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Frequency of the S65C mutation in the hemochromatosis gene in Brazil

V.C. Oliveira[1,2], F.A. Caxito[1], K.B. Gomes[3], A.M. Castro[1], V.C. Pardini[1] and A.C.S. Ferreira[1,2]

[1]Departamento de Genética Humana,
Instituto Hermes Pardini, Belo Horizonte, MG, Brasil
[2]Laboratório de Genética Animal e Humana,
Instituto de Ciências Biológicas,
Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil
[3]Faculdade de Farmácia,
Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil

Corresponding author: V.C. Oliveira
E-mail: vanessa.oliveira@labhpardini.com.br

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ABSTRACT. Development of hereditary hemochromatosis is associated with the C282Y, H63D or S65C mutations in the hemochromatosis gene. Though there is extensive knowledge about the former two, there is little information on the mechanism of action and the allelic frequency of the S65C mutation. We examined the prevalence of the S65C mutation of the hemochromatosis gene in Brazilians with clinical suspicion of hereditary hemochromatosis. Genotyping for this mutation was carried out in 633 individuals with clinical suspicion of hereditary hemochromatosis, using the polymerase chain reaction, followed by enzymatic digestion. The sample comprised 77.1% men and 22.9% women, giving a ratio of approximately 3:1; the mean age was 48.8 $\pm$ 13.8 years. More than half (57.3%) of the individuals in the sample were 41 to 60 years old. The frequency of heterozygotes for this mutation was 0.016; no homozygous mutant patients were found.

This is the first analysis of the S65C mutation in individuals suspected of having hereditary hemochromatosis in Brazil.

Key words: Hereditary hemochromatosis; HFE gene; S65C mutation; Brazil

INTRODUCTION

The progressive increase in plasma iron stockpiles can lead to complications such as heart failure, pituitary and gonadal dysfunction, diabetes, hepatic cirrhosis, and hepatocellular carcinoma (de Souza et al., 2001). This iron systemic overload can be genetically transmitted, which is then known as hereditary hemochromatosis (HH), an autosomal recessive disease, with a prevalence of 1:200 to 1:500 individuals in the Caucasian population (Bittencourt et al., 2002; Limdi and Crampton, 2004; Cimburová et al., 2005). Due to menstruation and pregnancy, HH occurs 2 to 4 times more in men than in women (Limdi and Crampton, 2004; US Preventive Services Task Force, 2006). Generally, HH appears between 40 to 60 years old, an age at which there is an excess of 20 to 40 g iron, accumulated slowly in the body throughout life (Pedersen et al., 2008), where the fifth decade is the most common age for the main signs and symptoms in women (US Preventive Services Task Force, 2006).

The development of HH is often related to the presence of C282Y, H63D and/or S65C mutations in the gene that expresses the protein HFE (Martinelli et al., 2005; Vizzi et al., 2005; Oliveira et al., 2006), whose activity is to regulate the intestinal absorption of iron (Bittencourt et al., 2002). C282Y and H63D mutations are known as the main ones responsible for HH (Guerreiro et al., 2006; Ferreira et al., 2008). S65C mutation, recently found to be related to milder HH, comes from an amino acid conversion of serine (S) to cysteine (C) at position 65, due to an adenine (A) to thymine (T) transversion at position 193 of the HFE gene (Oliveira et al., 2006).

Knowledge about this mutation is still scarce, with little information about its mechanism and its frequency in different populations. In the Brazilian population, it is around 0.0087 (Oliveira et al., 2006), while its frequency in Caucasians is around 0.005 to 0.03 (Cimburová et al., 2005). The Ecuadorian population shows S65C allelic frequency of 0.04, the highest found so far (Oliveira et al., 2006). HH of lesser severity is also associated with the presence of H63D/S65C and C282Y/S65C composed heterozygosity (Cimburová et al., 2005; Oliveira et al., 2006).

In Brazil, no study was yet conducted to determine S65C mutation frequency in a representative sample of the population. Given the different frequencies of this mutation in the world's different populations and the heterogeneity of the Brazilian population, the aim of this study was to determine the prevalence of S65C mutation of the HFE gene in Brazilian individuals with clinical suspicion of HH, according to gender and age.

MATERIAL AND METHODS

Material

The study was a retrospective analysis of data recorded from 633 individuals with clinical suspicion of hereditary hemochromatosis genotyped for S65C from 2005 to 2007, at the Department of Human Genetics of Instituto H. Pardini, in Belo Horizonte, Minas Gerais State, Brazil.

All patients had a clinical suspicion of hereditary hemochromatosis. The study population was composed of 77.1% (488) men and 22.9% (145) women, and had an average age of 47 ± 13 years old, where men and women had averages of 46 ± 16 and 55 ± 10 years, respectively. Among the group studied, 45 individuals were born in the Central West region of the country, 3 in the North region, 370 in the Northeast region, 93 in the Southeast region, and 122 in the South region. As this was an epidemiological and statistical survey, the procedures suggested by the National Commission on Ethics in Research (CONEP) for execution of these studies were followed.

Genotyping of S65C mutation

Genomic DNA was extracted from peripheral blood leukocytes, collected in EDTA, using FTA® Card methods. S65C mutation was examined by the PCR-RFLP technique, using the primers R: 5'-GCCACATCTGGCTTGAAATT-3' and F: 5'-ACATGGTTAAGGCCTGTTGC-3' and subsequent enzymatic digestion with *Hinf*I. In the presence of S65C mutation, the *Hinf*I recognition site is abolished resulting in only one fragment of 207 bp, while the normal allele is digested yielding two fragments of 60 and 147 bp. The digestion product was visualized on a 7% acrylamide gel after staining with SYBR Green.

Statistical analysis

The variables gender, age and S65C genotype were compared using the chi-square test in the BioStat 4.0 software. Differences were considered to be significant with a value of $P < 0.05$.

RESULTS

Heterozygous individuals were from the States of Rio Grande do Sul (3), Pernambuco (3), Minas Gerais (1), Rio de Janeiro (1), Bahia (1), and Ceará (1) (Table 1). It was impossible to determine the genotypic frequency by geographic region of the country due to the low frequency of heterozygotes.

Table 1. Profile of heterozygous subjects for S65C mutation in HFE gene.

Individual	Gender	Age (years)*	Birthplace
1	Female	56	Minas Gerais
2	Female	62	Pernambuco
3	Female	67	Ceará
4	Male	43	Rio Grande do Sul
5	Male	49	Rio Grande do Sul
6	Male	65	Bahia
7	Male	30	Pernambuco
8	Male	42	Rio de Janeiro
9	Male	49	Pernambuco
10	Male	59	Rio Grande do Sul

*Age on the date of molecular test collection.

The S65C heterozygous genotype was present in 1.6% (10 individuals), while 98.4% of the population studied showed the wild-type homozygous genotype (Table 2). No mutant

homozygous patient was found. There was no statistical difference in genotypic (P = 0.59) and allelic (P = 0.59) frequencies for S65C between males and females.

Table 2. Genotypic and allelic frequencies for S65C mutation, according to gender.

Population	Genotypic frequency		Allelic frequency	
	S65S	S65C	65S	65C
Female	0.979	0.021	0.990	0.100
Male	0.986	0.014	0.993	0.007
Total	0.984	0.016	0.992	0.008

The variable age was divided into 3 age groups: 0 to 40, 41 to 60 and >61 years, according to HH manifestation stages. The age group over 41 years old involved 78.8% of patients, of whom 57.3% were in the group of 41 to 60 years. The number of women (36.6%) over 61 years old with clinical suspicion of HH was statistically higher than the male population of the same age (17.0%; $P < 0.0001$), but the genotypic and allelic frequencies of S65C in the stratification by age and gender did not show a significant difference ($P > 0.05$).

DISCUSSION

The absence of important statistical differences in genotypic and allelic frequencies of S65C between males and females is due to the autosomal inheritance of HH. However, the higher proportion of men than women is explained by the lower iron plasma concentration in the female body through physiological blood loss (menstruation and childbirth) (Pietrangelo, 2004; Scotet et al., 2005). Consequently, women require more time in life to show a significant tissue iron deposition. This mechanism explains the observation that there were more women (37%) than men (17%) over 61 years, since in this age women in general are already in the climacteric period when there is no physiological blood loss.

The impossibility of determining differences in the genotypic frequency between the Brazilian states and also between geographic regions of the country is due to the necessity of expanding the studied population due to the low frequency of heterozygotes. The data relate to genotypic and allelic frequencies in the study group and cannot be accurately extrapolated to the population in general.

While the 633 patients with suspicion of HH analyzed in this study showed a genotypic frequency of 0.016 for heterozygosity and allelic frequency of 0.008 for mutation, other Brazilian studies on S65C showed an allelic frequency of 0.010 in 148 healthy individuals (Bueno et al., 2006), 0.0087 in 173 healthy individuals without hemoglobinopathies (Oliveira et al., 2006) and zero in 35 patients with iron overload (Cançado et al., 2006). This study had a limited sample size and low frequency of this mutation affects benchmarks that allow more detailed conclusions. Similar data are also reported on the international scene because, for example, in China and South Korea, there is no indication of the presence of the mutant S65C allele (Lin et al., 2007; Lee et al., 2009); in Venezuela, the allelic frequency is 0.009 (Vizzi et al., 2005), while individuals with hemochromatosis in Denmark and Spain were found to have respectively allelic frequencies of 0.018 and 0.02 (de Diego et al., 2004; Pedersen et al., 2008).

Given the allelic frequency of 0.008 and considering that the population is in Hardy-Weinberg equilibrium, it is estimated that the incidence of S65C mutation in homozygous, in the Brazilian population, is 1 in 15,625 individuals.

The data presented here are of great importance because this study was based on the largest sample ever evaluated in the Brazilian population and because it is the first S65C mutation analysis in individuals under suspicion of HH in Brazil. Therefore, the data presented here allow us to consider that this mutation genotyping should be recommended in cases of family history, genetic counseling involving heterozygous and homozygous individuals for S65C mutation, and consanguineous marriages. This study should contribute to the establishment of an HH genetic profile in the Brazilian population and, consequently, help in selecting appropriate examinations for the screening and diagnosis of this disease.

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