FREQUENCY OF THREE HEMOCHROMATOSIS GENE MUTATIONS IN ANTALYA, TURKEY

Oztürk S, Lülecı G, Keser I*

***Corresponding Author:** Associate Professor Dr. Ibrahim Keser, Department of Medical Genetics, School of Medicine, Akdeniz University, 07070 Antalya, Turkey; Tel.: +90-242-249-6000, Ext: 6973; Fax: +90-242-227-4484; Mobile Tel.: +90-532-788-7644; E-mail: keser@akdeniz.edu.tr

ABSTRACT

Hemochromatosis gene (HFE) mutations are associated with hereditary hemochromatosis. We used the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method to determine the frequency of the mutations (C282Y, S65C and H63D) of the HFE gene in DNA samples of 141 healthy Turkish adults in Antalya, Turkey. The mutant allele frequencies were 0.0, 0.0 and 0.12, respectively. The H63D mutation was found in the heterozygous state in 30 samples (21.27%) and in the homozygous state in two samples (1.41%). These results suggest that the H63D mutation may be responsible for the hereditary hemochromatosis in the Turkish population.

Key words: Hereditary hemochromatosis, Hemochromatosis gene (HFE), C282Y, S65C, H63D, Antalya.

INTRODUCTION

Hereditary hemochromatosis is an autosomal recessive disorder of iron regulation that results in excessive intestinal absorption of iron. This metabolism leads to early multi systemic and non specific signs such as liver cirrhosis, hepatocellular carcinoma, diabetes mellitus, skin pigmentation, hypogonadotrophic hypogonadism, arthropathy

and cardiomyopathy [1]. The hemochromatosis gene (HFE) is localized in human chromosome 6p21.3 [2]. Homozygosity for the C282Y mutation has been reported in 60-100% of individuals with hemochromatosis in European populations [3]. However, the H63D mutation has a frequency of approximately 16% in the European populations [2,3]. The S65C mutation is much rarer and is associated with a mild form of hemochromatosis [4]. In the general population in Europe, the prevalence of the C282Y mutation is estimated to be 9.2% (heterozygote) and 0.4% (homozygote). The prevalence of the C282Y mutation differs in different populations in the Slavic populations (1.9% in the former USSR and 10.0% in the Czech Republic). This mutation is absent in populations outside Europe and the USA [5].

The prevalence of the S65C mutation that has been associated with the development of a mild form of iron overload is 5.0% in the Republic of Macedonia [6], 2.5% in France [4], 1.5% in Denmark [7] and 1.1% in the USA [5]. The prevalence of the H63D mutation is equally high in northwestern and southern European populations, where 22.0% are estimated to be heterozygotes and 2.0% homozygotes. It is the highest in Spain (32.1%), lower in Norway and the former USSR (18.0%) and in India (15.0%). It is very rare outside Europe and the USA [5].

The aim of this study was to determine the frequency of HFE gene mutations (C282Y, S65C, and H63D) in Antalya, Turkey, which is a bridge between Asia and Europe.

* Department of Medical Genetics, School of Medicine, Akdeniz University, 07070 Antalya, Turkey

MATERIALS AND METHODS

A total of 141 random DNA samples from healthy adult volunteers (92 females, 49 males, age ranges from 20 to 61) who reside in Antalya, Turkey, were genotyped for HFE mutations (C282Y, S65C, and H63D). Written consent was obtained from each individual enrolled in this research.

Genomic DNA was isolated from peripheral blood leukocytes by a salting-out method [8]. The mutations were detected by a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method [2]. The PCR was carried out in a Thermal Cycler (Techne-Genius, Cambridge, Cambridgeshire, UK). Each exon was amplified using the primers designed by Feder *et al.* [2]. The amplified fragments were 208 bp for H63D and S65C and 390 bp for C282Y. The PCR products were digested with

the restriction enzymes *RsaI* for C282Y, *HinfI* for S65C and *MboI* for H63D. The restriction fragments were visualized after electrophoresis on a 3% agarose gel stained with ethidium bromide. Quality control of the enzyme digestions was confirmed by positive and negative control DNAs.

RESULTS

The allelic frequencies and genotypes of the mutations found are summarized in Table 1. The H63D mutation was found in the heterozygous state in 30 individuals (21.28%), and in the homozygous state in two individuals (1.42%). The frequencies of the H and D alleles of the H63D mutation were found to be 0.88 and 0.12%, respectively. These results are in keeping with other reports from different population studies (Table 2).

Table 1. Distribution of allelic frequencies of the HFE gene mutations in healthy Turkish individuals

HFE Mutations	Alleles Studied	Number of Individuals			Mutant Allele Frequency
		WT/WT	WT/Mut	Mut/Mut	
C282Y	282	141	0	0	0.0
S65C	282	141	0	0	0.0
H63D	282	109	30	2	0.12

WT: wild type, Mut: mutated type.

Table 2. Frequencies (%) of the HFE mutations C282Y, H63D and S65C in different populations

Population		Mutant Allele Frequency (%)		
	Reference(s)	C828Y	S65C	H63D
Europe:				
• Spain	9; 10	3.0	0.0	21.7
• France	4; 5	4.2	2.5	15.8
• Italy	5; 11	1.1	1.3	11.9
• Denmark	1; 13	5.7	1.5	13.3
• Sweden	5; 13	3.9	1.6	12.5
• Norway	5	6.4	0.0 ^a	11.2
• Faroe Islands	1	6.6	1.0	15.3
• Macedonia	6	1.0	5.0	21.0
The Americas:				
• Connecticut, USA	5	5.0	0.0 ^a	16.0
• Missouri, USA	5	4.9	0.0 ^a	15.4
• Alabama, USA	5	6.0	0.0 ^a	12.7
• Brazil	14	0.0	0.0 ^a	0.0
• Ecuador	15	0.0	4.0	3.5
Asia:				
• China	5	0.0	2.8	0.0 ^a
• Japan	16	0.0	1.5	0.0 ^a
• Turkey	Present study	0.0	12.0	0.0

^a These mutations were not tested for in their studies.

DISCUSSION

Hereditary hemochromatosis is the most prevalent monogenic disease in Caucasian European populations. It has been reported that the frequency of the C282Y mutation is high in people of Anglo-Celtic origin [2,5,12,17]. Heterozygotes are very common in Ireland (28.4%) [5], Denmark (13.7%) [12], Norway (12.8%) [5] and Iceland (10.0%), and less common in southern Europe: Greece (2.6%), Italy (2.2%) and Spain (4.5%) [5]. This mutation is less frequent in people from southern Europe and present at very low frequencies or absent in the Asiatic and African populations [5,20,21]. We did not find the C282Y and S65C mutations in Antalya, Turkey. This result is similar to those published for the Brazilian [14], Chinese [5] and Japanese [16] populations, excluding 4.0% for the frequency of the S65C mutation in the Ecuadorian population [15]. In addition, Bozkaya *et al.* [22], Merryweather-Clarke *et al.* [23], Simsek *et al.* [24] and Barut *et al.* [25], did not observe the C282Y mutation, and also did not test for the S65C mutation in their Turkish population. Prevalence of the H63D mutation was high in the present study, such as in the Macedonian population [6]. Therefore, we believe that our data are important due to being a bridge between Asia and Europe. Full phenotypic expression of hereditary hemochromatosis is very rare in the population of Antalya. However, the H63D mutation may be responsible for the hereditary hemochromatosis in the Turkish population.

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