**HFE Gene C282Y, H63D and S65C Mutations Frequency in the Transylvania Region, Romania**

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**Abstract**

**Background & Aims.** HFE-associated haemochromatosis is one of the most frequent autosomal recessive disorders in the Caucasian population. Although most of the cases are homozygous individuals for the C282Y mutation, another two mutations, H63D and S65C, have been reported to be associated with milder forms of the disease. This study was a first attempt to evaluate the distribution of these HFE gene mutations in the Transylvania region. **Methods.** Two-hundred and twenty-five healthy, unrelated volunteers originating from the Transylvania region, Romania, were screened for the HFE gene C282Y, H63D and S65C mutations, using molecular genetics assays (Polymerase Chain Reaction-Restriction Fragments Length Polymorphism). **Results.** For the C282Y mutation, 7 heterozygotes (3.1%) were found, but no homozygous individual. In the case of the H63D mutation, 40 heterozygotes (17.8%) and 4 homozygotes (1.75%) for the mutant allele were evidenced. We found a compound heterozygous genotype (C282Y/H63D) in one individual (0.45%). Thus, the allele frequencies of the C282Y and H63D were 1.75% and 10.9%, respectively. Three individuals (1.3%) were found to harbour the S65C mutation in a heterozygous state, but none in a homozygous state: the allele frequency of the mutant allele was 0.75%. **Conclusions.** The distribution of the HFE gene C282Y, H63D and S65C mutations found in our group matches the tendencies observed in other European countries: a decreasing gradient from Northern to Southern Europe for the C282Y mutation; high frequency for the H63D mutation, and low frequency for the S65C mutation in most of the countries.

**Key words**


**Introduction**

Hereditary haemochromatosis is one of the most frequent autosomal recessive genetic diseases in individuals of European origin. The highest incidence (1:300-1:400 affected individuals) is observed in populations of Northern European descent [1]. The disease is characterized by a progressive iron overload and subsequently its deposition mainly in parenchymal cells, due to a primary increase in the duodenal absorption of the iron and impaired release from the reticuloendothelial cells. The most damaged sites are: the liver, the heart, the pancreas, the joints and the pituitary gland. If untreated, serious complications, such as hepatic cirrhosis, heart failure or diabetes mellitus can occur [2,3]. Although classically described by Trousseau as “hepatic cirrhosis, diabetes mellitus and melanodermia”, haemochromatosis is a clinically heterogeneous condition and both genetic and environmental factors can modulate the evolution and the severity of the disease [4].

Classical hereditary haemochromatosis, also known as type I haemochromatosis, is associated with mutations in the HFE gene on chromosome 6, which encodes the HFE (high-iron) protein, a key limiting factor of the duodenal iron absorption, interacting with the TfR (transferrin receptor), and decreasing its affinity for the transferrin [5]. First described by Feder in 1996, the HFE gene mutation C282Y (a cysteine-to-tyrosine substitution), resulting from a G (guanine) to A (adenine) transition in position 845 of the HFE gene, accounts for 60-80% of the type I hereditary haemochromatosis cases, depending on the ethnic background [1, 5, 6]. A second mutation, H63D (a histidine-to-aspartate substitution), resulting from a transversion C (cytosine) to G in position 187 of the HFE gene, is associated with a milder form of the disease, as this mutation leads only to a partial decrease in the ability of the HFE protein to
reduce the TfR affinity for transferring [6]. Depending on the ethnic background, this mutation represents 40-70% of the \textit{HFE} non-C282Y haemochromatosis chromosomes [1]. It is notable that around 7% of the haemochromatosis patients are C282Y/H63D compound heterozygotes [7]. Finally, a third mutation, S65C (a serine-to-cysteine substitution), resulting from a tranversion A to T (thymine) in position 193 of the \textit{HFE} gene, although initially considered a polymorphism with no phenotypic effect, has been found to be associated with milder forms of haemochromatosis, especially in C282Y/S65C compound heterozygous individuals [7,8]. Other \textit{HFE} gene mutations are generally private and usually inherited in trans with the C282Y mutation [9].

The objective of the present study was to establish the frequency of the C282Y, H63D and S65C mutations in a cohort of Romanian healthy volunteers, originating from the Transylvania region (Western, North-Western and central regions of Romania), as there are no data regarding this region of the country.

\section*{Material and methods}

Two-hundred and twenty-five individuals were enrolled in the study; there were 142 women (63%) and 83 men (37%); the age of the participants ranged from 18 to 55 years (median 21 years). All the participants had a Romanian ethnic background and originated from 16 different counties located in Transylvania. Written consent was obtained from each participant prior to admission in the study.

After admission in the study, 3 ml of peripheral blood was taken on EDTA (ethylenediaminetetraacetic acid) from each participant by venous puncture. DNA was extracted from peripheral blood leukocytes using a commercially available kit (Wizzard Genomic DNA Purification Kit, Promega, MA, USA).

The \textit{HFE} C282Y, H63D and S65C gene mutations were studied by PCR-RFLP (Polymerase Chain Reaction-Restriction Fragments Length Polymorphism) assays, as previously described [5,7,10].

This study was reviewed and approved by the Ethics Committee of the University of Medicine and Pharmacy Iuliu Hațieganu, Cluj-Napoca.

\section*{Results}

The C282Y mutation was found in 7 individuals (3.1%), all of them heterozygotes; thus, the frequency of the mutated allele was 1.75%. The H63D mutation was seen in 40 individuals (17.8%) in a heterozygous state, while 4 individuals (1.75%) were homozygotes for this mutation; thus, the frequency of the mutated allele was 10.9%. One individual (0.45%) had a C282Y/H63D compound heterozygous genotype. S65C was the rarest mutation observed in the group studied; 3 individuals were heterozygous for this mutation, corresponding to an allele frequency of 0.75%. Observed genotypes frequencies for the three \textit{HFE} mutations studied were consistent with the Hardy-Weinberg equilibrium (data not shown). Table I shows in detail the distribution of the genotypes and the allele frequencies for the 3 mutations assessed in the study.

\begin{table}[h]
\centering
\caption{Genotypes and allele frequencies observed for the \textit{HFE} mutations in our study}
\begin{tabular}{ll}
\hline
Genotype & Frequency; n (%) \\
\hline
wt/wt & 170 (75.6) \\
wt/C282Y & 7 (3.1) \\
C282Y/C282Y & 0 (0) \\
w t/H63D & 40 (17.8) \\
H63D/H63D & 4 (1.75) \\
w t/S65C & 3 (1.3) \\
S65C/S65C & 0 (0) \\
C282Y/H63D & 1 (0.45) \\
C282Y/S65C & 0 (0) \\
H63D/S65C & 0 (0) \\
\hline
\end{tabular}
\end{table}

\begin{table}[h]
\centering
\caption{Allele frequency (N = 450 alleles)}
\begin{tabular}{ll}
\hline
Allele & Frequency; N (%) \\
\hline
wt & 390 (86.6) \\
C282Y & 8 (1.75) \\
H63D & 49 (10.9) \\
S65C & 3 (0.75) \\
\hline
\end{tabular}
\end{table}

\begin{table}[h]
\centering
\caption{Genotype distribution (n = 225 individuals)}
\begin{tabular}{ll}
\hline
Allele & Frequency; N (%) \\
\hline
wt & 390 (86.6) \\
C282Y & 8 (1.75) \\
H63D & 49 (10.9) \\
S65C & 3 (0.75) \\
\hline
\end{tabular}
\end{table}

\section*{Discussion}

The distribution of the \textit{HFE} C282Y, H63D and S65C mutations in different European countries are presented in Table II. By analyzing the data, we may conclude that our results match the tendencies observed in other European countries: a decreasing gradient from Northern to Southern Europe for the C282Y mutation, with the lowest allele frequency observed in South-Eastern Europe; high frequency for the H63D mutation, with allele frequency between 10 and 20%, and low frequency, in general between 1 and 2%, for the S65C mutation in most of the countries.

Voicu et al found allele frequencies of 1.75% and 13.25% for the \textit{HFE} mutations C282Y and H63D, in the Moldavian population (North-Eastern and Eastern regions of Romania); these values are very close to our findings from the Transylvanian population [21]. We can conclude that at least in Moldavia and Transylvania, the \textit{HFE} mutations have similar frequencies. It would be interesting to assess the frequencies of these mutations also in Southern regions of Romania (Oltenia, Muntenia and Dobrogea). At least for the C282Y mutation, a decreasing gradient Northern-Southern was observed in other countries, such as France [1]. This could have consequences regarding the variable incidence of hereditary haemochromatosis across the Romanian population living in different parts of the country.

Based on our findings, we were able to estimate the incidence of hereditary haemochromatosis in our region. Transylvania has around 7 million inhabitants, which represents roughly one third of Romania’s population.
We observed an allele frequency of 1.75% for the C282Y mutation in our study, so that we may anticipate that the frequency of the homozygous genotype for the C282Y mutation in our region, based on the Hardy-Weinberg law, is around 1/3,500. These individuals, especially the males, are at the highest risk of developing the classical clinical picture of the hereditary haemochromatosis. Meanwhile, a frequency of 1/225 for the compound genotype C282Y/H63D may be anticipated; these individuals have a moderate risk of developing the clinical picture of hereditary haemochromatosis. Finally, we may expect that one individual in around 55 harbours a homozygous genotype for the H63D mutation, which, especially in certain conditions, could lead to an iron overload.

Different authors emphasized the difference between the high frequencies of the \textit{HFE} gene mutations and the lower number of clinically manifested haemochromatosis cases. This is due to the incomplete penetrance of the \textit{HFE} mutations [22]. Therefore, population screening for \textit{HFE} mutations is still a matter of debate. However, there is no doubt that, once the diagnosis of haemochromatosis has been established in a proband, screening of family members is efficient and cost-effective. Another problem to consider regarding \textit{HFE} screening in a population is the existence, in rare cases, of non-\textit{HFE} hereditary haemochromatosis, related mainly to mutations in the hemojuvelin, hepcidin, transferrin receptor 2 and ferroportin genes [3]. The spectrum of these mutations would obviously not be covered by a \textit{HFE} mutations screening strategy. An additional aspect to consider would be to find the optimal genotyping method, with respect to the costs/effectiveness. The major benefit of \textit{HFE} mutations screening would be the early detection of individuals at risk of iron overload and, hence, periodical evaluation of biochemical markers of iron metabolism (ferritin and transferrin saturation) in those individuals. This would prompt early repeated phlebotomies in individuals with iron overload, before organ damage onset. However, a genetic generalized population screening for the \textit{HFE} mutations is not presently recommended [23].

\textbf{Conclusions}

This is the first report on a healthy Transylvanian population regarding the frequency of the \textit{HFE} gene

\begin{table}[h]
\centering
\begin{tabular}{|l|l|l|l|l|}
\hline
Country & Sample size & C282Y & H63D & S65C & Reference \\
\hline
Romania (Transylvania region) & 225 & 1.75 & 10.9 & 0.75 & Present study \\
\hline
Ireland (data pooled from 5 studies) & 1,119 & 10.1 & 14.7 & - & [1] \\
\hline
UK (data pooled from 10 studies) & 12,697 & 8.1 & 15.2 & - & [1] \\
\hline
Denmark (data pooled from 4 studies) & 876 & 7 & 12.1 & - & [1] \\
\hline
\hline
France (data pooled from 4 studies) & 10,395 & 7.1 & 16.7 & - & [1] \\
\hline
Germany (data pooled from 4 studies) & 425 & 3.8 & 13.2 & - & [1] \\
\hline
Poland (data pooled from 4 studies) & 871 & 3.1 & 16.2 & - & [13] \\
\hline
Czech republic & 481 & 3.4 & 14.9 & 1.25 & [14] \\
\hline
Austria & 271 & 3.7 & 12.9 & - & [15] \\
\hline
Hungary (data pooled from 4 studies) & 1719 & 3.4 & 12.3 & - & [1] \\
\hline
Slovenia & 200 & 4 & 14.5 & 0.5 & [16] \\
\hline
Croatia & 200 & 3.3 & 14.5 & 1.8 & [16] \\
\hline
Serbia and Montenegro & 318 & 1.6 & 15.7 & 1.6 & [17] \\
\hline
Bulgaria & 100 & 0 & 23 & - & [18] \\
\hline
Spain (data pooled from 8 studies) & 1,194 & 3.2 & 20.6 & - & [1] \\
\hline
Italy (Apulia region) & 500 & 1.5 & 14 & 0.5 & [20] \\
\hline
Greece & 196 & 1.3 & 13.5 & - & [19] \\
\hline
Turkey & 70 & 0 & 13.6 & - & [19] \\
\hline
Romania (Moldavia region) & 200 & 1.75 & 13.25 & - & [21] \\
\hline
\end{tabular}
\caption{Allele frequencies of the \textit{HFE} mutations in different European countries}
\end{table}

* 14% reported by Ryan et al, on 109 individuals [11]; * 1.95% reported by Mura et al, on 771 individuals [7]; * 30.4% reported by Merryweather-Clarke et al, on a Basque population of 28 individuals [19]
mutations C282Y, H63D and S65C. Overall, our results are consistent with the distribution pattern of these mutations in Europe. Even though hereditary haemochromatosis has an expected lower frequency in Romania than in other countries, and a general population screening would probably not be worthwhile, the diagnosis of hereditary haemochromatosis should be considered in patients presenting with disease-related manifestations. An interdisciplinary approach is warranted, with input from various medical specialists: gastroenterologists (those who usually are the first to encounter clinically manifest cases), geneticists, endocrinologists, rheumatologists and haematologists.

Conflicts of interest
None to declare.

References