Mutations in the \textit{HFE} gene (C282Y, H63D, S65C) in a Brazilian population

\textit{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, \textit{HFE}, was identified in 1996. Three allelic variants of the \textit{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 \textit{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.

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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′-
TGCTCTCTTTGGAAGTGACAC-3′ and reverse primer
5′-CTCAGGCACTCCTTCAAACC-3′ were utilized.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 94°C for 30 seconds, 67°C for 30 seconds and 72°C for 30
seconds and a final step at 72°C 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′-TCACTCCTCTGCACTACCTTTGATCC-3′ and
reverse primer 5′-TACACAGTGAACATGTAATCCCACC-3′
were utilized.10 The reactions were performed on a final volu-
me of 50 µL containing 2 mM MgCl2, 0.2 mM of each dNTP,
2 ng of the DNA template, 2 pmol of each primer, and 1 U of
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 203 and 140 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

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
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 miscenagation, we believe it is very important to perform larger multi-center studies to define the genetic characteristics of HH in Brazilian patients.

**Resumo**


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

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