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Studying the role of *GCLC* gene polymorphisms in predicting the clinical course of acute alcoholic pancreatitis

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ABSTRACT

The aim of the study was to evaluate the role of polymorphic loci rs12524494, rs17883901, rs606548, rs636933, rs648595, and rs761142 in the *GCLC* gene in predicting the clinical course of acute alcoholic pancreatitis (AAP).

Materials and methods. The material of the study was blood DNA samples obtained from 547 patients with AAP and 573 healthy individuals. The average age of patients was 48.9 ± 13.1 years, the average age of healthy individuals was 47.8 ± 12.1 years. Genotyping was performed using the MassARRAY 4 Analyzer. Plasma levels of total glutathione were determined using the OxiSelect™ Total Glutathione (GSSG/GSH) Assay Kit STA-312. The level of reactive oxygen species (ROS) was determined using the OxiSelect™ In Vitro ROS/RNS Assay Kit (Green Fluorescence) STA-347 (Cell Biolabs Inc., USA). The kinetic colorimetric assay was used to determine the level of amylase in the blood serum. Statistical data processing was performed using the Statistica 10.0 and SNPStats software.

Results. It was found that the polymorphic loci rs606548 (genotype C/C, odds ratio (OR) = 3.34, 95% confidence interval (CI) 1.29–8.66, $p = 0.007$), rs648595 (genotype G/T, OR = 1.56, 95% CI 1.04–2.36, $p = 0.029$), and rs12524494 (genotype A/G, $p = 0.021$) in the *GCLC* gene were predictors of an increased risk of necrotizing pancreatitis. For the genotype T/T of rs648595 (recessive model) in the *GCLC* gene, the lowest values of oxidized glutathione were found, whereas rs17883901 – G/A in the *GCLC* gene was associated with the highest ROS values in the blood. The rs761142 A/A genotype in the *GCLC* gene (OR = 1.70, 95% CI 1.12–2.59; $p = 0.010$) showed predisposition to acute peripancreatic fluid collection, and the rs648595 G allele (OR = 1.47, 95% CI 1.01–2.13; $p = 0.042$) in the *GCLC* gene exhibited predisposition to the formation of acute pancreatic pseudocysts. Predisposition to massive bleeding was associated with rs17883901 (G/A genotype, OR = 6.20, 95%CI 1.3–28.81; $p = 0.031$) in the *GCLC* gene.

Conclusion. The established genotype – phenotype associations will make it possible to predict the clinical course of AAP in a particular patient, taking into account their genetic makeup, as well as to determine the treatment strategy in a timely manner.

Keywords: acute pancreatitis, predicting, rs12524494, rs17883901, rs606548, rs636933, rs648595, rs761142, *GCLC* gene

Conflict of interest. The author declares the absence of obvious and potential conflicts of interest related to the publication of this article.

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Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by the regional Ethics Committee at Kursk State Medical University (Protocol No. 3 of 11.03.2013).

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Изучение роли полиморфизмов гена *GCLC* в прогнозировании клинического течения острого алкогольно-алиментарного панкреатита

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РЕЗЮМЕ

Цель исследования: оценить роль полиморфных локусов rs12524494, rs17883901, rs606548, rs636933, rs648595, rs761142 гена *GCLC* в прогнозировании клинического течения острого алкогольно-алиментарного панкреатита (ОААП).

Материалы и методы. Материалом исследования послужили образцы ДНК крови, полученные от 547 пациентов с ОААП и 573 здоровых индивидов. Средний возраст больных составил 48,9 ± 13,1 года, здоровых лиц – 47,8 ± 12,1 года. Генотипирование проводилось на анализаторе MALDI-TOF MassARRAY-4. Определение в плазме крови уровня общего глутатиона проводилось с помощью набора Oxi Select™ Total Glutathione (GSSG/GSH) Assay Kit STA-312, уровня активных форм кислорода – с помощью набора OxiSelect™ InVitro ROS/RNS AssayKit (Green Fluorescence) STA-347 (Cell Biolabs Inc., США). Для определения уровня амилазы сыворотки крови применяли кинетический колориметрический метод. Статистическая обработка данных проводилась с использованием программы Statistica 10.0 и SNPStats.

Результаты. Установлено, что полиморфные варианты rs606548 (генотип C/C; отношение шансов (OR) 3,34; 95-й доверительный интервал (95% CI) 1,29–8,66; $p = 0,007$), rs648595 (генотип G/T; OR = 1,56; 95% CI 1,04–2,36; $p = 0,029$) и rs12524494 (генотип A/G; $p = 0,021$) гена *GCLC* являются предикторами повышенного риска развития панкреонекроза. Для генотипа T/T rs648595 гена *GCLC* (рецессивная модель) установлены наиболее низкие значения окисленного глутатиона, а генотип G/A rs17883901 *GCLC* ассоциировался с наиболее высокими значениями активных форм кислорода в крови. Предрасположенность к формированию перипанкреатического инфильтрата показал генотип A/A rs761142 *GCLC* (OR = 1,70; 95% CI 1,12–2,59; $p = 0,010$), а псевдокисты – аллель G rs648595 (OR = 1,47; 95% CI 1,01–2,13; $p = 0,042$) гена *GCLC*. Предрасположенность к летальному исходу вследствие развития аррозивного кровотечения ассоциирована с rs17883901 (генотип G/A; OR = 6,20; 95% CI 1,3–28,81; $p = 0,031$) гена *GCLC*.

Заключение. Установленные нами генно-фенотипические ассоциации позволят прогнозировать клиническое течение ОААП у конкретного больного с учетом его генетического статуса и своевременно определять лечебную тактику.

Ключевые слова: острый алкогольно-алиментарный панкреатит, прогнозирование, rs12524494, rs17883901, rs606548, rs636933, rs648595, rs761142, ген *GCLC*

Конфликт интересов. Автор декларирует отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Автор заявляет об отсутствии финансирования при проведении исследования.

Соответствие принципам этики. Все лица, участвующие в исследовании, подписали добровольное информированное согласие. Исследование одобрено региональным этическим комитетом при Курском государственном медицинском университете (протокол № 3 от 11.03.2013).

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INTRODUCTION

The crucial role of oxidative stress in the pathogenesis of acute alcoholic pancreatitis (AAP) is beyond doubt [1, 2].

Glutathione is a powerful antioxidant for cell detoxification and cellular regeneration [3], which is synthesized via two ATP-dependent stages from the amino acids L-cysteine, L-glutamic acid, and glycine. At the first stage, γ-glutamylcysteine is synthesized by the enzyme γ-glutamylcysteine synthetase (or glutamate – cysteine ligase). At the second stage, the enzyme glutathione synthetase adds a glycine residue to the C-terminal group of γ-glutamylcysteine

in lymphocytes [4]. Glutathione depletion seen in acute pancreatitis is cause by deficient glutathione synthesis [4]. The study of genes encoding enzymes of glutathione metabolism is reasonable and relevant.

Glutamate – cysteine ligase (GCL) is the main glutathione-metabolizing enzyme. The GCL heterodimer consists of two subunits: catalytic (GCLC), which provides the catalytic activity of the enzyme, and modifier (GCLM), which increases the catalytic efficiency [3, 4].

Establishing the role of individual polymorphic loci in genes encoding enzymes of glutathione metabolism in the development and outcome of AAP will make it possible to predict the clinical course of the disease and determine the patient management strategy using their genetic status.

The aim of the study was to evaluate the role of polymorphic loci rs12524494, rs17883901, rs606548, rs636933, rs648595, and rs761142 in the *GCLC* gene in predicting the clinical course of AAP.

MATERIALS AND METHODS

We examined and treated 547 Russian individuals (ethnic self-identification) with AAP (154 women and 393 men), who received treatment at surgery units of Kursk hospitals – clinical sites of the Department of Surgical Diseases No. 2 in 2015–2021.

The material for the study was blood DNA samples obtained from 547 patients with AAP and 573 healthy individuals (161 women and 412 men). The average age of patients was 48.9 ± 13.1 years, the average age of healthy individuals was 47.8 ± 12.1 years. The diagnosis and severity of AAP were verified according to clinical guidelines elaborated by a working group of the Russian Society of Surgeons.

The study was performed in compliance with the ethical principles set out in the WMA Declaration of Helsinki "Ethical principles for medical research involving human subjects" (2000 revision) and Rules of Good Clinical Practice in the Russian Federation approved by the Order of the Ministry of Health of Russia No. 266 of 19.06.2003. All patients signed an informed consent to participate in the study.

The level of total glutathione was determined by the colorimetric method using the OxiSelect™ Total Glutathione (GSSG/GSH) Assay Kit (Cell Biolabs, USA). The level of reactive oxygen species (ROS) was quantified by the OxiSelect™ In Vitro ROS / RNS Assay Kit (Cell Biolabs, USA). The level of amylase in the blood serum was determined by the kinetic colorimetric assay.

Genomic DNA was extracted by the phenol – chloroform method. Genotyping of the polymorphisms was performed by the MALDI-TOF iPLEX technology using the MassArray System (Agena Bioscience Inc, USA).

To compare categorical variables between the groups, the chi-square test was used. To compare quantitative variables, the Student's *t*-test (for normally distributed variables) and the Mann – Whitney test were used. A pre-test for normal distribution was performed using the Kolmogorov – Smirnov test. The data were presented as absolute and relative values *n* (%) and as the median and the interquartile range *Me* (Q_1– Q_3).

The association of alleles and genotypes with the risk of AAP was evaluated by the value of odds ratio (OR), showing by how many times the odds of getting in the case group (disease) with exposure differs from the odds of getting in the control group (healthy individuals) without exposure. OR and 95% confidence interval (CI) were calculated by the multiple logistic regression analysis with adjustments for age and sex using the SNPStats software (http://bioinfo.iconcologia.net/snpstats/start.htm). The logistic regression analysis was also used to assess associations of DNA markers with clinical characteristics (clinical forms of the disease, symptoms, disease severity, treatment efficacy). Correction for multiplicity was carried out by permutation testing (P_{perm}) using PLINK.

RESULTS

Table 1 presents the characteristics of clinical forms and complications of AAP in the group of patients.

Table 1

Characteristics of clinical forms and complications of AAP in the main group

Parameter	Number of patients, *n* (%)
Clinical forms of AAP	
Interstitial edematous pancreatitis	281 (51.3)
Sterile pancreatic necrosis	143 (26.1)
Infectious pancreatic necrosis	123 (22.4)
Total	547 (100)
Complicated AAP	
Peritonitis	120 (21.9)
Acute peripancreatic fluid collection	154 (28.4)
Pancreatic pseudocyst	101 (18.5)
Pancreatic abscess and purulent necrotizing pancreatitis	111 (20.3)

Fifteen patients died, the causes of death were multiple organ failure, purulent septic complications (8), and life-threatening bleeding (7). The analysis of associations of the single-nucleotide polymorphisms

(SNPs) in the *GCLC* gene with the risk of AAP was carried out (Table 2).

The identification of genetic markers of an increased risk of pancreatic necrosis in patients with AAP at the onset of the disease seemed to be the most important. We analyzed the associations of the SNPs with the risk of pancreatic necrosis using data from patients with AAP only. As a result, it was found that polymorphic variants rs606548 (genotype C/C, OR = 3.34 95% CI 1.29–8.66, p = 0.007), rs648595 (genotype G/T, OR = 1.56, 95 %CI 1.04–2.36; p = 0.029), and rs12524494 (genotype A/G, p = 0.021) in the *GCLC* gene are predictors of an increased risk of pancreatic necrosis. Moreover, all the identified associations of DNA markers did not depend on the sex and age of the patients.

Table 2

Analysis of associations of the studied polymorphic variants in the *GCLC* gene with the risk of developing acute alcoholic pancreatitis

SNP ID	Genotype, allele	Number of individuals, *n* (%)		P_{perm}	p^1	$_{cor}$OR (95% CI)
		Healthy inividuals, *n* = 573	Patients with AAP, *n* = 547			
GCLC A>G (rs12524494)	A/A	397 (97.3)	420 (95)	0.01^R	0.014	1.00
	A/G	7 (1.7)	21 (4.8)			2.84 (1.19–6.74)
	G/G	4 (1)	1 (0.2)			0.24 (0.03–2.12)
	G	0.02	0.03		0.292	1.43 (0.74–2.75)
GCLC G>A (rs17883901)	G/G	472 (86.9)	469 (88)	0.5^D	0.422	1.00
	G/A	68 (12.5)	58 (10.9)			0.86 (0.59–1.25)
	A/A	3 (0.6)	6 (1.1)			2.01 (0.50–8.09)
	A	0.066	0.068		0.810	0.96 (0.69–1.35)
GCLC C>T (rs606548)	C/C	471 (95.7)	438 (93.4)	0.05^{AD}	0.160	1.00
	C/T	21 (4.3)	30 (6.4)			1.54 (0.87–2.72)
	T/T	0 (0)	1 (0.2)			–
	T	0.02	0.03		0.089	1.62 (0.93–2.83)
GCLC G>A (rs636933)	G/G	290 (60.8)	288 (61.8)	0.41^R	0.463	1.00
	G/A	172 (36.1)	157 (33.7)			0.92 (0.70–1.21)
	A/A	15 (3.1)	21 (4.5)			1.41 (0.71–2.79)
	A	0.21	0.21		0.912	1.01 (0.81–1.26)
GCLC G>T (rs648595)	T/T	153 (30.5)	126 (26.6)	0.17^D	0.342	1.00
	G/T	255 (50.9)	261 (55.1)			1.24 (0.93–1.66)
	G/G	93 (18.6)	87 (18.4)			1.14 (0.78–1.65)
	T	0.56	0.54		0.431	0.93 (0.78–1.11)
GCLC C>A (rs761142)	A/A	271 (53.8)	266 (54.9)	0.72^R	0.741	1.00
	C/A	212 (42.1)	195 (40.2)			0.94 (0.72-1.21)
	C/C	21 (4.2)	24 (5)			1.16 (0.63-2.14)
	A	0.75	0.75		0.913	1.01 (0.82-1.23)

[1] the level of significance of the association (codominant model) with the risk of developing AAP with adjustments for sex and age

Table 3

Established relationships of the studied polymorphic gene variants with quantitative parameters of the blood in patients with AAP

SNP ID	Genotypes	Oxidized glutathione, μmol / l			Reactive oxygen species, nM			Leukocytes, $\times 10^{-9}$			Amylase, U / l		
		Me	*Q1–Q3*	*p*	*Me*	*Q1–Q3*	*p*	*Me*	*Q1–Q3*	*p*	*Me*	*Q1–Q3*	*p*
1	2	5	6	7	8	9	10	11	12	13	14	15	16
GCLC (rs648595)	G/G	10.51	3.83–12.41	0.041^R	3.59	2.26–4.22	0.471	7.95	6.60–12.10	0.921	164.0	92.0–244.0	0.812
	G/T	4.59	3.16–8.51		2.32	1.60–3.39		8.20	6.60–12.90		168.0	87.0–300.0	
	T/T	4.61	2.03–8.08		2.73	2.18–3.65		7.90	6.60–12.60		148.0	84.0–280.0	
GCLC (rs17883901)	G/G	5.93	3.11–10.41	0.752	2.52	1.98–3.69	0.040^D	8.80	6.70–12.80	0.823	164.0	85.0–272.0	0.840
	G/A	5.74	2.89–8.50		5.00	1.19–5.25		7.80	6.40–12.35		96.0	68.0–195.0	
	A/A	3.29	3.29–3.29		2.18	2.18–2.18		8.30	6.70–9.90		–	–	

Note: R – recessive model, D – dominant model

However, we did not find an association of the SNPs in the *GCLC* gene with the severity of AAP (mild, moderate, and severe). We also studied the relationship of polymorphic loci in the *GCLC* gene with intermediate phenotypes of AAP in the main group: the level of oxidized glutathione (GSSG), the level of ROS, the level of amylase, and leukocyte count in the blood serum.

The influence of polymorphic variants of genes encoding the catalytic and modifier subunits of GCL on the level of oxidized glutathione in the blood serum was established. The lowest GSSG values were found for rs648595 T/T genotype in the *GCLC* gene (recessive model). The rs17883901 G/A genotypes in *GCLC* were associated with the highest levels of ROS in the blood serum. No significant effects of genotypes were established for other polymorphic loci.

We analyzed the associations of the SNPs in the *GCLC* gene with the risk of AAP complications: rs761142 A/A genotype in the *GCLC* gene (OR = 1.70, 95%CI 1.12–2.59; $p = 0.010$) showed predisposition to acute peripancreatic fluid collection, and G allele of rs648595 (OR = 1.47, 95%CI 1.01–2.13; $p = 0.042$) in the *GCLC* gene – to the formation of a pancreatic pseudocyst.

Predisposition to life-threatening bleeding and death was associated with rs17883901 (G/A genotype, OR = 6.20, 95%CI 1.3–28.81; $p = 0.031$) in the *GCLC* gene.

DISCUSSION

The depletion of reduced glutathione in the pancreas, observed in acute pancreatitis, is caused by its cleavage [5], which indicates insufficiently effective activation of the GSH synthesis in cells and contributes to destructive pancreatitis. However, within 12–24 hours, GSH is redistributed towards the inflammatory focus due to endogenous reserves [6], since beta cells demonstrate low expression of antioxidant enzymes [7].

J. Pereda et al. found that the GSH level remained low in pancreatic necrosis for several hours without an increase in the level of the enzyme or GCL subunit mRNA, despite the binding of RNA polymerase II to their promoters and coding regions. On the contrary, in edematous pancreatitis, the GSH level quickly recovered, and the expression of the protein increased significantly due to increased transcription mediated by the effect of c-MYC, NF-kB and SP-1 on promoters. At the same time, an increase in the activity of cytosolic ribonuclease was not found in this case [8].

As a result of the study, we found that the polymorphic variants rs606548 (genotype C/C, OR = 3.34 95%CI 1.29–8.66, $p = 0.007$), rs648595 (genotype G/T, OR = 1.56, 95%CI 1.04–2.36; $p = 0.029$), and rs12524494 (genotype A/G, $p = 0.021$) in the *GCLC* gene are predictors of an increased risk of pancreatic necrosis. For the rs648595 T/T genotype in the *GCLC* gene (recessive model), the lowest values of oxidized glutathione were found, and the rs17883901 G/A *GCLC* genotype was associated with the highest values of ROS in the blood serum. The rs761142 A/A *GCLC* genotype (OR = 1.70, 95%CI 1.12–2.59; $p = 0.010$) showed predisposition to acute peripancreatic fluid collection, and G allele of rs648595 (OR = 1.47, 95%CI 1.01–2.13; $p = 0.042$) in the *GCLC* gene – to the formation of a pancreatic pseudocyst. Predisposition to fatal bleeding was associated with rs17883901 (G/A genotype, OR = 6.20, 95%CI 1.3–28.81; $p = 0.031$) in the *GCLC* gene.

The effect of the polymorphic variants in the *GCLC* gene in AAP has not been studied before. However, the study on type 2 diabetes mellitus revealed that SNPs rs17883901, rs636933, and rs648595 in the *GCLC* gene have a protective effect on disease progression, and their effects are mediated by an increased level of GSH [9].

To analyze the effect of the SNPs on gene expression in the pancreas, liver, and blood, we used the bioinformatic resources of the GTEx database. The polymorphic variants rs636933, rs648595, and rs761142 were associated with increased expression of the *GCLC* gene in the pancreas ($p \leq 0.0002$) and liver, with the exception of rs636933 ($p \leq 0.02$).

CONCLUSION

The established genotype – phenotype associations will make it possible to predict the clinical course of AAP in a particular patient, using their genetic status, and to determine the treatment strategy in a timely manner.

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