Short Report: Genetic Variability in Platelet Integrin α2β1 Density: Possible Contributor to Plasmodium vivax–induced Severe Thrombocytopenia

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Abstract. Understanding the pathogenesis of Plasmodium vivax malaria is challenging. We hypothesized that susceptibility to P. vivax-induced thrombocytopenia could be associated with polymorphisms on relevant platelet membrane integrins: integrin α2 (C807T), and integrin β3 (T1565C). Although β3 polymorphism was not related with P. vivax malaria, α2 807T carriers, which show high levels of integrin α2β1, had a higher probability for severe thrombocytopenia than wild-type carriers. This evidence of the association of integrin polymorphism and P. vivax morbidity was further demonstrated by a moderate but significant correlation between clinical disease and surface levels of the integrin α2β1.

Plasmodium vivax infection is no longer considered a benign disease because it might cause severe or fatal episodes.1,2 Although the mechanisms underlying P. vivax-induced pathogenesis remain poorly studied, thrombocytopenia is frequently observed in P. vivax infection.3 Recent studies suggest an association between deep thrombocytopenia and severity of the illness.4 However, the mechanisms leading to thrombocytopenia, as well as its contribution to malaria pathogenesis, are not well understood.

Besides their central role in homeostasis, platelets contain a wide range of inflammatory, immune-modulating, and angiogenic factors. Consequently, it is not surprising that the role of platelets in the development of an array of disorders continues to emerge.5 Although P. vivax-induced thrombocytopenia has not been investigated in detail,6 several lines of evidence suggest that platelets participate actively in the pathogenesis of malaria.6 In P. vivax malaria, platelets release microparticles into the circulation, and these platelet-derived microparticles seem to be associated with acute inflammatory symptoms of disease.7 In addition, we have shown that levels of plasma cell-free circulating nucleic acids were closely correlated with platelet counts, increasing in a linear fashion with the spectrum of P. vivax malaria.8 On the basis of these observations, we hypothesized that platelet receptor polymorphisms that result in a gain of function in platelet adhesion and/or aggregation in vivo might place carriers at increased risk for P. vivax-induced thrombocytopenia.

We studied the association between P. vivax and polymorphisms of platelet integrins (cell-surface heterodimeric proteins that mediate cell-matrix and cell-cell interactions).5 The focus of this study was two gain-of-function platelet receptor single-nucleotide polymorphisms: the C807T polymorphism of integrin α2 (also known as platelet glycoprotein Ia, GPIa) and the T1565C of integrin β3 (platelet glycoprotein IIIa, GPIIIa). Both polymorphisms have been implicated in different clinical events, including those related to the coronary syndromes, probable because of their gain-of-function mechanisms.9,10 Integrin α2, a platelet receptor for collagen, forms a functional receptor with the integrin β3 subunit, which is essential for platelet function.11 The C807T single nucleotide polymorphism (single-nucleotide polymorphism no. rs126643; National Center for Biotechnology Information, Bethesda, MD) is considered a genetic marker of the integrin α2β1 density.12,13 Integrin β3, a common β subunit for β3-integrins, such as αIIβ3, has a key role in platelet function by binding fibrinogen and von Willebrand factor (vWF).14,15 The T1565C polymorphism (rs9518) seems to increase platelet aggregation.15 To the best of our knowledge, there has been no study that assessed the association between integrin polymorphisms and P. vivax malaria.

A total of 150 P. vivax patients 2–78 years of age were enrolled in the study after written informed consent, as specified by the Brazilian National Council of Health (Protocol CEPSH/CPqRR/03/2008). Antimalarial and supportive therapies were given according to standard protocols. The study included patients with symptomatic but uncomplicated P. vivax malaria, and all volunteers were negative for P. falciparum and/or P. malariae by microscopy and polymerase chain reaction. Demographic, clinico-epidemiologic, and hematologic data of P. vivax-infected volunteers are shown in Table 1.

Because there are no clear criteria to define P. vivax-induced morbidity, we used a previously validated semi-quantitative clinical assessment to enable numerical comparisons.8 In brief, scores of 0 or 1 were assigned to clinical and hematologic parameters reported as absent (or within reference ranges) or present (or outside reference ranges), respectively; the sum of scores provides the patient’s final clinical score (scores range from 0 to 5). Determination of α2 C807T and β3 T1565C integrin genotypes was performed by using polymerase chain reaction–restriction fragment length polymorphism assays with restriction enzymes TaqI (integrin α2) and MspI (integrin β3), as described.16

The prevalence of integrin α2 C807T genotypes in P. vivax patients were 35% CC, 55% CT and 10% TT, and overall allele frequencies were C = 63% and T = 37%. The frequency of P. vivax patients who showed the highest receptor density (807T allele) was similar to that reported for healthy persons in a meta-analysis of seven independent studies (approximately 1,000 healthy persons); 37% were 807T allele carriers.17

To determine the impact of α2 C807T polymorphic variation on susceptibility to morbidity, we stratified patients according
to clinical and hematologic data. Results showed that median platelet counts for *P. vivax* patients carrying the 807T allele (90,500/mm$^3$, interquartile range = 53,000–128,500/mm$^3$) was significantly lower than for patients carrying the wild-type allele (101,000/mm$^3$, interquartile range = 70,000–139,250/mm$^3$) ($P = 0.0228$, by non-parametric Wilcoxon Mann-Whitney test). There was a weak trend toward decreased platelet counts according to the number of mutated alleles (median platelet count = 85,000/mm$^3$ for two mutated alleles and 91,500 for one mutated allele). The general trend of low platelet counts among patients carrying one or two mutated alleles ($\chi^2$ for trend = 5.18, $P = 0.022$) is shown in Figure 1A. The 807T allele was predominantly associated with severe thrombocytopenia (platelets counts < 50,000/mm$^3$) but not with moderate thrombocytopenia (platelets counts = 50,000–100,000/mm$^3$).

Using the wild-type genotype CC as a reference, we found that the TT genotype was associated with an increased probability for severe thrombocytopenia (adjusted odds ratio [OR] = 10.31, 95% confidence interval [CI] = 2.13–49.79, $P = 0.004$) (Figure 2). Also, *P. vivax* patients with the variant genotypes (CT and TT) had a significantly higher risk for severe thrombocytopenia than patients with the CC genotype (adjusted OR = 4.44, 95% CI = 1.23–15.99, $P = 0.023$).

To make numerical comparisons between integrin $\alpha_2\beta_1$ levels and clinical spectrum of *P. vivax* malaria, we scored persons according to integrin $\alpha_2\beta_1$ levels as follows: score = 2 (homozygous for the 807T allele), score = 1 (heterozygous for the 807T allele), and score = 0 (no 807T allele). This criterion was used because 807 T/C dimorphism is a genetic marker of integrin $\alpha_2\beta_1$ levels, and platelets carrying the TT genotype express the highest levels, TC platelets express intermediate levels, and CC platelets express the lowest levels.$^{13,18}$

Using this semi-quantitative assessment, we demonstrated a moderate but significant correlation between intensity of clinical malaria and surface levels of the integrin $\alpha_2\beta_1$ (Spearman $r = 0.2$, $P = 0.011$). Nevertheless, the present study precludes any definite conclusion about clinical disease and integrin $\alpha_2\beta_1$ because it deals only with uncomplicated *P. vivax* malaria. To properly address this question, a follow-up study would be desirable, which included patients with different degrees of malaria.

The $\beta_3$ integrin genotype distribution among *P. vivax* patients showed that the frequencies of the heterozygous (TC) and homozygous (CC) mutations of T1565C were 18.7% and 0.8%, respectively, with a predominance of the TT genotype ($n = 99$, 80.5%). The allele frequencies (90% T and 10% C) we report confirm those of previous studies, which showed that usually more than 80% of the human population harbors the T1565 allele.$^{19}$ In *P. vivax* patients stratified according to platelet counts, we did not find evidence supporting an association between *P. vivax* thrombocytopenia and the T1565C polymorphism of integrin $\beta_3$ (Figure 1B); the T versus C allele comparison showed an adjusted OR of 1.55 (95% CI = 0.44–5.47, $P = 0.419$). No evidence of any gene-disease association was obtained comparing the T1565C polymorphism of the integrin $\beta_3$ among *P. vivax* patients. However, because of the low frequency of CC homozygotes in our sample and the high frequency of polymorphisms in integrin $\beta_3$, it is not appropriate to exclude a role for $\beta_3$ integrin in *P. vivax*-induced thrombocytopenia. In this context, it would be of interest to investigate polymorphisms in the major vWF receptor, glycoprotein-1b (GP1b), because studies have implicated endothelial cell activation with release of active vWF as one pathogenic mechanism contributing to malaria-induced thrombocytopenia.$^{21}$
The pathogenic mechanism involved in the association between hereditary variation in platelet integrin α2β1 density and *P. vivax*-induced thrombocytopenia is unknown. Nevertheless, our evidence showed that the integrin α2C807T polymorphism is associated with an increased risk for *P. vivax*-induced severe thrombocytopenia according to the fact that the coagulation-inflammation cycle acts as a cofactor for malaria morbidity. In this context, if one considers recent findings that suggest that *P. vivax* malaria is associated with excessive endothelial activation and inflammation, one could speculate that perturbed endothelia induced by malaria infection would potentiate an exacerbate platelet-collagen interaction in α2 C807T carriers, whose propensity for collagen-induced platelet aggregation/activation is increased.

Previous finding suggest that thrombocytopenia in persons with malaria might be related to endothelial damage. Furthermore, because platelet activation by agonists such as collagen or thrombin also leads to vesiculation of the platelet membrane and formation of microparticles, this mechanism might amplify *P. vivax*-induced inflammation and contribute to reduced platelet lifespan in malaria patients. We have demonstrated that platelet-derived microparticles might play a role in acute inflammatory symptoms of *P. vivax* malaria. However, a relationship between α2 polymorphism and *P. vivax* thrombocytopenia remains unclear because the contribution of endothelial activation in the pathophysiology of *P. vivax* malaria, as well as mechanisms involved in thrombocytopenia, are unknown. Thus, a useful field of investigation would be the role of platelets and surface integrin receptors in *P. vivax* cytoadhesion, which is a not well-defined phenotype traditionally associated with *P. falciparum* infection.

Because ancestry might play a role in the prevalence of integrin genotypes, it is important to explore whether regional differences in the hematologic status of *P. vivax* malaria are linked to differences in ancestral origins of the study population. Unfortunately, there is little information on integrin polymorphisms in malaria-endemic areas. Consequently, a more comprehensive search for polymorphisms/haplotypes of different integrins may provide additional insight into the role of integrin genetics in malaria.

In conclusion, the present study provides evidence that an integrin α2 C807T polymorphism is associated with an increased risk of *P. vivax*-induced severe thrombocytopenia. It remains to be clarified whether this polymorphism is linked to other relevant mutations. Because the number of cases was relatively small and hematologic variables were obtained at the time of diagnosis, our findings should be interpreted with caution before being confirmed in larger studies. This preliminary study provides a valuable guidance for the future studies in this area.

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