

Research Article



Frequency of C282Y, H63D, S65C Hereditary Hemochromatosis Gene Mutations in Syrian Population and Their Association with Serum Iron Overload

Taghrid Hammoud^{1,2}, Alia Al-assad³, Abir Kadar⁴, Hana Zarzour⁴, Ammar Madania⁵

¹ Department of Physiology and Pharmacology, Faculty of Medicine, Damascus University, Syria.

² Department of Medicine Interne, Endocrinology Service, Hospital Al-Mouassa, Faculty of Medicine, Damascus University, Syria.

³ University Blood Transfusion Center, Faculty of Medicine, Damascus University, Syria.

⁴ Department of Hematology, Faculty of Medicine, Damascus University, Syria.

⁵ Department of Radiation Medicine, Atomic Energy Commission, Damascus, Syria.

*Corresponding author's E-mail: taghridl@yahoo.fr

Accepted on: 30-07-2013; Finalized on: 30-09-2013.

ABSTRACT

Hemochromatosis is a common genetic disease, characterized by increased absorption of iron. If not treated it will cause excessive accumulation of iron in some organs (liver, heart, brain, pancreas), and consequently irreversible organ failure. The objective of this study is to explore the prevalence of HFE gene mutations (S282Y, H63D, S65C) in Syrian population and to investigate the relationship between the presence of mutation and serum iron overload. Our study involved 751 randomly selected blood donors from different Syrian regions reviewing University Blood Transfusion Center in Damascus. The subjects were analyzed for the presence of HFE mutations by real-time PCR assay based on TaqMan technology using 5'-FAM labeled MGB-probes. Among 751 blood donors tested, the observed S282Y, H63D and S65C allele frequency were 0.2%, 12.3% and 0.07%, respectively. We found three subjects heterozygous for S282Y mutation. Conversely the H63D mutation was more high frequent; there were 143 heterozygous and 21 homozygous subjects for H63D mutation. The S65C was uncommon or rare and we found one subject heterozygous for S65C mutation. We did not observe any subject with compound heterozygote mutation. Additionally, we measured serum iron level in 146 subject carriers of mutation and 156 normal subjects used as control group. We observed that there was not a statistical significance correlation between the serum iron levels and the presence of HFE mutations (p value = 0.10).

Keywords: Hereditary hemochromatosis, HFE gene, iron overload, S282Y mutation.

INTRODUCTION

Hemochromatosis is a hereditary autosomal recessive disease, caused by a mutation in the HFE gene, causing a disturbance in iron metabolism, thus leading to an increase in intestinal absorption of iron and consequent deposition of the extra iron in parenchymal cells of the important organs such as the liver, pancreas and heart. The accumulation of iron in cells leads to increased oxidative stress and damage cellular result in impairment in the function of the affected organs, which manifests in the form of cirrhosis of the liver or hepatic cell cancer, diabetes, cardiac failure and hyperpigmentation of skin¹⁻⁵. The phenotype expression starts in the form of fatigue, malaise, arthralgia non-specific, hypogonadism, diabetes and hepatomegaly. Hemochromatosis gene, HFE, was discovered in 1996⁶ and is characterized by the presence of three point mutations of the HFE protein: the first is resulting in amino acid change at position 282 from cyteine to tyrosine (C282Y mutation) and is present at 52-96% of hemochromatosis patients in European regions⁷. The second occurs within the exon 2 of the HFE gene where aspartate replaces histidine at amino acid position 63 (H63D mutation)^{8,9}. Studies have shown that this variant is most relevant to disease risk in the case of combined heterozygosity with C282Y variant (C282Y/H63D)¹⁰. The third mutation is resulting from the substitution of serine at position 65 of amino acid by the cysteine (S65C mutation). This variant is less common and

safe and may contributes to a mild type of disease if accompanied with C282Y mutation^{11,12}.

Hemochromatosis is predominantly associated with C282Y homozygous genotype which is more common in most northwestern European origin, approximately 1 in 200 people. The highest reported prevalence for C282Y homozygosity is 1 in 83 in Ireland^{13,14}. It is now accepted that C282Y homozygote is necessary for iron overload development, but not sufficient to develop the disease¹⁵⁻¹⁷. Identification of the genetic basis for the different types of hemochromatosis has assisted in enhancing the knowledge of the scientific concept of physiological mechanisms of iron metabolism and particularly since the discovery of the Hcpidine hormone in 2000, the product of the liver and its role in iron metabolism, which functions in a similar way to the role of insulin in regulating glucose levels¹⁸⁻²⁰.

As a result of the evolution of technical development in this century most countries started to adopt national programs in order to raise awareness and draw attention to the hemochromatosis disease, especially that this disease is undergoing a silent period for several years before the onset of symptoms. Therefore, early diagnosis is very important before the development of non-reversible organic lesions in order to begin appropriate preventive treatment which is simple and inexpensive, and is limited to phlebotomy therapy²¹.



Thus, the study of the frequency of HFE gene mutations in general population has to be established in order to implement appropriate genetic tests in diagnostic and screening procedures for hemochromatosis.

We do not have any data about the prevalence of HFE gene mutations in Syrian population. We aim through this study to explore the three point mutations in HFE gene: H63D, S65C, C282Y in a large random sample of Syrians. In the context of this research we will be measuring the concentration of serum iron level to know the impact of the presence of HFE mutations on iron overload.

MATERIALS AND METHODS

Subjects

A total of 751 healthy blood donors were recruited from University Blood Transfusion Center in Damascus (their ages range: 18–65 years, average: 19.5 years), (90% males, 10% females). The donors happened to belong to different geographical regions in Syria.

Blood Sample

Peripheral blood sample were collected into two tubes, the first is sterile tube (Vacutainer, 4 ml) in EDTA to isolate genomic DNA, and the second (Vacutainer, 4 ml) is in heparine to measures serum iron level.

Isolation of genomic DNA Genomic

DNA was isolated from 2 mL peripheral blood using a conventional phenol/chloroform procedure. Briefly, red blood cells were lysed by the addition of 3 volumes of RBC lysis buffer (5 mM MgCl₂, 10 mM NaCl, 10 mM Tris-HCl pH7) and eliminated by 3 consecutive washes in the same buffer. Leukocyte pellets were resuspended in 1 ML water and lysed by the addition of 20 µL 10% SDS. After addition of 80 µL proteinase K (1 mg/mL), cell lysates were incubated at 54° C for 30 min under shaking, and then extracted with an equal volume of Trissaturated phenol (pH 8). After three extractions with chloroform, genomic DNA was precipitated by the addition of 2.2 mL of cold ethanol, washed with 75% ethanol, air dried and dissolved in 500 µL high quality water.

Multiplex-PCR amplification of exons 2-4 of the HFE gene

We chose multiplexing PCR reactions in order to reduce labor and number of PCR tubes. The first multiplex-PCR reaction amplified primers 1 and 2 of exons 2, whereas the second reaction amplified primers 3 and 4 of exon 4 of the HFE gene. Each multiplex-PCR reaction (25 µl final volume) contained the following components (final concentrations): buffer (1x), MgCl₂ (3 mM), dNTPs (200 µM each), DMSO (5%), primers (300 nM each, see Table 1), Taq polymerase (1 unit GoTaq, Promega) and gDNA (30-100 ng). PCR cycling conditions were as follows: initial denaturation 94O C for 3 min, then 35 cycles of 94O C for 45 sec, 58O C for 45 sec and 72O C for 55 sec (final elongation cycle at 72O C for 5 min). After electrophoresis in a 2% agarose gel containing ethidium bromide, the

amplified products were visualized and photographed using a UV transilluminator.

Table 1: Primers used to amplify exon 2,4 of the HFE gene

Primer ID	Sequence(5' →3')	PCR fragment
HFE-EX2-Fwrd	ACAGGACTCGCAACTCACCTTCACAA	514 bp
HFE-EX2-Rev	CTCCCTCTCCCTGCTsCCCACAA	
HFE-EX4-Fwrd	CTGAAAAGGGTATTTCCTCCTCCAA	445 bp
HFE-EX4-Rev	AGGCACTCCTCTCAACCCCAA	

Screening of point mutations in the HFE gene by real time PCR

Single nucleotide changes in the HFE gene corresponding to 3 known mutations were detected using 5'-FAM labeled Taqman MGB-probes (minor groove binding probes) designed to hybridize to HFE DNA segments encompassing each one of the tested mutations. For each mutation tested, two Taqman MGB probes were designed; one probe was fully identical with the sequence of the "wild type" allele, whereas the other probe was fully identical with the sequence of the mutant allele. In order to detect a mutation in every sample, two real-time PCR reactions were set up, one reaction contained a probe specific for the wild type allele, and the other contained a probe specific for the mutant allele. Each real-time PCR reaction (20 µl final volume) contained the following components (final concentrations): buffer (1x), MgCl₂ (4 mM), dNTPs (200 µM each), DMSO (5%), two primers (300 nM each) flanking the tested mutation, MGB-probe (120 nM each), 4 µl diluted (1:100) PCR products, Taq polymerase (1unit GoTaq, Promega).

Real-time PCR was performed using a Quantica instrument (Techne, England) with the following cycling conditions (30 cycles): 94O C for 15 sec, 60O C for 1 min (annealing and extension in one step). Fluorescence acquisition was performed in the FAM channel at the end of each cycle. Data were analyzed using Quansoft software (Techne).

For each reaction tube, amplification curves were plotted against cycle number. The shape of both amplification curves were compared to each other. The presence of a mutation was suspected if the amplification curve corresponding to a "mutant" probe showed a significant increase in fluorescence while that of the "wild type" probe did not increase significantly with increasing cycle number.

Iron analysis principle

Iron (Fe³⁺) is separated from transferrin by means of guanidinium chloride in the weakly acidic PH range and

reduced to Fe²⁺ ascorbic acid. Fe²⁺ Then forms a colored complex with ferrozine.

Statistical analysis

The allele and genotype frequencies were calculated in Excel Microsoft Office 2007. The fit to the Hardy-Weinberg equilibrium was tested for each variant locus in population. To define the correlation between the Serum iron levels and HFE mutations, the statistical analysis were performed using the Chi-squared test. A p value <0.05 was considered to be statistically significant.

RESULTS

By screening of 751 subjects included in this study; we have found three subjects with C282Y heterozygous mutation, while we have not found any subject with C282Y homozygous mutation, the allele frequency was of 0.2%. In contrast, the H63D mutation was found in high

frequency, 143 subject carriers of heterozygous mutation and 21 subject carriers of homozygous mutation, the allele frequency was of 12.3%. The S65C mutation was very rare and only one subject had heterozygous mutation pattern, allele frequency was of 0.07% (Table 2,3). No compound heterozygotes were found in our study sample.

It is worth nothing that it is still not possible to distinguish those who will develop iron overload and those who will not because the variability of penetrance.

To establish a relationship between clinical penetrance and genotype, we compared the serum iron level between 146 blood donors carrier of mutations (whether homozygous or heterozygous) and 156 normal blood donors (without mutation) as a control group. It was observed that there was no significant correlation between the two groups.

Table 2: Genotype frequencies of H63D, S65C and C282Y mutation in HFE gene in the study population

Genotype	H63D			S65C			C282Y		
	Wild Type C/C	Mutant Heterogeneous C/G	Mutant Homogeneous G/G	Wild Type A/A	Mutant Heterogeneous A/T	Mutant Homogeneous T/T	Wild Type G/G	Mutant Heterogeneous G/A	Mutant Homogeneous A/A
Subjects Number	587	143	21	750	1	0	748	3	0
Subjects (%)	78	19	2.8	99.9	0.1	0	99.6	0.4	0

Table 3: Allele Frequencies of H63D, S65C and C282Y mutation in the HFE gene in Syrian population .

Allele	H63D		S65C		C282Y	
	C	G	A	T	G	A
Frequency (%)	87.7	12.3	99.93	0.07	99.8	0.2

Table 4: Allele Frequencies of HFE in different populations as compared to the Syrian population

Population	Subjects	Allele frequencies %			Reference
		C282Y	H63D	S65C	
Ireland, north	1001	11	15	-	(32)
Denmark	876	5.6	12.8	1.8	(31)
Italy, north	1132	3.2	13.4	1.3	(33)
Italy, south	500	1.5	14.0	0.5	(34)
France	126	3.97	8.2	-	(35)
Russia	-	3.7	13.3	1.7	(36)
Slovenia	1282	4.0	14.5	0.5	(37)
Spain, north	1146	3.0	20.0	1.0	(38)
Spain, central	125	2.0	16.0	-	(39)
Bulgaria	100	0	23.0	-	(30)
India	225	0	9.09	-	(29)
Turkey	141	0	12.06	0	(26)
Iran	400	0	8.2	-	(27)
Jordan	440	0	11.25	0.11	(22)
Tunisia	570	0.09	15.7	-	(25)
Libya	100	0	17	-	(28)
Lebanon	115	0	12	-	(23)
Syria	751	0.2	12.3	0.07	Present study

DISCUSSION

This study is the first to evaluate the three common variants of the HFE gene in a sample representative of general Syrian population. Our study included 751 subjects and this is considered to be as the largest number to be studied in the Arab countries and neighboring countries so far (Table 4), which make our statistics closer to reality. Additional, the S282Y mutation still has not been discovered in Jordan²², Lebanon²³, Turkey²⁶ Iran²⁷ and Libya²⁸, but in Tunisia the frequency of this allele was very rare²⁵. Suggesting that this mutation is rare in the Middle East and this is consistent with our results, which showed that the frequency of S282Y mutation in Syria is 0.2%.

Despite our plan at the beginning of the study to find 20 patients from the reviewing clinics at Assad University Hospital with Hemochromatosis, in order to investigate HFE mutations they have, we have only found one patient from reviewers who was diagnosed with this disease. This patient is 65 years old and had been suffering from mild cirrhosis of the liver, and had a percentage of transferrin saturation higher than 40% (60%) and ferritin blood more than 1 mg/ml. This patient has heterozygous H63D mutation, with the absence of S282Y and S65C mutations. It is likely that this patient carries another mutation in the HFE gene which was not studied, and therefore might have a compound heterozygous mutation pattern.

Syria is a Middle Eastern country with a variety of demographic composition in population. The S282Y mutation was discovered in three individual of 751 Syrian people. This indicates that this variant might be generated from the genetic mixture between European and Syrian population in the 11 th and 12 th centuries. In addition, our results confirm that the frequency of S282Y mutation in Arab societies is notably low and rare from north to South. In Jordanian study the S282Y mutation was completely absent in 440 Jordanian people²² and in samples of Libya, Turkey, Iran (Table 4). It is clear from Table 4 that the prevalence of the S282Y mutation is higher in northern Europe, where the highest frequencies are observed in the Anglo-Saxon countries(6%-14%), and less frequent in south of Europe (about 1.5% in the south of Italy)³⁴.

There are not enough studies for the S65C mutation and we did not have adequate information about its prevalence. In our study, we found only one individual carrying this mutation in form heterozygous mutation, and there were no homozygous mutation.

H63D mutation is much more frequency than S282Y mutation. The geographical distribution in Europe is the opposite of the geographical distribution of S282Y mutation, and follows low gradient from southern Europe toward the north⁷ and the frequency of this mutant allele in most European populations studies is 10-20%. Our results have shown that the frequency of H63D mutation in Syrian population was of 12.3%, the allele frequency which is very close to the values seen in the Arab

countries (17.5% to 11.25%, see Table 8). The H63D mutation has been linked to a mild form of hemochromatosis and showed incomplete penetrance.

In conclusion, we analyzed the frequency of three point mutations (S282Y H63D, S65C) in HFE gene related to hemochromatosis disease in general Syrian population. For the first time in Arabe countries, we discovered the presence of S282Y mutation in three individual or subject of 751 people, this mutation which is the main cause for the development of this disease. This is consistent with the finding that this disease is very rare in Syria and the Arab countries in general. For this we think that we could not find a sufficient number of diagnosed patients. We have found in our study that only one person is a carrier of S65C mutation in heterozygous pattern. While, H63D mutation was more common in the Syrian population but this mutation plays a secondary role in the development of hemochromatosis compared with the S282Y mutation.

Our results indicate that there were no a significant correlation between the presence of the HFE mutations and serum iron level. Considering that most of the blood donors included in this study were young an average age of 19.5 years. Additionally, we believe that the presence of any of three point mutations in heterozygous or homozygous pattern might be causing a slight defect in the HFE protein and thus unable to significantly rise the serum iron level.

Finally, We recommend the following: Search mutations causing hemochromatosis disease in individuals who suspect carrying the disease in order to confirm it and take appropriate medical procedures. Explore these mutations among all members of families that prove the diagnosis of disease in one of its members, and to take appropriate preventive procedures to carrier mutations (phlebotomy therapy). Draw the attention of doctors and health workers to the presence of this disease in Syria though it is rare. Availability of genetic testing to detect point mutations that cause hemochromatosis as a service work that benefits the public.

REFERENCES

1. Rochette J, Le Gac G, Lassoued K, Férec C, Robson K J H. Factors influencing disease phenotype and penetrance in HFE haemochromatosis, *Hum Genet*, 128, 2010, 233-248.
2. Bonekamp NA, Volkl A, Fahimi HD, Schrader M, Reactive oxygen species and peroxisomes: struggling for balance, *Biofactors*, 35, 2009, 346–355.
3. Nichols L, Dickson G, Phan PG, Kant JA. Iron binding saturation and genotypic testing for hereditary hemochromatosis in patients with liver disease. *Am J Clin Pathol*, 125, 2006, 236–240.
4. Davis TM, Beilby J, Davis WA, Olynyk JK, Jeffrey GP, Rossi E, Boyder C, Bruce DG, Prevalence, characteristics and prognostic significance of HFE gene mutations in type 2 diabetes: The Fremantle Diabetes Study, *Diabetes Care*, 31, 2008, 1795–1801.



5. Carroll GJ. Primary osteoarthritis in the ankle joint is associated with finger metacarpophalangeal osteoarthritis and the H63D mutation in the HFE gene: evidence for a hemochromatosis-like polyarticular osteoarthritis phenotype, *J Clin Rheumatol*, 12, 2006, 109–113.
6. Feder J N, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis, *Nat Genet*, 13, 1996, 399–408.
7. Hanson EH, Imperatore G, Burke W, HFE gene and hereditary hemochromatosis: a HuGE review. Human Genome Epidemiology, *Am J Epidemiol*, 154 (3), 2001, 193–206.
8. Best LG, Harris PE, Spriggs EL. Hemochromatosis mutations C282Y and H63D in cis phase, *Clinical Genetics*, 60, 2001, 68–72.
9. Ferreira ACS, Oliveira VC, et al. Prevalence of C282Y and H63D mutations in the HFE gene of Brazilian individuals with clinical suspicion of hereditary hemochromatosis. *Rev. Bras. Hematol*, 30, 2008, 379-83.
10. Gochee PA, Powell LW, Cullen DJ, Du Sart D, Rossi E, Olynyk JK: A population-based study of the biochemical and clinical expression of the H63D hemochromatosis mutation. *Gastroenterology*, 122(3), 2002, 646-651.
11. Mura C, Ragueneas O, Ferec C. HFE mutations analysis in 711 hemochromatosis probands: evidence for S65C implication in mild form of hemochromatosis, *Blood*, 93, 1999, 2502–2505.
12. Asberg A, Thorstensen K, Hveem K, Bjerve KS: Hereditary hemochromatosis: the clinical significance of the S65C mutation *Genet Test*, 6(1), 2002, 59-62.
13. Gleeson, F; Ryan, E; Barrett, S; Crowe, J: Clinical expression of haemochromatosis in Irish C282Y homozygotes identified through family screening. *Eur J Gastroenterol Hepatol*, 16, 2004, 850-79.
14. Olsson KS, Konar J, Dufva IH, Ricksten A, Raha-Chowdhury R. Was the C282Y mutation an Irish Gaelic mutation that the Vikings helped disseminate? *Eur J Haematol*, 86, 2011, 75-82.
15. Allen KJ, Gurrin LC, Constantine CC, et al. Iron-overload-related disease in HFE hereditary hemochromatosis, *N Engl J Med*, 358, 2008, 221–230.
16. Pedersen P, Milman N Genetic screening for HFE hemochromatosis in 6, 020 Danish men: penetrance of C282Y, H63D, and S65C variants, *Ann Hematol*, 88, 2009, 775–784.
17. Aguilar-Martinez P, Bismuth M, et al. The Southern French registry of genetic hemochromatosis: a tool for determining clinical prevalence of the disorder and genotype penetrance, *Hematologica*, 95, 2010, 551–556.
18. Gao J, Chen J, Kramer M, Tsukamoto H, et al. Interaction of the hereditary hemochromatosis protein HFE with transferrin receptor 2 is required for transferrin-induced hepcidin expression, *Cell Metab*, 9, 2009, 217–227.
19. Krause A, Neitz S, Magert HJ, et al. LEAP-1, a novel highly disulfide-bonded human peptide, exhibits antimicrobial activity, *FEBS Lett*, 480, 2000, 147–150.
20. Pigeon C, Ilyin G. A new mouse liver-specific gene, encoding a protein homologous to human antimicrobial peptide hepcidin, is overexpressed during iron overload, *J Biol Chem*, 276, 2001, 7811–7819.
21. Pietrangelo A, Hereditary Hemochromatosis: Pathogenesis, Diagnosis, and Treatment, *Gastroenterology*, 139, 2010, 393–408.
22. Alkhatib A, Uzrail A, Bodoor K, Frequency of the hemochromatosis gene (HFE) variants in Jordanian Arab population and in diabetics from the same region, *Dis Marker*, 27 (1), 2009, 17-22.
23. Mahfouz RA, Saredidine DS, Charafeddine KM, Abdul Khalik RN, Cortas NK, Daher RT, Should we screen for hereditary hemochromatosis in healthy Lebanese: a pilot study, *Mol Biol Rep*, 39, 2012, 753-9.
24. Merryweather-Clarke A T, Pointon J J, Shearman J D and Robson K J, Global prevalence of putative haemochromatosis mutations, *J Med Genet*, 34, 1997, 275–278.
25. Sassi R, Hmida S, Kaabi H, Hajje A, Abid A, Abdelkefi S, Yacoub S, Maamar M, Mojaat N, Ben Hamed L, Bellali H, Dridi A, Jridi A, Midouni B, Boukef MK, Prevalence of C282Y and H63D mutations in the haemochromatosis (HFE) gene in Tunisian population, 47, 2004, 325-30.
26. Oztürk S, DİKİCİ H, DİNİER D, LÜLECİ G, Keser I Screening of the HFE Gene Mutations in Turkish Patients with Cryptogenic Cirrhosis and Hemochromatosis, *Turkiye Klinikleri J Med Sci*, 30, 2009, 1891-1895.
27. Karimi M, Yavarian M, Delbini P, Harteveld CL, Farjadian S, Fiorelli G, Giordano PC Spectrum and haplotypes of the HFE hemochromatosis gene in Iran: H63D in beta-thalassemia major and the first E277K homozygous, *Hematol J*, 5, 2004, 524-7
28. Elmrghni S, Dixon RA, Williams DR, Frequencies of HFE gene mutations associated with haemochromatosis in the population of Libya living in Benghazi, 4, 2011, 200-4.
29. Garewal G, Das R, Ahluwalia J, Marwaha RK, Prevalence of the H63D mutation of the HFE in north India: its presence does not cause iron overload in beta thalassemia trait, *Eur J Haematol*, 74, 2005, 333-6.
30. Ivanova A, von Ahsen N, Adjarov D et al., C282Y and H63D mutations in the HFE gene are not associated with porphyria cutanea tarda in Bulgaria, *Hepatology*, 30, 1999, 1531–1532.
31. Pederson P, Melson G V and Milman N, Frequencies of the haemochromatosis gene (HFE) variants C282Y, H63D and S65C in 6,020 ethnic Danish men, *Ann Hematol*, 87, 2008, 735–740.
32. Byrnes V, Ryan E, Barrett S, Kenny P, Mayne P, Crowe J, Genetic hemochromatosis, a Celtic disease: is it now time for population screening?, 5, 2001, 127-30.
33. Mariani R, Slavioni A, Corengia C, Erba N, Lanzafame C, De Micheli V, Baldini V, Arosio C, Fossati L, Trombini P, Oberkanins C, A Prevalence of HFE mutations in upper Northern Italy: Study of 1132 unrelated blood donors, *Dig Liver Dis*, 35, 2003, 479–481.
34. A. Pietrapertosa, A. Vitucci, D. Campanale, Palma A, Renzi R, Delios G, Tannoia N HFE gene mutations in Apulian

- population: allele frequencies, *Eur J Epidemiol*, 18, 2003, 685–689.
35. Mercier G, Burckel A, Bathelier C, Boillat E, Lucotte G Mutation analysis of the HLA-H gene in French hemochromatosis patients, and genetic counseling in families, *Genet Couns*, 9, 1998, 181-6.
36. Mikhaïlova SV, Kobzev VF, Kulikov IV, Romashchenko AG, Khasnulin VI, Voevoda MI Polymorphism of the HFE gene associated with hereditary hemochromatosis in populations of Russia, *Genetika*, 39, 2003, 988–995.
37. Cukjati M, Vaupoti T, Ruprecht R, Čurin-Šerbec V, Prevalence of H63D, S65C and C 282Y hereditary hemochromatosis gene mutations in Slovenian population by an improved high-throughput genotyping assay, *BMC Medical Genetics*, 8, 2007, 69.
38. A. Altes, A. Ruiz, M.J. Barcelo., Prevalence of the C282Y, H63D, and S65C mutations of theHFEgene in 1,146 newborns from a region of northern Spain, *Genet Test*, 8, 2004, 407–410.
39. Alvarez S, Mesa MS, Bandrés F, Arroyo E, C282Y and H63D mutation frequencies in a population from central Spain, *Dis Markers*, 17, 2001,111–114.

Source of Support: Nil, **Conflict of Interest:** None.

