An ATP2B4 Polymorphism Protects Against Malaria in Pregnancy

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Polymorphisms of ATP2B4 encoding an ubiquitous Ca2+ pump protect against severe childhood malaria. We assessed the influence of a main polymorphism (rs10900585) on malaria among 834 delivering Ghanaian women. In homozygous primiparae, the odds of placental Plasmodium falciparum infection were reduced by 64%. No influence of the polymorphism on parasite density, low birth weight, or preterm delivery was discernible. However, malarial anemia was greatly reduced in primiparous carriers of the variant allele, paralleling the reduced impact of malaria on hemoglobin levels in this group. A common ATP2B4 polymorphism protects against malaria in pregnancy and related maternal anemia, suggesting ATP2B4 variant associated protection not to be limited to severe childhood malaria.

Keywords. malaria; pregnancy; ATP2B4; PMCA; Ghana.

Malaria has shaped the human genome, and in regions of high endemicity, it has driven the selection of protective polymorphisms, notable among which are erythrocyte variants, such as the sickle cell trait [1]. Genome-wide association studies have been performed to identify further protective traits, and a recent study found that a common single nucleotide polymorphism (SNP) of the ATP2B4 gene encoding the plasma membrane calcium-transporting ATPase 4 (PMCA4) reduces the odds of severe malaria in African children by 40% [2].

Apart from young children, pregnant women, particularly primiparae, have a high risk for Plasmodium falciparum infection and malaria. Notably, severe childhood malaria and malaria in pregnancy differ largely in terms of pathophysiology, immunity, and clinical manifestation [3–5]. In regions of high endemicity, malaria in pregnancy is frequently asymptomatic, but consequences include anemia, abortion, stillbirth, low birth weight (LBW), preterm delivery (PTD), and, annually, up to 200,000 infant deaths [3]. In pregnant women, specific expression variants of P. falciparum erythrocyte membrane protein 1 mediate adhesion to the placental syncytiotrophoblast (the epithelial lining of the intervillous space) and, thereby, placental sequestration of infected erythrocytes, which is frequently paralleled by the local accumulation of inflammatory cells [4]. In regions where P. falciparum infection and malaria are highly endemic, specific immunity against these pregnancy-associated parasites is particularly low in primigravidae and acquired only with successive pregnancies, with a concomitant decrease in the frequency of clinical manifestations [3–5]. Here, we examined the impact of rs10900585, the ATP2B4 SNP most strongly associated with protection against severe malaria in Ghanaian children [2], on malaria in pregnant Ghanaian women.

PATIENTS AND METHODS

Delivering women were recruited from January 2000 through January 2001 at the Presbyterian Mission Hospital in Agogo (population, 30,000), Ghana, where malaria is hyperendemic to holoendemic. The study protocol was approved by the Committee on Human Research Publications and Ethics, School of Medical Sciences, University of Science and Technology, Kumasi, and informed written consent was obtained. Study procedures and the characteristics of the largely asymptomatic participants have been described in detail elsewhere [5]. In brief, women were clinically examined, socioeconomic data were documented, and intervillous and venous blood samples was collected into tubes containing ethylenediaminetetraacetic acid. Malaria parasites in postdelivery placental and venous samples were counted microscopically on Giemsa-stained thick blood films per 100 high-power fields and 500 white blood cells, respectively. Placental parasite densities were expressed as parasites/100 fields, and peripheral parasite densities were expressed as parasites/microliter, assuming a mean white blood cell count of 8000 cells/μL. Leukocyte-associated hemozoin in placental samples was recorded. Following DNA extraction (QIAmp, Qiagen, Hilden, Germany), nested P. falciparum–specific polymerase chain reaction (PCR) assays were performed [6]. Plasma concentrations of pyrimethamine, at that time recommended for malaria chemoprophylaxis, were...
measured by enzyme-linked immunosorbent assays [7] with a limit of detection of 10 ng/mL. Hemoglobin levels were measured by a HemoCue photometer (Ångelholm, Sweden). Anemia was defined as a hemoglobin level of <11 g/dL, LBW as <2500 g, and PTD as a gestational age of <37 weeks, applying the Finnström score [8]. ATP2B4 rs10900585 (T > G) was typed by melting curve analysis, using the LightCycler 480 device (Roche Diagnostics, Mannheim, Germany) and commercially available primers and probes (TIB Molbiol, Berlin, Germany).

Geometric mean parasite densities and 95% confidence intervals (CIs) were calculated. Continuous variables were compared between groups by the t test, analysis of variance, the Mann-Whitney U test, and the Kruskal-Wallis test, as applicable. Associations of genotype with *P. falciparum* infection, anemia, LBW, and PTD were identified by the χ² test, and odds ratios (ORs) calculated. Adjusted ORs (aORs) were derived from logistic regression models. A P value of <.05 was considered statistically significant.

**RESULTS**

ATP2B4 genotyping was successful in 834 of 839 women (99.4%) with a live singleton delivery. The genotypes TT (wild type, comprising the “major” alleles), TG, and GG were present in 28.5% (238), 50.2% (419), and 21.2% (177), respectively, and were in Hardy-Weinberg equilibrium. *P. falciparum* in placental samples was detected by PCR in 59.2% (494). This figure was almost identical in women with ATP2B4 TT (61.3% [146 of 238]) and with TG (61.1% [256 of 419]) but considerably lower in GG homozygous women (52.0% [92 of 177], P = .06). This difference was pronounced and statistically significant in primiparous (Table 1) but could not be observed in women of higher parity (TT, 56.0% [84 of 150]; TG, 58.7% [148 of 252]; and GG, 52.4% [65 of 124]; P = .50).

Further analysis therefore focused on the 301 primiparae. Among these, the ATP2B4 genotypes were not associated with age, residence, number of antenatal care visits, delivery in the rainy season, or presence of plasma pyrimethamine levels (Table 1). *P. falciparum* as detected by microscopy only tended to be less common in women with the ATP2B4 G allele, and parasite densities did not differ with genotypes. However, on the basis of sensitive *P. falciparum*-specific PCR assays, the homozygous GG genotype was associated with significantly reduced odds of peripheral blood infection (OR, 0.35 [95% CI, 0.16–0.76], P = .004) and placental blood infection (OR, 0.39 [95% CI, 0.18–0.86], P = .01). In multivariate analysis that adjusted for years of age (aOR, 0.92 [95% CI, 0.86–0.99]), delivery in the rainy season (aOR, 1.74 [95% CI, 1.06–2.84]), and presence of pyrimethamine in plasma (indicating compliance with chemoprophylaxis; aOR, 0.48 [95% CI, 0.29–0.80]), the odds of peripheral blood *P. falciparum* infection were reduced by almost 70% in women with the homozygous GG genotype (aOR 0.31 [95% CI, 0.15–0.68]), P = .003) and, to a lesser extent, in women with a heterozygous genotype (aOR, 0.56 [95% CI, 0.31–1.01], P = .05; combined heterozygous and homozygous: aOR, 0.49 [95% CI, 0.28–0.86], P = .01). For placental infection, these estimates were similar (GG: aOR, 0.36 [95% CI, 0.17–0.77], P = .009; TG: aOR, 0.69 [95% CI, 0.38–1.25], P = .22; combined: aOR, 0.59 [95% CI, 0.33–1.04], P = .07).

Next, we analyzed whether the impact of placental hemoglobin, the most robust indicator of clinical manifestation in this group [5], on LBW, PTD, and anemia differed with genotype. LBW, PTD, and anemia occurred in 25.9% (78), 26.2% (79), and 38.2% (115) of 301 primiparae, respectively. For LBW and PTD, no influence of the ATP2B4 genotype was observed, irrespective of placental malaria (data not shown). However, with regard to maternal anemia, the G allele was associated with reduced odds in women with placental hemoglobin (heterozygous: OR, 0.17 [95% CI, 0.06–0.46], P < .0001; homozygous: OR, 0.32 [95% CI, 0.09–1.09], P = .04) but not in those without (Table 1). Correspondingly, by analysis of variance, the reduction of hemoglobin levels associated with placental hemoglobin was less than half in G allele carriers as compared to wild-type individuals (F = 8.9, P = .003). This was largely due to the respective effect in heterozygous women (F = 11.6, P = .0008).

**DISCUSSION**

The clinical manifestations of severe childhood malaria are distinct from those of malaria in pregnancy [3–5]. Notwithstanding the differences, we showed that an ATP2B4 variant recently reported to protect children from severe malaria [2] also reduces the odds of *P. falciparum* infection in pregnancy and, furthermore, mitigates the odds of associated maternal anemia.

The present study has some limitations. Protection from malaria was statistically significant only when based on PCR data (although microscopic data showed accordant trends). The sensitivity of peripheral blood microscopy for detection of malaria parasites during pregnancy is notoriously low, and infections below the detection threshold are frequent [5]. Notably, these submicroscopic infections were comparatively less frequent in women with the ATP2B4 variant. Statistically significant findings were observed in the relatively small group of 301 primiparae, of whom 52 were homozygous for the variant allele. Limited sample sizes in subgroups may thus have interfered with some of the analyses, such as influences of the variant allele on the less frequent outcomes of LBW and PTD. This does not preclude that respective effects are actually present but points out the necessity of verification in larger studies. Several ATP2B4 polymorphisms have shown genomewide association with protection against severe malaria [2]. In the present study, we examined the SNP with the reportedly strongest association, which does not necessarily mean that it represents the causal variant. Located in intron 2, this...
polymorphism has no apparent function, but its effects on gene regulatory elements or tissue-specific splicing still need to be elucidated. Also, close linkage with further ATP2B4 SNPs [2] may underlie the observed associations.

The product of ATP2B4, PMCA4, is a ubiquitous Ca\(^{2+}\) pump that is expressed in different splice variants in erythrocytes, platelets, endothelial cells, and the microvillus membranes of the syncytiotrophoblast, among other cells and tissues [9, 10]. ATP2B4 variants may modify the structure and function of PMCA4 and, thus, intracellular Ca\(^{2+}\) homeostasis. Timmann and colleagues [2] proposed various mechanisms by which altered intracellular Ca\(^{2+}\) concentrations may affect P. falciparum–related pathology, namely, impaired intraerythrocytic parasite reproduction and maturation [11]; modified activation of platelets, including their role in sequestration and parasite killing [12]; and altered activation of endothelium, influencing the adherence of P. falciparum–infected cells [13].

In pregnant women, P. falciparum–infected erythrocytes sequester in the inter villous space by adhering to ligands on the syncytiotrophoblast. This is frequently paralleled by the local accumulation of hemozoin, a product of hemoglobin degradation by the parasite, and of inflammatory cells. Specific antibodies capable of blocking parasite adhesion prevent this placent malaria only after successive pregnancies during which the mother is exposed to P. falciparum, Plasmodium falciparum

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All</th>
<th>TT</th>
<th>TG</th>
<th>GG</th>
<th>(P^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects, no. (%)</td>
<td>301 (100)</td>
<td>85 (28.2)</td>
<td>164 (54.5)</td>
<td>52 (17.3)</td>
<td>.62</td>
</tr>
<tr>
<td>Age, y, median (range)</td>
<td>20.5 (15–36)</td>
<td>20 (16–29)</td>
<td>20 (15–32)</td>
<td>21 (15–36)</td>
<td>.72</td>
</tr>
<tr>
<td>Rural residence</td>
<td>51.2 (154)</td>
<td>51.8 (44)</td>
<td>49.4 (81)</td>
<td>55.8 (28)</td>
<td>.72</td>
</tr>
<tr>
<td>&gt;3 antenatal care visits</td>
<td>47.3 (139/294)</td>
<td>49.4 (41/83)</td>
<td>46.3 (74/160)</td>
<td>47.1 (24/51)</td>
<td>.90</td>
</tr>
<tr>
<td>Delivery in rainy season</td>
<td>51.2 (154)</td>
<td>55.3 (47)</td>
<td>48.2 (79)</td>
<td>53.8 (28)</td>
<td>.52</td>
</tr>
<tr>
<td>Plasma pyrimethamine positive</td>
<td>35.8 (106/296)</td>
<td>42.4 (36)</td>
<td>31.9 (52/163)</td>
<td>37.5 (18/48)</td>
<td>.26</td>
</tr>
<tr>
<td>P. falciparum infection, peripheral blood</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microscopy positive</td>
<td>26.6 (80)</td>
<td>28.2 (24)</td>
<td>27.4 (45)</td>
<td>21.2 (11)</td>
<td>.62</td>
</tr>
<tr>
<td>Parasite density, parasites/µL, geometric mean (95% CI)</td>
<td>718 (463–1113)</td>
<td>809 (411–1592)</td>
<td>585 (309–1107)</td>
<td>1276 (420–3880)</td>
<td>.49</td>
</tr>
<tr>
<td>PCR positive</td>
<td>59.1 (178)</td>
<td>69.4 (59)</td>
<td>58.5 (96)</td>
<td>44.2 (23)</td>
<td>.01</td>
</tr>
<tr>
<td>P. falciparum infection, placental blood</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microscopy positive</td>
<td>45.8 (138)</td>
<td>48.2 (41)</td>
<td>46.3 (76)</td>
<td>40.4 (21)</td>
<td>.66</td>
</tr>
<tr>
<td>Parasite density, parasites/100 fields, geometric mean (95% CI)</td>
<td>116.8 (76–177)</td>
<td>119 (66–250)</td>
<td>108 (61–189)</td>
<td>143 (40–508)</td>
<td>.90</td>
</tr>
<tr>
<td>Hemozoin positive</td>
<td>42.2 (127)</td>
<td>47.1 (40)</td>
<td>37.8 (62)</td>
<td>48.1 (25)</td>
<td>.24</td>
</tr>
<tr>
<td>PCR positive</td>
<td>64.8 (195)</td>
<td>71.8 (61)</td>
<td>65.9 (108)</td>
<td>50.0 (26)</td>
<td>.03</td>
</tr>
<tr>
<td>Anemic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All women</td>
<td>38.2 (115)</td>
<td>51.8 (44)</td>
<td>32.3 (53)</td>
<td>34.6 (18)</td>
<td>.01</td>
</tr>
<tr>
<td>Hemozoin negative (n = 174)</td>
<td>25.3 (44/174)</td>
<td>26.7 (12/45)</td>
<td>27.5 (28/102)</td>
<td>14.8 (4/27)</td>
<td>.39</td>
</tr>
<tr>
<td>Hemozoin positive (n = 127)</td>
<td>55.9 (71/127)</td>
<td>80.0 (32/40)</td>
<td>40.3 (25/62)</td>
<td>56.0 (14/25)</td>
<td>.0004</td>
</tr>
<tr>
<td>Hemoglobin level, g/dL, mean ± SD</td>
<td>(11.25 ± 1.83)</td>
<td>(10.80 ± 1.97)</td>
<td>(11.41 ± 1.70)</td>
<td>(11.48 ± 1.88)</td>
<td>(0.03)</td>
</tr>
<tr>
<td>All women</td>
<td>(11.25 ± 1.83)</td>
<td>(10.80 ± 1.97)</td>
<td>(11.41 ± 1.70)</td>
<td>(11.48 ± 1.88)</td>
<td>(0.03)</td>
</tr>
<tr>
<td>Hemozoin positive (n = 127)</td>
<td>(10.44 ± 1.81)</td>
<td>(9.59 ± 1.61)</td>
<td>(10.94 ± 1.59)</td>
<td>(10.54 ± 2.17)</td>
<td>(0.0008)</td>
</tr>
</tbody>
</table>

Data are percentage (no.) or percentage (proportion) of subjects, unless otherwise indicated.

Abbreviations: CI, confidence interval; PCR, polymerase chain reaction; P. falciparum, Plasmodium falciparum.

\(a\) Global \(P\) values are presented.

\(b\) \(P < .05\), compared with TT genotype.

\(c\) \(P\) values based on genetic models: additive (\(\chi^2\) for trend test), \(P = .004\); dominant (TT vs [TG + GG]), \(P = .02\); recessive ([TT + TG] vs GG), \(P = .02\).

\(d\) \(P\) values based on genetic models: additive, \(P = .01\); dominant, \(P = .11\); recessive, \(P = .01\).
attraction of peripheral blood mononuclear cells to the intervil-
ous space [4, 14]. The role of ATP2B4-dependent intracellular Ca^{2+} homeostasis in this multifaceted response is unknown. In
general, the reduced prevalence of infection associated with the
ATP2B4 SNP may result from reduced susceptibility, impaired
establishment of infection, and increased immune responses,
leading to enhanced parasite elimination. Parasite densities did
not correlate with ATP2B4 genotypes, but the erratic nature of
this parameter precludes respective conclusions. Nevertheless,
it is noteworthy that at similar parasite density, anemia was less
often seen in women with the variant ATP2B4 G allele than in
those with the wild type and, related to that, the effect of malaria on hemoglobin levels was more than halved. The path-
ophysiology of malarial anemia is complex, involving intravas-
cular hemolysis and splenic clearance of infected erythrocytes
and even more uninfected erythrocytes, as well as suppressed erythropoiesis and dyserythropoiesis. The relative contribu-
tions of these factors seem to vary with age, immunity, and se-
verity and chronicity of infection, among other characteristics
[15]. It would be speculative to comment on potential mecha-
nisms of lessened malarial anemia in ATP2B4 variant allele car-
riers. The effect of mitigating malarial anemia was more
pronounced in heterozygous women than in homozygous
women, although the influence on infection was more distinct
for the latter. At present, we do not have conclusive arguments
to explain this inconsistency. Limited sample sizes in the sub-
groups may again be involved, but this finding may also indi-
cate diverse mechanisms of protection from malaria and from
malarial anemia per se and in individuals with the ATP2B4
SNP in particular.

In conclusion, the recently reported protection against severe
childhood malaria by a common ATP2B4 SNP [2] is not con-
fined to severe disease but also acts against the very different
condition of malaria in pregnancy. This suggests the involve-
ment of a broad or of various protective mechanisms which
need to be identified to potentially deduce preventive or ther-
apeutic measures.

Notes

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Conflicts of Interest. Conflicts that the editors consider relevant to the
content of the manuscript have been disclosed.

References

190:1006–9.

2. Timmann C, Thye T, Vens M, et al. Genome-wide association study indi-

Epidemiology and burden of malaria in pregnancy. Lancet Infect Dis
2007; 7:93–104.

4. Rogerson SJ, Hviid L, Duffy PE, Leke RF, Taylor DW. Malaria in

clinical manifestation of placental malaria in southern Ghana. Malar J
2006; 5:119.

of human malaria parasites by the use of nested polymerase chain reac-

7. Greenwood BM, Greenwood AM, Bradley AK, et al. ELISA tests for
dapsone and pyrimethamine and their application in a malaria che-
 moprophylaxis programme. Bull World Health Organ 1986; 64:
909–16.

8. Finnström O. Studies on maturity in newborn infants. IX. Further ob-
servations on the use of external characteristics in estimating gestation-

2009; 89:1341–78.

onstration of the presence of Ca-ATPase (PMCA) in both microvillous
and basal plasma membranes from syncytiotrophoblast of human term

11. Gazarini ML, Thomas AP, Pozzan T, Garcia CR. Calcium signaling in a
low calcium environment: how the intracellular malaria parasite solves

throcytic malarial parasites and mediate survival to infection. Science
2009; 323:797–800.

13. Bridges DJ, Bunn J, van Mounik IA, et al. Rapid activation of endotheli-
cal cells enables Plasmodium falciparum adhesion to platelet-decorated

14. Lucchi NW, Sarr D, Owino SO, Mwalimu SM, Peterson DS, Moore JM.
Natural hemozoin stimulates syncytiotrophoblast to secrete chemo-
kines and recruit peripheral blood mononuclear cells. Placenta 2011;
32:579–85.

15. Menendez C, Fleming AF, Alonso PL. Malaria-related anaemia. Parasit-
tol Today 2000; 16:469–76.