

## Letter to the Editor

# Hemochromatosis gene mutations in patients with alcoholic cirrhosis

### *To the Editor:*

Oxidative stress, which can be induced by iron overload, may play a pivotal role in the pathogenesis of alcoholic liver disease (ALD) which is characterized by fatty liver, hepatitis, fibrosis, cirrhosis and hepatocellular carcinoma (1). Increasing evidence indicates that ALD is a multifactorial disease in which both environmental and multiple genetic factors play a role. The hemochromatosis (HFE) protein is involved in iron metabolism and the synergistic effect between iron and alcohol is suggested in the progression of alcoholic cirrhosis (2). Therefore, it has been speculated that homo- or heterozygous mutations in the HFE gene which increase serum iron levels might contribute to the pathological process.

There have been five published reports investigating the relationship between HFE gene mutations and alcoholic cirrhosis in white populations and all of them have failed to detect any association between the C282Y mutation and susceptibility to ALD (3–7). On the other hand, the H63D mutation was investigated in only three studies, with contradictory results (5–7). A control group of alcoholics without liver disease has not been included in most of the studies cited. The effect of the third common mutation, S65C, on disease susceptibility has not been investigated.

The aim of our study was to investigate the contribution of these three HFE genetic polymorphisms to susceptibility for ALD in a sample of Croatian and Slovenian patients.

The study included 147 patients with alcoholic cirrhosis and two control groups: 66 alcoholics without cirrhosis and 350 healthy blood donors. The alcoholic groups were well matched for age, sex, origin, and alcohol intake (all consumed more than 80 g of ethanol per day for 5 or more years). There were no significant differences in the sex ratio and age between the patients and controls ( $p > 0.05$ ).

Patients were recruited from the internal clinics of University Medical Centers in Rijeka

(Croatia) and Ljubljana (Slovenia). The diagnosis of cirrhosis was based on the clinical, biochemical, and ultrasonographic features. These patients had severe liver disease: all had decompensated, with mean prothrombin time prolongation of 4.5 s on presentation, increasing the risk of liver biopsy. Patients with other causes of cirrhosis and also patients with anemias with ineffective erythropoiesis and hemolysis were excluded by appropriate serologic and biochemical investigations. Alcoholics without cirrhosis had no clinical signs of cirrhosis and had normal plasma levels of bilirubin, albumin, aspartate aminotransferase, and alanine aminotransferase and normal prothrombin time. On ultrasound examination, their liver was normal. They were attending alcoholic support groups in Rijeka and Ljubljana. Healthy control subjects were clinically unaffected individuals (without liver disease or anemia). The study design was approved by the institutional ethics committees of both centers and informed consent was obtained from all subjects.

Genotyping of HFE gene mutations was performed by the polymerase chain reaction/restriction fragment length polymorphism method as described previously (8, 9). Differences in the frequencies of various alleles between patients with alcoholic cirrhosis and control subjects were performed using the chi-square test and Fisher's exact test.

Table 1 shows the distribution of HFE genotypes and allele frequencies in ALD patients and in both control groups.

There were no significant differences in the frequencies of the C282Y and S65C mutations between the ALD patients and control groups. On the other hand, the frequency of H63D heterozygotes was significantly higher ( $p = 0.0019$ ) in ALD patients (31.98%) than in healthy controls (19.14%). The frequency of H63D heterozygotes was also higher in ALD patients (31.98%) than in alcoholics without cirrhosis (22.73%) but failed to reach statistical significance possibly due to the limited number of alcoholics without cirrhosis. There were no

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Table 1. Distribution of HFE genotypes and allele frequencies in patients with alcoholic cirrhosis, alcoholics without cirrhosis and healthy controls

HFE genotypes			Alcoholic cirrhosis, n (%)	Alcoholic controls, n (%)	Healthy controls, n (%)
C282Y	H63D	S65C			
-/-	-/-	-/-	83 (56.46)	44 (66.66)	240 (68.57)
-/-	+/-	-/-	47 (31.98)	15 (22.73)	67 (19.14)
-/+	-/-	-/-	10 (6.80)	4 (6.06)	18 (5.14)
-/-	-/-	-/+	4 (2.72)	2 (3.03)	8 (2.29)
-/-	+/+	-/-	3 (2.04)	1 (1.52)	12 (3.43)
+/+	-/-	-/-	0	0	1 (0.29)
-/-	-/-	+/+	0	0	0
+/-	+/-	-/-	0	0	4 (1.14)
-/-	+/-	+/-	0	0	0
+/-	-/-	+/-	0	0	0
Total			147 (100)	66 (100)	350 (100)
HFE gene mutations			% (CI)	% (CI)	% (CI)
C282Y			3.40 (1.85–6.14)	3.03 (1.18–7.53)	3.43 (2.31–5.05)
H63D			18.03 (14.05–22.82)	12.88 (8.19–19.65)	13.57 (11.23–16.31)
S65C			1.36 (0.53–3.44)	1.52 (0.41–5.35)	1.14 (0.58–2.24)

significant differences in serum iron parameters (iron, unsaturated iron-binding capacity, transferrin saturation, ferritin) between patients and controls with different HFE genotypes, within each patient or control group. Nevertheless, our data suggest a possible relationship between the pathogenesis of ALD and the H63D mutation.

Our results regarding the H63D mutation are in agreement with the results of Ropero et al. (6) in a Spanish population but opposite to those of Grove and Daly (5) and Gleeson et al. (7) in a British population. Iron parameters in southern European populations appear to be different from the northern European countries, which may explain the discrepancy between the studies (10). According to the geographic position in Europe, the prevalence of the C282Y mutation in Croatia and in Slovenia fits in an observed north/south gradient. H63D has a much broader distribution, with high frequencies throughout Europe, especially in the Mediterranean area, and the frequency in our healthy control was similar to those reported for the normal Croatian and Slovenian populations (11). It seems that in southern European populations, where the prevalence of the C282Y mutation is low, the H63D mutation could be a genetic predisposing factor when it is present with other unknown genetic or environmental factors (12–14).

Despite the minor effect of the H63D mutation in hereditary hemochromatosis, it should be noted that a pooled analysis of 14 case-control studies (15) showed that homozygosity for the H63D mutation was associated with a fivefold risk of iron overload (OR = 5.7) and heterozygosity with a 1.6-fold risk. In addition, several studies have reported that individuals carrying the

H63D mutation are at higher risk of developing chronic hepatitis (16), non-alcoholic steatohepatitis (14) and porphyria cutanea tarda (17).

In conclusion, we found no evidence that either the C282Y or S65C mutations contributed to susceptibility to ALD, while a higher prevalence of H63D heterozygotes was found in the group of ALD patients. Therefore, further studies in a larger series of patients are necessary to evaluate the role of H63D mutation in the development of ALD.

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## References

- Purohit V, Russo D, Salin M. Role of iron in alcoholic liver disease: introduction and summary of the symposium. *Alcohol* 2003; 30: 93–97.
- Britton RS, Bacon BR. Hereditary hemochromatosis and alcohol: a fibrogenic cocktail. *Gastroenterology* 2002; 122: 563–565.

3. Aldersley MA, Howdle PD, Wyatt JI, Robinson PA, Markham AF. Haemochromatosis gene mutation in liver disease patients. *Lancet* 1997; 349: 1025–1026.
4. Frenzer A, Rudzki Z, Norton I, Butler WJ. Heterozygosity of the haemochromatosis mutation, C282Y, does not influence susceptibility to alcoholic cirrhosis. *Scand J Gastroenterol* 1998; 33: 1324.
5. Grove J, Daly AK. Heterozygotes for HFE mutations have no increased risk of advanced alcoholic liver disease. *Gut* 1998; 43: 262–266.
6. Ropero GP, Villegas MA, Fernandez AM et al. C282Y and H63D mutations of HFE gene in patients with advanced alcoholic liver disease. *Rev Esp Enferm Dig* 2001; 93: 156–163.
7. Gleeson D, Evans S, Bradley M et al. HFE genotypes in decompensated alcoholic liver disease: phenotypic expression and comparison with heavy drinking and with normal controls. *Am J Gastroenterol* 2006; 101: 304–310.
8. Merryweather-Clarke AT, Pointon JJ, Shearman JD, Robson KJ. Global prevalence of putative haemochromatosis mutations. *J Med Genet* 1997; 34: 275–278.
9. Mura C, Ragueneas O, Ferec C. HFE analysis in 711 hemochromatosis probands: evidence for S65C implication in mild form of hemochromatosis. *Blood* 1999; 93: 2502–2505.
10. Cassanelli S, Pignatti E, Montosi G et al. Frequency and biochemical expression of C282Y/H63D hemochromatosis (HFE) gene mutations in the healthy adult population in Italy. *J Hepatol* 2001; 34: 523–528.
11. Ristić S, Makuc J, Starčević N et al. Hemochromatosis gene mutations in the Croatian and Slovenian populations. *Clin Genet* 2003; 64: 444–446.
12. Battaller R, North KE, Brenner DA. Genetic polymorphisms and the progression of liver fibrosis: a critical appraisal. *Hepatology* 2003; 37: 493–503.
13. de Diego C, Murga MJ, Martinez-Castro P. Frequency of HFE H63D, S65C, and C282Y mutations in patients with iron overload and controls from Toledo, Spain. *Genet Test* 2004; 8: 263–267.
14. Sampietro M, Piperno A, Lupica L et al. High prevalence of the His63Asp HFE mutation in Italian patients with porphyria cutanea tarda. *Hepatology* 1998; 27: 181–184.
15. Burke W, Imperatore G, McDonnell SM, Baron RC, Khoury MJ. Contribution of different HFE genotypes to iron overload disease: a pooled analysis. *Genet Med* 2000; 2: 271–277.
16. Erhardt A, Maschner-Olberg A, Mellenthin C 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: 335–342.
17. Bonkovsky HL, Jawaid Q, Tortorelli K et al. Non-alcoholic steatohepatitis and iron: increased prevalence of mutations of the HFE gene in non-alcoholic steatohepatitis. *J Hepatol* 1999; 31: 421–429.

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