

Frequency of HFE Gene Mutations C282Y and H63D in Bosnia and Herzegovina

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ABSTRACT

Genetic epidemiology studies of hereditary hemochromatosis (HHC) have shown a high prevalence of the C282Y mutation in individuals of the North Western European origin, whereas lower prevalence of HFE gene mutations was detected in the populations from southern European countries. However, no HFE mutation prevalence data have been provided for the population of Bosnia-Herzegovina so far. Therefore, the aim of this study was to determine the frequency of the C282Y and H63D HFE gene mutations in the population of Bosnia-Herzegovina. Among 200 analysed subjects 8 (4%) were C282Y heterozygotes; no C282Y homozygotes were found. The frequency of the H63D allele was 11.5%. There were 33 (16.5%) heterozygotes and 6 (3%) homozygotes for the H63D mutation. One (0.5%) compound heterozygote C282Y/H63D was identified. The observed C282Y and H63D allele frequency was 2.25% (95% confidence interval: 1.2–4.2) and 11.5% (95% confidence interval: 8.7–14.9), respectively. The prevalence of the C282Y and H63D mutations was estimated in Bosnia-Herzegovina, which fit well in the European northwest-to-southeast gradient of the C282Y mutation distribution. In addition, these results have an important implication for clinical evaluation of HHC in Bosnia-Herzegovina.

Key words: hereditary hemochromatosis, HFE gene, C282Y and H63D mutation, allele frequency

Introduction

Hereditary hemochromatosis (HHC) is one of the most common autosomal recessive inherited diseases in Caucasians with an estimated prevalence of 1:188 to 1:327¹. In Caucasians the disease leads to progressive iron accumulation in various organs (liver, pancreas, heart and joints) with reduced lifetime-expectancy if phlebotomy treatment is not instituted².

The hemochromatosis gene (HFE) was identified by positional cloning and located on chromosome 6. Two most frequent missense mutations were identified, namely a G to A transition at nucleotide 845 which leads to a substitution of cysteine for tyrosine mutation at the amino acid position 282 (C282Y) and a C to G change at nucleotide 187 that results in a substitution of histidine for aspartate at the amino acid position 63 (H63D)³. Over 80% of HHC patients from North European populations are homozygous for the C282Y mutation. A significant proportion of HHC patients who are not homozygous for C282Y are C282Y heterozygotes, compound heterozy-

gotes, H63D homozygotes, or H63D heterozygotes⁴. Hereditary hemochromatosis was initially described exclusively in individuals of the North Western European origin, whereas lower HFE gene mutations were detected in populations from southern Europe^{5–9}. However, no HFE mutation prevalence data have been reported on the Bosnia-Herzegovina population so far.

Therefore, the aim of this study was to determine the frequency of C282Y and H63D HFE gene mutations in the Bosnia-Herzegovina population.

Participants and Methods

The study population included two hundred (100 males and 100 females) unrelated healthy blood donors from Bosnia and Herzegovina, in the period from 2003 to 2005. All participants subscribed the informed written consent.

Genomic DNA was extracted from peripheral blood leukocytes using a standard procedure¹⁰. HFE mutation analysis was performed using PCR-RFLP analysis, as previously described¹¹. The amplified PCR fragments were digested with restriction enzyme *Rsa* I to identify the C282Y mutation and with *Mbo* I to detect the H63D mutation, as recommended by the manufacturer (Promega, USA). Restriction enzyme digest products were analysed on a 3.0% agarose gel and visualized after ethidium bromide staining.

Chi-squared (χ^2) test was used to compare the frequency of C282Y and H63D genotypes. A 95% confidence interval (CI) was calculated for the frequency of the alleles.

Results

Frequencies of the C282Y and H63D genotypes are shown in Table 1. Among 200 analysed subjects 8 (4%) were C282Y heterozygotes, 33 (16.5%) were H63D heterozygotes and 6 (3%) were homozygotes for the H63D mutation. One (0.5%) compound heterozygote C282Y/H63D was identified and no C282Y homozygotes were found.

The genotype distribution of C282Y was within Hardy-Weinberg equilibrium ($p=0.74$; $\chi^2=0.11$), whereas the H63D distribution was not ($p=0.02$; $\chi^2=5.43$).

In Table 2, the allelic frequencies of the two mutations are presented. The overall C282Y allele frequency in the population of Bosnia-Herzegovina population was 2.25% (95% CI: 1.2–4.2) and the observed H63D allele frequency was 11.5% (95% CI: 8.7–14.9). Taking into ac-

count the allele frequency of C282Y, the prevalence of homozygous C282Y was calculated as approximately 0.5 per 1000.

Discussion

Hereditary hemochromatosis HFE mutations have been studied in several European populations. Initially HFE mutations were described exclusively in populations of North Western European origin¹². It has been suggested that the C282Y mutation originates within the population of Celtic origin. On the other hand, there is evidence that the C282Y mutation originated from the Germanic Iron Age population in Southern Scandinavia and spread with the Vikings¹³. The allele frequencies are lowest in locations where the Vikings had been scarce such as Central Europe, the Balkans and the Mediterranean countries. As previously described, the frequency of the C282Y mutation declines from Northern to Southern Europe, thus allele frequencies of 6.5 to 8.2% was found in English blood donors¹⁴, intermediate allele frequencies (3.1–4.8%) are seen in the populations in Central Europe⁴, whereas low allele frequencies (0–3.1%) are recognized in populations in Southern Europe and the Mediterranean⁶. According to the geographic position in Europe, the prevalence of the C282Y mutation in Bosnia and Herzegovina (2.25%) fits in the observed north/south gradient. Recent studies have shown that in Slovenia the observed allele frequency of the C282Y mutation is 4.0%, in Croatia 3.3%, and in Greece 1.0%, which is comparable to the frequencies found in the Bosnia-Herzegovina population^{9,15}.

In the Bosnia-Herzegovina population, the H63D allele frequency is 11.5%. This frequency is comparable to European populations, in which the H63D mutation is present, and is more frequent than C282Y, ranging between 10% and 20%⁴. In the Slovenian and Croatian populations, the estimated prevalence of H63D is 14.5% and declines to Middle East where it is between 8% and 10%^{4,9}. There is a moderate deviation from Hardy-Weinberg equilibrium in the distribution of H63D genotypes, which might be explained as a consequence of genetic drift and/or migrational movements in the area of Bosnia and Herzegovina in the war and post-war period.

In conclusion, the estimated prevalence of the C282Y and H63D mutations in the Bosnia-Herzegovina population was lower than that in the Slovenian and Croatian populations, which supports the idea of the so-called gradient distribution of the C282Y mutation; a more definite proof on the origin of this mutation remains unclear. These results have an important implication to clinical evaluation of HHC in Bosnia and Herzegovina, and contribute to an easy identification of those at risk of developing the disease.

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TABLE 1
DISTRIBUTION OF HFE GENOTYPES IN BOSNIA-HERZEGOVINA

C282Y	H63D	N = 200	%
-/-	-/-	152	76.0
+/-	-/-	8	4.0
+/+	-/-	0	0
-/-	+/-	33	16.5
+/-	+/-	1	0.5
+/+	+/-	0	0
-/-	+/+	6	3.0
+/-	+/+	0	0
+/+	+/+	0	0

+ denotes the mutated allele, - denotes wild-type allele

TABLE 2
ALLELE FREQUENCIES AND 95% CONFIDENCE INTERVAL (CI) FOR C282Y AND H63D MUTATIONS IN BOSNIA-HERZEGOVINA

HFE gene mutations	Allele frequencies (%) and 95% CI
282Y	2.25 (1.2–4.2)
63D	11.5 (8.7–14.9)

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FREKVENCIJA C282Y I H63D MUTACIJA GENA HFE U BOSNI I HERCEGOVINI

SAŽETAK

Genetičko-epidemiološka istraživanja nasljedne hemokromatoze (HHC, hereditary hemochromatosis) pokazala su visoku učestalost mutacije C282Y kod osoba porijeklom iz sjeverozapadne Europe, dok je niža učestalost mutacija HFE gena opažena u populacijama južnoeuropskih zemalja. Međutim, do sada nisu postojali podaci o učestalosti mutacija tog gena za populaciju Bosne i Hercegovine. Zbog toga je cilj ovog istraživanja bio odrediti frekvenciju mutacija C282Y i H63D HFE gena u populaciji Bosne i Hercegovine. Među 200 analiziranih ispitanika bilo je 8 (4%) C282Y heterozigota i niti jedan C282Y homozigot. Frekvencija alela H63D bila je 11.5%. Zabilježena su 33 (16.5%) heterozigota i 6 (3%) homozigota za H63D mutaciju. Zabilježen je 1 (0.5%) složeni heterozigot C282Y/H63D. Opažene frekvencije alela C282Y i H63D bile su 2.25% (95%-tni interval pouzdanosti: 1.2–4.2) i 11.5% (95%-tni interval pouzdanosti: 8.7–14.9). Procijenjena učestalost mutacija C282Y i H63D u Bosni i Hercegovini dobro se uklapa u europski gradijent distribucije mutacije C282Y od sjeverozapada prema jugoistoku. Uz to, ovi rezultati imaju važnu ulogu za kliničku evaluaciju HHC-a u Bosni i Hercegovini.