

## Short Report

# Prevalence of HFE C282Y and H63D in Jewish populations and clinical implications of H63D homozygosity

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The frequencies of C282Y and H63D mutations of the *HFE* gene vary between different populations. A previous study showed an unexpectedly high H63D frequency in Chuetas (a population of Jewish descent). The present study addressed the question of the distribution of these mutations in Jewish populations from different origins and studied the possible causes of the high H63D frequency in Chuetas. Moreover, to improve the understanding of the controversial relationship between H63D homozygosity and iron overload, a group of patients with altered iron metabolism were studied. The high frequency of H63D mutation in Chuetas is not due to a high prevalence of this mutation in Sephardic Jews. Jewish populations have low C282Y and moderate H63D frequencies, suggesting slight gene flow from their surrounding populations. In accordance with historical and demographic data, genetic drift is the most probable cause for the singular H63D frequency in Chuetas. Clinically, this study of H63D homozygotes supports the conclusion that this genotype must be taken into account, because it confers an increased risk of iron overload and therefore genetic susceptibility to developing hereditary hemochromatosis or to aggravating other diseases.

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## Introduction

Hereditary hemochromatosis (HH) is an autosomal recessive trait, frequently found in Caucasian populations, in which the excess absorption of iron from the diet leads to severe organ damage. If the disorder is diagnosed before organ damage has occurred, reduction of body iron stores to normal will prevent organ disease and result in a normal life expectancy (1).

In 1996, two mutations in the *HFE* gene (C282Y and H63D) were identified as being responsible for the disease (2). The C282Y mutation has been found to be present in 64–100% of patients with HH (2, 3). The role of the second mutation (H63D) in HH pathogenesis remains controversial and unclear. Due to its high frequency in the general population (15–20%) (3, 4), H63D may represent a common polymorphism, although the

implication of the H63D mutation as a deleterious event on the *HFE* gene has been established by clinical and molecular studies. Firstly, an excess of H63D alleles was clearly demonstrated among HH patients (2, 5, 6), and further studies have pointed out that the H63D homozygous status could be a pre-disposing factor in other diseases with iron overload (7, 8). Variability in phenotypic expression is observed in many monogenic diseases, and although phenotypic variability for recessive conditions is usually not as striking as in dominant ones, HFE shows a marked phenotypic heterogeneity, as happens in other recessive traits, i.e. cystic fibrosis (9). This situation could explain the fact that the clinical expression of iron overload in H63D homozygotes is still a matter of debate and represents a recurring question for physicians (10).

Frequencies of HFE C282Y and H63D mutations have been analyzed worldwide (reviewed in 3, 11), and an unequal distribution profile of mutations has been found. The C282Y mutation is most abundant in North European populations and in those of North European descent (allele frequencies of 5–10%). Other European populations studied ranged from null to 5%. It has been proposed that the mutation C282Y first occurred on one Celtic (12, 13) or Viking chromosome (14). H63D has a much broader distribution, with high frequencies throughout Europe (10–30%), especially in the Mediterranean area, and moderate frequencies in North Africa, the Middle East, and parts of Asia (8–10%). The highest H63D frequencies have been detected in Spain, and this fact prompted some authors to hypothesize that the H63D mutation emerged in the Iberian peninsula (15).

The genetic structure of the present-day Jewish populations is the outcome of a common ancestral gene pool and the admixture with people the Jews lived among (reviewed in 16). Jews can be traced back to populations occupying a small geographic area, in the land of Israel, several thousand years ago. Nowadays, modern Jews constitute one ethnic group split into several groups, the most numerous of which are the Ashkenazim, who have resided in North-Eastern Europe for centuries; the Sephardim ('Spanish' in Hebrew), who, after their expulsion from Spain in 1492, lived in other Mediterranean countries, especially Turkey; the North African Jews, where evidence exists of Jewish communities as early as the first centuries AD that were augmented as a consequence of the Spanish expulsion; and the Oriental Jews, who have lived in Middle East countries throughout their history (17).

One population with an ancient Sephardic origin – the so-called Chuetas – resides in Majorca (Balearic Islands, Spain). Chuetas was the name given to the descendants of the accused Jews in the last Spanish Inquisition process (17th century) on the Island of Majorca. They were secluded from their immediate neighbors and have shown a strong endogamic behavior from this period until now (18). Genetic studies have indicated the singularity of the Chuetas in having an evident Jewish origin but also a substantial Spanish admixture (19–21, among others).

Guix et al. (22) studied the distribution of the HFE C282Y and H63D mutations in Chuetas. H63D mutation was the only one observed, and its allelic frequency has been one of the highest frequencies (26.6%) described to date. There are no data in the literature about Sephardic Jews,

but the frequencies found in Ashkenazi Jews for the H63D mutation (approximately 9%) (23, 24) differ significantly from that found in Chuetas. The genetic study of HFE mutations in non-Ashkenazi Jews would be of interest to explain the high H63D frequency detected in Chuetas.

Therefore, the aims of this study were (a) to determine the prevalence of the C282Y and H63D mutations in a sample of non-Ashkenazi Jews from different origins (Sephardic, North African, and Oriental Jews); (b) to study the possible causes of the high H63D frequency in Chuetas; and (c) to describe the clinical expression of iron overload associated with H63D homozygosity.

## Materials and methods

The genetic study of the C282Y and H63D mutations was carried out in 255 unrelated Jewish individuals (146 men and 109 women). DNA samples were obtained from the collection of The National Laboratory for the Genetics of Israeli Populations at Tel Aviv University, Israel. Following classical criteria, they were categorized into three groups: Oriental (26 Iranian and 27 Iraqi individuals), North African (29 Moroccan, 13 Tunisian, and 13 Libyan individuals), and Sephardic (76 Turkish and 71 Bulgarian individuals). Moreover, a set of data from the literature was used, which included the preceding studies of HFE mutations in populations of Jewish ancestry (Ashkenazi and Chuetas).

The C282Y and H63D mutations in the *HFE* gene were assessed on genomic DNA, using standard polymerase chain reaction and enzyme digestion with the primers described by Feder et al. (2), following the method of Lynas (25).

For the estimation of the genotype frequencies, taking into account that intragenic recombination is quite rare, three possible alleles in the *HFE* gene were considered: wild type (wt), C282Y, and H63D, as indicated by Fairbanks (26). Allele frequencies, Hardy–Weinberg equilibrium and genetic differentiation between populations ( $F_{ST}$ ) were calculated by means of the BIOSYS-1 package (27). To test genetic admixture, we used the formula indicated by Cavalli-Sforza and Bodmer (28).

The study of patients was carried out on 337 consecutive samples received in the Clinical Chemistry laboratory of Son Dureta Hospital (Majorca) for HFE genotyping. The genetic study had been solicited in patients with altered iron metabolism. All subjects were carefully

screened for secondary causes of iron overload. A routine chemistry panel and complete blood count were obtained for all patients. As appropriate, specific serological studies (Hepatitis B and C viruses, and HIV), plasma and/or urinary porphyrin levels, and tumoral markers (alpha-fetoprotein) were also undertaken. Liver biopsy was only available in selected cases.

## Results

Genotype and allele frequencies for the three groups of Jews studied are summarized in Tables 1 and 2. No subject was homozygous for the C282Y mutation, two were heterozygous and two were compound C282Y/H63D heterozygotes, that is, four in 510 chromosomes studied carried the C282Y mutation, giving a gene frequency of 0.78%. Thus, the expected prevalence of C282Y homozygotes was one in 86,505 in Sephardic Jews; one in 11,317 in Oriental Jews, and one in 3,019 in North African Jews. In the H63D mutation, homozygotes and heterozygotes were found in all three Jewish populations studied, with allelic frequencies ranging from 9.43 to 13.26%, which predicts that between one in 112 and one in 57 subjects will be homozygotes for the H63D mutation. The results agreed with the Hardy-Weinberg equilibrium, and no significant differences were found in the frequencies of both mutations between men and women in any of the populations studied.

The genetic differentiation (measured by means of the  $F_{ST}$ ) between the three non-Ashkenazi Jewish populations studied was not significant. Therefore, these populations can be considered homogeneous for the C282Y and H63D mutations. The comparison of these non-Ashkenazi populations with Ashkenazi Jews was also not significant (Table 3). However, the Chueta population was significantly different from Ashkenazi and from non-Ashkenazi ( $p < 0.001$ ) due to the H63D allele. Each Jewish population was compared with non-Jewish populations from the same geographic area. Ashkenazi and Sephardic Jews showed a clear difference in HFE mutation

frequencies with respect to their host population, but Oriental and North African Jews did not differ significantly from other Middle Eastern and North African populations because Jews, like other populations in these regions, show low and moderate frequencies for C283Y and H63D mutations, respectively.

The study of the patients was carried out on a total of 337 Spanish individuals referred through hematology (42%), gastrointestinal (40%), primary care (16%), and other clinical departments (2%). The main reason for consulting was altered iron metabolism (transferrin saturation (TSat)  $>45\%$  and/or elevated ferritin  $>300$  ng/ml in men and  $>200$  ng/ml in women). A total of 26 of the 337 patients were H63D homozygotes. Full data could be obtained for 24 of them, who were then included in the study.

The frequency of H63D homozygotes in patients (7.7%) was significantly higher ( $P < 0.005$ ) than in a control group (1.6%) from the same area (22) (Table 4). The patients studied were 14 males (58%) and 10 women (42%). The mean age of patients was 49 years, which is the age at which iron requirements decrease. They were classified into two groups (clinical and biological characteristics are described in Table 4).

### Group A

Patients with a secondary cause of iron overload. This group comprised of 12 patients (50%): 10 had chronic liver disease [non-alcoholic steatohepatitis (NASH), chronic viral hepatitis C, excessive alcohol consumption, cirrhosis, and hepatocarcinoma] and two had porphyria cutanea tarda (PCT). In the patients with chronic liver disease, 45.4% had ferritin  $>750$  ng/ml, associated with severe iron overload (4), 33.3% had moderately elevated ferritin levels ( $>500$  and  $<750$  ng/ml), and 21.3% had mildly elevated ferritin levels (between 300 and 500 ng/ml). The patients with PCT were two sisters, 26 and 29 years old, one of them on erythropoiesis treatment (body iron removed: 0.87 g).

Table 1. Genotype frequencies [n (%)] of C282Y and H63D mutations in Jewish populations

Population	n	wt/wt	C282Y/C282Y	wt/C282Y	H63D/H63D	wt/H63D	C282Y/H63D
Oriental	53	43 (81.13%)	0	1 (1.89%)	1 (1.89%)	8 (15.09%)	0
Sephardic	147	111 (75.51%)	0	0	3 (2.04%)	32 (21.77%)	1 (0.68%)
North African	55	41 (74.55%)	0	1 (1.82%)	1 (1.82%)	11 (20.00%)	1 (1.82%)
Total	255	195 (76.47%)	0	2 (0.78%)	5 (1.96%)	51 (20.00%)	2 (0.78%)

wt, wildtype.

Table 2. Allele frequencies (%) of C282Y and H63D mutations in populations of Jewish descent

	n	C282Y	H63D	wt	Reference
Non-Ashkenazi Jews					
Oriental	53	0.94 ± 0.94	9.43 ± 2.83	89.63 ± 2.96	Present study
Sephardic	147	0.34 ± 0.34	13.26 ± 1.98	86.40 ± 2.00	Present study
North African	55	1.82 ± 1.27	12.73 ± 3.18	85.45 ± 3.36	Present study
Total	255	0.78 ± 0.39	12.35 ± 1.46	86.87 ± 1.50	
Chuetas	137	0	26.60 ± 2.70	73.40 ± 2.70	Guix et al. (2002) (22)
Ashkenazi Jews	381	1.30 ± 0.41	9.70 ± 1.07	89.00 ± 1.13	Beutler and Gelbart(1997) (24)
	35	0	8.60 ± 3.35	91.40 ± 3.35	Merryweather et al. (1997) (23)
Total	416	1.20 ± 0.38	9.60 ± 1.02	89.20 ± 3.11	

## Group B

Patients without secondary cause of iron overload. This group comprised of 12 patients. Two of these presented a phenotypic diagnosis of HH (altered iron metabolism and clinical symptoms). The other 10 patients only had altered iron metabolism (persistent TSat >45% and/or elevated ferritin >300 ng/ml in men and >200 ng/ml in women) without clinical symptoms. One of them had ferritin >750 ng/ml, two had moderate, and four mildly elevated ferritin levels. Two only had elevated transferrin saturation (their ages were 14 and 25 years). One patient had normal iron parameters, because she was on erythropoiesis treatment (body iron removed: 0.3 g), and the same occurred in the two patients with clinical diagnosis of HH (body iron removed: 0.87 and 1.35 g, respectively).

## Discussion

One of the purposes of this study was to determine the prevalence of the C282Y and H63D

mutations in non-Ashkenazi Jewish populations from different geographic origins. The high frequency (26.6%) of the H63D mutation found in Chuetas (descendants of Majorcan Jews) in a previous study, and the null presence of C282Y, significantly different from those of their neighboring Balearic population, led us to question the distribution of HFE mutations in Jewish populations.

Modern Jews constitute one ethnic group, with Semitic origin, split into several groups that have lived in different geographic areas and, have therefore, been in contact with different host populations. The genetic similarities between Jewish populations and between them and their host peoples have been widely studied. The results have pointed out that the present-day Jewish populations still retain genetic evidence of a common Middle Eastern origin but also that the Jews have similarities with the people among whom they lived. However, the extent of this admixture is controversial, because some researchers have concluded that they have a substantial non-Jewish admixture, whereas others have maintained the existence of only a slight gene flow from their respective hosts or have suggested different admixture rates depending on the populations and/or the loci studied (19, 29–31). Moreover, other factors, such as founder effects, inbreeding, and/or selection, may have influenced the genetic differentiation of Jewish population groups.

Regarding HFE mutations, the Ashkenazi Jewish population was investigated by Merryweather-Clarke et al. (23), who found that in 35 individuals, the C282Y allele frequency was zero, and the H63D was 8.6%. Beutler and Gelbart (24), in a larger study, reported 1.3 and 9.7%, respectively.

C282Y frequencies found in the present study in the different non-Ashkenazi Jewish populations ranged from zero to 1.8%, and the  $F_{ST}$  values indicated that the Jewish populations (Ashkenazi and non-Ashkenazi) were

Table 3. Estimations of genetic differences between populations ( $F_{ST}$ )

Population 1	Population 2	$F_{ST}$
Ashkenazi Jews <sup>a</sup>	Ashkenazi Jews <sup>b</sup>	NS
Non-Ashkenazi Jews <sup>c</sup>	Ashkenazi Jews <sup>a,b</sup>	NS
Chuetas <sup>d</sup>	Non-Ashkenazi Jews <sup>c</sup>	***
Chuetas <sup>d</sup>	Ashkenazi Jews <sup>a,b</sup>	***
Chuetas <sup>d</sup>	Majorcans <sup>d</sup>	*
Ashkenazi Jews <sup>a,b</sup>	North-eastern Europeans <sup>b</sup>	***
Sephardic Jews <sup>c</sup>	Spaniards <sup>e</sup>	***
North African Jews <sup>c</sup>	North Africans <sup>f,g</sup>	NS
Oriental Jews <sup>c</sup>	Middle Easterns <sup>a,h</sup>	NS

NS, not significant; \* $p < 0.05$ ; \*\*\* $p < 0.001$

<sup>a</sup>Merryweather-Clarke et al., 1997 (23)

<sup>b</sup>Beutler and Gelbart, 1997 (24)

<sup>c</sup>Present study

<sup>d</sup>Guix et al., 2002 (22)

<sup>e</sup>Pooled Spanish populations revised in Merryweather-Clarke et al., 2000 (3)

<sup>f</sup>Roth et al., 1997 (52)

<sup>g</sup>Sassi et al., 2004 (53)

<sup>h</sup>Jazayeri et al., 2003 (54)

Table 4. Genetic and clinical features of H63D homozygote Spanish patients

Patients	Clinical characteristics	n	Mean age	Sex ratio (male : female)	AST $\pm$ SD (U/L)	ALT $\pm$ SD (U/L)	TSat $\pm$ SD (%)	Ferritin $\pm$ SD (ng/ml)	Liver biopsy		
									n	Fibrosis	Cirrhosis
Group A	Chronic liver disease	10	54	7:3	68 $\pm$ 33	95 $\pm$ 63	59 $\pm$ 17	641 $\pm$ 316	4	3	0
	Porphyria cutanea tarda	2	27.5	0:2	32 $\pm$ 20	60 $\pm$ 70	30.5 $\pm$ 16	143 $\pm$ 87	-	-	-
Group B	Altered iron metabolism <sup>a</sup>	10	48	6:4	25 $\pm$ 7	27 $\pm$ 7	41 $\pm$ 15	376 $\pm$ 227	1	1	0
	HH	2	59	1:1	26 $\pm$ 14	24 $\pm$ 6	26 $\pm$ 22	254 $\pm$ 347	-	-	-
Total		24	49	14:10	43	60	45	425			
Patients	n = 337			Allele frequency H63D (% $\pm$ SD) = 26.7 $\pm$ 1.7							
Control population <sup>b</sup>	n = 192			Allele frequency H63D (% $\pm$ SD) = 19.5 $\pm$ 2.0							

AST, aspartate aminotransferase; ALT, alanine aminotransferase; HH, hereditary hemochromatosis; SD, standard deviation; TSat, transferrin saturation.

Reference range: AST = 5–40 U/L; ALT = 5–41 U/L; TSat = 20–40%; Ferritin = men 30–300 ng/ml, women 15–200 ng/ml.

<sup>a</sup>TSat >45% and/or elevated ferritin >300 ng/ml in men and >200 ng/ml in women.

<sup>b</sup>Guix et al., 2002 (22).

homogeneous. Therefore, although the different Jewish populations have lived with host populations with very different C282Y frequencies (high in North-East Europe, almost null in North Africa and the Middle East), they have a low C282Y prevalence (average 0.9%), and these results are in agreement with the studies suggesting a slight gene flow from their hosts. The results of H63D prevalence also indicated low admixture rates, because Jewish populations are homogeneous, except for Chuetas who have a significantly higher frequency (26.6%).

An important question is why Chuetas have such a high frequency of the H63D mutation. The Chueta population has a particular history – different from other Jews – which has probably moulded their genetic structure. Despite their social isolation, their conversion to Christianity (15th century) allowed mixed marriages with the local population to take place more easily than in other European countries, where there were strict religious barriers between Jews and their host people. In fact, previous studies on classical and DNA genetic markers in Chuetas showed a substantial admixture rate (approximately 50%) (19, 32, 33). Taking into account this previous genetic information for Chuetas, which indicated that they are a hybrid population between Sephardic Jews and Majorcans, we have calculated the H63D frequency expected in Chuetas. The frequencies found in Majorca and Sephardic Jews were 19.5 and 13.3%, respectively. This, combined with an admixture rate of 50%, means Chuetas should have a H63D prevalence of around 16%, which differs significantly from the observed value (26.6%). Therefore, we can discard the fact that the high frequency observed is only due to admixture.

Different authors have pointed out that the HFE mutations might have been selected for because of the conferred resistance to iron deficiency due to infectious diseases, environmental conditions, or other genetic disorders, such as anaemia (34, 35). However, selection does not seem to be the main cause of the Chueta's H63D frequency, because they must have had similar selection pressures as their host population.

Therefore, genetic drift seems to be the most probable cause for the H63D frequency found in Chuetas. Although there is no historical knowledge about the number of founders of the Chueta population, it is reasonable to assume that the number of Sephardic settlers on the Island of Majorca would have been low. In addition, this population suffered different bottlenecks throughout its history, mainly due to the pursuit

of the Inquisition (36). These situations may have led to allelic frequencies different from those in ancestral populations (37). Moreover, social discrimination against Chuetas pushed them into important inbred behavior until the middle of the twentieth century (18), which could have increased the effect of the genetic drift and raised the H63D frequency up to the current value of approximately 27%.

In Spanish populations, high H63D frequencies are found (>15%), and in Chuetas and Basques, two genetically singular Spanish populations, the frequencies reach even higher values, almost 30% (3, 22, 38). Therefore, in Spanish populations a high number of H63D homozygotes are expected. Although the role of this mutation remains controversial, and some authors (39) think that without additional factors, H63D is insufficient for overt iron overload; other studies evidence that H63D homozygosity contributes to iron loading (10, 40–42). For these reasons, it is important to improve the understanding of the relationship between H63D homozygosity and iron overload.

On the Island of Majorca, the H63D allelic frequency is 19.5% and the observed H63D homozygotes were 1.6% (22). However, the frequency of H63D homozygotes in the patients studied with altered iron metabolism (7.7%) was clearly higher than in the control group. These results are similar to those found by other authors in other Mediterranean populations (10, 42–44), indicating a relationship between H63D homozygosity and the biological and/or clinical abnormalities of iron metabolism.

When the H63D homozygote subjects investigated in this study were screened for secondary causes of iron overload, an acquired cause was only found in 50% of patients, with clinical diagnosis of chronic liver disease and porphyria cutanea tarda (PCT). In this group, 45% of patients showed levels of ferritin greater than 750 ng/ml. These results support the synergistic role or a pre-disposing factor of the H63D genotype in these diseases, as found by different authors in NASH, PCT, and chronic hepatitis C (7, 8, 45, 46). However, Chiaverini et al. (47) did not find that the H63D mutation was a PCT associated factor. Along the same lines, Cauza et al. (48) found that H63D homozygotes do not increase the risk of developing hepatocarcinoma.

In the group of patients without any recognizable cause of secondary iron overload, the H63D homozygote genotype seems to lead to a mild-to-moderate iron overload, which in the patients without HH, was expressed only through altered iron parameters – especially through persistent

elevated ferritin. The different degrees of iron overload observed are probably due to the variable expressivity of this genotype, previously described by other authors (10, 44). This variability suggests the presence of modifiers, either environmental or genetic, which contribute to the clinical expression of HFE mutations. As far as environmental factors are concerned, alcohol intake has been shown to accentuate disease expression. Regarding possible genetic modifiers of the hemochromatosis phenotype, of the many genes investigated, polymorphisms in the hepcidin gene (HAMP) and the juvenile hemochromatosis gene (HJV) are the only ones to date found to be associated with HFE phenotypic heterogeneity (reviewed in 49).

From the results obtained in patients, we believe that in populations where a high H63D homozygosity is expected, this genotype must be taken in account when it occurs with altered iron metabolism and/or clinical manifestations. These patients should have a suitable follow-up to prevent severe iron overload and, where necessary, treatments such as erythropheresis must be considered. Recently, the H63D mutation has also been associated with other diseases, such as malignancies (50) and Alzheimer's disease (51). If the results of these studies are confirmed, this fact must be held in consideration in Spanish populations, due to the high prevalence of both the mutation and the aforementioned diseases.

In conclusion, our results indicate that the high frequency of the H63D mutation found in the Chueta population is not a consequence of a high prevalence of this mutation in Sephardic Jews. In fact, Jewish populations have low C282Y and moderate H63D frequencies, independently from the area where they have lived, thus suggesting a slight gene flow from their hosts. In accordance with historical and demographic data, genetic drift is the most probable cause for the singular H63D frequency in this Jewish descendant population. From a clinical point of view, the present study of H63D homozygotes supports the conclusions of other authors that this genotype must be considered because it confers an increased risk of iron overload and therefore genetic susceptibility to developing HH or to aggravating other diseases, especially in populations where a high H63D homozygosity is expected.

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