Matrix Metalloproteinase-9 Polymorphism 1562 C > T (rs3918242) Associated with Protection against Placental Malaria

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Abstract. Phagocytosis of malaria pigment (hemozoin) induces increased activity of matrix metalloproteinase (MMP)-9, an endopeptidase involved in cytokine regulation. In this study, we examined whether a common functional *MMP-9* promoter polymorphism (rs3918242) affects *Plasmodium falciparum* infection in pregnancy. Eighteen percent of Ghanaian primiparae carried the minor *T* allele. It was associated with reduced odds of placental hemozoin and of placental as well as peripheral blood parasitemia. The results indicate that a common *MMP-9* polymorphism protects against placental malaria indicating that this endopeptidase is involved in susceptibility to *P. falciparum*.

Pregnant women are a particular risk group for infection with *Plasmodium falciparum* and malaria. Although commonly asymptomatic at high endemicity, malaria in pregnancy may cause anemia, abortion, stillbirth, low birth weight (LBW), and preterm delivery (PTD), and contributes to high infant mortality. The increased susceptibility of pregnant women, particularly primigravidae, is largely due to parasites expressing specific variants of the *P. falciparum* erythrocyte membrane protein-1. Parasite adhesion via these variant surface proteins results in the sequestration of infected red blood cells (RBCs) in the placental intervillous space. Sequestration frequently is accompanied by local hemozoin (malaria pigment) deposition and accumulation of inflammatory cells, including monocytes/macrophages. Specific immune mechanisms targeting the pregnancy-associated parasites, particularly parasite-specific antibodies, are low in primigravidae. Only with successive pregnancies, these are acquired, and infection risk and manifestation decrease.^{1–3}

Risk and manifestation of malaria and of malaria in pregnancy are influenced by diverse factors including host genetics.^{4,5} The latter may involve variants of matrix metalloproteinases (MMPs), a family of metal ion-dependent endopeptidases that are involved in the breakdown of extracellular matrix and tissue remodeling.⁶ MMPs also contribute to the regulation of various cytokines and chemokines, thus playing an important role in host immune responses.⁷ In acute malaria, increased expression of MMP-9 (gelatinase B) has been observed.⁸ Moreover, in vitro, hemozoin phagocytosis by human monocytes and the exposure of endothelial cells to parasitized RBCs stimulate the release of MMP-9 and tumor necrosis factor α (TNF- α).^{9,10} A common *MMP-9* gene polymorphism (rs3918242, replacement of C by T at location 1562) increases the promoter activity of the *MMP-9* gene 1.5-fold because the associated transcriptional repressor protein has a reduced affinity to the *T* allelic promoter. The polymorphism, hence, has been associated with increased transcription activity and with altered risks of various diseases.^{11–13} We therefore examined whether this

polymorphism affects susceptibility to or manifestation of malaria in pregnancy.

The characteristics of the 304 primiparous pregnant women with live singleton delivery have been reported elsewhere.² Informed written consent was obtained from all women, and the study protocol was approved by the Committee on Human Research Publications and Ethics, School of Medical Sciences, University of Science and Technology, Kumasi, Ghana. In brief, delivering women were recruited at the Presbyterian Mission Hospital in hyper- to holoendemic Agogo, Ghana (population, 30,000), in 2000 and 2001. Women were clinically examined and sociodemographic data were documented. Intervillous and peripheral blood samples were collected into ethylenediaminetetraacetic acid (EDTA). Parasites were counted on Giemsa-stained blood smears per 100 high-power fields and per 500 white blood cells, respectively. Leukocyte-associated hemozoin in placental samples was recorded. Deoxyribonucleic acid (DNA) was extracted from blood (AS1 and QIAamp DNA Blood Mini Kit; Qiagen, Hilden, Germany), and nested *P. falciparum*-specific polymerase chain reaction (PCR) assays were performed.¹⁴ Present or past placental *P. falciparum* infection was defined by the presence of placental parasites or hemozoin in microscopy, or a positive placental *P. falciparum* PCR result. Pyrimethamine in plasma (indicating compliance with the chemoprophylaxis recommended at that time) was measured by enzyme-linked immunosorbent assays (ELISAs) with limits of detection of 10 ng/mL.¹⁵ Anemia was defined as hemoglobin (Hb) < 11 g/dL, measured by a hemoglobin photometer (HemoCue AB, Ängelholm, Sweden). LBW was defined as birth weight < 2,500 g, and PTD as gestational age < 37 weeks applying the Finnström score.¹⁶ Genotyping of rs3918242 was achieved by restriction fragment length polymorphism.¹⁷ Data were analyzed with Statview 5.0 (SAS Institute Inc., Cary, NC). Continuous variables were compared between groups by the Mann–Whitney *U* test or Student's *t* test as applicable. Associations of genotypes with, for example, *P. falciparum* infection were assessed by χ^2 test, and odds ratios (ORs) and 95% confidence intervals (95% CI) were determined. Adjusted ORs (aORs) were calculated in logistic regression models with stepwise backward removal of factors not associated in multivariate analysis (*P* > 0.05).

Typing of rs3918242 was successful in 302 of 304 primiparae. The major homozygous genotype (CC) was observed in

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TABLE 1
Characteristics of 302 Ghanaian primiparae with live singleton delivery according to MMP-9 genotype (rs3918242)

Parameter	All	rs3918242		P*
		Major genotype (CC)	Genotypes with T allele (CT, TT)	
No. (%)	302 (100)	248 (82.1)	54 (17.9)	
Age (years); median (range)	20.5 (15–36)	20 (15–36)	21 (15–30)	0.25
Rural residence (n, %)	154 (51.0)	126 (50.8)	28 (51.9)	0.89
>3 antenatal care visits (n, %)	139/295 (47.1)	106/241 (44.0)	33/54 (61.1)	0.02
Delivery in rainy season (n, %)	155 (51.3)	130 (52.4)	25 (46.3)	0.41
Pyrimethamine in plasma (n, %)	106/297 (35.7)	87/243 (35.8)	19 (35.2)	0.93
Anemia (n, %)	116 (38.4)	99 (39.9)	17 (31.5)	0.25
LBW (n, %)	79 (26.2)	65 (26.2)	14 (25.9)	0.97
PTD (n, %)	80 (26.5)	69 (27.8)	11 (20.4)	0.26
<i>Plasmodium falciparum</i> infection (peripheral blood)				
Microscopy positive (n, %)	80 (26.5)	75 (30.2)	6 (11.1)	0.004
Geometric mean parasite density/ μ L, (95% CI)	746 (476–1,171)	718 (451–1,141)	1,159 (173–7,773)	0.58
PCR positive (n, %)	179 (59.3)	152 (61.3)	27 (50)	0.13
<i>P. falciparum</i> infection (placental blood)				
Microscopy positive (n, %)	139 (46.0)	122 (49.2)	17 (31.5)	0.02
Geometric mean parasite density/100 high-power fields, (95% CI)	119 (78–181)	126 (83–191)	431 (73–2,544)	0.48
Hemozoin positive (n, %)	128 (42.4)	115 (46.4)	13 (24.1)	0.003
PCR positive (n, %)	196 (64.9)	166 (66.9)	30 (55.6)	0.11
Present or past placental infection (n, %)	205 (67.9)	175 (70.6)	30 (55.6)	0.03

CI = confidence interval; LBW = low birth weight; MMP = matrix metalloproteinase; PCR = polymerase chain reaction; PTD = preterm delivery.

*P values derived from Student's *t* tests, Mann-Whitney *U* tests, or χ^2 tests, as applicable. P value in bold indicate significance < 0.05. In the three TT homozygous individuals, prevalence was peripheral blood: microscopy positivity, 0/3; PCR positivity, 1/3; placental blood: microscopy positivity, 0/3; hemozoin positivity, 0/3; past or present placental infection, 1/3.

82.1% (248); 17.9% of the women exhibited the minor *T* allele (heterozygous, 51; homozygous, 3; grouped henceforth). Genotypes were in Hardy-Weinberg equilibrium.

Overall, 67.9% of the women had evidence of present or past *P. falciparum* infection. Placental hemozoin was observed in 42.4%, and PCR assays on placental samples were positive in 64.9% (Table 1). Nevertheless, fever occurred in only 2.5% (12/299). Women with the minor *T* allele had a significantly lower prevalence of present or past placental *P. falciparum* infection (OR = 0.52; 95% CI = 0.27–0.99; *P* = 0.03), placental hemozoin (OR = 0.37; 95% CI = 0.18–0.75; *P* = 0.003), placental parasitemia (OR = 0.47; 95% CI = 0.24–0.92; *P* = 0.02), and peripheral blood parasitemia (OR = 0.29; 95% CI = 0.10–0.79; *P* = 0.004). For infections detected by PCR, the difference did not reach statistical significance, and parasite densities did not differ between women with and without the minor *T* allele (Table 1). Adjusting for factors previously identified as being associated with placental malaria, that is, delivery in rainy season, age, and presence of plasma pyrimethamine,² women with the minor *T* allele tended to have reduced odds of present or past *P. falciparum* infection (aOR = 0.56, 95% CI = 0.30–1.03, *P* = 0.06; age (years), aOR = 0.92, 95% CI = 0.85–0.98; rainy season, aOR = 1.76, 95% CI = 1.06–2.92; plasma pyrimethamine, aOR = 0.58, 95% CI = 0.35–0.98). Further adjustment for the difference between groups in the use of antenatal care (itself not associated with malaria, Table 1) did not substantially change the estimate (aOR = 0.55, 95% CI = 0.29–1.02, *P* = 0.06). In the multivariate model, significantly reduced odds were observed for placental hemozoin (aOR = 0.38, 95% CI = 0.19–0.75, *P* = 0.005), placental parasitemia (aOR = 0.49, 95% CI = 0.26–0.93, *P* = 0.003), and peripheral blood parasitemia (aOR = 0.29, 95% CI = 0.12–0.73, *P* = 0.008).

Maternal anemia and PTD but not LBW tended to be less common in women carrying the minor *T* allele. However, there was no association of these outcomes with the

polymorphism (Table 1), irrespective of stratification into infected and noninfected women (data not shown).

MMP-9 has been shown to be upregulated in acute malaria,⁸ and specifically, both parasitized RBCs and hemozoin induce release of MMP-9 by monocytes and endothelial cells.^{9,10} The role of this endopeptidase in malaria is, nevertheless, controversial, potentially generating both protective and detrimental effects.¹⁸ Here, we show that a promoter single nucleotide polymorphism (SNP) increasing MMP-9 activity reduces the odds of placental malaria. The mechanisms involved are speculative: MMP-9 has an important role in the regulation of inflammatory processes including a complex influence on various chemokines. Increased MMP-9 levels may result in both increased and decreased chemotactic activities but in murine models they appear to promote leukocyte migration. In addition, MMP-9 modulates the activity of several pro-inflammatory mediators, for example, by inducing the release of TNF- α or activating pro-interleukin (IL)-1 β , thereby augmenting the pro-inflammatory response.⁷ Pro-inflammatory responses in malaria are double edged: they may contribute to pathophysiologic damage but initially increased release in particular contributes to accelerated parasite clearance.³ Conceivably, increased MMP-9 activity may affect leukocyte recruitment to the intervillous space and local pro-inflammatory responses thereby enhancing parasite elimination. Notably, the placental syncytiotrophoblast, the epithelium lining the intervillous space, has been shown to be immunoreactive to *P. falciparum*,¹⁹ and also to express MMP-9.²⁰ Moreover, part of the produced MMP-9 forms heteromers with chondroitin sulfate proteoglycans.²¹ Altered MMP-9 expression could affect heteromer formation, which in turn may decrease binding of parasitized RBCs to chondroitin sulfate (the main placental parasite ligand), resulting in reduced placental malaria.

As a limitation, this study was not a priori designed to assess an association between *P. falciparum* infection in

pregnancy and the *MMP-9* promoter SNP, and it comprised a relatively small group of 302 primiparae. We use the term placental malaria to describe the detection in placental blood samples of parasites by microscopy or PCR and of hemozoin by microscopy. This does not correspond to histologic classification as suggested by, for example, Muehlenbachs and others²² but is characterized by a high sensitivity due to the inclusion of PCR assays.² Although infection prevalence as assessed by PCR differed between genotypes, this did not reach statistical significance. This was partly because the proportion of submicroscopic infections (i.e., positive by PCR only, but negative by microscopy) among all infections was comparatively increased in individuals with the minor *T* allele (Table 1). Thus, infection prevalence as detected by PCR in *T* allele carriers was overall reduced even if not significantly so, and low-level infections prevailed. Statistical significance might have been present in case of a larger sample size. The same applies to the manifestation of infection, for example, malaria-associated anemia or PTD.

The MMPs have been considered as biomarkers and therapeutic targets in malaria.¹⁸ This study suggests that a common functional *MMP-9* polymorphism is associated with reduced odds of placental malaria, and thereby provides evidence for the in vivo relevance of *MMP-9* in human malaria.

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