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Association of polymorphic loci of the *GSS* gene with the risk of acute biliary pancreatitis

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ABSTRACT

Aim. To investigate the role of single nucleotide polymorphisms (SNPs) rs13041792, rs1801310, and rs6088660 in the *GSS* gene and environmental factors in the development of acute biliary pancreatitis (ABP) and its complications.

Materials and methods. The material for the study was blood samples obtained from 84 patients with ABP and 573 healthy individuals. Both groups were comparable in terms of gender and age. To diagnose ABP, we used the clinical guidelines recommended by the working group of the Russian Society of Surgeons. DNA was isolated by phenol / chloroform extraction. Multiplex genotyping of SNPs was performed by the iPLEX assay on the MALDI-TOF MassARRAY-4 genetic analyzer. Statistical data processing was performed using Statistica 10 and SNPStats software.

Results. We found that insufficient consumption of fresh vegetables and fruits increased the probability of ABP in carriers of genotypes G/A-A/A at rs1801310 in *GSS* ($p = 0.02$). The analysis revealed the association of the T allele at rs6088660 with the odds for developing acute pancreatitis ($p = 0.007$) and digestive fistulas ($p = 0.02$). A high probability of death was associated with rs1801310 (G/A genotype, $p = 0.002$) and rs6088660 (C/T genotype, $p = 0.01$) in the *GSS* gene.

Conclusion. SNPs rs6088660 and rs1801310 in the *GSS* gene can be used to predict the course of ABP.

Keywords: acute biliary pancreatitis, rs13041792, rs1801310, rs6088660 in the *GSS* gene

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Ассоциация вариантов нуклеотидной последовательности гена *GSS* с риском развития острого билиарного панкреатита

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РЕЗЮМЕ

Цель: исследовать вклад вариантов нуклеотидной последовательности rs13041792, rs1801310 и rs6088660 гена *GSS* и некоторых средовых факторов в развитие острого билиарного панкреатита (ОБП) и его осложнений.

Материалы и методы. От 84 пациентов с ОБП и 573 здоровых индивидов были получены образцы крови для выделения геномной ДНК. Обе группы были сопоставимы по полу и возрасту. Диагностику заболевания проводили с использованием клинических рекомендаций, разработанных рабочей группой Российского общества хирургов. Геномную ДНК выделяли стандартным методом фенольно-хлороформной экстракции. Мультиплексное генотипирование SNPs проводили по технологии iPLEX на генетическом анализаторе MALDI-TOF MassARRAY-4. Статистическую обработку данных осуществляли с использованием программы Statistica 10, SNPStats.

Результаты. Установлено, что недостаточное употребление свежих овощей и фруктов повышает риск развития ОБП у носителей генотипов G/A-A/A rs1801310 *GSS* ($p = 0,02$). Проведенный анализ установил ассоциацию аллеля T rs6088660 с вероятностью развития гнойного парапанкреатита ($p = 0,007$) и дигестивных свищей ($p = 0,02$). Высокая вероятность смертельного исхода была связана с носительством SNPs rs1801310 (генотип G/A, $p = 0,002$) и rs6088660 (генотип C/T, $p = 0,01$) гена *GSS*.

Заключение. Варианты нуклеотидной последовательности rs6088660 и rs1801310 гена *GSS* можно использовать для прогнозирования течения ОБП.

Ключевые слова: острый билиарный панкреатит, rs13041792, rs1801310 и rs6088660 гена *GSS*

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

Соответствие принципам этики. Все лица, участвующие в исследовании, подписали информированное согласие на участие в исследовании. Исследование одобрено региональным этическим комитетом при КГМУ (протокол № 3 от 11.03.2013).

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INTRODUCTION

Acute biliary pancreatitis (ABP) is a complication of cholelithiasis which occurs following an impairment of bile and pancreatic juice outflow. ABP is a multifactorial disease taking place due to the interaction of genetic and environmental factors. Most of the research in the world is devoted to studying the role of genes regulating the synthesis of pancreatic enzymes, the kallikrein – kinin system, and cytokines, that play the crucial the role in the pathogenesis of ABP [1–3]. Environmental risk factors include consumption of fatty, fried, spicy foods that stimulate the exocrine function of the pancreas, insufficient consumption of

fresh vegetables and fruits rich in vitamins and antioxidants, as well as alcohol abuse and smoking [4, 5].

Disturbances in redox homeostasis that develop in ABP due to premature intraductal activation of pancreatic enzymes and hypertension lead to an increase in the concentration of calcium ions in acinar cells. It is this mechanism that serves as a link between the activation of trypsinogen, nuclear factor kB (NF-kB), the development of mitochondrial dysfunction, and cell death [6].

The depletion of intracellular glutathione observed at the onset of acute pancreatitis [7, 8] prompted us to study the contribution of genes regulating glutathione metabolism to the pathogenesis of ABP. Only a few works on this issue are known in the literature [9].

The main enzyme for glutathione biosynthesis is glutathione synthetase, which is expressed in the liver and pancreas. No studies on its role in the development of ABP and its complications have been conducted in the world yet.

The aim of this study was to investigate the contribution of single nucleotide polymorphisms (SNPs) rs13041792, rs1801310. and rs6088660 in the *GSS* gene and environmental factors to the risk of ABP and its complications.

MATERIALS AND METHODS

We examined and treated 84 ethnically Russian (self-identification) patients with ABP (24 women and 60 men) who received inpatient treatment at the surgery departments of hospitals in Kursk (clinical sites of the Department of Surgical Diseases No. 2) in 2015–2021. The material of the study was blood samples obtained from 84 patients with ABP and 573 (161 women and 412 men) healthy individuals who were selected following regular health checkups carried out during the same period. The mean age of the patients was 48.9 ± 13.1 years, the mean age of healthy individuals was 47.8 ± 12.1 years. ABP was diagnosed using clinical guidelines recommended by the working group of the Russian Society of Surgeons [10]. All participants signed a voluntary 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). All study participants answered the questions of the questionnaire which helped analyze the effect of environmental risk factors on the disease [11].

Genomic DNA was isolated by the standard phenol / chloroform extraction. Multiplex genotyping of SNPs was performed by the iPLEX assay on the MALDI-TOF MassARRAY-4 genetic analyzer (AgenaBioscience, USA).

To compare categorical variables between the groups, the c^2 test was used; to compare quantitative variables, the Student's *t*-test (for normally distributed variables) and the Mann – Whitney test (for non-normally distributed variables) were used. Since the distribution of the studied quantitative blood parameters was statistically significantly different from the normal one ($p < 0.05$, Kolmogorov – Smirnov test), these parameters were presented as the median and the interquartile range. To assess the influence of the studied SNPs on the normalized quantitative parameters, the linear regression analysis was used.

Associations of alleles and genotypes with the probability of developing the disease were assessed by the odds ratio (OR). The OR and 95% confidence interval (CI) were calculated by the logistic regression analysis with adjustment for sex and age using the SNPStats statistical package. To assess the associations of DNA markers with clinical characteristics (clinical forms, symptoms, nature of the course, severity of the disease, treatment efficacy), we also used the logistic regression analysis. Multiplicity adjustment of tests was performed by the permutation test (Pperm) using the PLINK program.

RESULTS

The genotype frequencies of the studied SNPs in the *GSS* gene met the Hardy – Weinberg principles in both study groups. We did not find associations of the studied SNPs with ABP.

The analysis of the role of environmental risk factors (alcohol abuse by frequency, volume, and duration; smoking; the content of proteins, fats and carbohydrates in the food consumed) in the risk of developing the disease found that insufficient consumption of fresh vegetables (less than 27 g / day) increased the risk of ABP in carriers of G/A-A/A at rs1801310 in the *GSS* gene (Table 1). Only statistically significant results are presented.

The analysis of the effect of SNPs on laboratory parameters, such as the level of amylase, oxidized glutathione, and blood leukocytes, established an association of rs1801310 in *GSS* with leukocytosis (Table 2).

The study also found that the frequency of the GG (rs1801310) *GSS* genotype was the lowest in patients with severe acute pancreatitis ($p = 0.01$) compared to mild and moderate forms of the disease.

Table 1

The influence of environmental factors on the development of acute biliary pancreatitis in carriers of the studied SNPs in the *GSS* gene (rs1801310)

Genotype	No risk factor			Presence of a risk factor		
	Healthy individuals	Patients with ABP	OR (95% CI)[1], *P*[2]	Healthy individuals	Patients with ABP	OR (95% CI)[1], *P*[2]
G/G	61 (35.5)	13 (40.6)	0.74 (0.33–1.62) 0.6	13 (59.1)	16 (30.8)	4.12 (1.38–12.28) 0.02
G/A-A/A	111 (64.5)	19 (59.4)		9 (40.9)	36 (69.2)	

[1] odds ratio and 95% confidence interval of associations of SNPs with the likelihood of developing the disease;
[2] significance levels for the most significant genetic models of associations of SNPs with the likelihood of developing the disease.

Table 2

Associations of SNPs with quantitative parameters of the blood in ABP patients

Parameter	*Me*	*Q1/Q3*	*p**
GSS G>A (rs1801310)	7.50	6.50/11.80	0.0005[D]
	9.10	6.75/15.00	
	7.90	6.60/11.20	

Note. D – dominant model.
* statistical significance of the association of SNP with normalized blood parameters (linear regression analysis).

The analysis of the associations of SNPs in the *GSS* gene with the likelihood of complications found that T allele at rs6088660 (OR=1.62, 95%CI 1.14–2.29, $p = 0.007$) was associated with the development of purulent acute pancreatitis and the formation of digestive fistulas (allele T, OR=4.54, 95%CI 1.19–17.33, $p = 0.02$).

A high probability of death was observed in carriers of the G/A genotype at rs1801310 (OR=6.76, 95%CI 1.51–30.38, $p = 0.002$) and C/T genotype at rs6088660 (OR=4.01,95%CI 1.24–13.04, $p = 0.01$) in the *GSS* gene.

DISCUSSION

Disturbances of redox homeostasis underlie many acute and chronic diseases; therefore, the study of the role of genes regulating glutathione metabolism enzymes is of great interest for researchers. When studying the effect of rs1801310 in the *GSS* gene on the risk of developing uterine fibroids, O.Yu. Bushueva et al. found no association of the locus with the disease [12]. However, associations of rs1801310 and rs6088660 in the *GSS* gene with the likelihood of ischemic stroke in men and women have been established [13].

Yu.E. Azarova et al. in the study on type 2 diabetes established an association of rs13041792 and rs6088660 in the *GSS* gene with changes in fasting blood glucose in men, an association of rs6088660 in *GSS* with a decrease in hydrogen peroxide in women, as well as an association of rs1801310 in *GSS* with a decrease in total glutathione in women [14]. W. Tang et al. established an association of rs13041792 in the *GSS* gene with the level of protein C in the blood plasma [15].

The logistic regression analysis established correlations between the expression of genes encoding glutathione metabolism enzymes with each other and with the genes encoding antioxidant enzymes (*GPX2, GSTP1*). Their co-expression with candidate genes for pancreatitis also attracts attention. *GSS* gene expression was positively associated with the level of *GGT6* gene mRNA ($r = 0.283$, $p = 0.0001$). SNPs rs1801310 and rs6088660 were associated with increased transcriptional activity of the *GSS* gene in the pancreas ($p = 0.01$) and liver ($p \leq 0.05$). Numerous eQTLs associated with the expression of molecular chaperones in the pancreas have been identified for SNPs of genes encoding glutathione metabolism enzymes.

Allele A at rs13041792 in the *GSS* gene was associated with increased expression of the *HSPE1* ($p = 0.0056$, $\beta = 0.11$), *HSPA1A* ($p = 0.0032$, $\beta = 0.16$), *HSPBP1* ($p = 0.0037$, $\beta = 0.15$), *HSPA4* ($p = 0.034$, $\beta = 0.095$), *HSPH1* ($p = 0.026$, $\beta = 0.12$), and *DNAJA1* ($p = 0.019$, $\beta = 0.12$) genes and decreased expression of *HSPA12A* ($p = 0.0019$, $\beta = –0.27$). Allele T at rs6088660 in *GSS* was associated with reduced expression of the chaperone genes *HSPE1* ($p = 0.030$, $\beta = –0.071$), *HSPA1A* ($p = 0.016$, $\beta = –0.11$), *HSPA1B* ($p = 0.0019$, $\beta = –0.13$), *HSPH1* ($p = 0.021$, $\beta = –0.10$), and *DNAJB1* ($p = 0.022$, $\beta = –0.11$). Allele G at rs1801310 in the *GSS* gene was associated with an increase in mRNA of the *HSPB1* gene ($p = 0.023$, $\beta = 0.083$). Allele G at rs1801310 was positively correlated with the expression level of the *CTSG* gene ($p = 0.048$, $\beta = 0.13$). Allele G at rs1801310 in the *GSS* gene was associated with increased expression of *AMY2A* ($p = 0.05$, $\beta = 0.075$), *CTRL* ($p = 0.011$, $\beta = 0.081$), *PRSS1* ($p = 0.043$, $\beta = 0.046$), and *SPINK1* ($p = 0.032$, $\beta = 0.096$) genes. Allele A at rs13041792 in *GSS* was associated with an increase in the transcriptional activity of the *SPINK1* gene ($p = 0.032$, $\beta = 0.096$). Allele T at rs6088660 in

the *GSS* gene was associated with an increased level of *CPA3* gene expression ($p = 0.035$, $\beta = –0.12$).

CONCLUSION

In the course of the study, we found that insufficient consumption of fresh vegetables and fruits increases the likelihood of developing ABP in carriers of the G/A-A/A genotypes at rs1801310 in the *GSS* gene ($p = 0.02$). The analysis of associations of SNPs of the *GSS* gene with an increased risk of complications established an association of the T allele at rs6088660 with the development of purulent acute pancreatitis ($p = 0.007$) and the formation of digestive fistulas ($p = 0.02$). A high risk of death was observed in carriers of the G/A genotype at rs1801310 ($p = 0.002$) and C/T genotype at rs6088660 ($p = 0.01$) in the *GSS* gene.

SNPs rs6088660 and rs1801310 of the *GSS* gene can be used to predict the course of ABP.

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