Analysis of HFE Genes C282Y, H63D, and S65D in Patients With Hyperferritinemia From Northeastern Brazil

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Background: Hereditary hemochromatosis (HH) is a genetic disease caused by the high absorption and deposition of iron in several organs. This accumulation results in several clinical complications such as cirrhosis, arthritis, cardiopathies, diabetes, sexual disorders, and skin darkening. The H63D and C282Y mutations are well defined in the HH etiology. The objective of this article is identification of the H63D and C282Y mutations in the HFE protein gene and the frequency assessment of these mutations in patients with persistent increase of serum ferritin in patients from Natal City from state of Rio Grande do Norte, located in northeastern Brazil. **Results:** Of the 299 patients studied for C282Y and H63D, 48.49% showed absence of mu-

tation and 51.51% showed some sort of mutation: heterozygous C282Y mutation in 4.35% patients, homozygous C282Y mutation in 2.67% patients, heterozygous H63D mutation in 31.44% patients, homozygous H63D mutation in 8.03% patients, and heterozygous for the mutation in both genes (C282Y/H63D) in 5.02% patients. The S65C mutation was studied in 112 patients and heterozygous mutation (S65D/WT) in 2.67% of patients and double mutation (H63D/S65C) in 1.78% of patients were observed. **Conclusion:** Due to the high prevalence of hemochromatosis, its genetic diagnosis has become a challenge, especially in the high-risk group. J. Clin. Lab. Anal. 28:178–185, 2014. © 2014 Wiley Periodicals, Inc.

Key words: hereditary hemochromatosis; hyperferritinemia; C282Y mutation; H63D mutation; S65C mutation

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INTRODUCTION

Hereditary hemochromatosis (HH) is an autosomal recessive disorder of iron metabolism. Due to excessive intestinal absorption, iron accumulates in parenchymal cells of the liver, pancreas, heart, and other organs with resultant damage to their structure and impairment of their function. This accumulation results in several clinical complications such as cirrhosis, arthritis, cardiopathies, diabetes, skin darkening, and hypogonadism. It is one of the most common genetic diseases in Caucasians with a prevalence of nearly one in 300 individuals (1–6).

Although the symptoms of the disease are often non-specific, much of the organ damage is irreversible once it has occurred. The patients with hemochromatosis absorb more iron than the population, in general. However, the clinical manifestations caused by excessive deposition of this metal occur when the individuals reach at the age of 20–40, and the levels of iron accumulation reach at the concentration of around 20–40 g of iron (3). The clinical manifestations rarely appear before 20 years of age, when the iron stocks are still low, and a majority of patients are symptomatically aged between 40 and 50 years (1–6).

The classical HH, also known as type I hemochromatosis, is associated with mutations in the *HFE* gene located on chromosome 6, which encodes the *HFE* (high-iron) protein, a key limiting factor of the duodenal iron absorption, interacting with the transferrin receptor (TfR), and decreasing its affinity for the transferrin (7).

The *HFE*-C282Y gene mutation was first described by Feder in 1996 resulting from a guanine-to-adenine transition in position 845 of the *HFE* gene. About 60–80% of the type I HH cases depend on the ethnic background (1, 5–7).

A second mutation, H63D (a histidine-to-aspartate substitution), resulting from a transversion C (cytosine) to G in position 187 of the *HFE* gene, is associated with a milder form of the disease, as this mutation leads only to a partial decrease in the ability of the HFE protein to reduce the TfR affinity for transferrin. Depending on the ethnic background, this mutation represents 40–70% of the *HFE* non-C282Y hemochromatosis (5, 8).

Finally, a third mutation, S65C (a serine to cysteine substitution), resulting from a transversion A to T (thymine) in position 193 of the *HFE* gene, although initially considered a polymorphism with no phenotypic effect, has been found to be associated with milder forms of hemochromatosis, especially in C282Y/S65C compound heterozygous individuals (5, 9, 10).

Although the presence of the mutant gene is equally distributed between men and women, most studies report a higher prevalence in men than in women in the 4 to 10:1 proportion, due to “physiological losses” of iron that females show because of menstruation and pregnancy (1, 3–6).

Around 80–100% of individuals from North European descendency who show clinical manifestations for HH were homozygous for the C282Y mutation (6, 8). Although compound heterozygosity (C282Y/H63D) appears to be disease associated in such individuals with suspected iron overload, cofactors should be considered as a cause (11).

Around 5–6% of patients clinically diagnosed with HH do not show identifiable mutations in the *HFE* gene, suggesting that other genes are involved in the disease, especially in some populations, such as in southern Italy (5, 6). Every year, molecular studies point to the molecular investigations through genetical studies that are able to identify the C282Y and H63D mutations in the *HFE* gene in individuals with HH. The high frequency of the C282Y and H63D mutations in individuals affecting some populations encouraged the usage of molecular tracing techniques of these mutations such as a confirming exam to the clinical and laboratory diagnosis of HH (1, 6, 7).

Considering the lack of genetical studies in Brazil about hemochromatosis, especially in the northeast area, this article aimed at identifying and evaluating the frequency of mutation and determination of allele frequency of the *HFE* genes H63D, C282Y, and S65C in patients with persistent hyperferritinemia from Natal city from northeast of Brazil.

MATERIALS AND METHODS

Subjects

Samples of peripheral blood (PB) from 299 patients suspected of HH were studied. The main criterion for inclusion of such patients in the study was the increase of persistent serum ferritin in individuals aged between 18 and 89 years, both males and females. As to the exclusion criteria, individuals with hemolytic anemia, thalassemia, and previously did blood transfusion, did not take part in the study.

For the control, a group of 160 healthy patients of both sexes were selected, with serum ferritin levels within normal limits.

The study delimitation and its starting point occurred after its approval by ethics committee of Universidade Federal do Rio Grande do Norte, Brazil. The patients were informed about the study protocol, and only those who signed the consent term took part (CEP/UFRN; No. 0312.0.000-061-06).

Twenty milliliters of PB were collected from every patient by venous puncture after an 8-h fasting period, at least. Upon collecting, the PB was fractioned into two aliquots, one of them in a tube without anticoagulant destined to serum ferritin dosage and the other in a tube containing ethylenediaminetetraacetic acid (EDTA-K₂ 5,4 mg Plus Plastic; BD.Vacutainer) holding

up to 5 mL for studying the C282Y, S65C, and H63D mutations.

Serum Ferritin Dosage

The serum ferritin dosage was determined by the immune assay method with micro particles (Abbott IMX immunology analyser, MA). The normal serum ferritin levels were 15–300 ng/mL in male and 15–200 ng/mL in female (12).

DNA Extraction

The genomic DNA extraction was obtained from total blood according to the protocol described by Loparev et al. (13).

Detection of C282Y, H63D, and S65C mutations

The *HFE* gene C282Y, H63D, and S65C mutations were studied by polymerase chain reaction restriction fragments length polymorphism (PCR-RFLP) assays, as previously described (14). The fourth exon of the *HFE* gene flanking the *SnabI* recognition site for the C282Y substitution was amplified using the following primers: 5'-TGGCAAGGGTAAACAGATCC-3' and 5'-CTCAGGCACTCTCTCAACC-3'. The length of the amplified fragment of exon 4 of the C282Y *HFE* gene is 400 bp. The G to A transition at nucleotide 845 (amino acid 282) creates a cleavage site and fragments of the 290 and 110 bp are generated after enzymatic restriction with *SnabI*.

Amplification of the second exon of the *HFE* gene containing a *BclI* recognition site for H63D was done using the primers 5'-ACATGGTTAAGGCCTGTTGC-3' and 5'-GCCACATCTGGCTTGAAATT-3'. Approximately 800 and 200 ng of genomic DNA was used, respectively, for amplification of exon 2 and 4 of *HFE* gene, 0.6 μ M of each primer in a total volume of 50 μ L containing 200 mM each of dNTP (Gibco-BRL, New York, NY), 2 IU of Taq polymerase (Cenbiot, Porto Alegre, RS, Brasil), and PCR buffer containing 1.5 mM magnesium chloride. In the presence of the H63D mutation, only the undigested 208-bp fragment is observed, since the C to G transversion at nucleotide 187 (amino acid 63) disrupts a *BclI* cleavage site.

Amplification of S65C was done using the primers 5'-ACATGGTTAAGGCCTGTTGC-3' and 5'-GCCACATCTGGCTTGAAATT-3'. Approximately 800 and 200 ng of genomic DNA was used, respectively, for amplification of exon 2 and 4 of *HFE* gene, 0.6 μ M of each primer in a total volume of 50 μ L containing 200 mM each of dNTP (Gibco-BRL), 2 IU of Taq poly-

merase (Cenbiot), and PCR buffer containing 1.5 mM magnesium chloride.

The amplification was carried out in a PCR-100 Thermal cycle (MJ Research Inc., Watertown, MA, USA) and conditions were same for both amplifications: 35 cycles, each one composed of the denaturation step at 96°C, for 2 min, hybridization at 56°C, for 1 min, and extension at 72°C, for 1 min, with a final extension of 10 min at 72°C.

The amplified products were digested with the *BclI*, *SnabI*, and *Hinfl* enzymes, for the H63D, C282Y, and S65C mutations, respectively, and separated by electrophoresis in agarose gel at 2.5%. The band visualization was done under ultra-violet (UV) light in transilluminator.

The analysis of restriction fragments was done by using a DNA size marker (100 bp) and the genotypical profiles of the individuals studied were evaluated with positive control samples, including the absence of mutation in both alleles or wild type (WT/WT); homozygous mutant with the same mutation in both alleles for the H63D gene (H63D/H63D) and C282Y gene (C282Y/C282Y); heterozygous mutant with a mutation present in one allele H63D/WT, C282Y/WT, and S65D/WT and double heterozygous mutant H63D/C282Y and H63D/S65D.

Statistical Analysis and Determination of the Allele Frequencies

The variance analysis (ANOVA) was applied in order to know whether there is meaningful difference between the groups. It was performed by using the STATISTICS 6.0 software.

The variables were then analyzed descriptively. For the quantitative analysis, it was done by observing the minimum and maximum values and the mean and standard deviation equation. For the qualitative variables, we calculated the absolute and relative frequencies.

The determination of allele frequencies for the *HFE* genes C282Y, H63D, and S65D was performed according to the proposal by Trifa et al. (16).

RESULTS

The genotype distribution pattern and the allele frequency of C282Y and H63D mutations were determined in 299 patients with hyperferritinemia and 160 samples from control group (Table 1). In patients with hyperferritinemia, the genotype frequency observed was as follows: 94 cases of (31.44%) H63D/WT. Of the total 299 patients with hyperferritinemia and 160 samples from control groups, absence of H63D and C282Y mutations was observed in 48.49% and 68.75% of cases, respectively.

The investigation of the S65D mutation was performed in 112 patients with hyperferritinemia and 160 samples

TABLE 1. Distribution of Different Genotypes C282Y, H63D and S65C of the HFE Protein in Patients with Hyperferritinemia and Control Group

Distribution of genotypes	Control group		Patient group	
	No	%	Number	%
C282Y/C282Y	0	0	8	2.67
C282Y/WT	6	3.75	13	4.35
H63D/H63D	2	1.25	24	8.03
H63D/WT	41	25.62	94	31.44
C282Y/H63D	1	0.63	15	5.02
No mutation (WT)	110	68.75	145	48.49
Total (C282Y and H63D)	160	100	299	100
S65C/WT	1	0.62	3	2.67
S65C/S65C	0	0	0	0
S65C/H63D	0	0	2	1.78
S65D/C282Y	0	0	0	0
No mutation (WT)	159	99.38	107	95.53
Total S65D	160	100	112	100

Note: No mutation or WT (Wild Type); No (Number of patients); C282Y/WT (mutant C282Y heterozygous); C282Y/C282Y (mutant C282Y homozygous); H63D/WT (mutant H63D heterozygous); H63D/H63D (mutant H63D homozygous); C282Y/H63D (double heterozygous); S65C/WT (mutant S63C heterozygous).

from control group. The genotypic distribution observed was as follows: three cases (2.67%) and one case (0.62%) were of S65D/WT, in patients with hyperferritinemia and control group, respectively (Table 1).

With respect to the ferritin levels in PB, all patients were investigated taking into account the sex and presence or absence of mutation to the *HFE* gene. The maximum measures of serum ferritin found were 3,204 ng/mL and 2,057 ng/mL in male and female patients, respectively. In male patients, the highest serum levels of ferritin found were with average of 1,362 ng/mL with genotypes C282Y/C282Y, and in female patients serum levels of ferritin were 1,261 ng/mL, with genotypes C282Y/WT (Table 2).

As observed in male patients with hyperferritinemia, female patients with other genotypes also showed elevated levels of serum ferritin, but with average and maximum values lower than those observed in male patients, and the same happened also in female patients with hyperferritinemia without mutations (Table 2).

The allelic frequency for these genes in patients with serum hyperferritinemia was 7.36% for C282Y, 26.59% for H63D, and 2.23% for S63D (Table 3). In samples from control group, the allelic frequencies were 2.19% for C282Y, 14.37% for H63D, and 0.31% for S65C.

DISCUSSION

In spite of the genetics of HH and its clinical perspectives are known, the statement of alleles related with this

TABLE 2. Correlation between the H63D, C282Y and S65C Mutations and the Serum Ferritin Levels

Mutation		No	Serum ferritin levels (ng/mL)	
			Mean	Range
Male	No alteration (WT)	112	799.4	400–1,143
	C282Y/WT	11	905.7	654–1,198
	H63D/H63D	21	788	345–1,497
	H63D/WT	76	791.4	337–1,756
	C282Y/H63D	11	814.1	442–1,468
	C282Y/C282Y	6	1,362	625–3,204
	S65C/WT	1	515.5	515.5
Female	S65C/H63D	2	510.5	401–620
	No alteration (WT)	28	806.9	414–1,322
	C282Y/WT	2	1,261	1,200–1,322
	H63D/H63D	3	538.3	412–685
	H63D/WT	18	863.05	410–2,057
	C282Y/H63D	4	841	536–991
	C282Y/C282Y	2	628	526–730
	S65C/WT	2	460.1	323–597

Note: WT (Wild Type); No (Number of patients); C282Y/WT (mutant C282Y heterozygous); C282Y/C282Y (mutant C282Y homozygous); H63D/WT (mutant H63D heterozygous); H63D/H63D (mutant H63D homozygous); C282Y/H63D (double heterozygous); S65C/WT (mutant S63C heterozygous).

disease in populations is not clear. Here, we detected that because of the high heterogeneity *HFE* genes (C282Y, H63D, and S65C), it was not possible to elucidate how these alleles affects different populations (Tables 3 and 4).

This study, for the first time in Natal, city from north-eastern of Brazil, reports the genotypic frequencies of C282Y, H63D, and S65C mutations in *HFE* gene from individuals with hyperferritinemia and healthy controls in order to attempt to understand their role in predisposition to HH.

Population studies indicate that HH has originated from Northern Europe. The C282Y mutation is most common in Caucasians of North Western Europe, North America, Australia, and New Zealand. Some studies involving populations of the United States, Australia, New Zealand, and Transilvania showed that a frequency of homozygotes and heterozygotes for the C282Y mutation varies between 0.2 and 3% and 7 and 14%, respectively (15–18). The homozygosity for the C282Y mutation is now found in approximately five out of 1,000 patients from North European descendency (Table 4). Every homozygous person for the C282Y mutation has a genetical predisposition for an event chain that can trigger severe multiple organ damage (1, 3–7).

The H63D mutation is two or three times more frequent than C282Y mutation and the prevalence of heterozygous and homozygous for H63D mutation varies between 15 and 40% and 2.5 and 3.6%, respectively, and the frequency of C282Y/H63D genotype is approximately

TABLE 3. Allele Frequency of C282Y, H63D, and S65C HFE Gene in Brazilian Population and in Present Study

Study, year, reference, and place	Skin color	Allele frequency (%)		
		C282Y Mutation	H63D Mutation	S65C Mutation
Agostinho et al., 1999 (21)	W	(1.4)	(16.3)	NP
Campinas (No. = 227)	B	(1.1)	(7.5)	
	M	(1.1)	(1.1)	
Calado et al., 2000 (22)	W+B+M	(2.2)	(14.3)	NP
Ribeirão Preto (No. = 320)				
Pereira et al., 2001 (23)	W	(3.7)	(20.3)	NP
São Paulo (No. = 395)	B	(0.5)	(6.4)	
	M	(0.7)	(13.0)	
Oliveira et al., 2003 (24)	W	(1.4)	(8.6)	(0.6)
São Paulo (No. = 148)	B	(0.0)	(2.4)	(0.3)
Terada et al., 2009 (25)	NP	(2.1)	(13.6)	(0.6)
São Paulo (No. = 542)				
Present study (No. = 299)	NP	(7.36)	(26.59)	(2.23*)

Note: No (Number of participants in each study); W (white); B (black); M (mulatto); NP (not performed). *This Allele frequency was only made in 112 patients. Adapted from Cançado RD et al., 2010 (30).

2% (15, 16). Regarding the S65C mutation, the frequency in Caucasians varies between 1% and 4% (9, 10, 18, 19).

In Brazil, population studies performed by several authors have also demonstrated the predominance of H63D mutation, followed by C282Y, with the highest prevalence in white compared to black or mulatto population (Table 3) (14, 21–25), which was similar to that observed in this study and also in countries with strong influence in shaping the Brazilian population such as Portugal, Spain, France, and Italy (20).

The Brazilian population has a great miscegenation. During the time period from the Brazil's discovery and beginning of colonization to the present date, the percentage of the descendents of European immigrants is 58%, 40% of Africans, and 2% of Asians, besides the great influence of Amerindian (26–29). The miscegenation between people of different ethnicities during this period was a determinant factor in the genetic diversity of the population, reflecting a large variability of results in the investigation of biochemical, molecular, and immunological changes such as mutation or polymorphism of genes such as an HFE gene (21, 26–29).

Thus, some authors point out that the investigation of the frequency of HFE mutations in the Brazilian population, mainly in C282Y gene may present a wide range

TABLE 4. Allele Frequency of the HFE Mutations C282Y, H63D and S65C in Different Countries

Country	Study, year, and reference	Allele frequencies (%)		
		S65D	C282Y	H63D
Denmark	Milman et al., 2004 (36)	NP	5.7	13.4
Sweden	Holmstrom et al., 2002 (38)	1.6	6.2	11.4
Finns, Swedes, Swedish Saamis	Beckman et al., 2001 (39)	3.0	2.0	7.9
Faroe Islands	Milman et al., 2005 (37)	1.0	8.0	17.5
Ireland	Ryan et al., 1998 (40)	NP	14.0	17.9
UK	Merryweather-Clarke et al., 2000 (41)	NP	8.1	15.2
France	Mura et al., 1999 (9)	1.95	7.7	14.0
Germany	Merryweather-Clarke et al., 2000 (39)	NP	3.8	13.2
Poland	Moczulski et al., 2001 (42)	NP	3.1	16.2
Czech Republic	Cimburová M et al., 2005 (18)	1.25	3.4	14.9
Austria	Merryweather-Clarke et al., 2000 (41)	NP	3.7	12.9
Croatia	Ristic et al., 2003 (43)	1.8	3.3	14.5
Slovenia	Ristic et al., 2003 (43)	0.5	4.0	14.5
Russia	Mikhailova et al., 2003 (44)	1.7	3.7	13.3
Bulgaria	Merryweather-Clarke et al., 2000 (41)	NP	0.0	23.0
Northern Italy	Mariani et al., 2003 (45)	1.3	3.2	13.4
Italy, Apulian	Pietrapertosa et al., 2003 (46)	0.5	1.5	14.0
Greece	Merryweather-Clarke et al., 2000 (41)	NP	1.3	13.5
Northern Spain	Altes et al., 2004 (47)	1.0	3.0	20.0
Spain, Basque	Merryweather-Clarke et al., 2000 (41)	NP	3.6	30.4
Portugal	Porto et al., 1998 (48)	NP	2.8	23.0
USA, Caucasian	Beutler et al., 2000 (49)	1.6	6.3	15.2
USA, Hispanic	Beutler et al., 2000 (49)	0.6	2.7	12.4
USA, Asian	Beutler et al., 2000 (49)	0.0	0.2	3.3
USA, Black	Beutler et al., 2000 (49)	0.7	1.1	5.1
Transilvania, Romania	Trifa et al., 2012 (17)	0.75	1.75	10.9

NP, not performed.

of results, depending not only on the basis of population, but also on the criteria adopted in the selection of each sample, with such region, ethnicity, and the criteria used for the detection of iron overload (Table 3).

Cançado et al. in 2007 (30) conducted a study on the frequency of gene mutation C282Y, H63D, and S65D in patients with clinical suspicion of HH in São Paulo, Brazil, showing the presence of *HFE* gene mutations in 76% of this group of patients with genotype being 30.0% for C282Y/C282Y, 14.0% for C282Y/H63D, 2.0% for H63D/H63D, and 14.0% and 16.0% for C282Y/WT and H63D/WT, respectively; there were no cases with a mutation involving the gene S65C.

Bittencourt et al. (14), in a study performed to investigate the C282Y and H63D mutations of the gene in 15 Brazilian individuals with histologic diagnosis of HH, observed frequency of 53.0% patients with genotype C282Y/C282Y and 13.5% patients with genotypes C282Y/WT and H63D/WT.

In Latin America, some countries have conducted studies involving mutations in the *HFE* gene (31–35). In Ecuador, the allele frequency for C282Y, H63D, and S65C were 0%, 3.5%, and 4.0%, respectively (31). In a study conducted in Venezuela by Vizzi et al. (32), the allele frequency for C282Y and H63D were 3.8% and 21.1%, respectively. In Argentina and Chile, the allele frequency for C282Y and H63D was 2.6% and 6.7%, 2.4% and 23.0%, respectively (33, 34). In a study conducted in Mexico by Hanson et al. (35), the allele frequency for C282Y and H63D were 0% and 13.0%, respectively.

In Natal, city from state of Rio Grande do Norte, located in northeastern Brazil had strong genetic influence of the European peoples, mainly Portuguese, French, Dutch, and Spanish in addition to the indigenous peoples and Africans, constituting one of the largest regions of Brazil with index miscegenation.

As we evaluated the genotypes separately in our study, we noticed that 7.69% of the patients studied had both mutations that are considered important for the HH, which are the C282Y homozygous and the double heterozygous mutations (C282Y/H63D), from which 2.67% of the patients showed the homozygous C282Y mutation and 5.02% of the patients showed double heterozygous mutation (Table 1).

In the present study, we observed a predominance of mutations in the gene H63D ($n = 133$), 94 (31.44%) cases were H63D/WT, 24 (8.03%) cases were H63D/H63D, and also double heterozygote mutation (H63D/C282Y) in 15 cases (5.02%). The mutation C282Y involving the C282Y gene totaling 36 patients (12%) with the following distribution: 15 cases (5.02%) were double heterozygous mutant (H63D/C282Y), 13 (4.35%) cases were C282Y/WT, and 8 (2.67%) cases were C282Y/C282Y. The heterozygous genotype for S65C/WT was detected

in three out of 112 patients (2.67%) and heterozygous compounds with H63D in three cases (1.78%) (Table 1). Our results being consistent with that described by Brazilian authors and research from other countries (Tables 3 and 4).

In the control of healthy individuals, genotype frequency of mutation as 6 out of 160 individuals (3.75%) with genotype C282Y/WT, 2 (1.25%) H63D/H63D, 41 (25.62%) H63D/WT, and one case (0.63%) C282Y/H63D was observed. In this group, one case (0.62%) was found with the S65C/WT mutation and no case was found with C282Y/C282Y mutation (Table 1).

The allelic frequency found in our controls of healthy individuals was 2.19%, 14.37%, and 0.31% for C282Y, H63D, and S65C, respectively, lower than group of patients with hyperferritinemia that was 7.36%, 26.59%, and 2.23% for genes *HFE* C282Y, H63D, and S65C, respectively, corroborating with some international studies, mainly in populations from Portugal, France, Spain, and Italy (Table 4).

In this study, the frequency of the group of patients with hyperferritinemia detected the genotypic frequency of mutation as being 13 cases (4.35%) with genotype C282Y/WT, 8 (2.7%) C282Y/C282Y, 24 (8.0%) H63D/H63D, 94 (31.3%) H63D/WT, 15 (5.0%) C282Y/H63D, 3 (2.67%) S65C/WT, and 2 (1.78%) S65C/H63D (Table 1).

In this group, the highest levels of ferritin were found in male patients. It was found that 76 patients (46.9%) from this group were heterozygous for the H63D mutation, 11 (6.8%) patients were double heterozygous mutant (H63D/C282Y), 21 (13.0%) patients were homozygous for the H63D mutation, 11 (6.8%) patients were heterozygous for the C282Y mutation, and six patients (3.7%) were homozygous for the C282Y mutation with average ferritin value of 1,362 ng/mL.

Several reports show that among men and women, the C282Y homozygous have some tendency for high serum ferritin, downfollowed by the individuals with genotypes C282Y/H63D (double heterozygous), H63D/H63D, C282Y/WT, and H63D/WT, and these data were practically similar to our study, only highlighting an inversion of the C282Y/WT genotype position with H63D/H63D comparing to the papers reported (Table 2).

Approximately 5–10% of patients diagnosed with HH presents the compound heterozygous for C282Y/H63D (1, 3–7). In these studies, the compound heterozygous frequency was 5.0%, having the average ferritin values of 814.1 ng/mL for males and 841.0 ng/mL for females, all of them showed some clinical manifestation and one patient showed liver cirrhosis (1, 5–7). For compound heterozygous S65C/H63D, the frequency was 1.78%, found just in male patients with average ferritin values of 510.5 ng/mL.

When we compare the study group and the control group in relation to frequency, both had similar results and the H63D genotype had the highest frequency, confirming what has been published in literature (Tables 3 and 4). Since hemochromatosis is the most common genetical disease in the Caucasian population, even considering the genetical heterogeneity seen in Brazil, our control group showed molecular alteration for those below 40 years of age, but we should emphasize that they still showed no increased ferritin. As the molecular biology advances, it is now possible to detect specific genetical alterations responsible for several diseases, even before the individual show any symptoms. It is important to prevent iron overload, which happens silently and slowly, and detect individuals who are still healthy but who will or could show symptoms and signs of a hereditary disease or an increase susceptibility to certain common diseases of adult people.

In conclusion, with the HH is considered a public health matter with high frequency, it should be investigated in its early stages in patients suspected for this infirmity. This article brings a contribution for a better understanding of penetration and clinical expression of this gene in our population, especially in the high-risk group, since the H63D and C282Y mutations are well defined in the HH etiology.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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