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Molecular epidemiology of HFE gene polymorphic variants (C282Y, H63D and S65C) in the population of Espírito Santo, Brazil

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ABSTRACT. Hereditary hemochromatosis (HH) is an autosomal recessive disorder that leads to progressive iron accumulation and may cause cirrhosis, hepatocellular carcinoma, diabetes, and heart failure. Most cases of HH have been linked to mutations in genes associated with iron homeostasis. There have been three major variants in the high Fe (*HFE*) gene associated with the disease: C282Y, H63D and S65C. In this context, we aimed to evaluate the prevalence of the polymorphic variants (C282Y, H63D and S65C) of the *HFE* gene in the population of the Espírito Santo State (ES), Brazil by analyzing three different groups: general population (N = 120), Pomeranian descendants (N = 59), and patients with HH (N = 20). Using genomic DNA extracted from peripheral blood, polymorphic variant identification was performed by

polymerase chain reaction-restriction fragment length polymorphism. Statistically significant differences were observed for genotype distribution of C282Y ($P < 0.001$) and H63D ($P = 0.013$) between the general population and the patients diagnosed with HH. This is the first study to analyze *HFE* gene allele frequencies for the general population, Pomeranian subpopulation, and patients with HH of ES, Brazil.

Key words: Hereditary hemochromatosis; HFE gene; Polymorphisms; Iron overload; Iron metabolism disorders; Espírito Santo-Brazil

INTRODUCTION

Hereditary hemochromatosis (HH) is an autosomal recessive disorder of iron metabolism with incomplete penetrance and variable expressivity (Møller et al., 2010; Santos et al., 2012; Leão et al., 2014). It is characterized by a progressive iron accumulation and abnormal deposition in several organs, such as the liver, heart, pancreas, joints, and skin, leading to cirrhosis, heart failure, diabetes, arthritis, hypogonadism, skin pigmentation and hepatocellular carcinoma (Møller et al., 2010; Neghina and Anghel, 2011; Salgia and Brown, 2015).

Most cases of hemochromatosis have been linked to mutations in genes associated with cellular iron homeostasis, especially the high Fe (*HFE*) gene that is mutated in more than 80% of HH cases (Santos et al., 2012; Pietrangelo, 2015). This gene is located on the short arm of chromosome 6 (Feder et al., 1996) and influences iron absorption by modulating the expression of hepcidin, the main controller of iron metabolism (Barton et al., 2015). When the HFE protein is mutated, the expression of hepcidin decreases and intestinal iron absorption increases (Salgia and Brown, 2015).

The most common genetic variants in the *HFE* gene associated with HH are C282Y (p.Cys282Tyr, c.845G > A, rs1800562), H63D (p.His63Asp, c.187C > G, rs1799945), and S65C (p.Ser65Cys, c.193A > T, rs1800730). The C282Y polymorphic variant consists of a substitution in which cysteine is replaced by tyrosine at amino acid position 282 due to a guanine to adenine transition at position 845 of the *HFE* gene. A transversion of cytosine to guanine at position 187 of the *HFE* gene results in the H63D common substitution of histidine for aspartic acid at amino acid position 63. Finally, the substitution of the amino acid cysteine for serine at position 65 because of a transversion of adenine to thymine at position 193 of the *HFE* gene causes the S65C polymorphism (Santos et al., 2012; Leão et al., 2014; Salgia and Brown, 2015).

The major variant that has been associated with *HFE*-related HH is C282Y, particularly when homozygous (Santos et al., 2012). This polymorphism has different rates of incidence according to ethnic diversity, being highly prevalent in Caucasians and much less common in other ethnicities (Ekanayake et al., 2015; Pietrangelo, 2015). The H63D variant has higher prevalence than C282Y in the general population, and despite having clinical interest, is rarely pathological on its own and usually requires compound heterozygosity to be considered as a risk factor for the development of HH (Ekanayake et al., 2015; Silva and Faustino, 2015). S65C is a less common *HFE* variant and is of little clinical significance on its own, but when associated with compound heterozygosity with C282Y can cause symptomatic disease (Ekanayake et al., 2015; Silva and Faustino, 2015).

Considering that single nucleotide polymorphism (SNP) frequencies are population-specific and vary between different regions, this article aimed to characterize the general

population, Pomeranian subpopulation, and patients diagnosed with hemochromatosis of the Espírito Santo state (ES), Brazil. We also identified and evaluated the frequency of the polymorphic variants C282Y, H63D and S65C of the *HFE* gene.

MATERIAL AND METHODS

Sample

Five milliliters of peripheral blood were collected from 120 healthy volunteers of the ES general population and from 20 volunteer patients diagnosed with hemochromatosis and treated by phlebotomies at the Centro de Hemoterapia e Hematologia do Espírito Santo - HEMOES, a blood center located in the city of Vitória, ES. As for the 59 healthy Pomeranian volunteers of Santa Maria de Jetibá, ES, 3-5 peripheral blood drops were collected on FTA_ Elute Cards (Whatman, USA).

This study was approved by the Research Ethics Committee of the Federal University of Espírito Santo and all participants provided written informed consent.

Genotyping

Genomic DNA was isolated using phenol/chloroform extraction or following the FTA_ Elute Card manufacturer recommendations (Whatman, USA). Genotyping of *HFE* gene C282Y, H63D and S65C polymorphisms was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)-based methods as previously described (Cançado et al., 2007).

Statistical analysis

SNP allele and genotype frequencies were determined by direct counting. Fisher's exact test was performed using Epi-Info version 7.1.5.2 and P values < 0.05 were considered statistically significant.

RESULTS

The *HFE* gene polymorphisms were found in 26.65, 35.59 and 55% of samples from the general population, Pomeranian subpopulation and patients with hemochromatosis, respectively. The allele and genotype frequencies of C282Y, H63D and S65C found in each group are shown in Table 1 and Table 2.

Significant results ($P < 0.001$) were found for the genotype distribution of the C282Y polymorphic variant when comparing patients diagnosed with hemochromatosis and the general population by either combined analysis of homozygotes and heterozygotes or only heterozygotes. In addition, a significant association ($P = 0.013$) for the presence of compound heterozygote of the H63D polymorphism in patients diagnosed with hemochromatosis compared to the general population was found.

When considering sex, there was no statistically significant difference in genotype distributions between the general population and patients diagnosed with hemochromatosis. Unfortunately, it was not possible analyze sex for the Pomeranian subpopulation due to lack of data.

Table 1. Allele frequencies of C282Y, H63D and S65C polymorphisms in the *HFE* gene.

Allele	General population N = 120/A = 240	Pomeranian population N = 59/A = 118	Patients with hemochromatosis N = 20/A = 40
C282Y	4 (1.67%)	5 (4.24%)	10 (25%)
H63D	28 (11.67%)	18 (15.25%)	6 (15%)
S65C	2 (0.83%)	0 (0%)	0 (0%)

N = number of subjects examined; A = total number of alleles. The allele frequencies were obtained by dividing the number of mutated alleles by the total number of alleles.

Table 2. Genotype frequencies of C282Y, H63D and S65C polymorphisms in the *HFE* gene.

Genotypes	General population		Pomeranian population		Patients with hemochromatosis	
	N	(%)	N	(%)	N	(%)
C282Y/WT	4	3.33	4	6.78	5	25
C282Y/C282Y	0	0	0	0	1	5
H63D/WT	25	20.83	15	25.43	1	5
H63D/H63D	1	0.83	1	1.69	1	5
S65C/WT	1	0.83	0	0	0	0
S65C/S65C	0	0	0	0	0	0
C282Y/H63D	0	0	1	1.69	3	15
C282Y/S65C	0	0	0	0	0	0
H63D/S65C	1	0.83	0	0	0	0
WT/WT	88	73.35	38	64.41	9	45
Total	120	100	59	100	20	100

N = number of subjects examined; WT = wild type.

DISCUSSION

This is the first study to report the frequencies of the C282Y, H63D and S65C *HFE* gene variants in the population of the ES and the first to evaluate the prevalence of these polymorphisms in a Pomeranian subpopulation in Brazil. The molecular epidemiology of three polymorphic variants (C282Y, H63D and S65C) of the *HFE* gene associated with hemochromatosis was analyzed in a general healthy population, Pomeranian healthy subpopulation, and patients diagnosed with HH from the ES, Brazil.

In the present study, the frequency of C282Y, H63D and S65C polymorphisms in the general population was 1.67, 11.67 and 0.83%, respectively, and for the patient group was 25, 15 and 0%, respectively (Table 1). Studies performed by several authors in Brazil also demonstrated a higher frequency of H63D than other polymorphic variants in the healthy control group, whereas C282Y was more frequent in the patient group (Table 3). The prevalence of the *HFE* gene alleles C282Y, H63D and S65C in the general healthy population in Brazil ranges from 1.15-2.19%, 9.54-14.57 and 0.31-1%, respectively. At the same time, for the patient group, the alleles range from 7.36-50% for C282Y, 6.25-26.59% for H63D and 0-2.23% for S65C (Bueno et al., 2006; Oliveira et al., 2006; Cançado et al., 2006; Cançado et al., 2007; Santos et al., 2010; Santos et al., 2011; Leão et al., 2014; Dionísio Tavares Niewiadonski et al., 2015). The different number of samples and the region of each study can explain the variations in the frequency of the *HFE* gene polymorphisms in Brazil. Moreover, it is necessary to take into consideration the large amount of miscegenation existing in Brazil. Nevertheless, we found a significant correlation between the genotype distribution in patients and the general population for the C282Y and H63D variants in our study. Therefore, we have highlighted the need to characterize specific populations for SNPs associated with disease.

Table 3. Comparison of genotype and allele frequencies of C282Y, H63D and S65C polymorphisms in the HFE gene between studies of the Brazilian population.

	Population of Brazil											
	Control						Patients					
Reference	Present Study	Leão et al., 2014	Bueno et al., 2006	Santos et al., 2010	Oliveira et al., 2006	Dionisio Tavares Niewiadonski et al., 2015	Present study	Leão et al., 2014	Cançado et al., 2007	Bueno et al., 2006	Cançado et al., 2006	Santos et al., 2011
Genotypes (%)	(N = 120)	(N = 160)	(N = 148)	(N = 542)	(N = 173)	(N = 400)	(N = 20)	(N = 299)	(N = 50)	(N = 8)	(N = 35)	(N = 51)
C282Y/WT	3.33	3.75	2.7	3.46	0.58	3.75	25	4.35	14	12.5	17	7.8
C282Y/C282Y	0	0	0	0	0	0	5	2.67	30	37.5	14	21.6
H63D/WT	20.83	25.62	18.3	22.4	19.08	20	5	31.44	16	0	29	21.6
H63D/H63D	0.83	1.25	1.3	1.82	0	1.6	5	8.03	2	0	3	3.8
S65C/WT	0.83	0.62	1.3	1.1	0	1.9	0	2.67	0	0	0	0
S65C/S65C	0	0	0	0	0	0	0	0	0	0	0	0
C282Y/H63D	0	0.63	0	0.73	0	0.25	15	5.02	14	12.5	11	11.7
C282Y/S65C	0	0	0	0	1.73	0	0	0	0	0	0	0
H63D/S65C	0.83	0	0.7	0	0	0	0	1.78	0	0	0	3.8
Alleles (%)	(A = 240)	(A = 320)	(A = 296)	(A = 1084)	(A = 346)	(A = 800)	(A = 40)	(A = 598)	(A = 100)	(A = 16)	(A = 70)	(A = 102)
C282Y	1.67	2.19	1.4	2.1	1.15	2	25	7.36	44	50	28.6	31.4
H63D	11.67	14.37	10.8	13.6	9.54	13.1	15	26.59	17	6.25	22.86	23.5
S65C	0.83	0.31	1	0.6	0.87	1	0	2.23	0	0	0	2

N = number of subjects examined; A = total number of alleles; WT= Wild-type. The allele frequencies were obtained by dividing the number of mutated alleles by the total number of alleles.

This is the first report to evaluate the prevalence of *HFE* gene variants in Pomeranian descendants in Brazil and thus we could not compare the frequencies found for the Pomeranian subpopulation of the ES to other studies. However, if we compare the frequencies of the present study with those detected in control subjects from Northern Germany (a population that can be considered close to the origins of Pomeranians), it is possible to observe an approximate value for the allelic frequencies of C282Y and H63D. Our study showed a frequency of 4.24 and 15.25% and the German study demonstrated a frequency of 4.8 and 13% for C282Y and H63D polymorphisms, respectively (Nielsen et al., 1998). Analysis of the S65C allele frequencies in the Germany population was not performed, and we did not find it in the Pomeranian subpopulation of the ES. In addition, there was no statistically significant difference in allele and genotype distribution between the Pomeranian subpopulation and general population in our study. This result confirms other data that reported gene flow between the general and Pomeranian populations of ES (Stur et al., 2012; Dettogni et al., 2013).

Due to the high degree of miscegenation in Brazil, establishment of genetic traits associated with disease in the Brazilian population is very important. This study should contribute to the creation of an HH genetic profile in the Brazilian population, especially in the population of the ES.

Conflicts of interest

The authors declare no conflict of interest.

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