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GENETIC POLYMORPHISMS OF *HELICOBACTER PYLORI* CLINICAL ISOLATES IN ST. PETERSBURG, RUSSIA

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Abstract. *Introduction. Helicobacter pylori* was proved to be the principal causative agent of gastroduodenal disorders in human. Although Russian Federation is among the countries with a high prevalence of *H. pylori* infection (60–90%), currently there is a very limited number of studies evaluating *H. pylori* genotypes in Russia. *Objective.* Based on the assessment of virulence-associated *cagA, oipA*, and *vacA* genes, our study was aimed to determine *H. pylori* genotypes associated with the clinical outcomes in patients with *H. pylori* infection in St. Petersburg, Northwest Russia. *Materials and methods.* Using PCR for the detection of *cagA, oipA*, and *vacA* s, m, i allelic variants, we analyzed 61 *H. pylori* isolates isolated and cultured from biopsies collected during endoscopy of patients with chronic gastritis (G), duodenal ulcer (DU), and gastric cancer (GC). *Results.* The genetic diversity of *H. pylori* clinical isolates has been revealed (HGDI 0.88): 41 (67%) of 61 *H. pylori* isolates were *cagA*-positive, 38 (62%) — *oipA*-positive. The proportions of *cagA*+ isolates differed in patients with G (56.7%) and DU (80.9%) ($p = 0.06$). The s, m, and i allelic variants of the *vacA* gene were detected in all strains, although the *vacA* s1 allele was significantly dominant in patients with DU (95.2%) rather than with G (64.9%) ($p = 0.01$). The *vacA* alleles m1 and i1 in the isolates from patients with G and DU were found in almost equal proportions: 45.9% and 42.8% for m1 allele, 45.9% and 47.6% for i1 allele, respectively. Seven isolates (11.5%) were positive for different mixed combinations of vacA alleles s, m, and i. Noteworthy, all *vacA* s2 strains were *cagA*-negative and had the m2 allele. *OipA*+ strains were found in almost equal proportions in patients with G (62.2%) and DU (57.1%) ($p = 0.71$). All three *cagA*- and *oipA*-positive isolates from patients with GC carried *vacA* s1/m1/i1 alleles. Different combinations of virulence-associated determinants constituted 17 genetic profiles. The most common combined genotype *cagA+/oipA+/vacA* s1/m1/i1 comprised 18 (29.5%) *H. pylori* isolates. *Conclusion.* We have determined predominant genotypes in the *H. pylori* population in the Northwest of Russia. The significant association between *vacA* s1 genotype of the pathogen and clinical manifestations of *H. pylori* infection has been established in our study.

Key words: *Helicobacter pylori, cagA gene, vacA gene, oipA gene, gastritis, duodenal ulcer, gastric cancer, virulence determinants, genomic polymorphism.*

ГЕНОМНЫЙ ПОЛИМОРФИЗМ КЛИНИЧЕСКИХ ИЗОЛЯТОВ *HELICOBACTER PYLORI* В САНКТ-ПЕТЕРБУРГЕ, РОССИЯ

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Резюме. *Введение. Helicobacter pylori* — основной возбудитель гастродуоденальных заболеваний человека. Несмотря на то что Российская Федерация относится к числу стран с высоким уровнем распространенности

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инфекции *H. pylori* (60–90%), в настоящее время довольно ограниченное количество исследований посвящено генетическому разнообразию *H. pylori* в России. Цель — на основании оценки генов вирулентности *cagA, oipA* и *vacA* изучить геномный полиморфизм клинических изолятов *H. pylori*, полученных от различных групп больных на территории Санкт-Петербурга, Россия. *Материалы и методы.* Изучен 61 штамм *H. pylori*, выделенных от пациентов с хроническим гастритом (ХГ), язвой двенадцатиперстной кишки (ЯДК) и раком желудка (РЖ). Стандартный метод ПЦР использовали для детекции генов *cagA, oipA* и аллельных вариантов гена *vacA* (s, m, i). *Результаты.* Установлена генетическая неоднородность 61 штамма *H. pylori* (HGDI 0.88): 41 (67%) штамм был *cagA*-позитивным, 38 (62%) были *oipA*-позитивными. Доли *cagA*+ штаммов различались у пациентов с ХГ (56,7%) и ЯДК (80,9%) (p = 0,06). Ген *vacA* в различных s-, m-, i-аллельных вариантах выявлен у всех штаммов. Доля штаммов аллельного варианта *vacA* s1 существенно превалировала у пациентов с ЯДК (95,2%) по сравнению с больными ХГ (64,9%) (p = 0,01). Аллели *vacA* m1 и i1 у штаммов от пациентов с ХГ и ЯДК были обнаружены почти в равных пропорциях: 45,9 и 42,8% для аллеля m1, 45,9 и 47,6% для аллеля i1 соответственно. Семь штаммов (11,5%) имели смешанные s, m и i генотипы. Все штаммы аллеля *vacA* s2 являлись *cagA*-негативными и несли аллель m2. Штаммы *oipA*+ практически в равных долях были обнаружены у больных ХГ (62,2%) и ЯДК (57,1%) (p = 0,71). Все три штамма от пациентов с РЖ являлись *cagA*- и *oipA*-позитивными и несли аллели *vacA* s1/m1/i1. Анализ результатов генотипирования позволил выявить 17 вариантов профилей (комбинированных генотипов). Наиболее распространенный комбинированный генотип *cagA+/oipA+/vacA* s1/m1/i1 включал 18 (29,5%) штаммов *H. pylori*. *Выводы.* В результате анализа геномного полиморфизма клинических изолятов *H. pylori*, выделенных от больных хеликобактериозом, были выявлены доминирующие генотипы популяции *H. pylori* в Санкт-Петербурге, Россия. Установлена связь генотипа *vacA* s1 возбудителя с клиническими проявлениями инфекции *H. pylori*.

Ключевые слова: *Helicobacter pylori, ген cagA, ген vacA, ген oipA, гастрит, язва двенадцатиперстной кишки, рак желудка, гены вирулентности, геномный полиморфизм.*

Introduction

Helicobacter pylori, a microaerophilic gram-negative spiral-shaped bacteria, infects approximately 4.4 billion humans worldwide. Although most *H. pylori*-positive individuals remain asymptomatic, the infection may result in the development of gastritis, ulcer disease, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma [9].

The severity of gastroduodenal lesions in infected individuals depends on the environmental factors, host genetics, and the expression of a large variety of virulence factors in *H. pylori* strains that play a key role in the development of the infection. Presently, the most intensively studied are the vacuolating cytotoxin (VacA), cytotoxin-associated antigen A (CagA), and outer inflammatory protein (OipA) encoded by *vacA*, *cagA*, and *oipA* genes, respectively [9, 13].

The *vacA* gene found in the genome of all *H. pylori* strains encodes a cytotoxin (~140 kDa), inducing the vacuolization of gastric epithelial cells through the formation of anion-selective pores in the cytoplasmic membrane. The genetic diversity of *H. pylori* strains is associated with *vacA* allelic variants s (alleles s1/s2), i (alleles i1/i2/i3), and m (alleles m1/m2) due to the mosaic structure of the *vacA* gene [5, 23]. The product of *vacA* in *H. pylori* s1/m1/i1 genotype strains is considered the most cytotoxic and associated with ulcer disease and gastric carcinoma compared with strains of other genotypes [11].

The primary determinant of *H. pylori* virulence is the cag pathogenicity island (*cag*PAI) believed to contribute to clinical outcomes, which seems controversial. For instance, a strong association between *cagA* status and severity of the disease was reported in the developed European countries [15]. In Russia and most Asian countries, such contribution was not proved [18, 21]. The *cag*PAI genes encode for the type IV secretion system proteins that transport the immunogenic CagA protein to the epithelial cells of the gastric mucosa. Further phosphorylation of CagA by host protein kinases results in the morphological changes in epithelial cells that stimulate ulceration, atrophy, and stomach cancer [8]. The marker of the cagPAI is the *cagA* gene, which is present in the genome of 25–99% of *H. pylori* strains depending on their geographical origin [15, 18, 21].

The outer membrane protein OipA, a member of the HOP protein family (Helicobacter outer proteins), is encoded by the *oipA* gene, which can be functionally active (“on”) or inactive (“off”) due to regulation by the repeated CT motif in the nucleotide sequence. OipA protein provides adhesion of *H. pylori* to gastric epithelial cells and is associated with interleukin-8 induction and neutrophil infiltration of the gastric mucosa in inflammation and duodenal ulcer [6].

Although Russia belongs to countries with a high prevalence of *H. pylori* infection (70–90% depending on the region), currently there is a very limited number of studies evaluated *H. pylori* genotypes in Russia. Based on the assessment of virulence-associated *cagA*, *oipA*, and *vacA* genes, our study was aimed to determine *H. pylori* genotypes associated with the clinical outcomes in patients with *H. pylori* infection in St. Petersburg, Northwest Russia.

Materials and methods

Bacterial strains, culture conditions, and identification

A total of 240 patients with a confirmed diagnosis of *H. pylori* infection from three different hospitals (in St. Petersburg) were recruited between 2014 and 2019. From this cohort, only 122 biopsies from both the corpus and antral mucosa taken during endoscopy from 61 patients were available. The study group included 28 men (45.9%) and 33 women (54.1%). The median age was 44 years (range 17–88 years). Regarding endoscopic findings and histological routine results, 61 patients were distributed into chronic gastritis (n = 37, 60.7%), duodenal ulcer (n = 21, 34.4%) and gastric cancer (n = 3, 4.9%) groups. The retrospective study was approved by the Independent Ethics Committee of St. Petersburg Pasteur Institute, Russia (Protocol No. 50/04-2019, 22.06.2020).

Endoscopic biopsy specimens were homogenized and used for the culture. The *H. pylori* culture was carried out at St. Petersburg Pasteur Institute (Russia) on a medium containing Columbia agar base with the addition of 5–7% defibrinated horse blood and 1% IsoVitalex solution at 37°C under microaerophilic conditions (oxygen content ~ 5%) using anaerostats of the GasPak 100 System. Visible growth of bacteria was observed after 4–7 days. For primary identification, Gram-stained culture smears were studied by microscopy. The urease, catalase, and oxidase biochemical tests were used for species identification. The strains were identified as *H. pylori* if all tests were positive. Strain *H. pylori* NCTC 12823 was used as a reference.

DNA extraction and polymerase chain reaction (PCR) assays

Isolation of chromosomal DNA *H. pylori* was performed using a set of Helicopol II produced by Litech Laboratories (Moscow).

The PCR for the detection of *cagA*, *oipA*, and *vacA* genes in the DNA samples was performed in the Bio-Rad C1000 Thermal Cycler (USA). The nucleotide sequences of the primers, the annealing temperatures, and the lengths of amplification products are shown in Table 1.

PCR protocol: 95°C — 3 min.; 35 cycles: 94°C — 35 sec, annealing temperature — 35 sec, 72°C — 45 sec; 72°C — 5 min. PCR products were separated in a 2% agarose gel stained with ethidium bromide. The length of amplification products was determined using molecular weight markers of 50 bp and 100 bp DNA Ladder (LLC Interlabservis, Moscow). The results were visualized using the GelDoc gel documentation system (BioRad, USA).

Statistical analysis

The statistical analysis of group comparison was performed using SPSS for Windows statistical software (version 12; StatSoft Inc., Chicago, IL, USA) and the OpenEpi (a Web-based Epidemiologic and Statistical Calculator for Public Health [www.OpenEpi.com]) for two-by-two tables to calculate the odds ratio (OR) and 95% confidence interval (CI) and the Fisher exact test (one-tailed). A p-value < 0.05 was considered statistically significant.

To quantitatively evaluate the variability of *cagA*, *oipA*, and *vacA* genes, the Hunter–Gaston discriminatory index was calculated (HGDI) using a Discriminatory Power Calculator algorithm (http://insilico.ehu.es/mini_tools/discriminatory_power/index.php).

Results

The culture of biopsies on a selective nutrient medium at 37°C in microaerophilic conditions after 4–7 days resulted in the visible growth of typically small (about 1 mm diameter), round, smooth, transparent, moist colonies containing Gram-negative curved/S-shaped rods. Positive results of biochemi-

Table 1. Primers used for PCR detection of *oipA*, *cagA*, and *vacA* genes

Genes	Primers	Sequences of primers	Annealing temperature, °C	Length of the PCR product, bp	Reference
oipA	OipA-F OipA-R	GTTTTTGATGCATGGGATTTGTGCAT CTCTTATGGCTTT	53	401	[29]
cagA	CagA-F CagA-R	GATAACAGGCAAGCTTTTGAGGCTG CAAAAGATTGTTTGGCAGA	56	349	[26]
vacA s1/s2	VAI-F VAI-R	ATGGAAATACAACAAACACACCTGC TTGAATGCGCCAAAC	53	259/286	[5]
***vacA* m1/m2**	VAG-F VAG-R	CAATCTGTCCAATCAAGCGAGGCGT CAAAATAATTCCAAGG	52	570/645	[30]
***vacA* i1**	VacF1 VacA-C1R	GTTGGGATTGGGGGAATGCCGTTAA TTTAACGCTGTTTGAAG	52	426	[23]
***vacA* i2**	VacF1 VacA-C2R	GTTGGGATTGGGGGAATGCCGGAT CAACGCTCTGATTTGA	52	432	[23]

cal tests (the ability to produce catalase, oxidase, and urease) allowed us to identify 61 bacterial isolates as *H. pylori* species.

The PCR-based examination of DNA samples revealed the genetic diversity of *H. pylori* clinical isolates in terms of the presence of virulence-associated genes *cagA*, *oipA*, and the distribution of *vacA* allelic variants (HGDI 0.88) (Table 2). The 41 (67%) of 61 strains were *cagA*-positive, 38 (62%) — *oipA*-positive; the *vacA* gene in various allelic variants was detected in all strains. The s1 (77%), m2 (49%), and i1 (49%) alleles were the most frequent in polymorphic s, m, and i regions of the *vacA* gene. Seven isolates (11.5%) were positive for different mixed combinations of *vacA* alleles s, m, and i (Table 2). Such cases may indicate the presence of multiple strains in the human body.

Allelic variants of three regions of the *vacA* gene were grouped into five genotypes, among them *vacA* s1/m1/i1 was dominant (41%). The *vacA* s1/m2/i2 and vacA s2/m2/i2 genotypes included 10 and 12 strains (16% and 20%), respectively. Noteworthy, a rare s2/m1 genotype was not found in our study.

To assess the association of pathogen's virulence determinants with the severity of gastroduodenal lesions due to *H. pylori* infection, we analyzed the distribution of *cagA*, *oipA*, and *vacA* genes in *H. pylori* clinical isolates from patients diagnosed with chronic gastritis (G), duodenal ulcer (DU) and gastric cancer (GC) (Table 2).

The proportions of *cagA*+ *H. pylori* strains differed depending on the clinical manifestations. In patients with G it was 56.7%, while in patients with DU reached 80.9%, however, the difference was not statistically significant [p = 0.06; OR 3.24 (0.91; 11.52)].

The distribution of strains bearing *vacA* s1 allele significantly differed in patients with G (64.9%) and DU (95.2%): [p = 0.01; OR 10.833 (1.30; 90.14)]. The *vacA* alleles m1 and i1 in the isolates from patients with G and DU were found in almost equal proportions: p = 0.82 (for allele m1) and p = 0.90 (for allele i1).

Also, no statistical difference between the *oipA* status and severity of the disease was detected: the proportions of *oipA*+ strains in patients with G (62.2%) and DU (57.1%) were almost equal (p = 0.71).

All isolates from patients with GC were *cagA*-, *oipA*-positive, and carried vacA s1/m1/i1 alleles (Table 2).

Further analysis of the *vacA*- and *cagA*-associated polymorphism in *H. pylori* clinical isolates revealed a relationship between the *cagA*+ status and the allelic variant s1 of the *vacA* gene: among 41 *cagA*-positive strains 39 (95.1%) possessed the *vacA* s1 allele (two *cagA*+ strains had multiple genotype s1s2), while none of the *vacA* s2 bearing strains carried *cagA* gene. Noteworthy, all *vacA* s2 strains had the m2 allele (Table 3). Only 24 (58%) of *cagA*-positive strains were *vacA* m1. The majority (88%) of the *vacA* s1/m1/i1 allelic profile strains were *cagA*-positive. The majority of *oipA*-positive isolates (87%) were carriers of the *cagA* gene.

The proportion of *cagA*+/*vacA*s1 genotype strains in patients with G reached 51%, compared to larger proportions in patients with DU (81%) and GC (100%). Only one of the 21 isolates from patients with DU had the *cagA*-/*vacA*s2 genotype.

Table 2. Genotypes of *H. pylori* clinical isolates from different patient groups

H. pylori genotype	G, N (%) (n = 37)	DU, N (%) (n = 21)	GC, N (%) (n = 3)	Total, N (%) (n = 61)
***cagA*+**	21 (56.7%)	17 (80.9%)	3 (100%)	41 (67.2%)
***oipA*+**	23 (62.2%)	12 (57.1%)	3 (100%)	38 (62.3%)
***vacA* s1**	24 (64.9%)	20 (95.2%)	3 (100%)	47 (77.0%)
***vacA* s2**	11 (29.7%)	1 (4.8%)	–	12 (19.7%)
***vacA* s1s2**	2 (5.4%)	–	–	2 (3.3%)
***vacA* m1**	17 (45.9%)	9 (42.8%)	3 (100%)	29 (47.5%)
***vacA* m2**	18 (48.6%)	12 (57.1%)	–	30 (49.2%)
***vacA* m1m2**	2 (5.4%)	–	–	2 (3.3%)
***vacA* i1**	17 (45.9%)	10 (47.6%)	3 (100%)	30 (49.2%)
***vacA* i2**	17 (45.9%)	7 (33.3%)	–	24 (39.3%)
***vacA* i1i2**	3 (8.1%)	4 (19.0%)	–	7 (11.5%)
***vacA* s1/m1/i1**	17 (48.5%)	11 (47.8%)	3 (100%)	31 (50.8%)
***vacA* s2/m2/i2**	11 (31.4%)	1 (4.3%)	–	12 (19.7%)
***vacA* s1/m2/i2**	4 (11.4%)	9 (39.1%)	–	13 (21.3%)
***vacA* s1/m2/i1**	3 (8.5%)	2 (8.6%)	–	5 (8.2%)
***vacA* s1/m2/i1i2**	–	3 (14.3%)	–	3 (4.9%)
***vacA* s1/m1/i1i2**	–	1 (4.8%)	–	1 (1.6%)
***vacA* s1s2/m1m2/i1i2**	1 (2.7%)	–	–	1 (1.6%)
***vacA* s1s2/m1/i1i2**	1 (2.7%)	–	–	1 (1.6%)
***vacA* s1/m1m2/i1i2**	1 (2.7%)	–	–	1 (1.6%)

Different combinations of *cagA/oipA/vacA* alleles in 61 clinical *H. pylori* isolates were grouped in 17 profiles, five of which represented multiple genotypes (Table 4). The most common variant was *cagA*+/*oipA*+/*vacA*s1/m1/i1 which comprised 18 (30%) of the strains isolated from patients with G, DU, and GC. The remaining genotypes were represented by groups, including 1 to 6 strains.

Discussion

The populations of *H. pylori* appear heterogenic in different countries with variable ethnic, socioeconomic, and environmental characteristics. The polymorphisms in *cagA* and *vacA* genes associated with virulence are widely exploited for the genotyping of *H. pylori* strains. The presence of the *cagA* gene (a marker of the pathogenicity island, *cag*PAI) varies among *H. pylori* strains of different geographical origin: ~ 80–99% in East Asian countries [14, 21], Southeast and South Asia [20, 22, 27], South Africa [24]; ~ 50–70% in countries of Western Europe [7, 12, 15, 17]; ~ 50% and lower in the countries of the Middle East [10, 19]. According to the studies conducted in the Russian Federation, the presence of *cagA*-positive *H. pylori* strains varies in different regions: 80–90% in Moscow (Central region) [18] and Yekaterinburg (Ural Federal District) [3], 70–80% in Rostov-on-Don, Astrakhan (Southern Federal District) [2], 30–60% in Eastern Siberia [25], < 50% in Kazan (Volga Federal District) [1].

In this study, we detected about 67% of *cagA*-positive *H. pylori* strains among patients from St. Petersburg, which is consistent with data from Europe. In particular, in Finland, the proportion of *cagA*+ *H. pylori* strains reached 66%. The observed similarities may be partly explained by the territorial neighborhood and close communication between St. Petersburg Region, Russia, and Finland.

It is generally accepted that CagA-negative *H. pylori* strains are less virulent than CagA-positive strains causing severe gastrointestinal lesions in humans. The *cagA*-positive strains are reported in 80–100% of patients with DU and GC in Europe. In our study, the *cagA* gene was observed in *H. pylori* isolates from patients with DU (81%) and GC (100%), which is consistent with the previously published data [7, 15, 17]. In Asia, almost all strains of *H. pylori* carry the *cagA* gene, regardless of the infection severity [21], thus emphasizing the role of the CagA protein as a pathogen's virulence factor.

The *vacA* gene is known to be present in the genome of all *H. pylori* strains. However, different levels of cytotoxic activity of the VacA protein are associated with the diversity of allelic variants in the s-, m-, and i-regions of the *vacA* gene [11, 23].

We have established an association between the *vacA* s1 allele and DU since only one of the 21 *H. pylori* strains possessed an alternative *vacA* s2.

Table 3. The distribution of *vacA* and *oipA* profiles in *cagA*-positive and *cagA*-negative *H. pylori* clinical isolates

H. pylori genotype	*cagA*+, N (%) (n = 41)	*cagA*–, N (%) (n = 20)	Total, N (%) (n = 61)
***vacA* s1**	39 (95.1%)	8 (40.0%)	47 (77.0%)
***vacA* s2**	–	12 (60.0%)	12 (19.6%)
***vacA* m1**	24 (58.5%)	5 (25.0%)	29 (47.5%)
***vacA* m2**	15 (36.6%)	15 (75.0%)	30 (49.2%)
***vacA* i1**	26 (63.4%)	4 (20.0%)	30 (49.2%)
***vacA* i2**	8 (19.5%)	16 (80.0%)	24 (39.3%)
***vacA* s1/m1/i1**	22 (53.6%)	3 (15.0%)	25 (40.9%)
***vacA* s1/m2/i1**	4 (9.7%)	1 (5.0%)	5 (8.2%)
***vacA* s1/m2/i2**	8 (19.5%)	2 (10.0%)	10 (16.4%)
***vacA* s2/m2/i2**	–	12 (60.0%)	12 (19.7%)
***oipA*+**	33 (80.5%)	5 (25.0%)	38 (62.3%)
***oipA*–**	8 (19.5%)	15 (75.0%)	23 (37.7%)
***vacA* s1s2/ m1m2/i1i2**	1 (2.4%)	–	1 (1.6%)
***vacA* s1s2/m1/ i1i2**	1 (2.4%)	–	1 (1.6%)
***vacA* s1/m1m2/ i1i2**	1 (2.4%)	–	1 (1.6%)
***vacA* s1/m1/i1i2**	1 (2.4%)	–	1 (1.6%)
***vacA* s1/m2/i1i2**	3 (7.3%)	–	3 (4.9%)

Interestingly, that *vacA* s2 allele was predominant in *H. pylori* isolates from patients with G (~ 92%). No similar association was found in the m-variants of the *vacA* gene: the m1 and m2 alleles were distributed almost equally among clinical isolates from patients with G (45.9% and 48.6%, respectively) and DU (42.8% and 57.1%, respectively). In contrast to the widespread opinion on the leading role of the *H. pylori vacA* s1/m1 genotype in the development of a duodenal ulcer, our data did not confirm such association: we observed almost similar proportions of the s1/m1 and s1/m2 genotypes in patients with DU (42.8% and 52.4%, respectively). However, the s1/m1 genotype was detected in *H. pylori* isolates from patients with GC (though the number of such isolates was limited to three in our study), which is consistent with the reports from the Netherlands and Portugal [4, 28]. These data suggest a variety of *H. pylori* virulence determinants associated with the severity of lesions during infection of the gastrointestinal tract.

Polymorphism of the intermediate i region of the *vacA* gene is determined by alternative alleles i1/i2. According to the published data, the *vacA* i1 allele appears more informative than the s1/m1 allele and can be considered as an independent "marker" of gastric cancer [14].

We found that all *vacA* s1/m1 and *vacA* s2/m2 *H. pylori* isolates carried the i1 (*vacA* s1/m1/i1) and i2 (*vacA* s2/m2/i2) alleles, respectively. On the contrary, *vacA* s1/m2 genotype isolates appeared heterogeneous in the i-region (*vacA* s1/m2/i1 and *vacA* s1/m2/i2), which is in line with other reports [14, 21]. All *H. pylori*

Table 4. Combined genotypes of *H. pylori* clinical isolates from different patient groups

Combined *H. pylori* genotypes	G (n = 37)	DU (n = 21)	GC (n = 3)	Total (n = 61)
***cagA*+/*oipA*+/*vacA* s1/m1/i1**	10 (27.0%)	5 (23.8%)	3 (100%)	18 (29.5%)
***cagA*+/*oipA*+/*vacA* s1/m2/i2**	3 (8.1%)	3 (14.3%)	–	6 (9.8%)
***cagA*+/*oipA*+/*vacA* s1/m2/i1**	2 (5.4%)	–	–	2 (3.3%)
***cagA*+/*oipA*–/*vacA* s1/m1/i1**	2 (5.4%)	2 (9.5%)	–	4 (6.5%)
***cagA*+/*oipA*–/*vacA* s1/m2/i1**	1 (2.7%)	1 (4.8%)	–	2 (3.3%)
***cagA*+/*oipA*–/*vacA* s1/m2/i2**	–	2 (9.5%)	–	2 (3.3%)
***cagA*–/*oipA*+/*vacA* s2/m2/i2**	5 (13.5%)	–	–	5 (8.2%)
***cagA*–/*oipA*–/*vacA* s1/m1/i1**	2 (5.4%)	1 (4.8%)	–	3 (4.9%)
***cagA*–/*oipA*–/*vacA* s1/m1/i2**	2 (5.4%)	–	–	2 (3.3%)
***cagA*–/*oipA*–/*vacA* s1/m2/i2**	1 (2.7%)	1 (4.8%)	–	2 (3.3%)
***cagA*–/*oipA*–/*vacA* s2/m2/i2**	6 (16.2%)	1 (4.8%)	–	7 (11.5%)
***cagA*–/*oipA*–/*vacA* s1/m2/i1**	–	1 (4.8%)	–	1 (1.6%)
***cagA*+/*oipA*+/*vacA* s1s2/m1m2/i1i2**	1 (2.7%)	–	–	1 (1.6%)
***cagA*+/*oipA*+/*vacA* s1s2/m1/i1i2**	1 (2.7%)	–	–	1 (1.6%)
***cagA*+/*oipA*+/*vacA* s1/m1m2/i1i2**	1 (2.7%)	–	–	1 (1.6%)
***cagA*+/*oipA*+/*vacA* s1/m1/i1i2**	–	1 (4.8%)	–	1 (1.6%)
***cagA*+/*oipA*+/*vacA* s1/m2/i1i2**	–	3 (14.3%)	–	3 (4.9%)

isolates from patients with gastric cancer (n = 3) were carriers of the *vacA* i1 allele combined with s1/m1. However, there was no correlation of *vacA* i1 genotype with other forms of *H. pylori* infection: 45.9% *vacA* i1 isolates from patients with G versus 47.6% from patients with DU. Thus, a large-scale assessment of the *vacA* i1 allele as a putative marker of predisposition to gastric cancer is necessary.

Based on the *vacA* genotyping, our results suggest the coexistence of multiple genetically different *H. pylori* strains in various gastric sites resulting from the mixt infection in a considerable number of patients (7/61, 11.5%).

An analysis of the *H. pylori cagA* and *vacA* combined genotypes demonstrated, firstly, the association of the *cag*PAI region with the *vacA* s1 allele and the absence of *cag*PAI in *vacA* s2 strains; secondly, the association of DU with the *vacA* s1 genotype. The *vacA* s2 strains were unique for patients with G. These data support the generally accepted opinion that *vacA* s1 strains increase the risk of developing DU and GC, while *vacA* s2 strains are less virulent and rarely associated with the progress of *H. pylori* infection. The *vacA* i1 and *vacA* m1 genotypes of *H. pylori* isolates were not associated with DU.

It is believed that the functionally active *oipA* gene is associated with the presence of the *cagA* gene, which, in turn, is associated with the *H. pylori vacA* s-region [16, 30]. However, their relationships remain unclear, taking into account the mutual remoteness of the *oipA*, *cagA*, and *vacA* genes on the bacterial chromosome.

In our study, a functionally active *oipA*+ gene was found in 62% of *H. pylori* isolates, while several studies reported the presence of the *oipA* gene in 90–100% strains [6, 16]. Most *oipA*-positive isolates (80%) carried the *cagA* gene. We did not find links between the presence of *oipA* gene and *H. pylori*-mediated diseases: the frequency of *oipA*+ strains in patients with G and DU was similar (60%). At the same time, the *oipA*+ isolates have predominated in patients with GC (100%), though the low number of gastric cancer cases in our study did not allow us to confirm an association.

The present study revealed the dominant combined genotype *cagA*+/*oipA*+/vacA s1/m1/i1 in *H. pylori* clinical isolates (30%). Our results inspire to search for reliable genetic markers associated with various clinical manifestations of *H. pylori* infection.

Conclusion

In conclusion, the PCR-based analysis of virulence determinants in clinical isolates revealed heterogeneity and the predominant genotypes in the *H. pylori* population in St. Petersburg, Russia. Although Russia belongs to countries with a high prevalence of *H. pylori* infection, a relatively low proportion of the *cagA*-bearing isolates were detected, and they were not significantly associated with duodenal ulcer. The significant association between the *vacA* s1 genotype of the pathogen and clinical manifestations of *H. pylori* infection has been established. Despite the limitations in the number of specimens, this finding may serve as a potential predictor for the *H. pylori* disease progression. A large-scale assessment is a demand to reveal the actual risk in developing gastroduodenal diseases due to *H. pylori* infection in Russia. In general, our study gained new insights into the *H. pylori* genetic structure in St. Petersburg, thus contributing to Russian and global pathogen population characterizations.

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