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Hereditary hemochromatosis in a Brazilian university hospital in São Paulo State (1990-2000)

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ABSTRACT. Hereditary hemochromatosis (HH) is the most common genetic disease among individuals of European descent. Two mutations (845G→A, C282Y and 187C→G, H63D) in the hemochromatosis gene (HFE gene) are associated with HH. About 85-90% of patients of northern European descent with HH are C282Y homozygous. The prevalence of HH in the Brazilian population, which has a very high level of racial admixture, is unknown. The aims of the present study were to identify individuals with diagnostic criteria for HH among patients with a body iron overload attended at the university hospital of the Faculty of Medicine of Ribeirão Preto from 1990 to 2000, and to evaluate the prevalence of HFE mutations. We screened first-degree relatives for HFE mutations. Four of 72 patients (three men and one woman, mean age 47 years) fulfilled the criteria for HH. HFE mutations were studied in three patients [two C282Y homozygotes (patients 1 and 2) and one H63D heterozygote]. Patient 1 had four children (all C282Y heterozygotes with no iron overload) and seven brothers and sisters: two sisters (66 and 76 years old) were C282Y homozygotes and both had an iron overload (a liver biopsy in one showed severe iron deposits), one sister (79 years

old) was a compound heterozygote with no iron overload, one brother (78 years old) was a C282Y heterozygote with no iron overload, two individuals were H63D heterozygotes (one brother, 49 years old, obese, with a body iron overload and abnormal liver enzymes - a biopsy showed non-alcoholic steatohepatitis, and one 70-year-old sister with no iron overload). Patient 2 had two children (22 and 24 years old who were C282Y heterozygotes with no iron overload) but no brothers or sisters. These results showed that HH was uncommon among individuals attended at our hospital, although HFE mutations were found in all patients. Familial screening is valuable for the early diagnosis of individuals at risk since it allows treatment to be initiated before the onset of the clinical manifestations of organ damage associated with HH.

Key words: Body iron overload, Genetic diseases, European descent, Hereditary hemochromatosis, HFE mutations

INTRODUCTION

Hereditary hemochromatosis (HH) is the most common genetic disease in Europeans, with an estimated prevalence of 1 in 200 in the northern European population of Nordic or Celtic ancestry (Tavill, 2001). Although HH is concentrated primarily where people with this genetic background live, its distribution is worldwide (Tavill, 2001). The increased absorption of iron associated with HH may lead to the progressive development of severe complications such as cirrhosis, hepatocellular carcinoma, diabetes, and heart disease, all of which have a direct impact on life expectancy (Niederau et al., 1996).

Two mutations in the hemochromatosis gene (HFE gene) on chromosome 6 are associated with HH (Beutler et al., 1996; Feder et al., 1996; Merryweather-Clarke et al., 1997). The 845G→A (C282Y) mutation is the most clearly associated with HH. About 85-90% of patients of northern European descent with hemochromatosis are homozygous for the C282Y mutation (Beutler et al., 1996; Feder et al., 1996). The relationship of the second mutation (187C→G; H63D) to HH is less obvious. This mutation results in hemochromatosis in some patients when co-inherited with the C282Y mutation, and accounts for 3-5% of cases of HH (Beutler et al., 1996; Feder et al., 1996; Aguilar Martinez et al., 1997; Beutler, 1997). The rate of C282Y and H63D carriers in individuals of northern European descent is 8-10 and 24.8-26.8%, respectively (Roberts et al., 1997; Bonkovsky et al., 1998; Stuart et al., 1998). The possibility of using a genetic test to detect HH allows the diagnosis of patients at an early stage when there are no symptoms or significant iron accumulation and the disease is more easily treated. The availability of genetic testing has meant that strategies for the screening of populations can now be implemented (Tavill, 2001).

The prevalence of HH in the Brazilian population, which has a very high level of racial admixture, is unknown, although the percentage of people of Italian and Spanish descent is very high in southern Brazil. The rate of heterozygous carriers of C282Y and H63D has been reported in only two Brazilian studies, both from southeastern Brazil. One of these studies reported a prevalence of C282Y and H63D carriers of 1.2 and 31.1%, respectively (Martinelli et

al., 1999) while in the other the prevalence of C282Y and H63D carriers was 2.8 and 32.6%, respectively (Agostinho et al., 1999). Hence, the prevalence of C282Y is lower in the Brazilian than in the northern European population, whereas the prevalence of H63D is similar.

The university hospital of the Faculty of Medicine of Ribeirão Preto, University of São Paulo (UH-FMRP) is located in southeastern Brazil and is a reference medical center for this city and the surrounding region. The aims of the present study were to assess the frequency of HH among patients with a diagnosis of body iron overload who were attended at the UH-FMRP from 1990 to 2000 and who fulfilled the diagnostic criteria for HH, and to evaluate the prevalence of HFE mutations in these patients. We also screened the first-degree relatives of patients in whom HFE mutations were identified.

PATIENTS AND METHODS

Patients and study design

We analyzed the medical records of all patients attended at the UH-FMRP from 1990 to 2000 who had a diagnosis of body iron overload. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the local Ethics Committee. The diagnostic criteria for HH included evidence of an iron overload in serum as shown by elevated serum iron levels, transferrin saturation (>50%) or a high ferritin level (>300 ng/ml) and iron deposits in hepatocytes (grades III or IV siderosis) by Perls stain, with no evidence of other chronic liver disease or any secondary cause of iron overload (blood transfusions, iron intake, hemolytic anemia, thalassemia major, alcohol abuse, chronic liver disease due to hepatitis C or hepatitis B, non-alcoholic steatohepatitis) (Bacon, 2001). A blood sample to detect the HFE mutations C282Y and H63D was collected from those patients who fulfilled the diagnostic criteria for HH.

A liver biopsy was revisited and iron deposits (Perls staining) were assessed and scored [from 0 to 20] based on their amount and their cellular and lobular location (Sciot et al., 1989). The total iron score was classified as grade I (score of 1 to 5), grade II (score of 6 to 10), grade III (score of 11 to 15), or grade IV (score of 16 to 20). The slides were also scored for fibrosis using the scoring system proposed by Knodell et al. (1981). First-degree relatives of patients who were homozygous for the C282Y mutation and of patients with a compound mutation were tested for the HFE mutations.

The serum iron levels and activities of the liver enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyltransferase (GGT) were also examined in patients with HH and their first-degree relatives. The serum ALT, AST and GGT levels were obtained from the medical records. The reference values for the biochemical tests in the UH-FMRP laboratory were: AST = 10-34 U/l, ALT = 10-44 U/l, GGT = 11-50 U/l, serum iron 53-167 µg/dl (men) and 49-151 µg/dl (women), and ferritin = 18-370 ng/dl (men) and 9-120 ng/ml (women).

Analysis of HFE mutations

Genomic DNA was extracted from peripheral blood leukocytes by a standard method (Miller et al., 1988), and was amplified by PCR followed by digestion with the restriction en-

zymes *Rsa*I (for analysis of the C282Y mutation) and *Bcl*I (for analysis of the H63D mutation). The primers and PCR conditions used have been described elsewhere (Merryweather-Clarke et al., 1997).

RESULTS

Patients with HH

A review of the medical records of 72 patients showed no conclusive evidence of iron overload in 14 patients and secondary hemosiderosis in 54, so that only four patients (three men and one woman, mean age ± SD = 47 ± 12 years) fulfilled the diagnostic criteria for HH.

Patient 1

A 51-year-old male patient had a serum iron level of 293 µg/dl, a transferrin saturation of 90%, and grade III iron deposits in a liver biopsy. The patient's father and one brother had died in another hospital because of complications associated with liver cirrhosis of unknown etiology. This patient was homozygous for the C282Y mutation.

Patient 2

A 58-year-old male patient with a serum iron level of 192 mg/dl, a transferrin saturation of 71%, a ferritin level of 3230 µg/l, and grade IV liver iron deposits. This patient was homozygous for the C282Y mutation.

Patient 3

A 35-year-old female patient with a serum iron level of 202 µg/dl, a transferrin saturation of 96%, and grade III liver iron deposits. This patient was heterozygous for the H63D mutation.

Patient 4

A 54-year-old male patient with a serum iron level of 206 mg/dl, a transferrin saturation of 89%, a ferritin level of 1180 µg/l, and grade III liver iron deposits. No blood was available to test for the HFE mutation.

First-degree relatives

Familial screening was done only for patients 1 and 2. The two other families did not come for blood collection because of the great distance they had to travel to reach the hospital.

First-degree relatives of patient 1

This patient had four children, all of them heterozygous for C282Y, with no evidence of

iron overload or liver enzyme abnormalities. The patient had two brothers and four sisters who were still alive and one brother who had died in another hospital because of complications associated with cirrhosis of unknown etiology. Screening of the six relatives showed that two sisters were homozygous for C282Y (one was 76 years old, with a serum iron level of 224 µg/dl, a transferrin saturation of 98%, a ferritin level of 657 µg/l, and normal liver enzymes, and the other was 66 years old, with a serum iron level of 216 µg/dl, a transferrin saturation of 94%, a ferritin level of 1351µg/l, normal liver enzymes, and a liver biopsy that showed grade 3 fibrosis and grade IV iron deposits), another sister (79 years old) was a compound heterozygote, with a normal serum iron biochemistry and liver enzymes, and one brother (78 years old) was heterozygous for C282Y but had normal serum iron and liver enzyme levels. The other two siblings were heterozygous for H63D and included a 49-year-old brother, with a body mass index of 33.4, no history of alcohol ingestion, a serum iron level of 146 µg/dl, a transferrin saturation of 53%, a ferritin level of 1486 µg/l, abnormal liver enzymes (AST = 54 U/l, ALT = 62 U/l and GGT = 85 U/l), a liver biopsy with grade II iron deposits, and findings compatible with non-alcoholic steatohepatitis, and a 70-year-old sister with no iron overload or abnormal liver enzymes.

First-degree relatives of patient 2

This patient had two children (22 and 24 years), both of whom were heterozygous for C282Y and had no iron overload or abnormal liver enzymes. This patient had no brothers or sisters.

DISCUSSION

The university hospital where this study was done is a reference center for patients with liver disease. However, of the 2121 adult patients seen at the Gastroenterology Clinic of this hospital during the 10-year period surveyed here, only four were diagnosed with HH. This low frequency was not unexpected since HH is a disease strongly associated with populations of European descent whereas the general Brazilian population shows a high level of racial admixture.

The frequency of C282Y carriers in the general population in the geographic area where the UH-FMRP is located was 1.2% (Martinelli et al., 1999), which was similar to that reported in another Brazilian study (2.8%) (Agostinho et al., 1999) but lower than that found in North America, Australia and England (11-14%) (Roberts et al., 1997; Bonkovsky et al., 1998; Stuart et al., 1998). In our previous study (Martinelli et al., 1999), we also found a similar prevalence of C282Y carriers (4.4%) among HCV patients from the same geographic area. In addition, the frequency of the H63D mutation in the general population was 31.1% (Martinelli et al., 1999), which was similar to another Brazilian study (32.6%) (Agostinho et al., 1999) and that reported for North America, Australia and England (24.8-26.8%) (Roberts et al., 1997; Bonkovsky et al., 1998; Stuart et al., 1998).

One of our patients (patient 3) was heterozygous for H63D. Heterozygosity for H63D can be associated with liver fibrosis when other conditions, such as chronic hepatitis C, are concomitant (Smith et al., 1998; Martinelli et al., 1999). This was not the case for this patient. There is evidence of geographic variation in HFE mutations and of a role for a gene other than HFE in determining iron overload (Bacon et al., 1999). In Italy, the involvement of HFE muta-

tions in HH is lower than in northern Europe and there is north-to-south variation in the occurrence of HFE mutations in this country (Piperno et al., 1998). In addition, there are well-documented Italian families with an iron overload in which the C282Y and H63D mutations do not occur (Pietrangelo et al., 1999). Other mutations have been reported in a small number of patients (Barton et al., 1999; Mura et al., 1999; Piperno et al., 2000). In the present study, we did not search for mutations other than C282Y and H63D. Another Brazilian study involving 15 HH patients showed a 53% prevalence of C282Y homozygosity (Bittencourt et al., 2002).

Familial screening of patient 1 identified three of six siblings with an accumulation of body iron. Two of the three were homozygous for C282Y and one was heterozygous for H63D. The two homozygotes were clinically asymptomatic, but one had advanced liver disease with massive iron deposits. The liver iron concentration in this patient was 17,136 µg/g dry weight. Organ damage is usually observed in patients more than 40 years old when the parenchymal iron storage is >10,000 µg/g dry weight and a liver biopsy is likely to show fibrosis or cirrhosis (Basset et al., 1986). This patient was treated with sessions of phlebotomy. The serum liver enzyme activities of this patient were normal. Thus, these two patients were asymptomatic with no manifestation of HH, although the liver biopsy of the only patient who underwent this procedure showed advanced liver disease. The clinical condition of HH evolves over at least three stages that include insignificant iron accumulation (0-20 years of age), iron overload without organ damage (20-40 years of age) and iron overload with organ damage (>40 years of age) (Adams et al., 1997). Women have a slower rate of iron accumulation because of their greater loss of iron. For therapy to be effective, the diagnosis should identify the patients at risk before the last stage of the disease is reached so that phlebotomy to remove iron and prevent the progression to irreversible tissue damage can be initiated. Phlebotomy is a highly effective therapy that results in normal longevity if it is initiated before the onset of irreversible tissue damage (Niederau et al., 1996).

The other first-degree relative of patient 1 who had an iron overload was heterozygous for H63D. HH has been strongly associated with C282Y homozygosity and only a small percentage of patients have compound heterozygosity. However, this patient was obese and had steatohepatitis. H63D heterozygosity can be a co-factor contributing to iron accumulation in conditions such as non-alcoholic steatohepatitis (Bonkovsky et al., 1999), chronic hepatitis C (Smith et al., 1998; Martinelli et al., 1999) and porphyria cutanea tarda (Roberts et al., 1997; Martinelli et al., 2000).

We found only one compound heterozygote, a first-degree relative of patient 1, who showed no evidence of iron overload. Compound heterozygotes account for 3-5% of HH cases (Beutler et al., 1996). The absence of the HH phenotype can be explained by the variable penetrance of the C282Y gene (Olynk et al., 1999).

Since diagnostic screening strategies should target high-risk populations such as those with a family history of HH, it is recommended that individuals over 20 years of age who are first-degree relatives of known cases of HH be screened to assess their degree of transferrin saturation and to determine the genotype of the HFE mutations (Tavill, 2001).

In conclusion, HH was not common in the population attended at the UH-FMRP. However, HFE mutations were found in all of the patients tested who showed clinical evidence of iron overload in the absence of secondary iron accumulation. Familial screening is valuable for the early diagnosis of individuals at risk, and allows treatment to be initiated before the onset of the clinical manifestations of organ damage. Multicenter studies in Brazil involving a larger

numbers of patients are warranted to accurately determine the prevalence of HH and its association with HFE mutations.

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