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HFE GENE MUTATIONS IN PATIENTS WITH ALTERED IRON METABOLISM IN ARGENTINA

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Dedicated to the memory of our dear friend and colleague Dr. Susana Afonso

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Abstract – Hereditary Hemochromatosis (HH) is an iron overload syndrome caused by increased duodenal iron absorption, which leads to excesive iron deposition in parenchymal cells of the liver and mayor organs, causing cirrhosis, diabetes, cardiac failure, endocrine complications and arthritis. There are 6 types of HH related to mutations in the genes that encode proteins of iron metabolism. HH Type I is inherited as an autosomal recessive trait of mutations in *HFE* gene. We investigate the prevalence of C282Y, H63D and S65C mutations in 95 individuals (77 males, 18 females) bearing iron metabolism alterations to establish an early diagnosis of HH. Among this population, 58% carried mutations in the HFE gene (45 males, 10 females). H63D mutation was found in 32.6% of the subjects (29.5% in heterozygocity, 3.15% in homozygocity). S65C mutation was only detected in the heterozygous form (5.3% of the patients), 2 of them carried also H63D mutation. C282Y in heterozygocity was found in 15.8% of the individuals; but only 4.15% carried this mutation in homozygocity. Our findings are in agreement with the prevalence of the Mediterranean origin of most of our patients, where C282Y mutation is not as common as H63D mutation.

Key words: Hereditary Hemochromatosis, *HFE* gene, mutations, iron metabolism

INTRODUCTION

Hereditary hemochromatosis (HH) is one of the most common inherited metabolic diseases in Caucasian populations (4). The main cause of this disorder is the progressive overload of iron in several tissues and organs; such overload leads to a severe cellular damage in liver, pancreas and heart, where the iron concentration is higher (2,14). These complications usually manifest during adulthood (40-60 years), and patients show hepatic dysfunction, diabetes, hipogonadism, infertility, arthritis and cardiomiopathies. The frequency of HH is 5 to 10 times higher in men than in women (3, 22).

At present there are 6 types of HH, each one linked to a specific gene, and therefore, to a different protein involved in iron metabolism. In 1996, Feder et al (7), identified a candidate gene for HH in chromosome 6p that codifies HFE, protein involved in iron absorption, detecting two mutations, C282Y and H63D, associated to HH type 1. Later another mutation, S65C, was implicated in a mild form of HH (15). Individuals who are homozygous for the C282Y

Abbreviations: **HBV**, hepatitis B virus; **HCV**, hepatitis C virus; **HH**, hereditary hemochromatosis; **PCT**, porphyria cutanea tarda

mutation and compound heterozygous C282Y/H63D are predisposed to develop the disease, but it is currently impossible to predict its clinical course. C282Y mutation is more frequent in northern European population while H63D is more frequent in Mediterranean population. Other genotypes such as C282Y/S65C and H63D/S65C, lead to mild clinical expressions (18).

There also exists a severe type of juvenile hemochromatosis inherited as an autosomal recessive trait in which the clinical symptoms appear before 30 years old associated to mutations in hemojuvelin (*HJV*) and hepcidine (*HAMP*) genes , known as type 2A and 2B, respectively (17, 20, 21).

Hemochromatosis type 3 is due to mutations in *TFR2* gene, which codifies for receptor 2 of transferrin and it is an autosomal recessive condition (5).

Hemochromatosis due to mutations in *SLC40A1* gene (16) that codify for ferroportin is called HH type 4 and it is inherited as an autosomal dominant condition. This HH is also known as ferroportin disease because the distribution of hepatic iron overload is different to classical HH (19).

There is another type of HH due to mutations in H ferritin gene, only found in a japanesse family (10). In addition other types of HH due to mutations in more than one gene involved in iron metabolism have been described (1, 9, 11, 13).

The aim of this work was to investigate the prevalence of C282Y, H63D and S65C mutations in the HFE gene among subjects bearing iron metabolism alterations.

MATERIALS AND METHODS

Patients

Ninety five patients (77 males, 18 females) with alterations in iron metabolism were derived from several Health Services between 1999 and 2005 (Table 1). All patients were from different areas of Argentina and most of them were of Spanish or Italian ancestry. All individuals gave informed consent prior to inclusion in the study. The study was conducted in accordance with the Declaration of Helsinki principles and was approved by the Ethic Committee of the CIPYP.

DNA extraction

Peripheral blood was collected with EDTA as anticoagulant, and genomic DNA was extracted from the whole blood (200 µl) using the InstaGene Isolation kit (BioRad) following the manufacturer's instructions.

Table 1. Patients with altered parameters in iron metabolism

		MALE	FEMALE
Altered parameters	Serum iron	12.6%	3.2%
	Ferritin	32.6%	6.3%
	Transferrin	5.3%	1.0%
	Transferrin Saturation	15.8%	6.3%
Iron deposits in liver (Biopsy +)		5.3%	1.0%

The values of iron status ranged from: Serum iron (µg/dl) = 90-120 (normal value: 65-170); Ferritin (ng/ml)= 359-6,667 (normal value: 15-300); Transferrin (mg/dl)= 200-400 (normal value: 145-396); Transferrin saturation (%)= 47-91 (normal value: 29-45)

Analysis of the C282Y, H63D and S65C mutations in HFE gene

Exons 2 and 4 of HFE gene were amplified using the primers 5'-CAC ACT CTC TGC ACT ACC TCT TCA-3' and 5' CTT GCT GTG GTT GTG ATT TTC CAT A-3' for exon 2, and the primers 5'-CCT CCT TTG GTG AAG GTG ACA CAT-3' and 5'-AGA TCA CAA TGA GGG GCT GAT CCA-3' for exon 4. In both cases the amplification mixtures consisted of 200 ng of DNA, 0.2 mM of each dNTP, 25 pmol of each primer, 1.5 mM $MgCl_2$, and 1.5 units of Taq DNA polymerase (Gibco-BRL) in 1x buffer supplied by Gibco-BRL in a final volume of 50 µl. After an initial denaturation at 95 °C for 3 minutes, 30 cycles of 1 minute at 95 °C, 1 minute at 60 °C, and 30 seconds at 72 °C were performed. These mutations were detected by cycle sequencing employing the PCR primers.

RESULTS

HFE genotypes

As it is shown in Table 2, in our series, 58% (45 males, 10 females) carried at least one of the three HFE mutations studied. Only 4 patients were homozygous for C282Y mutation while 15 subjects were heterozygous. H63D mutation was observed in 3 patients in homozygosis and in 26 subjects in heterozygosis. S65C mutation was observed only in the heterozygous form in 3 patients and 2 patients were compound heterozygous for H63D and S65C mutations.

Although among the cohort studied there was a relationship of about 4:1 male: female, the percentage of individuals positive for at least one of the HFE mutations studied was similar in men and women (55.5% and 58.4% respectively) (Table 2).

Onset age and ancestry

The onset age of the subjects analyzed ranged between 11 and 75 years, however 40% of individuals were between 41 and 70 years (Fig. 1). In this range there are 4 patients

Table 2. *HFE* genotypes

	C282Y/C282Y	C282Y/+	H63D/H63D	H63D/+	H63D/S65C	S65C/+	+/+
Men (n=77)	3 (3.2%)	13 (13.7%)	3 (3.2%)	23 (24.2%)	2 (2.1%)	1 (1.0%)	32 (33.7%)
Women (n=18)	1 (1.0%)	2 (2.1%)	_	5 (5.3%)	_	2 (2.1%)	8 (8.4%)
Total (n=95)	4 (4.2%)	15 (15.8%)	3 (3.2%)	28 (29.5%)	2 (2.1%)	3 (3.1%)	40 (42.1%)

Individuals with at least one of the mutations studied: Men: 45/77 (58.4%) and Women: 10/18 (55.6%)

homozygous for C282Y mutation. Eleven subjects who were younger than 41 years old had not any mutation in heterozygosis (3 C282Y, 8 H63D). Moreover most of the patients (73.7%) had Spanish and Italian ancestors (Figure 2).

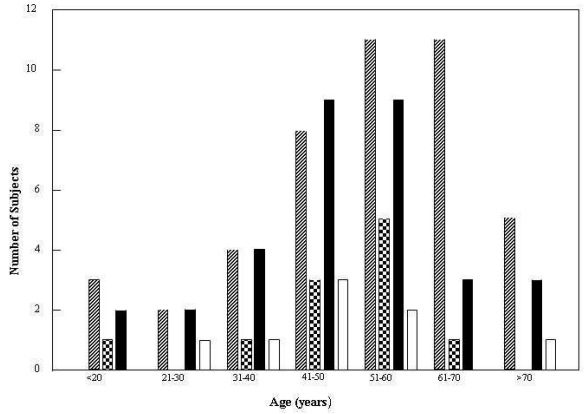

Figure 1. Onset Age

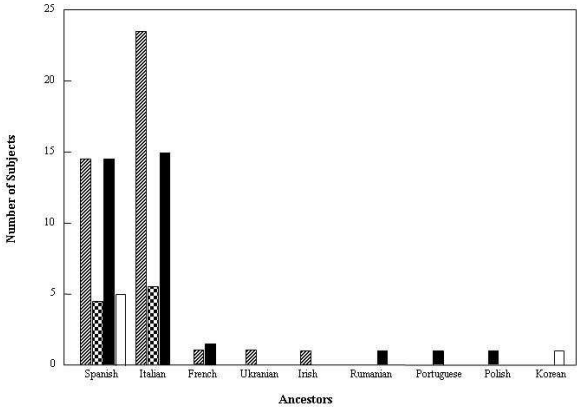

Figure 2. Ancestry
To calculate the frequency of the different ancestries we have considered the contribution of each parent as 0.5.

Associated pathologies

Forty two percent of the patients that were mutation positive presented another disorder: hepatitis (8/55, 15%), cirrhosis (11/55, 20%), Thalassemia minor (1/55, 2 %) and Porphyria Cutanea Tarda (PCT, 3/55, 5%) (Figure 3).

Each of the 3 PCT patients carried one of the C282Y, H63D and S65C mutations in the heterozygous form**.** From the patients with cirrhosis, 3 were homozygous for C282Y, 2 were homozygous and 5 heterozygous for H63D respectively and 1 patient carried S65C mutation in heterozygous form. In patients suffering hepatitis, 2 carried C282Y, 5 carried H63D and 1 S65C mutations, all in heterozygosis. The patient with Thalassemia was heterozygous for H63D mutation.

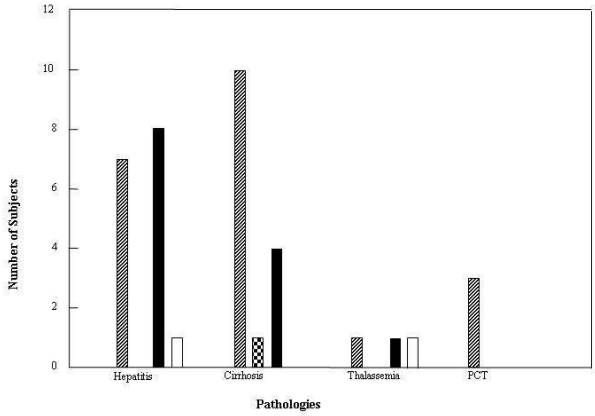

Figure 3. Associated pathologies
Patients with hepatitis: HCV (6), HBV (1) and HCV/HBV (1) Cirrhosis caused by: alcohol (4), HCV (2), unknown (5)

DISCUSSION

HFE mutations

From the total population studied, 58% (45 males, 10 females) carried one of the three mutations studied but only 7 patients were homozygous (4 for C282Y and 3 for H63D mutations) and 2 compound heterozygous H63D/S65C. H63D was the most frequent mutation found in at least one allele (33/55, 60%). Considering that 73.7% of our patients have Italian or Spanish ancestors our results are in accordance with the Mediterranean origin where H63D is more frequent than C282Y mutation (8,23). Similar results were previously found in PCT patients from Argentina (12).

All the patients analyzed were derived to our Centre for genetic study due to their iron metabolism alterations, however 42% of these subjects did not carry any of the three mutations studied in the HFE gene. These patients would have some *HFE* mutation in the region not yet analyzed and/or in other gene involved in iron metabolism.

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AB, MVR, VEP and EG are Superior, Independent and Associate Researchers in the Career of Scientific Researcher of CONICET. SG was Principal Member of the Assistant Research Career of CONICET. AM is chief of the Hepatology Service at the Hospital of Gastroenterology.

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