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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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ARTICLE INFO

Article history:
Received 20 March 2015
Received in revised form
27 July 2016
Accepted 13 September 2016
Available online xxx

Keywords:
HFE
C282Y
H63D
Liver cirrhosis

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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Peer review under the responsibility of the Lithuanian University of Health Sciences.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.medici.2016.09.004

Please cite this article in press as: Juzėnas S, et al. Association of HFE gene C282Y and H63D mutations with liver cirrhosis in the Lithuanian population. Medicina (2016), http://dx.doi.org/10.1016/j.medici.2016.09.004

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.

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

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