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Original Research Article

Association of *HFE* gene C282Y and H63D mutations with liver cirrhosis in the Lithuanian population

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ABSTRACT

Background and objective: Liver cirrhosis is the end-stage disease of chronic liver injury. Due to differences in the natural course of chronic liver diseases, identification of genetic factors that influence individual outcomes is warranted. *HFE*-linked hereditary hemochromatosis (HH) predisposes disease progression to cirrhosis; however, the role of heterozygous C282Y or H63D mutations in the development of cirrhosis in the presence of other etiological factors is still debated. The aim of this study was to determine the association between heterozygous C282Y and H63D mutations and non-HH liver cirrhosis in Lithuanian population.

Materials and methods: The patient cohort consisted of 209 individuals. Diagnosis of cirrhosis was confirmed by clinical, laboratory parameters, liver biopsy, and radiological imaging. Control samples were obtained from 1005 randomly selected unrelated healthy individuals. *HFE* gene mutations were determined using the PCR-RFLP method.

Results: The most common causes of cirrhosis were hepatitis C (33.9%), hepatitis B (13.6%), and alcohol (25.8%). C282Y allele was associated with the presence of cirrhosis (OR = 2.07; $P = 0.005$); this was also observed under recessive model for C282Y (OR = 2.06, $P = 0.008$). The prevalence of C282Y allele was higher in cirrhotic men than in controls (7.0% vs. 2.8%, $P = 0.002$). The carriage of H63D risk allele (OR = 1.54; $P = 0.02$), heterozygous C282Y/wt and homozygous H63D/H63D genotypes were associated with liver cirrhosis in males (OR = 2.48, $P = 0.008$, and OR = 4.13, $P = 0.005$, respectively).

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Conclusions: Heterozygous C282Y mutation of the *HFE* gene was associated with liver cirrhosis in the Lithuanian population. In gender-related analysis, heterozygous C282Y and homozygous H63D mutations were linked to liver cirrhosis in men, not in women.

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1. Introduction

Liver cirrhosis is the end-stage disease of chronic liver injury. Cirrhosis is caused by different etiological factors; however, progression of liver injury varies considerably among individuals independently of the cause [1]. Different research groups over the last decade have attempted to identify crucial cofactors that contribute to the development of liver damage [2,3]. A growing number of studies show that, apart from the main underlying causative agent in liver cirrhosis, the process may be reinforced by confounding factors such as diet, alcohol consumption, etc. [4–6]. Interindividual variation of time span from normal liver to fibrotic and cirrhotic stages suggested potential influence of congenital variations. Advances in genotyping techniques allowed to identify coexisting genetic alterations in relation to liver fibrosis [6] and cirrhosis of different etiologies [4,7].

C282Y and H63D mutations of the *HFE* gene are now recognized as the most common genetic disorders in populations of European ancestry. Carriage of heterozygous hemochromatosis (HH) gene mutations has been attributed as the risk factor of iron overload and liver damage, but equivocal conclusion on the role of these mutations has not been achieved [8,9]. The rationale that suggested iron as a susceptible hepatotoxic factor is based on the ability of this metal to induce oxidative stress by stimulating free radical formation in liver tissue [10,11]. Furthermore, increased contents of iron have been attributed to progression to liver cirrhosis of chronic viral hepatitis C (HCV) infection [12], nonalcoholic fatty liver disease (NAFLD) [11] or alcoholic liver disease (ALD) [13].

As noted above, development of liver cirrhosis regardless of etiology in separate individuals may have enormous variation in terms of time frame and severity. Carriage of *HFE* gene mutations has been linked with increased risk of liver fibrosis or liver cirrhosis; however, published studies report conflicting results [8]. The presence of the C282Y mutation was associated with more advanced degrees of fibrosis or cirrhosis [12,14], but these findings were not confirmed in other studies [11,15]. The prevalence of *HFE* C282Y mutations varies significantly across Europe, with highest estimated in Ireland (>10%), intermediate frequencies (2.7%–7%) in neighboring countries Latvia [16] and Poland [17], and very low rates of (0%–2%) in Mediterranean areas [18]. *HFE* H63D mutation also occurs at different frequencies in separate regions [18]. Therefore, the discordance among the findings in previous studies on association of *HFE* mutations with non-HH liver cirrhosis/fibrosis might be related to variations in study design and differences in *HFE* mutation prevalence in individual populations.

In this study we performed analysis of *HFE* gene C282Y and H63D mutations in consecutive 209 cirrhotic patients and 1005 voluntary, unrelated blood donors of the Caucasian ethnicity. The aim of this study was to determine the association between *HFE* gene C282Y and H63D mutations and liver cirrhosis in the Lithuanian population. This was the first study assessing the prevalence of *HFE* gene mutations in Lithuanian cirrhotic patients and adds additional insights on the impact of *HFE* mutations in development of non-HH cirrhosis.

2. Materials and methods

2.1. Patients and control subjects

A cohort of liver cirrhosis patients consisted of 209 consecutive patients referred to the Department of Gastroenterology, Hospital of the Lithuanian University of Health Sciences. The diagnosis and etiology of liver cirrhosis was confirmed by laboratory tests, clinical features, liver biopsy and radiological imaging tests. ALD was confirmed when daily consumption of alcohol was >30/20 g/day for males/females, respectively, as confirmed by at least 1 family member of affected individuals [19]. Control samples came from our previous genotyping study on the prevalence of *HFE* mutations in the Lithuanian population [20] and included 1005 voluntary, unrelated Lithuanian blood donors. The study design met ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Lithuanian Bioethics Committee (Protocol No. 2/2008) and Kaunas Regional Biomedical Research Ethics Committee (Protocol No. BE-2-10). Informed consent was obtained from all participants.

2.2. DNA extraction and genotyping

Genomic DNA was isolated from whole blood containing EDTA by using salting-out procedure. *HFE* mutations C282Y c.845 G>A (p.Cys282Tyr) and H63D c.187 C>G (p.His63Asp) were detected after DNA amplification by polymerase chain reaction and restriction with RsaI (for C282Y) and BclI (for H63D). For identification of the C282Y mutation, the fragment was amplified using primer forward 5′-TCCAGTCTTCCTGGCAA-3′ and primer reverse 5′-TTCTAGCTCCTGGCTCTCA-3′. The exon 2 containing S65C and H63D mutations were amplified with primer forward 5′-TGTGGAGCCTCAACATCCT-3′ and primer reverse 5′-TGAAAAGCTCTGACAACCTCA-3′. PCR amplification was performed in a total volume of 25 μl, which contained 100 ng of genomic DNA, 200 μM of each dNTP, 200 nM of each primer, 1.0 mM $MgCl_2$, 10× PCR buffer solution, and 2.5 U Taq polymerase (Thermo Scientific, Vilnius, Lithuania). PCR

consisted of an initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 56 °C for 30 s, and extension at 72 °C for 30 s with final extension at 72 °C for 10 min. The restrictions reactions were performed according manufacturer's protocol. The RFLP fragments were analyzed electrophoretically in 3% of agarose gel.

2.3. Statistical analysis

The distribution of *HFE* genotypes in both cases and controls was examined for deviation from Hardy Weinberg equilibrium (HWE) using the chi-square (χ^2) test in each single nucleotide polymorphism (SNP). Comparisons of carriage frequencies for alleles between cases and controls were analyzed by Pearson chi-square and Fisher exact tests. Association analysis based on the case-control design was performed for each SNP by using the Armitage trend test. To estimate relative risks for mutations, odds ratios (ORs) and 95% confidence intervals (95% CI) were calculated using recessive and dominant models. A *P* value of 0.05 was defined to be statistically significant. Statistical analysis was performed using statistical software for genetic association studies PLINK v2.050 [21].

3. Results

3.1. Characteristics of the study group

The characteristics of the study groups are presented in Table 1. The group of cirrhotic patients consisted of 209 individuals: 107 men and 102 women with a mean age of 54.0 years (range, 25–84 years). The most common cause of liver cirrhosis was HCV infection and alcohol consumption. Distribution of Child–Pugh classes A, B, and C in the cirrhotic group was 31.1%, 47.8%, and 21.1%, respectively. The control group consisted of 1005 individuals: 581 men and 424 women with a mean age of 37.1 years (range, 18–65 years). In more detail, the control group was described in our previous study [20].

Table 1 – Characteristics of the subject groups.

	Liver cirrhosis (*n* = 209)	Controls (*n* = 1005)
Gender, *n* (%)		
Male	107 (51.2)	581 (57.8)
Female	102 (48.8)	424 (42.2)
Age, years (SD)	54.0 (8.1)	37.1 (4.3)
Etiology of liver cirrhosis, *n* (%)[a]		
HCV	71 (33.9)	
HBV	28 (13.6)	
Autoimmune	15 (7.1)	
Alcohol	54 (25.8)	
Other causes	12 (5.7)	
Cryptogenic	19 (9.1)	
Child–Pugh class, *n* (%)		
A	65 (31.1)	
B	100 (47.8)	
C	44 (21.1)	

HBV, hepatitis B virus; HCV, hepatitis C virus.
[a] 17 of HCV and 5 of HBV patients had a mixed (viral and alcohol) etiology.

3.2. Association between HFE C282Y mutation and liver cirrhosis

The association between *HFE* C282Y mutation and liver cirrhosis is presented in Table 2. The carriage of C282Y risk allele was significantly more frequent in patients with liver cirrhosis than in controls (OR = 2.1, *P* = 0.005). This association was also evident in genotypic analysis where heterozygous genotype C282Y/wt and wt/wt carriers were compared (OR = 2.0, *P* = 0.01) and in recessive model (OR = 2.1, *P* = 0.007).

Table 2 – The frequencies of *HFE* C282Y and H63D mutations in cirrhotic patients and controls.

Genotype	Liver cirrhosis (*n* = 209) *n* (%)	Controls (*n* = 1005) *n* (%)	OR (95% CI)	*P*
H63D				
wt/wt	142 (67.9)	712 (70.84)	1.00 (reference)	
H63D/wt	58 (27.8)	267 (26.57)	1.09 (0.78–1.53)	0.618
H63D/H63D	9 (4.3)	26 (2.58)	1.74 (0.80–3.78)	0.161
wt/wt vs H63D/wt + H63D/H63D			1.15 (0.83–1.58)	0.403
wt/wt + H63D/wt vs. H63D/H63D			0.59 (0.83–1.58)	0.403
wt allele	342 (81.9)	1682 (84.47)		
H63D allele	76 (18.1)	320 (15.53)	1.18 (0.89–1.55)	0.244
C282Y				
wt/wt	189 (90.43)	956 (95.5)	1.00 (reference)	
C282Y/wt	19 (9.09)	48 (4.79)	2 (1.15–3.48)	**0.012**
C282Y/C282Y	1 (0.48)	1 (0.09)	5.06 (0.32–81.22)	0.203
wt/wt vs. C282Y/wt + C282Y/C282Y			2.07 (1.20–3.55)	**0.008**
wt/wt + C282Y/wt vs. C282Y/C282Y			0.21 (0.01–3.33)	0.219
wt allele	397 (94.98)	1952 (97.51)		
C282Y allele	21 (5.02)	50 (2.49)	2.07 (1.23–3.49)	**0.005**
C282Y/H63D	6 (2.87)	13 (1.29)	2.26 (0.85–6.00)	0.118

OR, odds ratio; CI, confidence interval; In bold, significant *p*-values.

Table 3 – The frequencies of *HFE* C282Y and H63D mutations in male cirrhotic patients and controls.

Genotype	Men with liver cirrhosis (n = 107) n (%)	Controls (n = 581) n (%)	OR (95% CI)	P
H63D				
wt/wt	67 (62.61)	415 (71.41)	1.00 (reference)	
H63D/wt	34 (31.77)	157 (27.02)	1.34 (0.85–2.11)	0.202
H63D/H63D	6 (5.62)	9 (1.57)	4.13 (1.42–11.98)	**0.005**
wt/wt vs H63D/wt + H63D/H63D			1.49 (0.97–2.30)	0.067
wt/wt + H63D/wt vs. H63D/H63D			0.27 (0.09–0.76)	**0.008**
wt allele	168 (78.51)	987 (84.94)		
H63D allele	46 (21.49)	175 (15.06)	1.54 (1.07–2.22)	**0.019**
C282Y				
wt/wt	93 (86.92)	549 (94.49)	1.00 (reference)	
C282Y/wt	13 (12.15)	31 (5.33)	2.48 (1.25–4.91)	**0.008**
C282Y/C282Y	1 (0.93)	1 (0.18)	5.90 (0.37–95.20)	0.155
wt/wt vs. C282Y/wt + C282Y/C282Y			2.58 (1.33–5.02)	**0.004**
wt/wt + C282Y/wt vs. C282Y/C282Y			0.18 (0.01–2.95)	0.178
wt allele	199 (92.99)	1129 (97.16)		
C282Y allele	15 (7.01)	33 (2.84)	2.58 (1.38–4.84)	**0.002**
C282Y/H63D	5 (4.67)	12 (2.06)	2.32 (0.80–6.73)	0.163

OR, odds ratio; CI, confidence interval; In bold, significant p-values.

Gender-based stratification analysis revealed significant gender-related differences in the carriage of C282Y mutation between liver cirrhosis patients and controls (Tables 3 and 4). The carriage of heterozygous C282Y/wt genotype in men was associated with liver cirrhosis (OR = 2.48, P = 0.008), whereas no significant associations were found in the female group (Table 4).

3.3. *Association between HFE H63D mutation and liver cirrhosis*

Genotyping analysis of *HFE* gene H63D mutation did not reveal significant association with liver cirrhosis (Table 2). The carriage of H63D alleles was distributed equally in the control (15.5%) and cirrhotic groups (18.1%, P = 0.244). However, significant gender-related differences were revealed in carriage of H63D mutation between liver cirrhosis patients and controls (Tables 3 and 4). The carriage of H63D risk allele (OR = 1.54, P = 0.02) and homozygous H63D/H63D genotypes (OR = 4.13, P = 0.005) were associated with liver cirrhosis in men, but not women.

3.4. *Association between the carriage of two or more HFE gene alleles and liver cirrhosis*

Carriage of two or more risk alleles was significantly higher in the group of patients with liver cirrhosis than in controls and resulted in significant association (7.66% vs. 3.98%, P = 0.021;

Table 4 – The frequencies of *HFE* C282Y and H63D mutations in female cirrhotic patients and controls.

Genotype	Women with liver cirrhosis (n = 102) n (%)	Controls (n = 424) n (%)	OR (95% CI)	P
H63D				
wt/wt	75 (73.53)	297 (70.05)	1.00 (reference)	
H63D/wt	24 (23.53)	110 (25.94)	0.86 (0.52–1.44)	0.573
H63D/H63D	3 (2.94)	17 (4.01)	0.70 (0.20–2.45)	0.573
wt/wt vs H63D/wt + H63D/H63D			0.84 (0.52–1.37)	0.488
wt/wt + H63D/wt vs. H63D/H63D			1.38 (0.40–4.80)	0.613
wt allele	174 (85.29)	704 (83.02)		
H63D allele	30 (14.71)	144 (16.98)	0.84 (0.55–1.29)	0.432
C282Y				
wt/wt	96 (94.12)	407 (95.91)	1.00 (reference)	
C282Y/wt	6 (5.88)	17 (4.01)	1.50 (0.58–3.90)	0.406
C282Y/C282Y	0 (0)	0 (0)	4.22 (0.08–214.11)	1
wt/wt vs. C282Y/wt + C282Y/C282Y			1.50 (0.58–3.90)	0.406
wt/wt + C282Y/wt vs. C282Y/C282Y			0.24 (0.01–12.24)	1
wt allele	98 (97.06)	407 (97.99)		
C282Y allele	6 (2.94)	17 (2.01)	1.48 (0.58–3.81)	0.412
C282Y/H63D	1 (0.98)	1 (0.24)	4.78 (0.30–77.10)	0.350

OR, odds ratio; CI, confidence interval.

OR = 2.00, 95% CI = 1.10–3.64). Gender-based analysis revealed that in male patients relative risk was increased when bearing two or more risk alleles compared with controls (11.22% vs. 3.78%, $P = 0.001$; OR = 3.22, 95% CI = 1.54–6.71), whereas no significant difference was observed in women. The prevalence of particular C282Y/H63D compound heterozygous genotype in Lithuanian cirrhotic patients is presented in Table 2. Only a small number of individuals were carriers of the C282Y/H63D genotype, and the difference between cirrhotic patients and controls was not significant.

4. Discussion

The major finding of our present study is a significant gender-related association of C282Y and H63D mutations in the *HFE* gene with liver cirrhosis in the Lithuanian population. The impact of *HFE* mutations was found to be significant in male, but not in female subjects. These data suggest that *HFE* mutations may contribute to hepatic fibrogenesis process during the natural history of chronic liver diseases. This is the first study to assess the prevalence of *HFE* gene C282Y and H63D mutations in Lithuanian cirrhotic patients.

Individuals with chronic liver diseases may have mild to moderate iron overload, but the mechanism behind this phenomenon in not fully understood [22]. Increased hepatic iron content is known to have the potential to exacerbate liver injury [10,11]. Furthermore, different groups have provided evidence that levels of iron near the upper limit of normal are associated with different pathological processes including cardiovascular diseases and even cancer [23–25]. C282Y and H63D are the most common mutations causing HH in Caucasians, but studies over the last years have revealed that these mutations have lower penetration than previously estimated, and cannot be advocated alone for the development of HH [26]. Elevated liver enzymes were observed only in 30% of males, while elevated transferrin saturation in combination with an elevated ferritin was present in 43.4% of males and 23.3% of females homozygous for C282Y [26]. Further studies showed that cirrhosis was diagnosed only in 6% of males and in 2% of females in a population-based screening setting among C282Y homozygotes [27]. Nevertheless, even carriage of heterozygous C282Y and H63D variants has been suggested to increase iron overload and exacerbate chronic non-HH related liver injury [12,14].

Studies in different populations examining the relationship between *HFE* mutations and chronic liver diseases have produced varying outcomes. A study including 587 patients from Italy with NAFLD and 184 control subjects did not find a link between *HFE* mutations and hepatocellular iron accumulation [11]. A Canadian study has demonstrated that Caucasian C282Y heterozygotes were more likely to have bridging fibrosis or cirrhosis in non-alcoholic steatohepatitis (NASH) [14], meanwhile this link was not present in a study by Chitturi et al. [15]. A Polish study conducted by Raszeja-Wyszomirska et al. showed a trend toward a more common occurrence of ALD in individuals homozygous for the H63D mutation [28], while another study found no differences in the prevalence of *HFE* mutations between Polish cirrhotic patients and healthy individuals [29]. C282Y or H63D heterozygosity was found as an independent risk factor for liver fibrosis and cirrhosis in a German study including 401 patients with chronic HCV infection and 295 healthy controls [30]. The presence of *HFE* mutations was independently associated with iron loading and advanced fibrosis in patients with HCV, especially after controlling for duration of disease [12]. Whereas, a Scottish study has shown that carriage of *HFE* mutations does not have any role in the accumulation of iron or the progression of liver disease in HCV infection [31]. Similar results were observed in the Czech study which has demonstrated that *HFE* mutations do not play an important role in the pathogenesis of chronic hepatitis B, chronic hepatitis C or alcoholic liver disease [32]. Interestingly, an Indian study has observed significant associations of common *HFE* mutations (C282Y and H63D) with HCV and ALD related liver cirrhosis, even though the mutations are relatively rare in this population [33]. Another study in non-European population has suggested that iron overload and *HFE* gene mutations do not play a primary role in cryptogenic cirrhosis in the south Iranian population [34].

Varying results between previous studies have urged us to examine the above-discussed associations in a cohort of Lithuanian cirrhotic patients. The results of our study support those studies that have revealed a significant role of C282Y and H63D mutations in non HH-related liver cirrhosis in Caucasian populations. Overall analysis has revealed that C282Y mutation is associated with liver cirrhosis in our study population, but this observation has not been found for H63D carriers. Gender based stratification analysis of our data revealed that carriage of *HFE* risk allele C282Y was associated with liver cirrhosis in males, but not in females. Association of liver cirrhosis in men was also evident among the carriers of heterozygous C282Y genotype. Furthermore, in males the carriage of homozygous H63D genotypes were also associated with liver cirrhosis, while this relationship was not present in females. There was a trend for increased risk of liver cirrhosis among female carriers of C282Y mutation, but due to a relatively small sample size the difference did not reach statistical significance. It is well known that the penetrance of *HFE* C282Y homozygous subjects is higher in males than in females, who, due to physiological mechanisms, are less likely to develop iron overload [22,27]. As pointed out by Fargion et al., discrepant results that have been reported on the association between *HFE* mutations and different liver diseases might be influenced by ethnic differences and small sample sizes of the individual studies, as well as by variable penetrance of *HFE* gene mutations [22]. The strength of our study is a large, well-selected control group, which offers a good representation of the overall Lithuanian population, and which has been used for determination of *HFE* gene mutation frequencies in Lithuania [20]. Overall, the ultimate role of *HFE* mutations for chronic liver injury has to be determined in further large-scale, well-designed prospective studies.

The major limitation of our research is the retrospective design of the study. For this reason, full-scale information on iron parameters including ferritin levels, transferrin saturation and hepatic iron content was available only for a small proportion of cirrhotic patients. Due to a relatively small sample size, subgroup analysis in different etiological entities of liver cirrhosis (hepatitis C and B, alcoholic liver disease, etc.) was not performed. In addition, the spectrum of etiology of

cirrhosis in a tertiary-level hospital (one having a liver transplantation unit) patients' cohort could be influenced by referral and selection bias. We admit that the relatively small and heterogeneous sample size of cirrhotic patients in our study does not carry high statistical power; however, significant associations between *HFE* mutations and liver cirrhosis determined by our group suggest a possible role of these genetic alterations in chronic liver diseases.

5. Conclusions

Heterozygous C282Y mutation of the *HFE* gene was associated with liver cirrhosis. In gender-related analysis, heterozygous C282Y and homozygous H63D mutations were linked with liver cirrhosis in men, but not in women.

Conflict of interest

All the authors declare to have no conflict of interest.

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Authors' contribution

S.J. and J.K.: performed genotyping experiments, analyzed data, and wrote the manuscript; I.V.: analyzed and collected data; J.Š., V.P., and J.K.: collected data; L.K.: performed genotyping experiments; J.S.: study design, performed genotyping experiments, and analyzed data; G.K. and L.K.: study design and project supervision.

REFERENCES

[1] Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. Lancet 1997;349(9055):825–32.
[2] Argo CK, Northup PG, Al-Osaimi AMS, Caldwell SH. Systematic review of risk factors for fibrosis progression in non-alcoholic steatohepatitis. J Hepatol 2009;51(2):371–9.
[3] Friedman SL. Mechanisms of hepatic fibrogenesis. Gastroenterology 2008;134(6):1655–69.
[4] Zimmer V, Lammert F. Genetics in liver disease: new concepts. Curr Opin Gastroenterol 2011;27(3):231–9.
[5] Stickel F, Buch S, Zoller H, Hultcrantz R, Gallati S, Österreicher C, et al. Evaluation of genome-wide loci of iron metabolism in hereditary hemochromatosis identifies PCSK7 as a host risk factor of liver cirrhosis. Hum Mol Genet 2014;23(14):3883–90.
[6] Kitamoto T, Kitamoto A, Yoneda M, Hyogo H, Ochi H, Nakamura T, et al. Genome-wide scan revealed that polymorphisms in the PNPLA3, SAMM50, and PARVB genes are associated with development and progression of nonalcoholic fatty liver disease in Japan. Hum Genet 2013;132(7):783–92.
[7] De Boer YS, Van Gerven NM, Zwiers A, Verwer BJ, Van Hoek B, Van Erpecum KJ, et al. Genome-wide association study identifies variants associated with autoimmune hepatitis type 1. Gastroenterology 2014;147(2):443–52.
[8] Wallace DF, Subramaniam VN. Co-factors in liver disease: the role of HFE-related hereditary hemochromatosis and iron. Biochim Biophys Acta 2009;1790(7):663–70.
[9] Andersen RV, Tybjaerg-Hansen A, Appleyard M, Birgens H, Nordestgaard BG. Hemochromatosis mutations in the general population: iron overload progression rate. Blood 2004;103(8):2914–9.
[10] Edgren G, Nyrén O, Melbye M. Cancer as a ferrotoxic disease: are we getting hard stainless evidence? J Natl Cancer Inst 2008;100(14):976–7.
[11] Valenti L, Fracanzani AL, Bugianesi E, Dongiovanni P, Galmozzi E, Vanni E, et al. HFE genotype, parenchymal iron accumulation, and liver fibrosis in patients with nonalcoholic fatty liver disease. Gastroenterology 2010;138(3):905–12.
[12] Tung BY, Emond MJ, Bronner MP, Raaka SD, Cotler SJ, Kowdley KV. Hepatitis C, iron status, and disease severity: relationship with HFE mutations. Gastroenterology 2003;124(2):318–26.
[13] Harrison-Findik DD, Klein E, Crist C, Evans J, Timchenko N, Gollan J. Iron-mediated regulation of liver hepcidin expression in rats and mice is abolished by alcohol. Hepatology 2007;46(6):1979–85.
[14] Nelson JE, Bhattacharya R, Lindor KD, Chalasani N, Raaka S, Heathcote EJ, et al. HFE C282Y mutations are associated with advanced hepatic fibrosis in Caucasians with nonalcoholic steatohepatitis. Hepatology 2007;46(3):723–9.
[15] Chitturi S, Weltman M, Farrell GC, McDonald D, Kench J, Liddle C, et al. HFE mutations, hepatic iron, and fibrosis: ethnic-specific association of NASH with C282Y but not with fibrotic severity. Hepatology 2002;36(1):142–9.
[16] Peculis R, Lace B, Putnina A, Nikitina-Zake L, Klovins J. HFE-related hemochromatosis risk mutations in Latvian population. Ann Hematol 2015;94(2):343–4.
[17] Raszeja-Wyszomirska J, Kurzawski G, Suchy J, Zawada I, Lubinski J, Milkiewicz P. Frequency of mutations related to hereditary haemochromatosis in northwestern Poland. J Appl Genet 2008;49(1):105–7.
[18] European Association for the Study of the Liver. EASL clinical practice guidelines for HFE hemochromatosis. J Hepatol 2010;53(1):3–22.
[19] Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al., American Gastroenterological Association; American Association for the Study of Liver Diseases; American College of Gastroenterology. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. Gastroenterology 2012;142(7):1592–660.
[20] Kucinskas L, Juzenas S, Sventoraityte J, Cedaviciute R, Vitkauskiene A, Kalibatas V, et al. Prevalence of C282Y, H63D, and S65C mutations in hereditary HFE-hemochromatosis gene in Lithuanian population. Ann Hematol 2012;91(4):491–5.
[21] Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81(3):559–75.
[22] Fargion S, Valenti L, Fracanzani AL. Beyond hereditary hemochromatosis: new insights into the relationship between iron overload and chronic liver diseases. Dig Liver Dis 2011;43(2):89–95.
[23] Robinson JP, Johnson VL, Rogers PA, Houlston RS, Maher ER, Bishop DT, et al. Evidence for an association between

compound heterozygosity for germ line mutations in the hemochromatosis (HFE) gene and increased risk of colorectal cancer. Cancer Epidemiol Biomarkers Prev 2005;14(6):1460–3.
[24] Yuan X-M, Li W. Iron involvement in multiple signaling pathways of atherosclerosis: a revisited hypothesis. Curr Med Chem 2008;15(21):2157–72.
[25] Agudo A, Bonet C, Sala N, Muñoz X, Aranda N, Fonseca-Nunes A, et al. Hemochromatosis (HFE) gene mutations and risk of gastric cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Carcinogenesis 2013;34(6):1244–50.
[26] Gleeson F, Ryan E, Barrett S, Crowe J. Clinical expression of haemochromatosis in Irish C282Y homozygotes identified through family screening. Eur J Gastroenterol Hepatol 2004;16(9):859–63.
[27] Powell LW, Dixon JL, Ramm GA, Purdie DM, Lincoln DJ, Anderson GJ, et al. Screening for hemochromatosis in asymptomatic subjects with or without a family history. Arch Intern Med 2006;166(3):294–301.
[28] Raszeja-Wyszomirska J, Kurzawski G, Zawada I, Suchy J, Lubinski J, Milkiewicz P. HFE gene mutations in patients with alcoholic liver disease. A prospective study from northwestern Poland. Pol Arch Med Wewnętrznej 2010;120 (4):127–31.
[29] Sikorska K, Romanowski T, Stalke P, Iżycka-Świeszewska E, Bielawski KP. Iron overload and HFE gene mutations in Polish patients with liver cirrhosis. Hepatobiliary Pancreat Dis Int 2011;10(3):270–5.
[30] Erhardt A, Maschner-Olberg A, Mellenthin C, Kappert G, Adams O, Donner A, et al. HFE mutations and chronic hepatitis C: H63D and C282Y heterozygosity are independent risk factors for liver fibrosis and cirrhosis. J Hepatol 2003;38(3):335–42.
[31] Thorburn D, Curry G, Spooner R, Spence E, Oien K, Halls D, et al. The role of iron and haemochromatosis gene mutations in the progression of liver disease in chronic hepatitis C. Gut 2002;50(2):248–52.
[32] Dostalikova-Cimburova M, Kratka K, Stransky J, Putova I, Cieslarova B, Horak J. Iron overload and HFE gene mutations in Czech patients with chronic liver diseases. Dis Markers 2012;32(1):65–72.
[33] Jain S, Agarwal S, Tamhankar P, Verma P, Choudhuri G. Lack of association of primary iron overload and common HFE gene mutations with liver cirrhosis in adult Indian population. Indian J Gastroenterol 2011;30(4):161–5.
[34] Jowkar Z, Geramizadeh B, Shariat M. Frequency of two common HFE gene mutations (C282Y and H63D) in a group of Iranian patients with cryptogenic cirrhosis. Hepat Mon 2011;11(11):887–9.