frontiers | Frontiers in Hematology

TYPE Original Research
PUBLISHED 25 April 2023
DOI 10.3389/frhem.2023.1150134

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Red Cells, Iron and Erythropoiesis, a section of the journal Frontiers in Hematology

RECEIVED 23 January 2023
ACCEPTED 10 April 2023
PUBLISHED 25 April 2023

CITATION
Lamptey H, Seidu Z, Lopez-Perez M, Kyei-Baafour E, Hviid L, Adjei GO and Ofori MF (2023) Impact of haemoglobinopathies on asymptomatic *Plasmodium falciparum* infection and naturally acquired immunity among children in Northern Ghana.
Front. Hematol. 2:1150134.
doi: 10.3389/frhem.2023.1150134

Impact of haemoglobinopathies on asymptomatic *Plasmodium falciparum* infection and naturally acquired immunity among children in Northern Ghana

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Background: The protective effect of certain haemoglobinopathies, such as HbS, HbC, and α-thalassaemia, against severe malaria has long been established; however, there is only limited and equivocal evidence regarding their impact on asymptomatic parasitaemia. Here, we investigated the effect of HbS, HbC, and α-thalassaemia on asymptomatic *P. falciparum* parasitaemia and acquired immunity among children in Northern Ghana.

Materials and methods: A cross-sectional study was conducted among 1,017 healthy children (1-17 years) in 13 malaria-endemic communities in Northern Ghana. The children were screened for structural Hb phenotypes using SickleSCAN, for P. falciparum infection using anti-HRP2 malaria RDT and subsequently confirmed by capillary electrophoresis and PCR, respectively. α-thalassaemia genotyping was done using PCR. Levels of IgG specific for six recombinant malaria antigens (*Pf*CSP, GLURP, MSP3, *Pfs*230, HB3VAR06, and IT4VAR60) and crude asexual blood-stage antigens were evaluated by ELISA.

Results: 266 out of the 1,017 participants had either HbAC (18%) or HbAS (8.4%), whereas 35% had α-thalassaemia. Twenty-five percent and 6% HbAC individuals co-inherited heterozygous and homozygous α-thalassaemia respectively. Similarly, 25% and 10.5% of HbAS co-inherited heterozygous and homozygous α-thalassaemia. Asymptomatic parasitaemia rates were 23%, 24%, and 19% in those with HbAA, HbAC and HbAS, respectively. The overall parasite carriage rates in heterozygous (21%) and homozygous α-thalassaemia (25%) individuals were similar to that of individuals without α-thalassaemia (23%). *P. falciparum*

parasite carriage risk was about three times higher among homozygous α-thalassaemia individuals with HbAC (OR = 2.97; 95% CI 0.83-10.62) and heterozygous carriers with HbAS variants (OR = 2.86; 95% CI 0.85-9.60) compared to the wildtype. In HbAS individuals, IgG levels to IT4VAR60 and HB3VAR06 were significantly lower, whereas anti-CSP levels were higher than in HbAA and HbAC.

Conclusions: Co-inheritance of HbAS and HbAC with α-thalassaemia increased the risk of asymptomatic parasitaemia, an indication of a negative epistatic effect between these Hb variants. Antibody levels against non-PfEMP1 antigens were slightly higher among HbAS children, but quite similar in all study groups, indicating differences in parasite exposure.

KEYWORDS

antibodies, asymptomatic infection, parasite carriage, *Plasmodium falciparum*, α-thalassaemia, haemoglobinopathies, Ghana

Introduction

Haemoglobinopathies are highly prevalent in malaria-endemic communities (1–3). Haldane in 1949 (4) and Allison in 1954 (5) first speculated about the protective effect of these, giving rise to the 'malaria hypothesis'. HbS, HbC, and HbE result from single amino acid substitutions in the β-globin gene in adult haemoglobin (HbA), whereas the thalassaemias are the consequence of reduced production of globins (6).

HbC (7, 8) HbS (9), HbE (10), α-thalassemia, and β-thalassaemia protect against severe malaria (1, 11) and are hence common in malaria-endemic areas (12). The protective effect against severe forms of malaria is most pronounced for HbS (1), with the highest level of protection in HbAS individuals (91%). The homozygous HbCC provides up to 73% protection, and 37% for homozygous and heterozygous α-thalassaemia, as well as 20% for the HbAC phenotypes (1). HbS is widely distributed across Africa, whereas HbC is restricted to parts of West Africa (13–15). In Ghana, HbC is most frequent in the northern part of the country (19.7% to 20.7%) (16, 17). α-thalassemia is highly prevalent in the sub-Saharan region (up to 50%) (18, 19) and in Southeast Asia (40%) (20).

The proposed protective mechanisms for these erythrocyte polymorphisms include reduction in parasite growth (21–23), parasite invasion (24, 25), and in the adhesion of parasitised erythrocytes to endothelial vessels and uninfected erythrocytes (26, 27). These last two phenotypes are mediated by PfEMP1 on the parasitised erythrocyte surface (28). However, the exact mechanism of protection is unknown, and little is known about the impact of these variants on uncomplicated malaria and asymptomatic infections (29, 30). In addition, HbS could have an indirect effect on malaria susceptibility because it enhances phagocytosis and activates inflammatory cytokines (31–33). Overall, the impact of these haemoglobinopathies on parasite carriage appears to be minimal (1), but little is known about how co-inheritance of these haemoglobinopathies affects parasite carriage, malaria pathogenesis, and naturally acquired immunity. We assessed the impact of HbS and HbC, α-thalassaemia and their co-inheritance in *P. falciparum* parasite carriage and acquired immune response in Ghanaian children.

Materials and methods

Ethics statement

This study was approved by the Ethics Review Committee of the Ghana Health Service (GHS; GHS-ERC 008/07/19) and by the Noguchi Memorial Institute for Medical Research (NMIMR) Institutional Review Board (Federal-wide Assurance FWA 00001824, NMIMR-IRB CPN 006/19). A declaration of free willingness to participate in the study and written informed consent were obtained from all participants or guardians before enrolment.

Study design and study site

A cross-sectional study was conducted between August and September 2020 in 13 rural communities in the Kumbugu, Nanton, and Tolon Municipalities, Northern Region, Ghana (Figure 1), to assess the prevalence of the above haemoglobinopathies and their association with the risk of *P. falciparum* infection among children. Detailed information on the study is given elsewhere (Seidu et al. submitted for publication). The Northern Region is the largest in Ghana, and situated in the savannah woodland zone (34). The region is relatively dry, with a single rainy season from April to September or October, a dry season from November to March/

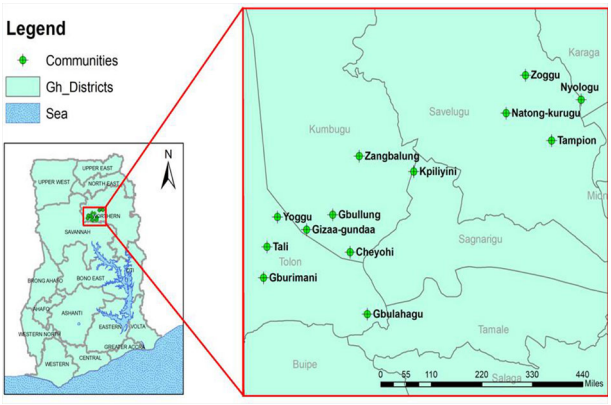

FIGURE 1
Location of the study sites. Ghana is shown on the left. The location of the study site in the Northern Region and the study communities are shown in the enlarged map. The GIS coordinates of the thirteen study communities were used to construct a graphical plot using the ArcMap tool employed in ArcGIS v10.1. (ESRI, Redlands, California, USA). The shapefiles used for constructing the map are publicly available at https://data.humdata.org/dataset/cod-ab-gha.

April, and severe harmattan winds between December and early February (35). The population of the municipalities is approximately 300,000.

Study population and data collection

One-thousand-and seventeen healthy children aged 1-17 years participated in the study (60% males and 40% females). Children from randomly selected households in the communities were recruited and assembled at community health facilities for sample collection and screening. A structured questionnaire was used for in-person interviews with children's guardians to collect socio-demographic information. Finger-prick blood samples were collected to measure Hb levels using a URIT-12 haemoglobin instrument (URIT Medical Electronic Ltd, China), and to test for *P. falciparum* infection and structural Hb phenotypes by RDT. Dried blood spots (DBS) on filter paper (Whatman, USA) were used for retrospective confirmation of *P. falciparum* infection and α-thalassaemia genotyping by PCR. In addition, venous plasma was stored at -20°C to determine malaria-specific IgG levels later.

Determination of *P. falciparum* infection with anti-HRP2 RDT and PCR

The First Response Malaria Antigen-HRP2 Card Test (Premier Medical Corporation, Gujarat, India) was used for on-site diagnosis of *P. falciparum* infection, following the manufacturer's instructions. DNA was extracted from DBS using the Chelex method (36), and *P. falciparum* infection was confirmed retrospectively by PCR as described (Seidu et al., submitted for publication). Children both RDT and PCR positive were classified as having asymptomatic infection.

Screening for structural Hb variants and α-thalassaemia status

The Hb phenotyping was carried out using the Sickle-SCAN (Bioline, USA), point-of-care test (37, 38) and retrospectively confirmed with iso-electric focusing (IEF) electrophoresis method, using a Multiphor II electrophoresis unit (GE Healthcare, Little Chalfont, England) as described (39). The α 3.7 deletion in the α globin gene was used for α-thalassaemia detection in this study, as described (40). Participants were categorised as wildtype, heterozygous α-thalassaemia, or as homozygous α-thalassaemia based on band size after genotyping.

Recombinant proteins

Recombinant glutamate-rich protein domain R0 (GLURP) was produced in *E. coli* (41), while the merozoite surface protein 3 (*Pf*MSP3) (42) and a 230-kDa sexual stage protein (*Pf*s230) (43) were produced in *Lactococcus lactis*. The entire ectodomain of two PfEMP1 proteins, HB3VAR06 *and* IT4VAR60, were produced in baculovirus-transfected *Sf9* insect cells, as described (44). Circumsporozoite protein (*Pf*CSP) was purchased from IPG Technologies (Germany) and a crude asexual *P. falciparum* lysate was prepared from the 3D7 clone in our laboratory.

Measuring *P. falciparum*-specific IgG levels

Levels of IgG specific for six recombinant *P. falciparum* proteins and crude 3D7 antigens were measured using an indirect ELISA, as reported (45, 46). Briefly, 96-well flat-bottom microtiter plates (Nunc MaxiSorp; Thermo Fisher Scientific) were coated with 0.5 μg/mL of recombinant proteins (CSP, GLURP, *Pf*MSP3, or *Pf*s230),

2μg/mL of the *Pf*EMP1-type recombinant proteins or 5×10^5 IEs/mL of the crude antigen lysate. After blocking with 1% BSA in PBS, plasma samples (1:300) were added in duplicate, followed by horseradish peroxidase-conjugated rabbit anti-human IgG (1:3,000; Dako, Denmark). Bound plasma antibodies were detected by adding TMB (Abcam, UK), and the reaction was stopped with 0.2 M H_2SO_4. The optical density (OD) was read at 450 nm (Varioskan LUX; Thermofisher, USA). The specific antibody levels were calculated in arbitrary units (AU) using the equation $[(OD_{SAMPLE} - OD_{BLANK})/(OD_{POSITIVE\ CONTROL} - OD_{BLANK})] \times 100$. Plasma samples from malaria-unexposed Danish individuals and malaria-exposed Ghanaian children were included as negative and positive controls, respectively.

Statistical analysis

Data were analysed using R version 3.6.3 (R Core Team, 2020) in Rstudio and GraphPad Prism version 9.3.1 (GraphPad Software, San Diego, CA, USA). The data were converted into frequencies, and subgroup proportion tables were generated using the "publish" package (47). The proportions between different categories were compared by Chi-square test or Fisher's exact test. The "glm" function in R and the "publish" package were used for multivariate analysis using a logistic regression model. P-values < 0.05 were considered statistically significant.

Results

Demographic and clinical characteristics of the study population

The study included 1,017 children aged 1-17 years, most of them with HbAA (wild type; 73%), whereas 18% and 8.4% had HbAC and HbAS, respectively (Table 1). Thirty percent had heterozygous α-thalassaemia, while 6% were homozygous. Additionally, 25% (45/181) and 6.1% (11/181) of HbAC individuals co-inherited heterozygous and homozygous α-thalassaemia respectively. Similarly, 25% (21/85) and 10.5% (11/85) of the HbAS individuals co-inherited heterozygous and homozygous α thalassaemia respectively. Overall, the mean Hb level was 10.9 g/dL. Children with homozygous α-thalassaemia had the lowest Hb levels (p = 0.04).

Association of haemoglobinopathies with *P. falciparum* parasite carriage

The association of the Hb phenotypes and α-thalassaemia with *P. falciparum* infection was determined by comparing parasite carriage by PCR among the different haemoglobinopathies groups. The parasite carriage rates were similar among individuals with HbAA (23%), HbAC (24%), and HbAS (19%; p = 0.73; Table 1). With respect to

TABLE 1 Demographic and clinical characteristics of the study population.

		Hb phenotypes				
Variable	Level	HbAA (n=751)	HbAC (n=181)	HbAS (n=85)	Total (n=1,017)	p-value
Gender	Male	450 (59.9)	105 (58.0)	57 (67.1)	612 (60.2)	
	Female	301 (40.1)	76 (42.0)	28 (32.9)	405 (39.8)	0.36
Age (years)	mean (SD)	7.3 (3.4)	7.2 (3.5)	6.9 (3.4)	7.2 (3.4)	0.63
Hb level g/dL	mean (SD)	10.9 (1.3)	10.8 (1.2)	10.7 (1.1)	10.9 (1.3)	0.56
	Yes	157 (21.0)	41 (23.6)	17 (20.0)	215 (21.4)	
Parasite carriage	No	590 (79.0)	133 (76.4)	68 (80)	791 (78.6)	0.72
		α-thalassaemia status				
Variable	Level	Wildtype (n=568)	Heterozygous (n=261)	Homozygous (n=50)	Total (n=879)	p-value
Gender	Male	352 (62.0)	150 (57.5)	30 (60.0)	532 (60.5)	
	Female	216 (38.0)	111 (42.5)	20 (40.0)	347 (39.5)	0.47
Age (years)	mean (SD)	7 (3.3)	7.7 (3.6)	7.8 (3.3)	7.2 (3.4)	**0.01**
Hb level g/dL	mean (SD)	10.9 (1.3)	10.9 (1.2)	10.4 (1.3)	10.9 (1.3)	**0.04**
	Yes	130 (22.9)	54 (20.8)	12 (25.0)	196 (22.4)	
Parasite carriage	No	437 (77.1)	205 (79.2)	36 (75.0)	678 (77.6)	0.73

[a]αα/-α; -α/-α. p-value was calculated using Pearson's chi-squared and Fisher's exact test.
Numbers in bold are statistically significant p-values.

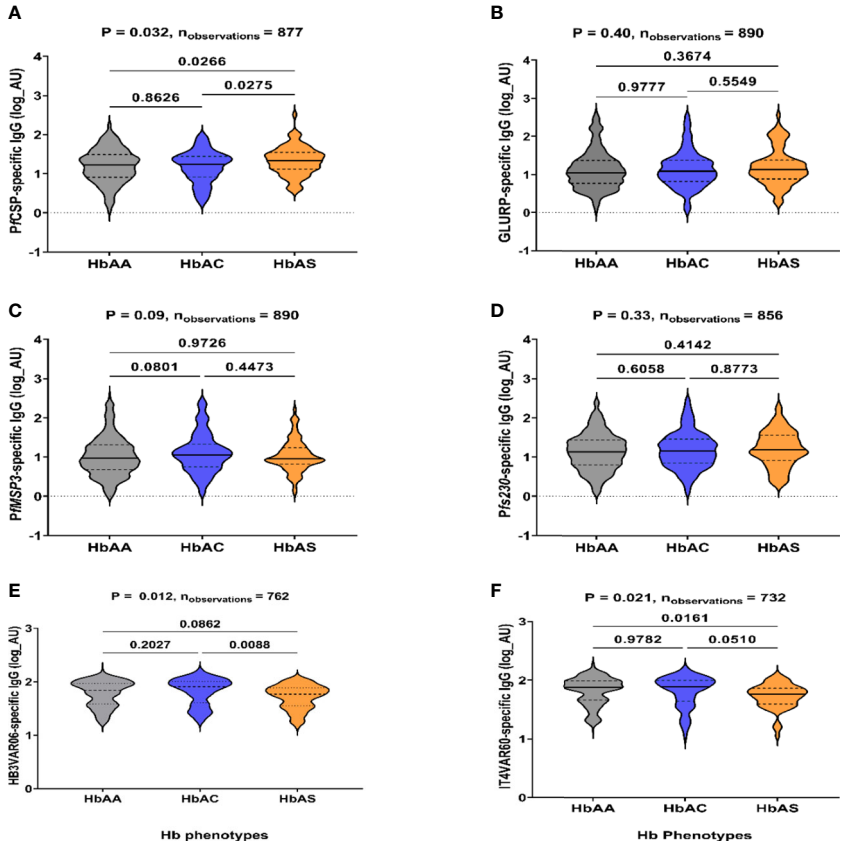

FIGURE 2
IgG-specific levels to *P. falciparum* antigens in HbAA, HbAS, and HbAC individuals. IgG levels to **(A)** *Pf*CSP, **(B)** GLURP, **(C)** *Pf*MSP3, **(D)** *Pfs*230, **(E)** HB3VAR06, and **(F)** IT4VAR60 determined by ELISA, respectively. The x-axis is the Hb phenotypes (HbAA, HbAC and HbAS). IgG levels are expressed in arbitrary log units (AU) on the y-axis. P-values using Kruskal-Wallis test followed by *Dunnett's* test for multiple comparisons are shown.

α-thalassaemia, parasite carriage rates were similar among homozygous (25%) and heterozygous (21%), and wildtype individuals (23%) (p = 0.75; Figure 2B). However, using a logistic regression model with interaction, it was observed that parasite carriage was about three times higher among homozygous α-thalassaemia HbAC individuals (OR = 2.97, p = 0.09) and among heterozygous HbAS individuals (OR = 2.86, p = 0.09; Table 2). However, for the HbAS individuals carrying the homozygous α-thalassaemia phenotype, no significant difference was observed with the risk of parasite carriage (OR = 0.71, p = 0.77; Table 2).

Levels of *P. falciparum* antigen-specific IgG as a measure of malaria exposure

To further explore the effect of the haemoglobinopathies on malaria parasite exposure, IgG specific levels were measured against recombinant *P. falciparum* proteins from several parasite stages, sporozoite (*Pf*CSP), merozoite (GLURP, MSP3), gametocyte (*Pfs*230), and infected erythrocytes (PfEMP1) (Figure 2) and crude antigens (Supplementary Figure 1). Children with HbAS had significantly higher IgG levels to *Pf*CSP than in HbAA (p = 0.03) and HbAC (p = 0.03; Figure 2A). In contrast, levels to HB3VAR06 (p = 0.09) and IT4VAR60 (p = 0.02) were lower in HbAS compared to HbAA (Figures 2E, F). No significant differences in IgG levels to GLURP, MSP3, and *Pfs*230 (non-

TABLE 2 Interaction among HbAA, HbAS, HbAC, α-thalassaemia, and parasite carriage.

Interaction variables	Odds Ratio [95% CI]	p-value
αα/αα: (HbAC *vs* HbAA)	0.88 [0.52-1.47]	0.62
αα/αα: (HbAS *vs* HbAA)	0.55 [0.24-1.26]	0.16
-α/-α: (HbAC *vs* HbAA)	1.33 [0.60-2.94]	0.49
-α/-α: (HbAS *vs* HbAA)	2.12 [0.80-5.63]	0.13
αα/-α: (HbAC *vs* HbAA)	3.06 [0.69-13.57]	0.14
αα/-α: (HbAS *vs* HbAA)	0.46 [0.05-4.42]	0.50
HbAA: (αα/-α *vs* αα/αα)	0.74 [0.48-1.13]	0.16
HbAA: (αα/-α *vs* αα/αα)	0.85 [0.34-2.16]	0.74
HbAC: (αα/-α *vs* αα/αα)	1.11 [0.48-2.60]	0.80
HbAC: (-α/-α *vs* αα/αα)	2.97 [0.83-10.62]	0.09
HbAS: (αα/-α *vs* αα/αα)	2.86 [0.85-9.60]	0.09
HbAS: (-α/-α *vs* αα/αα)	0.71 [0.08-6.63]	0.77

Multivariate analysis with interaction using logistic regression. P value using Chi-square test. Hetero-mutant: heterozygous (αα/-α); Homo-mutant: homozygous mutant (-α/-α). 95% CI: 95% confidence interval.

PfEMP1-type) antigens were observed among the HbAA, HbAC, and HbAS individuals.

The IgG specific levels to all the antigens except *Pf*s230 and IT4VAR60 positively correlated with age in the HbAA individuals (Figure 3A). There was no correlation between age and the IgG levels to HB3VAR06, IT4VAR60, *Pf*S230 and *Pf*MSP3 in HbAC individuals (Figure 3B). Additionally, among the HbAS individuals, there was no correlation between age and the IgG levels to all antigens (Figure 3C).

A principal component analysis (PCA) indicated variation in the IgG levels among HbAA, HbAC, and HbAS individuals (Figure 3D) but did not differentiate the Hb phenotypes. The PCA also revealed that PfEMP1-specific IgG levels are completely different from the non-PfEMP1 antigens. Among the non-PfEMP1 antigens, levels against *Pf*CSP, the only pre-erythrocytic stage antigen, were also different.

Individuals with homozygous α-thalassaemia had significantly higher IgG levels to *Pf*CSP (p=0.01) and GLURP (p=0.02) and lower IgG levels to HB3VAR60 (p=0.07) and IT4VAR06 (p=0.03) than both the heterozygous mutants and wildtype individuals. They also had slightly higher IgG levels to MSP3 and *Pf*s230 (Figure 4).

There is virtually no correlation between age and IgG levels against all the antigens in both the wildtype α-thalassaemia individuals and homozygous mutation (Figures 5A, C). In contrast, IgG levels against GLURP, *Pf*MSP3 and the crude antigens positively correlated with age (Figure 5B).

The PCA for α-thalassaemia status revealed that IgG levels to the PfEMP1 antigens differed from the non-PfEMP1 antigens, with the IgG levels against the *Pf*CSP differentiated from the rest of the antigens (Figure 5D), as observed for Hb phenotype.

Discussion

HbAS, HbAC, and α-thalassaemia have been reported to protect against severe *P. falciparum* malaria (1, 48); however, the effect on these highly frequent haemoglobinopathies on the risk of *P. falciparum* parasite carriage is less clear. Here, we show lower but not statistically significant parasite carriage (19%) in individuals carrying the HbAS phenotype compared to HbAA (23%) and HbAC (24%) as evidenced in literature, that this could be an indication of enhanced parasite clearance in those carrying the sickle cell trait (16). In contrast, individuals carrying homozygous α-thalassaemia had higher parasite carriage (25%) compared to the heterozygous (21%) and wild type (23%). We also report that co-inheritance of homozygous α-thalassaemia with HbAC and heterozygous α-thalassaemia with HbAS increased the risk of *P. falciparum* asymptomatic parasitaemia about threefold compared to wild-type. Our results agree with previous reports of protection against severe malaria but not asymptomatic infections conferred by HbAS and HbAC phenotypes (1, 8, 16, 39, 49–51), and protection against severe malaria anaemia but not against parasite carriage in homozygous α-thalassaemia (52, 53). Taken together, our findings and previous studies reinforce the hypothesis that reduced cytoadhesion of infected RBCs (54) rather than a parasitaemia reduction *in vivo* is the most likely explanation for the protection afforded by these haemoglobinopathies.

The increased risk of parasite carriage among individuals that co-inherited α-thalassaemia, with HbAC or HbAS suggests a negative epistasis between α-thalassaemia and HbAC/HbAS in Ghana, as reported previously in a study in Kenya on negative epistasis associated with co-inheritance of HbAS and α-

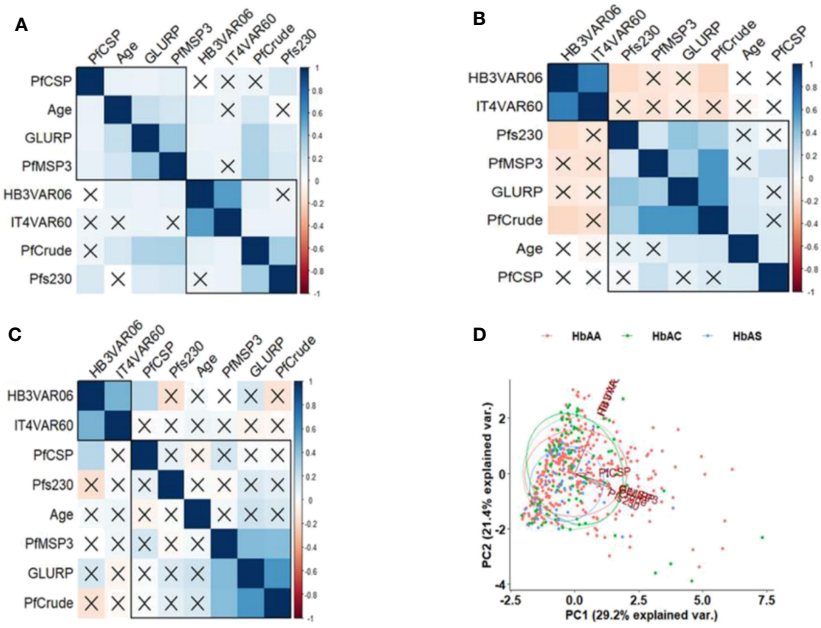

FIGURE 3
Correlation of IgG levels and age according to Hb phenotypes. Pearson's correlation matrixes of specific IgG levels and age in **(A)** HbAA, **(B)** HbAC, and **(C)** HbAS individuals. The values displayed are r values, deep blue colour shows strength of the variation among the variables. Insignificant correlations are crossed. **(D)** principal component analysis (PCA) based on the IgG levels to the antigens. The x-axis and y-axis on the PCA show the proportion of variance explained by Principal Component (PC) 1 and 2, respectively.

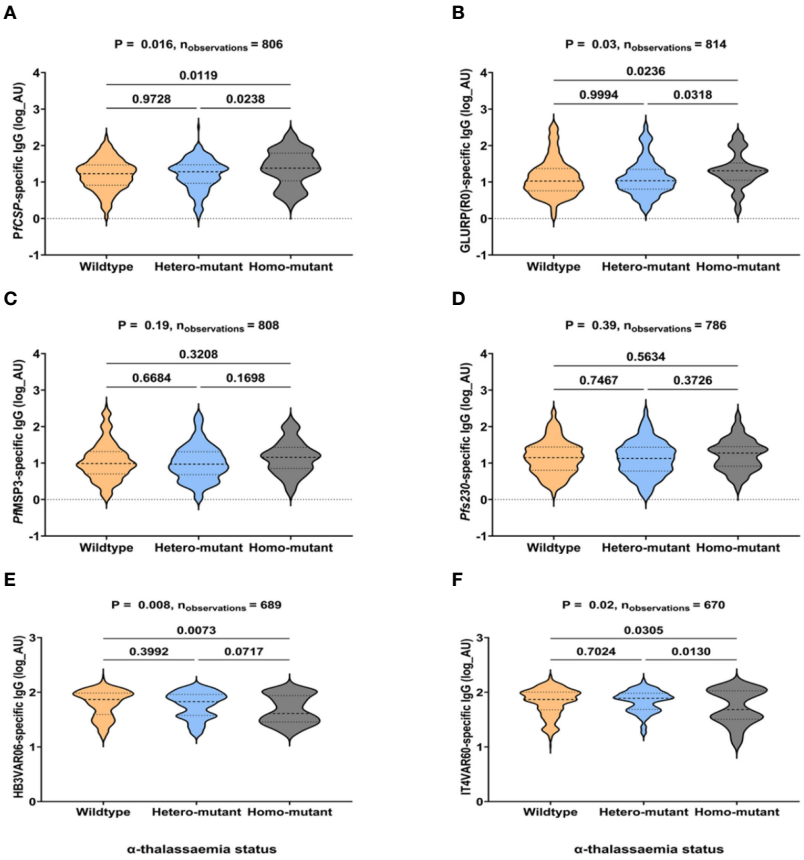

FIGURE 4
IgG levels to non-PfEMP1 and PfEMP1 antigens in individuals with α-thalassaemia. **(A–F)** shows total IgG responses to *Pf*CSP, GLURP(R0), *Pf*MSP3, *Pf*s230, HB3VAR06, and IT4VAR60, respectively. The x-axis is the α-thalassaemia status of participants (wildtype, heterozygous, and homozygous mutants). IgG levels are expressed in arbitrary log units (AU) on the y-axis. P-values were determined using Kruskal-Wallis test followed by D*unnett's* test for Multiple Comparisons.

thalassaemia (55). It is also consistent with reports of chronic parasite carriage in HbAS and HbAC individuals (56, 57). Previously, reduction in *Pf*EMP1 expression and cytoadhesion of HbAS erythrocytes has been reported (27); however, co-inheritance of HbAS with α-thalassemia might reverse this effect (58), which might explain the loss of protection observed *in vivo* (55). We observed no significant risk of parasite carriage among HbAS individuals co-inherited with the homozygous α-thalassaemia phenotype, this could be due to sample size in our cohort. The conflicting effects of the HbAS heterozygous and HbAS homozygous on parasite carriage could be that the AS-heterozygous α-thalassaemia co-inheritance supports preferential persistence of parasites, but AS-homozygous α-thalassaemia co-inheritance may retard parasite growth (58). However, this hypothesis was not tested in this work, we hope to test this in future studies.

Naturally acquired immunity to malaria is sustained by premunition (59), and the clinical and epidemiological consequences of reduced parasite exposure on protective immunity are reported, with rebounds of malaria in previously eliminated areas (60, 61). On the other hand, haemoglobinopathies might act like a 'double-edged' sword in malaria-endemic areas, operating against severe malaria on the one hand and promoting asymptomatic parasitaemia on the other. Hence, such chronic parasite carriage could facilitate parasite transmission (57) but also sustain naturally acquired protective immunity to malaria.

To assess the impact of these haemoglobinopathies on naturally acquired immunity, we measured antibody levels to several recombinant *P. falciparum* antigens spanning the various life cycle stages. The selected antigens have been associated with protection against malaria and are potential vaccine candidates (62–64). Here, we found higher IgG levels against *Pf*CSP and lower to IT4VAR60 in HbAS individuals than in HbAA and HbAC. Similarly, children carrying the homozygous α-thalassaemia mutation had significantly higher IgG levels to *Pf*CSP and GLURP but lower to HB3VAR06 and IT4VAR60 than the heterozygous α-thalassaemia and wildtype individuals.

The higher antibody responses observed to some malaria antigens measured (non-PfEMP1 antigens) in our study among the HbAS, HbAC compared to the wild type, and the homozygous α-thalassaemia agrees with previous studies, which found higher antibody responses among individuals with various Hb variants and these have been associated with clinical protection from malaria (31, 33, 65). This finding could also be associated with the higher

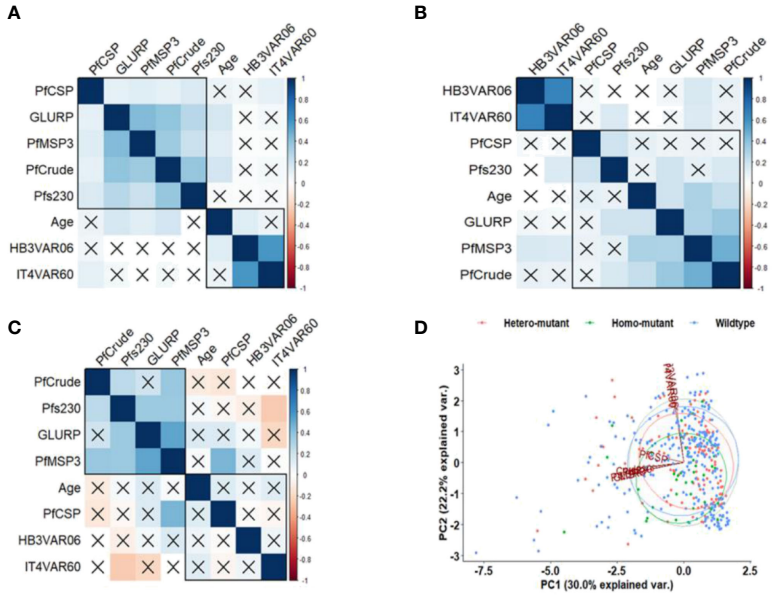

FIGURE 5
Correlation of IgG levels, age, and α-thalassaemia status. Pearson correlation matrixes of **(A)** wildtype, **(B)** heterozygous, and **(C)** homozygous mutant individuals. The values displayed are r values, deep blue colour shows strength of the variation among the variables. Insignificant correlations are crossed. **(D)** PCA based on the IgG levels to the antigens. The x-axis and y-axis on the PCA show the proportion of variance explained by Principal Component (PC) 1 and 2, respectively.

asymptomatic infection rate in haemoglobinopathy individuals, as chronic parasitaemia could boost antibody responses (46, 63, 66, 67). Studies have proposed that HbAS and HbAC have similar mechanisms of conferring protection through impaired cytoadhesion, which enhances the acquisition of protective IgG responses to clear parasites (31, 68). However, studies found no association with acquisition of protective IgG responses (69) or lower IgG levels (70) in HbAS compared to HbAA individuals, perhaps due to limited exposure to malaria. Since acquiring IgG antibodies only to merozoite antigens does not reduce the risk of malaria in HbAS individuals (69–71), other factors concerning transmission intensity and different repertoire of antigens should also be considered.

In contrast with our findings using two recombinant proteins, studies conducted in Gabon and Burkina Faso found higher antibody levels in HbAS children compared to HbAA to variant surface antigens expressed by clinical isolates (31, 68). However, no differences were found in Mali using a few PfEMP1 recombinant proteins (72). A recent study among HbAS and HbAC Beninese pregnant women showed no differences in the IgG levels to selected PfEMP1 variants (46, 73). Hence analysis with a broader range of PfEMP1 variants would be more informative. Less is known in α-thalassaemia, but our data indicate a lower exposure to those specific proteins used here. The differences in IgG levels to Pf-EMP1 could be due to transmission intensity.

We acknowledge that the nature of our study may bias the results with respect to protection in certain Hb variants, since a longitudinal study may provide a better estimate of the exposure status. However, this study confirmed the lack of protection for asymptomatic parasitaemia conferred by Hb variants and the increased risk of parasite carriage among children carrying on α-thalassaemia and HbAS or HbAC. Therefore, screening for these haemoglobinopathies in malaria-endemic communities is important to help reduce the risk it might pose to chronic parasite carriage. Differences in IgG levels to the antigens tested here also indicate changes in the exposure and potentially reduced exposure of *Pf*EMP1 on the infected erythrocytes of HbAS and α-thalassaemia individuals. Identifying *P. falciparum* antigens that would induce protective antibody responses in future vaccine development must consider these Hb variants in endemic populations since they influence naturally acquired immunity in these individuals.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This study was approved by the Ethics Review Committee of the Ghana Health Service (GHS; GHS-ERC 008/07/19) and by the Noguchi Memorial Institute for Medical Research (NMIMR) Institutional Review Board (Federal-wide Assurance FWA 00001824, NMIMR-IRB CPN 006/19). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

HL, ZS, ML-P, LH, GA and MO conceived and designed the study. HL, ZS and EK-B collected data. HL, ZS and EK-B performed laboratory experiments. HL and ZS analysed the data and wrote the manuscript with inputs from all authors. All authors contributed to the article and approved the submitted version.

Funding

This work was funded by DANIDA (grant 17 02 KU) and by the Independent Research Fund Denmark (grant 0134-00123B). HL and ZS were supported by a Postdoctoral Fellowship and a PhD scholarship from the DANIDA-sponsored Building Stronger Universities initiative grant (BSUIII-UG), respectively. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgments

We thank the parents of the participants for consenting to join this study, and the leaders and household heads for allowing us to enter their communities and homes to conduct this study. We are grateful for the support from GHS staff, our field research team, Alex Danso-Coffie, Nicholas Atsu, Naporo Mathias, and Bernard Tornyigah for technical support. We thank Michael Theisen (University of Copenhagen, Denmark), Asamoah Kusi, and Linda Eva Amoah (University of Ghana) for providing the GLURP, *Pf*CSP, *Pf*MSP3, and *Pfs*230 recombinant proteins, respectively.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/frhem.2023.1150134/full#supplementary-material

SUPPLEMENTARY FIGURE 1
IgG levels against crude antigens. **(A)** crude-specific IgG levels among HbAA, HbAC, and HbAS individuals. **(B)** crude-specific IgG levels among wildtype, heterozygous, and homozygous α-thalassaemia individuals. IgG responses are expressed in log arbitrary units (AU) on the y-axis. P-values were determined using Kruskal-Wallis test followed by Dunnett's test for Multiple Comparisons.

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