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ASSOCIATION BETWEEN DUFFY BLOOD GROUP SYSTEM VARIANTS AND SUSCEPTIBILITY AND RESISTANCE TO MALARIA IN THE BRAZILIAN AMAZON

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ABSTRACT: The interaction between Duffy blood group proteins and *Plasmodium vivax* is necessary for the establishment of the erythrocytic phase of malaria, because these molecules act as receptors for the protozoan. This study showed significant associations between Duffy blood group variants and susceptibility and resistance to vivax malaria. In the present study, Duffy blood group genotyping and phenotyping were carried out for 244 individuals living in a Brazilian area where malaria is endemic and *P. vivax* identification was performed by thick blood smears. Our results showed that 80 individuals were positive and 164 were negative to *P. vivax*. We registered a high frequency of genotype *FYAFYB* (47.5%), followed by *FYBFY* (15.6%), *FYAFYA* (14.3%), *FYBFYB* (11.5%), *FYAFY* (8.6%), and *FYFY* (2.5%). The frequencies of *FYA*, *FYB*, and *FY* alleles were 55%, 38.8%, and 6.3% in infected individuals, respectively, whereas in non-infected ones they were 36.3%, 45.1%, and 18.6%, respectively. These results demonstrate that *FYA* allele is more frequent in individuals infected with vivax malaria. The null genotype was not found in infected subjects, but was present in 3.7% of the non-infected individuals. We also found 11.5% discordance between genotype and phenotype. The estimated genetic frequency proved that the population participating in the study did not show genetic balance according to Hardy-Weinberg.

KEY WORDS: Duffy blood group system, malaria, *Plasmodium vivax*.

ASSOCIAÇÃO ENTRE O SISTEMA DE GRUPO SANGUÍNEO DUFFY E A SUSCEPTIBILIDADE E RESISTÊNCIA À MALÁRIA NA AMAZÔNIA BRASILEIRA

RESUMO: A interação entre as proteínas do grupo sanguíneo Duffy e o *Plasmodium vivax* é necessária para o estabelecimento da fase eritrocitária da malária, pois essas moléculas funcionam como receptores do protozoário. Este estudo mostrou associações significativas entre as variantes do grupo sanguíneo Duffy e a suscetibilidade e resistência à malária vivax. No presente estudo, 244 indivíduos, residentes em área onde a malária é endêmica no Brasil, foram fenotipados e genotipados para o sistema de grupo sanguíneo Duffy e também submetidos ao exame da gota espessa para pesquisa de *P. vivax*. Os resultados mostraram que 80 indivíduos foram positivos e 164 negativos para *P. vivax*. Foi registrada alta frequência do genótipo *FYAFYB* (47,5%), seguida de *FYBFY* (15,6%), *FYAFYA* (14,3%), *FYBFYB* (11,5%), *FYAFY* (8,6%) e *FYFY* (2,5%). As frequências dos alelos *FYA*, *FYB* e *FY* foram 55%, 38,8% e 6,3% em indivíduos infectados, respectivamente, ao passo que nos não infectados foram 36,3%, 45,1% e 18,6%, respectivamente. Esses resultados demonstram que o alelo *FYA* é mais frequente no grupo de indivíduos infectados com malária vivax. O genótipo nulo não foi encontrado nas pessoas infectadas, porém estava presente em 3,7% dos indivíduos não infectados. Também houve 11,5% de não concordância entre fenótipo e genótipo. Os cálculos de frequência gênica demonstraram que a população estudada não se encontra em equilíbrio gênico de acordo com Hardy-Weinberg.

PALAVRAS-CHAVE: Sistema de grupo sanguíneo Duffy, malária, *Plasmodium vivax*.

INTRODUCTION

Malaria is one of the main illnesses that occur in Brazil and it is endemic in certain areas, such as the Amazon Region, due to the climate, hydrography, rain, frequent floods, and poor housing conditions. Because of these factors, this region becomes a good breeding environment for the parasite, causing the disease to spread (Tadei et al., 1998).

In the Amazon Region, malaria caused by *Plasmodium vivax* (Grassi & Feletti, 1989) is the most prevalent, responsible for 80% of all known cases of the disease. This high occurrence of vivax malaria was observed by Tadei et al. (1998) when studying the Amazon anopheline mosquitoes. They reported that the mosquitoes were infected by *P. vivax* two and a half times more frequently than by *Plasmodium falciparum* (Welch, 1897) and almost thirty times more than by *Plasmodium malariae* (Laveran, 1922).

One of the interesting aspects of the Duffy blood group system is that it functions as a recipient to *P. vivax* and *Plasmodium knowlesi* merozoites, responsible for different types of malaria that affect both humans and primates (Miller et al., 1975, 1976). In 1989, researchers reached a consensus that the erythrocyte glycoprotein, which carries the determinants of Duffy, is necessary as a connector in the process of erythrocyte invasion by *P. vivax* merozoites (Barnwell et al., 1989). The antigens Fya and Fyb are codified by two allelic variants of the FY gene. Fya antigen differs from the Fyb antigen as a result of a single nucleotide difference (A131 or G) encoding amino acid Gly44 (Fya) or Asp (Fyb) in the N-terminal extracellular domain of the glycoprotein (Mallinson et al., 1995). FY allele, characterized by a single replacement of the nucleotide -33T>C in the promoting region GATA-box from de FYB gene, influences the non-expression of antigen Fyb in the membrane of the erythrocytes. This allele can appear in heterozygosis or homozygosis: FYAFY, FYBFY, and FYFY. When in homozygosis, it corresponds to the null phenotype Fy(a-b-), resulting in the absence of the antigen Fyb only in the erythrocytes, but not altering the presence of this protein

in other tissues (Tournamille et al., 1995). The results of a natural relationship between the GATA-box region and FYA^{null} allele was found in endemic regions of *P. vivax* in Papua New Guinea (Zimmerman et al., 1999).

FYX allele was described in more details by Lewis et al. (1972), who showed that it reacts more weakly to anti-Fyb antibody. The relatively rare mutation C265T in FYB allele characterizes FYX allele and appears in 2% of the Caucasian population (Parasol et al., 1998; Tournamille et al., 1998).

The Duffy blood group system has been the subject of many researches and it has long been considered a likely target of natural selection, due to the extreme pattern of geographic differentiation of its three major alleles (Hamblin et al., 2002). Tournamille et al. (1998) concluded that the Duffy blood group system is controlled by four alleles.

In this study, we compared the frequency of occurrence of Duffy alleles in a population from the state of Amazonas, in Brazil, an area where malaria is endemic, relating it to individual vulnerability and resistance to this disease.

MATERIALS AND METHODS

This study was approved by the Ethics Committee of the Instituto Nacional de Pesquisas da Amazônia (INPA) (023/2006) in June 6, 2006.

LOCATION

All materials and samples used in this study were collected in Presidente Figueiredo, a town approximately 100 km from Manaus by highway BR 174, located in the state of Amazonas.

BLOOD SAMPLES

A total of 244 individuals who were attended in the local hospital presenting with the symptoms of malaria participated in this study. After signing their consent, we collected 5 mL of their blood in tubes containing EDTA (ethylenediaminetetraacetic acid) and 1 µL of blood by digital puncture to diagnose malaria using the thick blood smear test.

BLOOD GROUP SEROLOGY

We used the Duffy phenotype method to study the Duffy protein in the surface membrane of the erythrocytes. These data were used to contrast the phenotype and the genotype as well as to evaluate the percentage of similarity between them. Phenotypes were determined by hemagglutination in gel cards, according to the manufacturer's specifications (DiaMed AG, Morat, Switzerland).

DNA PREPARATION

As soon as the blood samples were collected, DNA was extracted using the Easy-DNA Kit (Invitrogen) and kept at -20°C to be used later.

BLOOD GROUP GENOTYPING

The molecular methodology described by Olsson et al. (1998) was applied in this study for Duffy blood group genotyping since it is a very simple and quick way to identify the four possible alleles at the FY locus through polymerase chain reaction (PCR). For the amplification of FYA, FYB, FY, and FYA^{null} alleles, the following primers were used: GATAFY2, FYAB2, FYAREV, and FYBREV. The nucleotide sequences are shown in Table 1.

STATISTICAL ANALYSIS

The results obtained in this study were analyzed using the Epi Info program. The comparisons between the genotypic and phenotypic frequencies were performed using Pearson's chi-square test (χ^2) at a 5% significance level, considering significant differences concerning p-values (P) smaller than the significance level ($P < \alpha$).

The method brought forward by Bernstein to estimate the frequencies of the alleles researched in the population under study starting from the knowledge of the phenotypic distribution of the Duffy blood group was also applied (Cabello & Krieger, 1997).

After the analysis, we verified whether the distribution of the phenotypes in the sample studied matched the genetic hypothesis, that is, if the sample really represented a balanced population, according to the Hardy-Weinberg principle, using Pearson's chi-square test at a 5% significance level.

RESULTS

The samples were collected between November 2007 and June 2008. The average age of the participants was 32 years. Among the individuals researched, 80 tested positive for vivax malaria (32.79%) and 164 yielded negative results by smear (67.21%).

The following Duffy phenotypes, in decreasing order of prevalence, were serologically determined: Fyab (36.07%), Fya (34.43%), Fyb (27.05%), and Fy (2.46%).

In Table 2 we compare Duffy blood group genotypes and deduced phenotypes as well as the allelic frequency of individuals infected and non-infected with *P. vivax*.

The frequencies of FYA, FYB, and FY alleles were 55%, 38.8%, and 6.3%, respectively, in the individuals positive to vivax malaria, whereas in the group tested negative, they were 36.3%, 45.1%, and 18.6%, respectively.

None of the patients infected with malaria but 3.7% of the individuals that tested negative to the disease were homozygous for FY (FYFY) allele. This allele was significantly more prevalent in the patients negative to vivax malaria ($P < 0.05$).

Table 1 - Sequence of specific primers used for PCR.

Primer	Nucleotide sequence 5' 3'
GATAFY2	5' CTCATTAGTCCTTGGCTCTTAC 3'
FYAB2	5' CTCATTAGTCCTTGGCTCTTAT 3'
FYAREV	5' AGCTGCTTCCAGGTTGGCAC 3'
FYBREV	5' AGCTGCTTCCAGGTTGGCAT 3'

Source: Olsson et al. (1998).

DISCUSSION

The frequencies of Duffy blood group system alleles vary in different populations (Castilho et al., 2004; Cavasini et al., 2001; Cavasini et al., 2007a; Cavasini et al., 2007b). Researches carried out in regions where malaria is endemic detected the presence of defense mechanisms to *P. vivax*, determining the appearance of phenotypes that were different from expected.

Duffy allele frequency in the present study differed from data described in the literature, a fact that may have occurred due to the pressure present in the selection.

In this study, we found 11.5% discrepancy between the phenotype and genotype in relation to Fyb antigen. It is important to emphasize the importance of Duffy genotyping in patients presenting phenotype Fyb(-), due to the weakened expression of Fyb antigens in the erythrocyte surface, which are not detected by the antibodies, only analyzing the presence of FYX gene.

This study showed significant associations between Duffy blood group variants and susceptibility and resistance to malaria. FYA allele was prevalent in infected individuals, while FYB allele appeared in higher frequency in individuals tested negative for malaria, al-

though not statistically significant. Homozygous FYB allele did not indicate statistically significant differences ($P > 0.05$) comparing the two groups, but when FYB allele was associated with FY allele in heterozygous subjects, the difference between the two groups was statistically significant. FYB allele did not present protection to the infection in homozygotes (FYBFYB). However, its protective effect appeared when associated to FY allele ($P < 0.05$) in heterozygous individuals (FYBFY).

Our data suggest that individuals homozygous for FYA allele have higher susceptibility to *P. vivax* infection, while in FYAFY heterozygous individuals this susceptibility is reduced, since the presence of FY allele confers protection against this infection, as already mentioned. Regarding genotype FYAFY, no difference was found between the groups ($P > 0.05$).

On the other hand, individuals homozygous for FY allele were not found in the infected group, and the presence of FY allele showed a decrease in susceptibility to vivax malaria or an increase in protection against this infection when associated with FYA and FYB alleles, respectively. These findings suggest that this mutation could be an advanta-

Table 2 – Comparison of Duffy blood group genotypes and deduced phenotypes and allelic frequency of individuals from the Brazilian Amazon testing positive and negative to *P. vivax* infection, in 2007-2008.

Duffy blood group		<i>P. vivax</i> infection		
Genotype	Deduced phenotype	Negative (n = 164)	Positive (n = 80)	P ¹
FYA FYA	Fy(a+b-)	12	23	0.00001*
FYB FYB	Fy(a-b+)	20	8	0.61420
FYA FYB	Fy(a+b+)	77	39	0.79210
FYA FY	Fy(a+b-)	18	3	0.05938
FYB FY	Fy(a-b+)	31	7	0.04047*
FY FY	Fy(a-b-)	6	0	0.08942
Allele		Allelic frequency		
FYA		60 (36.3%)	44 (55.0%)	0.00643*
FYB		74 (45.1%)	31 (38.8%)	0.34632
FY		30 (18.6%)	5 (6.3%)	0.00904*

¹ p-value (P) calculated by Pearson's chi-square test (χ^2), using the Epi Info program, at a significance level (α) of 0.05.

* Significant differences between the two groups.

geous selection in the population from areas where *P. vivax* is endemic.

Our results showed a high frequency of phenotype Fyab (47.5%), whereas genotype FYAFYB, differently than expected, did not present significant differences between the groups studied ($P > 0.05$).

The null genotype was not found in infected subjects, but it occurred in 3.7% of the individuals tested negative, as it was already expected, since this phenotype presents resistance to the infection caused by *P. vivax*, as described previously (Barnwell et al., 1989; Cavasini et al., 2001).

Some authors reported the occurrence of vivax malaria in individuals homozygous for FY allele (Castilho et al., 2004; Cavasini et al., 2007b; Ryan et al., 2006). FY allele presented significantly increased frequency in the group that tested negative to vivax malaria, showing that this numerical mutation might be an advantageous selection in the population from areas where this disease is endemic. We also observed a decrease in the occurrence of infected individuals among those homozygous for FY allele. Although Zimmerman et al. (1999) reported the presence of FY^{null} allele among the participants in their research, in the present study we found no occurrence of this allele, which is in accordance with the findings of Cavasini et al. (2001).

Based on the concepts of gene frequency and evolution, we analyzed the genetic balance of the population under study using the Hardy-Weinberg principle. The results obtained in this research were significantly different from expected. Our results show that the population under study is not in genetic balance according to the Hardy-Weinberg principle. There is natural selection pressure in this population and this natural adaptation in a region where malaria is endemic may lead to partial defense mechanisms against *P. vivax*.

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