

TOPICAL ISSUE

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GOTOVTSEV Nyurgun Naumovich – scientific researcher, Laboratory of Molecular Genetics, Federal State Budgetary Scientific Institution "Yakut Science Center of Complex Medical Problems". Address: 677010, Sakha Republic, Yakutsk, Sergelyakhskoye Shosse, 4. Phone: 89141062015, e-mail: Donzcrew@mail.ru <https://orcid.org/0000-0002-4710-1592>, **BARASHKOV Nikolay Alekseevich** – Candidate of biological sciences, Head of laboratory of Molecular genetics, Federal State Budgetary Scientific Institution "Yakutsk Scientific Center of Complex Medical Problems". Address: 677010, Sakha Republic, Yakutsk, Sergelyakhskoye Shosse, 4. Phone: 8-(4112) 32-19-81; E-mail: barashkov2004@mail.ru <https://orcid.org/0000-0002-6984-7934>, **BORISOVA Tuyara Valerievna** – student, Institute of Natural Sciences, Federal State Autonomous Educational Institution of Higher Education "North-Eastern Federal University. M.K. Ammosov", 677000, Yakutsk, Kulakovskiy st. 48. Phone: 89644290223, e-mail: borisovaty96@gmail.com, **PAK Maria Vladimirovna** – endoscopist of the endoscopic department of the Republican Hospital No. 1 National Center of Medicine. Address: 677010, Sakha Republic, Yakutsk, Sergelyakhskoye Shosse, 4. e-mail: pakmv@mail.ru, **ALEXEEVA Mavra Pavlovna** – endoscopist of the endoscopic department of the Republican Hospital No. 1 National Center of Medicine. Address: 677010, Sakha Republic, Yakutsk, Sergelyakhskoye Shosse, 4, **INNOKENTYEVA Natalya Nikolaevna** – Post-graduate student, Medical Institute, Federal State Autonomous Educational Institution of Higher Education "North-Eastern Federal University. M.K. Ammosov", 677010, Yakutsk, Oyunsky st. 27. Phone: 8-(4112) 36-30-46, e-mail: natalia_inn@mail.ru, **LOSKUTOVA Kunnai Savvichna** – Candidate of Medical Science, Head of the Pathoanatomical department of the Republican Hospital No. 1 National Center of Medicine. Address: 677010, Sakha Republic, Yakutsk, Sergelyakhskoye Shosse, 4. e-mail: loskutovaks@mail.ru, **PSHENNIKOVA Vera Gennadiyevna** – Candidate of Biological Sciences, Head of laboratory of Population Genetics, Federal State Budgetary Scientific Institution "Yakutsk Scientific Center for Complex Medical Problems". Address: 677010, Sakha Republic, Yakutsk, Sergelyakhskoye Shosse, 4. Phone: 8-(4112) 32-19-81, e-mail: pshennikovavera@mail.ru <https://orcid.org/0000-0001-6866-9462>, **LEKHANOVA Sargylana Nikolaevna** – Candidate of Medical Science, Associate Professor, Medical Institute, Federal State Autonomous Educational Institution of Higher Education "North-Eastern Federal University M.K. Ammosov", 677010, Yakutsk, Oyunsky st. 27. Phone: 8-(4112) 36-30-46, e-mail: lehanovash@mail.ru, **FEDOROVA Sardana Arkadiyevna** – Doctor of biological sciences, Head of the Research Laboratory of Molecular Biology Institute of Natural Sciences, Federal State Autonomous Educational Institution of Higher Education "North-Eastern Federal University M.K. Ammosov". Address: 677010, Sakha Republic, Yakutsk, Kulakovskiy st. 46., e-mail: sardanaafedorova@mail.ru <https://orcid.org/0000-0002-6952-3868>

N.N. Gotovtsev, N.A. Barashkov, T.V. Borisova, M.V. Pak, M.P. Alekseeva, N.N. Innokentiyeva, K.S. Loskutova, V.G. Pshennikova, S.N. Lekhanova, S.A. Fedorova

ANALYSIS OF CLINICAL OUTCOMES IN PATIENTS WITH GASTRODUODENAL DISEASES DEPENDING ON *HELICOBACTER PYLORI* *vacA* GENE VARIANTS IN YAKUTIA

Vacuolating cytotoxin (VacA) is an important virulence factor coding for vacuolizing toxin and present in all *Helicobacter pylori* strains. There is a significant difference in vacuolating activity among strains due to the heterogeneity of the sequence in the m- and s- regions of the *vacA* gene of *Helicobacter pylori* strains. The m region has allelic types *m1* and *m2*, and the s region located at the 5'-end of the gene has alleles *s1* or *s2*. Strains carrying the mosaic combination of the *vacAs1m1* gene demonstrate higher levels of cytotoxic activity than *s1m2* strains, while *s2m2* strains do not secrete vacuolating cytotoxin. The effect of the *vacA* gene alleles on gastroduodenal diseases in patients in Yakutia has not been studied. The aim of this study is analysis of clinical outcomes in patients with gastroduodenal diseases depending on the variants of the *Helicobacter pylori vacA* gene in Yakutia.

According to the results of the histological report, 154 patients (out of 322 examined) were included in the study, in whom the presence of *Helicobacter pylori* in the antrum of the stomach was revealed. The patients were divided into two groups: chronic gastritis (n=65) and chronic gastritis with erosive and ulcerative lesions (n=89). The average age was 26.6 years (range 5 to 70). The diagnosis of chronic gastritis was established in 65 cases (42.2%), and chronic gastritis with erosive and ulcerative lesions was diagnosed in 89 cases (57.7%). The *vacAs1m1* allele was found in the sample with erosive gastritis in 30 patients among 39 (76.9%) and 9 out of 39 patients in the sample with chronic gastritis (23.1%) (p<0.01). In relation to other allelic variants of *vacA*, no statistically significant differences were found. The high rate of the *vacAs1m1* allele in Yakut patients confirms the hypothesis that the *vacAs1m1* alleles of *Helicobacter pylori* are the most cytotoxic and are more common in the sample of patients with more severe gastritis in peptic ulcer disease.

Keywords: *Helicobacter pylori*, gastroduodenal diseases, *vacA* gene, Yakutia.

Introduction. *Helicobacter pylori* (*H. pylori*) is a spiral-shaped gram-negative bacterium that infects various areas of the stomach and duodenum. Many cases of gastric and duodenal ulcers, gastritis, duodenitis, gastric cancer, and possibly some cases of gastric lymphomas are etiologically associated with *H. pylori* infection. However, most infected carriers of *H. pylori* show no symptoms of disease [2]. The genome of *H. pylori* strain "26695" is represented by a circular double-stranded DNA molecule with a size of 1667867 base pairs, and contains 1630 genes, of which 1576 encode proteins [19]. The results of two sequences of *H. pylori* genomes were published, in which a large family of 32 bound outer membrane proteins (HOP) was discovered, including the most famous *H. pylori* adhesins [19].

It is found that *H. pylori* has a number of the most characteristic adhesins that have focused on the action of human gastric epithelial cells. *H. pylori* produces specific proteins that can presumably be attributed to virulence and pathogenicity factors. Vacuolating cytotoxin (VacA) is

an important virulence factor encoding vacuolizing toxin and is present in all strains of *H. pylori* [7]. There is a significant difference in vacuolizing activity among *H. pylori* strains [1, 3] due to the heterogeneity of the sequence in the m and s regions of the *vacA* gene. Region m has allelic types *m1* and *m2*, and s region located at the 5'-end of the gene has alleles *s1* or *s2*. Strains carrying a mosaic combination of the *vacAs1m1* gene demonstrate higher levels of cytotoxic activity than *s1m2* strains, while *s2m2* strains do not secrete vacuolizing cytotoxin [15, 20]. Infection with vacuolizing cytotoxin-positive strains is associated with certain gastroduodenal diseases [19, 21].

Most *H. pylori* strains deliver the 95kDa vacuolating cytotoxin VacA secreted by the exotoxin. The toxin is injected into the epithelial cell membranes and forms hexameric anion-selective, voltage-gated channels through which bicarbonate and organic anions can be released, with the ability to provide nutrients. VacA toxin causes the release of cytochrome C and induces apoptosis [19].

In addition to vacuolization of gastric epithelial cells, VacA inhibits acid secretion in the stomach, increases the secretion of pepsinogen, inhibits cell proliferation, damages mitochondria, disorganizes the cytoskeleton of gastric epithelium cells, and provides nutrients to the bacteria. *H. pylori* can "feed" on the exudate of the host tissue, while certain importance has the inflammatory process in the gastric mucosa, which is caused by the bacterium. Damage to the gastric mucosa is facilitated by the formation of proinflammatory cytokines: IL-1 β , IL6, IL-8, and tumor necrosis factor (TNF- α) [18, 22].

The production of *H. pylori vacA* exotoxins causes vacuolization, damage and death of cells of the gastric mucosa. The clinical outcomes of gastroduodenal diseases, depending on the variants of the *H. pylori vacA* gene, common in Yakutia, have not been previously studied. The aim of this study is analysis of clinical outcomes in patients with gastroduodenal diseases depending on the variants of the *Helicobacter pylori vacA* gene in Yakutia.

Materials and methods. Gastric biopsy specimens were obtained from 322 patients that admitted to the endoscopic department for fibrogastroduodenoscopy (FGDS) in endoscopic department of State autonomous institution of Republic Sakha (Yakutia) «Republican Hospital No. 1 – National Center of Medicine» (RH No.-1 NCM). To confirm the presence of *H. pylori* infection, gastric biopsy specimens were sent for histological examina-

patients were divided into two groups: chronic gastritis and chronic gastritis with erosive and ulcerative lesions.

Genomic DNA of *H. pylori* was isolated from frozen gastrobiopsies of the examined patients by using phenol-chloroform extraction [10].

Fibrogastroduodenoscopy was performed in the morning on an empty stomach. Biopsy specimens were taken from the antrum in amount of 2-3 pieces during endoscopic examination using a GIF-P3 fiberscope (Olympus, Japan).

Obtained biopsies of the gastric mucosa were fixed in 10% formalin solution. Dewaxing of sections and staining with hematoxylin and eosin were carried out according to the standard technique. For targeted bacterioscopy, sections are stained according to the Romanovsky-Giemsa method. The study was performed under magnification x100, X400 and X1000 on the "Axioskop" microscope "Opton" company. The morphological criteria of chronic gastritis were assessed in accordance with the visual analogue scale according to the modified Sydney system (Houston, USA, 1996).

The genomic DNA of *H. pylori* was isolated from frozen gastrobiopsy specimens from patients with a confirmed histological diagnosis of chronic gastritis and chronic gastritis with erosions and ulcers using phenol-chloroform extraction.

Amplification of the required *H. pylori* DNA fragments was performed using the Bio-Rad PCR amplifier. The detection of alleles of the *vacA* gene was carried out

using the original sequences of oligonucleotide primers previously proposed [Hou et al., 2000], that flank the DNA region of the *H. pylori* containing *vacA* gene. Separation of amplification products was carried out in horizontal electrophoresis chambers in 3% agarose gel. Visualization of PCR products was carried out using a Bio-Rad gel-video documentation device using Image Lab™ Software.

Differences between groups were evaluated by x-square test using Biostat software (McGraw-Hill, Inc. Version 3.03). Differences were considered statistically significant at $p < 0.05$.

The surveys, provided by the framework of research work, were carried out strictly after the informed consent of participants, parents or legal representatives of minor patients without violations of ethical standards. This study was approved by the local committee on biomedical ethics of the Yakutsk Scientific Center for Complex Medical Problems. Protocol No. 41 of November 12, 2015. Decision No. 5.

Results and Discussion. Analysis of the frequency of *H. pylori vacA* gene variants in patients with gastroduodenal diseases

Endoscopic and histological examinations carried out at the first stage of the work showed that only 154 (out of 322 patients) were confirmed to have the *H. pylori*. The diagnosis of chronic gastritis was established in 65 cases (42.2%), and chronic gastritis with erosive and ulcerative lesions was diagnosed in 89 cas-

Table1

Design of oligonucleotide primers for the detection of alleles of the *vacA* gene

Gene	Primer	Sequence from 5' → 3' end	Fragment size	Reference
<i>vacA</i>	<i>vacAs1</i>	F5'-ATGGAAATACAACAAACACAC-3'	259 п.н.	[13]
		R5'-CTGCTTGAATGCGCCAAAC-3'		
	<i>vacAs2</i>	F5'-ATGGAAATACAACAAACACAC-3'	286 п.н.	
		R5'-CTGCTTGAATGCGCCAAAC-3'		
	<i>vacAm1</i>	F5'-GGTCAAAATGCGGTCATGG-3'	290 п.н.	
		R5'-CCATTGGTACCTGTAGAAAC-3'		
	<i>vacAm2</i>	F5'-GGAGCCCCAGGAAACATTG-3'	352 п.н.	
		R5'-CATAACTAGCGCCTTGCAC-3'		

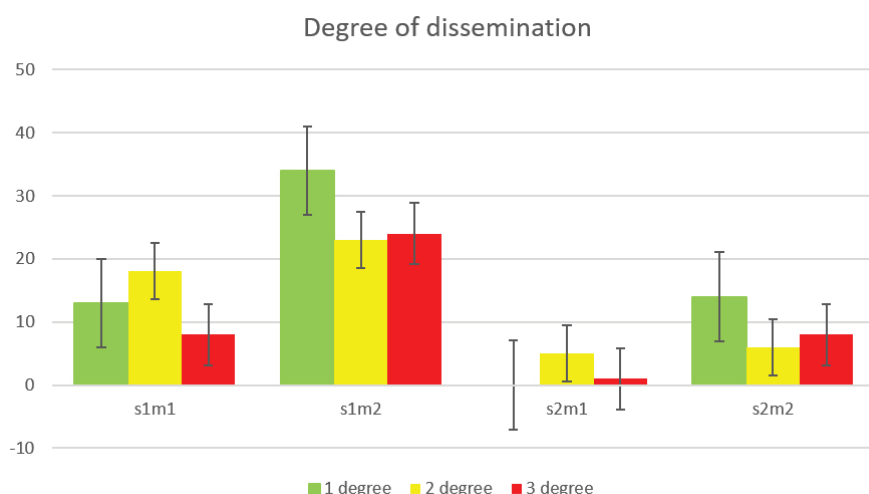
tion to the pathoanatomical department of RH No.-1 NCM.

According to the results of histological analysis, 154 patients (out of 322) were included in the study, who had the presence of *H. pylori* in the antrum. The average age was 26,6 years (from 5 to 70). In accordance with macroscopic analysis of the mucosa and histological results,

Table2

Prevalence of *H. pylori* genotypes in detected strains

Gene	<i>H. pylori vacA</i> strains	Prevalence
<i>vacA</i> (154)	<i>s1m1</i>	39 (25.2)
	<i>s1m2</i>	81 (52.4)
	<i>s2m1</i>	6 (4.0)
	<i>s2m2</i>	28 (18.3)



Comparative analysis of *vacA* gene with dissemination degree in patients with gastroduodenal diseases.

es (57.7%). Next, we analyzed the frequencies of *H. pylori vacA* gene variants among 154 samples with histologically confirmed *H. pylori* infection. Regions of the s- and m- genotype *vacA* were determined in all studied strains of *H. pylori*, among which four genotypes were found: *s1m1* (25.2%), *s1m2* (52.4%), *s2m1* (4.0%) and *s2m2* (18.3%) (Table 2).

Comparison of the occurrence of allelic variants of the H. pylori vacA gene in patients depending on the course of gastroduodenal diseases (erosive or chronic gastritis)

It was found that the *vacAs1m1* gene variant was significantly more frequent in the sample of patients diagnosed with chronic gastritis with erosive and ulcerative lesions of the stomach and duode-

Table3

Comparison of the frequency of *vacA* genes depending on the presence of erosions and ulcers, age and sex and demographic factors

Factors	n (154)	CG with erosions and GU/DU	CG	χ^2	<i>p</i>
Dependence on the presence of erosions and ulcers					
<i>vacAs1m1</i>	39	30 (76.9)	9 (23.1)	8.215	<0.01
<i>vacAs1m2</i>	81	40 (49.3)	41 (50.6)		
<i>vacAs2m1</i>	6	4 (66.6)	2 (33.3)	0.334	>0.05
<i>vacAs2m2</i>	28	15 (53.5)	13 (46.4)		
Dependence of age					
Factors	n (154)	Children	Adults	χ^2	<i>p</i>
<i>vacAs1m1</i>	39	20 (51.3)	19 (48.6)	0.755	>0.05
<i>vacAs1m2</i>	81	46 (59.7)	35 (40.2)		
<i>vacAs2m1</i>	6	4 (60.0)	2 (40.0)	0.170	>0.05
<i>vacAs2m2</i>	28	14 (51.8)	14 (48.1)		
Comparison by gender					
Factors	n (154)	♂	♀	χ^2	<i>p</i>
<i>vacAs1m1</i>	39	20 (51.2)	19 (48.7)	0.201	>0.05
<i>vacAs1m2</i>	81	38 (46.9)	43 (53.1)		
<i>vacAs2m1</i>	6	0 (0)	6 (100)	5.100	<0.05
<i>vacAs2m2</i>	28	14 (50.0)	14 (50.0)		
Comparison on place of residence					
Factors	n (154)	Urban population	Rural population	χ^2	<i>p</i>
<i>vacAs1m1</i>	39	6 (15.3)	33 (84.6)	0.179	>0.05
<i>vacAs1m2</i>	81	15 (18.5)	66 (81.4)		
<i>vacAs2m1</i>	6	0 (0)	6 (100)	1.561	>0.05
<i>vacAs2m2</i>	28	6 (21.4)	22 (78.5)		

num (76.9%) than in patients diagnosed with chronic gastritis, in whom the *vacAs1m2* gene variant was more common (50.6%) ($p < 0.01$) (Table 3).

Comparison of the occurrence of allelic variants of the H. pylori vacA gene in patients depending on the degree of contamination

No statistically significant differences were found when comparing groups of patients with different degrees of contamination and the presence of variants of the gene *vacAs1m1*, *vacAs1m2*, *vacAs2m1* and *vacAs2m2* *H. pylori*. However, there was a slight predominance of grade 2 contamination in patients with the *vacAs1m1* gene variant (рис. 1).

Comparison of allelic variants of the H. pylori vacA gene in group of patients depending on gender, age, place of birth and residence

There were no significant differences depending on age, place of birth and residence in patients with *vacA* gene variants of the *H. pylori* ($p > 0.05$) however, statistically significant differences were found when comparing groups of men and women ($p < 0.05$) (Table 3). Thus, the frequency of occurrence of the *vacAs2m1* gene variant in the group of female patients was 100% ($p < 0.05$), while in male patients this allelic variant was not recorded (0%) (Table 3).

In this study the incidence of gene variants *vacA H. pylori* has been studied and analyzed for the first time in patients with chronic gastritis in Yakutia. It was found that when analyzing a sample of patients with different variants of the *H. pylori vacA* gene, statistically significant differences were found in the group of patients, depending on the presence of erosive and ulcerative manifestations (Table 3). Thus, in patients with erosive and ulcerative lesions, the *H. pylori vacAs1m1* variant was significantly more often identified than in patients with chronic gastritis (76.9%) ($p < 0.01$). Obtained results are consistent with the results of similar studies in other populations, where the frequency of the *vacAs1m1* gene variant was higher than the frequency of *vacAs1m2* in patients with chronic gastritis complicated by erosions and ulcers of the stomach and duodenum. (In Russia: Rostov-on-Don – 48.0%, Vladivostok – 57.2%, St. Petersburg – 55.5%; in Thailand – 58.0%, in Southern Mexico – 61.4%) [4-6, 14, 17].

This result may indicate a more pronounced pathogenic potential of the *H. pylori vacAs1m1* gene variant. The results of our study are in accordance with the previously obtained data that the presence of the *vacAs1m1* gene variant

in the *H. pylori* genome is associated with a higher incidence of duodenal ulcer disease, complicated course of *H. pylori* infection, as well as with gastric adenocarcinoma. [8, 12, 14, 16].

When comparing *vacA* gene variants with inflammation activity, no statistically significant differences were found. However, a slight difference was found when comparing the *vacAs1m1* gene variant with the 2nd degree of contamination (Fig. 1). The insignificant difference is most likely explained by the small number of compared subgroups in the studied sample.

When comparing *H. pylori vacA* gene variants in patients depending on age, place of birth and residence, no statistically significant differences were obtained. ($p > 0.05$). However, when analyzed according to gender in women, the allelic variant *vacAs2m1* of *H. pylori* was 100% and most likely due to stochastic reasons ($p < 0.05$) (Table 3). The *vacAs2m1* gene variant is very rare and also belongs to non-virulent variants. Previous studies indicate a very low cytotoxic activity and a very low frequency of this allelic variant [9, 11].

With help of the obtained results for the alleles of the *vacA* gene and it will be possible to obtain data that will make it possible to determine how virulent and pathogenic the identified strain of *H. pylori* is, thereby making it possible to choose a more appropriate tactics for treating patients with chronic gastritis in Yakutia.

Conclusion. We have shown a relationship between a high rate of the *vacAs1m1* gene variant with a more severe clinical course of gastroduodenal diseases in Yakut patients. The obtained result confirms the hypothesis that the *vacAs1m1* alleles are the most cytotoxic and are more common in patients with more severe gastritis in peptic ulcer disease.

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