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ASSOCIATION OF POLYMORPHISM rs1495741 *NAT2* GENE WITH INFLAMMATORY LIVER DISEASE DEVELOPMENT UNDER EXPOSURE TO EXTERNAL FACTORS

DOI 10.25789/YMJ.2024.85.03

UDC 575.167[616.36-04+616.36-003.826]

Slow acetylation of substrate is associated with drug-induced liver damage and transformation of viral and alcohol hepatitis in cirrhosis. Increasing xenobiotic load is a significant factor in development of metabolic associated liver diseases. This interaction between genotype and environment should be studied to reveal disease pathogenesis. We analysed polymorphism rs1495741 genotypes in control group and in patients with cryptogenic liver cirrhosis and non-alcohol fatty liver disease to evaluate association of acetylation type with liver disease development. As part of the study, patients filled the questionnaire to assess xenobiotic load. The rs1495741 polymorphism was detected by real-time PCR. Significant differences were revealed in the criptogenic liver cirrhosis and non-alcoholic fatty liver disease groups in patients consuming fried and smoked foods (OR: 5,49 at p<0,05); in combination with older age (>55) the risk increases by 7.57 times (p<0,05). However, no association of the rs1495741 polymorphism with the development of liver diseases was identified.

Keywords: N-acetyltransferase 2, polymorphism, cryptogenic liver cirrhosis, non-alcoholic fatty liver disease.

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Introduction. One of the causes for high mortality in the Russian Federation is the liver cirrhosis, associated with hepatitis virus infection and alcohol abuse. Previously, in approximately 10% of cases, it was not possible to identify the etiological cause of the disease, which led to a diagnosis of cryptogenic liver cirrhosis (CLC). The development of molecular genetic diagnostics in combination with laboratory and instrumental methods has led to a decrease in the proportion of CLC in the structure of cirrhosis [4]. In addition, studies have shown that most people with CLC are likely to have an outcome of active fibrosis in non-alcoholic steatohepatitis, a severe form of non-alcoholic fatty liver disease (NAFLD) [18].

Currently, the population is faced with a variety of foreign chemicals (xenobiotics): pharmaceuticals, household chemicals and products of human economic activity, including food additives. The liver plays the main role in the neutralization and biotransformation of xenobiotics. One of the enzymes in the second phase of detoxification is N-acetyltransferase 2 (NAT2), which is involved in the acetylation of arylamines and hydrazines [9]. The enzyme gene is localized on chromosome 8 (8p22) and has several single nucleotide polymorphisms (SNP), the combination of which led to the existence of two haplotypes, slow and rapid acetylation, in the population [8]. The *NAT2* genotype can be identified by SNP detection in polymerase chain reaction (PCR) or gene sequencing. The simplest and the most sensitive methods is real-time PCR genotyping of tagSNP (rs1495741), which correlates with acetylation type [2, 5].

Scientists described the role of slow acetylation alleles in the development of drug-induced hepatitis when using anti-tuberculosis drugs. It is known that rapid acetylation alleles are associated with a high risk of transformation of hepatitis into cirrhosis and hepatocellular carcinoma (HCC) in the presence of provoking environmental factors in patients with chronic viral and alcoholic hepatitis [10, 12, 13]. Considering that the expression of NAT2 has the highest level in the liver, it can be assumed that, depending on the rate of acetylation, toxic substances accumulate in liver cells, which form a focus of chronic inflammation under conditions of constant increased load of xenobiotics [19]. Taking into account the increasing influence of exogenous chemicals on the human body, it seems relevant to study the role of gene polymorphisms that determine the activity of xenobiotic metabolic enzymes in the development of CLC and NAFLD.

The aim of this study is to evaluate

the influence of external factors (age, gender, smoking, regular consumption of fried and smoked foods) and genetically determined type of acetylation on the risk of developing CLC and NAFLD under conditions of high xenobiotic load.

Materials and methods. The study included 47 patients who signed informed voluntary consent and had liver disease: CLC or NAFLD. Healthy individuals without a history of liver cirrhosis (N=17) were selected as controls. As part of the study, patients were surveyed in groups to assess xenobiotic load (smoking, including electronic devices, frequent consumption of fried and smoked foods 5 times or more in a week, the presence of harmful factors at work). This study was approved by the Ethics Committee of ISMU (protocol No. 1 of the Ethics Committee of ISMU dated April 9, 2023).

Buccal epithelium was used as the material for the study. Rs1495741 polymorphism genotype was determined in the Research Institute of Biomedical Technologies of the Irkutsk State Medical University according to the method described earlier [2].

Statistical analysis was carried out by the R programming language for statistical data processing and graphics [14]. The sample size was not previously calculated. Differences in age in the groups were determined by the Mann-Whitney test for small samples; gender differences were calculated by Fisher's exact test. The distribution of allele and genotype frequencies was compared with the expected distribution according to the Hardy-Weinberg law by the Chi-square test. The odds ratio (OR) for disease development under the influence of factors was calculated by Fisher's exact test with confidence interval (CI) 95%. The rs1495741 polymorphism genotype association with the disease development was calculated in the SNPassoc package [17]. Differences between groups were considered significant at p<0,05.

Results. The average age of patients in the clinical group is 60,68 (56,79 - 64,57) years, in the control group it is 49,23 (43,96 - 54,51) years (Table 1). The age of patients was significantly higher in the clinical group compared to the control group (Mann-Whitney test W=615,5, p=0,001037). There were no significant differences between the groups by gender (Chi-square=0,0647, p=0,799). The small sample size should be noted, which is caused by incomplete questionnaires of patients included in the groups.

Despite the small sample size, the distribution of genotype and allele frequencies in the groups does not differ from expected, according to the Hardy-Weinberg law (Chi-square, p>0.05) (Table 2), and are similar to the frequencies observed in the European population (1000 Genomes Project, p>0.05).

When comparing the genotype frequencies of the rs1495741 polymorphism in the groups, no significant association of slow or rapid acetylation genotypes with the development of NAFLD or CLC was found (Table 2).

To assess the effect of environmental factors on the disease development, we used Fisher's exact test at a confidence level of 95%. As a result, in the group of people who consume fried and smoked foods 5 or more times in a week, the risks of the disease development are significantly higher by 5,5 times compared to the control group (p = 0,004) (Table 3).

According to the differences in age between the clinical and control groups, we constructed a logistic regression model that included the following factors: age over 55 years, consumption of fried and smoked foods more than 5 times a week, and the rs1495741 polymorphism. As a result, the rs1495741 *NAT2* genotype was not considered as a significant predictor and was removed from the model. The final model included the effects of age and fried food consumption (p=0,0001). After assessment of predictor value, we found that the chances of developing inflammatory liver diseases increase by 7.57 times when consuming fried and smoked foods in combination with age >55 years (Table 4).

Discussion. Among the environmental factors, the consumption of fried and smoked foods (more than 5 times in a week) has the greatest importance in the development of metabolic liver diseases. The chances of disease development are 5.5 times higher in the case group compared to controls; in combination with age >55 years, the chances increase by 7.57 times. The small sample size should be noted, which may indicate randomness of the obtained results and the sample size should be increased. In addition, there are differences in age between the groups, which arose due to the peculiarities of the patient sampling: the group included patients with a newly diagnosed liver cirrhosis and patients of an

Table 1

Distribution of patients by gender and age in the groups

Characteristic	Control (CLC–, NAFLD–)	Case (CLC+, NAFLD+)	P value
Age, M±m (95% CI)	49.23±2.69 (43.96 – 54.51)	60.68±1.98 (56.79 – 64.57)	0.001037
Women	11	32	0.799
Men	6	15	
Total	17	47	

Table 2

Distribution of frequencies of genotypes and alleles of polymorphism rs1495741 in the studied group

Group	Genotype frequency. absolute (relative)			Allele frequency. absolute (relative)		Hardy-Weinberg equation. p value
	AA	*AG*	*GG*	*A*	*G*	
Control	9 (0.529)	7 (0.412)	1 (0.059)	25 (0.735)	9 (0.265)	1
Case	20 (0.4255)	20 (0.4255)	7 (0.149)	60 (0.638)	34 (0.362)	0.5454
Odds ratio (95% CI)	1.00	1.29 (0.4 – 4.13)	3.15 (0.34 – 29.53)	1.57 (0.62 – 4.28)		AIC=78.9
p value for OR	0.5383			0.3977		

older age group who had previously been observed with a diagnosis of NAFLD.

It is known that heterocyclic aromatic amines are formed during frying and smoking foods. They are absorbed in the intestine and then metabolized in the liver under the action of enzymes of the first and second phases of detoxification. During the first phase of biotransformation, under the influence of the cytochrome P450 system, active carcinogens with genotoxic effects can be produced. These active metabolites are subsequently inactivated by the NAT2 enzyme [3, 6, 7]. Thus, individuals with a slow acetylation pattern have a higher chance of accumulating active toxic metabolites leading to liver damage. As a result, an inflammation is formed, which, under conditions of constant exposure to xenobiotics, leads to chronic liver damage with further transformation into fibrosis and cirrhosis. Consumption of fried foods is associated with higher risk for the progression of fibrosis in NAFLD and the development of HCC with red meat consumption [13, 15, 16]. The main pathogenetic role belongs to saturated fats and trans fats deposited in the liver. It is possible that heterocyclic aromatic amines activated by the cytochrome system are slowly metabolized by the NAT2 enzyme and accumulate in hepatocytes, which also makes a significant contribution to the development of steatohepatitis, subsequent fibrosis and liver cirrhosis. According to our results, the chances of developing liver diseases under conditions of regular consumption of fried and smoked foods increases by 5,5 times, but no reliable results have been found on the association of slow or rapid acetylation type with the development of metabolic-associated liver diseases. But in the control group, only one patient had a genotype associated with the rapid acetylation type, which does not allow us to reliably determine the role of acetylation in the development of liver diseases under the influence of environmental factors. Therefore, it is necessary to increase the number of individuals with the rapid acetylation type in the control group. However, in Russia, the frequency of the rapid acetylation genotype varies from 0,05 to 0,5 depending on the region [1, 11]. This factor significantly affects the probability of the presence of volunteers with the rapid acetylation type in the sample.

Thus, increased consumption of fried and smoked foods more than 5 times a week should be considered as a risk factor for the development of metabolic-associated liver diseases especially in association with elder age over 55 years.

Table 3

Effect of environmental factors on the development of liver diseases

Factor	Control	Case	Odds ratio (95% CI)	P value
Smoking				
No	15	39	1.00	1
Yes	2	8	1.53 (0.26 – 16.41)	
Fried and smoked food				
No	12	14	1.00	0.004
Yes	5	33	5.49 (1.47 – 23.89)	

Table 4

Factors included in the model and their role in the development of liver diseases

Factor	Coefficient	Odds ration (95% CI)	P value
Age >55 years	2.0246	7.57 (3.79 – 14.63)	0.00436
Fried and smoked food consumption	2.0246	7.57 (3.79 – 14.63)	0.00436
Constant	-0.9993	–	0.09725

Increasing the groups, as well as including patients from other regions of Russia in the study, will allow us to assess the significance of genotype associated with acetylation rate and role of different environmental factors. Non-infectious liver diseases are multifactorial diseases, therefore, determining the genotype in combination with multifactorial analysis will improve the effectiveness of treatment and prevention, which is a relevant area in personalized medicine.

The work was carried out within the framework of the state assignment of the Ministry of Health of the Russian Federation (topic No. 121031000062-2 Study of the role of NAT2 polymorphism in the development of cryptogenic liver cirrhosis under conditions of xenobiotic load).

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