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# The frequency of HLA-DRB1 polymorphisms in Brazilian *Plasmodium vivax* malaria patients and in blood donors from the Amazon Region

Frequência de polimorfismos HLA-DRB1 em pacientes brasileiros com malária por *Plasmodium vivax* e em doadores de sangue da Região Amazônica

Frecuencia de polimorfismos HLA-DRB1 en pacientes brasileños con malaria por *Plasmodium vivax* y en donantes de sangre de la Región Amazónica

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## ABSTRACT

We evaluated the frequency of different HLA-DRB1 alleles in *Plasmodium vivax*-infected individuals and in healthy blood donors from malaria endemic areas of Brazil. Low-resolution human leukocyte antigen-DRB1 genotyping was performed for 73 malaria patients and 29 healthy blood donors. The most frequent alleles in individuals from northern Brazil were human leukocyte antigen-DRB1*04, *08, *07 and *13. The frequency of human leukocyte antigen-DRB1*07 was higher in malaria-infected individuals than in the control group, which reinforces the theory that this allele plays an important role in susceptibility to malaria. This study offers new information about a potential susceptibility factor for *P. vivax* malaria in a Brazilian population that is naturally exposed to malaria.

**Keywords:** Malaria; *Plasmodium vivax;* MHC Class II genes.

## INTRODUCTION

Human leukocyte antigen (HLA) class II genes were originally described as genes involved in immune response because different alleles of these genes are known to influence antibody production[1]. Studies have associated these alleles with diseases of the immune system, such as leukemia[2] and vitiligo[3]. Moreover, population studies have reported the association of certain HLA alleles with susceptibility or resistance to infectious diseases, including leprosy[4], mucosal leishmaniasis[5], tuberculosis[6] and hepatosplenomegaly in schistosomiasis[7]. Few studies have examined the influence of HLA alleles on immunity to malaria. The authors of two studies (published in 1989) were unable to establish a relationship between the HLA-DR types and the immune response to circumsporozoite protein (CSP) of *Plasmodium falciparum*[8] and to sporozoite and gametocytes epitopes in Papua New Guinea[9]. However, two years later, protection against severe *P. falciparum* malaria was found to be associated with the presence of the HLA-Bw53, DRB1*1302 and DQB1*0501 alleles in African children[10]. By the turn of the 21st century,

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some HLA-DR alleles had been associated with an increased antibody response to Nt47 (p126 amino-terminal portion)[11], to apical membrane antigen-1 (AMA-1)[12] of *P. falciparum* and to the VK247 CSP repetition of *P. vivax*[13].

The majority of studies investigating the influence of HLA alleles on the immune response to malaria have been conducted with *P. falciparum* antigens because of the higher mortality attributed to malaria caused by this parasite. However, *P. vivax* is the species responsible for the majority of malaria cases in South and Central America. In Brazil, this species accounts for more than 80% of the clinical cases reported in the Amazon Region[14]. Thus, it is important to understand the factors that control the immune response to vaccine candidate antigens, such as the asexual-blood stage proteins of *P. vivax*, in Brazilian people naturally exposed to the parasite. In this study, we evaluated the HLA-DRB1 allelic frequency in samples from individuals infected with *P. vivax* and from healthy blood donors in malaria endemic areas of Brazil.

## MATERIALS AND METHODS

### STUDY SUBJECTS AND LOCATIONS

The patients who were enrolled in this study met the following criteria: they sought medical assistance for clinical malaria symptoms, were over 18 years old and had a positive *P. vivax* malaria diagnosis by thick blood film. Signed, written informed consent was obtained before peripheral blood samples were collected from each patient. The study subjects were distributed among four Brazilian malaria endemic areas: Macapá, Amapá State (00°02'20" S, 51°03'59" W); Novo Repartimento, Pará State (04°19'50" S, 49°47'47" W); Porto Velho, Rondônia State (-08°45'43" S, 63°54'14" W); and Plácido de Castro, Acre State (10°16'33" S, 67°09'00" W). Macapá is the capital of the State of Amapá, which is located on the banks of the Amazon River, in a tropical forest region. The estimated population of Macapá is 366,486 inhabitants, and its annual parasitic index (API) was 6.0 in 2009. Porto Velho is in the State of Rondônia, located in the upper Amazon River Basin. In 2010, Porto Velho had approximately 383,425 inhabitants and an API of 53.7. Plácido de Castro is located at the border of the States of Rondônia and Amazonas, and it has a population of 18,235 inhabitants. The API of Plácido de Castro was 20.6 in 2009. Novo Repartimento is a gold mining area in the southeast of the State of Pará. Its population was approximately 55,759 inhabitants and it had an API of 15.4 in 2010. These areas are characterized by having a tropical climate without a dry season; the mean monthly precipitation level is at least 60 mm.

Subjects in the control group were blood donors who, according to the Brazilian blood bank policy and the inclusion requirements for this study, met the following criteria: they were over 18 years old; their place of birth was within the study area; they reported never having suffered from malaria attacks and had no signs of malaria during the initial interview; and they had negative results for thick blood film. Molecular diagnosis methods were used for HLA-DRB frequency comparisons. The subjects of the two groups showed no statistically significant differences in mean age or ethnicity, indicating a well-matched population (Fisher's exact test, $p > 0.05$). DNA samples were extracted from frozen peripheral blood, using the Easy-DNA™ extraction kit (Invitrogen, Carlsbad, CA, USA); malaria diagnosis was confirmed using a semi-nested polymerase chain reaction (PCR) with specific small-subunit rDNA primers[15]. The protocol for this study was reviewed and approved by the Research Board of the Faculdade de Medicina de São José do Rio Preto, São Paulo State, Brazil (Process number 235/2006).

### HLA CLASS II ALLELES

DNA samples from the malaria patients ($n = 73$) and the control group ($n = 29$) were subjected to genotyping of the HLA-DRB1 alleles. The DNA concentration was measured using a spectrophotometer at 260 and 280 nm, and a concentration of 100 ng/mL was used for low-resolution genotyping of HLA-DRB1 by PCR with sequence-specific primers (PCR-SSP), as previously described[16].

### STATISTICAL ANALYSIS

Allele frequencies were calculated with the formula $AF = a/N$, in which $a$ represents the number of positive samples for a specific allele and $N$ represents the total number of alleles in the study population[17]. R version 2.8.1 statistical software (The R Foundation for Statistical Computing, Vienna, Austria, http://www.r-project.org) was used for the statistical analyses. The Hardy-Weinberg equilibrium was tested according to Guo and Thompson[18]. The heterogeneity of HLA allelic frequencies between control and malaria-infected groups was evaluated using a Chi-square analysis, with Yate's correction test or Fisher's exact test. P-values less than 0.05 were considered statistically significant.

## RESULTS

HLA-DRB1 genotyping was performed in a total of 102 individuals, including malaria-infected individuals and uninfected controls, from four endemic areas in the north of Brazil. As summarized in table 1, 13 different alleles were found; the frequencies observed for each individual and for each group are also described in table 1. The most frequent alleles for both groups were HLA-DRB1*04, *08, *07 and *13. In malaria-infected individuals, HLA-DRB1*04, *08 and *13 were the most frequent alleles, followed by HLA-DRB1*07. In the control group, HLA-DRB1*04, *08 and *13 were also the most frequent, but HLA-DRB1*07 was one of the least frequent alleles in this group. When we compared the malaria-infected group with the control group, the HLA-DRB1*07 frequency was higher in the infected group than in the control group; this difference was statistically significant ($p = 0.006$, Chi-square test with Yate's correction). The loci were in Hardy-Weinberg equilibrium in the studied population (Pearson's $x^2 = 0.5$, $p = 0.799$).

**Table 1** – Frequencies of HLA-DRB1 alleles in samples from the Brazilian Amazon Region, including control and malaric groups

| HLA-DRB1 alleles | Allele frequencies | | |
|---|---|---|---|
| | All samples (n = 102) | Malaric group (n = 73) | Control group (n = 29) |
| *01 | 0.147 | 0.151 | 0.138 |
| *03 | 0.196 | 0.192 | 0.207 |
| *04 | 0.304 | 0.315 | 0.276 |
| *07 | 0.255 | 0.342† | 0.034 |
| *08 | 0.265 | 0.260 | 0.276 |
| *09 | 0.029 | 0.014 | 0.069 |
| *10 | 0.078 | 0.068 | 0.103 |
| *11 | 0.098 | 0.110 | 0.069 |
| *12 | 0.029 | 0.028 | 0.034 |
| *13 | 0.235 | 0.220 | 0.276 |
| *14 | 0.147 | 0.136 | 0.172 |
| *15 | 0.127 | 0.110 | 0.172 |
| *16 | 0.088 | 0.054 | 0.172 |

n = number of samples; † Chi-square test with Yate's correction, $x^2 = 7.521$, p = 0.006, compared control and malaric groups.

## DISCUSSION

HLA class II molecules are crucial components of the adaptive immune response, and a correlation between HLA alleles and susceptibility to several diseases, including malaria, has been documented[2,3,4,5,6,7,8,9,10,11,12,13]. Moreover, ethnic and/or geographic variations apparently play a major role in this correlation. The nation of Brazil is as large as an entire continent, and its inhabitants are genetically diverse. This diversity results in a mixed population formed by the contributions of three important groups: Caucasians, Africans and Amerindians[19]. Due to the diverse history of colonization, different regions of the country have different levels of prevalence of the three ethnic groups[20,21]. We found a high frequency of HLA-DRB1*04 in our subjects, which is similar to the results seen in previous studies[22,23]. Because this allele is characteristic of indigenous Americans, its prevalence reflects the Amerindian contribution to the Brazilian population, especially in the north of the country[24]. Additionally, the high frequency of HLA-DRB1*04 that was observed in both malaria-infected and healthy individuals indicates that this allele is not associated with susceptibility to or protection from *P. vivax* malaria. On the other hand, the significantly higher frequency of the HLA-DRB1*07 in the infected group suggests a role for this allele in susceptibility to malaria. Oliveira-Ferreira et al[13] detected a poor immune response induced by HLA-DRB1*07 against the VK210 repetitive region of *P. vivax* CSP. In fact, studies with viral vaccine[22,25] and vaccine trials with CSP of *P. falciparum*[26] have shown that this allele fails to trigger a consistent immune response.

Although differences have been observed in the HLA-DRB1*07 frequencies between malaria-infected and uninfected individuals, we have not established a mechanism by which this allele may function in malaria susceptibility. There may be multiple HLA molecules associated with human malaria, although we were only able to analyze the HLA-DRB1 alleles in this study. Analysis of a single HLA molecule cannot fully reflect how multiple genetic variations impact the MHC region. One possibility is that MHC alleles could be in linkage disequilibrium with other relevant genes involved in susceptibility to *P. vivax* malaria. Additionally, patients homozygous for the HLA-DRB1*07 allele could not be distinguished from carriers due to the sampling limitations of this study.

In the Brazilian Amazon Region, malaria predominates in mesoendemic conditions with a wide variation in transmission[14,27]. Thus, malaria endemicity could be viewed as a selective pressure for maintenance of the genotype frequencies of the HLA molecules. Investigation of other malaria-associated HLA alleles, using serological, clinical and epidemiological analysis of a large sample size, is necessary. Such a study should also evaluate the influence of other infectious diseases. The epidemiology of infectious diseases is conditioned by a complex interrelationship between the parasite, its vector and the human being. The parasite-host coevolutionary process can be viewed as an arms race, in which adaptive genetic changes in one are eventually matched by alterations in the other, in this case, within the genetically diverse Amazonian populations[28].

## CONCLUSION

We found that the HLA-DRB1*07 allele frequency was higher in malaria patients than in the control group, which could suggest a role for this allele in susceptibility of the immune system to *P. vivax* malaria. Our results reinforce the need for studies that focus on genes linked to the histocompatibility complex; there is a particular need for studies using a larger sample size, as well as individuals from areas with different rates of infection, to better establish the role of the HLA in susceptibility to *P. vivax* parasites in this region.

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## Frequência de polimorfismos HLA-DRB1 em pacientes brasileiros com malária por *Plasmodium vivax* e em doadores de sangue da Região Amazônica

**RESUMO**

Este estudo avaliou a frequência de diferentes alelos HLA-DRB1 em indivíduos infectados por *Plasmodium vivax* e em doadores de sangue saudáveis provenientes de áreas endêmicas de malária do Brasil. Foi realizada uma genotipagem de baixa resolução dos alelos HLA-DRB1 em 73 pacientes com malária e em 29 doadores de sangue saudáveis. Os alelos mais frequentes em indivíduos do norte do Brasil foram HLA-DRB1 *04, *08, *07 e *13. A frequência de HLA-DRB1 *07 foi maior nos indivíduos infectados com malária do que no grupo controle, o que reforça a hipótese de que esse alelo desempenha um papel importante na suscetibilidade à malária. Esta pesquisa fornece novas informações sobre um fator potencial de suscetibilidade à malária por *P. vivax* em uma população brasileira naturalmente exposta à doença.

**Palavras-chave:** Malária; *Plasmodium vivax;* Genes Classe II do Complexo de Histocompatibilidade (MHC).

## Frecuencia de polimorfismos HLA-DRB1 en pacientes brasileños con malaria por *Plasmodium vivax* y en donantes de sangre de la Región Amazónica

**RESUMEN**

Este estudio evaluó la frecuencia de diferentes alelos HLA-DRB1 en individuos infectados por *Plasmodium vivax* y en donantes de sangre saludables provenientes de áreas endémicas de malaria de Brasil. Se realizó un genotipado de baja resolución de los alelos HLA-DRB1 en 73 pacientes con malaria y en 29 donantes de sangre saludables. Los alelos más frecuentes en individuos del norte de Brasil fueron HLA-DRB1 *04, *08, *07 y *13. La frecuencia de HLA-DRB1 *07 fue más grande en individuos infectados con malaria que en el grupo control, lo que refuerza la hipótesis de que ese alelo desempeña un papel importante en la susceptibilidad a la malaria. Esta investigación suministra nuevas informaciones sobre un factor potencial de susceptibilidad a la malaria por *P. vivax* en una población brasileña naturalmente expuesta a la enfermedad.

**Palabras clave:** Malaria; *Plasmodium vivax;* Genes Clase II del Complejo de Histocompatibilidad (MHC).

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