ORIGINAL ARTICLE

HFE gene mutations in patients with alcoholic liver disease

A prospective study from northwestern Poland

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KEY WORDS

alcoholic liver disease, gene mutations, hereditary hemochromatosis

ABSTRACT

INTRODUCTION Hereditary hemochromatosis has been linked with C282Y and H63D mutations of the *HFE* gene encoding human hemochromatosis protein. It is genetic disorder of iron metabolism, leading to iron accumulation and increased liver fibrosis. The association between alcoholic liver disease (ALD) and *HFE* gene mutations remains unclear and requires clarification.

OBJECTIVES The aim of the study was to determine the prevalence of C282Y and H63D mutations in patients with ALD and healthy individuals and to analyze laboratory data in the context of *HFE* gene mutation in ALD patients.

PATIENTS AND METHODS We analyzed 119 patients with ALD. The control group comprised 1516 DNA samples obtained either from cord blood or healthy subjects from the records of general practitioners. *HFE* mutations were detected using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

RESULTS Among the ALD patients, 0.84% were homozygous and 3.36% were heterozygous for the C282Y mutation, while 5.04% were homozygous and 21.85% heterozygous for the H63D mutation. There was 1 C282Y/H63D compound heterozygote in the ALD group. In the control group, 2 homozygotes and 117 heterozygotes for the C282Y mutation were identified. As for the H63D mutation, 2.5% homozygotes, 25% heterozygotes, and 1.4% compound heterozygotes were found. There was a trend towards a more common occurrence of ALD patients homozygous for the H63D mutation. Patients with H63D genotype had higher total and low-density lipoprotein cholesterol.

CONCLUSIONS The prevalence of *HFE* mutations in ALD patients is similar to that observed in healthy subjects and comparable to the prevalence in other Central European countries. Our findings on lipid disturbances in the H63D heterozygotes are potentially interesting and require further studies on larger patient groups.

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INTRODUCTION It is estimated that as few as up to 20% of subjects with heavy alcohol consumption develop alcoholic liver disease (ALD).[1] Therefore, some other, most likely genetic factors play an important role in the predisposion to liver damage in ALD. ALD has been associated with heterozygosity for the mutation of the *HFE* gene (encoding human hemochromatosis protein), but the data in the literature remain inconclusive. A number of studies showed an overrepresentation of C282Y and H63D heterozygotes in patients with ALD.[2] However, the results have not been confirmed by others.[3,4] As iron overload precipitates the progression of alcohol-induced liver damage in animal models, *HFE* mutations may play a role in the development and

progression of ALD in humans. *HFE* mutations have also been linked with progression of hepatic damage in patients with nonalcoholic fatty liver[5] and hepatitis C.[6]

The aim of the study was to analyze the frequency of C282Y and H63D mutations of the *HFE* gene in patients with alcohol-induced liver damage and in healthy individuals. We also aimed to analyze laboratory data in the context of *HFE* gene mutation in ALD patients and to investigate whether *HFE* mutations produced hepatic iron overload, which in turn could exert a deleterious fibrogenic effect in chronic ALD.

PATIENTS AND METHODS

Patients

A cohort of 119 patients with clinical and laboratory features of ALD were included in the study. Patients presented with signs and symptoms of alcoholic hepatitis or liver cirrhosis caused by daily consumption of more than 20 g alcohol. The diagnosis of liver cirrhosis was confirmed either by liver biopsy or appropriate radiological imaging tests. The diagnosis of decompensated cirrhosis was based on imaging tests, including abdominal ultrasound and computed tomography scan, and on clinical features of portal hypertension after excluding prehepatic portal hypertension. The group included 85 men and 34 women aged 50 ±6 years. All analyzed patients tested negative for HBs and anti-HCV. Patients at the age of 40 and less had normal ceruloplasmin levels. Control samples were obtained from the DNA bank of the Department of Genetics and Pathology, Pomeranian Medical University, Szczecin, Poland. They comprised 1000 samples obtained from patients registered with the local general practitioners and 516 samples from cord blood. The study was approved by the local Ethics Committee.

Genetic analysis

HFE gene analysis was performed by polymerase chain reaction (PCR) amplification of total genomic DNA of 2 regions of the *HFE* gene carrying the mutations C282Y and H63D. The PCR primers for amplifications of C282Y locus were 5'-TCCGTCTTA GCTGAGTGGAACTACTACCCCCAGAACATC ACC-3' and 5'-AGGCAGAATCGACTCACCTG-GCTCTCATCAGTCACATACCC-3'. For H63D detection we used sens primer 5'-ATGGTT AAGGCCTGTTGCTCTGTC-3' and antisens primer 5'-CCCTTGCTGTGGTTGTGATTTTC-3'. PCR amplification was performed in total 12.5 uL that contained 10 to 20 ng genomic DNA, 2.5 nmol of each deoxynucleotide triphosphate, 4 pmol of each primer, 1.5 mmol/l magnesium chloride, 1 × PCR buffer solution and 0.3U Taq polymerase (MBI Fermentas, Vilnius, Lithuania). PCR amplification consisted of initial denaturation for 3 min at 94°C, followed by 35 cycles of denaturation at 94°C for 30 s, and annealing at 56°C for 30 s, and extension at 72°C for 30 s. The terminal elongation was performed at 72°C for 7 min. PCR products were digested with RsaI (MBI Fermentas, Vilnius, Lithuania) for detection C284Y and with MboI (MBI Fermentas, Vilnius, Lithuania) for H63D. The G to A transition (aa282) creates a new RsaI site; there is a second RsaI site in this fragment which acts as internal control in the restriction fragment length polymorphism. The C to G transversion (aa63) results in the loss of 1 of the two MboI sites in the amplified product. DNA fragments generated after digestion were separated on 3% agarose gel.

Statistical analysis was performed with Stat View® program using the χ^2, χ^2 with Yates modification, Fisher, and Student's t-test. $P < 0.05$ was considered statistically significant.

RESULTS

The prevalence of C282Y and H63D mutations in the analyzed cohort of patients with ALD and the control group is presented in TABLE 1. In patients with ALD, we identified 1 homozygote (0.84%) and 4 heterozygotes (3.36%) for C282Y mutations, and 6 homozygotes (5.04%) and 26 heterozygotes (21.85%) for H63D mutations. One compound heterozygote (0.84%) was detected in the ALD group. In controls, we identified 2 homozygotes (0.13%) for the C282Y mutation and 117 heterozygotes (7.8%) for this mutation. As for the H63D mutation (1505 DNA samples), 38 subjects (2.5%) were homozygotes and 380 heterozygotes (25%). There were 21 compound heterozygotes (1.4%) in the control group. H63D homozygocity tended to occur more often in ALD patients ($P = 0.09$) but we did not observe a statistically significant difference in the frequency of *HFE* mutations between ALD patients and controls (TABLE 1). The biochemical data in ALD patients in respect to C282Y or H63D status are summarized in TABLE 2. We did not observe a significant difference between these groups in aspartate transaminase, alanine transaminase, alkaline phosphatase, γ-glutamyltransferase, total iron-binding capacity, and unsaturated iron-binding capacity. Patients with W/H63D genotype had significantly higher low-density lipoprotein values than patients with W/W ($P = 0.039$) and borderline significantly higher cholesterol levels ($P = 0.05$). Patients with W/C282Y had significantly higher iron values than W/H63D ($P < 0.0001$), H63D/H63D ($P = 0.003$), and W/W ($P = 0.0001$).

DISCUSSION

The *HFE* gene is located on a short arm of chromosome 6 at location 6p21.3. The product encoded by this gene is a membrane protein similar to MHC class I (major histocompatibility complex class I) proteins and associates with β_2-microglobulin. It is thought that this protein regulates iron absorption by influencing the interaction between transferrin receptor and transferrin. The iron storage disorder, hereditary hemochromatosis type 1, is a recessive genetic disorder that results from defects in this gene. The *HFE* gene has 2 mutant alleles at different loci, H63D and C282Y, which follow an autosomal recessive inheritance pattern and influence iron levels. Iron overload affects liver and other organs

TABLE 1 Prevalence of *HFE* mutations in patients with alcoholic cirrhosis and the control group

Mutation	Alcoholic liver disease (n = 119)	Control group (n = 1516)	*P*
C282Y/C282Y, n (%)	1 (0.84)	2 (0.13)	0.1
W/C282Y, n (%)	4 (3.36)	117 (7.8)	0.5
H63D/H63D, n (%)	6 (5.04)	38 (2.5)	0.09
W/H63D, n (%)	26 (21.85)	380 (25)	0.5
C282Y/H63D, n (%)	1 (0.84)	21 (1.4)	0.6
W/W, n (%)	81 (68.07)	958 (63)	0.7

TABLE 2 Laboratory findings in patients with alcoholic cirrhosis in relation to their *HFE* status

Laboratory test	C282Y/C282Y (n = 1)	W/C282Y (n = 4)	H63D/H63D (n = 6)	W/H63D (n = 26)	C282Y/H63D (n = 1)	W/W (n = 81)
Fe (5.37–28.64 µmol/l)[a]	18.258	59.61 ± 31.50	22.02 ±6.80	16.11 ±1.97	2.51	18.8 ±2.15
TIBC (42.3–66.2 µmol/l)[a]	43.32	43.14 ± 10.92	43.86 ±5.91	36.9 ±3.4	48.3	38.66 ±2.69
UIBC (40.8–76.6 µmol/l)[a]	19.51	18.97 ±9.5	20.2 ±5.0	20.9 ±4.3	45.8	22.2 ±2.69
ferritin (292.1–3370.5 pmol/l)[a]	2247	2004.3 ± 512.3	633.7 ±337.05	1157.2 ± 238.2	47.19	1166.2 ±211.2
AST (5–40 U/l)[a]	47	144 ±56	69 ±17	148 ±44	155	124 ±16
ALT (5–40 U/l)[a]	47	43 ±2	27 ±5	71 ±19	55	51 ±6
GGT (5–40 U/l)[a]	425	554 ±191	269 ±104	491 ±168	981	485 ±94
ALP (40–120 U/l)[a]	133	235 ±93	171 ±30	181 ±23	227	201 ±15
total cholesterol (mmol/l)	8.65	3.98 ±0.73	3.76 ±0.39	5.23 ±1.11	3.78	3.8 ±0.23
LDL cholesterol (mmol/l)	6.22	3.34 ±0.44	2.36 ±0.36	3.5 ±0.96	2.36	2.31 ±0.18
HDL cholesterol (mmol/l)	1.11	0.44 ±0.18	0.93 ±0.16	0.853 ±0.18	0.8	0.75 ±0.08
TG (mg/dl)	408	199 ±66	95 ±21	159 ±46	72	136 ±14

a normal values

Abbreviations: ALP – alkaline phosphatase, ALT – alanine transaminase, AST – aspartate transaminase, GGT – γ-glutamyltransferase, HDL – high-density lipoprotein, LDL – low-density lipoprotein, TG – triglycerides, TIBC – total iron-binding capacity, UIBC – unsaturated iron-binding capacity

such as the pancreas, heart, pituitary gland, as well as the skin and joints.

Liver histology in patients with ALD typically shows iron overload that has been found to negatively correlate with patients' survival.[7] Only a relatively small proportion of heavy alcohol drinkers develop ALD. Therefore, other, probably genetic factors facilitate liver injury in these subjects. *HFE* gene mutations may potentially play a role in the progression of liver damage or development of ALD in heavy drinkers because increased iron accumulation may facilitate liver injury via enhanced oxidative stress and augmented lipid peroxidation. A possible association between ALD and heterozygosity for H63D mutations has been suggested by one study, but not confirmed by others. Similarly, for the C282Y heterozygosity an association with ALD has been suggested[2] and then questioned by other studies.[3,4,8] On the other hand, C282Y homozygous patients who consume large amounts of alcohol have significantly higher serum levels of iron, transferrin saturation, and transaminases, which may suggest that they are at a higher risk of disease progression and liver cirrhosis.[9]

The prevalence of common *HFE* mutations varies across Europe. The C282Y mutations occur more commonly in northern countries leading to a dispute on its Celtic vs. Viking origin.[10] The highest prevalence of H63D mutation is found in Brittany.[11] We have recently demonstrated that the prevalence of common *HFE* mutations in northwestern Poland is comparable to that observed in other Central European countries.[12] The prevalence of these mutations has not been analyzed so far in patients with ALD living in northwestern Poland. In our study we prospectively investigated a homogenous, white population with ALD from this region. The prevalence of these mutations in our patients did not differ from that of the general population; however, homozygotic mutation H63D seemed to be more common in ALD patients. It would definitely be worthwhile to evaluate *HFE* gene mutations in a larger cohort of white patients with ALD to validate this tendency. This cohort should be at least as large as our control group, because the frequencies of *HFE* gene mutations were similar. In their detailed study in patients with decompensated ALD, Gleeson et al.[13] showed no significant difference in the prevalence of these mutations between their patients with ALD and heavy alcohol drinkers without liver disease, suggesting that most likely *HFE* mutations do not contribute to an increased risk of alcohol-induced liver damage. On the other hand, our results regarding

lipid disturbance in W/H63D subjects could be of interest, and if validated in larger cohorts of patients, could help to elucidate the pathogenesis of liver damage in at least a proportion of ALD patients.

In summary, an interesting hypothesis that common *HFE* mutations increase the risk of liver cirrhosis in heavy alcohol drinkers could not be substantiated by the results of our study, supporting previous observations from the United Kingdom and New Zealand.

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ARTYKUŁ ORYGINALNY

Mutacje genu *HFE* u pacjentów z alkoholową chorobą wątroby

Prospektywne badanie w północno-zachodniej Polsce

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SŁOWA KLUCZOWE

alkoholowa choroba wątroby, mutacje genetyczne, wrodzona hemochromatoza

STRESZCZENIE

WPROWADZENIE Wrodzona hemochromatoza jest związana z mutacjami C282 i H63D genu *HFE* kodującego ludzkie białko hemochromatozy (*human hemochromatosis protein*). Jest to genetyczne zaburzenie metabolizmu żelaza, prowadzące do gromadzenia się żelaza w wątrobie, z nasilonym włóknieniem narządu. Związek między mutacjami genu *HFE* a alkoholową chorobą wątroby (*alcoholic liver disease* – ALD) pozostaje wciąż niejasny i wymaga wyjaśnienia.

CELE Celem pracy była ocena częstości występowania mutacji C282Y oraz H63D genu *HFE* wśród chorych z ALD oraz w grupie osób zdrowych, a także analiza danych laboratoryjnych w kontekście mutacji genu *HFE* w grupie chorych z ALD.

PACJENCI I METODY Badano 119 pacjentów z ALD. Grupa kontrolna objęła 1516 próbek DNA pozyskanych z krwi pępowinowej lub od osób zdrowych z rejestrów lekarzy rodzinnych. Analizę molekularną mutacji genu *HFE* przeprowadzono metodą oceny polimorfizmu długości fragmentów restrykcyjnych (PCR-RFLP).

WYNIKI W grupie chorych z ALD wykryto 0,84% homozygot i 3,36% heterozygot mutacji C282Y, a także 5,04% homozygot i 21,85% heterozygot mutacji H63D oraz 1 heterozygotę złożoną. Wśród badanych z grupy kontrolnej stwierdzono 2 homozygoty i 117 heterozygot mutacji C282Y. Dla mutacji H63D było to odpowiednio 2,5% homozygot i 25% heterozygot, a także 1,4% złożonych heterozygot. Zaobserwowano trend w kierunku częstszego występowania homozygotycznej mutacji H63D wśród chorych z ALD. U chorych z ALD i mutacją H63D stężenie cholesterolu całkowitego i cholesterolu LDL było większe.

WNIOSKI Częstość występowania mutacji genu *HFE* jest zbliżona w populacji polskiej z ALD i zdrowych ochotników i porównywalna z częstością obserwowaną w innych krajach Europy Środkowej. Stwierdzony związek mutacji W/H63D a poziomem cholesterolu jest interesujący i wymaga potwierdzenia w większej grupie badanych.

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