Artículo de investigación

Search for risk markers of development of pathology. Hepatobiliary system based on genetic polymorphism of xenobiotic detoxification system genes study

ПОИСК МАРКЕРОВ РИСКА РАЗВИТИЯ ПАТОЛОГИИ ГЕПАТОБИЛИАРНОЙ СИСТЕМЫ НА ОСНОВЕ ИЗУЧЕНИЯ ГЕНЕТИЧЕСКОГО ПОЛИМОРФИЗМА ГЕНОВ СИСТЕМЫ ДЕТОКСИКАЦИИ КСЕНОБИОТИКОВ

Búsqueda de marcadores de riesgo de desarrollo de patología. Sistema hepatobiliar basado en el polimorfismo genético del estudio de genes del sistema de desintoxicación xenobiótico

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Abstract

The aim of this study was to compare the frequencies of genotypes and alleles of polymorphic variants of the *CYP2E1* and *GSTA1* genes in patients with hepatobiliary system pathology who were examined at the Ufa Scientific Research Institute of Occupational Medicine and Human Ecology and healthy individuals living in the Republic of Bashkortostan who have no hepatobiliary system pathology.

Materials and methods: 81 patients with pathology of the hepatobiliary system and 502 practically healthy individuals living in the Republic of Bashkortostan were examined. The material for molecular genetic analysis was DNA samples isolated from the peripheral venous blood of the examined individuals by phenol-chloroform extraction. The study of polymorphic loci of the *CYP2E1* and *GSTA1* genes was carried out by the method of polymerase chain reaction

Аннотация

Целью данного исследования явилось изучение частот полиморфных вариантов генов *CYP2E1* и *GSTA1* у больных с патологией гепатобилиарной системы, проходивших обследование в ФБУН «Уфимский НИИ медицины труда и экологии человека» и здоровых индивидов, проживающих в Республике Башкортостан, которые не имеют заболеваний гепатобилиарной системы. Также проводился анализ возможных ассоциаций генотипов этих генов с развитием патологии гепатобилиарной системы.

Материалы и методы. Обследован 81 пациент с патологией гепатобилиарной системы и 502 практически здоровых индивида, проживающих на территории Республики Башкортостан. Проведен анализ ассоциаций полиморфных вариантов генов

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of DNA synthesis. Mathematical processing of the results of the study was carried out using the statistical program Statistics.
Results. A comparative analysis revealed statistically significant differences between the group of patients with ASD and healthy individuals in the frequency distribution of the genotypes of the polymorphic locus rs3957357 of the *GSTA1* gene. The AA genotype was more common in patients with ASD with a frequency of 16.05%, compared with the control group – 8.37% ($\chi 2 = 3.96$; $p = 0.047$). Conclusion As a result of the study, it was shown that the AA genotype (OR = 2.09; 95% CI 1.07 - 4.10) of the polymorphic locus rs3957357 of the *GSTA1* gene is a marker of the risk of pathology of the hepatobiliary system.

Keywords: Pathology of the hepatobiliary system, detoxification system genes, genetic markers.

CYP2E1 и *GSTA1*. Материалом для молекулярно-генетического анализа служили образцы ДНК, выделенные из лимфоцитов периферической венозной крови обследуемых индивидов методом фенольно-хлороформной экстракции. Изучение полиморфных локусов генов *CYP2E1* и *GSTA1* проводилось методом полимеразной цепной реакции синтеза ДНК. Математическую обработку результатов исследования проводили с использованием статистических программы Statistica.
Результаты. Сравнительный анализ выявил статистически достоверные различия между группой больных с патологией гепатобилиарной системы и здоровыми индивидами в распределении частот генотипов полиморфного локуса rs3957357 гена *GSTA1*. Генотип АА чаще встречался у больных с патологией гепатобилиарной системы с частотой 16,05%, по сравнению с группой контроля–8,37% ($\chi 2=3,96$; $p=0,047$). Заключение. В результате проведенного исследования было показано, что маркером риска развития патологии гепатобилиарной системы является генотип АА (OR=2,09; 95% CI 1,07 – 4,10) полиморфного локуса rs3957357 гена *GSTA1*.

Ключевые слова: патология гепатобилиарной системы, гены системы детоксикации, генетические маркеры

Resumen

El objetivo de este estudio fue comparar las frecuencias de genotipos y alelos de variantes polimórficas de los genes CYP2E1 y GSTA1 en pacientes con patología del sistema hepatobiliar que fueron examinados en el Instituto de Investigación Científica de Medicina Ocupacional y Ecología Humana de la Ufa e individuos sanos que viven en el República de Bashkortostán que no tienen patología del sistema hepatobiliar.
Materiales y métodos: se examinaron 81 pacientes con patología del sistema hepatobiliar y 502 individuos prácticamente sanos que viven en la República de Bashkortostán. El material para el análisis genético molecular fue muestras de ADN aisladas de la sangre venosa periférica de los individuos examinados mediante extracción con fenol-cloroformo. El estudio de los loci polimórficos de los genes CYP2E1 y GSTA1 se llevó a cabo mediante el método de reacción en cadena de la polimerasa de la síntesis de ADN. El procesamiento matemático de los resultados del estudio se realizó mediante el programa estadístico Statistics.
Resultados Un análisis comparativo reveló diferencias estadísticamente significativas entre el grupo de pacientes con TEA y los individuos sanos en la distribución de frecuencia de los genotipos del locus polimórfico rs3957357 del gen GSTA1. El genotipo AA fue más común en pacientes con TEA con una frecuencia de 16.05%, en comparación con el grupo control –8.37% ($\chi 2 = 3.96$; $p = 0.047$). Conclusión Como resultado del estudio, se demostró que el genotipo AA (OR = 2.09; IC del 95%: 1.07 - 4.10) del locus polimórfico rs3957357 del gen GSTA1 es un marcador del riesgo de patología del sistema hepatobiliar.

Palabras clave: Patología del sistema hepatobiliar, genes del sistema de desintoxicación, marcadores genéticos.

Introduction

Chronic toxic hepatitis is a chronic liver disease that develops as a result of prolonged exposure the hepatotropic substances on the organism. Chronic toxic hepatitis is characterized by a gradual development of the disease, starting with dyspeptic complaints, biliary syndrome, moderate enlargement of the liver and finally impaired liver function (Agzamova, 2009).

In recent years, the number of toxic liver lesions caused by environmental pollution and harmful production factors has increased. The group of occupational toxic hepatitis includes liver diseases that occur when exposed to industrial toxicants. In industrial conditions, many chemicals with hepatotoxicity are used as starting, intermediate or final products. One of the most toxic substances in the air of a working zone used for the production of liquid fuel is nitrosodimethylhydrazine (heptyl). Among occupational diseases of workers in petrochemical enterprises, toxic hepatitis is one of the most common pathologies (Valeeva, 2009).

The processes of biotransformation of xenobiotics take place in the liver with the participation of cytochrome P450 and associated with the formation of highly reactive intermediates and the initiation of free radical processes which can cause liver damage and induce the development of toxic hepatitis.

One of the representatives of the cytochrome P450 family is *CYP2E1*. The *CYP2E1* gene is mapped on chromosome 10 in the 10q24.3 region, and is expressed mainly in the liver. The most widely studied genetic polymorphisms in the *CYP2E1* gene are PstI / RsaI restriction endonuclease polymorphisms located in the 5' flanking region of the gene, in which the mutant allele contributes to increased transcriptional and enzymatic activity, as well as DraI polymorphism localized in the 6th intron (Tang, 2010).

Glutathione S-transferases (GSTs) are a leading component of the second phase of xenobiotic detoxification. In humans, several isoforms of glutathione-S-transferase (A1, M1, P1, T1, etc.) have been described. These enzymes catalyze the inclusion of glutathione to the electrophilic center of various chemical compounds, which leads to the loss of toxicity and the formation of more hydrophilic products (Limborskaya, 2011). For the *GSTA1* gene, a widespread polymorphism in the promoter region (rs3957357) that reduces expression has been described.

The purpose of this study was to investigate the frequency of polymorphic variants of the *CYP2E1* and *GSTA1* genes in workers with pathology of the hepatobiliary system and healthy individuals in the Republic of Bashkortostan, as well as to analyze the possible associations of the genotypes of these genes with the development of pathology of the hepatobiliary system.

Materials and research methods

81 patients with pathology of the hepatobiliary system hospitalized in the clinic Ufa Research Institute of Occupational Medicine and Human Ecology in Ufa were examined. The experimental group included workers with an increased risk of developing occupational chronic hepatitis. The selection criterion was the presence of characteristic complaints, individual symptoms of a lesion from the hepatobiliary system (biliary dyskinesia), changes in certain indicators of homeostasis (increased bilirubin, increased alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transpeptidase, alkaline phosphatinemide diaprotenemide), dysprotinemide diaprotenemide).

As control, we used DNA samples from 502 healthy individuals, living in the Republic of Bashkortostan, selected selected according to age, gender and ethnicity. The material for molecular genetic analysis was DNA samples isolated from peripheral venous blood of the examined individuals by phenol-chloroform extraction. The study of polymorphic loci of the *CYP2E1* and *GSTA1* genes was carried out by polymerase chain reaction using locus-specific oligonucleotide primers developed using PrimerQuest (Integrated DNA Technologies, Inc.). Mathematical processing of the results of the study was carried out using statistical programs IBM, SPSS, Statistics. The significance of differences in the frequency distribution of alleles and genotypes between

groups was revealed by comparing samples using the $\chi 2$ criterion with Yates correction. The differences were considered statistically significant at $p < 0.05$. The relative risk of disease for a particular symptom was calculated as the odds ratio (OR), the confidence interval for the relative risk (95% Cl).

Research results and discussion

Table 1 shows the frequencies of polymorphic variants of the rs2031920 locus of the *CYP2E1* gene of the pathology of the hepatobiliary system group and healthy individuals. As a result of the analysis of the frequency distribution of genotypes and alleles of the marker rs2031920 of the *CYP2E1* gene, no statistically significant differences were found between the groups of patients and healthy people. In the patient sample, as in the control group, the CC genotype ($\chi 2 = 0.00$; $p = 0.962$; OR = 0.89; 95% CI 0.38-2.07) and the C allele ($\chi 2 = 0.01$; $p = 0.919$; OR = 1.14; 95% CI 0.50-2.59) were predominant.

Table 1. Frequency distribution of genotypes and alleles of the polymorphic locus rs2031920 of the *CYP2E1* gene in pathology of the hepatobiliary system patients and in the control

Genotype and alleles	Case (81)		Control (502)		$\chi 2$	p	OR (95%CI)
	абс.	%	абс.	%			
CC	74	91,36	463	92,23	0,00	0,962	0,89 (0,38 – 2,07)
CT	7	8,64	39	7,77	0,00	0,962	1,12 (0,48 – 2,61)
TT	0	0,00	0	0,00			
C	156	95,71	765	95,15	0,01	0,919	1,14 (0,50 – 2,59)
T	7	4,29	39	4,85	0,01	0,919	0,88 (0,39 – 2,01)

When comparing the frequencies of genotypes and alleles of the marker rs6413432 of the *CYP2E1* gene in the sample of patients with pathology of the hepatobiliary syste M and healthy individuals, no significant associations were found (Table 2). The AT genotype ($\chi 2 = 1.00$; $p = 0.318$; OR = 1.37; 95% CI 0.80-2.36) and the allele A ($\chi 2 = 1.84$; $p = 0.176$; OR = 1.43; 95% CI 0.89-2.28) were predominated in the patients group, while the TT genotype and the T allele ($\chi 2 = 1.84$; $p = 0.176$; OR = 0.70; 95% CI 0.44-1.17) were more common in a sample of healthy individuals ($\chi 2 = 1.52$; $p = 0.218$; OR = 0.69; 95% CI 0.41 -1.17).

Table 2. Frequency distribution of genotypes and alleles of the polymorphic locus rs6413432 of the *CYP2E1* gene in pathology of the hepatobiliary system patients and in the control

Genotype and alleles	Case (81)		Control (502)		$\chi 2$	p	OR (95%CI)
	абс.	%	абс.	%			
AA	2	2,47	6	1,20	0,16	0,689	2,09 (0,42–10,56)
AT	21	25,93	102	20,32	1,00	0,318	1,37 (0,80–2,36)
TT	58	71,60	394	78,49	1,52	0,218	0,69 (0,41 – 1,17)
A	25	15,43	114	11,35	1,84	0,176	1,43 (0,89–2,28)
T	137	84,57	890	88,65	1,84	0,176	0,70 (0,44–1,12)

A comparative analysis revealed statistically significant differences between the group of patients with ASD and healthy individuals in the frequency distribution of the genotypes of the polymorphic locus rs3957357 of the *GSTA1* gene (Table 3). The AA genotype was more common in patients with ASD with a frequency of 16.05%, compared with the control group – 8.37% ($\chi 2 = 3.96$; $p = 0.047$). It was shown that the AA genotype of the rs3957357 locus of the *GSTA1* gene is a risk marker of developing pathology of the hepatobiliary system (OR = 2.09; 95% CI 1.07 - 4.10).

Table 3. Frequency distribution of genotypes and alleles of the polymorphic locus rs3957357 of the *GSTA1* gene in pathology of the hepatobiliary system patients and in the control group

Genotype and alleles	Case (81)		Control (502)		$\chi 2$	p	OR (95%CI)
	абс.	%	абс.	%			
GG	35	43,21	231	46,02	0,12	0,726	0, 89 (0,56 – 1,43)
AG	33	40,74	229	45,62	0,49	0,485	0,82 (0,51 – 1,32)
AA	13	16,05	42	8,37	3,96	0,047	2,09 (1,07 – 4,10)
G	103	63,58	691	68,82	1,53	0,217	0,79 (0,56 – 1,12)
A	59	36,42	313	31,18	1,53	0,217	1,27 (0,89 – 1,79)

Discussion

Chemicals or their metabolites are known to have direct and indirect toxic effects on hepatocytes and also trigger the activation of cytotoxic immune T cells (Abdel-Rahman, 2010). According to several authors, an indirect effect of xenobiotics on cell organelles is possible through the activation or inhibition of various kinases, transcription factors, and expression of profile genes. The outcome may be the launch of a necrotic or apoptotic process or an increase in the effect of cytokines on the immune system (Podprasart, 2007).

The toxic effect of industrial poisons depends on many factors, such as the structure of the chemical compound, blood flow in the liver, genetic factors, various cellular factors, age, nutritional characteristics, and drug and alcohol abuse. The current area in the study of occupational pathology is genetic research. The data of such studies in combination with clinical and functional characteristics help to identify a predisposition to the disease and identify people with an individual predisposition to certain industrial toxic substances.

More and more attention is being paid to polymorphic genes of the xenobiotic biotransformation system. Genes of the cytochrome P-450 system (*CYP2E1*) and glutathione-S-transferases (*GSTA1*) belong to this category. Different variations of these genes can lead to different susceptibilities to harmful industrial factors.

Cytochrome *CYP2E1* is a main enzyme that metabolizes a wide range of industrial chemical compounds, some drugs, ethanol and nitrosamines from tobacco smoke.

In our study of the polymorphic locus of the *CYP2E1* gene, no statistically significant differences were found between the group of patients and healthy individuals. This suggests that, apparently, the polymorphisms of the *CYP2E1* gene do not make a significant contribution to the formation of toxic liver damage. Although, according to some authors, polymorphic variants of the *CYP2E1* gene are associated with the development of drug hepatitis in people taking anti-TB drugs (Sun, 2008). The role of *CYP2E1* gene polymorphism in the development of alcoholic liver disease, cirrhosis, and liver cancer has been shown in previous studies (Kravchenko, 2004).

It is known that the *GSTA1* gene is involved in the exchange of bilirubin, heme and steroid hormones. Due to the fact that *GSTA1* takes part in the metabolism of carcinogens in the liver, an assumption is made about the possible role of this gene in the development of malignant neoplasms (Coles, 2001).

During this study, we found an association of the polymorphic locus rs3957357 of the *GSTA1* gene with the risk of developing pathology of the hepatobiliary system (Deng, 2015). The revealed regularity is consistent with the results of individual studies, which established the

relationship of *GSTA1* gene polymorphism with the development of colorectal cancer (Komiya, 2005) and prostate cancer (Ning, 2014, Vodicka, 2012).

Conclusion

As a result of the study, it was shown that the AA genotype (OR = 2.09; 95% CI 1.07 - 4.10) of the polymorphic locus rs3957357 of the *GSTA1* gene is a marker of the risk of developing pathology of the hepatobiliary system. The identification of genetic risk markers for the development of the disease opens up the possibility of early diagnosis of the pathology and the implementation of the necessary preventive measures to prevent it.

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