0.016 and OR 0.08; 95% CI 0.009, 0.72, p = 0.025, respectively), but not for children with severe anemia (OR 0.66; 95% CI 0.35, 1.23, p = 0.193 and OR 0.55; 95% CI 0.21, 1.44, p = 0.226, respectively). G6PD A- was not associated with protection against severe malaria or against specific types of severe malaria in both females and males.

Association between host polymorphisms and risks of severe malaria compared to community controls

Hemoglobin S heterozygotes demonstrated protection against severe malaria when compared to community controls (OR 0.23; 95% CI 0.11, 0.50, p <0.001; Table 3). Similarly, _α/αα demonstrated protection against severe malaria (OR 0.49; 95% CI 0.32, 0.76, p = 0.002), whereas _α/_α did not demonstrate this protection (OR 1.04; 95% CI 0.45, 2.41, p = 0.918). Upon sub-group analysis by severe malaria phenotype, when compared to community controls, HbAS protection was limited to children with severe anemia (OR 0.17; 95% CI 0.04, 0.65, p = 0.009). In contrast, the protective effect of _α/αα was limited to children with impaired consciousness (OR 0.24; 95% CI 0.09, 0.59, p = 0.002). The _α/_α genotype was not protective against any of the severe malaria phenotypes. G6PD A- was not associated with protection against severe malaria or against specific types of severe malaria in both females and males.

Interaction between HbAS and _α/αα

Based on the regression model, compared to wild type individuals, HbAS (OR 0.28; 95% CI 0.10, 0.76, p = 0.013) and _α/αα (OR 0.45; 95% CI 0.29, 0.70, p<0.001) demonstrated significant protection against severe malaria, whereas the co-inheritance of both HbAS and _α/αα did not (OR 0.45; 95% CI 0.11, 1.84, p = 0.269), all compared to uncomplicated malaria controls (Table 4). Upon recoding the outcome such that the exposure variables became risk and not protective factors, the three measures of additive interaction, relative excess risk due to interaction (-2.07; 95% CI -6.40, 2.24), attributable proportion (-0.84; 95% CI -3.42, 1.73) and synergy index (0.41; 95% CI -3.44, 1.73) all indicated significant negative interaction on the additive scale. Inclusion of the product of HbAS and _α/αα in the regression model was not statistically significant (Wald statistic 0.175), possibly due to small sample size. Overall, negative epistasis was observed, whereby combined HbAS and _α/αα genotype modified the protective effects of each individual polymorphism.
Polymorphisms that alter the sequence of hemoglobin, decrease hemoglobin production, or decrease the activity of G6PD have been associated with changes in malaria risk, but results from a range of studies have varied. We evaluated the impacts of common erythrocyte polymorphisms on malaria risk, taking advantage of a trial that included children with clinically well-characterized severe malaria and two sets of matched controls, children with uncomplicated malaria and healthy community members. Overall, HbAS, \( \alpha / \alpha \alpha \), and \( \alpha / \alpha \) were protective against severe malaria, but protection against different severe malaria phenotypes varied. Specifically, HbAS was protective against severe malarial anemia whereas \( \alpha / \alpha \alpha \) and \( \alpha / \alpha \) protected against impaired consciousness. In addition, a negative epistatic effect was seen between HbAS and \( \alpha / \alpha \alpha \) such that individuals with both polymorphisms were not protected against severe malaria.

HbAS demonstrated a trend toward protection against uncomplicated malaria compared to community controls, a finding consistent with previous case control [4] and prospective [5–9, 37] studies. \( \alpha / \alpha \alpha \) was not protective against uncomplicated malaria, consistent with most studies [5, 8, 22, 38–40], but differing from one study in Tanzania [41]. In contrast, \( \alpha / \alpha \) was associated with increased risk of uncomplicated malaria, as demonstrated previously in Vanuatu [39] and Tanzania [42]. As in those prior studies, the association between \( \alpha / \alpha \) and increased risk of uncomplicated malaria was more pronounced in younger children (OR 3.33; 95% CI 2.20, 5.03).

### Table 4. Association between \( \alpha \)-thalassemia heterozygote (\( \alpha / \alpha \alpha \)) and Hemoglobin AS including interaction terms.

| Variables | Odds Ratio (95%CI) | p-value |
|-----------|--------------------|---------|
| **Model A. Analysis of individual and joint categories of HbAS and \( \alpha / \alpha \alpha \)**. Outcome: severe (1) vs. uncomplicated (0) malaria | | |
| Normal (HbAA and \( \alpha \alpha \alpha \)) | Ref | 1 |
| HbAA and \( \alpha / \alpha \alpha \) | 0.45 (0.29, 0.70) | <0.001 |
| HbAS and \( \alpha \alpha / \alpha \alpha \) | 0.28 (0.10, 0.76) | 0.013 |
| HbAS and \( \alpha / \alpha \alpha \) | 0.45 (0.11, 1.84) | 0.269 |
| **Model B. Analysis of individual and joint categories of HbAS and \( \alpha / \alpha \alpha \)**. Outcome: uncomplicated (1) vs. severe malaria (0) | | |
| Normal (HbAA and \( \alpha \alpha \alpha \)) | Ref | 1 |
| HbAA and \( \alpha / \alpha \alpha \) | 2.18 (1.41, 3.38) | <0.001 |
| HbAS and \( \alpha \alpha / \alpha \alpha \) | 3.47 (1.30, 9.27) | 0.013 |
| HbAS and \( \alpha / \alpha \alpha \) | 2.19 (0.54, 8.89) | 0.269 |
| **Model C. Including an interaction term between HbAS and \( \alpha \alpha / \alpha \)**. Outcome: severe (1) vs. uncomplicated (0) malaria | | |
| Hemoglobin S heterozygote (HbAS) | 0.28 (0.10, 0.76) | 0.013 |
| \( \alpha \)-thalassemia heterozygote (\( \alpha / \alpha \alpha \)) | 0.45 (0.29, 0.70) | <0.001 |
| HbAS\#\( \alpha / \alpha \alpha \) | 3.45 (0.57, 20.7) | 0.175 |

Measures of interaction on an additive scale (based on Model B)
- Relative excess risk due to interaction: -2.07 (95% CI, -6.40, 2.24)
- Attributable proportion due to interaction: -0.84 (95% CI -3.42, 1.73)
- Synergy index: 0.41 (95% CI 0.04, 3.88)

* Interaction term of HbAS and \( \alpha / \alpha \alpha \)
# Operator symbol for creating an interaction between two variables.

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### Discussion

Polymorphisms that alter the sequence of hemoglobin, decrease hemoglobin production, or decrease the activity of G6PD have been associated with changes in malaria risk, but results from a range of studies have varied. We evaluated the impacts of common erythrocyte polymorphisms on malaria risk, taking advantage of a trial that included children with clinically well-characterized severe malaria and two sets of matched controls, children with uncomplicated malaria and healthy community members. Overall, HbAS, \( \alpha / \alpha \alpha \), and \( \alpha / \alpha / \alpha \) were protective against severe malaria, but protection against different severe malaria phenotypes varied. Specifically, HbAS was protective against severe malarial anemia whereas \( \alpha / \alpha \alpha \) and \( \alpha / \alpha / \alpha \) protected against impaired consciousness. In addition, a negative epistatic effect was seen between HbAS and \( \alpha / \alpha \alpha \) such that individuals with both polymorphisms were not protected against severe malaria.

HbAS demonstrated a trend toward protection against uncomplicated malaria compared to community controls, a finding consistent with previous case control [4] and prospective [5–9, 37] studies. \( \alpha / \alpha \alpha \) was not protective against uncomplicated malaria, consistent with most studies [5, 8, 22, 38–40], but differing from one study in Tanzania [41]. In contrast, \( \alpha / \alpha / \alpha \) was associated with increased risk of uncomplicated malaria, as demonstrated previously in Vanuatu [39] and Tanzania [42]. As in those prior studies, the association between \( \alpha / \alpha / \alpha \) and increased risk of uncomplicated malaria was more pronounced in younger children (OR 3.33; 95% CI 2.20, 5.03).
95% CI 0.85, 13.0, p = 0.083 for children under 4 years of age; OR 1.85; 95% CI 0.16, 21.2, p = 0.618 for those over 4 years of age). These findings suggest an age dependent effect of immunity contributing to the interaction of _α/α with risk of severe malaria [41]. A similar age dependent effect on HbAS protection against uncomplicated malaria has been demonstrated, with protection more pronounced in older children [37, 43].

HbAS demonstrated protection against severe malaria when compared to both uncomplicated malaria and community controls. These findings are consistent with prior case controls studies, in which HbAS demonstrated protection against severe malaria when compared to uncomplicated malaria [37, 44] and healthy community [10–12, 44] controls. Two cohort studies in Kenya also demonstrated that HbAS protected against severe malaria when incidence was compared to that in healthy controls [5, 6]. α-thalassemia heterozygotes (_α/αα) were also protected against severe malaria when compared to both uncomplicated and community controls, consistent with findings from previous case control [12, 23, 24] and cohort [5] studies. α-thalassemia homozygotes (_α/α) were protected against severe malaria, but only when compared to uncomplicated malaria controls, unlike other studies that also demonstrated protection compared to community controls [12, 23]. The lack of association between _α/α and severe malaria when compared to community but not uncomplicated malaria controls might be explained by different selection biases for the different control groups, with community controls biased toward the null.

Upon stratification by severe malaria phenotype, the protective effect of HbAS was limited to cases with severe anemia. This association might be attributed to the HbAS phenotype limiting multiplication of parasites in erythrocytes, thereby limiting erythrocyte destruction and progression to severe anemia [45]. These results contrast with results from a cohort study conducted in Kenya [6], and two case control studies, one in Ghana and the other a multicenter centers African study, which demonstrated HbAS protection against both severe malarial anemia and cerebral malaria [12, 44]. Differences may be explained by larger sample sizes for the other studies. For _α/αα, the protective effect was limited to children with impaired consciousness. This association may be attributed to mechanisms of malaria protection by α-thalassemia, including abnormal expression of Pfemp1, which mediates adherence of parasitized erythrocytes to brain endothelial cells [46]. However, other studies from East and West Africa [12, 22] showed that protection with _α/αα was limited to patients with severe anemia. Lack of _α/αα protection against severe anemia in our study might be explained by _α/αα protection against rapid, but not slowly progressive anemia [12], with slow progression more prevalent in our study setting characterized by high transmission.

Among children with HbAS, co-inheritance of _α/αα was more common (26.1%) among community controls; the same prevalence was observed in healthy blood donors in Cameroon [47]. Importantly, a negative epistatic effect was seen between HbAS and _α/αα for protection against severe malaria. This negative interaction between HbAS and _α/αα has been described previously [48] and is potentially explained by reversal of some protective changes attributed to HbAS in the setting of α-thalassemia. Additionally, α-thalassemia has been associated with reduced intraerythrocytic concentrations of HbS, potentially counteracting protective effects of HbAS against malaria [49]. Studies of associations between the G6PD A- genotype and severe malaria have been less consistent than for the other studied polymorphisms, with reports of protective effects in females [50], in males [51], in both [52], or no protection [53]. Reasons for discrepancies are uncertain, but the lack of significant findings in our study may have been explained by a relatively small sample size.

Our study findings add to the literature demonstrating that HbAS, _α/αα and _α/_α protect against severe malaria. However, the effects of these polymorphisms varied depending on the type of severe malaria. Co-inheritance of HbS and _α/αα had a negative epistatic effect for
protection against severe malaria. Longitudinal studies with larger sample sizes should be helpful in better characterizing the complex and differing impacts of these polymorphisms. Consideration of additional human polymorphisms of interest including those regulating expression of endothelial receptors such as CD36 and endothelial protein C receptor [54], and those regulating the inflammatory response, including complement receptor 1, nitric oxide synthase 2, and tumor necrosis factor-α[55] will enable a fuller understanding of the impact of human genetics on risks of uncomplicated and severe malaria.

Supporting information

S1 Table. Adherence to Hardy-Weinberg equilibrium among community controls.
(TIF)

S1 Dataset. Severe malaria case control study dataset.
(DTA)

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Author Contributions

Conceptualization: Arthur Mpimbaza, Andrew Walakira, Grace Ndeezi, Anne Katahoire, Charles Karamagi, Philip J. Rosenthal.

Data curation: Arthur Mpimbaza.

Formal analysis: Arthur Mpimbaza, Charles Karamagi.

Funding acquisition: Arthur Mpimbaza, Philip J. Rosenthal.

Investigation: Arthur Mpimbaza, Andrew Walakira, Samuel L. Nsobya, Stephen Tukwasibwe, Victor Asua, Philip J. Rosenthal.

Methodology: Arthur Mpimbaza, Grace Ndeezi, Charles Karamagi, Philip J. Rosenthal.

Project administration: Arthur Mpimbaza, Samuel L. Nsobya, Philip J. Rosenthal.

Resources: Arthur Mpimbaza, Philip J. Rosenthal.

Software: Arthur Mpimbaza.

Supervision: Arthur Mpimbaza, Charles Karamagi, Samuel L. Nsobya, Philip J. Rosenthal.

Validation: Arthur Mpimbaza, Charles Karamagi, Philip J. Rosenthal.

Writing – original draft: Arthur Mpimbaza, Charles Karamagi, Stephen Tukwasibwe, Philip J. Rosenthal.

Writing – review & editing: Arthur Mpimbaza, Grace Ndeezi, Anne Katahoire, Charles Karamagi, Stephen Tukwasibwe, Philip J. Rosenthal.
References

1. Weatherall DJ. Hemoglobinopathies worldwide: present and future. Curr Mol Med. 2008; 8(7):592–9. Epub 2008/11/11. PMID: 18991645.

2. Williams TN. Balancing act: haemoglobinopathies and malaria. Lancet Infect Dis. 2012; 12(6):427–8. https://doi.org/10.1016/S1473-3099(12)70067-1 PMID: 22445353.

3. Taher AT, Weatherall DJ, Cappellini MD. Thalassaemia. Lancet. 2017. https://doi.org/10.1016/S0140-6736(17)31822-6 PMID: 28774421.

4. Modiano D, Luoni G, Sirima BS, Simporé J, Verra F, Konate A, et al. Haemoglobin C protects against clinical Plasmodium falciparum malaria. Nature. 2001; 414(6861):305–8. https://doi.org/10.1038/35104556 PMID: 11713529.

5. Williams TN, Mwangi TW, Wambua S, Peto TE, Weatherall DJ, Gupta S, et al. Negative epistasis between the malaria-protective effects of alpha+-thalassemia and the sickle cell trait. Nat Genet. 2005; 37(11):1253–7. https://doi.org/10.1038/ng1660 PMID: 16227994; PubMed Central PMCID: PMC3521056.

6. Williams TN, Mwangi TW, Wambua S, Alexander ND, Kortok M, Snow RW, et al. Sickle cell trait and the risk of Plasmodium falciparum malaria and other childhood diseases. The Journal of infectious diseases. 2005; 192(1):178–86. https://doi.org/10.1086/430744 PMID: 15942909; PubMed Central PMCID: PMC3545189.

7. Clark TD, Greenhouse B, Njama-Meya D, Nzurubara B, Maiteki-Sebuguzi C, Staedke SG, et al. Factors determining the heterogeneity of malaria incidence in children in Kampala, Uganda. The Journal of infectious diseases. 2008; 198(3):393–400. Epub 2008/06/05. https://doi.org/10.1086/589778 PMID: 18522503.

8. Crompton PD, Traore B, Kayentao K, Doumbo S, Ongoiba A, Diakite SA, et al. Sickle cell trait is associated with a delayed onset of malaria: implications for time-to-event analysis in clinical studies of malaria. The Journal of infectious diseases. 2008; 198(9):1265–75. https://doi.org/10.1086/592224 PMID: 18752444; PubMed Central PMCID: PMC2574881.

9. Kreuels B, Kreuzberg C, Kobbé R, Ayim-Akonor M, Apiah-Thompson P, Thompson B, et al. Differing effects of HbS and HbC traits on uncomplicated falciparum malaria, anemia, and child growth. Blood. 2010; 115(22):4551–8. Epub 2010/03/17. https://doi.org/10.1182/blood-2009-09-241844 PMID: 20231425.

10. Mockenhaup t FP, Ehrhardt S, Cramer JP, Otchwema RN, Anemana SD, Goltz K, et al. Hemoglobin C and resistance to severe malaria in Ghanaian children. The Journal of infectious diseases. 2004; 190(5):1006–9. Epub 2004/08/06. https://doi.org/10.1086/422847 JID32155 [pii]. PMID: 15295709.

11. Ackerman H, Usen S, Jallow M, Sisay-Joof F, Pinder M, Kwiatkowski DP. A comparison of case-control and family-based association methods: the example of sickle-cell and malaria. Ann Hum Genet. 2005; 69(Pt 5):559–65. https://doi.org/10.1111/j.1529-8817.2005.00180.x PMID: 16138914.

12. May J, Evans JA, Timmann C, Ehmen C, Busch W, Thyre T, et al. Hemoglobin variants and disease manifestations in severe falciparum malaria. JAMA. 2007; 297(20):2220–6. https://doi.org/10.1001/jama.2007.20.2220 PMID: 17519411.

13. Jallow M, Teo YY, Small KS, Rockett KA, Deloukas P, Clark TG, et al. Genome-wide and fine-resolution association analysis of malaria in West Africa. Nat Genet. 2009; 41(6):657–65. https://doi.org/10.1038/ng.388 PMID: 19465909; PubMed Central PMCID: PMC2889040.

14. Aidoo M, Terlouw DJ, Kolczak MS, McElroy PD, ter Kuile FO, Kariuki S, et al. Protective effects of the sickle cell gene against malaria morbidity and mortality. Lancet. 2002; 359(9314):1311–2. https://doi.org/10.1016/S0140-6736(02)08273-9 PMID: 11965279.

15. Gong L, Parikh S, Rosenthal PJ, Greenhouse B. Biochemical and immunological mechanisms by which sickle cell trait protects against malaria. Malar J. 2013; 12(1):317. Epub 2013/09/13. doi: 10.1186/1475-2875-12-317 [pii] https://doi.org/10.1186/1475-2875-12-317 PMID: 24025776.

16. Albiti AH, Nsiah K. Comparative haematological parameters of HbAA and HbAS genotype children infected with Plasmodium falciparum malaria in Yemen. Hematology. 2014; 19(3):169–74. https://doi.org/10.1179/1607845413Y.00000000113 PMID: 24074341.

17. Roth EF Jr., Friedman M, Ueda Y, Tellez I, Trager W, Nagel RL. Sickling rates of human AS red cells infected in vitro with Plasmodium falciparum malaria. Science. 1978; 202(4368):650–2. PMID: 360396.

18. Pasvol G. The interaction between sickle haemoglobin and the malarial parasite Plasmodium falciparum. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1980; 74(6):701–5. PMID: 7010693.

19. Cyrklaft M, Sanchez CP, Kilian N, Bisseye C, Simpore J, Frischknecht F, et al. Hemoglobins S and C interfere with actin remodeling in Plasmodium falciparum-infected erythrocytes. Science. 2011; 334(6060):1283–6. https://doi.org/10.1126/science.1213775 PMID: 22075726.
20. LaMonte G, Philip N, Reardon J, Lacsina JR, Majoros W, Chapman L, et al. Translocation of sickle cell erythrocyte microRNAs into Plasmodium falciparum inhibits parasite translation and contributes to malaria resistance. Cell host & microbe. 2012; 12(2):187–99. https://doi.org/10.1016/j.chom.2012.06.007 PMID: 22901539; PubMed Central PMCID: PMC3442262.

21. Archer NM. Resistance to Plasmodium falciparum in sickle cell trait erythrocytes is driven by oxygen dependent growth inhibition. PNAS. 2018.

22. Wambua S, Mwangi TW, Kortok M, Uyoga SM, Macharia AW, Mwacharo JK, et al. The effect of alpha+ thalassaemia on the incidence of malaria and other diseases in children living on the coast of Kenya. PLoS medicine. 2006; 3(5):e158. https://doi.org/10.1371/journal.pmed.0030158 PMID: 16605300; PubMed Central PMCID: PMC1435778.

23. Williams TN, Wambua S, Uyoga S, Macharia A, Mwacharo JK, Newton CR, et al. Both heterozygous and homozygous alpha+ thalassemias protect against severe and fatal Plasmodium falciparum malaria on the coast of Kenya. Blood. 2005; 106(1):368–71. https://doi.org/10.1182/blood-2005-01-0313 PMID: 15769889.

24. Mockenhaupt FP, Ehrhardt S, Gellert S, Otchwemah RN, Dietz E, Anemana SD, et al. Alpha(+)-thalassemia protects African children from severe malaria. Blood. 2004; 104(7):2003–6. Epub 2004/06/17. https://doi.org/10.1182/blood-2003-11-4090 2003-11-4090 [pii]. PMID: 15198952.

25. Allen SJ, O’Donnell A, Alexander ND, Alpers MP, Peto TE, Clegg JB, et al. alpha+-Thalassemia protects children against disease caused by other infections as well as malaria. Proceedings of the National Academy of Sciences of the United States of America. 1997; 94(26):14736–41. Epub 1998/02/07. PMID: 9405682; PubMed Central PMCID: PMC251062.

26. Taylor SM, Parobek CM, Fairhurst RM. Haemoglobinopathies and the clinical epidemiology of malaria: a systematic review and meta-analysis. Lancet Infec Dis. 2012; 12(6):457–68. Epub 2012/03/27. doi: S1473-3099(12)70055-5 [pii] https://doi.org/10.1016/S1473-3099(12)70055-5 PMID: 22445352; PubMed Central PMCID: PMC3404513.

27. Carter N, Pamba A, Duparc S, Wailumbe JN. Frequency of glucose-6-phosphate dehydrogenase deficiency in malaria patients from six African countries enrolled in two randomized anti-malarial clinical trials. Malar J. 2011; 10:241. https://doi.org/10.1186/1475-2875-10-241 PMID: 21849081; PubMed Central PMCID: PMC3188486.

28. Clarke GM, Rockett K, Kivinen K, Hubbart C, Jeffreys AE, Rowlands K, et al. Characterisation of the opposing effects of G6PD deficiency on cerebral malaria and severe malarial anaemia. eLife. 2017; 6. https://doi.org/10.7554/eLife.15085 PMID: 28067620; PubMed Central PMCID: PMC5225559.

29. Manjurano A, Sepulveda N, Nadjm B, Mtove G, Wangai H, Maxwell C, et al. African glucose-6-phosphate dehydrogenase alleles associated with protection from severe malaria in heterozygous females in Tanzania. PLoS genetics. 2015; 11(2):e1004960. https://doi.org/10.1371/journal.pgen.1004960 PMID: 25671784; PubMed Central PMCID: PMC4335500.

30. Mbanefo EC, Ahmed AM, Titouna A, Elmaraezy A, Trang NT, Phuoc Long N, et al. Association of glucose-6-phosphate dehydrogenase deficiency and malaria: a systematic review and meta-analysis. Scientific reports. 2017; 7:45963. https://doi.org/10.1038/srep45963 PMID: 28382932; PubMed Central PMCID: PMC5382680.

31. Mpimbaza A, Ndezei G, Katahoire A, Rosenthal PJ, Karamagi C. Demographic, Socioeconomic, and Geographic Factors Leading to Severe Malaria and Delayed Care Seeking in Ugandan Children: A Case-Control Study. The American journal of tropical medicine and hygiene. 2017; 97(5):1513–23. https://doi.org/10.4269/ajtmh.17-0056 PMID: 29016322.

32. Genton B, al-Yaman F, Alpers MP, Mokele D. Indicators of fatal outcome in paediatric cerebral malaria: a study of 134 comatose Papua New Guinean children. International journal of epidemiology. 1997; 26 (3):670–6. PMID: 9222795.

33. Poole C. Controls who experienced hypothetical causal intermediates should not be excluded from case-control studies. Am J Epidemiol. 1999; 150(6):547–51. PMID: 10489992.

34. Higdon MM, Hammitt LL, Deloria Knoll M, Baggett HC, Brooks WA, Howie SRC, et al. Should Controls With Respiratory Symptoms Be Excluded From Case-Control Studies of Pneumonia Etiology? Reflections From the PERCH Study. Clin Infect Dis. 2017; 64(suppl_3):S205–S12. https://doi.org/10.1093/cid/cix076 PMID: 28575354; PubMed Central PMCID: PMC5447853.

35. Walakira A, Tukwasibwe S, Kiggundu M, Verra F, Kakeeto P, Ruhamanyaaka E, et al. Marked variation in prevalence of malaria-protective human genetic polymorphisms across Uganda. Infection, genetics and evolution: journal of molecular epidemiology and evolutionary genetics in infectious diseases. 2017; 55:281–7. https://doi.org/10.1016/j.meegid.2017.09.021 PMID: 28939159; PubMed Central PMCID: PMC5685907.
49. Wambua S, Mwacha R, Uyoga S, Macharia A, Williams TN. Co-inheritance of alpha+-thalassaemia.

50. Rumaney MB, Ngitoungui VJ, Vorster AA, Ramares R, Kengne AP, Ngogang J, et al. The co-inheritance of alpha-thalassemia and sickle cell anemia is associated with better hematological indices and lower consultations rate in Cameroonian patients and could improve their survival.

Lin E, Tavul L, Michon P, Richards JS, Dadob E, Beeson JG, et al. Minimal association of common red blood cell polymorphisms with Plasmodium falciparum infection and uncomplicated malaria in Papua New Guinean school children. The American journal of tropical medicine and hygiene. 2010; 83 (4):828–33. https://doi.org/10.4269/ajtmh.2010.09-0713 PMID: 20889674; PubMed Central PMC: PMC2946751.

39. Williams TN, Maitland K, Bennett S, Ganczakowski M, Petö TE, Newbold CI, et al. High incidence of malaria in alpha-thalassaemic children. Nature. 1996; 383(6600):522–5. https://doi.org/10.1038/383522a0 PMID: 9849722.

40. Rosanas-Urgell A, Senn N, Rarau P, Aponte JJ, Reeder JC, Siba PM, et al. Lack of associations of alpha(+) thalassemia with the risk of Plasmodium falciparum and Plasmodium vivax infection and disease in a cohort of children aged 3–21 months from Papua New Guinea. Int J Parasitol. 2012; 42 (12):1107–13. https://doi.org/10.1016/j.ijpara.2012.10.001 PMID: 23065147.

41. Enevold A, Lusingu JP, Mbande B, Alfrangis M, Lemnge MM, Bygbjerg IC, et al. Reduced risk of uncomplicated malaria episodes in children with alpha+-thalassemia in northeastern Tanzania. The American journal of tropical medicine and hygiene. 2008; 78(5):714–20. PMID: 18458302.

42. Veenemans J, Andango PE, Mbugi EV, Kraaijenhagen RJ, Mwaniki DL, Mockenhaupt FP, et al. Alpha+-thalassemia protects against anemia associated with asymptomatic malaria: evidence from community-based surveys in Tanzania and Kenya. The Journal of infectious diseases. 2008; 198(3):401–8. Epub 2008/06/28. https://doi.org/10.1086/589884 PMID: 18582194.

43. Gong L, Maitelki-Sebuguzi C, Rosenthal PJ, Hubbard AE, Drakeley CJ, Dorsey G, et al. Evidence for both innate and acquired mechanisms of protection from Plasmodium falciparum in children with sickle cell trait. Blood. 2012; 119(16):3808–14. https://doi.org/10.1182/blood-2011-08-371062 PMID: 22327223; PubMed Central PMC: PMC3353834.

44. Reappraisal of known malaria resistance loci in a large multicenter study. Nat Genet. 2014; 46 (11):119–204. Epub 2014/09/30. doi: ng.3107 [pii]. https://doi.org/10.1038/ng.3107 PMID: 25261933.

45. Dicko A, Klion AD, Thera MA, Sagara I, Yalcouye D, Niambele MB, et al. The etiology of severe anemia and sickle trait results in specific effects on hematological parameters. Br J Haematol. 2006; 133 (2):206–9. https://doi.org/10.1111/j.1365-2451.2006.06006.x PMID: 16611313; PubMed Central PMC: PMC4397954.

46. Opi DH, Ochola LB, Tendwa M, Siddonbo BR, Ocholla H, Fanjo H, et al. Mechanistic Studies of the Negative Epistatic Malaria-protective Interaction Between Sickle Cell Trait and alpha-thalassemia. EBioMedicine. 2014; 1(1):29–36. https://doi.org/10.1016/j.ebiom.2014.10.006 PMID: 25893206; PubMed Central PMC: PMC4076272.

47. Wambua S, Mwacharo J, Uyoga S, Macharia A, Williams TN. Co-inheritance of alpha+-thalassaemia and sickle cell trait results in specific effects on haematological parameters. Br J Haematol. 2006; 133 (2):206–9. https://doi.org/10.1111/j.1365-2451.2006.06006.x PMID: 16611313; PubMed Central PMC: PMC4397954.

48. Manjurano A, Clark TG, Nadjim B, M'tove G, Wangai H, Sepulveda N, et al. Candidate human genetic polymorphisms and severe malaria in a Tanzanian population. PloS one. 2012; 7(10):e47463. Epub 2012/11/13. https://doi.org/10.1371/journal.pone.0047463 [pii]. PMID: 23144702; PubMed Central PMC: PMC3483265.

49. Guindo A, Traore K, Diakite S, Wellemes TE, Doumbo OK, Diallo DA. An evaluation of concurrent G6PD (A-) deficiency and sickle cell trait in Malian populations of children with severe or uncomplicated P. falciparum malaria. Am J Hematol. 2011; 86(9):795–6. https://doi.org/10.1002/ajh.22093 PMID: 21786288; PubMed Central PMC: PMC4795173.
52. Ruwende C, Khoo SC, Snow RW, Yates SN, Kwiatkowski D, Gupta S, et al. Natural selection of hemi- and heterozygotes for G6PD deficiency in Africa by resistance to severe malaria. Nature. 1995; 376(6537):246–9. Epub 1995/07/20. https://doi.org/10.1038/376246a0 PMID: 7617034.

53. Toure O, Konate S, Sissoko S, Niangaly A, Barry A, Sall AH, et al. Candidate polymorphisms and severe malaria in a Malian population. PloS one. 2012; 7(9):e43987. Epub 2012/09/08. https://doi.org/10.1371/journal.pone.0043987 PONE-D-12-10698 [pii]. PMID: 22957039; PubMed Central PMCID: PMC3434208.

54. Cabrera A, Neculai D, Kain KC. CD36 and malaria: friends or foes? A decade of data provides some answers. Trends in parasitology. 2014; 30(9):436–44. https://doi.org/10.1016/j.pt.2014.07.006 PMID: 25113859.

55. Lopez C, Saravia C, Gomez A, Hoebeke J, Patarroyo MA. Mechanisms of genetically-based resistance to malaria. Gene. 2010; 467(1–2):1–12. https://doi.org/10.1016/j.gene.2010.07.008 PMID: 20655368.