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MAJOR ARTICLE

PfEMP1-Specific Immunoglobulin G Reactivity Among Beninese Pregnant Women With Sickle Cell Trait

Mary Lopez-Perez,[1] Firmine Viwami,[2,3] Zakaria Seidu,[1,4,5] Anja T. R. Jensen,[1] Justin Doritchamou,[3,a] Nicaise Tuikue Ndam,[2,3,4] and Lars Hviid[1,6]

[1]Centre for Medical Parasitology, Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark, [2]UMR 261 MERIT, Université de Paris, Institut de Recherche pour le Développement, Paris, France, [3]Institut de Recherche Clinique du Benin, Abomey Calavi, Benin, [4]Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana, [5]West Africa Centre for Cell Biology of Infectious Pathogens (WACCBIP), Department of Biochemistry, Cell and Molecular Biology, University of Ghana, Accra, Ghana, [6]Centre for Medical Parasitology, Department of Infectious Diseases, Rigshospitalet, Copenhagen, Denmark

Background. Sickle cell trait (HbAS) protects against severe *Plasmodium falciparum* malaria but not against placental malaria (PM). In this study, *P falciparum* erythrocyte membrane protein (PfEMP1)-specific antibodies were measured in HbAA and HbAS Beninese pregnant women as a proxy of exposure to specific PfEMP1 variants.

Methods. Plasma samples collected at delivery from 338 HbAA and 63 HbAS women were used to measure immunoglobulin (Ig)G levels to 6 recombinant PfEMP1 proteins and 3 corresponding native proteins expressed on the infected erythrocyte (IE) surface. Immunoglobulin G-mediated inhibition of VAR2CSA$^+$ IEs adhesion to chondroitin sulfate A (CSA) was also tested.

Results. Levels of PfEMP1-specific IgG were similar in the 2 groups, except for native IT4VAR09 on IEs, where IgG levels were significantly higher in HbAS women. Adjusted odds ratios for women with positive IgG to HB3VAR06 and PFD1235w suggest a lower risk of infection with these virulent variants among HbAS individuals. The percentage of IEs binding to CSA did not differ between HbAA and HbAS women, but it correlated positively with levels of anti-VAR2CSA and parity. Women with PM had lower levels of anti-VAR2CSA-specific IgG and lower IgG-mediated inhibition of IE adhesion to CSA.

Conclusions. The findings support similar malaria exposure in HbAA and HbAS women and a lack of HbAS-dependent protection against placental infection among pregnant women.

Keywords. Benin; PfEMP1; placental malaria; *Plasmodium falciparum*; sickle cell trait.

Malaria continues to be an important public health problem in the developing world. Despite intensive global efforts, there has been no major change in the burden of malaria worldwide in the past 7 years. In 2019, an estimated 229 million cases of malaria and 409 000 deaths occurred worldwide. In the same year, 12 million pregnant women in Africa were exposed to *Plasmodium falciparum* infection during pregnancy [1], a major cause of mother-offspring severe morbidity and mortality [2].

Although substantial protective immunity is acquired during childhood and adolescence, pregnant women are at high risk of malaria [3]. Protection is mainly antibody mediated, and members of the *P falciparum* erythrocyte membrane protein 1 (PfEMP1) family expressed on the surface of the infected erythrocytes (IEs) are important targets [3]. PfEMP1 enables adhesion of IEs to endothelial host receptors such as intercellular adhesion molecule 1 (ICAM-1) [4, 5] and endothelial protein C receptor (EPCR) [5, 6]; phenotypes that have been associated with severe malaria. In pregnant women, the IEs bind to oncofetal chondroitin sulfate A (CSA) in the placenta [7, 8] via VAR2CSA-type PfEMP1 [9, 10], a condition known as placental malaria (PM). Because oncofetal CSA expression is normally restricted to the placenta, and because VAR2CSA is immunologically distinct from other types of PfEMP1, women become highly susceptible to malaria when they get pregnant, particularly for the first time, despite clinical immunity acquired earlier in life. Indeed, antibody levels to VAR2CSA increase with parity [11–13], including antibodies that inhibit the binding of IEs to CSA [14].

Malaria parasites have exerted a strong evolutionary pressure on the human genome, and selected for host polymorphisms that protect against *P falciparum* malaria [15]. Sickle hemoglobin (HbS) is a prominent example that results from a single point mutation within codon 6 of the β-globin gene of adult hemoglobin ([HbA] wild type). Individuals who are homozygous for hemoglobin S (HbSS) suffer from sickle cell disease, whereas heterozygous carriers, ie, sickle cell trait

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[a]Present affiliation: Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Disease, National Institute of Health, Bethesda, Maryland, USA.

Correspondence: Mary Lopez-Perez, PhD, Panum Institute 07-11-38, Blegdamsvej 3B, 2200 Copenhagen N, Denmark (mlopez@sund.ku.dk).

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(HbAS) individuals, are generally asymptomatic. In vitro, *P falciparum* invasion of HbAS erythrocytes seems unimpaired. However, reduced parasite growth has been observed at the low oxygen tensions [16–18] prevailing in most postcapillary venules, where IE cytoadhesion mainly occurs. Likewise, reduced cytoadhesion of HbAS IEs to human microvascular endothelial cells has been demonstrated [19, 20] and associated with abnormal and reduced display of PfEMP1 on the IE surface [19, 21–23], which could limit their sequestration in the microcirculation. Multiple studies in Africa have shown that HbAS provides significant protection against severe malaria (up to 90%) [24], but it does not protect against asymptomatic and low-density infection [25]. Moreover, it seems that HbAS does not protect against PM, because the prevalence of placental *P falciparum* infection [26] and the plasma levels of VAR2CSA-specific immunoglobulin (Ig)G [27] are similar in HbAS and HbAA women. Thus, it appears that both groups are equally exposed to IEs expressing VAR2CSA. We hypothesized that HbAS selectively protects against PfEMP1 variants causing IEs sequestration in low-oxygen tissues such as those associated with severe disease in children. Under that hypothesis, HbAS would not be expected to protect against PfEMP1 variants causing IEs sequestration in high-oxygen tissues, for example, VAR2CSA mediating IE sequestration in the placenta. To test this, the quantity and profile of the IgG antibody response to PfEMP1 variants were measured in a cohort of Beninese pregnant women with or without sickle cell trait as a proxy of exposure to those PfEMP1-specific variants.

METHODS

Patient Consent Statement

The original study, from which the samples used here were obtained, was approved by the Comité Consultatif de déontologie et d'éthique of the Institut de Recherche pour le Développement ([IRD] Marseille, France) and the Comité d'éthique de la Faculté des Sciences de la Sante (Université d'Abomey Calavi, Benin; FSS 026/2007/CE/FSS/UAC). Declaration of free willingness to participate in the study and written informed consent was obtained from all study participants before enrollment.

Study Site and Participants

Stored plasma samples collected within the frame of STOPPAM study conducted in Comè District, Benin, between 2008 and 2011 were used [28]. A detailed description of the study area has been given elsewhere [28]. In the original cohort, 15.8% of women carried the HbAS genotype [29]. In this study, plasma samples collected at delivery from 401 randomly selected women with HbAA or HbAS were analyzed. Plasma samples from 8 nonpregnant Danish women without malaria exposure and a pool of nonpregnant Ghanaian women previously exposed to *P falciparum* infection during pregnancy were included as negative and positive controls, respectively.

Women enrolled in the STOPPAM study received 2 doses of sulfadoxine-pyrimethamine as intermittent preventive treatment during pregnancy (IPTp) per national guidelines. At enrollment, each antenatal visit, and unscheduled visits for health reasons, a rapid diagnostic test (Parascreen; Zephyr Biomedicals) for *P falciparum* infection was performed on capillary blood. Samples were also tested by real-time polymerase chain reaction (PCR) assay to detect submicroscopic *P falciparum* infection. At delivery, blood and biopsies from placenta were collected and used for diagnosis of PM [28].

Recombinant Parasite Proteins

The entire ectodomain of the VAR2CSA-type PfEMP1 protein IT4VAR04 and the non-VAR2CSA-type PfEMP1 proteins HB3VAR06, IT4VAR09, and IT4VAR60 were produced in baculovirus-transfected Sf9 insect cells, as described previously (Figure 1) [30]. An additional recombinant VAR2CSA protein expressed in suspension-adapted CHO cells (ExpiCHO; ThermoFisher Scientific) was also used [31]. Finally, 2 recombinant DBLβ domains containing an ICAM-1 binding motif, HB3VAR34 and PFD1235w [32, 33], and the domain R0 of glutamate-rich protein (GLURP) [34], produced in *Escherichia coli*, were included.

Antibody Reagents

For in vitro selection of particular PfEMP1 proteins expressed on the IE surface (see below), the human monoclonal antibody PAM1.4, specific for a conformational epitope in several VAR2CSA-type PfEMP1 proteins [35], was used. In addition, rabbit antisera raised against HB3VAR06 and IT4VAR09 [30] were used to select IEs expressing the corresponding non-VAR2CSA-type PfEMP1 on their surface.

Assessment of the Immunoglobulin G Antibody Response to Recombinant Proteins

Immunoglobulin G reactivity against recombinant proteins was measured by enzyme-linked immunosorbent assay as described elsewhere [31]. In brief, 96-well, flat-bottom, microtiter plates (Nunc MaxiSorp; Thermo Fisher Scientific) were coated with 100 ng/well recombinant protein in Dulbecco's phosphate-buffered saline ([PBS] Sigma-Aldrich) and incubated overnight at 4°C. After blocking (washing buffer with 1% Ig-free bovine serum albumin [BSA]), plasma samples (1:400) were added in duplicate, followed by horseradish peroxidase-conjugated rabbit antihuman IgG (1:3000; Dako). Bound antibodies were detected by adding TMB PLUS2 (Eco-Tek), and the reaction was stopped by adding 0.2 M H_2SO_4. The optical density (OD) was read at 450 nm (VERSAmax microplate reader; Molecular Devices), and the specific antibody levels were calculated in

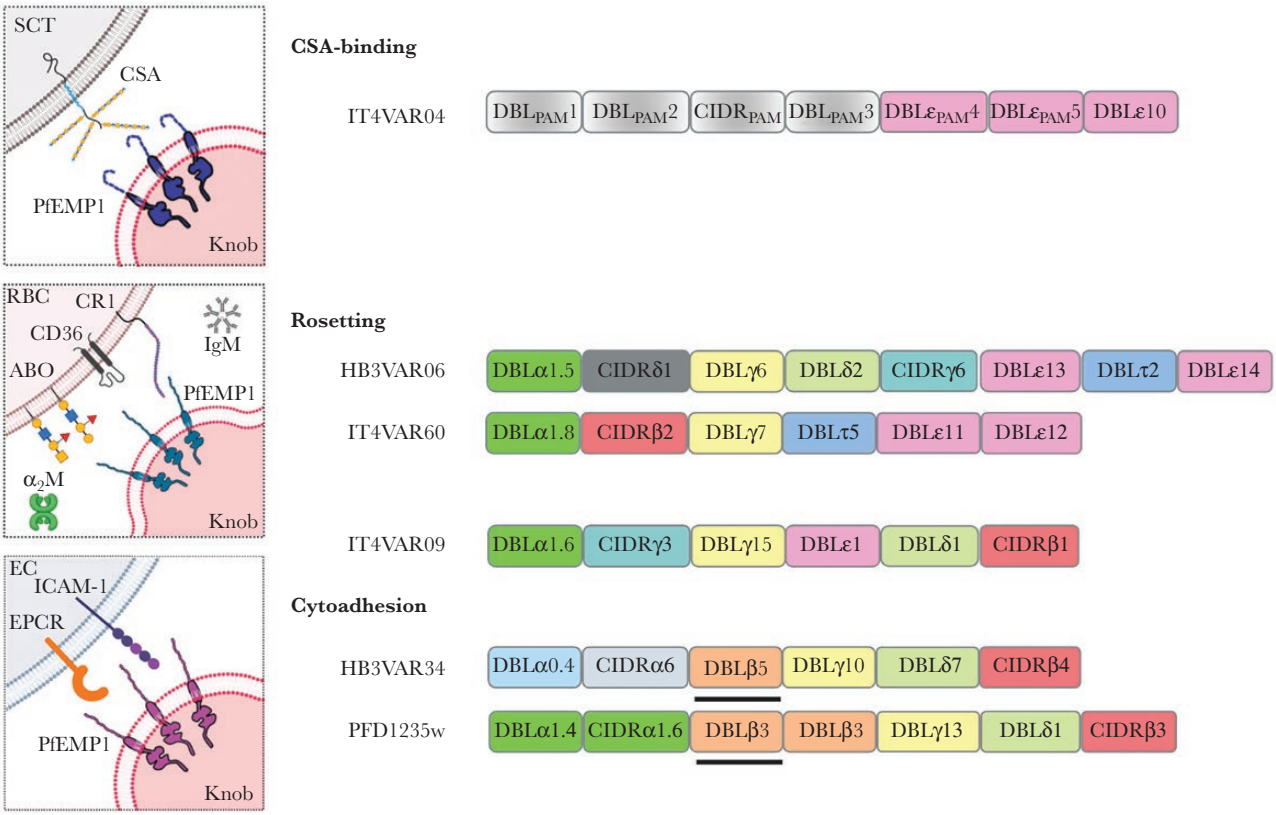

Figure 1. Domain structures of the PfEMP1 proteins. IT4VAR04 is a VAR2CSA-type PfEMP1 protein involved in chondroitin sulfate A (CSA)-binding, causing placental malaria [9, 10]. HB3VAR06 and IT4VAR60 bind uninfected erythrocytes (rosetting), considered a marker of parasites causing severe malaria complications. HB3VAR34 and PFD1235w are involved in cytoadhesion via intercellular adhesion molecule 1 (ICAM-1) and endothelial protein C receptor (EPCR) [5]. IT4VAR09 cause rosetting and binds to human brain microvascular endothelial cells [50]. Recombinant PfEMP1 single constructs used in the present study are indicated by underlining. Domain structures are color-coded as shown in https://services.healthtech.dtu.dk/service.php?VarDom-1.0. α_2M, α_2-macroglobulin; ABO, ABO blood group; CR1, complement receptor 1; EC, endothelial cell; IgM, immunoglobulin M; RBC, red blood cell; SCT, syncytiotrophoblasts. Created with BioRender.

arbitrary units (AUs) using the equation $100 \times [(OD_{SAMPLE}\text{-}OD_{BLANK})/(OD_{POS.CTRL}\text{-}OD_{BLANK})]$, essentially as described elsewhere [31]. Negative cutoff values were calculated as the mean AU values plus 2 standard deviations (SD) obtained with the negative control samples described above. Individuals were considered responders if their specific antibody level was higher than the cutoff. The breadth of antibody response was defined as the number of antigens recognized by an individual [36].

Immunomagnetic Selection of *Plasmodium falciparum*-Infected Erythrocytes

Erythrocytes infected by late-stage IT4 parasites were selected for surface expression of VAR2CSA or IT4VAR09 using protein A-coupled DynaBeads coated with PAM1.4 or specific rabbit antiserum, as described previously [37]. A similar approach was used to select HB3-IEs for surface expression of HB3VAR06, using a specific rabbit antiserum. Transcription of the relevant *var* genes and IE surface expression of the corresponding PfEMP1 protein were monitored by quantitative real-time PCR and flow cytometry [37], respectively. The genotypic identity of the parasites and the absence of *Mycoplasma* contamination using the MycoAlert Mycoplasma Detection Kit (Lonza) were verified regularly.

Antibody Reactivity With the Surface of *Plasmodium falciparum*-Infected Erythrocytes

Immunoglobulin G reactivity of plasma samples (1:20) against intact and unfixed late-stage IEs expressing VAR2CSA, IT4VAR09, or HB3VAR06 were analyzed by flow cytometry as described elsewhere [37]. A Beckman Coulter FC500 flow cytometer was used for data acquisition, and FlowLogic software (Inivai Technologies, Mentone, Australia) was used for data analysis. To normalize data between plates, IgG binding to IEs is presented as normalized median fluorescence intensity (nMFI) using the equation $MFI_{SAMPLE}/MFI_{NEG.CTRL}$.

Antibody-Mediated Inhibition of Infected Erythrocyte Adhesion to Chondroitin Sulfate A

The inhibition of IE adhesion to CSA by plasma samples collected at delivery was evaluated using a static adhesion assay as described elsewhere [38]. In brief, a petri dish was coated overnight with PBS containing 1% BSA and 5 µg/mL decorin (chondroitin sulfate proteoglycan [CSPG]; Sigma-Aldrich).

After blocking, a 20% parasite suspension of late-stage VAR2CSA IEs was incubated with plasma (1:5) or soluble CSA (500 μg/mL). Nonadhering erythrocytes were removed using an automated washing system. The remaining bound cells were fixed with 1.5% glutaraldehyde in PBS and stained with 10% Giemsa. Adhering cells were quantified by light microscopy as the number of IEs bound/mm^2. The positivity threshold was defined as the mean plus 3 SD obtained with plasma from 30 pregnant French women without malaria exposure.

Statistical Analysis

Data were analyzed and plotted using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA). The number of samples is reported in each figure. The Mann-Whitney *U* test was used to compare 2 groups. Spearman's rank correlation (r_s) was used to assess the association between numeric variables. Fisher's exact test was used to compare proportions. Multiple linear and logistic regression models were used to evaluate the effect of potential confounders on the relationship between antibody responses and relevant independent factors. Models used for analysis were adjusted by hemoglobin genotype, parity, infection during pregnancy, and previous malaria exposure. $P < .05$ were considered statistically significant.

RESULTS

Study Population

In this study, plasma samples collected at delivery from 401 pregnant women were analyzed. Of these, 63 (15.7%) carried the HbAS genotype. Overall, the women had a median age of 28 years (interquartile range, 22–32 years) and 4 children on average (range, 1–12 children). Forty-two percent of the women had at least 1 malaria episode during the current pregnancy, and 11.7% had PM. Women with PM had a higher number of infections during pregnancy (median 2 vs 0; $P < .0001$; Mann-Whitney test). No significant differences were observed in the demographic and clinical characteristics of the 2 groups (Table 1).

Table 1. Characteristics of Pregnant Women According to Hemoglobin Genotype

Characteristics[a]	HbAA (n = 338)	HbAS (n = 63)	*P* Value[b]
Age (years)	28 [22–32]	27 [22–32]	.86
Parity	3 [2–5]	3 [2–5]	.76
Infection during pregnancy	0 [0–1]	0 [0–1]	.39
Primigravidae	49 (14.5)	12 (19)	.34
Infection at enrolment (yes)	29 (8.6)	6 (9.5)	.43
Infection during pregnancy (yes)	132 (30.1)	23 (36.5)	.78
Placental malaria[c] (yes)	34 (11.3)	8 (13.8)	.66
Parasite infection[d] (yes)	143 (42.3)	25 (39.7)	.78

[a]Median and interquartile range are shown in brackets. Number and percentages are shown in parenthesis.

[b]*P* value using Mann-Whitney test for numerical variables and Fisher's exact test for proportions.

[c]Placental malaria data were available for 358 women (300 HbAA and 58 HbAS).

[d]Women having at least 1 of the following: infection at enrollment, during pregnancy, or placental malaria.

Similar Levels of VAR2CSA-Specific Immunoglobulin G in HbAA and HbAS Pregnant Women

Previous studies reported similar IgG levels to VAR2CSA-type PfEMP1 proteins [27] and prevalence of *P falciparum*-IEs in the placenta [29] in HbAS and HbAA pregnant women, suggesting that both groups are equally exposed to VAR2CSA antigen, despite the protection against severe malaria enjoyed by HbAS individuals. As reported previously [27], levels of VAR2CSA-specific IgG among the Beninese women studied here did not differ significantly between HbAA and HbAS individuals (Figure 2A and Supplementary Figure 1A). A very high positive correlation for VAR2CSA-specific IgG levels against recombinant proteins expressed in 2 different systems was found ($r_s = 0.96$; $P < .0001$) (Supplementary Figure 1B), documenting that the findings were not affected by the platform used to generate the recombinant full-length PfEMP1 proteins, as has previously been found in other cohorts [31]. Therefore, further analyses used VAR2CSA expressed in insect cells only.

To support the findings using recombinant proteins, we next measured IgG reactivity to *P falciparum*-IEs previously selected in vitro to express native VAR2CSA. All 63 HbAS samples and a random subset of 110 HbAA plasma samples were tested by flow cytometry. No statistically significant differences in the reactivity to native VAR2CSA were observed between HbAA and HbAS (Figure 2B). Antibody levels to the native and recombinant VARCSA antigen correlated positively ($r_s = 0.44$; $P < .001$).

Levels of anti-VAR2CSA were positively correlated with parity ($r_s = 0.26$; $P < .001$), and the association remained after adjusting for hemoglobin genotype and previous malaria exposure ($\beta = 0.22$; $P < .0001$, respectively). Likewise, IgG levels to native VAR2CSA were associated with parity and infection during pregnancy ($\beta = 0.27$ and $\beta = 0.20$ [$P < .001$], respectively).

No Impairment in the Ability of VAR2CSA-Specific Immunoglobulin G From HbAS Women to Inhibit Adhesion of Infected Erythrocytes to Chondroitin Sulfate A

Abnormal display of VAR2CSA on [21, 23], and low antibody binding to [27], the surface of HbAS IEs have been documented previously. Whether this affects the functionality of antibodies elicited in response to these antigens has not been reported. Hence, we evaluated IgG-mediated inhibition of IE adhesion to CSA using a static adhesion assay as described elsewhere [38]. The percentage of IEs binding to CSA in the presence of plasma did not differ significantly between HbAA and HbAS women (Figure 3A). Overall, 72% of the plasma samples inhibited

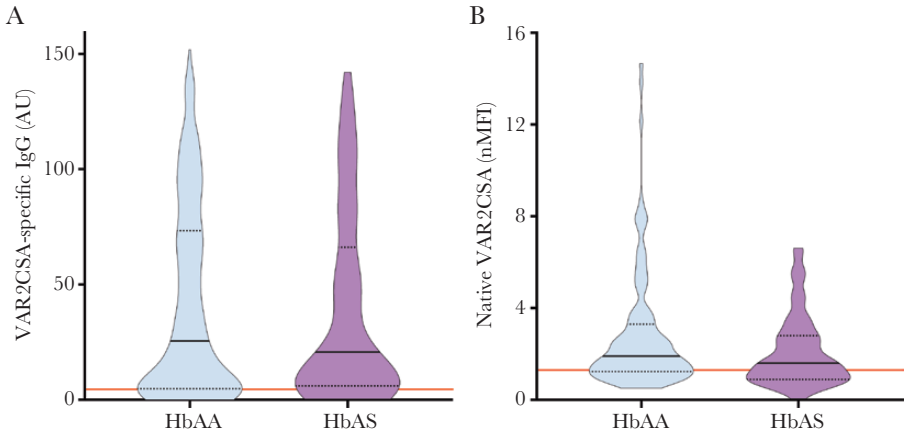

Figure 2. Antibody response to VAR2CSA. (A) Immunoglobulin (Ig)G levels against a full-length recombinant VAR2CSA protein in HbAA (n = 338) and HbAS (n = 63) were determined by an enzyme-linked immunosorbent assay. (B) The IgG levels specific for the native VAR2CSA expressed on the surface of infected erythrocytes and measured by flow cytometry in HbAA (n = 110) and HbAS (n = 63). The violin plots show medians (solid line) and interquartile ranges (dotted line). Solid red line indicates the negative cutoff values (mean values plus 2 standard deviation with negative controls). Values are expressed in arbitrary units (AU) or normalized mean fluorescence intensity (nMFI).

CSA adhesion (above the positivity threshold), with no differences between HbAA and HbAS (P = .88; Fisher's exact test). Percentage of inhibition correlated positively with levels of anti-VAR2CSA (Figure 3B) and parity (Figure 3C). Association with antibody levels remained after adjusting for hemoglobin genotype and the number of infections during pregnancy (β = 0.30; P < .0001). Lower, but nonsignificant, levels of anti-VAR2CSA (17.6 vs 27.0; P = .34) and percentage of inhibition of CSA adhesion (42% vs 46%; P = .95) were observed in women with PM in comparison with non-PM, respectively.

Similar Levels of Immunoglobulin G to Malaria Antigens Other Than VAR2CSA in HbAS and HbAA Women

On the assumption that HbAS protects specifically against PfEMP1 variants causing IEs sequestration in low-oxygen tissues, including those associated with severe disease in children, we next evaluated non-VAR2CSA-specific IgG responses in pregnant women with HbAA and HbAS. Immunoglobulin G levels against 3 full-length recombinant PfEMP1 proteins, HB3VAR06 (P = .11), IT4VAR09 (P = .23), and IT4VAR60 (P = .55) not restricted to parasites infecting pregnant women [31, 39], were not significantly different between HbAS and HbAA women (Figure 4A).

Likewise, no differences were found for levels of IgG specific for 2 ICAM-1-binding DBLβ domains, HB3VAR34 and PFD1235w (P = .80 and P = .66, respectively) (Figure 4B). As a proxy of previous malaria exposure, we also tested the antibody response to GLURP, a merozoite-specific antigen expressed in asexual blood stages. No differences (P = .20) were observed among groups of study (Figure 4B), suggesting that all

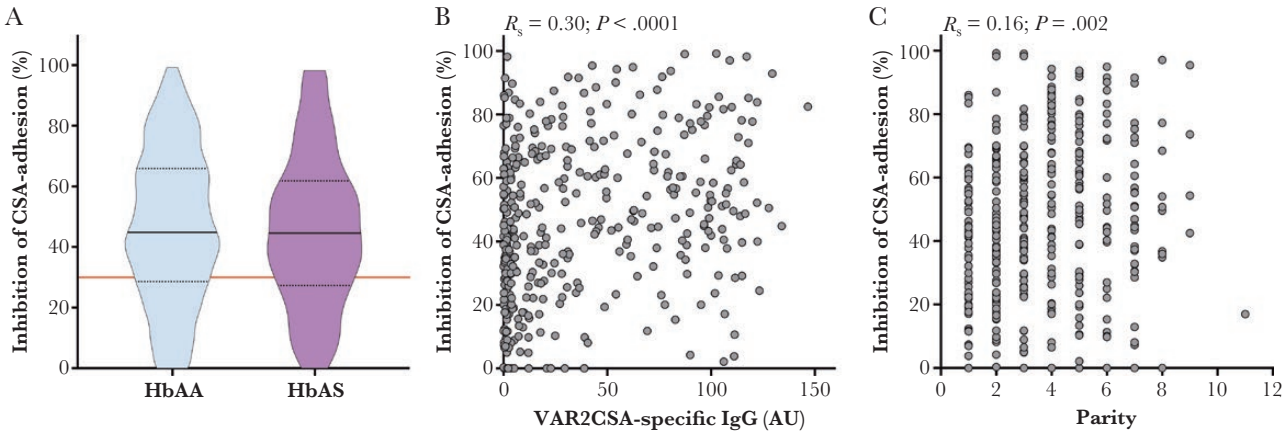

Figure 3. VAR2CSA-specific immunoglobulin (Ig)G from HbAS and HbAA women inhibit adhesion of infected erythrocytes (IEs) to chondroitin sulfate A (CSA). (A) Percentage of inhibition of IEs adhesion to CSA in HbAA (n = 325) and HbAS (n = 60) pregnant women at delivery. The threshold of positivity is indicated with a solid red line. The violin plot shows the median (solid line) and interquartile ranges (dotted line). P = .69 using Mann-Whitney test. (B) VAR2CSA-specific IgG levels and (C) parity are positively correlated with the percentage of inhibition of IEs adhesion to CSA. Spearman's rank correlation (r_s) and P values are shown.

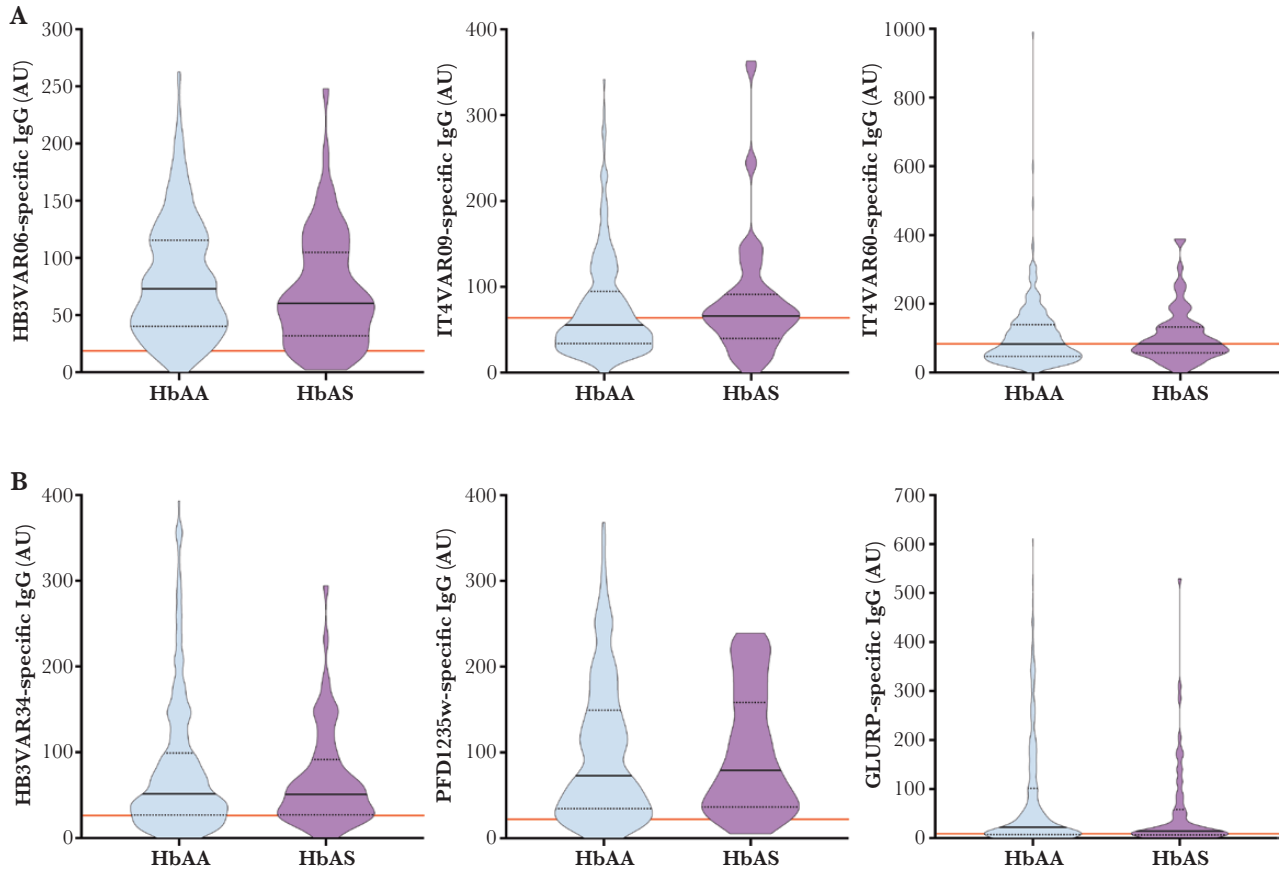

Figure 4. Antibody response to recombinant non-VAR2CSA PfEMP1 and GLURP. Immunoglobulin G (IgG) levels against (A) full-length recombinant proteins HB3VAR06, IT4VAR09, and IT4VAR60, or (B) single domains, HB3VAR34 DBLβ, PFD1235w DBLβ, and R0 of GLURP in HbAA (n = 338) and HbAS (n = 63) were determined by an enzyme-linked immunosorbent assay. The violin plots show medians (solid line) and interquartile ranges (dotted line). Solid red line indicates the negative cutoff values. $P > .05$ using Mann-Whitney test. In all panels, the values are expressed in arbitrary units (AU).

women had been similarly exposed to *P falciparum*. Antibody levels to the tested antigens were associated with having an infection during pregnancy, and these associations remained after adjusting for hemoglobin genotype and parity.

Additional analysis of the antibody response to recombinant antigens supported similar responses in HbAA and HbAS individuals ($F = 0.46$; $P = .52$) (Supplementary Figure 2A). The breadth of the antibody response, defined as the sum of recognized antigens per individual, was not significantly different between HbAA and HbAS individuals, with a median of 5 in both groups ($P = .85$; Mann-Whitey test) (Supplementary Figure 2A). Likewise, no significant differences in the percentage of positive responders were observed between HbAA and HbAS ($P > .05$; Fisher's exact test). A multiple logistic regression model was used to evaluate the effect of HbAS on positive responses to the tested antigens (Supplementary Figure 3). The adjusted odds ratio <1 for HB3VAR06 and PFD1235w with borderline significance suggests that HbAS individuals were less likely to be positive for those antigens after adjusting for parity and having an infection during pregnancy.

We used a subset of 173 plasma samples to measure IgG reactivity to IT4VAR09 and HB3VAR06 protein expressed on the surface of IEs. No differences in the reactivity to native HB3VAR06 were observed between HbAA and HbAS ($P = .68$) (Figure 5A). However, the reactivity to native IT4VAR09 was significantly higher in HbAS women ($P = .005$) (Figure 5B). This association remained after adjusting for parity, infection during pregnancy, and previous malaria exposure ($\beta = 0.34$; $P < .001$). Antibody levels to the native and recombinant antigen correlated positively among the samples (Supplementary Figure 4).

DISCUSSION

Sickle cell trait (HbAS) is associated with protection from severe *P falciparum* malaria [24, 40]. This confers a selective advantage over the wild type (HbAA) and offsets the disadvantage caused by sickle cell disease (HbSS), which has substantial morbidity and mortality. HbAS individuals are thus infected by *P falciparum* at similar rates as HbAA individuals, but infections rarely proceed to severe malaria. The protective mechanism is

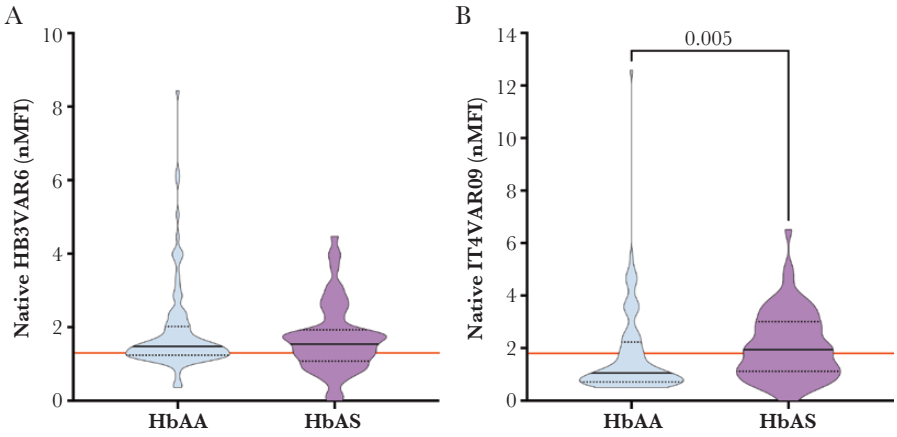

Figure 5. Antibody response to native PfEMP1. Specific immunoglobulin G levels to (A) HB3VAR06 and (B) IT4VAR09 expressed on the surface of infected erythrocytes and measured by flow cytometry in HbAA (n = 110) and HbAS (n = 63) samples. The violin plots show medians (solid line) and interquartile ranges (dotted line). Solid red line indicates the negative cutoff values. *P* values using Mann-Whitney test are also shown. Values are expressed in normalized mean fluorescence intensity (nMFI).

unclear but appears to be related to HbAS erythrocytes' inability to sustain parasite growth at the low oxygen tension generally preferred by *P falciparum* [18]. Despite the general protection against severe malaria afforded to HbAS individuals, the prevalence of peripheral *P falciparum* parasitemia [41] and PM [26] is similar between HbAA and HbAS pregnant women. Moreover, no protection for poor pregnancy outcomes associated with malaria in pregnancy was observed in Malawi and Benin [26, 29]. Previous studies showed that the degree of protection in HbAS individuals increases with age [25, 42], suggesting that acquired immunity to malaria in those individuals plays an important role. However, neither the magnitude nor the breadth of *P falciparum*-specific IgG appears to differ between HbAS and HbAA children [43]. Comparable plasma levels of VAR2CSA-specific IgG in HbAA and HbAS pregnant women were also recently reported [27]. However, IgG specific for PfEMP1 variants causing IEs sequestration in low-oxygen tissues such as those associated with severe disease have not been examined so far in this context.

In agreement with Chauvet et al [27], we found similar levels of VAR2CSA-specific IgG in HbAA and HbAS pregnant women from Benin. Likewise, infection during pregnancy and PM were equally frequent, suggesting similar exposure of the groups and supporting the hypothesis of a lack of HbAS-mediated protection in pregnant women. Whereas the antibody response to VAR2CSA at delivery might both be a marker of infection and protection [44], the ability of IgG to inhibit adhesion of IEs to CSA is considered to be an important protective mechanism [14, 45, 46]. Thus, a high degree of IgG-mediated inhibition of IE-binding to CSA has been associated with lowered risk of PM, premature birth, and low birthweight [46]. Data in this study show that VAR2CSA-specific IgG is a mixture of neutralizing (IE adhesion-inhibitory function) and nonneutralizing antibodies, but the data did not reveal any indication that the inhibition activity of such IgG is different between HbAS and HbAA women.

Neither the magnitude nor the breadth of the IgG response to non-VAR2CSA recombinant antigens differed between HbAS and HbAA women, although levels of IgG specific for HB3VAR06, a rosetting variant, tended to be lower among HbAS women than among HbAA women. Adjusted odds ratio for individuals with positive IgG responses to HB3VAR06 and PFD1235w (a variant associated with ICAM-1 and EPCR binding [5]) also tended to be lower in HbAS. No other *P falciparum* asexual-stage antigens were tested because several studies addressing this question did not find differences in the prevalence or levels of antibodies, at least in children [43, 47, 48]. The findings support similar exposure to the malaria parasites involved in severe malaria. Moreover, finding higher levels of antibodies in women with any kind of infection during pregnancy indicates boosting of previously acquired antibody repertoires.

On the other hand, reactivity to native IT4VAR09 protein expressed on the surface of IEs was significantly higher in HbAS women. In previous studies, a higher IgG antibody response directed to variant surface antigens expressed on IEs was reported in Gambian [48] and Gabonese children [49] with HbAS. It is interesting to note that IT4VAR09-expressing parasites can bind uninfected erythrocytes (rosetting) and to human brain microvascular endothelial cells [50]. However, the study population type and the cross-sectional nature of the present study do not allow any definitive conclusion regarding current or previous protection from severe malaria in the pregnant women studied.

Chauvet et al [27] reported significantly lower IgG binding to HbAS-IEs than to HbAA-IEs, in line with the aberrant expression of VAR2CSA on the surface of HbAS-IEs [21, 23]. Whether donor HbAS-IEs expressing diverse PfEMP1 variants are also differentially recognized by the same donor

plasma should be addressed in future studies. Despite the relatively large size of the study, no statistical significance was observed. This is probably an inherent consequence of the substantial interclonal variability of PfEMP1 antigens, which may be challenging to overcome. However, its consequences for the statistical power of studies of this kind may be ameliorated by adding additional VAR2CSA and non-VAR2CSA variants.

CONCLUSIONS

Placental malaria is a severe form of *P falciparum* malaria, because it is the direct cause of substantial mortality and severe morbidity among the offspring of pregnant women with PM. This study confirms evidence regarding the lack of protection against malaria in pregnancy and PM in women with HbAS, despite the protection they enjoy against other forms of severe *P falciparum* malaria morbidity. In this study, IgG responses to a limited set of PfEMP1 proteins were measured. HbAS-IEs have been shown to have abnormal display [21] and low copy number [23] of VAR2CSA molecules per cell. It appears likely that similar alterations occur with other PfEMP1 proteins, and it is reasonable to expect that hemoglobinopathies like HbAS modulate the acquisition of PfEMP1-specific IgG-quantitatively and/or qualitatively. Since the differences could be more evident in *P falciparum*-exposed children, we are currently investigating this hypothesis using a larger panel of PfEMP1 variants.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Author contributions. M. L.-P. and L. H. conceived and designed the study. M. L.-P., F. V., Z. S., and J. D. carried out the experiments. A. T. R. J. provided recombinant proteins. N. T. N. designed the original study and carried out the fieldwork. M. L.-P. analyzed data. M. L.-P. and L. H. wrote the manuscript. All authors reviewed and approved the final manuscript.

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