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PREVALENCE OF THE EAST ASIAN CAGA AND WESTERN CAGA GENES OF HELICOBACTER PYLORY IN YAKUTIA

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There are several alleles of the *cagA* gene, in which a change in the carboxyl end of its protein is the main distinguishing feature between different alleles. Polymorphisms at the C-terminus occur in the so-called EPIYA region and usually involve changes in amino acid sequences flanking repetitive five amino acid sequences – Glu-Pro-Ile-Tyr-Ala. In Yakutia, the estimation of prevalence of the East Asian *cagA* and Western *cagA* genes in *Helicobacter pylori* strains has not been studied. The aim of this work is to analysis of the prevalence of the Western *cagA* and East Asian *cagA* genes among *Helicobacter pylori* strains in Yakutia. The study sample consisted of 30 *Helicobacter pylori* DNA samples isolated from biopsy specimens of patients with gastroduodenal diseases. As a result of the endoscopic and histological examination, the presence of *Helicobacter pylori* infection was confirmed in 30 patients. The Western *cagA* was identified in 12 of 30 samples (40%). In 18 samples (60%) the *cagA* gene variant was not identified, since it did not belong to either the Western *cagA* or the East Asian *cagA*. The analysis of the prevalence of East Asian and Western *cagA* among *Helicobacter pylori* strains in Yakutia revealed that the prevailing variant of the *cagA* gene is the Western *cagA* and the East Asian *cagA* was not found in none of the samples. In 60% of cases, the samples did not belong to either the Western or the East Asian *cagA* gene, which may indicate the presence of their own *cagA* gene sequence among *Helicobacter pylori* strains in Yakutia.

Key words: *Helicobacter pylori,* East Asian *cagA,* Western *cagA*, EPIYA motif, Yakutia

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Introduction. *Helicobacter pylori* (*H. pylori*) – is a gram-negative, spiral-shaped bacterium that colonizes the gastric mucosa of the human stomach and duodenum, causing various gastroduodenal diseases (chronic gastritis, gastric and duodenal ulcers, MALT lymphoma, and stomach cancer) [5, 20]. The family is the basic unit of transmission for *H. pylori*. Children often become infected with a strain that is genetically identical to one of the parent's strain [23]. Consequently, transmission is likely to occur more frequently in the family or among infants. Once the infection has entered the human body, subsequent infection with other strains of *H. pylori* becomes unlikely [11]. According to some estimates, more than half of the world's population is infected with *H. pylori. H. pylori* infection is often not clinically apparent [19]. Only a certain part of the infected (20%) over time develop clinically significant symptoms of the disease: chronic gastritis, gastric and duodenal ulcer, stomach cancer [1, 24].

H. pylori is genetically more diverse than other types of bacteria. The characteristics of DNA samples and the sequence of any different DNA fragments almost always differ between independent pairs of isolates, and a comparison of the genomes of two strains in one study showed that 7% of genes are specific for each strain [23]. *H. pylori* DNA sequences can be used to distinguish between closely related human populations and are superior in this respect to classical human genetic markers [13, 28].

There are several alleles of the *cagA* gene, in which a change in the carboxyl end of its protein is the main distinguishing feature between different alleles. Polymorphisms at the C-terminus occur in the so-called EPIYA region and usually involve changes in amino acid sequences flanking repeated amino acid sequences – Glu-Pro-Ile-Tyr-Ala. In 2006, based on the deep sequence of the *cagA* gene was built a phylogenetic tree, and it was noted that the most spread motifs are EPIYA -A, -B, -C and -D and are in two different combinations by geographic location [4, 6, 12, 17]. The combination of EPIYA-A, -B and -C motifs (identified up to five motifs EPIYA-C) belongs to the Western *cagA*, while the combination of EPIYA-A, -B and -D motifs belongs to the East Asian *cagA* [6, 8, 9, 22]. The EPIYA-C motif is found everywhere (Iran, India, Kazakhstan, Greece, Italy, Sweden, Ireland, USA, Costa Rica, and Colombia) [3], as well as from 8 to 40% in samples of Southeast Asian countries (Japan, China, Korea, Thailand and Malaysia) [4]. The EPIYA-D motifs were clustered separately from strains isolated in Europe and found only in the countries of Southeast Asia (Malaysia, Vietnam, Thailand, Korea, China, and southern Japan) [14, 25, 29]. Subsequently, it was noted that both East Asian *cagA* and Western *cagA* circulate in Southeast Asia [26, 27, 30]. Interesting, that Truong et al., in 2009 among the inhabitants of the Okinawa island, Japan, there were found *H. pylori* strains with the motif of the *cagA* gene is very similar to the "Western *cagA*" [14], but it forms separate isolated cluster, that locate between two branches on the phylogenetic tree – Western CagA и East Asian CagA [14]. Thus, authors conclude, that there is Japanese subtype of the Western CagA from an enclave of populations from the Okinawa island (J-Western *cagA* subtype) [14, 19].

In Yakutia, the estimation of the frequency of the East Asian *cagA* and Western *cagA* genes in *Helicobacter pylori* strains has not been performed. The aim of this work is to analysis of the frequencies of the Western *cagA* and East Asian *cagA* gene strains of *Helicobacter pylori* in Yakutia.

Materials and methods. The study sample consisted of 30 *H. pylori* DNA samples isolated from biopsy specimens of patients with gastroduodenal diseases. The diagnosis was confirmed by histological and cytological methods in the endoscopy department of the Republican Hospital No. 1 - National Center of Medicine of the Ministry of Health of the Republic of Sakha (Yakutia). Among the studied patients 12 were women (40%), 18 were men (60%). Distribution by age – 21 patients were children and adolescents (from 8 to 17), 9 were adults (21-57 years old), the median age was 19.16 years. Among 30 patients 26 were Yakuts (86.6%), 2 were Russians (6.6%), 1 were Yukaghir (3.3%) and in one patient (3.3%) nationality was not established.

Genomic DNA of *H. pylori* was isolated from frozen gastrobiopsies of the examined patients by using phenol-chloroform extraction [16]. 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 microscope "Axioskop" ("Opton", Germany). 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 confirmed histological diagnosis of chronic gastritis and chronic gastritis with erosions and ulcers using phenol-chloroform extraction. To perform genotyping of the DNA fragments of the *H. pylori* East Asian *cagA* and Western *cagA*, oligonucleotide primer sequences were used that flanking required marker regions of this gene (Table 1). Visualization of PCR products was carried out using a gel-video documentation device ("Bio-Rad") using Image Lab ™ Software.

Sequences of oligonucleotide primers for two variants of the cagA gene: East Asian cagA and Western cagA of H. pylori

Gene	Sequences of oligonucleotide primers	Size (b.p.)
East Asian *cagA*	F: 5’- AAAGGAGTGGGCGGTTTCA-3’ R: 5’- CCTGCTTGATTTGCCTCATCA-3’	91
Western *cagA*	F:5’- AGGCATGATAAAGTTGATGAT-3’ R:5’- AAAGGTCCGCCGAGATCA-3’	88

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. 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. In the course of the endoscopic and histological examination, the presence of *H. pylori* infection was confirmed in 30 patients. Further, a molecular genetic analysis of the prevalence of *H. pylori* East Asian and Western *cagA* gene circulating in Yakutia was carried out. The East Asian *cagA* variant was not detected among 30 *H. pylori* DNA samples. The Western *cagA* was identified in 12 out of 30 samples (40%) (Fig. 1). 18 samples (60%) did not belong to either the Western *cagA* or the East Asian *cagA*.

It is known that on the basis of the amino acid sequence of the repeated fragments of the EPIYA motif in the *cagA* gene, it is possible to estimate the geographical origin of the *H. pylori* studied strain. Repeated EPIYA-D motifs in the *cagA* gene were not detected in our samples, that indicates the absence of the East Asian *cagA* in studied sample. In our study, it was noted that *H. pylori* strains with Western *cagA* dominate in Yakutia, since have repeating EPIYA-C motifs.

It is known from previously published works that in almost all countries, except the countries of Southeast Asia, the Western *cagA* gene variant is the predominant with EPIYA-C motifs (Canada – 95%, USA – 98%, Mexico – 73.8%, Colombia – 83, 7%, France – 95%, Italy – 100%, Greece – 74.8%, Iran – 88.1%, Mongolia – 79.6%) [7, 10, 26, 31, 32, 34]. The East Asian *cagA* with EPIYA-D motifs dominates in the Japanese Islands (98.4%), the Korean Peninsula (86.6%), China (42%), as well as in some countries washed by the South China Sea (Vietnam – 56.7% and Malaysia – 37.2%) (Fig. 2) [4, 12, 26, 27, 30]. In other regions of Asia, the East Asian *cagA* with EPIYA-D motifs occurs with lower frequencies (Thailand – 14% and Mongolia

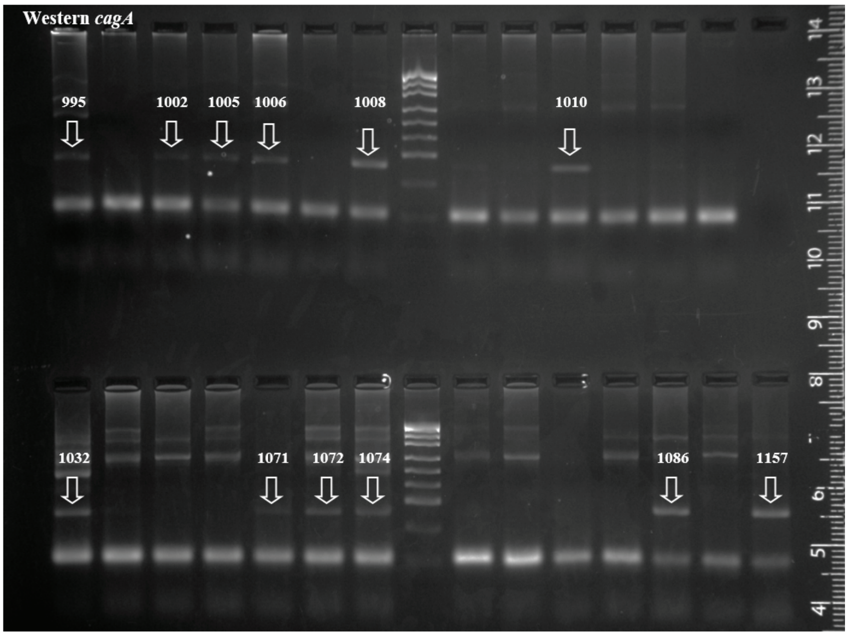
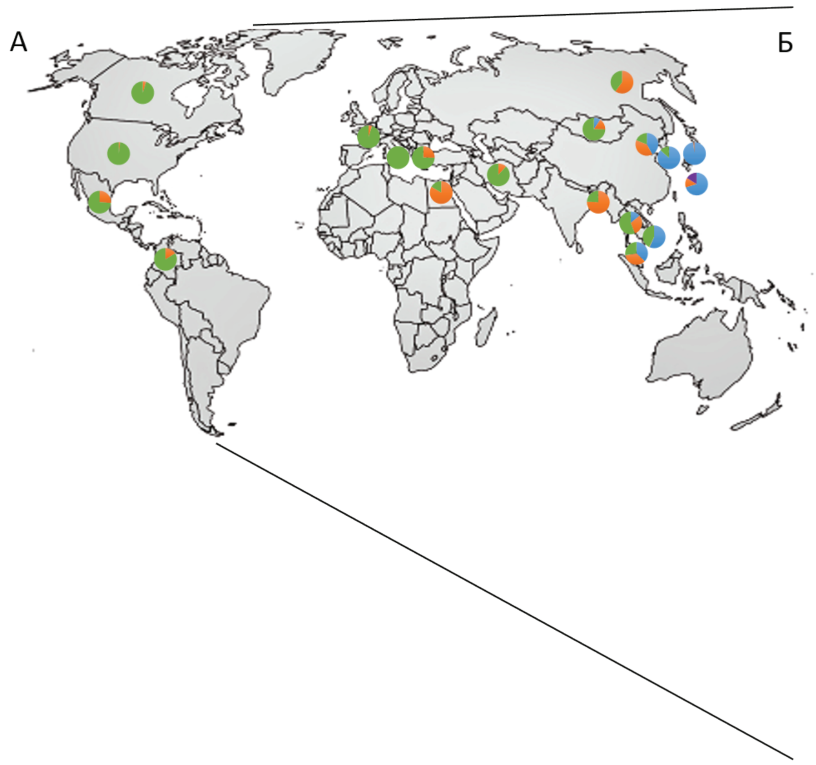


Fig 1. Electrophoregram of DNA samples with Western cagA gene (only positive samples are numbered).



Страна	Количество образцов	Western *cagA*	East Asian *cagA*	Неопределенный вариант *cagA*	Ссылка
Япония (Окинава)	337	54 16,1%	237 70,2%	46 13,6%	[5]
Япония (Фукуи)	65	0 0%	64 98,4%	1 1,5%	[4]
Китай (Далянь)	141	27 19%	59 42%	55 39%	[27]
Корея	45	6 13,3%	39 86,6%	0 0%	[12]
Таиланд	50	28 56%	7 14%	15 30%	
Вьетнам	171	73 42,6%	97 56,7%	1 0,6%	[26]
Малайзия	578	160 27,6%	215 37,2%	203 35,1%	[30]
Иран	159	140 88,1%	0 0%	19 11,8%	[10]
Египет	60	10 16,6%	0 0%	50 83,3%	[23]
Бангладеш	78	19 24,3%	0 0%	59 75,6%	[17]
Россия (Якутия)	30	12 40%	0 0%	18 60%	Данная работа
Монголия	368	293 79,6%	12 3,2%	63 17,1%	[34]
Греция	135	101 74,8%	0 0%	34 25,1%	[31]
Италия	20	20 100%	0 0%	0 0%	[26]
Франция	100	95 95%	0 0%	5 5%	
США	100	98 98%	0 0%	2 2%	
Канада	20	19 95%	0 0%	1 5%	
Колумбия	80	67 83,7%	0 0%	13 16,2%	[7]
Мексика	287	212 73,8%	0 0%	75 26,1%	[32]

Fig 2. The prevalence of two cagA gene variants among H. pylori strains in the world: A – The distribution of East Asian cagA and Western cagA gene variants in the world; B – initial data; Note: blue color – East Asian cagA, green color – Western cagA, violet color – J-Western cagA, orange color – undefined cagA.

3.2%), giving way to the Western *cagA* with EPIYA-C motifs (Thailand – 56% and Mongolia 79.6%) [12, 34]. A rather high percentage of detection of the Western *cagA* in Yakutia (40%) is consistent with the available information about the European origin of most *H. pylori* lines (hpEurope haplotype – 89.3%) circulating in Yakutia, according to the three housekeeping genes *atpA*, *mutY*, *ppa* [2].

The presence of undefined variants of the *cagA* gene (60%) in Yakutia is most likely explained by the fact that the *cagA* gene may have a sequence that differs from the sequences of East Asian and Western *cagA*. Undefined variants of the *cagA* gene were also found in Mexico (26.1%), Colombia (16.2%), Greece (25.1%), Egypt (83.3%), Iran (11.8%), Bangladesh (75.6%), Thailand (30%), Malaysia (35.1%), China (39%), Mongolia (17.1%) and Japan (13.6%), which may also indicate about local variants that differ from the previously identified East Asian and Western *cagA*.

Conclusion. Analysis of the prevalence of East Asian and Western *cagA* among *Helicobacter pylori* strains in Yakutia revealed that the prevailing variant of the *cagA* gene is the Western *cagA* and the East Asian *cagA* was not found in none of the samples. In 60% of cases, the samples did not belong to either the Western or the East Asian *cagA* gene, which may indicate about the presence of their own *cagA* gene sequence among *Helicobacter pylori* strains circulating in Yakutia.

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