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

F.R. Torres$^1$, W.C. Souza-Neiras$^{1,2}$, A.A. D’Almeida Couto$^1$, V.S.C. D’Almeida Couto$^1$, C.E. Cavasini$^2$, A.R.B. Rossit$^2$, R.L.D. Machado$^2$ and C.R. Bonini-Domingos$^1$

$^1$Laboratório de Hemoglobinas e Genética das Doenças Hematológicas, Departamento de Biologia, Instituto de Biociências, Letras e Ciências Exatas (IBILCE-UNESP), São José do Rio Preto, SP, Brasil
$^2$Departamento de Doenças Dermatológicas, Infecciosas e Parasitárias, Centro de Investigação de Microrganismos, Faculdade de Medicina de São José do Rio Preto, São José do Rio Preto, SP, Brasil
$^3$Faculdade SEAMA, Macapá, AP, Brasil

Corresponding author: C.R. Bonini-Domingos
E-mail: bonini@ibilce.unesp.br

Received October 18, 2007
Accepted December 11, 2007
Published January 25, 2008

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 MgCl₂, 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 RsaI (Invitrogen) for the C282Y polymorphism at 50°C for 7 h and BclI (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.
Frequency of the \textit{HFE} polymorphisms in Brazil

The Brazilian population displays an elevated degree of miscegenation. Our data show low \textit{HFE} gene genotypic and allelic frequencies in malaria patients (both for \textit{Plasmodium falciparum} and \textit{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 \textit{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.

\textit{HH} has several clinical features and the occurrence of the \textit{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 \textit{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 \textit{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 \textit{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 \textit{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 \textit{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 \textit{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.

ACKNOWLEDGMENTS

The authors wish to thank all individuals enrolled in this study and the managing directors of the Government Medical Centers and Blood Bank Managing of the States of Acre, Amapá, Pará, and Rondônia for their help in the collection of malaria samples. Professor Luiz Hildebrando Pereira da Silva provided facilities at CEPEM. Financial support was provided by FAPESP and CAPES.

REFERENCES


