Ann Hematol (2007) 86:17–21
DOI 10.1007/s00277-006-0179-0

ORIGINAL ARTICLE

Frequency of the hemochromatosis gene (*HFE*) 282C→Y, 63H→D, and 65S→C mutations in a general Mediterranean population from Tarragona, Spain

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Received: 12 June 2006 / Accepted: 12 July 2006 / Published online: 30 September 2006

Abstract Three mutations have recently been detected in the hereditary hemochromatosis *HFE* gene (282C→Y, 63H→D, and 65S→C). To determine their prevalence in a northeastern Spanish Mediterranean population, we studied 812 subjects between 18 and 75 years of age, randomly selected from the electoral roll of three villages. There were no homozygotes for the 282C→Y or S65D mutations in this sample. For the 63H→D mutation, 4.8% were homozygotes; 4.3, 32.3, and 2% were heterozygotes for the 282C→Y, 63H→D, and 65S→C mutations, respectively. The prevalence of compound heterozygotes was 2% for 282C→Y/63H→D and 0.6% for 63H→D /65S→C. We found no significant differences between men and women. In conclusion, 46% of this Mediterranean population of Spain are carriers of at least one of the three mutations that can increase iron absorption.

Keywords Hemochromatosis · *HFE* gene · Epidemiology · Genetics · 282C→Y · 63H→D · 65S→C mutations prevalence

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Introduction

Hereditary hemochromatosis (HH) is a genetic disorder of iron metabolism caused by increased intestinal iron absorption leading to severe iron overload [6, 34]. Three mutations, which have all been associated to varying extents with iron overload, have been detected in the *HFE* gene (282C→Y, 63H→D, and 65S→C). The first, 282C→Y, involves a guanine-to-adenine substitution at nucleotide 845 of exon 4, which causes a cysteine-to-tyrosine substitution during protein synthesis at position 282. This mutation destroys a disulfide bond between the transmembrane protein and the ß2-M, causing an excessive increase in cellular iron uptake [17, 38, 39]. In a recent review, Hanson et al. [21] observed that between 80 and 90% of people with HH are homozygotes for this mutation, while only 3.6% are heterozygotes. European studies have reported that the prevalence of the homozygous 282C→Y mutation is <1.5% and that of the heterozygous mutation is <29%, being highest in northern Europe [21, 28].

In the second variant, 63H→D, on exon 2 of the *HFE* gene, cytosine is substituted by guanine at nucleotide 187, causing a histidine-to-aspartic acid substitution during protein synthesis at position 63. This mutation alters the tertiary structure of the HFE protein, which also affects the regulation of iron absorption; although, in this case, the phenotypic expression is much less than that observed with the homozygous 282C→Y mutation [17, 29]. Homozygotes and heterozygotes for the 63H→D polymorphism constitute <8 and <38.8%, respectively, of the populations studied so far in Europe [15, 21]. This polymorphism is most prevalent in southern Europe and the prevalence of the 282C→Y/63H→D compound is <4% [21]. Approximately 5% of the subjects with HH are compound

heterozygotes (282C→Y/63H→D), 1.5% are homozygous for 63H→D, and 5.2% are heterozygous for 63H→D [21].

The third variant, S65D, has recently been associated with HH. It involves the replacement of adenine by thymidine at nucleotide position 193, with the subsequent substitution of serine by cysteine at position 65 of the protein (65S→C) [4, 22, 32]. Although this mutation has been studied much less than the previous ones, it has also been associated with a moderate or slight iron overload [22] (Table 1).

The aim of this study was to determine the prevalence of the three main variants in the *HFE* gene (282C→Y, 63H→D, and 65S→C) in northeast Mediterranean Spain.

Material and methods

The sample was randomly selected from the electoral rolls of three municipalities in Tarragona and was stratified for age and sex in the source population. Inclusion criteria were Caucasian origin, capability of understanding the nature of the study, absence of serious diseases that prevented participation, and absence of pregnancy or having given birth in the last 6 months.

The study was approved by the Ethics Committee of the Sant Joan University Hospital and the Jordi Gol Gorina Foundation. Letters of invitation to participate in the study were sent to a total of 1,325 people. Willingness to participate was confirmed by phone call. If someone refused or could not be contacted following three different phone calls (at different times on different days), they were replaced by the next available candidate of the same age and sex on the list. A total of 812 healthy subjects (423 women and 389 men) were studied. Signed informed consent in accordance with the Declaration of Helsinki was obtained. They were all between 18 and 75 years old.

Determination of genetic variants

DNA was extracted from peripheral blood leukocytes, obtained from blood collected in EDTA-K_3 tubes [24] with a DNA purification kit (Puregene®, Gentra Systems, Minneapolis, MN, USA). *HFE* genotypes were determined as described previously: 282C→Y (845 G→A mutation) [24], 63H→D (187 C→G mutation) [24], and 65S→C (193 A→T mutation) [32], using the polymerase chain reaction (PCR)-restriction fragment length polymorphism technique. Relevant exons were amplified in a Perkin Elmer (Wellesley, MA, USA) DNA thermal cycler 2400 using the following amplification primers: 5′-TGGCAAGGGTAAACAGATCC-3′ and 5′-CTCAGGCACTCCTCTCAACA-3′ for 282C→Y and 5′-ACATGGTTAAGGCCTGTTGC-3′

Table 1 *HFE* genotype frequencies

Study population	No. of subjects (studies with more than 100 subjects)	Randomly selected general population	C282Y/C282Y %	H63D/H63D %	S65/S65 %	C282Y/X %	H63D/X %	S65C/X %	C282Y/H63D %	C282Y/S65C %	H63D/S65C %
Donosti (Spain) [15]	116	No	0	7.8	0	6.9	38.8	4.3	3.4	0	1.7
Cantabria (Spain) [16]	213	No	0	ND	ND	9	ND	ND	ND	ND	ND
Madrid (Spain) [3]	125	No	0	1.6	ND	4	28.8	ND	ND	ND	ND
Mallorca (Spain) [19]	210	No	0	ND	ND	2.8	ND	ND	ND	ND	ND
Mallorca (Spain) [20]	192	No	0	1.6	ND	4.7	34.9	ND	1	ND	ND
Ibiza	169	No	0	4.7	ND	6.5	31.4	ND	1.8	ND	ND
Menorca	167	No	0	3.6	ND	1.2	27.5	ND	0	ND	ND
Mallorca	137 (Jews)	No	0	5.1	ND	0	43.1	ND	0	ND	ND
Barcelona (Spain) [35]	420	No	0.2	4.1	ND	4.5	33.8	ND	1.4	ND	ND
Barcelona (Spain) [36]	5370	No	0.1	4.6	ND	4.6	31	ND	1.4	ND	ND
Tarragona (Spain) (this study)	812	Yes	0	4.8	0	4.3	32.3	2	2	0	0.6

ND not determined

and 5′-GCCACATCTGGCTTGAAATT-3′ for 63H→D and 65S→C, respectively. Amplifications were performed on 20 ng genomic DNA with the following conditions: 1 mM $MgCl_2$ for amplification of the 282C→Y region or 4 mM $MgCl_2$ for amplification of the 63H→D or 65S→C regions, 0.2 mM deoxyribonucleotide triphosphates, 0.5 mM each primer, 0.025 U/μL Ampli Taq® Gold with geneAmp (5 U/μL. Applied Biosystems, Branchburg, NJ, USA) in the buffer supplied by the manufacturer. An initial denaturation step for 5 min at 94°C was followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min. A final 10-min extension was performed at 72°C. The 282C→Y, 63H→D, and 65S→C mutations were screened using enzymatic digestion of PCR products encompassing the mutation sites. As the 282C→Y mutation creates a new *Rsa*I restriction site, the 387-bp PCR product digested with *Rsa*I shows two fragments of 247 and 140 bp in normal DNA, while three fragments of 247, 111, and 29 bp were generated in mutated DNA. The 63H→D mutation destroys a *Mbo*I site in the 208-bp PCR product, while normal DNA is cut into two fragments of 138 and 70 bp. The 65S→C mutation destroys a *Hinf*I site in the 208-bp PCR product, while normal DNA is cut into two fragments of 147 and 61 bp. Digestion products were separated on 12% polyacrylamide gels (29:1.25 Acryl-bis) and visualized by staining with silver nitrate. Genotyping results were stored in an Access database and analyzed with the statistical package SPSS for Windows (version 11.5). To compare proportions, the chi-square statistical test was used with a level of significance of $p<0.01$.

Results

The mean (95% confidence interval) age of the population studied was 42.4 (41.4, 43.5) years. The prevalence of the ten genotypes of the 282C→Y, 63H→D, and 65S→C mutations according to sex are shown in Table 2. There were no homozygotes for the 282C→Y or 65S→C mutations in our population. The prevalence of the 63H→D homozygotes was 4.8%. We observed that the most common heterozygous genotype was 63H→D/wild type (WT), which was found in 32.3% of the population, followed by 282C→Y/WT (4.3%) and 65S→C/WT (2%). The compound heterozygous genotype 282C→Y/63H→D was found in 2% of the population and was more common in men than in women (2.8 and 1.2%, respectively, $p<0.001$). The compound 63H→D/65S→C had a prevalence of 0.6%. Differences between men and women were not significant. The allelic frequencies of the 282C→Y, 63H→D, and 65S→C mutations were 3.1% (51/1,624), 22.3% (362/1,624), and 1.2% (20/1,624), respectively. The *HFE* genotype distribution was in Hardy–Weinberg equilibrium ($X^2=4.5$, df=9, $p=0.87$).

Table 2 Prevalence of the *HFE* gene genotypes and their allelic frequencies in the Mediterranean population from the Northeast of Spain

HFE genotype	Women *n*=423	Men *n*=389	Total *n*=812
C282Y/C282Y	0	0	0
C282Y/wild type	4.7 (2.9 to 7.2)	3.9 (2.2 to 6.3)	4.3 (3.0 to 5.9)
C282Y/S65C	0	0	0
C282Y/H63D	1.2 (0.4 to 2.7)	2.8 (1.4 to 5.0)	2.0 (1.1 to 3.2)
H63H/H63D	5.9 (3.9 to 8.6)	3.6 (2.0 to 6.0)	4.8 (3.4 to 6.5)
H63D/wild type	32.9 (28.4 to 37.3)	31.7 (27.0 to 36.2)	32.3 (29.1 to 35.5)
H63D/S65C	0.7 (0.1 to 2.1)	0.5 (0.1 to 1.8)	0.6 (0.2 to 1.4)
S65C/S65C	0	0	0
S65C/wild type	1.7 (0.7 to 3.4)	2.3 (1.1 to 4.3)	2.0 (1.1 to 3.2)
wild type/wild type	53.0 (48.2 to 57.7)	55.4 (50.3 to 60.2)	54.0 (50.6 to 57.5)
Allelic frequencies			
C282Y	2.9 (1.9 to 4.1)	3.3 (2.2 to 4.9)	3.1 (2.3 to 4.1)
H63D	23.4 (20.6 to 26.2)	20.9 (18.2 to 23.9)	22.3 (20.2 to 24.3)
S65C	2.4 (1.4 to 3.4)	1.4 (0.7 to 2.5)	1.2 (0.8 to 2)

Values are prevalence (lower and upper limits of the 95% confidence interval of the prevalence) in percentage.

Discussion

We analyzed the prevalence of the three main mutations of the HH (*HFE*) gene in 812 randomly selected, apparently healthy subjects, representative of the population of Tarragona in Mediterranean Spain. Like previous studies, in primary care laboratories, in this country [19, 20] we found no homozygotes for the 282C→Y mutation. A greater prevalence of 282C→Y homozygotes has been reported previously, but only in studies of blood donors [15, 16]. This may be because subjects with iron deficiency anemia, who are less likely to have genetic disorders that increase iron absorption, cannot give blood [35, 36].

The *HFE* 282C→Y mutation seems to have originated from a single ancestor in the north of Europe some 6,000 years ago [25, 29]. This genetic defect, which was no serious obstacle to reproduction and may even have had some advantages such as resistance to iron deficiency and to certain infectious diseases, may have been spread by the migration of this population. Currently, the homozygous 282C→Y mutation is found in approximately five out of every 1,000 descendents from northern Europe. Approximately 80–90% of individuals with clinical HH are

homozygotes for 282C→Y [21, 24], indicating the important genetic origin of the disease. However, while the biochemical penetrance of the homozygous 282C→Y mutation occurred in about 75% of adult men, it only occurred in 40% of adult women in a large study [8]. Estimates of clinical penetrance of the 282C→Y mutation, expressed as the presence of disease attributable to the genetic abnormality (strict phenotypic expression), vary from less than 1% [9] to as high as 25% [1, 40].

The combined penetrance for the three mutations (defined by transferrin saturation >45% and serum ferritin >200 and 300 μg/L in women and men, respectively) was 1.6% in our study (Arija et al., paper in preparation). The prevalence of the 282C→Y mutation follows a north–south gradient in Europe [21, 28, 30], except in the northwest of the Iberian Peninsula, where it is similar to northern Europe [14–16, 37]. This may be due to the northern origin of the population in this region [29].

Studies of Caucasian populations worldwide have reported the prevalence of the 282C→Y mutation to be similar to that in northern Europe [7, 11, 12, 33]. However, it is considerably lower in other ethnic groups, such as Asians and blacks [2, 7]. Although iron overload affects 10% of the black population, studies carried out in South Africa conclude that its cause is not associated with mutations in the *HFE* gene [18, 26]. Other factors must account for HH in the small percentage of individuals in which it is not due to mutations in the *HFE* gene. Possibilities are rare genetic disorders involving mutations in the ferroportin, hepcidin, hemojuvelin, and transferrin receptor-2 [10] and epigenetic factors such as diet, alcohol intake, and source of bioavailable iron in foods or supplements [5, 18].

In our study, we found one of the highest prevalences of the 63H→D mutation in Europe. Similar results have been reported previously for blood donors in the northeast of Spain [35, 36] and in Caucasians in general. The geographical distribution of the 63H→D variant in Europe is the reverse of that observed for 282C→Y, following a south–north gradient [21, 28]. High prevalences of individuals with the 63H→D mutation are found in Spain [21]. This is especially so in descendants of Majorcan Jews (the Chuetas), whose genes have remained relatively homogenous over time as a result of endogamy [20].

There were no 65S→C homozygotes in our study and heterozygotes made up only 2% of our population. These results are similar to previous reports from the Faroe Islands [31] and Sweden [22] but different from a study from northeast Spain [15], which found twice our prevalence of heterozygotes but, like us, no homozygotes.

Compound heterozygotes (282C→Y/63H→D), which occurred in 2% of our population, are less prevalent than in northeast Europe (between 2.6 and 3.7%) [13, 21, 23, 27] and in the north of Portugal (3%) [14], but similar to that observed in the USA [2, 7, 11]. Compound heterozygotes with 65S→C are less frequent. In line with a previous report from the Faroe Islands [31], we found no 282C→Y/65S→C and only 0.6% of 63H→D/65S→C compound heterozygotes.

We conclude that 46% of our representative sample of our population carry at least one of the three *HFE* mutations studied, with 63H→D being the most common. They may be susceptible to iron overload and the associated health risks.

Acknowledgments This study was supported by three grants from the Instituto de Salud Carlos III, Fondo de Investigación Sanitaria, Ministerio de Sanidad y Consumo, Madrid, Spain (PI021131); Red de Centros RCMN, Madrid, Spain (C03/08); and Red de Grupos, Madrid, Spain (G03/140), and by NIH National Center on Minority Health and Health Disparities grant IP60MD000222-03 (for Dr. Viteri).

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