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Hemochromatosis gene mutations in the general population of Slovakia

Research Article

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Abstract: This is an epidemiologic study of the Slovak population with the aim of determining the frequencies of three hemochromatosis gene (HFE) variants C282Y, H63D and S65C known to be associated with manifestation of hereditary hemochromatosis and to assess deviations of these frequencies from those reported elsewhere. Mutations were detected in 359 ethnic Slovaks by real-time PCR assay based on TaqMan technology. The allelic frequencies were 4.03% for C282Y, 12.67% for H63D and 1.25% for S65C mutation. We observed 0.28% of C282Y/C282Y homozygotes, 3.34% H63D/H63D homozygotes, 0.84% of C282Y/H63D compound heterozygotes and 0.56% of H63D/S65C compound heterozygotes. This is the first time the frequencies of H63D and S65C mutations have been reported in the general population in Slovakia. The observed allelic frequencies are consistent with the previous studies of Slavic and Central European populations.

Keywords: *Hemochromatosis • HFE • Slovakia • Epidemiology*

1. Introduction

Hereditary hemochromatosis (HH) is a genetic disorder caused by failure of the iron metabolism mechanism and the resultant accumulation of iron in the liver, pancreas, heart and joints. If left untreated, it can lead to liver cirrhosis, hepatocellular carcinoma, cardiomyopathies and diabetes mellitus. Recognition of the disease in the early stage when the symptoms are nonspecific is crucial, because beginning the treatment before tissue damage can markedly reduce the risk of further serious complications [1,2].

In the last decades, our knowledge of the regulation of the iron level in the organism has greatly evolved. Hepcidin has been identified as a key molecule of iron metabolism. The amount of hepcidin produced by hepatocytes is regulated according to the iron level in the body through three proteins: hemojuvelin, HFE and transferrin receptor 2. Mutations of all the main components of this pathway have been proven to cause HH, the most common type being HFE-associated hereditary hemochromatosis [3].

The most frequent genotype found in HH patients is homozygosity for C282Y mutation in HFE gene. This genotype comprises 52 to 96% of clinically diagnosed patients [4,5]. A fraction of the patients is compound heterozygous for C282Y and H63D mutations or homozygous for H63D mutation (5% and 1.5% respectively) [2]. These two mutations are considered to be the main ones responsible for HH. The role of the third polymorphism, S65C has been disputed since it was discovered in 1999. Even though S65C mutation has been repeatedly found to be more frequent in affected probands, the clinical significance of S65C mutation is still uncertain and it is considered to be a risk factor rather than a causative mutation [6,7]. However, when

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combined with C282Y mutation, S65C is associated with a higher risk of HH [7,8].

The distribution of the three HFE mutations in general populations is variable. C282Y is found almost strictly in the populations of European origin. The highest frequencies were reported in Ireland, UK and Scandinavia (7.5 – 14.2%) and the lowest in Italy, Greece and Bulgaria (0 – 1.3%) [9]. C282Y is considered to be of Northwestern European origin. H63D is older than C282Y and has probably arisen more than once in history [10]. It is found in allelic frequencies more than 5% in Europe, Middle East and Indian subcontinent. Within Europe, the frequencies of most populations studied range from 11 – 17%, with an extreme value of about 30% in the Basque population [10,11]. Although many epidemiological studies concerned with HFE did not include frequency data for S65C, a frequency range 0.05 – 3% was reported for this mutation in Caucasians [6,12-14].

HFE-associated HH is one of the most common monogenetic diseases in Caucasians [15]. An estimation of mutation frequencies in common diseases in each population is important for the implementation of appropriate genetic tests to diagnostics and for the assessment of the need for the screening process. The aim of this study was to determine the frequency of C282Y, H63D and S65C mutations in a cohort of randomly selected individuals residing in Slovakia.

2. Material and Methods

We investigated a cohort of 359 unrelated volunteers from Eastern Slovakia, controls from our previous studies. Individuals were chosen randomly, irrespective of their health status. Informed consent was obtained from all participants of the study. The local institutional ethical committee approved the study. DNA was extracted from buccal swabs by commercial kit JetQuick (Genomed GmbH, Germany) using standard protocol. Genotyping was performed by the real-time PCR method on RotorGene 6000 (Corbett Research, Australia). TaqMan based technology was used for mutation detection. Primers and probes sequences and reaction conditions were described previously [16]. The frequencies of HFE genotypes were compared between genders using Chi square test with Yates' correction for continuity. The significance level was set to 0.05.

Table 1. Frequencies of HFE alleles and genotypes. N - number of individuals, CI - confidence interval, names of genotypes are formed of respective abbreviated amino acids.

HFE gene variants	N	%	95% CI
alleles			
C282Y		4.0	2.81-5.76
H63D		12.7	10.77-15.32
S65C		1.3	0.62-2.40
genotypes			
HH/CC/SS	248	69.1	64.11-73.64
HH/CY/SS	24	6.7	4.49-9.79
HH/YY/SS	1	0.3	<0.01-1.72
HD/CC/SS	62	17.3	13.70-21.54
DD/CC/SS	12	3.3	1.86-5.81
HD/CY/SS	3	0.8	0.17-2.55
HH/CC/SC	7	1.9	0.87-4.05
HD/CC/SC	2	0.6	0.02-2.14
total	359	100	

3. Results

The genotype and allele frequencies are shown in Table 1. Frequencies of C282Y, H63D and S65C alleles were 4.04%, 12.67% and 1.25% respectively. One individual was homozygous for C282Y mutation (0.28%) and 12 individuals for H63D mutation (3.34%). No S65C homozygotes were identified. On notice, we also identified three compound heterozygotes with H63D/C282Y genotype (0.84%) and two with H63D/S65C genotype (0.56%). No significant differences were found for the gender percentage distribution of HFE mutations (data not shown).

4. Discussion

Individuals homozygous for C282Y mutation comprise on average two-thirds of all HH patients in Europe [2]. Genotyping of HFE gene has become a common part of HH diagnostic procedure. Knowledge of the data on prevalence of mutations is necessary for management of HH. Nowadays the frequencies of the C282Y, H63D and S65C mutation are known for most of the European countries. This is the first study reporting the frequency of all three HFE mutations in the Slovak population.

The frequency of C282Y shows high variability within Europe. The estimated frequency in the Slovak population is 4.04%. This number is comparable to the neighboring countries (Austria 3.7% [17], Germany 3.8%, Hungary 3.4% [11]) and to European populations of Slavic origin,

where the frequencies range from 3.1 to 4% in the general populations [12,13,18-20]. It differs significantly from the populations in Northern and Southern Europe [11], thus the data fit into the established Northwest to Southeast gradient. The only study concerned with the frequency of the HFE mutation in healthy individuals in Slovakia estimated the frequency of C282Y allele to be 3% [21]. The difference in observed values may point out to regional variations in C282Y frequencies within Slovakia. Further studies analyzing particular regions are needed to confirm this assumption.

H63D mutation is more equally distributed within Europe. The frequency of minor allele in Slovakia (12.67%) falls within the European range. In Central Europe, the estimated frequencies vary from 12.3% (Hungary) [11] to 16.2% (Poland) [20], and thus are similar to the frequency reported by this study. We can conclude that H63D mutation frequency in Slovakia corresponds to its geographical position.

Many population studies focus only on C282Y and H63D mutations and for many European countries we lack the data involving S65C mutation prevalence. Our data greatly expand the knowledge on the frequency of this mutation in Central Europe. The 1.25% allelic frequency in our sample appears to be lower than other reported frequencies. Recent studies have reported S65C prevalence in Slavic populations as follows: 1.2% in the Czech Republic [13], 1.6% in Serbia [22] and 1.8% in Slovenia [16] and Croatia [12]. Due to the size of the examined sample the difference does not prove to be significant.

Despite the identification of the genetic causes of HH residing in HFE gene mutations and the availability of genetic methods for mutational analysis, HH remains under diagnosed. The clinical manifestation in the early stage is nonspecific (fatigue, joint pain, abdominal pain, weight loss) and makes it difficult to diagnose this disease correctly before organ damage has occurred. Therefore, a better understanding of HH mutations and their frequencies in patients as well as in the general populations is necessary in order to implement appropriate tests in diagnostics and population based screening.

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