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Research Article

Frequency of the HFE gene mutation C282Y and H63D in patients with hepatobiliary disease from Lviv region (Ukraine)

Ivanna Haiboniuk, Nataliya Kitsera, Zoriana Osadchuk, Bogdan Tretyak, Halyna Makukh

[1]*Institute of Hereditary Pathology, National Academy of Medical Sciences of Ukraine, Lviv, Ukraine, 79008, Lviv, Lysenko str.31-a*

Abstract Background: Genetic testing for HFE mutation is carried out in people with high levels of iron and suspected hemochromatosis or liver disease.

Aim of our study was to establish the frequency of the HFE gene mutation C282Y and H63D among patients with hepatobiliary disease of unknown origin from Lviv region (Ukraine) during 2006-2017 yy.

Materials and methods. The analysis of clinical, some instrumental and laboratory data of 172 patients aged 18-63 years, with chronic hepatobiliary pathology, who were hospitalized in a few gastroenterology departments of hospitals in Lviv (Ukraine) during 2006-2017 yy have been performed. The control group consisted of 280 people without hepatiobiliary disorders of the appropriate age and sex.

Results: There was also a significant difference among women (9.1%) with chronic hepatobiliary diseases of unknown origin from Lviv region (Ukraine) in the age category over 60 years ($p<0.05$) than among men (2%). Genetic testing of HFE gene mutations c.845G>A and c.187C>G among patients with idiopatic hepatobiliary diseases in 5.8% cases confirmed the diagnosis of hereditary hemochromatosis. The heterozygous C282Y/N mutation among patients with hepatobiliary diseases 17(9.9%) was significantly more often ($p<0.05$) than in the control group – 7 (2.5%) cases.

Conclusions: The main criteria for selection in the high risk group for hemochromatosis are chronic liver pathology, the corresponding hereditary burden in the proband's family, certain clinical and instrumental changes and elevated serum iron levels. Introduction of testing of genetic markers predisposition to hereditary human pathology, in particular to hereditary hemochromatosis, is promising direction that needs to be developed.

Keywords: hepatobiliary disease, hemochromatosis, HFE gene, C282Y, H63D, Ukraine

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Introduction

Genetic testing for HFE mutation is carried out in people with high levels of iron and suspected hemochromatosis or liver disease [1, 2]. Genetic disorders develops as a result of mutation of a gene linked to the A-locus of the HFE complex on the short arm of the 6th chromosome (6p22.2) - C282Y. The most common is the substitution of cysteine for tyrosine. This disease can have different clinical consequences [3, 4]. In the 6 chromosome, the major histocompatibility complex is localized, in which there are more than 100 genes that regulate the immune response. [5].

Two missense mutations C282Y (*c*.845G> A, rs1800562) and H63D (c.187C> G, rs1799945) are relatively common. C282Y is most common in Northern European populations and H63D has a global distribution. Whereas the

* Correspondence: Dr. Nataliya Kitserai, Institute of Hereditary Pathology, National Academy of Medical Sciences of Ukraine, Lviv, Ukraine, 79008, Lviv, Lysenko str.31-a. Email: nkitsera@gmail.com

prevalence of these mutations is high, the clinical penetrance of the disease they cause is low [5].

Due to the lack of early diagnosis and adequate treatment, the disease can lead to severe clinical manifestations and complications - liver cirrhosis, heart failure, diabetes, arthritis and hepatocellular carcinoma [1].

No effective prevention of the development of the disease and its complications are based on these a family of anamnesis and molecular -genetic testing of patients and their relatives [6].

The estimated prevalence of C282Y homozygosity was 0.44% in non-Hispanic white individuals, 1.2% in Ireland but 0.012% in individuals of Pacific Island and extremely low in Asian individuals - 0.000039% [7, 8].

Aim of our study was to establish the frequency of the HFE gene mutation C282Y and H63D among patients with hepatobiliary disease of unknown origin from Lviv region (Ukraine) during 2006-2017 yy.

Materials and methods. The analysis of clinical, some instrumental and laboratory data of 172 patients aged 18-63 years, with chronic hepatobiliary pathology, who were hospitalized in a few gastroenterology departments of hospitals in Lviv (Ukraine) during 2006-2017 yy have been performed. The control group consisted of 280 people without hepatiobiliary disorders of the appropriate age and sex, whose data are entered into the database of the Institute of Hereditary Pathology, National Academy of Medical Sciences of Ukraine, Lviv, Ukraine.

Inclusion criteria to the study group the presence of hepatobiliary pathology of unknown origin, exlusion criteria – chronic viral hepatitis B and C, chronic autoimmune hepatitis, cirrhosis, Gilbert, Wilson and Addison diseases.

We were studied the C282Y and H63D mutations in the HFE gene by polymerase chain reaction, restriction analysis, and agarose gel electrophoresis [9].

In order to find out how reliably the indicators of one sample differ from another, the criterion for estimating the significance of the difference was used - the probability coefficient (Student's t-test) as one of the parametric methods widely used in biomedical research [10].

Results

During the period (2006–2017 yy) of study, there were 172 patients aged 18-63 years with chronic hepatobiliary disease according to the medical records from Lviv hospitals (Ukraine), in particular 22 (15.7%) females and 150 (84.3%) males (table 1). The average age at diagnosis was 42.7±6.8 years in men and 36.9±5.3 years in women.

There is a significant difference in gender in this pathology. The ratio between women and men was 1:6.8. The inhabitants of the city were 122 (70.9%), the inhabitants of the villages – 50 (29.1%). Gender and age characteristics of patients with chronic hepatobiliary disorders are shown in table 1.

The largest number of cases was registered among men (98) and women (14) aged 35-60 years, the smallest – 2 cases among men 1 and one among women (table 1).

Table 1: Age and sex characteristics of patients with chronic hepatobiliary diseases (hepatopathy of unknown origin) from Lviv region (Ukraine) during 2006-2017 yy.

Age	Males		Female		P
	n	%	n	%	
5-18	2	1.3	1	4.5	<0.05
19-34	47	31.3	5	22.7	>0.05
35-60	98	65.4	14	63.6	>0.05
>60	3	2	2	9.1	<0.05
All	150	100	22	100	

Among 150 men in the age group of 5-18 years, the chronic hepatobiliary diseases (hepatopathy of unknown origin) were detected only in 2 boys (1.3%), while among 22 girls of the same age category, the pathology occurred in 1 (4.5%) case which has a significant difference ($p<0.05$). No significant difference ($p>0.05$) was found in the age group 19-34, although the frequency among men (31.3%) was higher than among women (22.7%). In the age group 35-60, the frequency among women (63.6%) and men (65.4%) was almost the same and no significant difference was found ($p>0.05$). There was also a significant difference in the age category over 60 years ($p<0.05$) where the frequency among women was 9.1% (two cases), and among men-2% (3 cases).

In medical history in the main group of patients with hepatobiliary pathology of unknown origin (172 people) different concomitant diseases were diagnosed: 58 (33.7%) cholelithiasis, 27 (15.7%) gastric and duodenal ulcer, 21 (12,2%) - chronic pancreatitis. Most commonly heart disease and cardiology pathology were diagnosed. 63 (36.6%) patients had hypertension disease, 42(24.4%) - ischemic heart disease and 3(1.7%) - infection myocarditis. Diffuse and nodular goiter was observed in 65(37.7%) patients.

Complaints were most often filed with patients for general weakness- 137 (79.7%), pain in the joints – 125 (72.7%), headache -102 (59.3%) and fatigue -97 (56.4%). The most frequent manifestations were an increase in the size of the liver palpation - 151 (87.8%), pigmentation of the skin -112 (65.1%), an increase in the size of spleen - 103 (59.9%).

Analysis of ultrasounds revealed that patients were more likely to have echography signs of enlargement and/or structural changes of the liver 158 -(91.9%), pathological changes of the gallbladder – 88 (51.2%), and hyperplasia and/or changes in the structure of thyroid gland – 104 (60.5%). Violation indicators of liver function in the laboratory was manifested by increased levels of alkaline phosphatase (125 cases -72.7%), ALT (112 cases - 65.1%) and AST (119 cases -69.2%). ECG disorders were detected in 108 (62.8%) patients, especially this pathology increased with age.

Serum ferritin (SF) values in blood males with chronic hepatobiliary deseases, as in blood females, were defined as not elevated: for men- 218.5+56.7 (norm <300mg/l) and for women-137+19.4 (norm <200mg/l).

Genetic analysis of *HFE* c.187C> G and c.845G> mutations A was performed in 280 practically healthy inhabitants of the Western regions of Ukraine aged from 25 to 35 years. The C282Y mutation in the HFE gene, which is the most common cause of hemochromatosis, was detected in 12 individuals in the heterozygous state, which determines the prevalence of heterozygous carriers in the cohort as 1:40, or 2.5%. More often this allele was found in males than females. The results of the analysis of the mutation c.187C> G indicate its high frequency in the studied groupe. Mutation c.187C> G (H63D) of *HFE* gene is widespread in Slavic populations. Every fourth person is a heterozygous carrier of this mutation, which allows us to interpret it as a neutral polymorphism [11].

After clinical and laboratory studies, we estimated the frequency of c.845G> A and c.187C> G mutations of the *HFE* gene among patients with chronic idiopathic hepatobiliary disorders. The results of the study are shown in table 2.

Table 2. Frequency of mutation of HFE gene genotype among patients with chronic hepatobiliary disorders from Lviv region (West Ukraine)

Genotype *HFE*	Frequ-ency among women, % (n=22)	Frequ-ency among men,% (n=150)	Frequency, % (n=172)	Prevalence among chronic hepatobiliary disorders (n=172)	Prevalence in the control group (n=280)	OR (CI)
c.845G> A/N	4(18,2%)	13 (8,7%)	17 (9,9%)	1:10	1:40	1.4834 (1.00- 2.20) *p = 0,05*
c.187C> G/N	16 (72,7%)	37 (24,7%)	53 (30,8%)	1:4	1:4	
c.187C> G/ 845G> A	0	2 (1,3%)	2 (1,1%)	1:86	<1:280	5,494 (1,49-20, 23) *p = 0,01*
c.187C> G/187C>G	1 (4,5%)	4 (2,7%)	5 (2,9%)	1:35	1:93	
c.845G> A/845G>A	0	3 (2%)	3 (1,7%)	1:58	<1:280	

The results of molecular genetic studies indicate a high frequency of different allelic variants of the *HFE* gene among individuals with hepatobiliary disorders of unknown origin. The acquired genotypes results c.187C>G/845G> A (two patients), c.845G>A/845G>A (three patients), c.187C>G/c.187C>G (five patients) give genetically conformation the diagnosis of hereditary hemochromatosis in case of the clinical symptoms presence. It was established the etiology of disorders and prescribed therapy as periodic phlebotomy. It is worth noting that men were referred to this study 5 times more often than women. Only once hemochromatosis diagnosis was confirmed in woman because homozygous c.187C>G mutation was detected.

Most of the patients with hepatobiliary disease and controls with heterozygous mutations had a normal iron level. When determining serum iron, the level fluctuated within the age normal -23.8±1.9 mg for men (normal - 11,6-30,4 μmol/l) and for women 19.7±3.5 (normal - 8,9-30, 4 μmol/l).

Discussion

In view of the significance of these differences, doctors of medical specialties, the problem of hemochromatosis with the current task is to improve the diagnosis of this hereditary disease. The identification of hemochromatosis cases could lead to the prophylaxis of its severe effects, including cardiomiopathy, gastric and breast cancer, hepatocellular carcinoma [12-15]

All additional laboratory and instrumental steps depends on the course and symptoms disease [1, 3, 4].

A high frequency of mutation c.187C>G in the heterozygous (72.7%) was found among females which deserves a separate study. It can be assumed that those patients have another, unidentified mutation within the *HFE* gene. Mutations in the HFE gene have been shown to be an etiologic factor in hepatobiliary disorders in 5.8% of patients, despite the high frequency of heterozygous

carriers of these variants in the population.

Clinical manifestations of haemochromatosis occur predominantly at the age of 30-50 years old, women - after 50 years old. In the population up to 30% cases have with asymptomatic course of the disease. If the haemochromatosis diagnosis had not been established in time, irons accumulates in the tissues and organs, that cause the lesion of pancreas and thyroid gland, heart rhythm disturbance, arthropathy, early menopause, chronic pain [8, 13, 16].

The risk of hemochromatosis is chronic liver pathology in the patient, due to hereditary burden in the family, certain clinical and instrumental results and increased level of ferritin in the blood of patients. In this study, we investigated the probable association of *HFE* gene mutations in a group of patients with hepatobiliary diseases.

Analysis of the *HFE* gene (using genetic testing) can confirm the clinical diagnosis of hemochromatosis in children or adults in the preclinical (latent) stage of the disease, especially as known cases of the disease among family members.

Conclusions

1. We evaluated 172 patients with hepatobiliary disease aged 5–63 years from Lviv region (Ukraine) who were diagnosed hepatobiliary diseases of unknown origin. The average age at diagnosis was 42.7±6.8 years in men and 36.9±5.3 years in women.

2. There was also a significant difference among women (9.1%) with chronic hepatobiliary diseases of unknown origin from Lviv region (Ukraine) in the age category over 60 years ($p<0.05$) than among men (2%).

3. Genetic testing of *HFE* gene mutations c.845G>A and c.187C>G among patients with idiopatic hepatobiliary diseases in 5.8% cases confirmed the diagnosis of hereditary hemochromatosis.

4. The heterozygous C282Y/N mutation among patients with hepatobiliary diseases 17(9.9%) was significantly more often ($p<0.05$) than in the control group – 7 (2.5%) cases.

The main criteria for selection in the high risk group for hemochromatosis are chronic liver pathology, the corresponding hereditary burden in the proband's family, certain clinical and instrumental changes and elevated serum iron levels. Introduction of testing of genetic markers predisposition to hereditary human pathology, in particular to hereditary hemochromatosis, is promising direction that needs to be developed.

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None

Conflict of Interest

None

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