

CLINICAL SCIENCE

ANALYSIS OF HFE AND NON-HFE GENE MUTATIONS
IN BRAZILIAN PATIENTS WITH HEMOCHROMATOSIS

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BACKGROUND: Approximately one-half of Brazilian patients with hereditary hemochromatosis (HH) are neither homozygous for the C282Y mutation nor compound heterozygous for the H63D and C282Y mutations that are associated with HH in Caucasians. Other mutations have been described in the *HFE* gene as well as in genes involved in iron metabolism, such as transferrin receptor 2 (TfR2) and ferroportin 1 (SCL40A1).

AIMS: To evaluate the role of HFE, TfR2 and SCL40A1 mutations in Brazilian subjects with HH.

PATIENTS AND METHODS: Nineteen male subjects (median age 42 [range: 20-72] years) with HH were evaluated using the Haemochromatosis StripAssay A®. This assay is capable of detecting twelve HFE mutations, which are V53M, V59M, H63D, H63H, S65C, Q127H, P160delC, E168Q, E168X, W169X, C282Y and Q283, four TfR2 mutations, which are E60X, M172K, Y250X, AVAQ594-597del, and two SCL40A1 mutations, which are N144H and V162del.

RESULTS: In our cohort, nine (47%) patients were homozygous for the C282Y mutation, two (11%) were heterozygous for the H63D mutation, and one each (5%) was either heterozygous for C282Y or compound heterozygous for C282Y and H63D. No other mutations in the HFE, TfR2 or SCL40A1 genes were observed in the studied patients.

CONCLUSIONS: One-third of Brazilian subjects with the classical phenotype of HH do not carry HFE or other mutations that are currently associated with the disease in Caucasians. This observation suggests a role for other yet unknown mutations in the aforementioned genes or in other genes involved in iron homeostasis in the pathogenesis of HH in Brazil.

KEYWORDS: Hereditary hemochromatosis; Iron overload; HFE mutations; Gene mutations; Brazil.

INTRODUCTION

Hereditary hemochromatosis (HH) is a recessive inherited disorder of iron metabolism that is characterized by systemic iron overload as a result of enhanced intestinal absorption.^{1,2} The disease usually affects males in the fourth

to fifth decade of life and may lead to cirrhosis, diabetes mellitus, panhypopituitarism, cardiomyopathy, arthritis and skin hyperpigmentation.^{1,2} In European and North-American Caucasians, HH has been linked to two missense mutations in the *HFE* gene, which maps to the 4 kb telomeric HLA-A locus on the short arm of chromosome 6.¹⁻³ Furthermore, 80% to 100% of Caucasian patients of Northern European descent with HH were shown to be homozygous for the C282Y mutation, whereas 4% to 6% were reported to be compound heterozygotes for the C282Y and H63D mutations.⁴

The frequencies of these HFE mutations are lower in Italian and Brazilian patients with HH,⁵⁻⁷ and approximately half of Brazilian patients with the classical disease phenotype are neither homozygous for C282Y nor compound heterozygous for the C282Y and H63D mutations.⁶ In

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addition, none of these mutations were observed in African Americans with HH or in patients with African iron overload.^{8,9}

Other mutations in the HFE gene, particularly *S65C*, have been implicated in HH and are generally found in a compound heterozygous state with either *C282Y* or *H63D*.^{3,10-12} Furthermore, mutations have been found in genes that have been recently implicated in iron homeostasis, including the transferrin receptor 2 (*TfR2*) gene on chromosome 7, the hepcidin antimicrobial peptide (*HAMP*) gene on chromosome 19, the hemojuvelin (*HJV*) gene on chromosome 1 and the ferroportin 1 (*Fpn1*, also known as *SCL40A1*) gene on chromosome 2. The identification of causative mutations for HH in these genes has improved our understanding of iron homeostasis as well as suggested a genetic basis for a new classification of iron overload disorders.^{1,3,13}

HH has different phenotypic presentations. Types 1 and 3 HH are characterized by the classical HH phenotype, which includes parenchymal iron overload and the presence of HFE and *TfR2* mutations, respectively. Type 2 HH is associated with the previously described phenotype of juvenile hemochromatosis and mutations in the *HAMP* and *HJV* genes, whereas type 4 HH is associated with heterogeneous clinical and laboratory findings of iron overload that are more pronounced in the reticuloendothelial system.¹³

Mutations in the *TfR2* gene, particularly the *Y250X* variant, and several different mutations in the *SCL40A1* gene have been reported in Caucasian, African and Asian patients with non-HFE linked iron overload.¹³⁻¹⁸ The prevalence of these recently described gene mutations in Brazilian patients with HH has not been assessed, and the purpose of the present study is to determine the frequency of non-classical *HFE* as well as *TfR2* and *SCL40A1* mutations in Brazilian subjects with the classical phenotype of HH.

PATIENTS AND METHODS

Subjects: Sixteen unrelated male patients from the metropolitan area of São Paulo, Brazil and three other subjects from Salvador, Bahia were studied. All had a diagnosis of HH based upon several criteria: 1) the absence of secondary causes of iron overload, including chronic hemolytic anemia, thalassemia major, sideroblastic and spur cell anemia, parenteral or dietary iron overload, alcohol abuse and chronic liver disease due to either hepatitis C or non-alcoholic steatohepatitis; 2) transferrin saturation greater than 50% and ferritin levels greater than 400 g/L; and 3) grade III or IV siderosis by Perls stain and no other evidence of liver disease upon liver biopsy.² Fifteen patients had been enrolled in a previous study and were already typed for the HLA-A *C282Y* and *H63D* mutations.⁶ All patients gave

Table 1 - Clinical and laboratory features of Brazilian patients with HH

Clinical and laboratory features	
Age at onset	Median [range] 42 [20-72] years
Signs and Symptoms	Number (%)
Chronic liver disease	14 (74)
Diabetes	4 (21)
Impotence	3 (19)
Skin hyperpigmentation	1 (15)
Panhypopituitarism	1 (15)
Cardiac Insufficiency	1 (15)
Arthritis	1 (15)
Laboratory features	Median [range]
Iron saturation (%)	93 [55-100]
Ferritin (µg/mL)	1102 [563-13.170]
ALT IU/L (normal: ≤ 20)	57 [20-187]
Bilirubin mg/dL (normal ≤ 1.1)	1.5 [0.6-7.2]
Albumin g/dL (normal 3.5-5.0)	4.3 [2.7-4.9]
Liver biopsy	Number (%)
Grade III Siderosis	8 (42)
Grade IV Siderosis	11 (58)
Cirrhosis	14 (73)

informed consent for participation in this study.

The median age of the patients was 42 (range: 20-72) years. The clinical, laboratory and histological features of the patients are summarized in table 1.

DNA extraction: Genomic DNA was extracted from peripheral blood leukocytes using the DTAB/CTAB technique.¹⁹

Determination of HFE and non-HFE mutations: DNA samples from all patients, with the exception of those who were previously confirmed as homozygous for the *C282Y* mutation,⁶ were tested with a reverse transcriptase-based assay, the Haemochromatosis StripAssay A® (Vienna Lab, Labordiagnostika, GmbH, Vienna, Austria), according to the manufacturer's instructions. This assay can detect twelve mutations in the *HFE* gene, which are *V53M*, *V59M*, *H63D*, *H63H*, *S65C*, *Q127H*, *P160delC*, *E168Q*, *E168X*, *W169X*, *C282Y* and *Q283P*, four mutations in the *TfR2* gene, which are *E60X*, *M172K*, *Y250X* and *AVAQ594-597del*, and two mutations in the *SCL40A1* gene, which are *N144H* and *V162del*.

Statistical analysis: Clinical and laboratory data are shown in the text and tables as median and range. Differences in the frequencies of clinical and laboratory parameters were compared using the Fisher exact probability test when appropriate. A p value < 0.05 was considered to be significant.

RESULTS

The *C282Y* and *H63D* mutation status for each patient in our cohort is shown in Table 2.

Table 2 - Clinical and laboratory features of Brazilian patients with hemochromatosis with and without homozygosity for C282Y mutation

	C282Y (+/+) (n=9)	C282Y (+/-) or (-/-) (n=10)
Age at onset	Median [range] 40 [36-47] years	Median [range] 44 [20-72] years
Signs and symptoms of chronic liver disease	Number (%) 8 (80)	Number (%) 6 (66)
Laboratory features	Median [range]	Median [range]
Iron saturation (%)	96 [36-100]	93 [58-100]
Ferritin (µg/mL)	1351 [576-13.170]	1057 [700-2333]
ALT IU/L (normal: ≤ 20)	63 [36-187]	52 [20-98]
Liver biopsy	Number (%)	Number (%)
Grade III Siderosis	3 (33)	4 (40)
Grade IV Siderosis	6 (66)	6 (60)
Cirrhosis	6 (66)	8 (80)

Numbers in parentheses are percentages

Nine (47%) patients were homozygous for the C282Y mutation. In addition, two (11%) patients were heterozygous for H63D, and one each (5%) was either heterozygous for C282Y or compound heterozygous for C282Y and H63D.

None of the other ten HFE mutations and none of the Tfr2 or SCL40A1 mutations that were included in the assay were found in these patients.

A comparison of the clinical and laboratory profiles of patients with and without homozygosity for the C282Y mutation revealed that the former had higher ferritin levels and a lower age of onset for the disease when compared to their counterparts who were non-homozygous for C282Y. However, this difference was not significant (Table 2).

DISCUSSION

Mutations in the HFE gene other than the C282Y substitution are uncommon in subjects with HH.^{1,3,10,13} In fact, the majority of patients who are not homozygous for the C282Y mutation are usually found to be compound heterozygotes for the C282Y and H63D mutations. Rare HFE mutations, such as S65C, E168X, W169X and Q238P, have been found in HH subjects who are heterozygous either for C282Y or H63D.^{1,3,10-12,20,21} The occurrence of the S65C mutation, as well as homozygosity for H63D,^{11,12,22,23} has recently been associated with a milder form of iron overload disease. In the present study, no other mutation in the HFE gene, apart from C282Y and H63D, was identified in subjects with HH. Similar results were also reported by Cancado et al.,⁷ who were unable to find subjects with the S65C mutation in their cohort of Brazilian patients with iron overload. These findings are also in accordance with several other reports from Europe and North America,^{1,3} in which the S65C or another HFE mutation was found without the additional presence of either the C282Y and H63D mutations

in only a few patients with HH or African iron overload.

Non-HFE linked HH has been particularly associated with mutations in the ferroportin 1, transferrin receptor 2 and hepcidin genes.¹³ Hpcidin is a pleiotropic protein that plays a major role in cellular iron trafficking. Mutations in the HAMP gene have been associated with type 2 HH or juvenile iron overload, which is characterized by severe iron loading with end-stage organ damage in the second to third decade of life.^{1,3,13} Given that our patients do not share clinical features that are compatible with type 2 HH, HAMP gene variants were not assessed in the present study.

On the other hand, our search for several common mutations in the Tfr2 and SCL40A1 genes also yielded negative results. Tfr2 variants have been implicated in HH type 3 HH, which exhibits a phenotype that is indistinguishable from classical disease phenotype of type 1 HH.¹³ Unexpectedly, none of our patients with HH carried any one of the four most common Tfr2 gene mutations, E60X, M172K, Y250X and AVAQ594-597del, that had been previously associated with non-HFE HH in Italy, Portugal and Japan.¹³⁻¹⁷ In addition, two mutations in the SCL40A1 gene, which have been correlated with type 4 HH in Europe, North America and Australia,¹³ were not encountered in our cohort of Brazilian patients with HH. This is not surprising due to the stringent criteria we employed in this study, selecting only patients with parenchymal iron overload and grade III or IV hepatic siderosis. Type 4 HH is mainly associated with iron loading in reticuloendothelial cells, especially macrophages,¹³ and none of our patients had histopathological features that were consistent with type 4 HH upon liver biopsy.

None of the individuals in our cohort of Brazilian patients with the classical phenotype of HH had any of the aforementioned non-C282Y mutations in the HFE gene or any mutations in the Tfr2 and SCL40A1 genes. Less than

two-thirds of Brazilian subjects with either iron overload or HH were previously reported to be either homozygous for the *C282Y* mutation or compound heterozygous for the *C282Y* and *H63D* mutations.^{6,7,24} Moreover, the frequency of both of these *HFE* mutations was reported to be lower in Brazilians when compared to healthy subjects from Europe and North America.^{25,26}

The absence of mutations in the *TfR2* and *SCL40A1* genes in subjects with non-HFE HH has also been reported in other of European and North American patient cohorts, particularly in African-Americans and in patients with African iron overload.²⁷⁻³⁰ This observation suggests a possible role for other mutations in the known HH genes or even for other undiscovered genes that are also involved in iron homeostasis. In this regard, mutations in the iron responsive element of the ferritin heavy chain polypeptide 1 gene (*FTH1*) have been recently reported in association with an autosomal dominant form of HH in Japan.¹⁷

Due to the ethnic background of the Brazilian population, other strategies, including direct sequencing or denaturing HPLC screening of candidate genes, may be more valuable in order to identify novel variants in the steadily increasing group of candidate genes for HH.

As previously shown in our first report,⁶ patients homozygous for the *C282Y* mutation tended to be younger and to have a more severe iron overload when compared to their counterparts who were non-homozygous for *C282Y*. Even after the inclusion of four additional subjects from the present cohort of HH patients, the difference is still not significant. Interestingly, *C282Y* homozygotes have been shown to have more pronounced iron overload when compared to heterozygotes or to patients without the *C282Y* mutation in other populations.³¹

In summary, only 52% of Brazilian HH patients who were analyzed in this study were either homozygous for *C282Y* (n=9) or compound heterozygous for the *C282Y* and *H63D* mutations (n=1). None of these patients had another HFE variant or any mutation in the *TfR2* and *SCL40A1* genes.

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