

Artigo / Article

## Prevalence of C282Y and H63D mutations in the HFE gene of Brazilian individuals with clinical suspicion of hereditary hemochromatosis

### *Prevalência das mutações C282Y e H63D no gene HFE em indivíduos brasileiros com suspeita clínica de hemocromatose hereditária*

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Classical hereditary hemochromatosis is a recessive autosomal disease related to a systemic iron overload that is frequently related to C282Y and H63D mutations in the HFE gene. In Brazil, reports on HFE gene mutation frequencies are rare, mainly in regards to a representative sample population. This study intended to determine the prevalence of C282Y and H63D mutations among individuals with clinical suspicion of hereditary hemochromatosis. A total of 1955 patients were studied with C282Y and H63D mutations being detected by the polymerase chain reaction technique followed by enzymatic restriction. The sample consisted of 76.6% men and 23.4% women. The highest percentage of analyzed individuals (56.9%) was concentrated in the 41 to 60-year-old age group. Although there were no genic or genotypic differences between genders, a higher number of over 60-year-old women was observed. The C282Y mutation was found as homozygous in 2.9% of the cases and as heterozygous in 10.1%, while the H63D was homozygous in 4.3% and heterozygous in 30.6%. The C282Y and H63D mutant allele frequencies were 0.079 and 0.196, respectively. The highest frequency was observed for H63D which was in genetic equilibrium. This work is important to determine the genetic profile of the population with hereditary hemochromatosis in Brazil. *Rev. Bras. Hematol. Hemoter.* 2008;**30**(5):379-383.

**Key words:** Hemochromatosis; genetic anomaly; HFE mutation.

### Introduction

Classical hereditary hemochromatosis (HH) is a recessive autosomal disease with a prevalence of between 1:200 and 1:500 individuals and is characterized by a systemic iron overload due to an inappropriate absorption by the intestine.<sup>1-4</sup> The progressive iron accumulation generally causes structural damage and functional harm to several organs followed by inflammatory and oxidative events. Clinical manifestations predominate in men (two to three times more common in men than in women) while the absence of this phenotype is common in women due to monthly blood loss which decreases iron deposits in the body.<sup>2,5,6</sup> HH presents a long term latency<sup>7</sup> and is divided into 3 stages: from 0 to 20

years old, in which there is no iron accumulation; from 20 to 40, in which there is an iron overload, however, with no physiological damage, and over 40, when there is an iron overload and damage to organs, initially with fibrosis and/or hepatic cirrhosis.<sup>8</sup> Generally, clinical manifestations appear between the ages of 40 and 60. The fifth decade is, in particular, the most common period among women for the onset of signs and symptoms.<sup>5,6</sup> Classical hereditary hemochromatosis (Type 1) is associated to the presence of genetic variations in the HFE gene located in the short arm of the 6p21.3 chromosome.<sup>1,9,10</sup> An average of 20 mutations have been identified in this gene;<sup>5</sup> nevertheless, there are two missense mutations, C282Y and H63D, which are more commonly associated to HH.<sup>1,8,11</sup>

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The C282Y mutation, in exon 4, consists in a transition from guanine (G) to adenine (A) in nucleotide 845, which determines the replacement of a cysteine (C) by a tyrosine (Y) in amino acid 282, incapacitating the interaction among HFE, transferrin and  $\beta$ 2-microglobulin.<sup>1,4</sup> These data support clinical findings which report that C282Y mutant homozygotes have a two-fold higher probability of presenting with hepatic diseases.<sup>7,12,13</sup> Most studies identify C282Y as the main mutation responsible for HH, with HH frequencies being influenced by ethnical variables.<sup>2,4,6,8</sup> A transversion from cytidine (C) to guanine (G) in nucleotide 187 reflects in the replacement of histidine (H) by aspartic acid (D) in amino acid 63, thereby determining the H63D mutation, which consequently results in a change in HFE protein conformation minimizing its binding affinity to transferrin.<sup>3,10</sup> Generally, this is associated to mild forms of HH.<sup>1,4,11</sup> A minority of C282Y/H63D heterozygotes (1 to 2% on average) develop clinical symptoms of hemochromatosis;<sup>2,9</sup> Beutler *et al.*<sup>14</sup> reported that, generally, Caucasians with clinical evidence of HH are either homozygous for C282Y or compound heterozygous for C282Y/H63D.

It is a scientific consensus that HFE gene mutations are more frequent in the Caucasian population, however, there are discrepancies among the diverse world populations. In Brazil, reports on the frequencies of these mutations and their relationship to clinical manifestations are rare, in particular when analyzing a representative sample population. The purpose of the present study was to determine the prevalence of C282Y and H63D mutations in the HFE gene among Brazilian individuals with clinical suspicion of hereditary hemochromatosis, according to the age and gender.

## Material and Method

### *Clinical material*

The study is the retrospective statistical analyses of data registered on 1955 individuals who were submitted to molecular studies of the C282Y and H63D mutations at the Instituto Hermes Pardini, Human Genetics Department, Belo Horizonte, Minas Gerais state in 2006. All patients had a clinical suspicion of hereditary hemochromatosis. The age average was  $48.8 \pm 13.8$  years old. The sample consisted of 1498 men and 457 women. Gender, age and genotype for C282Y and H63D were analyzed for these individuals.

### *C282Y and H63D mutation genotyping*

Genomic DNA was extracted from leukocytes of peripheral blood collected in EDTA utilizing the FTA Card®. C282Y and H63D mutations were detected by the Polymerase Chain Reaction technique followed by enzymatic restriction (PCR-RFLP); utilizing specific oligonucleotides C282YR: 5' CTCAGGCACTCCTCTCAACC 3', C282YF: 5' TGG CAA GGGTAAACAGATCC 3', H63DR: 5' GCCACATC TGGCT TGAAATT 3' and H63DF: 5' ACATGGTTAAGGCC TGTTC

3'. The PCR products for C282Y and H63D were digested, respectively, by the restriction endonucleases RsaI and BclI, specific for each site of mutations.<sup>15</sup> The denominations 282CC (63HH), 282CY (63HD) and 282YY (63DD) refer to the wild allele, and heterozygous and homozygous mutations of C282Y, respectively. Fragments of 247 and 140pb are encountered for the wild allele (282CC) and of 247, 111 and 29pb for the homozygous allele (282YY). In the presence of the homozygous allele (63DD), only one fragment of 208pb is observed, while fragments of 138 and 70pb correspond to the wild allele (63HH). The products were viewed in 7% acrylamide gel and evinced by SyBR® Safe DNA gel stain (Invitrogen®).

### *Statistical analysis*

The clinical and molecular characteristics of the studied individuals were analyzed utilizing the chi-square test (BioStat 4.0 Software). The Hardy-Weinberg Equilibrium was analyzed with the Genepop Software (on-line version.)<sup>16</sup> Values for  $p < 0.05$  were considered significant.

## Results

### *Prevalence of mutations*

This study evaluated the data registered for 1955 individuals with clinical suspicion of hereditary hemochromatosis, who were submitted to genotyping examinations for C282Y and H63D mutations. The C282Y mutation was presented as homozygous in 2.9% of the individuals and heterozygous in 10.1%, while the H63D mutation was homozygous in 4.3% and heterozygous in 30.6%. The allele frequency was 0.196 for H63D and 0.079 for C282Y. Compound heterozygosity (C282Y/H63D) was observed in 3% of the cases. It is interesting to note that the 56 (2.8%) homozygous individuals for the C282Y (282YY) mutation presented with the wild genotype for H63D in the same way that the 84 (4.3%) homozygous for the H63D (63DD) mutation had the wild genotype for C282Y.

### *Gender*

This sample consisted of 76.6% (1.498) men and 23.4% (457) women, resulting in a 3:1 proportion. The allele frequency of the C282Y mutation was 0.095 in women and 0.074 in men. The allele frequency of the H63D mutation was 0.206 in women and 0.194 in men. There was no significant genic differences between the genders on analyzing the presence of the mutations ( $p = 0.1$ ). The studied population, independently of the gender, did not present genotypic differences when the two loci, C282Y and H63D, were analyzed together ( $p = 0.18$ ) or separately (C282Y,  $p = 0.09$ ; H63D,  $p = 0.46$ ).

### *Age*

The individuals were divided into four age groups (0 to 20, 21 to 40, 41 to 60 and  $\geq 61$  years). The highest percentage

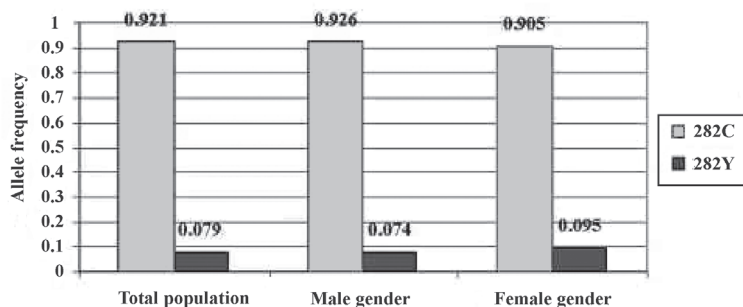


Figure 1. Allele frequencies of mutation C282Y according to the population type

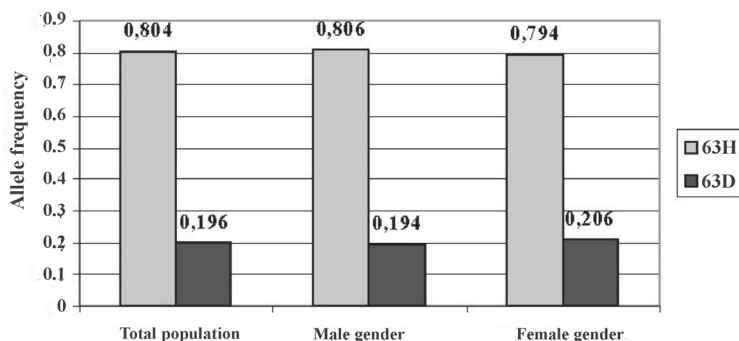


Figure 2. Allele frequencies of mutation H63D according to the population type

of individuals analyzed for C282Y and H63D (56.9%), with suspicion of HH were concentrated in the 41 to 60 age group, with 44.6% being female and 60.6% male. Almost 31.5% of women presented with ages equal to or greater than 61 years old, against 14.4% of men, and 5.5% of female individuals against 2.5% of male were in the 0 to 20 age group. In the 21-40 age group 44.6% were women and 60.6% men. The 0 to 20 age group was significantly smaller when compared to the 21 to 40 age group (p=0.0006). It is also significantly smaller than the 41 to 60 age group (p<0.0001). The size of the 21 to 40 and 41 to 60 age groups did not present any significant difference; but the number of over 61-year-old women (31.5%) was statistically greater (p<0.001) than over 61-year-old men

(14.4%). Individuals classified by age group and gender did not represent significant differences between the C282Y and H63D genotype frequencies (p>0.05). (Table 1)

*Hardy-Weinberg equilibrium*

On analyzing this study sample, the genotypic frequencies of C282Y and H63D mutations together do not follow the Hardy-Weinberg equilibrium (p = high significance). However, when the loci were analyzed separately, the C282Y mutation is not in the Hardy-Weinberg equilibrium (p = high significance) while the H63D mutation is in equilibrium (p = 0.46).

**Discussion**

The frequencies of C282Y and H63D mutations found in this study are greater than those reported for the general Brazilian population,<sup>1,8,11,13,15,17-22</sup> but are smaller than the genotypic frequencies among patients with HH.<sup>2,3,4</sup> It is unquestionable that C282Y is the main mutation responsible for HH in all studied populations, as the majority of published reports worldwide cite its prevalence in more than 80% of individuals with clinical manifestations of HH,<sup>1,4-6,12,15,17,23</sup> thus several other diagnostic guides are based on the result of C282Y mutation genic testing.<sup>2,6,7,15</sup> It is not different for the Brazilian population, as in the paper by Bittencourt *et al.*,<sup>1</sup> homozygous individuals for this mutation present an earlier onset for pathological aspects compared to heterozygous individuals with an onset at an earlier age than the wild allele, 282CC. The allele frequency of 282Y in the present study was 7.9%; a rate considerably greater than other Brazilian studies (2% in general population),<sup>11</sup> indicating a relationship between this mutation and clinical suspicion of HH.

The proportion of 3:1 between the men and women genotyped for the two mutations, is in accordance with several published studies that, in unanimity, justify this fact

Table 1. Genotypic frequencies for C282Y and H63D mutations, according to the gender and age of the studied population

Age Group	All population							Female population						male population							
	Total	C282Y		H63D			Total	C282Y		H63D			Total	C282Y		H63D					
	Genotyping Frequency																				
% <sup>a</sup>	CC	CY	YY	HH	HD	DD	% <sup>b</sup>	CC	CY	YY	HH	HD	DD	% <sup>c</sup>	CC	CY	YY	HH	HD	DD	
0 to 20 years	3.2	0.839	0.113	0.048	0.516	0.419	0.065	5.5	0.800	0.120	0.080	0.520	0.440	0.04	2.5	0.865	0.108	0.027	0.514	0.41	0.08
21 to 40 years	21.5	0.850	0.124	0.026	0.637	0.316	0.048	18.4	0.821	0.143	0.04	0.643	0.321	0.036	22.5	0.858	0.208	0.021	0.635	0.32	0.05
41 to 60 years	56.9	0.836	0.088	0.026	0.668	0.299	0.032	44.6	0.868	0.098	0.03	0.652	0.324	0.025	60.6	0.89	0.086	0.024	0.671	0.29	0.03
≥ 61 years	18.4	0.850	0.114	0.036	0.636	0.297	0.067	31.5	0.847	0.125	0.04	0.625	0.29	0.083	14.4	0.856	0.106	0.037	0.644	0.3	0.06
Total frequency <sup>d</sup>	100	0.87	0.101	0.029	0.651	0.306	0.043	100	0.847	0.116	0.04	0.635	0.32	0.046	100	0.877	0.117	0.025	0.656	0.3	0.04
Sampling N <sup>e</sup>	1955	1701	198	56	1272	599	84	1498	387	53	17	290	146	21	457	1314	145	39	982	453	63

a, b, c: They refer to the percentage of each group age for the total population, female population and male population, respectively  
 d: It corresponds to the frequency of each genotype according to the population under study  
 e: It refers to the sampling number for each genotype

by the "protective effect" of monthly bleeding and pregnancy by which the plasma concentration of iron is reduced because of the periodic physiological blood loss.<sup>3,5</sup> As it was expected, when assessed together, there was no statistical difference in the genotypic frequencies of C282Y and H63D between genders due to the fact that both are autosomally inherited.

Remembering that the studied population has clinical suspicion of HH and that in the majority of the cases the request for genic examinations is in order to confirm diagnosis, it is plausible that the concentration on individuals in the 41 to 60-year age group is evidence of the late development of HH. Generally, after 40 years old, the individual has lived long enough to allow iron accumulation to a pathological level.<sup>3,6,17</sup> Younger patients usually present milder clinical manifestations when compared to the advanced age group.<sup>3</sup> The fact that 31.5% of women present with ages equal to or greater than 61 years old against 14.4% of men is due to the intrinsic iron metabolism of the female organism and also because the menstrual cycle has finished. It is important to observe that there is scientific agreement regarding the onset of clinical manifestations of HH normally during or after the age of 50 among women<sup>5,6</sup> and, also manifestations of HH becoming apparent later than for men and are often milder.<sup>16</sup> Therefore, the presence of more female individuals than male in the 0 to 20 age group might be of a random nature, since, at this age the genetic testing of the HFE gene is generally to screen children with affected parents or might indicate the necessity of genetic tests for other genes, a situation that could not be confirmed in this study.

The fact that C282Y mutation was not in Hardy-Weinberg equilibrium may indicate the existence of natural selection of homozygous individuals.<sup>18</sup> Nevertheless, a study of 41,038 individuals considered Caucasians, from the USA, reported that the C282Y mutation is in balance.<sup>14</sup> Additionally, there is a deficit of heterozygous individuals for C282Y ( $p > 0.05$ ), a fact that could be intrinsic in this study as the studied population was under clinical suspicion of HH and the symptoms rarely manifest in C282Y heterozygous individuals who are thus, generally, not submitted to genetic testing. This fact may show the failure of the heterozygous C282Y genotype, in an isolated form, to cause a phenotypic expression of HH. On the other hand, H63D obeys the Hardy-Weinberg equilibrium and does not present with a heterozygous deficit, probably because this mutation is common in our population.

Homozygous and heterozygous genotypes for the H63D mutation may show minor penetration at the onset of HH clinical manifestations. Although having a minor penetration, H63D is of great importance in the development of HH, whether homozygous or simultaneous with C282Y. Hence, it is considered an essential part of genetic tests which investigate the cause of HH.<sup>3</sup> In the present study, the allele frequency of H63D was 0.196 and in accordance with the majority of the other world populations, in which the

frequency ranges from 0.1 to 0.2; it is important to note that Spain and Portugal, countries that greatly influenced colonization of Brazil, present frequencies greater than 0.2 in their general populations.<sup>4,11</sup>

The frequency of 0.03 for C282Y/H63D compound heterozygotes is in accordance with data already reported for the American population,<sup>14</sup> but it does not concur with Brazilian data,<sup>1,11,15,21,22</sup> probably because of the quantitative difference of the studied populations and because of the fact that this paper assessed individuals with clinical suspicion of HH. Thus, this frequency values the role of the C282Y/H63D genotype in the development of HH. A Pietrangelo<sup>3</sup> reported that about 1 to 2% of C282Y/H63D compound heterozygous individuals are predisposed to the expression of HH, a fact that provides them a distinct position in iron overload disease diagnostic guidelines. Another study cites that 11% of compound heterozygotes clinically manifest HH.<sup>2</sup>

The greater significance of heterogeneity of the C282Y mutation compared to the H63D mutation might be caused by the higher frequency of homozygous and heterozygous genotypes for the latter mutation in the population and its lesser importance in the onset of HH clinical manifestations. Population studies of HFE gene mutations indicate that the allele frequency of C282Y is 3 to 8 times lower in the Brazilian population than in north Europe, while these two populations have similar frequencies for H63D. The frequencies of HFE gene mutations encountered in Brazilian publications endorse the variability of the results found, as well as the inherent variables of each experiment (sample size, geographic inclusion of the population).<sup>1,8,11,13,15,19-22</sup>

Brazil is constituted by one of the most heterogeneous world populations, which reflects in the genetic parameters of different hereditary diseases, providing a genic diversity in the population and, also, the necessity of well conducted studies which assist in clarifying the enigmas of these diseases. With this in mind, the present paper shows the frequencies of C282Y and H63D mutations, according to gender and age aiming at helping the selection of diagnostic examinations, in respect to the populational profile of studied individuals, which could act as an important clinical benefit for health in Brazil.

## Resumo

*A hemocromatose hereditária clássica (HH) é uma doença autossômica recessiva caracterizada por uma sobrecarga sistêmica de ferro, a qual está freqüentemente relacionada às mutações C282Y e H63D no gene HFE. No Brasil, registros das freqüências das mutações no gene HFE são raros, principalmente envolvendo uma amostra representativa da população. Este estudo teve como objetivo a determinação da prevalência das mutações C282Y e H63D em indivíduos com suspeita clínica de HH. Para isto, foram estudados 1955 pacientes para os quais as mutações C282Y e H63D foram pesquisadas pela técnica de Reação em Cadeia da Polimerase seguida de digestão enzimática. A amostra consistiu de 76,6% ho-*

mens e 23,4% de mulheres. A maioria dos indivíduos analisados (56,9%) estava concentrada no grupo de 41 a 60 anos. Embora não tenham sido observadas diferenças gênicas e genotípicas entre os gêneros, foi observado um maior número de mulheres na faixa etária acima dos 60 anos. A mutação C282Y estava presente em homozigose em 2,9% dos indivíduos e em heterozigose em 10,1%, enquanto H63D estava presente em homozigose em 4,3% e em heterozigose em 30,6% dos indivíduos estudados. As frequências dos alelos mutantes C282Y e H63D foram de 0,079 e 0,196, respectivamente. Além de mais freqüente entre a população estudada, a mutação H63D mostrou equilíbrio genético, ao contrário da mutação C282Y. Este trabalho tem como importância a determinação do perfil genético da população acometida pela HH no Brasil. *Rev. Bras. Hematol. Hemoter.* 2008;30(5):379-383.

**Palavras-chave:** Hemocromatose hereditária; anormalidade genética; mutação HFE.

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