An ATP2B4 Polymorphism Protects Against Malaria in Pregnancy

George Bedu-Addo, Stefanie Meese, and Frank P. Mockenhaupt

1Department of Medicine, School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana; and 2Institute of Tropical Medicine and International Health, Charité–Universitätsmedizin Berlin, Germany

Polymorphisms of ATP2B4 encoding an ubiquitous Ca\(^{2+}\) pump protect against severe childhood malaria. We assessed the influence of a main polymorphism (rs10900585) on malaria among 834 delivering Ghanaian women. In homoygous 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 involve 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 (Angelholm, 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 primiparous. 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 (homozygous: 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]. 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 genome-wide 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...
In pregnant women, *P. falciparum*-infected erythrocytes sequester in the intervillus space by adhering to ligands on the syncytiotrophoblast. This is frequently paralleled by the local accumulation of hemoglobin, a product of hemoglobin degradation by the parasite, and of inflammatory cells. Specific antibodies capable of blocking parasite adhesion prevent this placental malaria only after successive pregnancies during which the mother is exposed to *P. falciparum* infection [4]. This could explain why our finding of protection against *P. falciparum* infection due to the ATP2B polymorphism is limited to primiparae, a group that is relatively immune naive with respect to the specific strains causing placental malaria. At increased parity, the effects of adaptive immunity may override the protection afforded by ATP2B variation. As with severe malaria [2], the actual mechanisms involved remain to be elucidated. Both *P. falciparum*-infected erythrocytes and hemoglobin activate the syncytiotrophoblast, which, in turn, elicits the
attraction of peripheral blood mononuclear cells to the intervillous space [4, 14]. The role of ATP2B4-dependent intracellular Ca²⁺ 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 pathophysiology of malarial anemia is complex, involving intravascular hemolysis and splenic clearance of infected erythrocytes and even more uninfected erythrocytes, as well as suppressed erythropoiesis and dyserythropoiesis. The relative contributions of these factors seem to vary with age, immunity, and severity and chronicity of infection, among other characteristics [15]. It would be speculative to comment on potential mechanisms of lessened malarial anemia in ATP2B4 variant allele carriers. 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 subgroups may again be involved, but this finding may also indicate 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 confined to severe disease but also acts against the very different condition of malaria in pregnancy. This suggests the involvement of a broad or of various protective mechanisms which need to be identified to potentially deduce preventive or therapeutic measures.

**Notes**

**Acknowledgments.** We thank the participating women and the midwives and administration at Agogo Hospital.

**Financial support.** This work was supported by Charité-University Medicine Berlin (grants 2000-512, 2001-613) and Deutsche Forschungsgemeinschaft (grant GRK1673/B7 to S. M.)

**Potential conflicts of interest.** All authors: No reported conflicts.

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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