The Journal of Infectious Diseases

BRIEF REPORT

The Rapid and Spontaneous Postpartum Clearance of *Plasmodium falciparum* Is Related to Expulsion of the Placenta

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Parasitemia among pregnant women with protective immunity to *Plasmodium falciparum* malaria is often dominated by VAR2CSA-positive infected erythrocytes (IEs). VAR2CSA mediates sequestration of IEs in the placenta. We hypothesized that the previously observed spontaneous postpartum clearance of parasitemia in such women is related to the expulsion of the placenta, which removes the sequestration focus of VAR2CSA-positive IEs. We assessed parasitemias and gene transcription before and shortly after delivery in 17 Ghanaian women. The precipitous decline in parasitemia postpartum was accompanied by selective reduction in transcription of the gene encoding VAR2CSA. Our findings provide a mechanistic explanation for the earlier observation.

Keywords. acquired immunity; VAR2CSA; pfEMP1; placental malaria; *Plasmodium falciparum*; pregnancy; *var* genes; variant-specific immunity.

Residents of areas with stable transmission of *Plasmodium falciparum* parasites acquire substantial protective immunity to malaria in childhood, reviewed in reference [1]. *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) is a family of parasite antigens, encoded by approximately 60 *var* genes per genome, and mediating sequestration of mature infected erythrocytes (IEs) to host vascular receptors, reviewed in reference [2]. Despite the importance of PfEMP1 in acquired protective immunity, women in such areas nevertheless become highly susceptible to malaria during pregnancy. This has been related to infections with parasites expressing VAR2CSA-type PfEMP1, which leads to accumulation of IEs in the intervillous space, causing placental malaria [2, 3].

VAR2CSA mediates adhesion of IEs to low-sulfated chondroitin sulfate (CSA), a host receptor that is normally restricted to the placenta [4]. Receptor-specific sequestration of IEs is an important parasite survival strategy, as it prevents splenic destruction of mature IEs [2]. VAR2CSA-positive IEs are therefore at a huge survival disadvantage in nonpregnant hosts, and VAR2CSA-specific immunoglobulin G (IgG) is not acquired before the first pregnancy [2, 3]. This explains the high susceptibility of pregnant women (and particularly primigravidae) to *P. falciparum* malaria, even in areas with stable parasite transmission, where substantial protective immunity is acquired well before reaching childbearing age [3]. *P. falciparum* infections in pregnant women are indeed often dominated by parasites expressing VAR2CSA [5], although parasites expressing other PfEMP1 types (and presumably sequestering outside the placenta) are also commonly found [6]. On this basis, we propose that 2 fundamentally different types of malaria in pregnant women can be defined. One is placental malaria, which is caused by parasites expressing VAR2CSA and which occurs mainly in women of low parity without substantial VAR2CSA-specific protective immunity. The other type is malaria caused by parasites that express other PfEMP1 types and where the IEs sequester in nonplacental tissues. This latter type is not restricted to pregnant women and can occur in women of any parity. It simply reflects gaps in variant (mainly PfEMP1)-specific immunity in general [7].

With these points in mind, we hypothesized that earlier reports of spontaneous resolution of *P. falciparum* parasitemia in pregnant women in Africa within a few of days of delivery [8, 9] reflect the sudden disappearance of the sequestration focus of VAR2CSA-positive IEs as the placenta is expulsed during childbirth.

METHODS

Study Site and Participants

The study was conducted at 5 health facilities (Abura Dunkwa, Biriwa, Mankessim, Moree, and Saltpond) in the Central Region of Ghana. Transmission of *P. falciparum* in the study area is stable, but with substantial seasonal variation.

Received 21 October 2022; editorial decision 28 January 2023; accepted 02 February 2023; published online 6 February 2023

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Presented in part: American Society of Tropical Medicine and Hygiene 2022 Annual Meeting, Seattle, WA, 30 October to 3 November 2022, poster LB-5192.

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https://doi.org/10.1093/infdis/jiad031

Near-term pregnant women, who consented in writing to participate in the study after receiving oral and written information about its aims, were recruited between November 2020 and February 2021. Women with any form of pregnancy complications were excluded from participation. Although most of the women received intermittent preventive treatment as part of current antenatal care policy in Ghana (Supplementary Table 1), none of them received any form of antimalarial treatment for the duration of the study (ie, from shortly before parturition until the end of their participation a few days later). The study was approved by the institutional review board at Noguchi Memorial Institute for Medical Research (NMIMR-IRB CPN 005/20-21) and by Ghana Health Service research ethics committee (GHS-ERC 005/08/20).

Detection and Quantification of *P. falciparum* Infection

Infection by *P. falciparum* parasites was determined by rapid diagnostic test, by microscopy of Giemsa-stained blood smears, and by quantitative polymerase chain reaction (qPCR) essentially as previously described [10, 11], using the peripheral blood samples collected. DNA extracted from six 10-fold dilutions of an in vitro culture of ring-stage *P. falciparum* IT4 at 1% parasitemia was used to establish a standard curve to allow qPCR quantification of parasitemia.

Analysis of *var* Gene Transcription

Parasite RNA was preserved in the field and used for quantification of *var* gene transcript levels essentially as described [11, 12], but using universal 1-step reverse transcription-qPCR (RT-qPCR) kits (E3005X, NEB Inc), a Quant Studio 5 thermal cycler system and fructose-bisphosphate aldolase as endogenous control. Data points were not considered if the cycle threshold (Ct) value for the endogenous control was >30 and/or if the Tm for the *var* gene amplicon for each pair of primers diverged >2°C from the expected value. Transcript abundance was calculated using $2^{-\Delta Ct}$, where mean $\Delta Ct = \text{mean } Ct_{var \text{ primer pair}} - \text{mean } Ct_{\text{endogenous control}}$.

Statistical Analysis

Associations between *var2csa* transcription and clinical variables were tested by Spearman rank correlation with correction for multiple testing. The correlation between parasitemias detected by microscopy and by PCR was evaluated by Pearson product-moment correlation test. Data obtained at different time points were compared by repeated measures analysis of variance (RM-ANOVA) or by RM-ANOVA on ranks (RM-ANOVA-R) as appropriate, followed by post hoc testing for pairwise differences. SigmaPlot version 14 software (Systat Inc) was used to analyze the data, and P values <.05 were considered significant.

RESULTS

Study Participants

A total of 377 consenting women were screened for *P. falciparum* infection, while in labor. Of these, 15 (4.0%) were parasite-positive by microscopy of peripheral finger-prick blood smear, 24 (6.4%) by rapid diagnostic test, and 50 (13.3%) by PCR. Based on these results, 17 rapid diagnostic test-positive women were enrolled and selected for follow-up at 8–16 hours postdelivery, 24–48 hours postdelivery, and 48–72 hours postdelivery. Clinical information of the study participants is summarized in Supplementary Table 1. There were no statistically significant associations between the proportion of *var2csa*-transcribing parasites and any of the clinical variables listed.

Postpartum Clearance of Parasitemia

At the time of delivery, all study participants were parasitemic by blood smear and/or qPCR (Supplementary Table 1). By 8 to 16 hours postdelivery, microscopically detectable parasitemia had declined spontaneously (ie, without therapeutical intervention) in 14/17 women (Figure 1*A*). By 24–48 hours after giving birth, parasitemias had dropped further and were very low or undetectable in the majority of the women ($P = .03$ compared to predelivery, Friedman RM-ANOVA-R). Another 24 hours later, all the women but 1 were without detectable parasitemia ($P = .005$ compared to predelivery, RM-ANOVA-R). With respect to parasitemias detected by PCR (Figure 1*B*), postpartum parasitemias were significantly lower by 24–48 hours and 48–72 hours than predelivery ($P < .001$, RM-ANOVA-R). Levels were also significantly lower by 48–72 hours postpartum than by 8–16 hours postpartum ($P < .001$, RM-ANOVA-R). Parasitemias estimated by microscopy and qPCR correlated significantly ($P < .001$). These data confirm previous reports of rapid and spontaneous postpartum clearance of *P. falciparum* parasitemia among women living in areas with stable transmission of this parasite [8, 9].

Parasite Transcription of *var* Genes

To identify the likely sequestration focus of the IEs detected, we measured parasite transcription of the PfEMP1-encoding *var* gene groups. Prior to delivery, *var2csa*, which encodes VAR2CSA-type PfEMP1 mediating placental IE sequestration [2, 3], was the primary transcript (up to >90% of total *var* transcription) in 9/17 women and *var2csa* transcription could be detected in all (Figure 2 and Supplementary Figure 1). Transcription of *var* genes that are not associated with placental malaria was thus detected (in addition to *var2csa*) in all the participants prepartum and dominated in some women, in accordance with previous observations [6].

The proportion of *var2csa* transcripts decreased during postpartum follow-up in all the women (Figure 2 and Supplementary Figure 1), in parallel with the decreases in

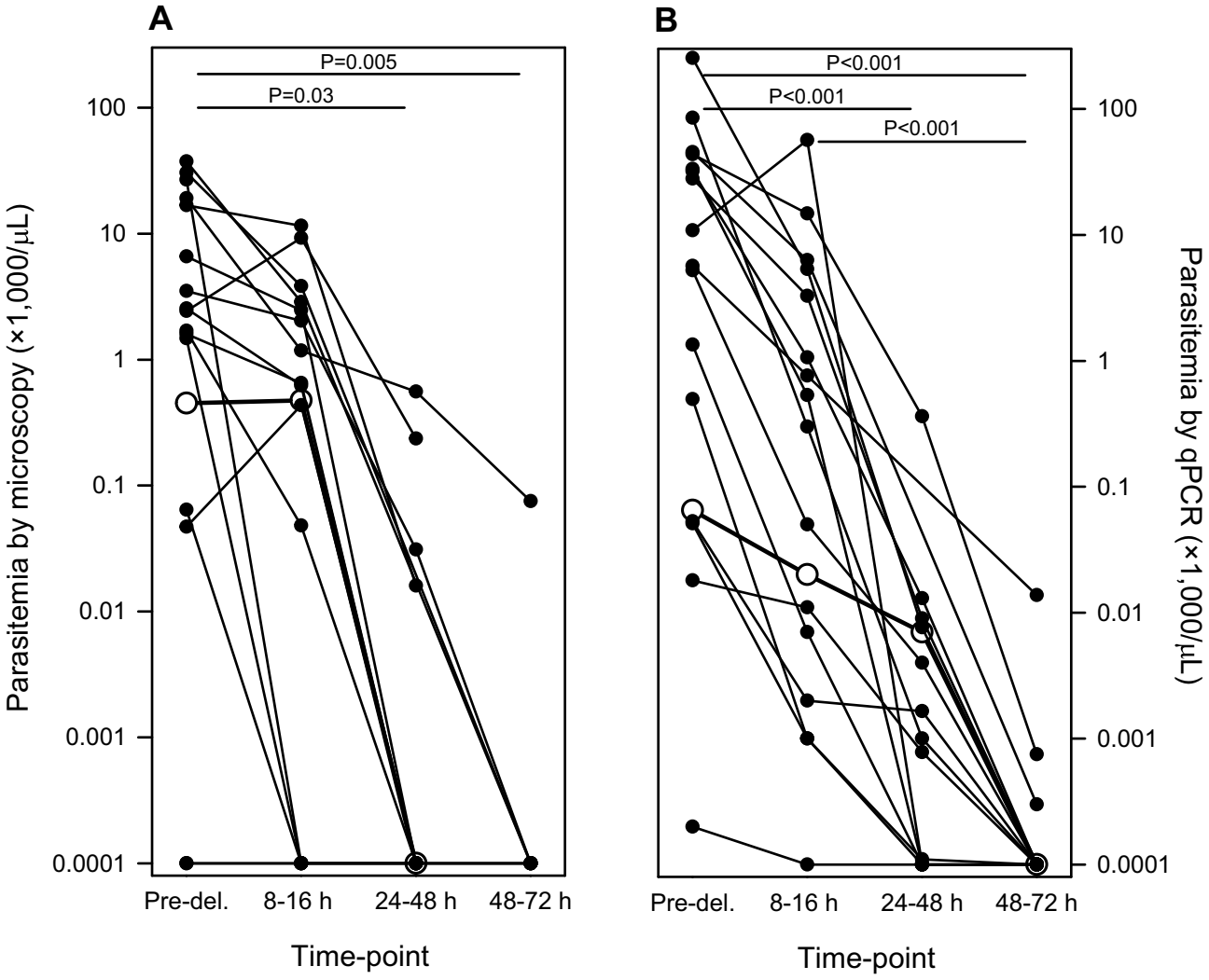

Figure 1. Short-term postpartum follow-up of asexual peripheral *Plasmodium falciparum* parasitemia among women who were parasitemic at the time of delivery. Parasitemias were estimated by microscopy of Giemsa-stained thin blood films (*A*) and by qPCR (*B*). Women with undetectable parasitemia were arbitrarily assigned a parasitemia of 0.1/μL. Datapoints from individual women are joined by lines. Data for a representative participant (MK002) is highlighted by enlarged, open symbols. Overall, the differences in levels of parasitemia at the different time points were statistically significant ($P = .002$ [microscopy] and $P < .001$ [PCR], RM-ANOVA-R). Statistically significant pairwise differences, assessed post hoc by Tukey test, are indicated along the top of the figure. Abbreviations: qPCR, quantitative polymerase chain reaction; RM-ANOVA-R, repeated measures analysis of variance on ranks.

parasitemias (Figure 1). There was thus a marked shift away from transcription of *var2csa* before delivery towards transcription of other *var* genes postpartum. The only exceptions (among those women where postpartum transcription data were available), were participants B213, S003, S001, and B201 (Supplementary Figure 1). However, based on the observation that the postpartum parasitemias also declined markedly among those women (Figure 1), we consider it likely that a corresponding shift away from transcription of *var2csa* would have been seen if data from later time points had been available. Overall, the data are fully consistent with a selective disappearance of VAR2CSA-positive IEs as we had hypothesized.

DISCUSSION

PfEMP1 is a family of proteins exported by intraerythrocytic *P. falciparum* parasites to the surface of the IEs, where they function as adhesion ligands for different host vascular receptors [2]. These ligands are encoded by the clonally variant *var* gene family with about 60 members per haploid parasite genome. The genes are transcribed and translated in a mutually exclusive manner, ensuring that only a single PfEMP1 is expressed on the IE surface at any given time. However, each parasite has the potential to switch among its different *var* genes, enabling changes in IE adhesion specificity and antigenic properties [2].

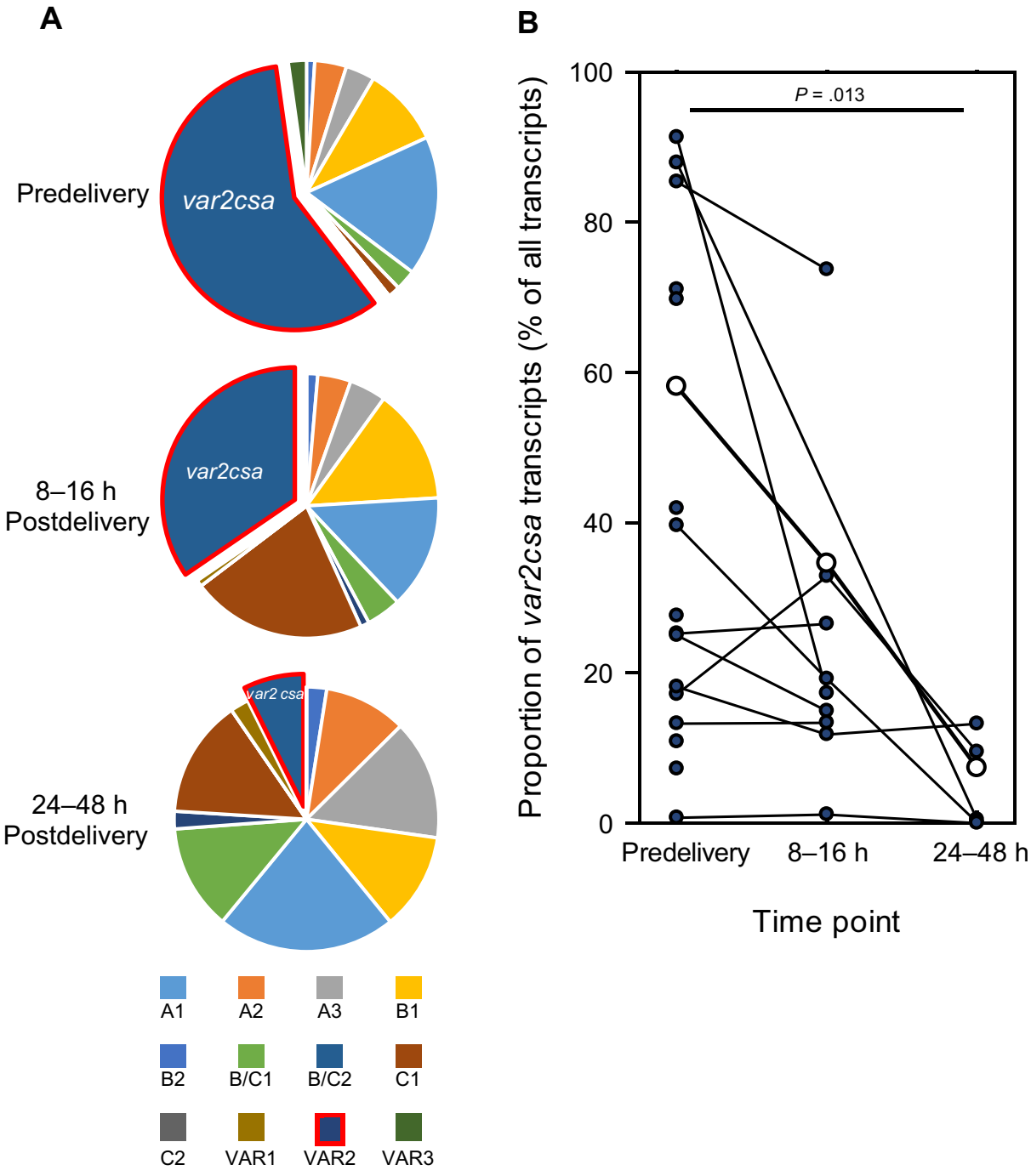

Figure 2. Transcription of *var* genes among peripheral blood parasites pre- and postpartum. *A*, Transcription of *var* gene groups (color-coded as indicated) in a representative participant (MK002) immediately predelivery (top), 8–16 hours (center), and 24–48 hours (bottom). Transcription of *var2csa* is emphasized by offsetting and red borders of the *var2csa* section in each diagram. *B*, Relative transcription of *var2csa* in all study participants (data for participant MK002 are by enlarged, open symbols). Overall, differences in *var2csa* transcription levels at the different time points were statistically different ($P = .01$; RM-ANOVA), and the difference between predelivery and 48–72 hours postpartum remained significant by post hoc pairwise comparisons ($P = .013$; Bonferroni *t* test).

The PfEMP1 proteins can be grouped and subgrouped according to a set of structural criteria, which influence their functional characteristics [2]. Thus, group A PfEMP1 (encoded by about a dozen *var* genes per genome) mediate IE adhesion to host receptors such as EPCR and ICAM-1, and have been implicated in the pathogenesis of severe childhood malaria in general, and cerebral malaria in particular [2]. In contrast, transcription of genes belonging to group B and C appears to be associated

with uncomplicated malaria and asymptomatic infections. Finally, VAR2CSA-type PfEMP1, encoded by just one or a few of *var2csa* genes per parasite genome, is functionally and antigenically completely distinct from all other PfEMP1, and is strongly implicated in the pathogenesis of, as well as in acquired immunity to, placental malaria [2, 3, 13]. Furthermore, the bulk of available evidence points to VAR2CSA as the major and likely only bona fide CSA ligand. Finally, it has previously been shown that peripheral parasitemia in pregnant women with lifelong *P. falciparum* exposure includes, and is often dominated by, IEs that are VAR2CSA-positive and have the general characteristic phenotype of placental *P. falciparum* infections [5, 13–15]. On this basis, we hypothesized that the most cogent explanation for the spontaneous clearance of parasitemia in pregnant women within a few days of delivery [8, 9] is selective splenic destruction of VAR2CSA-positive IEs following the expulsion of the placenta during delivery, which effectively removes their cognate adhesion receptor.

The results presented here confirm the previously reported [8, 9] rapid postpartum clearance of peripheral *P. falciparum* parasitemia (Figure 1). They furthermore support observations that peripheral parasitemia in African pregnant women includes, and is often dominated by, parasites transcribing *var2csa* [6, 15] (Figure 2 and Supplementary Figure 1). Most importantly, however, our data extend these earlier observations by providing first-time compelling evidence for a mechanistic explanation for the rapid postpartum clearance of peripheral parasites in women with naturally acquired immunity to *P. falciparum* malaria. Apart from a numerically very minor, and only borderline significant, difference in transcription of *var* group A1 (Supplementary Figure 2), the only major and statistically significant change in transcription of *var* genes following parturition was reduced relative transcription of *var2csa* (Figure 2*B* and Supplementary Figure 2).

Our study has limitations. The sample size is small. This is mainly because the successful implementation of intermittent preventive treatment of malaria for pregnant women, which has made placental malaria much less prevalent and necessitated the screening of many women to find eligible participants. Furthermore, the limited facilities at our study site prevented direct assessment of the adhesion receptor specificity of the collected IEs or their immunological phenotype. Nevertheless, the current evidence and earlier findings combine seamlessly.

In conclusion, we have presented evidence that the abrupt disappearance of the sequestration focus of VAR2CSA-positive IEs when the placenta is expulsed contributes significantly to the rapid and spontaneous postpartum clearance of *P. falciparum* parasitemia in women with natural exposure to these parasites. Parasites transcribing other *var* genes and displaying non-VAR2CSA–type PfEMP1 on the IE surface are often unable to thrive and cause disease in adults in endemic areas, because of protective PfEMP1-specific immunity to malaria acquired in childhood [2]. The continued survival of non-VAR2CSA IEs postpartum may be further compromised by parturition-associated physiological processes such as proinflammatory immune responses. Finally, undisclosed intake of antimalarial drugs immediately prior to delivery could conceivably contribute. Whatever the reason, parasitemia did decline markedly postpartum in some women, where *var2csa*-transcribing parasites constituted only a minority of the predelivery parasitemia, and this finding deserves further study. Our study underpins the key role of PfEMP1 in acquired immunity to malaria in general, and specifically of VAR2CSA in placental malaria pathogenesis and immunity.

Supplementary Data

Supplementary materials are available at *The Journal of 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.

Notes

Author contributions. N. G. A., M. P. Q., M. F. O., and L. H. contributed study conception and design. N. G. A. performed sample collection. N. G. A., M. P. Q., and L. H. performed data analysis. N. G. A., M. P. Q., and L. H. prepared the manuscript. All authors read and approved the final version of the manuscript.

Acknowledgments. We are grateful to all the women agreeing to participate in our study. We also thank all the midwives, medical doctors, physician assistants, and nurses in the various health facilities for their help in collecting samples.

Disclaimer. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. Funding to pay the Open Access publication charges for this article was provided by Danida.

Financial support. This work was supported by Danida (grant number 17-02-KU to MAVARECA-II project), including a PhD studentship to N. G. A. The Research Executive Agency of the European Commission supported M. P. Q. (grant number 101028915, Marie Skłodowska-Curie Individual Fellowship 2020, PAMSEQ project).

Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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