*Artigo / Article*

# Mutations in the *HFE* gene (C282Y, H63D, S65C) in a Brazilian population

## *Mutações no gene HFE (C282Y, H63D, S65C) em uma população brasileira*

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*Hereditary hemochromatosis (HH) is the most common genetic disorder occurring in individuals of northern European descent. The clinical characteristic of this disease is the gradual accumulation of iron in internal organs, which ultimately leads to organ failure and death. The defective gene in the majority of cases, HFE, was identified in 1996. Three allelic variants of the HFE gene have been correlated with HH: C282Y is significantly associated with HH; H63D and S65C have unclear relationships. In this report, these mutations were analyzed in 8 patients with HH and in 148 healthy individuals (blood donors). To detect the mutations, exons 2 and 4 of the HFE gene were amplified by PCR followed by restriction endonucleases cleavage. In patients with HH, three individuals were homozygous for the C282Y mutation, one showed compound heterozygous (C282Y/H63D), one was heterozygous for the C282Y and 3 presented with no mutations. In healthy individuals, the allele frequency observed was 0.014 for C282Y, 0.108 for H63D and 0.010 for S65C. The frequency of mutations was significantly higher in Caucasians compared with non-Caucasians. These data are concordant with the previous literature and with the ethnical origin of the population studied. Rev. bras. hematol. hemoter. 2006;**28**(4):293-295.*

***Key words:*** *HFE gene; polymorphisms; hereditary hemochromatosis.*

Hereditary hemochromatosis (HH) is an iron metabolism disorder characterized by increased iron absorption. Iron is progressively deposited in various tissues, particularly in the liver, pancreas, heart, joints and pituitary gland.[1] Phenotypic expression and severity of HH are variable and appear to depend on a complex interaction of genetic defect, age, gender and such environmental influences as dietary iron, the extent of iron loss from other processes, and the presence of other diseases and toxins (e.g., alcohol).[2] This inherited iron metabolism disorder is one of the most common genetic diseases in northern European descendents, affecting one in every 200-300 individuals.[3]

The gene responsible for HH was discovered in 1996 by Feder *et al*.[4] who described the novel gene and two sense mutations, C282Y and H63D.[2,5] The most important molecular mutation associated to clinical features, C282Y, has different frequencies in different populations. For example, the frequency of this mutation in Australian HH patients is 100% and in Italian HH patients it is 64%.[6] The other implicated mutation, H63D, does not seem to have such a strong association to iron overload, although the heterozygous C282Y/H63D is responsible for about 6% of HH cases in Europe and 4% in the USA.[6]

To date, 37 allelic variants of the *HFE* gene have been reported, but, except for C282Y and H63D, only S65C has been implicated in a moderate form of HH.[2] This mutation is observed in 2-3% of Caucasians and initially, it was considered to be a neutral polymorphism. Recent reports however, have implicated this mutation in mild iron overload when inherited in the compound heterozygous state.[6-8]

There are few reports in Brazil on this subject, and there is only one study investigating S65C in Brazilian patients with HH.[9] Thus, the aim of this work was to determine the allelic frequencies of C282Y, H63D and S65C of the *HFE* gene

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in healthy Brazilian individuals (blood donors) and in patients with HH.

## Material and methods

We analyzed blood samples of 148 healthy individuals (blood donors) and 8 patients with clinical and laboratory diagnoses of HH. Diagnosis of iron overload was achieved using the elevated transferrin saturation index (≥ 55%) and elevated serum ferritin concentration (≥ 500 ng/mL). Informed consent was obtained from each individual included in this study. The Ethics Committee of Unifesp approved the protocol used in this investigation.

Human genomic DNA was extracted from peripheral blood leukocytes; HFE mutations were detected by restriction enzyme analysis of polymerase chain reaction-amplified DNA. To detect the C282Y mutation, the forward primer 5'-TGCCTCCTTTGGTGAAGGTGACAC-3' and reverse primer 5'-CTCAGGCACTCCTCTCAACC-3' were utilized.[3,10] The reactions were performed on a final volume of 25 µL containing 2 mM MgCl2, 0.2 mM of each dNTP, 2 ng of template DNA, 4 pmol of each primer and 1 U of Taq Polymerase (Biotools) using the following sequence: 35 cycles at 94ºC for 30 seconds, 67ºC for 30 seconds and 72ºC for 30 seconds and a final step at 72ºC for 5 minutes. The length of the amplified fragment observed is 343 bp. After digestion with Rsa I, the digested wild-type resulted in fragments of 203 and 140 bp, whereas the digested mutant produced fragments of 203, 111 and 29 bp.

To detect the H63D and S65C mutations, the forward primer 5'-TCACACTCTCTGCAGTACCTCTTCATGG-3' and reverse primer 5'-TACACAGTGAACATGTGATCCCACC-3' were utilized.[10] The reactions were performed on a final volume of 50 µ L containing 2 mM MgCl2, 0.2 mM of each dNTP, 2 ng of the DNA template, 2 pmol of each primer, and 1 U de Taq Polymerase (Biotools) using the same thermal sequence as described above. The length of the amplified fragment observed is 223 bp.

For H63D detection, we digested it with Dpn II with wild-type digestion resulting in fragments of 118 and 105 bp, whereas mutant digestion showed only the 223 fragment. For the S65C mutation, the Hinf I enzyme was utilized and wild-type digestion resulted in fragments of 112, 69 and 42 bp, and the mutant digestion in fragments of 181 and 42 bp.

## Results

Five HH patients (62.5%) presented with molecular changes of the *HFE* gene: 3 were homozygous for the C282Y mutation (C282Y/C282Y), one patient presented with a compound heterozygous C282Y/H63D mutation and the fifth patient was heterozygous for the C282Y mutation (C282Y/WT). Three patients did not have any molecular changes. Thus, the C282Y was the most frequent mutation in our group of HH patients, corresponding to 8 of 16 (50%) of the alleles analyzed.

The allelic frequencies of the 3 mutations in the blood donor group were: 0.014 for the C282Y allele, 0.108 for H63D and 0.010 for the S65C mutation. When we calculated the genetic frequency for each mutation according to ethnic origin, the C282Y mutation was found only in Caucasians with an allelic frequency of 0.014. The H63D was seen in Caucasians and African descendants with frequencies of 0.086 and 0.024, respectively. This difference was considered significant (p=0.030). Only three S65C mutations were found: 2 (0.006) in Caucasians and 1 (0.003) in the African-Brazilian group.

## Discussion

There are few data on HH genotypes in Brazil, but all showed different patterns to those found in northern Europe.[11,12] This difference can be explained by the Brazilian ethnic miscegenation: predominant migration from southern Europe, where mutations are less frequent, and a high influence of Africans and Indians, populations that present with low mutation rates.[6,13,14]

As shown in Table 1, our results demonstrated that HFE genotypic frequencies in blood donors in São Paulo are very close to those of the Italian population, in agreement to the immigration observed in our state.[2]

Table 1
Genotype frequencies of HFE mutations in different population.

| | C282Y/ WT | C282Y/ C282Y | H63D/ WT | H63D/ H63D | C282Y/ H63D | H63D/ S65C | S65C/ WT |
|---|---|---|---|---|---|---|---|
| Our Results | 2.7% | 0 | 18.3% | 1.3% | 0 | 0.7% | 1.3% |
| Italy[2] | 2.2% | 0 | 20.9% | 1.4% | 0 | - - | - - |
| USA[2] | 11.4% | 1.0% | 20.9% | 2.9% | 3.2% | - - | - - |
| Japan[2] | 0 | 0 | 2.0% | 0 | 0 | - - | - - |
| Africa[2] | 0.2% | 0 | 5.4% | 0 | 0 | - - | - - |

The S65C mutation has not been investigated much and there is little information about its genetic frequency. In Spain and Italy its frequency is low.[14,15] In a study of Mediterranean populations a frequency of 0.15% was found and in the United Kingdom it was 0.9%.[16,17] We found that the S65C genetic frequency in blood donors from São Paulo is similar to the C282Y frequency (0.010), although in 35 Brazilian patients with iron overload the S65C mutation was not observed.[9] We consider these data relevant and meritorious for further investigation due to the importance of this mutation in iron overload according to the literature.[18-20]

Bittencourt *et al.*[21] analyzed the HFE gene in 15 HH patients and found 53% of C282Y homozygous and 7% of heterozygous individuals. None had the compound

heterozygous C282Y/H63D. Our results showed there were 37.5% of homozygous subjects for C282Y and 25% of heterozygous individuals and one heterozygous with C282Y/H63D. Those data are different, maybe because both studies involved a small number of patients (15 and 8, respectively).

## Conclusion

The studies carried out in Brazil involving the *HFE* gene, present the results of a small number of patients and controls with this being the first investigation of the S65C mutation in our general population. Because of our specific racial miscegenation, we believe it is very important to perform larger multi-center studies to define the genetic characteristics of HH in Brazilian patients.

***Resumo***

*A Hemocromatose hereditária (HH) é a alteração genética mais comumente encontrada em descendentes de Europeus, em especial da região Norte. A alteração clínica característica é a acúmulo gradual do ferro em órgãos internos, que evolui para lesão orgânica e morte. Na maioria dos casos, o gene alterado é o HFE, que foi identificado em 1996. Três variantes alélicas do gene HFE foram correlacionados com a HH: a C282Y, significativamente associada com a HH; e a H63D e a S65C, que apresentam uma relação obscura com esta doença. Neste relato, foi analisada a presença destas mutações em 8 pacientes com HH e em 148 indivíduos saudáveis (doadores de sangue). Para detecção das mutações, foi realizado PCR dos exons 2 e 4 do gene HFE, seguido pela clivagem com endonucleases específicas. No grupo de pacientes com HH, observou-se 3 indivíduos homozigotos para a mutação de C282Y, um heterozigoto composto (C282Y/H63D), um heterozigoto para C282Y e ausência de mutações nos outros 3 pacientes. Nos indivíduos saudáveis, a freqüência observada foi de 0,014 para o alelo C282Y, 0,108 para o H63D e 0,010 para o S65C. A presença de mutações foi significantemente maior nos indivíduos brancos, comparando com os não brancos. Estes dados são concordantes com a literatura prévia e com a origem étnica da população estudada. Rev. bras. hematol. hemoter. 2006;**28**(4):293-295.*

***Palavras-chave:*** *Gene HFE; polimorfismos; hemocromatose hereditária.*

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