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Am J Hematol. Author manuscript; available in PMC 2025 January 01.

Published in final edited form as:
Am J Hematol. 2024 January ; 99(1): 113–123. doi:10.1002/ajh.27149.

Sickle cell allele *HBB*-rs334(T) is associated with decreased risk of childhood Burkitt lymphoma in East Africa

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SMM, MDO, SJR, and PK conceived the idea, designed the study and supervised fieldwork. IDL, IO, PK, HN, MDO, KB, SJR, CNT, PAW, RTK, WNW, NM, EK, TK, HD, MM, NM, EB, GNL, SK, NM, CM, EMM, RN, and CG conducted and monitored field work. MM, TPL, AH, MY, XW, FB, TO, SLT, WL, BH, and NC conducted genetic testing and processed genetic data. MHG and HGH analyzed genetic data and developed primary and sensitivity models; AAA, CNR, and WTJ advised on statistical or genetic analyses; HGH completed statistical analyses; JW performed literature review and prepared figures and tables; JZ reviewed and formatted tables; KB, JJG, LPO, JFF, SJC contributed to the design, analysis, and interpretation of data. All authors read and approved the final manuscript.

Ethical approvals and patient consent
Ethical approvals for the EMBLEM study were granted by the Uganda Virus Research Institute (GC/127), Uganda National Council for Science and Technology (H816), Tanzania National Institute for Medical Research (NIMR/HQ/R.8c/Vol. IX/1023), Moi University/Moi Teaching and Referral Hospital (000536), and National Cancer Institute (10-C-N133). Ethical approvals for the Childhood Infections and Cancer study were granted by Malawi College of Medicine (P.03/04/277R), Oxford University, and National Health Sciences Research Committee (2405). Guardians of participants provided written informed consent. Children aged 7 years old gave written assent.

Permission to reproduce material from other sources: not applicable.

Clinical trial registration. Not applicable.

DISCLOSURE OF CONFLICT OF INTEREST
None declared

Conflict of interest: The authors declare no competing financial interests.

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Abstract

Burkitt lymphoma (BL) is an aggressive B-cell lymphoma that significantly contributes to childhood cancer burden in sub-Saharan Africa. *Plasmodium (P.) falciparum*, which causes malaria, is geographically associated with BL, but the evidence remains insufficient for causal inference. Inference could be strengthened by demonstrating that mendelian genes known to protect against malaria —such as the sickle cell trait variant, *HBB*-rs334(T)—also protect against BL. We investigated this hypothesis among 800 BL cases and 3,845 controls in four East African countries using genome-scan data to detect polymorphisms in 22 genes known to affect malaria risk. We fit generalized linear mixed models to estimate odds ratios (OR) and 95% confidence intervals (95% CI), controlling for age, sex, country, and ancestry. The ORs of the loci with BL and *P. falciparum* infection among controls were correlated (Spearman's $\rho = 0.37$, $p = 0.039$). *HBB*-rs334(T) was associated with lower *P. falciparum* infection risk among controls (OR = 0.752, 95% CI 0.628–0.9; p = 0.00189) and BL risk (OR=0.687, 95% CI 0.533–0.885; p=0.0037). *ABO*-rs8176703(T) was associated with decreased risk of BL (OR=0.591, 95% CI 0.379–0.992; p=0.00271), but not of *P. falciparum* infection. Our results increase support for the etiological correlation between *P. falciparum* and BL risk.

Graphical Abstract

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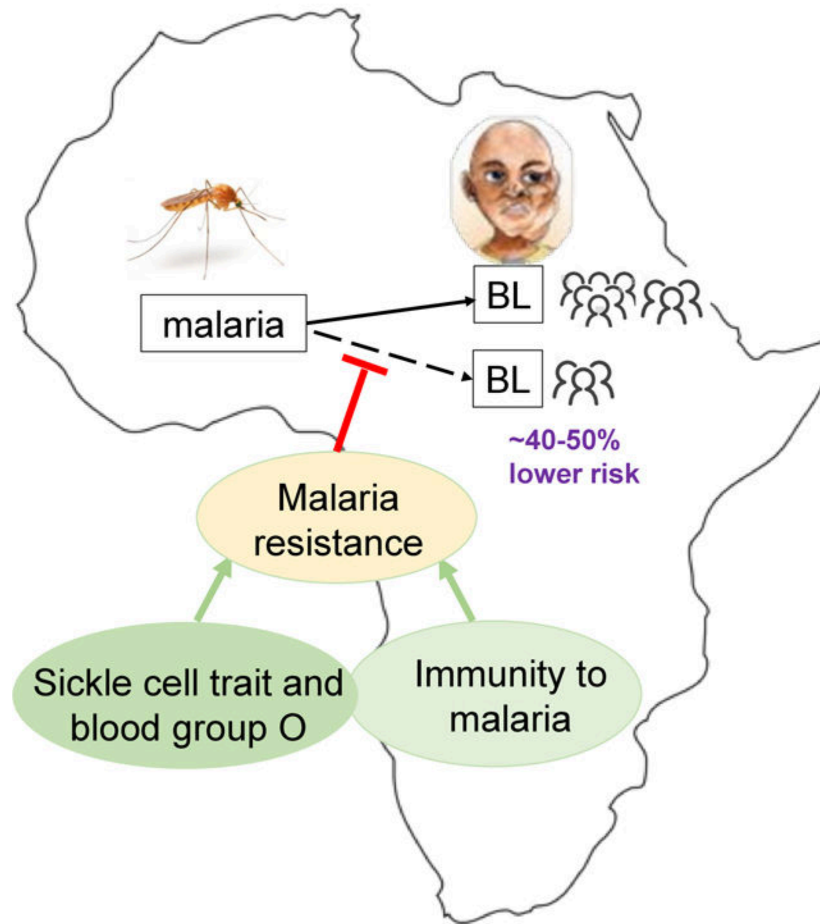

The association between Burkitt lymphoma (BL) with malaria resistance was analyzed in a case-control study conducted in children from four countries in Africa. Results indicate that the odds of BL were lower in children with genetic resistance due to the sickle cell trait or a variant linked to blood group O and apparent immunity indicated by loa grade asymptomatic infection.

Keywords

Plasmodium falciparum ; Burkitt lymphoma; non-Hodgkin lymphoma; malaria resistance loci; East Africa

INTRODUCTION

Burkitt lymphoma (BL) is a B-cell lymphoma that occurs worldwide, but with a marked excess in sub-Saharan African (SSA) children in whom it is one of the leading cancers [1]. This geographic pattern, which mirrors that of *Plasmodium (P.) falciparum* infection[2], which causes the deadliest malaria, suggested a possible causal role of malaria in BL. This hypothesis is supported by case-control associations of BL with serological [3, 4] [5, 6] and molecular markers of *P. falciparum* infection [7, 8]. It is also supported by immunological data indicating that *P. falciparum* infection impairs control of Epstein-Barr virus (EBV) [9],

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a known oncogenic factor for BL [10, 11]. EBV and *P. falciparum* parasites induce activation induced cytosine deaminase (AID), an enzyme that is involved in somatic hypermutation and *IG*::*MYC* translocation, genomic instability considered an early or primary abnormality in BL [11, 12]. However, this evidence remains insufficient for causal inference [13, 14].

A pathogenic variant in the β-hemoglobin gene, *HBB*-rs334 (Glu6Val), which causes the sickle cell disease in homozygotes, is associated with a 90% decrease in risk for severe malaria in heterozygotes (carriers), i.e., those with sickle cell trait (SCT) [15]. Demonstration that SCT—or others malaria protective loci— also protect against BL could strengthen inferences about the causal role of malaria in BL. SCT was associated with a reduced risk of BL in three studies [16–18] but not in three others [19–21]. All of these studies were small, lacked data to control for genetic ancestry [22], and did not evaluate effects of other loci known to affect malaria risk [23].

In 2017, we tested the SCT-BL hypothesis in the Epidemiology of Burkitt Lymphoma in East-African Children and Minors (EMBLEM) study in Uganda [24] using a larger sample (200 BL cases and 800 controls) and controlling for ancestry using genetic data. We found significant protection against BL among those with SCT, as well as suggestive protective associations with *IL10*-rs1800896(C), *IL1A*-rs2856838(A), and *SEMA3C*-rs4461841(C) [24]. Here, we expanded that analysis to 800 BL cases and 3,845 BL-free controls in Uganda, Tanzania, Kenya, and Malawi to evaluate associations of malaria loci with BL. In addition to SCT, we evaluated 31 other loci in 22 malaria-related genes. We included $\alpha^{3.7}$-thalassemia because co-inheritance of $\alpha^{3.7}$-thalassemia with SCT has been previously found to affect SCT phenotype, including malaria resistance [25], osmolality, and hemoglobin S (HbS) percentage [26]. We also controlled for contemporaneous asymptomatic *P. falciparum* detection, which we previously found to have an inverse association with BL, independent from prior history of and treatment for malaria [27, 28]. Asymptomatic *P. falciparum* detection indicates that naturally acquired immunity (NAI) is sufficient to control malaria symptoms but not to prevent infection [29, 30]. Thus, asymptomatic *P. falciparum* is an indicator of NAI. We tested whether contemporaneous asymptomatic *P. falciparum* infection (or NAI) was protective against BL when controlling for the identified genetic associations. We hypothesized that in children with basic NAI (i.e., sufficient to prevent symptomatic malaria [29, 30]), there exists another threshold above which NAI reduces *P. falciparum* infection to levels below the threshold sufficient to increase the risk of BL.

Methods

Study population

Our study was conducted using samples from the EMBLEM study conducted in Uganda, Tanzania, and Kenya [27], and from the Childhood and Infections Cancer study conducted in Malawi [4]. Briefly, the EMBLEM study was conducted in six geographical areas (Figure 1a). The average child in these areas' experiences 100–400 *P. falciparum* infections per year and a 39% risk of BL per 100 *P. falciparum* infections versus those with 50 lifetime infections [31]. For both BL cases and controls, eligibility was limited to usual residents (4 months prior to enrollment) of a village in the defined study areas who were aged 0–15 years. Eligible BL cases were enrolled at six participating hospitals with capacity to

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diagnose and treat BL. BL diagnosis was confirmed by local histology/cytology, whenever possible, or by clinical diagnosis, with supporting imaging and laboratory results for those lacking histology or cytology. The controls were a random sample of apparently healthy community children residing in 295 villages that were randomly selected from a list of all villages in the study areas constructed from national census files [27]. The primary controls were selected to match the age and sex frequency of BL cases diagnosed in their area over the past 10 years before the current study [27]. Other community controls were enrolled using household survey methods without any frequency-matching in 12 villages in Uganda (i.e., all children in the selected households who were eligible were enrolled) [32]. A small set of health-facility controls were enrolled from the same 12 villages in Uganda. These controls were selected to match the age and sex frequency of BL cases diagnosed in their area over the past 10 years before the current study.

In Malawi, participants were enrolled at Queen Elizabeth hospital, a tertiary cancer care facility in Blantyre [4]. Children with BL were designated as cases, while children with non-BL cancers were used as controls. No matching on age or sex was performed as was done in EMBLEM. In both studies (EMBLEM and Malawi), pre-treatment venous blood was collected in EDTA tubes at enrollment. In EMBLEM, the samples were processed into plasma and buffy coats, then stored at −80°C until shipment to the National Cancer Institute (NCI). In Malawi, samples were stored as whole blood at −20°C until shipment to NCI. In EMBLEM, *P. falciparum* was detected using microscopy of Giemsa stained thick-film blood smears and antigen capture rapid diagnostic tests (RDTs) [27]. In Malawi, *P. falciparum* was detected using *P. falciparum*-specific polymerase chain reaction (PCR) [8].

Ethical Approvals

Ethical approvals for the EMBLEM study were granted by the Uganda Virus Research Institute (GC/127), Uganda National Council for Science and Technology (H816), Tanzania National Institute for Medical Research (NIMR/HQ/R.8c/Vol. IX/1023), Moi University/Moi Teaching and Referral Hospital (000536), and National Cancer Institute (10-C-N133). Ethical approvals for the Childhood Infections and Cancer study were granted by Malawi College of Medicine (P.03/04/277R), Oxford University, and National Health Sciences Research Committee (2405). Guardians of participants provided written informed consent. Children aged 7 years old gave written assent. Ethical approval for genetic research was granted primarily to establish whether BL is a phenotype associated with malaria, in order to strengthen evidence efforts to control BL by targeting malaria before agnostically searching for other genetic factors associated with BL.

Selection and genotyping of malaria loci

DNA extracted from buffy coat aliquots using the Qiagen QIAsymphony automated instrument were genotyped at approximately 4.6 million single nucleotide positions (SNPs) using the Infinium Omni5Exome-4 v1.3 BeadChip (Illumina, San Diego, CA, USA) at the NCI Cancer Genomics Research (CGR) Laboratory (Figure S1). We used standard genome-wide association study quality control pipelines, including sample completion call rate >0.95; contamination rate <0.1; exclusion of unexpected, duplicated samples; and SNPs with less than a 95% completion rate. Phased genome scan data were imputed using the

African Genome Resources panel with 6 230 African genomes on the Sanger imputation server (https://imputation.sanger.ac.uk/). We retained SNPs imputed with high confidence (INFO 0.9) and with a minor allele frequency (MAF) 0.01, and those passing the Hardy-Weinberg equilibrium tests in the controls $p<10^{-6}$. We performed technical validation of the Illumina chip results for thirteen SNPs, including the classical *HBB*-rs334 for SCT, in 900 samples with 100% concordance [24]. We genotyped copy numbers of *HBA* 3.7 kb deletions using droplet digital polymerase chain reaction (ddPCR) (Bio-Rad QX200 ddPCR system) (Figure S2).

Statistical analysis

After QC filtering, we had an analytic set with 800 BL cases and 3,845 controls (Figure 1b). We analyzed associations between BL with 32 loci in 22 genes known to affect malaria risk (Figure 1c). We excluded seven loci that did not pass the quality control metrics mentioned above (Table S1). The analyzed loci included the classical SCT allele (*HBB*-rs334(T), which is known to reduce malaria risk [33] and previously shown to reduce BL risk in four [16–18, 24] of seven studies [16–21, 24]. We reported *HBB*-rs334 results according to the minus strand (i.e., sickle mutation is A to T) following the practice in sickle cell disease scientific literature.

We calculated principal components (PC) using 787,731 genotyped uncorrelated ($r^2 < 0.3$) SNPs outside the *HLA* region to assess ancestry patterns. The PCs showed extensive population substructure among participants across the four countries (Figure S3), with minimal relatedness in controls from Kenya and Tanzania but a high degree of relatedness in the Uganda controls (Figure S4 and Figure S5). Only two related BL cases, previously reported in Uganda [34], were identified. We used country-specific PCs and a genetic relationship matrix (GRM), based on the probability that two individuals i and j share 0, 1, or 2 alleles identical by descent (IBD) [35], to adjust for ancestry. We fit a generalized linear mixed model, controlling for sex, age, *P. falciparum* detection, country, and ancestry as fixed effects, and GRM as a random variable. *P. falciparum* was included in the models as an indicator for current *P. falciparum* exposure with NAI (detection of infection). Statistical significance for the loci was based on two-sided Wald tests with a p-value < 0.05 considered significant to detect concordant effects of the malaria loci against BL. We performed sensitivity analyses to assess robustness of effects. In Sensitivity 1, we adjusted for loci significantly associated with BL; In sensitivity 2, we excluded 24 participants with sickle cell disease *HBB*-rs334(TT). Additionally, we ran analyses when dropping country, ancestry, and *P. falciparum* detection (Sensitivity 3–5) from the primary model. We examined the independent contribution of *P. falciparum* detection, which is an indicator of NAI, by comparing unrestricted models (with *P. falciparum*) to restricted models (without *P. falciparum*) using likelihood ratio tests. Because co-inheritance of $\alpha^{3.7}$ thalassemia with *HBB*-rs334(T) modifies the phenotype of SCT [25], we analyzed the associations between BL and $\alpha^{3.7}$ thalassemia genotypes stratified by *HBB*-rs334 genotypes (AA versus T/A).

We leveraged available genome scan data to explore associations between BL and 2,735 loci in the 22 genes with linked malaria (Figure 1c; Table S1). We applied a modified Bonferroni correction based on the effective degrees of freedom as previously described

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to assess statistical significance [36] (see Supplementary methods and Table S2). Analyses were performed using computational resources of the NIH HPC Biowulf cluster (http://hpc.nih.gov).

RESULTS

Study participants' characteristics are shown in Table 1. The median age was 7 years for both BL cases and controls, with similar distributions (Figure S6); 64% of the participants were 6 years or older. The BL cases in the analytic set included 592 (74%) who were confirmed by local histology or cytology and 208 (26%) based on clinical diagnosis, usually with a consistent opinion from a fine needle aspirate read by local clinicians. Compared to the histologically diagnosed cases, those clinically diagnosed were more likely to be younger (6.5 years versus 7.4 years, p=0.0009), more likely to report a history of fever other than malaria in the past 6 months (attributed to BL symptoms: 63% versus 42%, p<0.001), and to report a history of treatment for outpatient malaria in the past 12 months (54% versus 40%, p=0.001), attributed to less well developed immunity to malaria due to their younger age (Table S3). However, no differences were observed by sex, presence of current fever, or history of fever due to malaria in the past 6 months or 12 months, and history of admission for severe malaria. There were more males among BL cases than among the controls (63.2% versus 52.1%, *p*<0.0001). HIV status was known for 770 (96.2%) of 800 BL cases and unknown for 30 (3.8%). The BL cases with HIV resembled those without regarding sex, mean age, having current fever or histories of fever in the past 12 months, fever due to malaria or unelated to malaria in the past 6 months, and history of outpatient treatment for malaria (Table S4). However, compared to those who were HIV-negative, HIV positive BL cases had a lower prevalence of asymptomatic *P. falciparum* infection (12% versus 37%, p=0.013) and were more likely to report an admission in the past 12 months when compared to HIV-negative BL cases (26% versus 9%, p=0.029). As previously reported [27], HIV infection was associated with being a BL case (OR~4), but the number of HIV positive participants was small [24 BL cases, 15 controls (Table S5)] and their impact on the overall findings was considered negligible. BL cases tended to live in areas likely to have heavier *P. falciparum* exposure, such as rural villages or villages close to surface water (i.e., <500m) (Table S1). Despite this heightened exposure, and consistent with our earlier findings [27], BL cases were less likely to have asymptomatic *P. falciparum* detection than controls (282 [32.5%] versus 1 857 [48.3%], *p*<0.0001) (Table 1). When *P. falciparum* was detected among EMBLEM participants (but not in Malawi, which used PCR rather than blood microscopy/RDT), BL cases displayed lower geometric parasite density compared to their control counterparts (Table S5). Similarly, EMBLEM BL cases were less likely than controls to report a fever that was diagnosed as or treated as malaria in the past 6 months before enrollment (Table S5). Also of relevance and arguing against poor recall of malaria-related fevers, EMBLEM BL cases were more likely than controls to report fever that was diagnosed as due to other causes other than malaria (Table S5); these fevers were interpreted as indicating presence of B-symptoms, which are defined as fever of unknown origin in patients with neoplasia [37].

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Effects of loci against *P. falciparum* infection and Burkitt lymphoma were correlated

The effects of the 32 loci against BL and against *P. falciparum* detection in the controls were found to be correlated ($\rho = 0.37$, $p = 0.039$; Figure 2). Two loci showed statistically significant protection against BL in models adjusting for age, sex, country, *P. falciparum* detection, ancestry, and genetic relatedness—namely, *HBB*-rs334(T) with an OR=0.687 (95% CI 0.533–0.885; p=0.0037) and *ABO*-rs8176703(T) with an OR=0.591 (95% CI 0.379–0.992; p=0.00271; Figure 2 and Table S6, Table S7). The associations of BL with *HBB*-rs334 and with *ABO*-rs8176703 persisted in models with both loci mutually adjusted for each other (Table S7), and in sensitivity analyses excluding children with sickle cell disease, country, population-specific PCs, or *P. falciparum* detection (Table S7). The association of the sickle cell *HBB*-rs334(A/T) with BL was similar in individuals without HIV infection (OR = 0.486) and those with HIV infection (OR=0.590) compare with the reference group (*HBB*-rs334(A/A), but the results were only significant in those without HIV, which had a large sample size. The protective effects of *HBB*-rs334(T) against severe malaria have been known to vary in different countries [38] and, following a similar pattern, the ORs for BL with *HBB*-rs334(T) in the four countries ranged from 0.21 to 0.94 (Figure S7), but with overlapping CIs. This pattern was similar for *ABO*-rs8176703(T) (Figure S7), with overlapping CIs.

In addition to its protective effect against BL, *HBB*-rs334 was also protective against *P. falciparum* detection among controls [OR= 0.752 (95% CI 0.628–0.9; p = 0.00189); Figure 2 and Table S8]. The OR for *P. falciparum* detection with *ABO*-rs8176703(T) was 1.122 (95% CI 0.83 – 1.517, p=0.543). The effects of three *IL10* loci against BL and against *P. falciparum* detection among controls were concordant, but statistically significant only for *P. falciparum* infection: *IL10*-rs1800896(C) (OR for infection = 0.886, 95% CI 0.799 – 0.982, p= 0.0208), *IL10*-rs3024496(G) (OR=0.894, 95% CI 0.810 – 0.987, p=0.0259), and *IL10*-rs1518110(C) (OR= 0.862, 95% CI 0.779 – 0.953, p= 0.0037; Figure 2 and Table S8).

Association of Burkitt lymphoma with $\alpha^{3.7}$-thalassemia

Among BL-free children, 47% had normal alpha genotypes ($\alpha\alpha/\alpha\alpha$), 41% were heterozygous for the $\alpha^{3.7}$ thalassemia deletion ($-\alpha^{3.7}/\alpha\alpha$), 10% were homozygous for the deletion ($-\alpha^{3.7}/-\alpha^{3.7}$), and approximately 1% had five copies of the normal gene ($\alpha\alpha\alpha/\alpha\alpha$). Compared to homozygous normal participants, the ORs for BL ranged from 0.778 in those with two copies of the thalassemia deletion gene to 1.655 in those with five copies of the normal gene, although none of these results reached statistical significance (all p>0.05, as shown in Table S9).

Association of asymptomatic *P. falciparum* infection with Burkitt lymphoma

In agreement with previously published results [27, 28], the OR for BL was 0.526 (95% CI 0.438–0.633, $p=9.64\times10^{-12}$; Table 2) among those with *P. falciparum* detection. This decreased OR for BL persisted (OR=0.524, 95% CI 0.435–0.630, 7.29×10^{-12}) in models further adjusting for the *HBB*-rs334(T) and *ABO*-rs8176703(T) alleles. Using the LRT, we confirmed that unrestricted models (i.e., with *P. falciparum* detection) were statistically better than restricted models (i.e., without the *P. falciparum* detection) for estimating associations with BL (Table 2). However, as noted above, the association of *P. falciparum*

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detection with BL was inverse in EMBLEM countries (Uganda, Tanzania, and Kenya) and positive in Malawi. This was most likely a reflection of different detection methods — PCR versus blood microscopy/RDT —with different analytic sensitivities (10 parasites/ul versus 100–200 parasites/ul) [39]. Because parasites can remain detectable at low levels for up 365 days [40, 41], detection only by PCR likely reflects both heavier exposure and chronic low-grade infection as risk factors for BL.

Exploratory analysis of associations of loci in malaria genes with Burkitt lymphoma

In exploratory analyses, using a modified Bonferroni procedure to correct for multiple comparisons after accounting for correlation between loci in the same gene (Table S2) [36], we observed suggestive associations for BL with 10 loci in two genes (*ATP2B4* and *PECAM1*) (Table S10). Nine of the loci, all with elevated ORs for BL, were in *ATP2B4* in a haplotype spanning a ~12 kb region, including *ATP2B4*-rs11808688(G) (OR=1.276 (95% CI 1.106–1.472; $p = 0.0008$), which has been associated with significantly decreased *ATP2B4* gene expression in whole blood (z-score = −11.84, $p = 2.40 \times 10^{-32}$) [42]. The tenth locus associated with BL was *PECAM1*-rs45495798(A) with an OR for BL=1.366 (95% CI 1.160– 1.656; p= 0.0015, Table S10).

DISCUSSION

This study was conducted to investigate whether carriage of one copy of the abnormal *HBB* gene (the cause of SCT), which has been shown to be protective against BL in Uganda [24], remained protective in a large study with children from multiple countries. This association has been reported before [16–18], but not confirmed three other studies [19–21]. Our study controlled for multiple confounders, including *P. falciparum*, other malaria loci that affect malaria risk, and ancestry, which was not possible before. SCT is a reliable marker of resistance against *P. falciparum* burden [43] that has ben useful in identifying malaria phenotypes whose risk decreased in those with SCT-mediated genetic resistance against malaria [44]. For example inverse associations of SCT with invasive bacteremia [45], iron-deficiency anemia [46], and hypertension in African individuals [47] provided strong inferential evidence that malaria is linked to those phenotypes. Our findings here (i.e., that SCT reduces the risk for both BL and detection of *P. falciparum*) thereby demonstrate that BL is a phenotype of *P. falciparum* infection. Demonstration of this correlation has important translational value because it could justify closer coordination between malaria and pediatric cancer programs to increase awareness about the link between BL and malaria particularly at primary care centers where most patients are seen first [48].

Our observation that carriers of *ABO*-rs8176703(T) were at a lower risk of BL is intriguing because this variant has shown protection against severe malaria in Ghana [49]. However, in contrast to the SCT allele, this variant did not concordantly protect against *P. falciparum* detection among controls. Thus, it is not clear whether the effect of this *ABO* locus on BL reflects a protective effect on *P. falciparum*-susceptibility in those with blood group O erythrocytes [15, 50]. Such a mechanism would not be supported by the low linkage disequilibrium (r^2=0.051) between ABO-rs8176703(T) and rs8176719(delG), the allele most correlated with blood group O status in the LWK population in the 1000 Genomes reference

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panel. However, such a mechanism is supported by the strong normalized disequilibrium coefficient (D′ =1.00) between them, which implies that both alleles residing on the same haplotype.

Our results for α-thalassemia with BL were not significant; however, this may not be surprising because although α-thalassemia genotypes are selected by malaria [51], the protective effect of α-thalassemia on malaria is less than that of SCT on malaria. Therefore, additional analysis of this relationship may require larger sample sizes to be observed against BL.

Our findings suggest that the effects of the 32 loci against BL and *P. falciparum* detection among controls are concordant but not equal. The inequality of effects fits the classical malaria model for BL [12], which implies a complex interplay of multiple factors, including EBV [2]. It also fits with different mechanisms of genetic control of malaria. For example, the effects of loci in *IL10* against BL and *P. falciparum* infection among controls were concordant in direction but significant only for *P. falciparum* infection. This unequal pattern of effects suggests that some loci may have stronger effects on aspects of malaria pathophysiology (e.g., such as fever and cytokine storm), which was the basis of their selection, but weaker effects on direct *P. falciparum* infection control, which is the relevant biological risk factor for BL. It is possible that some host genes, such as *IL10* loci, are selected because they reduce severe malaria symptoms due to fever and inflammation without directly reducing parasitemia. If so, such loci may protect individuals from severe malaria death without an equal impact on recurrent asymptomatic *P. falciparum* infections, which persist as multiclonal infections from super- or supra-infection [52, 53].

Our finding of strong inverse association between BL and *P. falciparum* detection agrees with our previous results [27, 28] and those reported by others [54, 55]. We note that this seeming paradox points to the importance of host immune responses against *P. falciparum*—i.e., natural acquired immunity to host survival by mediating clearance of a parasite clones [56] before developing BL and to a possible mechanism of BL pathogenesis. Specifically, children who develop BL, typically at approximately age 7 as in the current study, survive malaria in no small part thanks to NAI, i.e., an immune response that senses, reacts to, and controls parasitemia [56, 57]. NAI initially develops quickly against severe malaria, after the first one or two infections, [58, 59] then increases progressively to suppress symptoms (i.e., anti-disease immunity), and then *P. falciparum* infection (i.e., anti-parasite immunity) [60, 61]. NAI is mediated by circulating memory B cells, which represent a T-helper 2 (Th2) response characterized by up-regulation of antibody production to fight extracellular organisms [62], including *P. falciparum*. NAI against *P. falciparum* transmission is durable [63] and the half-life of the produced antibodies can range between several years to life-long persistence [64, 65]. It is possible that children prone to BL develop strong Th-2 type anti-inflammatory response, mediated by cytokines such as interleukin 10 [66], both controls parasitemia to limit severe disease while increasing tolerance of low levels of parasitemia without triggering severe symptoms [62].

We note that NAI could also be modulated by co-infection with EBV, which encodes BCRF-1, a viral interleukin-10-homolog [67] with capacity to down-regulate Th1-cytokines

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while upregulating Th-2 cytokines [68]. Because EBV infection occurs by 6 months in ~35% of children in areas where BL is endemic [69, 70], it has been suggested that EBV may protect against severe malaria via interleukin 10 mimicry [71], while the low levels of *P. falciparum* parasitemia promote BL by upregulating EBV infection [72]. Interestingly, in a recent study of BL cases reported localized disease with prominent granulomatous reaction and an unexplained high propensity for regression despite receiving none or minimal treatment [73]. These cases, exhibiting tumor cellular features characterized by Th1 lymphocytes and M1 macrophages, which is different from the usual Th2 phenotype described above, highlighted the importance of host immunity in BL pathogenesis. Is it possible that while most BL cases develop under a Th2-type response, a small fraction of BL cases with Th1 type response might be transient or have a good prognosis [73]. Additional research is needed to identify the close interaction between oncogenic factors, host immunity, including NAI that could be permissive for BL development or conversion to Th1-type response, which promotes BL regression or indolence.

The strengths of our study include the largest study conducted to date and the first multicenter study of BL, with confounder data, including *P. falciparum* detection, prior history of malaria and treatment, and genetically defined ancestry and relatedness. The limitations include incomplete histological verification of BL cases (about one quarter of cases were not confirmed), which could introduce biases. However, our results are within range of current capacity in poor resource settings where histological services are not always readily available. We also note that our analysis did not include malaria loci on the X chromosome (e.g., *G6PD* and *CD40LG*) as well as those that could not be imputed from GWAS data (e.g., *EPB41, GYPB,* and *FREM3/GYPE*). Our results for SNPs in the 22 malaria genes are exploratory but provide a useful baseline. Our inferences about NAI are not based on immune markers, but identify NAI as an important concept to be explored in BL. Finally, parasite evolution may be a factor. Recent discoveries indicate that certain parasite strains, such as those with *P. falciparum* sickle-cell associated alleles (*Pfsa*), have evolved to infect and cause severe malaria in individuals with SCT [74]. These discoveries suggest that parasite factors should be considered in future studies of BL.

In conclusion, we report that SCT is protective both against BL and against *P. falciparum* detection in controls when controlling for confounders. In addition, we observed protective associations between *ABO*-rs8176703(T) loci and BL, but not against infection. Our results increase support for the hypothesis that BL is a phenotype of *P. falciparum*. This inference opens a window to encourage closer coordination between malaria and pediatric oncology programs to improve the control of BL in malaria-endemic areas in Africa.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

We thank the study population and communities for their participation. We thank Ms. Janet Lawler-Heavener at Westat Inc, (Rockville, MD, USA) and Mr. Erisa Sunday at the African Field Epidemiology Network (Kampala, Uganda) for managing the study. We are grateful to Daniel Shriner at NHGRI (Bethesda, Maryland) for serving as

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Mateus H. Gouveia's mentor and for assistance with statistical genetics and computational analyses. We are grateful to the leadership of the collaborating countries and institutions for hosting local field offices and laboratories and supporting the fieldwork. We thank Ms. Laurie Buck, Dr. Carol Giffen, Mr. Greg Rydzak and Mr. Jeremy Lyman at Information Management Services Inc. (Calverton, MD, USA) for coordinating data, and preparing data analysis files, and David Check of the Division of Cancer Epidemiology and Genetics, National Cancer Institute (Bethesda, Maryland) for drawing forest plots.

Funding statement

The study was funded by the Intramural Research Program, Division of Cancer Epidemiology and Genetics, National Cancer Institute (NCI) (Contracts HHSN261201100063C and HHSN261201100007I), Cancer Genomics Research Laboratory (75N910D00024), National Institute of Allergy and Infectious Diseases (SJR), the Sickle Cell Branch, National Heart, Lung and Blood Institute; and Center for Research on Genomics and Global Health (Z01HG200362), National Human Genome Research Institute, National Institutes of Health (NIH), Department of Health and Human Services.

Support

The study was funded by the Intramural Research Program of the Division of Cancer Epidemiology and Genetics, National Cancer Institute (NCI) (Contracts HHSN261201100063C and HHSN261201100007I), the Intramural Research Program, National Institute of Allergy and Infectious Diseases (SJR), and the Intramural Research Program, Sickle Cell Branch, National Heart, Lung and Blood Institute; Center for Research on Genomics and Global Health (Z01HG200362), National Human Genome Research Institute; National Institutes of Health (NIH), Department of Health and Human Services. The authors acknowledge the research contributions of the Cancer Genomics Research Laboratory for their expertise, execution, and support of this research in the areas of project planning, wet laboratory processing of specimens, and bioinformatics analysis of generated data. from the NCI, NIH, under NCI Contract No. 75N910D00024. We acknowledge support to FB from the NCI Continuing Umbrella of Research Experiences (NCI-iCURE). The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government. The content of this manuscript is the sole responsibility of the authors.

Data availability

The genetic data will be deposited in dbGaP at: https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs001705.v1.p1. Access to covariate data for EMBLEM and Malawi studies can be requested directly from the corresponding author. The study forms are available for download from the EMBLEM website at: https://emblem.cancer.gov/resources/index.html.

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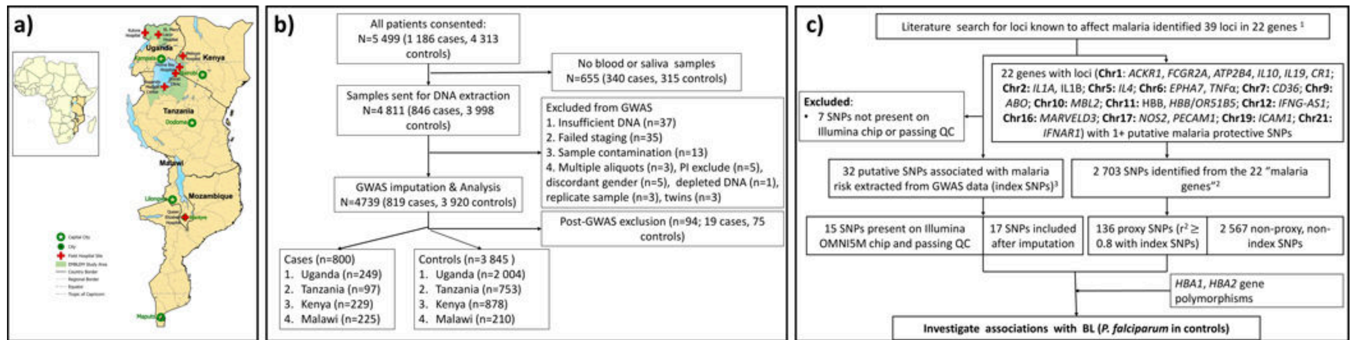

Figure 1. a) Map of the study sites: a locator map for Africa and a zoom out of EMBLEM study sites in Uganda, Kenya, and Tanzania and the Infections and Childhood Cancer Study site in Malawi.

Red crosses mark location of hospitals where cases were enrolled, and a green shade marks the 6 regions where cases and controls were enrolled in EMBLEM. Stars mark location of country capitals to show the relative distance between the participating hospitals versus enrollment hospitals. b) Flow chart showing participants enrolled and reasons for exclusion from the final set included in case-control analysis. c) Flow chart showing the process followed to identify polymorphisms associated with protection from or risk of severe malaria in epidemiological studies. Significantly associated polymorphisms were considered "malaria index" and the identified genes labeled "malaria index genes". The genotypes are analyzed are based on genotype results from the Omni 5M array or from imputed results. Non-index polymorphisms were extracted and analyzed for hypothesis generation. Genotypes for *HBA1 and HBA2* were determined using droplet digital PCR were included in the analysis. The 3.7 kb deletion ($-\alpha^{3.7}/$), which has been reported to interact with the sickle cell trait, is the most prevalent in our population (details in Supplementary data).

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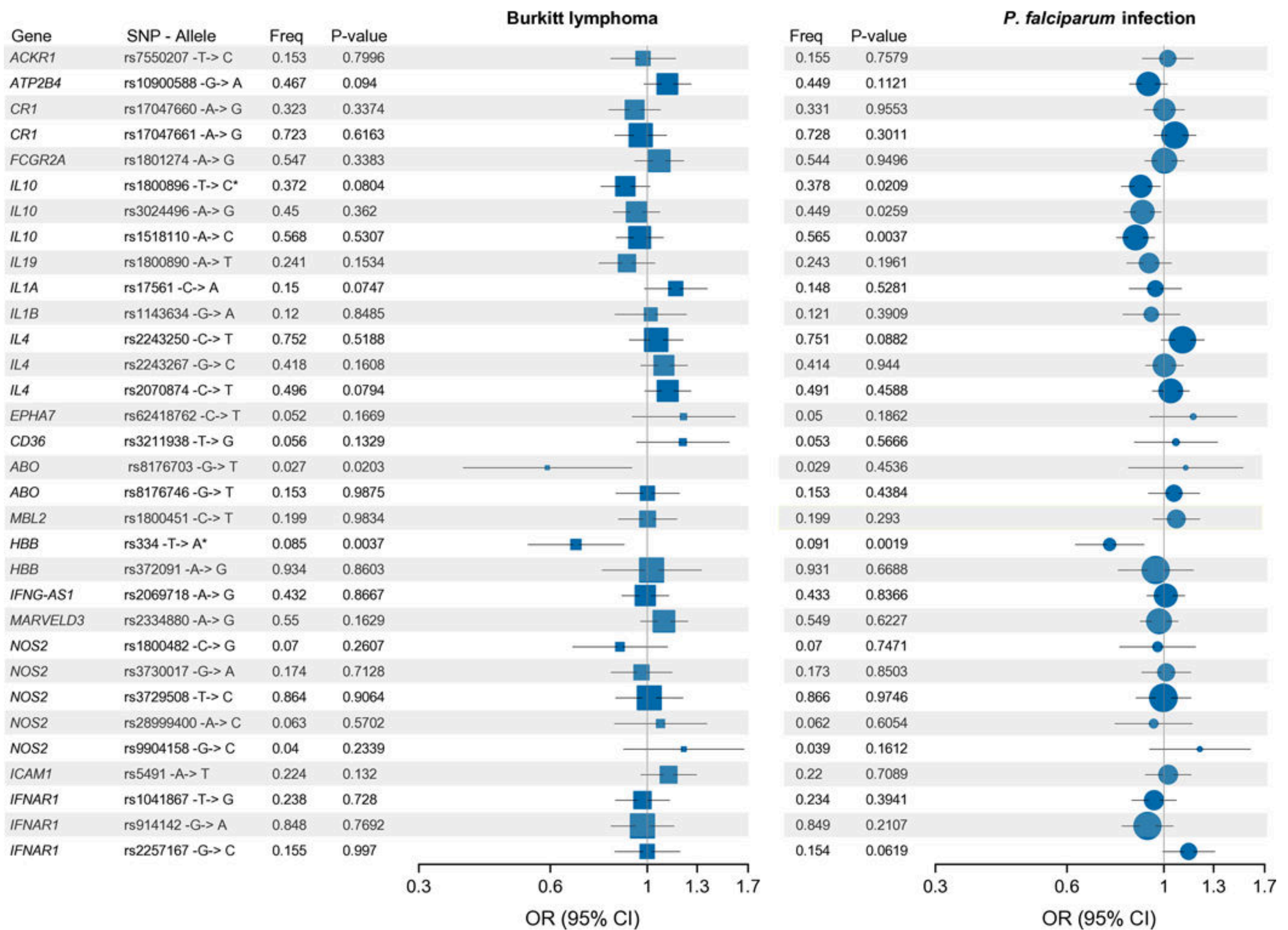

Figure 2. Forest plots showing associations between 32 malaria-risk index SNPs and endemic Burkitt lymphoma (left) or *P. falciparum* infection (right).

Forest plots show OR values (95% confidence intervals) and *p*-values from the mixed-effects logistic regression model analyses for association with BL among 4 581 BL cases and controls using model: BL case status~ SNP_ij+factor(*P. falciparum*)+ age+sex+pop-specific PC1+ pop-specific PC2+ pop-specific PC3+ factor(country)+[genetic relation matrix, random effect], and for association with *P. falciparum* infection among 3800 controls using model *P. falciparum* ~ SNP_ij+ age+sex+pop-specific PC1+ pop-specific PC2+ pop-specific PC3+ factor(country)+[genetic relation matrix, random effect]. $p < 0.05$ was considered statistically significant, * effect of SNP on BL concordant with effects against malaria in the literature and infection among the controls (see methods). The area of the squares/circles is proportional to the frequency (freq) of the variant in the population. The minor alleles are reported based on the sequence on the plus strand as reported in dbGAP, however results for *HBB*-rs334 are reported in the test based on the minus strand to be consistent with conventional usage across scientific literature on sickle cell disease.

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Table 1.

Participant demographic and clinical characteristics at enrollment

Characteristic	BL Cases, n (%)	Controls, n (%)	p value
All subjects	800 (100)	3 845 (100)	
Median age, (IQR) *	7·00 (4–10)	7.00 (5–10)	0·07
Age group±**, years**			0·12
0–2	67 (8·4)	342 (8·9)	
3–5	219 (27·4)	914 (23·8)	
6–8	239 (29·9)	1,130 (29·4)	
9–11	165 (20·6)	820 (21·3)	
12–15	110 (13·7)	639 (16·6)	
Sex			<0·0001
Males	503 (62.9)	2,005 (52·1)	
Females	297 (37·1)	1,840 (47·8)	
Country			<0·0001
Uganda	249 (31·1)	2 004 (52·1)	
Tanzania	97 (12·1)	753 (19·6)	
Kenya	229 (28·6)	878 (22·8)	
Malawi	225 (28·1)	210 (5·5)	
***P. falciparum* infection**¶			<0·0001
Negative	499 (63·3)	1 943 (50·5)	
Positive	282 (35·2)	1 857 (48·3)	
Unknown (missing)	19 (2·4)	45 (1·2)	

Abbreviation: BL Burkitt lymphoma; SD standard deviation

* The age distribution of cases and controls was not normally distributed, so median and inter-quartile ranges are used to describe central tendency (See supplementary Figures S3)

± The age groups were selected to reflect the age distribution of Burkitt lymphoma in the study area..

¶ Asymptomatic *P. falciparum* infection was determined using thick film microscopy or rapid diagnostic test results as in the EMBLEM study (Uganda, Tanzania, and Kenya) and using quantitative polymerase chain reaction tests in Malawi. As such, while parasitemia positivity is combined, the TFM/RDT results are at a lower sensitivity, while the PCR results are more sensitive (see methods and supplementary methods for more detailed discussion).

Differences for continuous variables were assessed by unpaired *t*-test. Differences in categorical variables were assessed by Chi-squared test.

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Table 2.

Associations between childhood Burkitt lymphoma status and *Plasmodium falciparum* infection at enrollment

Characteristic	OR (95% CI) *	*p*-value	OR (95% CI) ±	*p*-value
All Countries combined	0·526 (0·438–0·633)	9.64E-12	0·524 (0·435–0·630)	7·29E-12
Likelihood ratio test $\chi 2$ (p-value) ¶		6·11 (0·0134)		6·16 (0·0131)
Uganda§	0·410 (0·299–0·562)	3·09E-08	0·400 (0·291–0·549)	1·493E-08
Likelihood ratio test $\chi 2$ (p-value) ¶		3·12 (0·0774)		2·96 (0·0853)
Kenya§	0·302 (0·203–0·449)	3·53E-09	0·302 (0·203–0·450)	3·81E-09
Likelihood ratio test $\chi 2$ (p-value) ¶		4·55 (0·0329)		4·73 (0·0296)
Tanzania§	0·451 (0·289–0·726)	0·0010	0·452 (0·281–0·727)	0·0011
Likelihood ratio test $\chi 2$ (p-value) ¶		1·45 (0·2282)		1·.43 (0·2315)
Malawi§§	1·793 (1·203–2·673)	0·0041	1·829 (1·221–2·739)	0·0034
Likelihood ratio test $\chi 2$ (p-value) ¶		2·61 (0·1061)		2·57 (0·1090)

* Model: BL case status~factor(*P. falciparum*)+ age+sex+pop-specific PC1+ pop-specific PC2+ pop-specific PC3+ factor(country)+[genetic relation matrix, random effect]. Country was not included in country-specific models.

± Model: BL case status ~ factor(*P. falciparum*)+age+sex+pop-specific PC1+ pop-specific PC2+ pop-specific PC3+ factor(country)+[genetic relation matrix, random effect] + *HBB*-rs334(T)+*ABO*-rs8176703 (T). Country was not included in country-specific analyses.

¶ $\chi 2$ goodness-of-fit (*p*-value) testing differences in nested models with *P. falciparum* (unrestricted model) versus without *P. falciparum* (restricted model).

§ *P. falciparum* infection was determined by thick film microscopy or rapid diagnostic tests. Analytic sensitivity of giemsa-stained blood films and rapid diagnostic test is 100–200 parasites/μl and may detect 55% of the infectious reservoir population (See methods)

§§ *P. falciparum* infection was determined by polymerase chain reaction (PCR) in Malawi. Analytic sensitivity of PCR is 10 parasites per ul of blood and may detect 83% of the infectious reservoir population, including submicroscopic infections (See methods).