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Frequency of the *HFE C282Y* and *H63D* polymorphisms in Brazilian malaria patients and blood donors from the Amazon region

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ABSTRACT. Malaria is an endemic parasitosis and its causitive agent, *Plasmodium*, has a metabolism linked to iron supply. *HFE* is a gene with the polymorphisms *C282Y* and *H63D*, which are associated with a progressive iron accumulation in the organism leading to a disease called hereditary hemochromatosis. The aim of the present study was to determine the allelic and genotypic frequencies of the *HFE* gene polymorphisms in malaria patients and blood donors from the Brazilian Amazon region. We screened 400 blood donors and 400 malaria patients for the *HFE C282Y* and *H63D* polymorphisms from four states of the Brazilian Amazon region by polymerase chain reaction and restriction fragment length polymorphism analysis. We did not find any *C282Y* homozygous individuals, and the only five heterozygous individuals detected were from Pará State. The most frequent genotype in the North region of Brazil was the *H63D* heterozygote, in both study groups. Our

results contribute to the concept that the Brazilian Amazon region should not be regarded as a single entity in South America. These polymorphisms did not influence the symptoms of malaria in the population studied, as neither severe signs nor high parasitemia were observed. Therefore, different hereditary hemochromatosis diagnostic and control measures must be developed and applied within its diverse locations. Investigations are currently being carried out in our laboratory in order to determine the importance of the coexistence of hereditary hemochromatosis in patients affected by parasitic diseases, such as malaria.

Key words: Malaria; *HFE* gene polymorphisms; Blood donors; Brazilian Amazon region

INTRODUCTION

HFE gene mutations are one cause of a recessive autosomal disease called hereditary hemochromatosis (HH) characterized by an increase in the intestinal absorption of iron, leading to a gradual accumulation in the parenchymatous cells of the liver, heart and endocrine glands. Reports have described the influence of HH on the clinical expression of infectious parasitic diseases, specifically in patients who present infections caused by metabolic pathogens associated with iron supply (Martinelli et al., 2000). Hemochromatosis seems to be rare in Brazil, accounting for only 1% of the causes of liver disease in the country (Farias et al., 2000). Bittencourt et al. (2002) showed a genotypic frequency of 53% and allelic frequency of 56.7% for homozygous *C282Y* in HH patients from the Northeast region of Brazil. In the present study, no compound heterozygosity for the *C282Y* and *H63D* polymorphisms was detected. Additionally, Agostinho et al. (1999) observed a frequency of 16.3% for the *H63D* polymorphic allele in a healthy Caucasian population from the Brazilian Southeast region, whereas a 7.5% frequency of the same allele was detected in African descendents from the same area. On the other hand, allelic frequencies of the *C282Y* polymorphism were lower in both Caucasian (1.4%) and African descendents (1.1%) and absent in an Amerindian group. Pereira et al. (2001) observed an allelic frequency of 3.7% for *C282Y* and 20.3% for *H63D* in Euro-Brazilians from the Southeast region. These authors also detected allelic frequencies of 13.0 and 6.4% for the *H63D* polymorphism in the admixed population and Afro-Brazilians from the same population, respectively. For the *H63D* polymorphism, they observed a genotypic frequency of 63.5% for homozygous *C282Y* in Euro-Brazilians, 75.7% for admixed individuals and 87.6% for Afro-Brazilians. In addition, for the *C282Y* polymorphism, the frequencies were 92.7% for Euro-Brazilians, 98.6% for the admixed population and 99.0% for Afro-Brazilians. Recently, the allelic frequencies found in individuals with alpha-thalassemia and beta-thalassemia heterozygotes from different Brazilian regions were 0.98 and 0.29% for the *C282Y*, and 13.72 and 9.54% for the *H63D* polymorphism, respectively (Oliveira et al., 2006).

Malaria is an infectious disease caused by parasites of the genus *Plasmodium*, whose metabolic processes are dependent on iron. More than 99% of malaria cases in Brazil are registered in the Amazon Basin, characterizing this area as the endemic area of the country. As it is multi-systemic, this protozoan disease attacks organs including the brain, kidneys, liver, and spleen (Weatherall et al., 2002). Here, we report allelic and genotypic frequencies of two *HFE* genetic variants in patients with malaria and blood donors from the Brazilian Amazon.

SUBJECTS AND METHODS

After given written informed consent, 800 peripheral blood samples were drawn from malaria patients (N = 400) and volunteer blood donors (N = 400) from the four Brazilian Amazon States (Acre, Amapá, Pará, and Rondônia) who were invited to participate in the study. All individuals studied were classified as a unique admixed ethnic group (African American, Caucasian and/or Amerindian descendents). The malaria patients had laboratory diagnosis of thick blood film and symptoms compatible with malaria. The blood donors were matched to the patients with respect to age (± 5 years), gender and ethnicity. All subjects were not related and were considered genetically independent. The overall mean age of the population was 37 years old. A comparison of mean ages of the four groups studied was performed and no statistical differences were detected. DNA was extracted from the blood samples using a modified phenol-chloroform method (Pena et al., 1991). *HFE* polymorphisms were detected by restriction enzyme digestion of polymerase chain reaction products as described by Lynas (1997). In the first step, the DNA samples were processed by polymerase chain reaction using 25-µL reaction volumes (50 ng of template DNA, 1X *Taq* buffer, 0.25 µM of each primer, 200 mM of dNTP mix, 1.6 mM $MgCl_2$, and 1 U *Taq* DNA polymerase). DNA amplification was carried out under the following conditions: 95°C for 3 min, 32 cycles of 45 s at 95°C, 58°C for 30 s and 72°C for 1 min, followed by a final extension at 72°C for 5 min. The amplified *HFE* fragments were digested using *Rsa*I (Invitrogen) for the *C282Y* polymorphism at 50°C for 7 h and *Bcl*I (Invitrogen) for the *H63D* polymorphism at 37°C for 7 h, and the digested fragments were separated by 2% agarose gel electrophoresis. Analyses were performed using the Epi-Info version 6.0 statistical software. To obtain the independence among the proportions, the Fisher exact test was applied at a significance level of $P < 0.05$. The protocol for this study was approved by the Research Board Ethics Committee of the São Paulo State University (UNESP).

RESULTS AND DISCUSSION

To our knowledge, this is the first study to report the allelic and genotypic frequencies of the *HFE C282Y* and *H63D* polymorphisms in malaria patients and blood donors from the four states that make up the Brazilian Amazon region. We did not identify any homozygous (C282Y/C282Y) individuals for the *C282Y* polymorphism. Five heterozygous individuals for the *C282Y* polymorphism (WT/C282Y) were identified only for Pará State. The genotype frequencies obtained among the groups studied were in Hardy-Weinberg equilibrium. Genotypic and allelic frequencies of the *H63D* polymorphisms in malaria patients and blood donors are summarized in Table 1. In the Brazilian Amazon region, 0.5% (4/800) homozygous individuals for *H63D* polymorphism were detected. In this same area, we identified 0.13% (107/800) heterozygous individuals. Regarding the genotype WT/WT, 0.86% (689/800) was detected. In the Brazilian Amazon, the allelic frequency of *H63D* and *C282Y* polymorphisms was 0.07 and 0.003, respectively. The prevalence of the *H63D* allele was significantly higher ($P < 0.05$) among blood donors. For heterozygotes (WT/H63D), our results demonstrated a significant difference ($P < 0.05$), which was lower for malaria patients than for blood donors.

Table 1. Allelic and genotypic frequencies of the *H63D* polymorphism in malaria patients and blood donors from Brazilian Amazon region.

Genotype	North region (N = 800)	Amapá State (N = 200)	Pará State (N = 200)	Acre State (N = 200)	Rondônia State (N = 200)
Malaria patients (N = 400)					
H63D/H63D	-	-	-	-	-
WT/H63D	10.75	7.5	4.5	4.0	5.5
WT/WT	89.25	42.5	45.5	46.0	44.5
Allele *H63D*	0.053	0.0375	0.0225	0.02	0.0275
Blood donors (N = 400)					
H63D/H63D	1.0	0.5	-	0.5	1.0
WT/H63D	16.0*	10.5	7.0	6.0	8.5
WT/WT	83.0	39.0	43.0	43.5	40.5
Allele *H63D*	0.09	0.00575	0.035	0.035	0.0525

*P < 0.05. WT = wild type.

The Brazilian population displays an elevated degree of miscegenation. Our data show low *HFE* gene genotypic and allelic frequencies in malaria patients (both for *Plasmodium falciparum* and *P. vivax*) and blood donors from North Brazil, corroborating previously described studies for populations from the Southeast (Martinelli et al., 2000) and Northeast of Brazil (Bittencourt et al., 2002). The presence of the WT/H63D genotype may be a selective advantage in the population, reducing the rate of infection by *Plasmodium* in this region. Our data show higher allelic and genotypic frequencies of the *H63D* compared to the *C282Y* polymorphism, as observed by Oliveira et al. (2006), since in the present study we assessed the genotypes of a healthy population, and not those from individuals affected by HH. The allelic and genotypic frequencies found here were similar to those observed by Agostinho et al. (1999) and Pereira et al. (2001) in a population from the Brazilian Southeast region and different from previously published results of Oliveira et al. (2006) in Brazilian thalassemic patients.

HH has several clinical features and the occurrence of the *HFE* genotypes is not a predictive factor for clinical manifestations, and thus, other factors possibly involved in the expression of the genotype such as gender, age, diet, chronic alcoholism, blood transfusions, or infectious and parasitic diseases cannot be excluded. Our results showed low frequencies of *HFE* gene polymorphisms in uncomplicated cases of malaria in the Brazilian Amazon. This fact demonstrated that these polymorphisms did not influence the symptoms of malaria in the studied population, as neither severe signs nor high parasitemia were observed. However, these data are insufficient to discount totally the possibility of this parasitosis aggravating the symptoms in patients who have the *HFE* gene polymorphisms, specifically in mid- and high-epidemic areas, such as Africa and Asia, where cases of severe malaria are found. In these cases, individuals can possibly present greater hepatic involvement, aggravating the clinical outcome due to the parasitosis, as the presence of just one polymorphic allele of the *HFE* gene is capable of contributing to increases in the levels of organic iron. This condition would favor not only the appearance of hepatocellular carcinoma, but other illnesses too. Additionally, the high ferrous levels may stimulate the multiplication and the development of *Plasmodium*, elevating its pathogenicity in the organism. On the other hand, the great accumulation of iron in individuals with hemochromatosis may become toxic and even lethal for *Plasmodium*, as the parasite cannot manage the metabolism of great amounts of the metal (Oppenheimer, 2001). The fact that we did not find associations between *HFE* gene polymorphisms and the clini-

cal signs of malaria may also suggest that this disease can coexist with alterations in the iron levels caused by the presence of *HFE* gene polymorphisms.

Our results contribute to the concept that the Brazilian Amazon region should not be regarded as a single entity in South America. Therefore, different HH diagnostic and control measures must be developed and applied within the diverse Brazilian locations.

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