

Low frequency of HFE gene mutations in Croatian patients suspected of having hereditary hemochromatosis

Milić, Sandra; Ristić, Smiljana; Starčević-Čizmarević, Nada; Brajenović-Milić, Bojana; Crnić-Martinović, Marija; Kapović, Miljenko; Peterlin, Boris; Štimac, Davor

Source / Izvornik: **Medical science monitor, 2011, 17, 552 - 556**

Journal article, Published version

Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

<https://doi.org/10.12659/MSM.881980>

Permanent link / Trajna poveznica: <https://um.nsk.hr/um:nbn:hr:184:296406>

Rights / Prava: [In copyright](#)/[Zaštićeno autorskim pravom.](#)

Download date / Datum preuzimanja: **2024-11-29**



Repository / Repozitorij:

[Repository of the University of Rijeka, Faculty of Medicine - FMRI Repository](#)



Received: 2011.02.20
Accepted: 2011.05.30
Published: 2011.10.01

Authors' Contribution:

- A** Study Design
- B** Data Collection
- C** Statistical Analysis
- D** Data Interpretation
- E** Manuscript Preparation
- F** Literature Search
- G** Funds Collection

Low frequency of *HFE* gene mutations in Croatian patients suspected of having hereditary hemochromatosis

Sandra Milić^{1ADEF}, Smiljana Ristić^{2ACDEF}, Nada Starčević-Čizmarević^{2B},
Bojana Brajenović-Milić^{2B}, Marija Crnić-Martinović^{3B}, Miljenko Kapović^{2B},
Borut Peterlin^{4A}, Davor Štimac^{1A}

¹ Department of Gastroenterology, University Hospital Rijeka, Rijeka, Croatia

² Department of Biology and Medical Genetics, School of Medicine, University of Rijeka, Rijeka, Croatia

³ Department of Transfusion Medicine, University of Rijeka, Rijeka, Croatia

⁴ Division of Medical Genetics, Department of Obstetrics and Gynecology, University of Ljubljana, Ljubljana, Slovenia

Source of support: Departmental sources

Background:

Hereditary hemochromatosis (HH) is a common autosomal recessive disorder in populations of European descent. It is characterized by a variable prevalence of mutations in the hemochromatosis gene (*HFE*) in different countries and a complex relationship between the *HFE* genotype and the HH phenotype. Genetic analysis has not been conducted in Croatian patients with iron overload. The aim of this study was to determine whether *HFE* mutations, C282Y, H63D, and S65C were correlated with clinical and biochemical parameters in Croatian patients with suspected HH.

Material/Methods:

Clinical examination, biochemical analysis, and genotyping were performed in 175 patients suspected of having HH. The control group consisted of 350 healthy blood donors.

Results:

Among the patients, 20% had genotypes related to HH – 7.4% were homozygous for C282Y, 6.3% were compound heterozygous for C282Y and H63D, 5.7% were homozygous for H63D, and 0.6% was compound heterozygous for C282Y and S65C. The allelic frequencies were 14.6% for C282Y mutation, 23.7% for H63D mutation, and 1.4% for S65C mutation. A comparison of the clinical and laboratory profiles of patients revealed that C282Y homozygotes had higher frequencies of all clinical symptoms and higher levels of biochemical parameters than others. The C282Y/H63D compound heterozygotes and H63D homozygotes were found to be clinically important, despite the fact that they were associated with less severe disease.

Conclusions:

Our results show that *HFE* mutations are responsible for only about 20% of Croatian patients with suspected HH. Screening with biochemical methods and *HFE* genotyping may be not sufficient for diagnoses in the Croatian population.

key words:

hemochromatosis • *HFE* mutations • iron overload • Croatia

Full-text PDF:

<http://www.medscimonit.com/fulltxt.php?ICID=881980>

Word count:

2485

Tables:

2

Figures:

–

References:

28

Author's address:

Sandra Milić, Department of Gastroenterology, University Hospital Rijeka, Krešimirova 42, Rijeka-51000, Croatia, e-mail: smilic05@gmail.com

BACKGROUND

Hereditary hemochromatosis (HH) is a common autosomal recessive disorder in populations of European descent and is characterized by iron accumulation in the body. The clinical features of HH are directly related to the level of body iron overload. In fully established disease, organ structure and function are impaired, and this may result in cirrhosis, hepatocellular carcinoma, diabetes mellitus, cardiomyopathy, sexual dysfunction due to hypogonadotropic hypogonadism, arthritis, and generalized skin pigmentation.

In 1996, 2 major mutations were identified in the hemochromatosis gene (*HFE*) – C282Y and H63D – which were significantly correlated with clinically manifested HH [1]. Homozygosity for the C282Y mutation was associated with a high risk for iron overload and this genotype was found to be present in approximately 60–100% of patients with HH. On the other hand, homozygosity for the H63D mutation, which was detected in 1–2% of patients with HH, was associated with slightly increased body iron status, but not a clinically significant iron overload. In addition, a significant proportion (4–6%) of patients with HH was found to be compound heterozygous for both mutations (C282Y/H63D) [2]. Later, a third rare mutation, S65C, was identified and found to be implicated in a mild form of the disease, often in patients that were compound heterozygous for both S65C and C282Y (C282Y/S65C) [3].

It is clear that the relationship between the hemochromatosis phenotype and the *HFE* genotype is complex. Incomplete penetrance of the *HFE* mutations and variable expression of the disease in patients that exhibit similar levels of iron overload indicated that the phenotypic expression of hemochromatosis may be influenced by modifier gene(s) and/or environmental factors [4].

Population studies have shown a variable prevalence of *HFE* gene mutations in different countries and ethnic groups. The distribution of the C282Y mutation frequency decreases from 9.7% in the population of northwestern Europe to zero in the population of southeastern Europe [5,6]. The prevalence of the C282Y mutation among healthy Croatian subjects (3%) is consistent with this north/south gradient [7]. The H63D mutation frequency is widely distributed in European populations (15–40%) and the S65C mutation is found with a frequency of about 1–2% [3,6]. In the Croatian population, the H63D and S65C mutations had prevalences of 14.5% and 1.8%, respectively [7].

A genetic analysis has not been performed in Croatian patients with iron overload. Therefore, the aim of this study was to determine the frequencies of C282Y, H63D, and S65C mutations in individuals with suspected HH and analyze correlations between genotypic and phenotypic characteristics.

MATERIAL AND METHODS

Patients

This study included a series of 175 (111 males and 64 females) patients admitted to the Division of Gastroenterology, University Hospital Rijeka, Croatia, from 2003 to 2008. All patients had high serum iron parameters (serum iron and/or

ferritin and/or transferrin saturation >45%) and at least twice the upper level of alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST); moreover, some showed HH-related symptoms. The median age of the patients was 58 years (range, 31–67).

The study exclusion criteria were previous conditions of thalassemia major, anemia (sideroblastic and hemolytic), transfusional iron overload, alcoholic liver disease, chronic viral hepatitis, steatosis, cirrhosis, porphyria cutanea tarda, portocaval shunt, or chronic hemodialysis.

The control group consisted of 350 healthy blood donors without liver disease or anemia. For each patient, 2 control subjects were selected of the same sex and age ± 5 years.

The Ethics Committee of the School of Medicine at the University of Rijeka approved the study, and written informed consent was obtained from each subject. The study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

Methods

The initial check-up consisted of a questionnaire and a clinical examination. The questionnaire recorded the details of age, sex, and medical history, including liver disease, arthralgias, cardiovascular disease and arrhythmias, treated or untreated diabetes, impotence, and premature amenorrhea. Dark skin pigmentation was established by clinical examination.

Biochemical tests included measurements for ALT, AST, serum iron (SF), serum unbound iron binding capacity (UIBC), serum total iron binding capacity (TIBC), and transferrin saturation (TS). The levels of SF, ALT, AST, ferritin, UIBC, and TIBC were measured on an Olympus AU 640 (Tokyo, Japan). Liver enzymes (AST and ALT) were measured with an optimized UV method without pyridoxal phosphate and at pH 7.8 for AST and pH 7.3 for ALT. SF was measured with 2,4,6-tris(2-pyridyl)-5-triazine and UIBC was measured on an Olympus AU 640 (Tokyo, Japan) with a colorimetric method. Turbidimetric latex immunoassay was used for serum ferritin measurement. The TIBC value was calculated as follows: TIBC = Fe + UIBC. The TS values were calculated as follows: TS (%) = serum iron/TIBC $\times 100$. The normal values among healthy adults were: ALT < 37 U/L; AST < 37 U/L; Fe = 8.7–26.9 $\mu\text{mol/L}$; UIBC = 27.6–53.6 $\mu\text{mol/L}$; TIBC = 45–63 $\mu\text{mol/L}$; ferritin = 10–120 $\mu\text{g/L}$ (female), 20–300 (male); and TS < 45%.

Genomic DNA was extracted from whole blood with a QIAmp Blood Kit (Qiagen, Hilden, Germany). Genotyping of *HFE* gene mutations (C282Y, H63D, and S65C) was performed with the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method, as described previously [3,5].

Statistics

Allele and genotype frequencies were estimated by gene counting. Differences in genotype and allele frequencies between the patient and control groups were analyzed for statistical significance with the χ^2 test or Fisher's exact test, as appropriate.

CR

Table 1. The frequencies of *HFE* genotypes and alleles in patients with high serum iron and healthy controls.

Genotype	Patients (N=175)		Healthy controls (N=350)		P value
	n	Frequency (%)	n	Frequency (%)	
wt/wt	71	40.6	233	66.6	<0.0001
C282Y/wt	13	7.4	11	3.1	0.0314
H63D/wt	52	29.7	85	24.3	0.1825
S65C/wt	4	2.3	9	2.6	0.8427
C282Y/C282Y	13	7.4	0	0	<0.0001
H63D/H63D	10	5.7	9	2.6	0.0764
C282Y/H63D	11	6.3	3	0.8	0.0006
C282Y/S65C	1	0.6	0	–	0.3333
Total	175	100.0	350	100.0	
Allele/total alleles					
C282Y	51/350	14.6	14/700	2.0	<0.0001
H63D	83/350	23.7	106/700	15.1	0.0007
S65C	5/350	1.4	9/700	1.3	1.0000

The differences in clinical manifestations of disease among different groups of patients were analyzed with the χ^2 test and Fisher's exact test.

Descriptive analysis was used for biochemical parameters (means, standard deviations). Biochemical variables were transformed into logarithmic values to establish the normal distribution of population parameters. Univariate analysis was performed with the analysis of variance (one-way ANOVA) for quantitative variables. Logistic regression was used for assessing the associations between biochemical parameters and different genotypes.

The level of significance was set at $p < 0.05$.

RESULTS

The *HFE* genotypes and corresponding allelic frequencies of the 3 mutations in patients and healthy controls are presented in Table 1. Among the 175 patients, 35 (20%) had genotypes that have been associated with HH (C282Y/C282Y, C282Y/H63D, H63D/H63D, and C282Y/S65C); however, 71 (40%) patients did not have any mutations in the *HFE* gene (wt/wt). Homozygosity for the C282Y mutation (7.4%) occurred significantly more frequently in patients than in healthy controls (no observations in controls; $p < 0.0001$). Furthermore, compound heterozygosity for C282Y/H63D occurred significantly more often in patients (6.3%) than in healthy controls (0.8%; $p = 0.0006$). Homozygosity for the H63D mutation also occurred more frequently in patients (5.7%) than in healthy controls (2.6%), but the difference was not significant ($p = 0.0764$). One patient was a compound heterozygote for C282Y/S65C (0.6%), but no healthy controls had that genotype. Heterozygosity for the C282Y mutation occurred significantly more frequently ($p = 0.0314$) in patients than in controls, but heterozygosity for H63D occurred with similar frequency in patients (29.7%) and controls (24.3%) ($p = 0.1825$).

The frequencies of alleles in patients and healthy controls showed that the C282Y allele (14.6%) was significantly more common in patients than in healthy controls (2.0%; $p < 0.0001$). The H63D allele mutation was also significantly more common in patients (23.7%) than in controls (15.1%; $p = 0.0007$). There were no differences in the allele frequencies of the S65C mutation between patients and controls ($p = 1.000$).

The clinical and biochemical parameters of the patients with iron overload are presented in Table 2. The frequencies of liver disease, diabetes, hyperpigmentation, arthralgia, and cardiovascular disease and arrhythmia were significantly higher in C282Y homozygotes than in wt/wt patients ($p = 0.0320$, $p = 0.0191$, $p = 0.0024$, $p < 0.001$, and $p < 0.001$, respectively). Furthermore, the HH-related symptoms occurred with similar frequency in C282Y homozygotes and C282Y/H63D compound heterozygotes. However, only 2 symptoms occurred significantly more frequently in the C282Y homozygotes compared to the H63D homozygotes: arthralgia ($p = 0.0097$) and cardiovascular disease ($p = 0.0089$). The C282Y/H63D compound heterozygotes also had arthralgia significantly more frequently than H63D homozygotes ($p = 0.0287$) and wt/wt patients ($p = 0.0004$). Liver disease was more common in H63D homozygotes than in wt/wt patients, but the difference was not statistically significant ($p = 0.0604$). Cardiovascular disease was more frequent in H63D heterozygotes than in wt/wt patients ($p = 0.0267$).

Both the SF and TS were significantly higher in C282Y homozygotes compared to wt/wt patients ($p = 0.0091$ and $p < 0.0001$, respectively) and C282Y/H63D heterozygotes ($p = 0.0011$ and $p = 0.0009$, respectively). The TS was higher in C282Y homozygotes than in H63D homozygotes ($p = 0.0044$). However, the biochemical parameters of C282Y/H63D heterozygotes and H63D homozygotes were not statistically different.

Table 2. Clinical features of patients with iron overload, grouped according to *HFE* genotype.

Genotype	n	Clinical symptoms N (%)						Biochemical parameters mean±SD		
		LD	DM	DS	A	CD	IA	SF	Fer	%TS
wt/wt	71	51 (71.8)	19 (26.8)	13 (18.3)	8 (11.3)	3 (4.2)	0	33.8±7.0	782.0±1464.5	51.1±14.5
C282Y/wt	13	11 (84.6)	2 (15.4)	4 (30.8)	2 (15.4)	0	0	32.0±5.2	800.5±1034.8	55.1±14.5
H63D/wt	52	41 (78.8)	13 (25.0)	16 (30.8)	12 (23.1)	9 (17.3)	1 (1.9)	36.6±9.0	359.0±335.9	53.9±15.5
S65C/wt	4	3 (75.0)	0	0	1 (25.0)	0	0	37.4±7.5	210.0±28.5	61.2±23.7
C282Y/C282Y	13	13 (100.0)	8 (61.5)	8 (61.5)	9 (69.2)	6 (46.1)	0	39.3±5.7	1022.7±880.5	73.0±13.5
H63D/H63D	10	10 (100.0)	4 (40.0)	4 (40.0)	1 (10.0)	1 (10.0)	0	34.8±6.7	396.5±490.0	54.2±14.7
C282Y/H63D	11	9 (81.8)	4 (36.4)	4 (36.4)	7 (63.6)	1 (9.0)	0	29.7±6.8	841.7±1674.7	53.4±11.0
C282Y/S65C	1	0	0	0	0	0	0	40.3	305.0	40.0

LD – liver disease; DM – diabetes; DS – dark skin pigmentation; A – arthralgia; CD – cardiovascular disease; IA – impotence or premature amenorrhea; SF – serum iron concentration; Fer – ferritin concentration; %TS – transferrin saturation.

DISCUSSION

This is the first study to investigate *HFE* gene mutations in Croatian patients with suspected HH. Croatia is in south-eastern Europe and borders the Mediterranean Sea. Its population is mainly of Slavic origin. A few years ago, a population analysis of the frequency of *HFE* mutations showed that the allelic frequency of C282Y (3.3%) was in accordance with the geographical position of Croatia in Europe; however, the allelic frequencies of H63D (14.5%) and S65C (1.8%) were similar to those reported in other European populations [7].

In this study, a genetic analysis of patients with iron overload showed that only 20% had HH-related genotypes and only 60% carried at least 1 *HFE* mutation. Among the HH related genotypes, we found that 7.4% were C282Y homozygotes, 6.3% were C282Y/H63D compound heterozygotes, 5.7% were H63D homozygotes, and 0.6% were C282Y/S65C compound heterozygotes. These findings differed from those reported for a similar population of patients in northern Europe, where over 90% of patients with HH were either C282Y homozygotes or C282Y/H63D compound heterozygotes [8–12]. However, our findings were consistent with data reported for other similar populations of patients in southern Europe, where approximately one-third of the patients did not carry either a C282Y or a H63D mutation [13–19]. A thorough comparison of patients from the neighboring countries of former Yugoslavia was not possible because no similar studies had been published. However, 1 study from a group in Slovenia found that 71.4% of patients with HH were C282Y homozygotes and 4.1% were C282Y/H63D compound heterozygotes [20].

Our results cannot be directly compared with other studies that have investigated the frequency of *HFE* mutations among patients diagnosed with HH. Nevertheless, we did observe relatively low frequencies of C282Y homozygosity, H63D homozygosity, and C282Y/H63D compound heterozygosity in patients with suspected HH. There are several possible explanations for these observations, which are not mutually exclusive. First, the C282Y mutation is less frequent

in the general population of Croatia than in most general populations of northern and central Europe. Therefore, the lower incidence of C282Y mutation may be characteristic of Croatian patients. Second, other mutations may exist in the *HFE* gene, or in other genes involved in iron homeostasis, that might be responsible for HH in Croatia.

Another explanation could be related to the selection of patients enrolled in the study. Specifically, patients were selected for high serum iron parameters (serum iron and/or ferritin and/or TS >45%) and at least twice the normal upper level of ALT and/or AST. We chose these criteria because these values suggested that these patients could be readily differentiated from individuals with elevated transaminase levels that were disease-free. However, these selection criteria may have excluded patients with iron overload and *HFE* mutations that had normal ALT/AST levels; thus, this selection may have contributed to the low frequency of *HFE* mutations observed in our sample of patients.

Also, by selecting patients with elevated ALT/AST, we may have included patients with an increased chance of liver disease of unknown etiology that did not carry *HFE* mutations. However, all patients with secondary iron overload were excluded from the study on the basis of clinical histories and detailed clinical analyses performed at the beginning of the study [21]. Only the cases of nonalcoholic fatty liver disease (NAFLD) were somewhat controversial, because they were excluded after examination by abdominal ultrasound, and not by biopsy; therefore, this is a potential weakness of this study. Abdominal ultrasonography has been shown to have a sensitivity of 60%–94% and a specificity of 84%–95% for detecting a fatty liver. However, ultrasonography cannot detect small amounts of hepatic steatosis and cannot establish the diagnosis of non-alcoholic steatohepatitis or determine the stage of hepatic fibrosis, which may have increased the levels of ferritin and/or serum iron [22].

The manifestations of iron overload are known to develop gradually in 3 distinct stages: the latency stage, the biochemical expression stage, which corresponds to elevated serum iron parameters (observed in young adults), and the

clinical expression stage. However, the onset of the disease is often insidious, and affected individuals display widely variable biochemical signs, clinical expression, and evolution of the disease.

In our sample of patients, all the clinical symptoms associated with HH were observed in C282Y homozygotes at higher frequencies than in wt/wt patients. On the other hand, the HH-related symptoms were observed among C282Y homozygotes at frequencies similar to those observed in C282Y/H63D compound heterozygotes and H63D homozygotes, who are also considered to be at risk for HH. This clinical expression of the disease with similar frequency in all 3 genotypes suggests that C282Y/H63D compound heterozygosity and H63D homozygosity are clinically important, despite their association with lower levels of iron overload. We found only 1 patient that carried the S65C mutation, and it was in compound heterozygosity with the C282Y mutation. This patient exhibited a mild phenotype, but the rarity of the mutation precluded making any conclusions about this variant.

In this study, a relatively high incidence of clinical symptoms was observed in all categories of patients, including the wt/wt patients. This was in contrast to most other recent studies, which reported a low clinical penetrance, even in the C282Y homozygote groups [23–28]. In patients with iron overload, the severity of the phenotype is known to depend on a complex interplay of genetic and epigenetic factors, age, sex, and environmental conditions such as diet, alcohol intake, and blood loss. However, the most likely explanation for the discrepancies between our results and those of other studies was that our study involved genotyping patients suspected of having HH based on elevated iron parameters levels and/or clinical symptoms. In contrast, other studies enrolled participants without regard to any clinical conditions or symptoms typically associated with HH at the time of screening. In addition, the selection of patients in our study included only those with high ALT/AST; this certainly may have contributed to the high incidence of clinical symptoms observed among all genotypes.

CONCLUSIONS

Our results showed that *HFE* mutations were responsible for about 20% of Croatian patients with suspected HH. Thus, for patients with chronic liver disease or suspected iron overload, screening with biochemical methods and *HFE* genotyping may be not sufficient for diagnoses in the Croatian population, and further research is needed to identify other non-*HFE* genetic causes of HH. On the other hand, 20% is not a negligible percentage in everyday clinical practice. This emphasizes the importance of screening patients to enable the prediction of symptoms before they appear and also screening family members that may not yet show signs of HH. The results of this study should be taken into account in the diagnosis, screening, and management of HH in Croatia.

REFERENCES:

- Feder JN, Gnirke A, Thomas W et al: A novel MHC class I-like is mutated in patients with hereditary haemochromatosis. *Nat Genet*, 1996; 13: 399–408
- Hanson EH, Imperatore G, Burke W: *HFE* gene and hereditary hemochromatosis: a HuGE review. *Am J Epidemiol*, 2001; 154: 193–205
- Mura C, Raguene O, Ferenc C: *HFE* analysis in 711 hemochromatosis probands: evidence for S65C implication in mild form of hemochromatosis. *Blood*, 1999; 93: 2502–5
- Beutler E: Iron storage disease: facts, fiction and progress. *Blood Cells Mol Dis*, 2007; 39: 140–47
- Merryweather-Clarke AT, Pointon JJ, Shearman JD, Robson KJH: Global prevalence of putative haemochromatosis mutations. *J Med Genet*, 1997; 34: 275–78
- Merryweather-Clarke AT, Pointon JJ, Jouanolle AM et al: Geography of the *HFE* C282Y and H63D mutations. *Genet Test*, 2000; 4: 183–98
- Ristić S, Makuc J, Starčević N et al: Hemochromatosis gene mutations in the Croatian and Slovenian populations. *Clin Genet*, 2003; 64: 444–46
- The UK Haemochromatosis Consortium. A simple genetic test identifies 90% of UK patients with haemochromatosis. *Gut*, 1997; 41: 841–44
- Nielsen P, Carpinteiro S, Fischer R et al: Prevalence of the C282Y and H63D mutations in the *HFE* gene in patients with hereditary haemochromatosis and in control subjects from Northern Germany. *Br J Haematol*, 1998; 103: 842–45
- Cardoso EMP, Stal P, Hagen K et al: *HFE* mutations in patients with hereditary hemochromatosis. *J Intern Med*, 1998; 243: 203–8
- Ryan E, O'Keane C, Crowe J: Hemochromatosis in Ireland and *HFE*. *Blood Cells Mol Dis*, 1998; 24: 314–20
- Milman N, Koefoed P, Pedersen P et al: Frequency of the *HFE* C282Y and H63D mutations in Danish patients with clinical haemochromatosis initially diagnosed by phenotypic methods. *Eur J Haematol*, 2003; 71 (6): 403–7
- Carella M, D'Ambrosio L, Totaro A et al: Mutation analysis of the HLA H gene in Italian hemochromatosis patients. *Am J Hum Genet*, 1997; 60: 828–32
- Camaschella C, Fargion S, Sampietro M et al: Inherited *HFE* unrelated hemochromatosis in Italian families. *Hepatology*, 1999; 29: 1563–64
- Piperno A, Sampietro M, Pietrangelo A: Heterogeneity of hemochromatosis in Italy. *Gastroenterology*, 1998; 114: 996–1002
- Neghina AM, Anghel A, Sporea I et al: Mutant *HFE* genotype leads to significant iron overload in patients with liver diseases from western Romania. *J Appl Genet*, 2009; 50: 173–76
- Gómez-Llorente C, Miranda-León MT, Blanco S et al: Frequency and clinical expression of *HFE* gene mutations in a Spanish population of subjects with abnormal iron metabolism. *Ann Hematol*, 2005; 84: 650–55
- de Diego C, Murga MJ, Martínez-Castro P: Frequency of *HFE* H63D, S65C, and C282Y mutations in patients with iron overload and controls from Toledo, Spain. *Genet Test*, 2004; 8: 263–67
- Papanikolaou G, Politou M, Terpos E et al: Hereditary hemochromatosis: *HFE* mutation analysis in Greeks reveals genetic heterogeneity. *Blood Cells Mol Dis*, 2000; 26: 163–68
- Stepec S, Makuc J, Markovic S et al: Distribution of *HFE* gene mutations in Slovenian patients with hereditary hemochromatosis. *Ann Hematol*, 2008; 87: 667–69
- Sikorska K, Stalke, Izycka-Swiczewska E et al: The role of iron overload and *HFE* gene mutations in the era of pegylated interferon and ribavirin treatment of chronic hepatitis C. *Med Sci Monit*, 2010; 16(3): 137–43
- Mishra P, Younossi ZM: Abdominal ultrasound for diagnosis of non-alcoholic fatty liver disease (NAFLD). *Am J Gastroenterol*, 2007; 102(12): 2716–17
- McLaren GD, McLaren CE, Adams PC et al: Clinical manifestations of hemochromatosis in *HFE* C282Y homozygotes identified by screening. *Can J Gastroenterol*, 2008; 22: 923–30
- Ellervik C, Birgens H, Tybjaerg-Hansen A, Nordestgaard BG: Hemochromatosis genotypes and risk of 31 disease endpoints: meta-analyses including 66,000 cases and 226,000 controls. *Hepatology*, 2007; 46: 1071–80
- Asberg A, Hveem K, Thorstensen K et al: Screening for hemochromatosis: high prevalence and low morbidity in an unselected population of 65 238 persons. *Scand J Gastroenterol*, 2001; 36: 1108–15
- Olynyk JK, Cullen DJ, Aquilia S et al: A population-based study of the clinical expression of the hemochromatosis gene. *N Engl J Med*, 1999; 341: 718–24
- Beutler E, Felitti VJ, Koziol JA et al: Penetrance of 845G-A (C282Y) *HFE* hereditary hemochromatosis mutation in the USA. *Lancet*, 2002; 359: 211–18
- Pedersen P, Milman N: Genetic screening for *HFE* hemochromatosis in 6,020 Danish men: penetrance of C282Y, H63D, and S65C variants. *Ann Hematol*, 2009; 88: 775–84