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Impact of haemoglobinopathies on the clinical epidemiology of malaria: a systematic review and meta-analysis

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SUMMARY

Background—Haemoglobinopathies variously reduce the risk of developing malaria syndromes. Quantifying these relationships may strengthen the foundation for translational studies of malaria pathogenesis and immunity.

Methods—The databases of MEDLINE and Embase (1950 – September 9, 2011) were searched to identify studies that estimated the risk of malaria in patients with and without haemoglobinopathies. Additional studies were identified from reference lists. Included outcomes were *Plasmodium falciparum*-related outcomes of severe malaria, uncomplicated malaria, asymptomatic parasitaemia, or pregnancy-associated malaria, and *P. vivax* malaria. Two independent reviewers identified studies, assessed their quality, and extracted data; data were meta-analyzed when outcomes were reported in more than one study.

Findings—Of 62 identified studies, 44 reported on HbAS, 19 on HbAC and HbCC, and 18 on α-thalassaemia. Case-control studies showed a decreased risk of severe malaria for HbAS (summary

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Author contributions
SMT conceived of and designed the study, conducted the literature search, analyzed and interpreted data, and wrote the manuscript. CMP conducted the literature search, analyzed and interpreted data, and contributed to the drafting of the manuscript. RMF conceived of and designed the study, interpreted the data, drafted the manuscript, and supervised the study conduct. SMT had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Conflicts of interest
All authors declare that they have no conflicts of interest relevant to the subject of this manuscript.

Additional Contributions
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Odds Ratio [OR] 0.09; 95% confidence interval [CI] 0.06 – 0.12), HbCC (summary OR 0.27; 95% CI 0.11 – 0.63), homozygous α-thalassaemia (summary OR 0.63; 95% CI 0.48 – 0.83), HbAC (summary OR 0.83; 95% CI 0.74 – 0.92), and heterozygous α-thalassaemia (summary OR 0.83; 95% CI 0.74 – 0.92). Only HbAS was consistently associated with protection from uncomplicated malaria (summary Incidence Rate Ratio 0.69; 95% CI 0.61 – 0.79); none demonstrated protection from asymptomatic parasitaemia. There was a paucity of clinical studies investigating β-thalassaemia, HbE, *P. vivax* malaria, and pregnancy-associated malaria.

Interpretation—Protection from severe malaria syndromes is significant for HbAS, HbCC, HbAC, and homozygous and heterozygous α-thalassaemia, but these haemoglobinopathies differ substantially in the degrees of protection. Protection from uncomplicated malaria and asymptomatic parasitaemia is mild or absent. By attenuating the severity of malaria, haemoglobinopathies serve as a model for investigating the mechanisms of malaria pathogenesis and immunity.

INTRODUCTION

Haemoglobinopathies are highly prevalent in some human populations currently or historically exposed to the malaria parasite *Plasmodium falciparum*. In the major haemoglobinopathies, adult haemoglobin – normally composed of two α-globin and two β-globin chains – is altered by genetic polymorphisms that encode single amino acid substitutions in β-globin (as in HbS, HbC, and HbE) or reduce production of α- and β-globin chains (in α- and β-thalassaemia, respectively).[1] These haemoglobin variants and thalassaemias are postulated to have been naturally selected for their protection from malaria, as evidenced by a broad spectrum of investigations. These include experimental *P. falciparum* infection protocols, *in vitro* laboratory experimentation, ecological epidemiologic studies, and cartographic modeling.[2] Nevertheless, confirmation and quantification of malaria risk reductions due to haemoglobinopathies requires clinical studies.

Correlates of both malaria pathogenesis and immunity to disease can be identified by studying patterns of differential susceptibility to malaria. Investigations of increased susceptibility to *P. falciparum* malaria during pregnancy[3,4] and resistance to *P. vivax* infection in West Africans lacking erythrocyte expression of Duffy Antigen Receptor for Chemokines (DARC)[5,6] have unearthed fundamental mechanisms of both malaria pathogenesis and acquired immunity. These molecular mechanisms – adumbrated by careful epidemiologic studies – are foundations for leading vaccine candidates against pregnancy-associated malaria[7] and vivax malaria.[8] While some falciparum malaria vaccines are showing partial efficacy,[9,10] malaria's pathogenic mechanisms are not understood sufficiently to inform the rational design of future therapeutics and preventive measures.

The clinical manifestations of *P. falciparum* malaria display a broad spectrum of severity from asymptomatic parasitaemia to severe malaria syndromes.[11] Differential protection from specific syndromes owing to genetic resistance may constitute a "natural experiment" that helps to identify the mechanisms of malaria pathogenesis that cause clinical morbidity. Toward this end, we conducted a systematic review of published studies to estimate the direct clinical effects of haemoglobinopathies on malaria syndromes.

METHODS

Search strategy & review criteria

We performed our review and meta-analysis in accordance with the PRISMA guidelines (Supplementary methods, Table S1).[12] Two authors (SMT and CMP) independently performed the database searches (through September 9, 2011), appraised study quality, and

extracted study data. Additional references were selected from the reference lists of identified studies. To appraise the quality of the observational studies, we adapted the principles of the Newcastle-Ottawa scale;[13] in order to base analyses on robust data, we only included studies that scored at least seven stars on the scale's assessment of patient selection, comparability, and exposure/outcome. When reported data were not sufficient for estimation of desired comparisons, we contacted study authors. Overall, we selected studies that reported the frequency of clinical outcomes in patients with and without a haemoglobinopathy.

Study participants—We included studies that principally enrolled children; the exceptions were studies that investigated pregnancy-associated malaria. We included studies conducted in any level of malaria endemicity, but did not consider studies of non-immune travelers.

Study designs—For the incident outcomes of severe malaria, uncomplicated malaria, asymptomatic parasitaemia, and vivax malaria, we included data from both prospective cohort and case-control studies. For asymptomatic parasitaemia (with either *Plasmodium* species), we also included data from cross-sectional studies. For pregnancy-associated malaria outcomes, we included data from cross-sectional studies of pregnant women. For case-control studies, we required a clear description of the selection of controls. We excluded case reports.

Exposure assessment—We only considered papers in which haemoglobin typing employed electrophoresis, chromatography, or DNA analysis.

Outcome assessment—We investigated clinical outcomes owing to infection with either *P. falciparum* or *P. vivax. P. falciparum*-related outcomes were severe malaria (including cerebral malaria and severe malarial anaemia),[14] uncomplicated malaria, asymptomatic parasitaemia, and pregnancy-associated malaria; vivax malaria was also included (Supplementary methods).

Definitions

The human genome normally contains four copies of the α-globin gene and two copies of the β-globin gene. Individuals with deletions of one a-globin gene (–α/αα) and two α-globin genes (–α/-α or αα/--) are referred to as α-thalassaemia heterozygotes and homozygotes, respectively. β-thalassaemia refers to individuals with impaired production of a single β-globin gene (β-thalassaemia trait, or β-thalassaemia minor). We did not investigate HbSS, HbSC, the deletion of three α-globin genes (α−/−-), or the impaired production of two β-globin genes (β-thalassaemia major) because these genotypes typically manifest severe clinical sequelae which complicate any assessment of malaria-specific clinical morbidity. Additionally, we did not explore haemoglobin mutations with low global population prevalences, including haemoglobins D, Constant Spring, and Lepore. Odds Ratios (ORs) and Incidence Rate Ratios (IRRs) reflect comparisons between patients with haemoglobin variants and those with HbAA, or between patients with thalassaemias and those without.

Data analysis

For studies that did not report comparisons of interest, we extracted raw data to either 1) compare prevalences of parasitaemia between patient groups with the chi-squared test (in cross-sectional studies); 2) compare prevalences of haemoglobin variants between groups of patients with malaria syndromes with unadjusted ORs (in case-control studies); or 3) compute Risk Ratios (RRs) or IRRs of malaria syndromes between groups of patients with

and without haemoglobinopathies (in prospective studies). All comparisons were calculated with exact confidence intervals.

Because case-control and prospective cohort studies estimate relative risk using distinct statistical methodologies, we employed separate analyses to meta-analyze ORs and IRRs. When individual-level case-control data were available for two or more studies that compared the prevalence of a haemoglobinopathy for the same case and control groups, we meta-analyzed the data to produce summary ORs. Meta-analyses were computed using random-effects models employing the DerSimonian & Laird method (metan in Stata/IC); the I^2 statistic for heterogeneity was calculated using the Mantel-Haenszel method for meta-analyzed data within subgroups (haemoglobinopathy and malaria syndrome). Similarly, when data were available for two or more prospective studies which compared incidence rates of the same outcome, we meta-analyzed the data to produce summary IRRs. Meta-analyses of IRRs were computed using random-effects Poisson meta-regression.[15]. We assessed publication bias in case-control studies using funnel plots and Begg's test (Supplementary methods). All single-study and summary analyses were calculated with Stata/IC (version 11, Stata Corp, College Station, TX).

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author (SMT) had full access to all the data in the study and had final responsibility for the decision to submit for publication.

RESULTS

The search strategy identified 2664 studies for review, and we selected 62 for inclusion (Figure 1): 44 studies of HbAS, 19 of HbAC, eight of HbCC, 18 of α-thalassaemia (all except one included homozygotes), three of HbE, and two of β-thalassaemia (some studies examined more than one haemoglobinopathy). Of the 62 studies, 18 were prospective cohorts, 15 were case-control, and 31 were cross-sectional studies (two studies reported more than one design). Five studies investigated pregnancy-associated malaria, and two studies included patients with *P. vivax* malaria. There was no evidence of reporting bias amongst comparable studies (Supplementary methods, Figure S1).

Severe *P. falciparum* malaria syndromes

Haemoglobin S—Compared to healthy controls, the summary OR for severe malaria was 0.09 (95% CI 0.06 – 0.12; I^2 10.6%) across five studies which together enrolled more than 10,000 patients,[16–20] and was similar to summary ORs for the specific syndromes of cerebral malaria (0.07; 95% CI 0.04 – 0.14; I^2 0%)[18,19] and severe malarial anaemia (0.09; 95% CI 0.06 – 0.13; I^2 0%)(Figure 2, Table 1).[18,19] Compared to children with uncomplicated malaria, ORs for severe malaria in three studies[17,21,22] were heterogeneous and a summary OR estimate was non-significant (0.52; 95% CI 0.20 – 0.38; I^2 50.0%).

Only two cohort studies have reported the incidence of severe malaria (Figure 3, Table 2). The incidence of severe malaria was reduced by 71% (95% CI 38% – 88%)[23] and 83% (95% CI 60% – 93%)[24] in similar cohorts of Kenyan children. In the second cohort, the incidence of cerebral malaria was nonsignificantly reduced by 86% (95% CI -17% – 98%), while that of severe malarial anaemia was reduced by 60% (95% CI 40% – 70%)[25] and 89% (95% CI 3% – 99%).[24] Taken together, data from both case-control and prospective cohort studies indicate that HbAS is consistently associated with large reductions in the risk of severe malaria syndromes.

Haemoglobin C—HbC appears to protect against severe malaria to a lesser degree than HbAS and in proportion to allele frequency (Figure 2). Compared to healthy children in four studies[16,17,19,20] that together enrolled over 9,000 patients, the summary ORs for severe malaria were 0.27 (95% CI 0.11 – 0.63; I^2 0%) for HbCC and 0.83 (95% CI 0.74 – 0.92; I^2 10.2%) for HbAC (Figure 2; Table S1). Protection from specific severe malaria syndromes has not been fully investigated in HbCC; in one study,[19] HbAC showed mild protection from cerebral malaria (OR 0.64; 95% CI 0.45 – 0.91) and severe malarial anaemia (OR 0.87; 95% CI 0.68 – 0.11). When compared to children with uncomplicated malaria, protection from severe malaria is inconsistent: non-significant protection is reported from severe malaria in three studies[17,21,26] of HbCC (summary OR 0.12; 95% CI 0.12 – 10.70; I^2 0.7%) and HbAC (summary OR 0.76; 95% CI 0.32 – 0.79; I^2 60.7%), and from severe malarial anaemia in two studies[21,26] that combined homozygotes and heterozygotes (summary OR 0.35; 95% CI 0.04 – 0.73; I^2 0%). Significant protection from cerebral malaria was reported in one study of Malian children that combined homo- and heterozygotes (OR 0.15; 95% CI 0.004 – 0.93).[21]

Prospective studies have not reported the incidence of severe syndromes in HbC children (Table 2). Thus, convincing evidence for protection from severe malaria owing to HbC derives largely from few case-control studies.

Haemoglobin E—Meta-analysis of two studies[27,28] in Myanmar and Thailand that compared the prevalence of HbE in severe and uncomplicated malaria cases demonstrated no evidence of protection (summary OR 0.41; 95% CI 0.04 – 0.95), though this should be interpreted cautiously given the significant heterogeneity of the findings (I^2 70.5%, p=0.027) and the highly-selected settings of the studies.

α-thalassaemia—Four studies[19,29–31] investigated α-thalassaemia in healthy children and children with severe malaria: summary ORs were 0.63 (95% CI 0.48 – 0.83; I^2 20.6%) for homozygotes and 0.83 (95% CI 0.74 – 0.92; I^2 0%) for heterozygotes. Protection from cerebral malaria was nonsignificant in one study[19] for heterozygotes (OR 0.80; 95% CI 0.64 – 1); protection from severe malarial anaemia was reported in two studies,[19,29] with summary ORs of 0.50 (95% CI 0.35 – 0.72; I^2 0%) for homozygotes and 0.86 (95% CI 0.75 – 0.996; I^2 0%) for heterozygotes. One prospective study from Kenya documented a decreased incidence of severe disease in α-thalassaemia homozygotes (IRR 0.54; 95% CI 0.30 – 0.99) and heterozygotes (IRR 0.60; 95% CI 0.39 – 0.90) (Table 2; Figure 3).[23] Additionally, protection from severe malarial anaemia among heterozygotes (IRR 0.33; 95% CI 0.14 – 0.78) was similar to protection from cerebral malaria (IRR 0.48; 95% CI 0.24 – 0.97).[32]

β-thalassaemia—No studies have investigated the risk of severe malaria in patients with β-thalassaemia.

Uncomplicated *P. falciparum* malaria

Haemoglobin S—In two West African studies,[17,33] compared to healthy children the summary OR for children with uncomplicated malaria was 0.30 (95% CI 0.20 – 0.45; I^2 0.8%) (Table 1; Figure 2). Multiple prospective studies have characterized the risk reduction in malaria attributable to HbS (Table 2; Figure 3). Meta-analysis of five studies[23,34–37] produced a summary IRR estimate of 0.69 (95% CI 0.61 – 0.79), which likely approximates the risk reduction owing to HbAS more closely in these malaria hyperendemic settings.[38]

Haemoglobin C—Few studies have reported the risk of uncomplicated malaria associated with HbC. Two studies in West Africa compared healthy children and children with

uncomplicated malaria: for HbCC, the OR for malaria was 0 (95% CI 0 – 0.41) owing to the absence of HbCC in the case patients,[17] and for HbAC the summary OR was 0.16 (95% CI 0.26 – 0.23; I^2 80.9%).[17,33] Three prospective studies have yielded conflicting results (Table 2; Figure 3): meta-analysis of two studies[35,37] yielded a summary OR of 0.05 (95% CI 0.88 – 0.26). Thus, definitive evidence of protection from uncomplicated malaria afforded by HbCC and HbAC has not been established.

Haemoglobin E—No identified studies quantified susceptibility to malaria by HbE.

α-thalassaemia—Several prospective studies have assessed the incidence of uncomplicated malaria in α-thalassaemic children (Table 2; Figure 3), with conflicting results. In Vanuatu, the incidence of falciparum malaria was higher in α-thalassaemia homozygotes (IRR 0.3; 95% CI 0.32 – 0.07) and heterozygotes (IRR 0.1; 95% CI 0.77 – 0.61);[39] in contrast, the incidence of uncomplicated malaria was lower in homozygotes (IRR 0.83; 95% CI 0.70 – 0.97) and heterozygotes (IRR 0.93; 95% CI 0.82 – 0.04) in Kenya,[23] as well as homozygotes (RR 0.12; 95% CI 0.02 – 0.83) and heterozygotes (RR 0.30; 95% CI 0.10 – 0.85) in Tanzania.[40] Meta-analysis of three studies[23,35,39] suggests lack of protection for both homozygotes (summary IRR 0.12; 95% C.I. 0.69 – 0.81) and heterozygotes (summary IRR 0.98; 95% C.I. 0.87 – 0.11).

β-thalassaemia—In one case-control study in Liberia, the prevalence of β-thalassaemia was lower in cases of uncomplicated malaria than in community controls (OR 0.56; 95% CI 0.36 – 0.86) (Table 1; Figure 2).[33]

P. falciparum parasitaemia

Haemoglobin S—Cross-sectional studies have reported conflicting data on the prevalence of *P. falciparum* parasitaemia in asymptomatic HbAS children (Table S3). Compared with HbAA children, a lower prevalence of parasitaemia in HbAS children was reported in four studies,[41–44] similar prevalence in ten studies,[45–54] and higher prevalence in two studies.[55,56] In these surveys, parasite densities were reported in HbAS children as lower[41,46,49,56,57] or similar[45,50,52,55] to those in HbAA children. One case-control study reported similar prevalences of HbAS in parasitized (23%) and unparasitized (24%) asymptomatic children (Table 1).[20] In two prospective studies,[58,59] parasitaemia rates were similar in HbAS and HbAA children (Table 2). Taken together, HbAS does not consistently protect from *P. falciparum* parasitaemia.

Haemoglobin C—In cross-sectional surveys of adults and of children, HbC has not been associated with a reduced prevalence of *P. falciparum* parasitaemia[45–47,49,55,60] or *P. falciparum* density.[37,45,46,49,55] The incidence of asymptomatic parasitaemia did not differ between HbAC and HbAA children in Mali.[37] Thus, HbC does not appear to modify the risk of *P. falciparum* parasitaemia.

Haemoglobin E—One cross-sectional study in India reported a significantly lower prevalence of *P. falciparum* parasitaemia in patients with HbE (AE or EE) (0.6%) compared with patients with HbAA (20.5%; p = 0.005 by chi-squared test).[61]

α-thalassaemia—In cross-sectional studies, α-thalassaemia was not associated with the prevalence of parasitaemia in children[32,62–66] or, in several studies, the density of parasitaemias.[56,62–64] In one prospective study of children in Papua New Guinea, both α-thalassaemia homozygotes (IRR 0.51; 95% CI 0.32 – 0.81) and heterozygotes (IRR 0.56; 95% CI 0.36 – 0.87) had fewer episodes of PCR-detectable parasitaemia than those without α-thalassaemia,[67] though this outcome has not been investigated in other studies.

β-thalassaemia—In one cross sectional study in Liberia, *P. falciparum* prevalence was similar in children with (78%) and without (82%) β-thalassaemia.[68]

Pregnancy-associated *P. falciparum* malaria

Compared to women with HbAA, the prevalence of peripheral *P. falciparum* parasitaemia was similar in women with HbAS among Nigerian primigravidae[69] and Gabonese primi- and secundigravidae,[70] and significantly higher in Ugandan women of all gravidities (Table S4).[71] In two studies in Ghana there was no association between HbS, HbC, or α-thalassaemia and *P. falciparum* prevalence.[72] In one study in Papua New Guinea that assessed birth outcomes, α-thalassaemia was not associated with placental malaria, birth weight, placental parasite density, maternal peripheral parasitaemia, or maternal anaemia.[73] On the whole, there are few data on the effect of haemoglobin variants on pregnancy-associated malaria or placental parasitization.

P.vivax malaria: Is protection species-specific?

No studies investigated an effect of HbAS, HbAC, or HbCC on *P. vivax* infection. In a prospective study in Vanuatu, the incidence of *P. vivax* malaria was significantly increased in homozygous α-thalassaemic children less than 5 years old (IRR 0.4; 95% CI 0.40 – 0.30) and nonsignificantly increased in children greater than 5 years old (IRR 0.0; 95% CI 0.42 – 0.14) (a similar pattern of increased malaria susceptibility was reported for *P. falciparum* malaria).[39] In a cross-sectional study investigating HbE in India, *P. vivax* parasitaemia was significantly less prevalent in HbEE/AE (0.7%) than in HbAA individuals (20.1%; $p < 0.001$).[61]

DISCUSSION

Genetic polymorphisms that affect the structure and production of the β- or α-chains of haemoglobin are variously associated with protection from a range of clinical manifestations of *P. falciparum* infection. The degree of protection varies between haemoglobinopathies, but in general is greatest against severe malaria, moderate against uncomplicated malaria, and absent against asymptomatic *P. falciparum* parasitaemia. The degrees of protection against severe malaria by HbAS (91%; 95% CI 88 – 94), HbCC (73%; 95% CI 37 – 89), and homozygous α-thalassaemia (37%; 95% CI 17 – 52) compare favorably with those reported for current large-scale malaria-control efforts, including intermittent preventive antimalarial therapy in children (87% to 69%)[74,75] or infants (38%)[76] and the use of insecticide-treated bed nets (45%).[77]

HbS and to a lesser extent HbC protect from malaria but not from parasitaemia, suggesting that these haemoglobin variants prevent the transition from asymptomatic parasitaemia to malaria. This transition is poorly understood. This protective effect may derive from the abnormal display of parasite virulence factors on the surface of parasitized HbC and HbS erythrocytes,[78,79] possibly owing to the disruption of the parasite's remodeling of erythrocyte's intracellular trafficking network by HbS and HbC.[80] Additionally, the age-dependent nature of malaria protection owing to HbAS[81,82] and α-thalassaemia[83] among children in recent reports support a protective mechanism based upon an enhanced acquisition of malaria immunity. Though HbS does not generally enhance IgG responses to a diverse array of *P. falciparum* proteins,[84] HbS may yet enhance IgG responses specifically to the parasite's major cytoadherence ligand and virulence factor Plasmodium falciparum erythrocyte membrane protein (PfEMP1).[85] Additional possible mechanisms for protection owing to haemoglobinopathies include an enhanced clearance of parasitized erythrocytes,[86] impaired parasite growth,[87] or the induction of protective immunomodulatory mechanisms by parasitized erythrocytes.[88] Data supporting these various molecular mechanisms are

complex [reviewed in [89,90]], and because these possibilities are not mutually-exclusive, the relative contribution of mechanisms may vary between haemoglobinopathies. By allowing parasitization while attenuating the pathogenic mechanisms that lead to disease and fatal outcomes, haemoglobin variants offer a model system to explore the cellular events involved in causing morbidity (Panel 1).

Panel 1

Unanswered questions for future clinical and translational research

1. Does HbCC protect from uncomplicated malaria and asymptomatic parasitaemia, or only from severe falciparum malaria?
2. Does α-thalassaemia reduce the risk of disease from specific non-*Plasmodium* pathogens?
3. Do haemoglobinopathies influence the risk of uncomplicated or severe *P. vivax* malaria?
4. Do haemoglobinopathies influence the risk of pregnancy-associated malaria?
5. Do HbE and β-thalassaemia confer protection from uncomplicated or severe falciparum malaria?
6. Does α-thalassaemia exert negative epistatic effects on malaria protection by HbC and HbE?
7. Do haemoglobinopathies confer malaria protection to non-immune populations?
8. How do co-inherited G6PD deficiency variants and ABO blood groups influence the malaria-protective effects of haemoglobinopathies? 9. Does HbAS confer protection against falciparum malaria outside of sub-Saharan Africa, (e.g., India)?

The attenuation of malaria by haemoglobinopathies has important implications for non-randomised analyses of clinical malaria studies. While randomised trials may achieve balance of underlying protective polymorphisms, comparisons of non-randomised groups may be compromised by differential prevalences of haemoglobinopathies or other risk modifiers.[91] Such potential bias could impact the differential efficacy of therapies, vaccines, or other preventive measures in ecological analyses that compare populations that are not defined by randomisation and in analyses of predictors of individual-level risk. Our data endorse HbS as an important covariate in such analyses owing to its consistent protection from uncomplicated malaria (IRR 0.69; 95% CI 0.61 – 0.79), which is a common outcome in vaccine trials.[9,10]

Our review highlights several gaps in our basic understanding of how *Plasmodium* parasites cause the symptoms and life-threatening manifestations of malaria. The paucity of outcome investigations of pregnancy-associated malaria is striking, considering that this disease model has revealed fundamental mechanisms of both parasite virulence and host adaptive immunity.[92] Similarly, the effect of haemoglobinopathies on *P. vivax* parasitaemia and malaria incidence is relatively unknown despite geographic overlap in South Asia. Additionally, given the measurable incidence of severe *P. vivax* malaria,[93] case-control studies may explore associations between haemoglobinopathies and severe vivax malaria syndromes. Finally, clinical investigations have relatively neglected HbE, β-thalassaemia, and HbCC. This is surprising given the high prevalence (up to 50%) of HbE in Cambodia and HbC in parts of West Africa, as well as Haldane's 60-year-old 'malaria hypothesis' that heterozygous β-thalassaemia protects against severe and fatal falciparum malaria.[94]

Two further points merit attention. First, though our systematic review was specifically designed to assess malaria outcomes, within the identified studies we found some evidence that while HbAS conferred malaria-specific protection[22,24] α-thalassaemia protected against other mild and severe infectious syndromes, including pneumonia.[29,32] Because malaria itself may counfound the relationship between haemoglobinopathies and other infections – as recently reported for the effect of HbAS on bacteremia[95] – myriad individual and epidemiologic factors could account for this difference, in addition to biological differences in the mechanisms of protection. The identification of these mechanisms may be aided if this phenomenon is confirmed by future clinical studies or meta-analyses. Second, the dissimilarity of estimates from prospective studies of the risk of uncomplicated falciparum malaria in homozygous α-thalassaemic children is striking, with significantly increased risk on the southwestern Pacific island of Vanuatu[39] but either slightly decreased or unchanged risk in Africa and Papua New Guinea (Table 2).[31,35,40,67,96] Other data have suggested an increased Plasmodium prevalence in homozygous α-thalassaemics in Papua New Guinea,[97] underscoring that haemoglobinopathies may have variable effects in different settings upon different outcomes. Future studies are needed to more definitively characterize these effects and define their relationship with host genetics, malaria epidemiology, and acquired immunity to malaria.

This systematic review is subject to several limitations. We may have failed to identify relevant studies, though the independent selection of studies by two independent reviewers who each assessed over 2600 studies suggests adequate identification. Secondly, risk estimates for malaria may be influenced by unmeasured or unreported host factors, such as G6PD deficiency and ABO blood groups. Nevertheless, heterogeneity was low for most meta-analyzed comparisons, suggesting a consistent effect of haemoglobinopathies upon malaria risk. Finally, the clinical epidemiology of malaria results from poorly-understood interactions between host, parasite, and environmental factors which vary between included studies. We therefore employed random-effects meta-analysis models, and heterogeneity in risk estimates was generally low.

Despite previous successes in exploiting innate malaria protective-factors to investigate malaria pathogenesis, recent reports highlight the complexity of the co-evolution of host and parasite. *P. vivax* infection is now recognized in Malagasy individuals who lack DARC expression on their erythrocytes that were previously thought to be resistant to vivax malaria,[98] suggesting alternate erythrocyte invasion pathways. Additionally, a-thalassaemia can attenuate the malaria-protective effect of HbAS when co-inherited,[23] emphasizing the need to integrate investigations of genetic resistance. Nevertheless, by attenuating the virulence of malaria parasites, haemoglobinopathies offer an attractive “natural experiment” to help elucidate malaria’s pathogenic mechanisms and potentially translate models of pathogenesis and immunity into clinical application.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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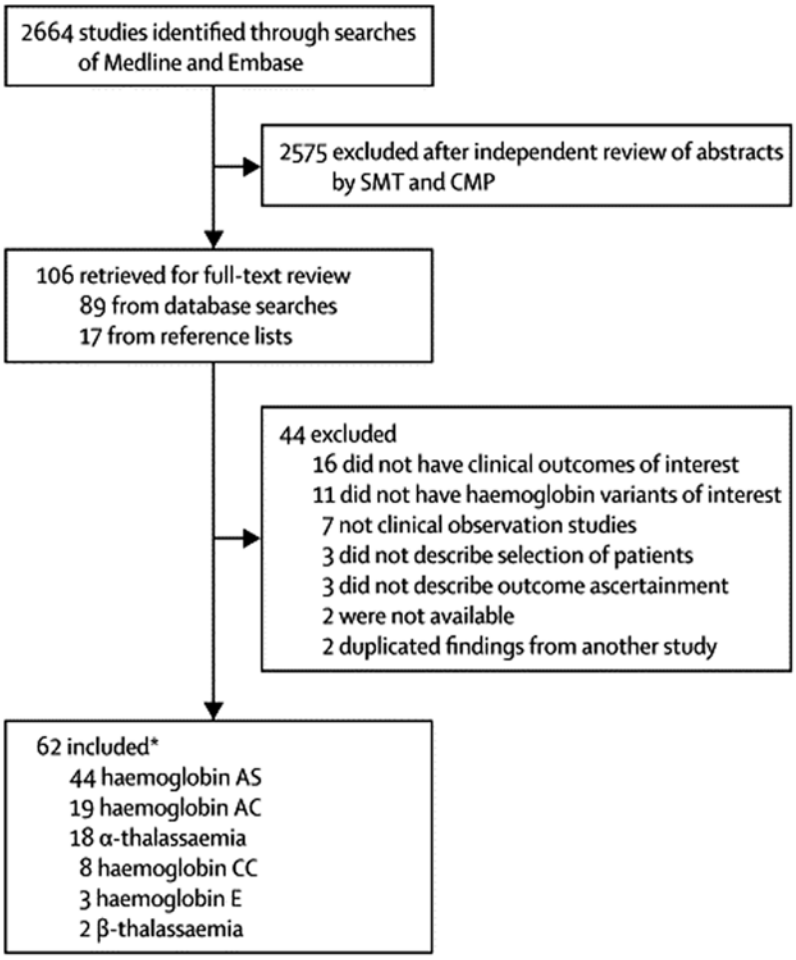

Figure 1.
Study identification flowchart

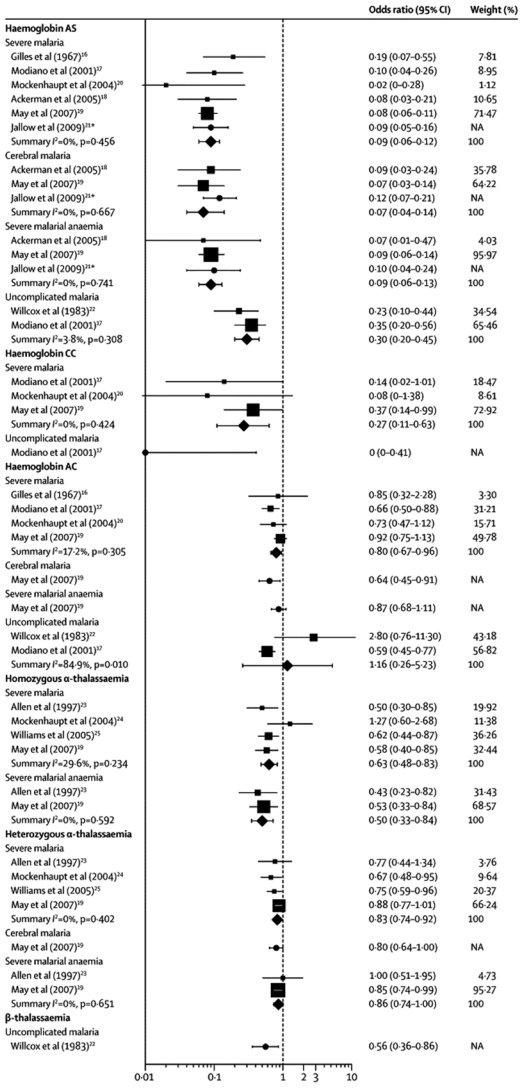

Figure 2.

Unadjusted individual and summary Odds Ratios for specific malaria syndromes by haemoglobin variants

Abbreviations: CI, confidence interval; Hb, haemoglobin

[a] Not included in meta-analyses because prevalences of HbAS in cases and controls were not reported. All Odds Ratios (ORs) are for comparison with healthy controls. The data markers represent either ORs from individual studies (circles) or summary ORs for subgroup data (diamonds) that were generated by random-effects meta-analysis of individual studies (squares). For individual studies included in meta-analyses, the size of the square data marker is relative to the weight of the study. The I^2 statistic is a measure of the heterogeneity of the individual study estimates which were meta-analyzed, and was calculated using the Mantel-Haenszel method. ORs for individual studies may differ from those in Table S1 or in the original published studies because they were calculated from raw data and are thus unadjusted.

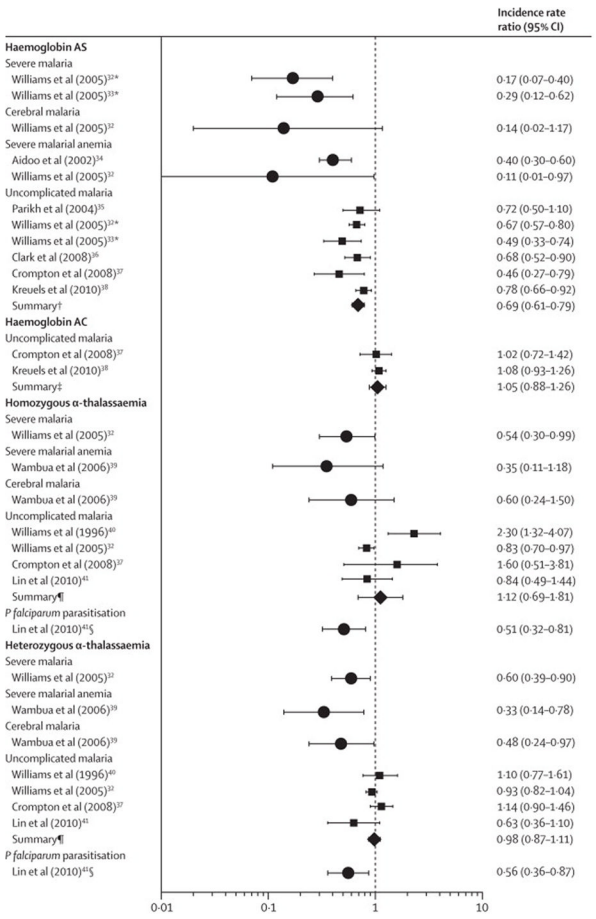

Figure 3.

Individual and summary incidence rate ratios of *P. falciparum* syndromes in prospective studies of children with haemoglobinopathies

Abbreviations: CI, confidence interval; Hb, haemoglobin. All incidence rate ratios (IRRs) compare patients with the variant listed to patients with either HbAA or the αα/αα genotype. Summary measures (diamonds) were computed using random-effects Poisson meta-regression of individual studies (squares).

[a] Studies had overlapping cohorts. Because the cohort in Williams et al (2005)[23] subsumes that of Williams et al (2005),[24] only data from Williams et al (2005)[23] was included in the meta-analyzed summary IRR for uncomplicated malaria in HbAS children.

[b] Summary IRR of uncomplicated malaria in HbAS compared with HbAA children from five studies.[23,34-37]

[c] Summary IRR of uncomplicated malaria in HbAC compared with HbAA children from two studies.[35,37]

[d] Summary IRR of uncomplicated malaria in homozygous or heterozygous α-thalassaemic compared with non-thalassaemic children from three studies [Williams 1996, Williams 2005 (NG), Crompton 2008].[23,35,39]

[e] Detected by polymerase chain reaction.

Table 1

Case-control studies investigating the effect of haemoglobinopathies on *P. falciparum* malaria

Source	Location	Syndrome[a]	No. patients	Age	Comment	% Haemoglobin opathy
Haemoglobin AS						
Gilles et al (1967)[16]	Nigeria	Severe malaria	100	Range 6m - 4y		4.0
		Healthy	100	Range 6m - 4y	Aparasitaemic outpatients	18.0
Willcox et al (1983)[33]	Liberia	Uncomplicated malaria	518	Range 2m - >15y	Outpatients	1.9
		Healthy	1027	Range 2m - >15y	Community surveys	7.9
Hill et al (1991)[22]	The Gambia	Severe malaria	619	Median 3.0y Range <10y		1.2
		Severe non-malaria illness	332	Median 2.2y Range <10y	Inpatients with severe non- malaria illness	10.9
		Mild non-malaria illness	510	Median 1.9y Range <10y	Mild outpatient illness without malaria parasites	12.9
		Uncomplicated malaria	354	Median 3.0y Range <10y		2.8
Agarwal et al (2000)[21]	Mali	Severe malaria	67	Mean 4.3y		4.5
		Cerebral malaria	34	Mean 3.8y	Subset of severe malaria patients with coma, obtundation, or convulsions	5.9
		Severe malarial anaemia	12	Mean 1.0y	Subset of severe malaria patients with Hct <15%	0
		Uncomplicated malaria	391	Mean 9.1y		2.7
Modiano et al (2001)[17]	Burkina Faso	Severe malaria	359	Mean 4y Range 0.5 -15y		1.1
		Uncomplicated malaria	476	Mean 4y Range 0.5 -15y		4.0
		Healthy	3513	Mean 11y Range 1- 40y	Community cross-sectional survey	9.5
Mockenhaupt et al (2004)[20]	Ghana	Severe malaria	290	Median 24m Range 6 - 102m		0
		Healthy, parasitaemic	290	NA	Community cross-sectional survey, matched to severe cases	7.9
		Healthy aparasitaemic	290	NA	Community cross-sectional survey, matched to severe cases	8.3

Source	Location	Syndrome[a]	No. patients	Age	Comment	% Haemoglobin opathy
Ackerman et al (2005)[18]	The Gambia	Severe malaria	315	NA		1.1
		Cerebral malaria	235	NA	Subset of severe malaria patients with BCS <3	1.3
		Severe malarial anaemia	80	NA	Subset of severe malaria patients with Hb <5g/dL	0.6
		Newborns	583	NA	Cord blood specimens	8.7
May et al (2007)[19]	Ghana	Severe malaria	2591	Median 20m		1.4
		Cerebral malaria	581	NA	Subset of severe malaria patients with BCS <3	1.2
		Severe malarial anaemia	1649	NA	Subset of severe malaria patients with Hb <5g/dL	1.6
		Healthy	2048	Median 30m	Community cross-sectional survey, matched to severe cases	14.8
Jallow et al (2009)[99]	The Gambia	Severe malaria	1060	NA		NA
		Cerebral malaria	869	NA	Subset of severe malaria patients with BCS <3	NA
		Severe malarial anaemia	318	NA	Subset of severe malaria patients with Hb <5g/dL	NA
		Newborns	1500	NA	Cord blood specimens	8.0
Haemoglobin C						
Gilles et al (1967)[16]	Nigeria	Severe malaria	100	Range 6m - 4y		He: 6.0
		Healthy	200	Range 6m - 4y	Clinic attendance, aparasitaemic	He: 7.0
Willcox et al (1983)[33]	Liberia	Uncomplicated malaria	518	Range 2m - >15y		He: 1.3
		Healthy	1027	Range 2m - >15y	Community surveys	He: 0.4
Guinet et al (1997)[26]	Mali	Severe malaria	110	Range 6m - 9y		10.0
		Severe malarial anaemia	6	Range 6m - 9y	Subset of severe malaria patients with Hct <15% or Hb <5g/dL	0
		Uncomplicated malaria	55	Range 6m - 9y	Matched to severe cases	9.0
Agarwal et al (2000)[21]	Mali	Severe malaria	67	Mean 4.3y		Ho: 0 He: 4.5
		Cerebral malaria	34	Mean 3.8y	Subset of severe malaria patients with coma, obtundation, or convulsions	Ho: 0 He: 2.9
		Severe malarial anaemia	12	Mean 1.0y	Subset of severe malaria patients with Hct <15%	Ho: 0 He: 0
		Uncomplicated malaria	391	Mean 9.1y		Ho: 1.5 He: 15.9
Modiano et al (2001)[17]	Burkina Faso	Severe malaria	359	Mean 4y		Ho: 0.3 He: 17.6

Source	Location	Syndrome[a]	No. patients	Age	Comment	% Haemoglobin opathy
				Range 0.5 – 15y		
		Uncomplicated malaria	476	Mean 4y Range 0.5 – 15y		Ho: 0 He: 15.6
		Healthy	3513	Mean 11y Range 1- 40y	Community cross-sectional survey	Ho: 1.7 He: 21.7
Mockenhaupt et al (2004)[20]	Ghana	Severe malaria	290	Median 24m Range 6 – 102m		Ho: 0 He: 16.6
		Healthy, parasitaemic	290	NA	Community cross-sectional survey, matched to severe cases	Ho: 1.0 He: 24.5
		Healthy, aparasitaemic	290	NA	Community cross-sectional survey, matched to severe cases	Ho: 1.7 He: 19.0
May et al (2007)[19]	Ghana	Severe malaria	2591	Median 20m		Ho: 0.2 He: 9.4
		Cerebral malaria	581	NA	Subset of severe malaria patients with BCS <3	Ho: NA He: 7.2
		Severe malarial anaemia	1649	NA	Subset of severe malaria patients with Hb <5g/dL	Ho: NA He: 9.2
		Healthy	2048	Median 30m	Community cross-sectional survey, matched to severe cases	Ho: 0.5 He: 8.7
Haemoglobin E						
Oo et al (1995)[27]	Myanmar	Severe malaria	200	19 - 45y		28.0
		Uncomplicated malaria	109	19 - 45y	Hospitalized	27.5
Hutagalung et al (1999)[28]	Thailand	Severe malaria	33	14 - 45y		3.0
		Uncomplicated malaria	184	14 - 45y	Hospitalized	22.3
α-thalassaemia						
Allen et al (1997)[29]	Papua New Guinea	Severe malaria	249	NA		Ho: 44.6 He: 37.3
		Severe malarial anaemia	155	Median 2.1y	Subset of severe malaria patients with Hb <5g/dL	Ho: 36.1 He: 43.9
		Healthy	249	NA	Community survey, matched to severe cases	Ho: 57.0 He: 31.3
Lell et al (1999)[100]	Gabon	Severe malaria	100	Mean 44m		Ho: 10.0 He: 37.0
		Uncomplicated malaria	100	Mean 44m		Ho: 10.0 He: 43.0

Source	Location	Syndrome[a]	No. patients	Age	Comment	% Haemoglobin opathy
Mockenhaupt et al (2004)[30]	Ghana	Severe malaria	261	<5y		Ho: 5.0 He: 23.7
		Healthy, parasitaemic	614	<5y	Community cross-sectional survey, matched to severe cases	Ho: 3.3 He: 33.2
		Healthy, aparasitaemic	479	<5y	Community cross-sectional survey, matched to severe cases	Ho: 3.6 He: 31.9
Williams et al (2005)[31]	Kenya	Severe malaria	655	NA		Ho: 12.7 He: 47.0
		Fatal malaria	72	NA	Death in hospital	Ho: 8.4 He: 10.4
		Healthy	648	<7y	Community survey, matched	Ho: 16.7 He: 50.6
May et al (2007)[19]	Ghana	Severe malaria	2591	Median 20m		Ho: 2.0 He: 25.2
		Cerebral malaria	581	NA	Subset of severe malaria patients with BCS <3	Ho: 2.2 He: 23.8
		Severe malarial anaemia	1649	NA	Subset of severe malaria patients with Hb <5g/dL	Ho: 1.8 He: 24.7
		Healthy	2048	Median 30m	Community cross-sectional survey, matched to severe cases	Ho: 2.0 He: 27.3
β-thalassaemia						
Willcox et al (1983)[33]	Liberia	Uncomplicated malaria	526	2m - >15y		5.7
		Healthy	1132	2m - >15y	Community surveys	9.7

Abbreviations: Hb: haemoglobin; NA: data not available; Ho: homozygote; He: heterozygote; BCS: Blantyre Coma Score; Hct: hematocrit.

[a]Unless otherwise stated, patients with severe malaria, severe malarial anaemia, cerebral malaria and uncomplicated malaria met definitions described in the Methods section. Healthy patients were recruited as described in the Comments column.

Table 2

Prospective studies investigating the effect of haemoglobinopathies on *P. falciparum* malaria

Source	Outcome	Location	n	Ages	Incidence in unexposed[a]	Incidence Rate Ratio (95% C.I.)
Haemoglobin AS						
Williams et al (2005)[23,b]	Severe malaria	Kenya	2655	< 5y	0.022/y	0.17 (0.07 – 0.40)
Williams et al (2005)[24,b]	Severe malaria	Kenya	2104	< 5y	0.020/y	0.29 (0.12 – 0.62)
Williams et al (2005)[23]	Cerebral malaria	Kenya	2655	< 5y	0.004/y	0.14 (0.02 – 1.17)
Aidoo et al (2002)[25]	Severe malaria anaemia	Kenya	1022	< 5y	0.048/y	0.40 (0.30 – 0.60)
Williams et al (2005)[23]	Severe malarial anaemia	Kenya	2655	< 5y	0.005/y	0.11 (0.01 – 0.97)
Parikh et al (2004)[34]	Uncomplicated malaria	Uganda	307	6m – 5y	2.04/y	0.72 (0.5 – 1.1)
Williams et al (2005)[23,b]	Uncomplicated malaria	Kenya	370	< 11y	2.36/y	0.67 (0.57 – 0.80)
Williams et al (2005)[24,b]	Uncomplicated malaria	Kenya	323	< 8y	3.06/y	0.49 (0.33 – 0.74)
Migot-Nabias et al (2006)[96]	Uncomplicated malaria	Senegal	169	2 – 10y	NA	Reduced incidence in HbAS (IRR not reported)
Clark et al (2008)[36]	Uncomplicated malaria3	Uganda	558	1 – 10y	0.82/y	0.68 (0.52 – 0.90)
Crompton et al (2008)[35]	Uncomplicated malaria3	Mali	176	2 – 10y	1.76/y	0.46 (0.27 – 0.79)
Enevold et al (2008)[40]	Uncomplicated malaria	Tanzania	159	6m – 19y	35%/6m	RR: 1.55 (0.51 – 4.77)
Kreuels et al (2010)[37]	Uncomplicated malaria3	Ghana	852	3m – 2y	1.2/y	0.78 (0.66 – 0.92)
Summary[23,34-37]	*Uncomplicated malaria*					*0.69 (0.61 – 0.79)*
Le Hesran et al (1999)[59]	*P. falciparum* parasitaemia	Cameroon	240	< 5y	NA	No difference (IRR not reported)
Stirnadel et al (1999)[58]	*P. falciparum* parasitaemia	Tanzania	204	< 1y	NA	No difference (IRR not reported)
Haemoglobin C						
Rihet et al (2004)[101]	Uncomplicated malaria3	Mali	256	1 – 24y	NA	Reduced incidence with HbC (IRR not reported)
Crompton et al (2008)[35]	Uncomplicated malaria3	Mali	176	2 – 10y	1.76/y	He: 1.02 (0.72 – 1.42)
Kreuels et al (2010)[37]	Uncomplicated malaria3	Ghana	852	3m – 2y	1.2/y	He: 1.08 (0.93 – 1.26)
Summary[35,37]	*Uncomplicated malaria*					*He: 1.05 (0.88 – 1.26)*
α-thalassaemia						
Williams et al (2005)[31]	Severe malaria	Kenya	2104	< 5y	0.061/y	Ho: 0.54 (0.30 – 0.99) He: 0.60 (0.39 – 0.90)
Wambua et al (2006)[32]	Severe malarial anaemia	Kenya	2104	< 5y	0.010/y	Ho: 0.35 (0.11 – 1.18)

Source	Outcome	Location	n	Ages	Incidence in unexposed[a]	Incidence Rate Ratio (95% C.I.)
						He: 0.33 (0.14 – 0.78)
Wambua et al (2006)[32]	Cerebral malaria	Kenya	2104	< 5y	0.002/y	Ho: 0.60 (0.24 – 1.50) He: 0.48 (0.24 – 0.97)
Williams et al (1996)[39]	Uncomplicated malaria3	Vanuatu	544	< 5y	0.7/y	Ho: 2.30 (1.32 – 4.07) He: 1.10 (0.77 – 1.61)
Williams et al (2005)[31]	Uncomplicated malaria	Kenya	370	< 11y	2.46/y	Ho: 0.83 (0.70 – 0.97) He: 0.93 (0.82 – 1.04)
Migot-Nabias et al (2006)[96]	Uncomplicated malaria3	Senegal	169	2 – 10y	NA	No difference (IRR not reported)
Enevold et al (2008)[40]	Uncomplicated malaria	Tanzania	159	6m – 19y	45%/6m	Ho: RR 0.12 (0.02 – 0.83) He: RR 0.30 (0.10 – 0.85)
Crompton et al (2008)[35]	Uncomplicated malaria3	Mali	176	2 – 10y	1.76/y	Ho: 1.60 (0.51 – 3.81) He: 1.14 (0.90 – 1.46)
Lin et al (2010)[67]	Uncomplicated malaria3	Papua New Guinea	206	5 – 14y	NA	Ho: 0.84 (0.49 – 1.44) He: 0.63 (0.36 – 1.10)
Summary[23,35,39]	*Uncomplicated malaria*					*Ho: 1.12 (0.69 – 1.81)* *He: 0.98 (0.87 – 1.11)*
Lin et al (2010)[67]	*P. falciparum* parasitaemia (PCR)	Papua New Guinea	206	5 – 14y	NA	Ho: 0.51 (0.32 – 0.81) He: 0.56 (0.36 – 0.87)

Abbreviations: NA: data not available; IRR: incidence rate ratio; RR: risk ratio; Ho: homozygote; He: heterozygote; PCR: polymerase chain reaction. No prospective studies investigated the effects of HbE or β-thalassaemia on malaria.

[a]Annual incidence rate in the unexposed population (i.e., HbAA or αα/αα genotype) of the indicated outcome.

[b]Studies with overlapping cohorts. Because the cohort in Williams et al (2005)[23] subsumes that of Williams et al (2005),[24] only data from Williams et al (2005)[23] was included in the meta-analyzed summary IRR for uncomplicated malaria in HbAS children.