Mutations in HFE and TFR2 genes in a Spanish patient with hemochromatosis

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ABSTRACT

Iron overload disease has a wide variety of genotypes. The genetic study of this disease confirms its hereditary nature and enables us to provide genetic counseling for first-degree relatives. We performed magnetic resonance imaging and liver biopsy in an asymptomatic patient with more than 1,000 µg/L of serum ferritin and studied the genes involved in this condition. The phenotype of iron overload is confirmed by a predominantly periportal pattern of iron deposits in the liver suggestive of genetic disease. In the case we present the molecular study revealed a double heterozygosity for the mutations c.187C>G (p.H63D) and c.840C>G (p.F280L) in the HFE and transferrin receptor 2 (TFR2) genes, respectively.

Key words: Hemochromatosis. HFE. Transferrin receptor 2.

INTRODUCTION

Clinicians have been aware of the clinical syndrome of hemochromatosis for more than 100 years; however, the most important advances in knowledge of this condition have occurred in the last two decades, especially with the discovery of the HFE gene (1). Hemochromatosis presents marked heterogeneity in genetic etiology, type of inheritance, environmental factors, and clinical and analytical manifestations (2-5).

Although the homozygous c.845G>A (p.C282Y) mutation in the HFE gene is responsible for the classic form of hereditary hemochromatosis, known as type 1 hemochromatosis, other genotypes, such as compound heterozygous c.845G>A (p.C282Y)/c.187C>G (p.H63D) in the HFE gene, are associated with iron overload (6). The heterozygous c.187C>G (p.H63D) mutation does not seem to be responsible for iron overload or hemochromatosis; therefore, it is necessary to analyze associated factors such as alcohol intake, hepatitis viruses, or mutations in genes involved in iron homeostasis (7,8). In rare cases, molecular abnormalities in the TFR2 and HFE genes in patients with iron overload are responsible for digenically inherited hemochromatosis (9,10).

Type 3 hemochromatosis is caused by mutations in the transferrin receptor 2 (TFR2) gene. The first cases were described in two Sicilian families with a homozygous c.750C>G (p.Y250X) mutation in this gene (11). TFR2 is homologous to TFR1, it is mainly expressed in liver, and the affinity of the TFR2 protein for transferrin is 30-fold higher than that of TFR1 (11). Patients with heterozygous mutations in the TFR2 gene do not have iron overload, unlike patients with heterozygous mutations in the HFE gene. The clinical manifestations of type 3 hemochromatosis are comparable to those of type 1, although there have been reports of more severe cases with earlier onset (12).

We report the case of a patient with mutations in HFE and TFR2 genes and a clinical phenotype of iron overload.
This report confirms the genotypic and phenotypic heterogeneity of hemochromatosis.

CASE REPORT

A 50-year-old man with 2-year history of serum ferritin higher than 1,000 µg/L (normal range, 26-370) was referred to our department. He had increased ferritin (1,277 µg/L) and the γGT (116 U/L; normal range, 10-60) and ultrasonography of the liver led us to suspect fatty liver disease. Complementary analytical and serological testing revealed no abnormalities. The patient was asymptomatic. He had no toxic habits, and his personal/family history and physical examination were unremarkable.

We analyzed iron metabolism markers (serum levels of iron, ferritin, soluble transferrin receptor, and transferrin saturation) and hepatic iron and tissue architecture by liver biopsy. Magnetic resonance imaging (MRI) was performed and the allelic variations c.845G>A (p.C282Y) and c.187C>G (p.H63D) were identified in the HFE gene. Five-micrometer tissue sections were stained with hematoxylin and eosin and Perls Prussian blue to detect the presence of iron.

Genomic DNA was extracted from peripheral blood and polymerase chain reaction (PCR) was performed using specific oligonucleotides to amplify the entire coding regions, splice junctions and 5’ and 3’ untranslated regions (UTR) of the HFE, hemojuvelin (HJV), hepcidin (HAMP), TFR2 and ferroportin (SLC40A1) genes. PCR products were sequenced bidirectionally using the ABI Prism 3130x1 DNA sequencer and compared with the reference sequence (GenBank Accession numbers: NG_008720.1, NG_011568.1, NG_011563.1, NM_003227.3, and NG_009027.1, respectively, for the aforementioned genes).

Written informed consent was obtained according to local guidelines for the genetic study, liver biopsy, and iron challenge. The study protocol was in accordance with the ethical guidelines of the Declaration of Helsinki (2008).

The patient’s iron parameters were as follows: serum ferritin, 1,070 µg/L (normal range, 26-370 µg/L); serum iron, 160 µg/dL (normal range, 59-158 µg/dL); transferrin saturation, 48% (normal range, 20-45%); and soluble transferrin receptor, 0.90 mg/L (normal range, 0.83-1.76 mg/L). Hepatic MRI showed diffuse intensity abnormalities in the parenchyma associated with an increase in iron; hepatic iron content was estimated to be 160 µmol/g (normal values < 36 µmol/g). Ultrasound-guided percutaneous liver biopsy was performed. Histopathologic evaluation showed normal lobular architecture, narrow portal spaces without inflammation or fibrosis, and no ductal or vascular lesions. The lobe was composed of hepatocytes with regular morphology and low-intensity hepatocellular hemosiderin deposits with the typical hemochromatosis pattern, namely, iron deposits located almost exclusively in the parenchymal cells in periporal areas with a granular pattern and a pericanicular distribution (Fig. 1).

The patient underwent phlebotomy, and, after 20 phlebotomies fortnightly, more than 5 g of iron were removed and these γGT and ferritin concentrations returned to normal values. The patient currently undergoes maintenance phlebotomy twice per year.

The molecular study of the genes involved in iron metabolism showed the following heterozygous allelic variants: c.187C>G (p.H63D) in the HFE gene and c.840C>G (p.F280L) in the TFR2 gene (Fig. 2).

DISCUSSION

Hemochromatosis is a clinically and genetically heterogeneous disease caused by an inappropriate increase in in-
testicular absorption of iron. Although most patients are homozygous for the C282Y mutation in the HFE gene, genetic and environmental factors can modify the hemochromatosis phenotype. Patients with double heterozygous c.845G>A/c.187C>G (p.C282Y/p.H63D) mutations in the HFE gene showed a common phenotype of hemochromatosis, and a wide variety of clinical manifestations have been reported in patients with monogenically and digenically inherited mutations in other genes such as SLC40A1, TFR2, HIV, and HAMP (9,13).

Type 3 hemochromatosis is an unusual recessive disease caused by mutations in the TFR2 gene. This alteration has been described in a small group of Italo-French families, as well as in Japanese, Portuguese, and Scottish families, and it causes clinical hemochromatosis that is indistinguishable from type 1 hemochromatosis. However, diagnosis of type 3 hemochromatosis is made at a significantly younger age than type 1 hemochromatosis (12,14). Type 3 hemochromatosis is an intermediate syndrome between the typical adult type 1 hemochromatosis and two juvenile forms, type 2a and type 2b, which are caused by mutations in the HIV and HAMP genes; therefore, mutations in the TFR2 gene should be borne in mind in younger patients with adult-onset hemochromatosis (10,14,15).

The low level of circulating hepcidin, the hormone that controls intestinal iron absorption, is a common feature of type 1, 2, and 3 hemochromatosis (16). HFE, TFR2, and HIV are considered modulators of synthesis/activity of hepcidin, which in turn regulates the release of iron into blood from duodenal cells and macrophages, thus maintaining appropriate iron levels to satisfy the body’s needs (17,18). The combination of mutations in the HFE and TFR2 genes and mutations in HAMP and HIV genes lead to more severe forms of hemochromatosis (due to a dysregulation in intestinal iron absorption), an increase of iron influx into the blood, and iron storage in tissues (10).

There are reports of type 3 hemochromatosis in patients with a heterozygous mutation in the TFR2 gene, and with or without mutations in the HFE gene. Mendes et al. (19) reported a case with heterozygous mutations, namely, c.187C>G in the HFE gene and c.840C>G in the TFR2 gene, and alcoholic habits, which are responsible for iron overload disease. This double heterozygosity was also observed in our patient, without toxic habits, who presented an iron overload phenotype confirmed by MRI and liver biopsy. The patient’s condition was mild and responded to phlebotomy. Although we did not find relevant mutations in other genes studied in the patient (HJV, HAMP and SLC40A1), other environmental factors and modifiers genes might be involved in the hemochromatotic patients iron storage.

Early identification of patients with iron overload is very important for prognosis. Therefore, liver biopsy, measurements of the hepatic iron index, and a genetic study are necessary to clarify the diagnosis, classification, and treatment of patients with hemochromatosis.

REFERENCES


