THE ROLE OF MTHFR AND MTRR GENE POLYMORPHISMS IN THE DEVELOPMENT OF CHOLELITHIASIS

Yu.Yu. Fedorova[1*], *A.Kh. Nurgalieva*[1], *S.G. Petrova*[1], *R.R. Murzina*[2], *M.A. Dzhaubermezov*[1], *N.V. Ekomasova*[1], *E.K. Khusnutdinova*[1], *D.S. Prokofieva*[1]

[1] Federal State Budgetary Educational Institution of Higher Education «Ufa University of Science and Technology», 32 Zaki Validi St., Ufa, 450076, Russia;
[2] Federal State Educational Institution of Higher Education «Bashkir State Medical University», 3 Lenin St., Ufa, 450008, Russia.

* Corresponding author: fedorova-y@yandex.ru

Abstract. Cholelithiasis is a multifactorial process that is influenced by both environmental and genetic factors. Some evidence suggests that total plasma homocysteine correlates with the presence of gallstones, suggesting that hyperhomocysteinemia is a risk factor for cholelithiasis. The aim of this work was to analyze the association of polymorphic variants of the methylenetetrahydrofolate reductase *MTHFR* (rs1801133 (677C > T), rs1801131 (1298A > C)) and methionine synthase reductase *MTRR* (rs1801394 (66A > G)) genes with the development of gallstone disease in individuals from the Republic of Bashkortostan. DNA samples from 196 patients with cholelithiasis and 274 healthy individuals aged 23-87 years living in the Republic of Bashkortostan were used as research material. Genotyping was performed using the real-time PCR method. It has been established that the rs1801133**T* allele and the rs1801133**TT* genotype of the *MTHFR* gene are markers of an increased risk of developing cholelithiasis. An association was established between the rs1801133**TT* genotype of the rs1801133 polymorphic variant of the *MTHFR* gene and the moderate severity of cholelithiasis and hereditary burden in patients with cholelithiasis. A study of the polymorphic variant of the *MTRR* gene revealed that the rs1801394**G* allele increases the risk of cholelithiasis. Analysis of associations of the polymorphism rs1801131 of the *MTHFR* gene with the development of cholelithiasis did not reveal statistically signif-icant differences between the compared groups of patients and controls. Determination of homocysteine levels and genetic testing of *MTHFR* and *MTRR* polymorphisms in patients with cholelithiasis may be useful in clinical practice.

Keywords: cholelithiasis, polymorphic variant, gene, methylenetetrahydrofolate reductase, methionine synthase reductase.

List of Abbreviations

MTHFR – methylenetetrahydrofolate reductase
MTRR – methionine synthase reductase
PEMT – phosphatidylethanolamine-N-methyltransferase
BMI – body mass index
SNP – single nucleotide polymorphism

Introduction

Gallstone disease is a disease of the digestive system affecting the hepatobiliary system, characterized by disturbances in lipid metabolism and cholesterol metabolism, accompanied by the formation of gallstones. In Russia, the annual attendance for cholelithiasis is 5-6 people per 1000 population, and the number of cholecystectomies performed exceeds 500 thousand (Merzlikin *et al.*, 2009). Cholelithiasis is a multifactorial disease that is influenced by both environmental and genetic factors. Recent advances in the analysis of human genome have identified that cholesterol levels in the pathogenesis of cholelithiasis are significantly influenced by polymorphisms in ABC protein superfamily genes (*ABCG8*, *ABCG5*, *ABCB4* and *ABCB11*), as well as genes of the apolipoprotein family (*ApoB100*, *ApoE*) and *MUC* family genes. Bilirubin levels in cholelithiasis are associated with polymorphic variants of liver protein genes (*UGT1A1*, *MRP2*, *MRP3*, *CFTR*) (Costa *et al.*, 2024).

Folate metabolism is a complex cascade process that is controlled by a number of enzymes in multiple pathways that produce active tetrahydrofolate derivatives (Nazki *et al.*, 2014). MTHFR and MTRR are key enzymes in homocysteine and folate metabolism (Li *et al.*, 2017). The most frequently studied single nucleotide polymorphisms of the *MTHFR* gene are C677T (Ala222Val, rs1801133) and A1298C (Glu429Ala, rs1801131). These polymorphisms reduce the activity of the MTHFR enzyme, which leads to increased levels of 5,10-methylenetetrahydrofolate and homocysteine (Li *et al.*, 2017). Methionine synthase reductase may contribute to carcinogenesis by altering DNA methylation, leading to nucleotide imbalance, increased uracil misincorporation into DNA, DNA strand breaks, and impaired DNA repair mechanisms. As a result,

DNA susceptibility to mutations and damage may increase (Wang *et al.*, 2022). Some evidence suggests that total plasma homocysteine correlates with the presence of gallstones, suggesting that hyperhomocysteinemia is a risk factor for gallstone disease (Tazuma, 2005).

The purpose of this work is to analyze the association of polymorphisms of the methylenetetrahydrofolate reductase *MTHFR* (rs1801133 (677C > T), rs1801131 (1298A > C)) and methionine synthase reductase *MTRR* (rs1801394 (66A > G)) genes with the development of gallstone disease in individuals from the Republic of Bashkortostan.

Materials and Methods

DNA samples from 196 patients with cholelithiasis of various ethnicities (99 Russians, 87 Tatars, 10 individuals of other nationalities) aged 23-87 years living in the Republic of Bashkortostan were used as research material. The gender distribution among the patients was: men – 46, women – 150. All patients underwent treatment in the surgical and gastroenterological departments of the City Clinical Hospital No. 21 (Ufa). 123 people were diagnosed with cholelithiasis of moderate severity, and 65 were diagnosed with mild severity. Diagnosis of cholelithiasis was based on data from a general clinical examination, ultrasound examination of the gallbladder, and analysis of the lipid profile of the blood serum. Anthropometric studies included measurements of weight and height, on the basis of which body mass index (BMI, kg/m2) was calculated. Quantitative parameters of clinical abnormalities included cholesterol measurements (mmol/L). As a control, we studied a group of healthy individuals without any signs of gastrointestinal diseases, cardiovascular pathology caused by atherosclerosis, consisting of 274 people of different ethnicities (135 Russians, 103 Tatars, 36 individuals of other nationalities). Among the individuals in the control group, there were 202 men and 72 women. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research bioethical committee of the Institute of Biochemistry and Genetics, Ufa Federal Research Center of the Russian Academy of Sciences and with the Helsinki declaration and its later amendments or comparable ethical standards.

Genomic DNA was isolated from peripheral blood lymphocytes using phenol-chloroform extraction. Genotyping of polymorphisms in *MTHFR* (rs1801133 (c.665C > T, p.Ala222Val), rs1801131 (c.1286A > C, p.Glu429Ala)), *MTRR* (rs1801394 (c.66A > G, p.Ile22Met)) genes were performed by real-time polymerase chain reaction (PCR), using TaqMan technology on the platform of the CFX96 device (Bio-Rad, USA).

All SNPs were tested for departure from Hardy-Weinberg equilibrium in the study group HWE ($\chi 2$). When pairwise comparison of allele and genotype frequencies in the groups of patients and controls, the x2 criterion was used. In the case of statistically significant differences, the strength of associations was evaluated in the odds ratio (OR). The normality of distribution of quantitative characteristics was checked. Comparison of quantitative characteristics (body mass index, cholesterol levels) was carried out using the Mann-Whitney test (in the case of two groups) and the Kruskal-Wallis test (in the case of three groups). Statistical processing of the results was carried out using application packages SPSS v.23.0, MS Office Excel 2013 (Microsoft).

Results

We studied polymorphic variants rs1801133, rs1801131 of the *MTHFR* gene and rs1801394 of the *MTRR* gene in patients with cholelithiasis and in the control group living in the Republic of Bashkortostan. The distribution of genotype frequencies corresponded to the Hardy–Weinberg equilibrium ($p > 0.05$) for the studied polymorphisms. As a result of the study, an association of the polymorphism rs1801133 (677C > T) of the *MTHFR* gene with the risk of cholelithiasis in the general group was identified (Table 1).

It was found that the rs1801133**T* allele and the rs1801133**TT* genotype of *MTHFR* gene polymorphism are markers of an increased risk of cholelithiasis ($\chi 2 = 4.62$, $p = 0.03$, OR = 1.37, 95%CI 1.03-1.82 and $\chi 2 = 6.9$, $p = 0.008$, OR = 2.18, 95%CI 1.21-3.93, respectively).

Analysis of the distribution of allele and genotype frequencies for the *MTHFR* gene rs1801133 polymorphism in individuals with cholelithiasis and control group was carried out. As a result, it was revealed that the rs1801133**TT* genotype is a marker of increased risk of moderate severity of cholelithiasis ($\chi 2 = 5.68$, $P = 0.02$, OR = 2.2, 95%CI 1.14-4.26). An association was found between the rs1801133**TT* genotype and hereditary burden in patients with cholelithiasis ($\chi 2 = 7.31$, $P = 0.007$, OR = 2.88, 95%CI 1.3-6.37).

Analysis of associations of *MTHFR* gene rs1801131 (1298A > C) polymorphism with the development of cholelithiasis did not reveal statistically significant differences between the groups of patients and controls, both in the general group and when divided by ethnicity ($p > 0.05$).

Table 1

Genotype and allele frequencies of *MTHFR* gene polymorphisms rs1801133, rs1801131 and *MTRR* gene polymorphism1801394 in patients with cholelithiasis and in the control group

		Genotypes			Alleles		N
		n (%)	n (%)	n (%)	n (%)	n (%)	
rs1801133		**CC**	**CT**	**TT**	**C**	**T**	
Patients with cholelithiasis	Russians	48 (48.5)	32 (32.3)	19 (19.2)	128 (64.6)	70 (35.4)	99
	Tatars	48 (55.2)	28 (32.2)	11 (12.6)	124 (71.3)	50 (28.7)	87
	Total	100 (51.0)	66 (33.7)	30 (15.3), **p = 0.008, OR = 2.18**	266 (67.9), **p = 0.03, OR = 0.73**	126 (32.1), **p = 0.03, OR = 1.37**	196
Controls	Russians	66 (50.0)	52 (39.4)	14 (10.6)	184 (69.7)	80 (30.3)	132
	Tatars	62 (62.6)	34 (34.3)	3 (3.0)	158 (78.6)	43 (21.4)	99
	Total	154 (56.2)	99 (36.1)	21 (7.7)	407 (74.3)	141 (25.7)	274
rs1801131		**AA**	**AC**	**CC**	**A**	**C**	
Patients with cholelithiasis	Russians	38 (39.2)	44 (45.4)	15 (15.5)	120 (61.9)	74 (38.1)	97
	Tatars	35 (39.8)	43 (48.9)	10 (11.4)	113 (64.2)	63 (35.8)	88
	Total	77 (39.5)	92 (47.2)	26 (13.3)	246 (63.1)	144 (36.9)	195
Controls	Russians	60 (45.5)	56 (42.4)	16 (12.1)	176 (66.7)	88 (33.3)	132
	Tatars	43 (44.3)	41 (42.3)	13 (13.4)	127 (65.5)	67 (34.5)	99
	Total	122 (45.0)	114 (42.1)	35 (12.9)	358 (66.1)	184 (33.9)	271
rs1801394		**AA**	**AG**	**GG**	**A**	**G**	
Patients with cholelithiasis	Russians	12 (12.8), **p = 0.01, OR = 0.39**	45 (47.9)	37 (39.4)	69 (36.7), **p = 0.04, OR = 0.67**	119 (63.3), **p = 0.04, OR = 1.49**	94
	Tatars	18 (21.9)	34 (41.5)	30 (36.6)	70 (42.7)	94 (57.3)	88
	Total	33 (17.6), **p = 0.03, OR = 0.59**	88 (46.8)	67 (35.6)	154 (40.9), **p = 0.03, OR = 0.75**	222 (59.0), **p = 0.03, OR = 1.33**	188
Controls	Russians	33 (27.3)	46 (38.0)	42 (34.7)	112 (46.3)	130 (53.7)	121
	Tatars	25 (26.0)	45 (46.9)	26 (27.1)	95 (49.5)	97 (50.5)	99
	Total	67 (26.6)	108 (42.9)	77 (30.6)	242 (48.0)	262 (51.9)	252

Note: n – number of groups; N – sample size; % - allele (genotype) frequency; p – significance level, indicated only if there is statistical significance ($p < 0.05$); OR – odds ratio.

Table 2

Variability of clinical and metabolic parameters in patients with cholelithiasis, carriers of different genotypes of *MTHFR* gene rs1801133, rs1801131 polymorphisms and *MTRR* gene rs1801394 polymorphism

Gene/polymorphic variants	Genotype	BMI (kg/m2), M ± SE	p	Cholesterol level (mmol/l), M ± SE	p
MTHFR/rs1801133	rs1801133**CC*	26.8 ± 0.5	0.6	5.7 ± 0.1	0.4
	rs1801133**CT*	26.0 ± 0.5		5.7 ± 0.1	
	rs1801133**TT*	25.6 ± 0.9		5.8 ± 0.2	
MTHFR/rs1801131	rs1801131**AA*	26.0 ± 0.5	0.7	5.7 ± 0.1	0.7
	rs1801131**AC*	26.5 ± 0.5		5.6 ± 0.1	
	rs1801131**CC*	27.1 ± 1.1		5.6 ± 0.3	
MTRR/rs1801394	rs1801394**AA*	26.3 ± 1.0	0.7	5.7 ± 0.2	0.7
	rs1801394**AG*	26.4 ± 0.5		5.8 ± 0.1	
	rs1801394**GG*	26.3 ± 0.6		5.6 ± 0.2	

Note: M ± SE – mean value and standard error of the mean; p – significance level.

An analysis of the variability of quantitative indicators (body mass index, cholesterol level) depending on the genotypes of *MTHFR* gene rs1801133, rs1801131 polymorphisms in patients with cholelithiasis was performed. As a result, no statistical differences were established between the compared groups of patients, carriers of different genotypes for these polymorphisms (Table 2).

We studied of the rs1801394 polymorphic variant of the *MTRR* gene in patients with cholelithiasis and in the control group. It was revealed that the rs1801394**G* allele of the *MTRR* gene polymorphism is a marker of an increased risk of cholelithiasis ($\chi2 = 4.33$, p = 0.03, OR = 1.33, 95%CI1.0-1.74). Association analysis of the *MTRR* gene rs1801394 polymorphic variant with the risk of cholelithiasis in individuals, considering ethnicity, was carried out. An association was found between the rs1801394**G* allele and the development of the disease in patients of Russian ethnicity ($\chi2 = 3.98$, P = 0.04, OR = 1.49, 95% CI1.0-2.19).

An analysis of the variability of quantitative indicators (body mass index, cholesterol level) depending on the genotypes of the polymorphic variant 66A > G of the *MTRR* gene was performed. As a result, no statistical differences were found between the compared groups of cholelithiasis patients, carriers of different genotypes for this polymorphism (Table 2).

Discussion

Cholelithiasis is a multifactorial disease that is influenced by both environmental and genetic factors. Some evidence suggests that total plasma homocysteine correlates with the presence of gallstones, suggesting that hyperhomocysteinemia is a risk factor for cholelithiasis (Merzlikin *et al.*, 2009). We genotyped the functionally significant polymorphisms rs1801133 and rs1801131 of the *MTHFR* gene in patients with cholelithiasis and healthy donors. It has been established that the rs1801133**T* allele and the rs1801133**TT* genotype of the *MTHFR* gene are markers of an increased risk of cholelithiasis. An association was established between the rs1801133**TT* genotype of the *MTHFR* gene rs1801133 polymorphic variant and the moderate severity of cholelithiasis and hereditary burden in patients with cholelithiasis. Analysis of associations of the *MTHFR* gene rs1801131 polymorphisms with the development of cholelithiasis did not reveal statistically significant differences between the compared groups of patients and controls.

The MTHFR enzyme plays a key role in folic acid metabolism, catalyzing the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is the active form of folic acid necessary for the formation of methionine from homocysteine and further S-adenosylmethionine, which plays a key role in the process of DNA methylation. One protein that requires the transfer of a methyl group from S-adenosylmethionine is the enzyme phosphatidylethanolamine-N-methyltransferase (PEMT) as a cofactor for the production of phosphatidylcholine.

Phosphatidylcholine and other phospholipids stimulate bile secretion, which in turn improves

liver function, eliminates biliary dyskinesia, and prevents the formation of gallstones. Increases in homocysteine and PEMT enzyme levels were observed during gallstone formation in transgenic mice susceptible to cholelithiasis (Zhang *et al.*, 2013). According to the literature, an association of *MTHFR* gene polymorphisms with cholelithiasis and kidney stones in people of Caucasian origin has been established (Beksac *et al.*, 2021). In a study of individuals who were diagnosed with gallbladder cancer and gallstones, an association of the polymorphism A1298C of the *MTHFR* gene with the risk of developing gallbladder cancer was established. There was no association of the *MTHFR* gene C677T polymorphism with the risk of developing these diseases (Dixit *et al.*, 2016).

In our work we genotyped the functionally significant polymorphism rs1801394 of *MTRR* gene in patients with cholelithiasis and healthy donors living in the Republic of Bashkortostan. A study of the *MTRR* gene polymorphic variant revealed that the rs1801394**G* allele increases the risk of developing cholelithiasis. Altered MTRR function may contribute to carcinogenesis through altered DNA methylation and impaired thymidylate synthesis, leading to nucleotide imbalance, increased uracil misincorporation into DNA, DNA strand breaks, and impaired excision repair. As a result, DNA susceptibility to mutations and damage may increase (Lu *et al.*, 2021). A study of *MTRR* gene polymorphisms in patients with gastric cancer and healthy individuals revealed an association of the *MTRR* gene rs1801394 polymorphism with an increased risk of developing gastric cancer (Wang *et al.*, 2017). An association of the rs1801394 polymorphism with relapse-free survival in patients with colorectal cancer was found (Zhou *et al.*, 2012). Female mice with mutations in the *MTRR* gene exhibited abnormal liver morphology, which was accompanied by changes in glycogen metabolism (Sowton *et al.*, 2020). The enzymes MTHFR and MTRR play a key role in DNA methylation, a process that controls and promotes a wide range of essential body functions, including regulation of gene activity, neurotransmitter synthesis, and hormone (estrogens) metabolism. Metabolic disorders in the body, including increased hormone levels, are considered one of the main factors in the development of cholelithiasis (Skvortsov & Khalilova, 2018).

Based on the results of the study, the role of *MTHFR* and *MTRR* genes polymorphisms in the development of cholelithiasis was established. The data obtained allow us to further study the mechanisms and molecular basis of the pathogenesis of cholelithiasis, as well as to identify important molecular genetic markers of the risk of developing cholelithiasis. Determination of homocysteine levels and genetic testing of polymorphic variants of *MTHFR* and *MTRR* in patients with cholelithiasis may be useful in clinical practice.

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The authors declare no conflict of interest.

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