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GENOTYPES OF THE *HELICOBACTER PYLORI* ISOLATES AND THE IL-1 GENES IN KAZAN CITIZENS (KAZAN, RUSSIA) WITH GASTRIC AND DUODENAL ULCER

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ABSTRACT

The prevalence of the virulence genes (*iceA*, *cagA*, *babA2*, *vacA*) in the *Helicobacter pylori* strains isolated from patients with clinic and histologically proved diagnosis of gastric and duodenal ulcer as well as the distinction of the IL-1 genes in the helicobacter carriers were studied. *VacA s1/m2 iceA1 cagA+* and *vacA s1/m2 iceA1 cagA+ babA2* genotypes of *H. pylori* were shown to be the prevalent in the clinic isolates. Four patients were found to be infected with *Ureaplasma urealyticum*. The combination of the *IL-1B-511*?/IL-1B+3954*C/IL-1RN*2* alleles was shown to be the prevalent among the patients with gastric and duodenal ulcer. The correlation between the *H. pylori* genotypes, the IL-1 genes and the ulcer particularity was not found.

Key words: *Helicobacter pylori*, virulence genes, IL-1 genes, genotyping, ulcer disease, persistence, *Ureaplasma urealyticum*

INTRODUCTION

An expressed genetic heterogeneity of the *Helicobacter pylori* strains determines the distinct clinical implications of the infection. In most of the *H. pylori*-infected people, clinical presentations are absent. In some individuals, however, colonization of the stomach mucous tunic with this bacterium may be the possible reason for development of chronic gastritis, peptic ulcer as well as gastric adenocarcinoma and B-cell lymphoma¹.

Pathogenicity of *H. pylori* is connected with products of the *urel*, *iceA*, *cagA*, *babA2* as well as *vacA* genes². Distribution of the *H. pylori* genotypes depends on the ethnogeographic peculiarities³. Moreover, polyphathia (*H. pylori*+*Ureaplasma urealyticum* infection) may modify the clinical aspects of the infection⁴.

It is known that pathogenesis depends on the biology of the pathogen, the interaction between the infective agent and the defense systems of the host⁵.

Recently, it was found that genetic predilection to various infections depends on the polymorphism of the genes for interleukins^{6, 7}. Furthermore, allelic variability in IL-1 genes may be the reason for the distinct susceptibility to the *H. pylori* infection⁸.

The subject of the study was to elucidate the genotypes of the *Helicobacter pylori* isolates and the IL-1 genes in Kazan citizens (Kazan, Russia) with gastric and duodenal ulcer.

MATERIALS AND METHODS

We obtained gastric biopsy specimens from 21 *H. pylori*-infected patients (20-78 years old citizens of Kazan, Russia) with gastric and duodenal ulcer. Biopsy and its analysis were performed as described by Momynaliev and co-workers⁹. For the extraction and purification of DNA, "HelicoPol" reagents (Litech Corporation, Moscow, Russia) were used. *H. pylori* and *U. urealyticum* were revealed by PCR assay. Genotyping of the *cagA*, *iceA* and *babA2* genes was performed with the specific reagents of Litech Corporation (Moscow, Russia). The results were documented using the "DNA Analyzer" vision system.

PCR analysis of multiform locuses for IL-1Ra and IL-1b genes (at minus 511 and plus 3954 position) was performed with a programmable thermal cycler "Tercyc" (DNA-technology, Russia). Variants of C-511T and C+3954T for *IL-1B* and VNTR for *IL-1RN* (alleles 1 and 2) genes were determined as described by Garcia-Gonzales and co-workers⁸. The amplified regions were treated with *AvaI* (C511T) and *TaqI* (C+3954T) restrictases (Fermentas, Lithuania). The lengths of the restriction fragments were as follows: 135 and 114 b.p. (presence of the restriction site, allele C) and 249 b.p. (absence of the restriction site, allele T) for *TaqI*; 190 and 115 b.p. (presence of the restriction site, allele C) and 305 b.p. (absence of the restriction site, allele T) for *AvaI*. The length of *IL-1RN*1* (IL-1Ra allele 1) was 410 b.p., and for *IL-1RN*2* (IL-1Ra allele 2) was 240 b.p.

The electron-microscopic analysis was performed with the use of Hitachi HU-125 transmissible device (Hitachi, Japan).

The data were analyzed using the χ^2 test implemented in a commercially available computer program. A value of $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Helicobacters (*H. pylori*) were revealed in all patients. Moreover, *U. urealyticum* was also detected in 4 patients (19%). Due to genotyping of *vacA* gene, both s1 and s2 alleles were identified in two samples from people with duodenal ulcer. Two distinct strains of *H. pylori* were possibly the reason for this finding. In order to avoid difficulties in interpretation of the results, these two samples were eliminated from the analysis.

Urease is the universal factor of *H. pylori* pathogenicity¹⁰. Products of the *cagA*, *vacA* (s1, s2, m1, and m2), *iceA* (A1, A2) and *babA* (A1, A2) genes are believed to contribute an additional input into *H. pylori* pathogenicity and clinical presentation^{2, 11}.

cagA+ isolates of *H. pylori* were obtained in 14 cases (73.7%) out of the remaining 19 biopsy specimens. This frequency of *cagA*+ is somewhat lower than that of observed in Europe and Central Russia⁹. High frequency variability of the gene as well as alterability in genetically determined susceptibility of the host organism to various *H. pylori* strains¹² is probably the explanation for the above-mentioned fact. The presence of the *cag* and *vac* pathogenicity islets in the *H. pylori* strains is believed to prevent the engulfing of these bacteria and favors their persistence¹³.

All the *cagA*+ *H. pylori* strains contained *vacA* s1 allele. It should be noted that distribution of the *vacA* alleles is different in various ethnopopulations. However, infection with *vacA* s1/m1 *H. pylori* is associated with more defined inflammation. *H. pylori* strains with *vacA* s1/m1 and *vacA* s1/m2 has maximal or median level of cytotoxin secretion while the *vacA* s2/m2 has not¹⁴. Indeed, *vacA* s1/m2 genotypes were revealed in 94.7% ulcer cases regardless of the disease location.

babA2 gene is thought to be a marker for duodenal ulcer and adenocarcinoma of stomach¹⁵. In our case, this gene was determined in 8 (42%) clinical isolates of *H. pylori*.

Infiltration of the stomach mucous membrane with polymorphonuclear leukocytes is more expressed in *iceA1* *H. pylori*-infected people¹¹. However, there is no a well-defined link between development of ulcer disease and presence of the *iceA1* gene^{11, 16}. We revealed *iceA1* gene in 73.7% of the investigated patients while the *iceA2* gene was not determined at all.

K. Momynaliev and co-workers⁹ suggested a hypothesis about the leading role of combination of the pathogenicity

factors in development of the *H. pylori* infection. Thus, in general, *H. pylori* have about 28 genotypes. In our study, 6 genotypes of *H. pylori* were revealed (Table 1). Previously, we did not obtain any correlation between combination of the revealed genotypes and ulcer location¹⁷.

Table 1. *H. pylori* and IL-1 genotypes in patients with gastric and duodenal ulcer

Patient n°	Disease	Size of the ulcer	Genotype of IL-1B-511/ IL-1B+3954/ IL-1RN	Genotype of <i>H. pylori</i>
1*	GU	0,4	IL-1B-511*T/T / IL-1B+3954*T/T / IL-1RN*2	vac s2/m2 cag-
2	GU	0,4/0,8	IL-1B-511*T/T / IL-1B+3954*C/C / IL-1RN*1/2	vac s1/m2 iceA1 cag-
3	GU	1,5	IL-1B-511*T/C / IL-1B+3954*C/C / IL-1RN*1/2	vac s1/m2 iceA1 cag+
4	GU	2,5	IL-1B-511*T/T / IL-1B+3954*C/C / IL-1RN*2	vac s1/m2 cag-
5	DU	0,9/1,0	IL-1B-511*T/T / IL-1B+3954*C/C / IL-1RN*2	vac s1/m2 iceA1 cag+ bab
6	DU	0,4/0,8	IL-1B-511*C/C / IL-1B+3954*T/C / IL-1RN*2	vac s1/m2 iceA1 cag+
7	DU	0,4/0,6	IL-1B-511*T/C / IL-1B+3954*C/C / IL-1RN*2	vac s1/m2 iceA1 cag+ bab
8	DU	0,4/0,8	IL-1B-511*T/C / IL-1B+3954*C/C / IL-1RN*1/2	vac s1/m2 iceA1 cag+
9	DU	0,6	IL-1B-511*T/C / IL-1B+3954*C/C / IL-1RN*2	vac s1/m2 iceA1 cag+
10	DU	0,8	IL-1B-511*T/T / IL-1B+3954*C/C / IL-1RN*2	vac s1/m2 iceA1 cag+ bab
11*	DU	0,8	IL-1B-511*C/C / IL-1B+3954*T/C / IL-1RN*2	vac s1/s2 /m2 iceA1 cag-
12	DU	0,9	IL-1B-511*T/T / IL-1B+3954*C/C / IL-1RN*1	vac s1/m2 iceA1 cag+ bab
13*	DU	1	IL-1B-511*T/C / IL-1B+3954*C/C / **	vac s1/m2 iceA1 cag+
14*	DU	1,2	IL-1B-511*T/T / IL-1B+3954*C/C / IL-1RN*1/2	vac s1/m2 iceA1 cag+ bab
15	DU	1,2	IL-1B-511*T/C / IL-1B+3954*C/C / IL-1RN*1	vac s1/m2 iceA1 cag-
16	DU	2,5	IL-1B-511*T/C / IL-1B+3954*C/C / IL-1RN*1	vac s1/s2 /m2 iceA1 cag-
17	DU	0,5	IL-1B-511*T/T / IL-1B+3954*C/C / IL-1RN*2	vac s1/m2 cag+ bab
18	DU	0,5	IL-1B-511*T/C / IL-1B+3954*C/C / IL-1RN*1/2	vac s1/m2 iceA1 cag+ bab
19	DU	0,5/0,4	IL-1B-511*C/C / IL-1B+3954*T/C / IL-1RN*1	vac s1/m2 iceA1 cag+
20	DU	0,3	IL-1B-511*T/C / IL-1B+3954*T/C / IL-1RN*2	vac s1/m2 cag-
21	DU	1,5	IL-1B-511*C/C / IL-1B+3954*C/C / **	vac s1/m2 cag+ bab

Abbreviations: GU=gastric ulcer, DU=duodenal ulcer

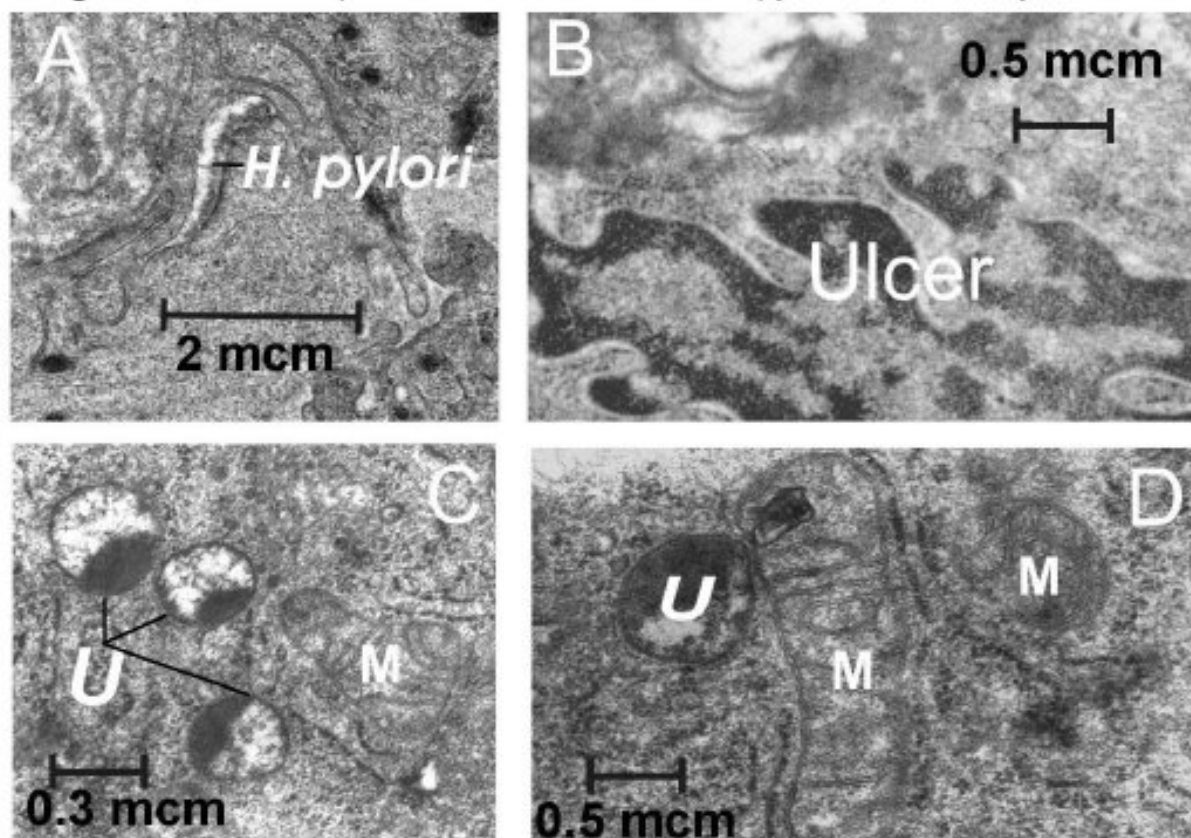
* *U. urealyticum* cells were revealed in the biopsy samples ** The presence of the *IL-1RN* gene was not tested

As far as it is known infection with bacteria and/or tissue damage result in activation of IL-1 expression. IL-1 family includes two agonists (IL-1A and IL-1B) and antagonist IL-1Ra. The susceptibility of individuals to some pathology might be connected with allele combination of the IL-1b and IL-1Ra genes^{6,7}.

Distribution of the IL-1 genes (*IL-1B-511*, *IL-1B+3954*, *IL-1RN*) in patients with gastric and duodenal ulcer is presented in Table 1. It is clear from the Table 1 that the combination of the following alleles was the most frequent: *IL-1B-511*T/ IL-1B+3954*C/IL-1RN*2*.

According to the data of Garcia-Ganzales et al., combination of *IL-1B-31*T/IL-1B-511*T/ IL-1B+3954*C/ IL-1RN*2* is important for duodenal ulcer progression in Europeans⁸. The data differences concerning the *IL-1B-511* might be connected with ethnogeographic aspects. It is likely that other factors including polypathia (in particularly, *H. pylori*+*U. urealyticum* infection) may promote to ulcer progression in people with other combination of IL-1 alleles (Table 1, Fig 1).

Figure 1. Stomach epithelial cells infected with *H. pylori* and *U. urealyticum*.



A – *H. pylori* (Hp) in the epithelial cell;
 B – Flemming's tingible corpuscle within the stomach epithelial cell in patient with *H. pylori*-associated gastric ulcer
 C and D – contact between *U. urealyticum* (U) and mitochondrion (M) in the *H. pylori*-infected stomach epithelial cell. *H. pylori* is localized near the nucleus.

We could not observe a well-defined correlation between specific *H. pylori* genotypes and features of ulcer disease. This may point out the presence of additional factors promoting *H. pylori* virulence and connected with the peculiarities of microbiota in the pathology seat as well as with polymorphism of various genes determining the specific and the nonspecific signal pathways of the "parasite-host" system.

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We think that the article under consideration it is a good work. This contribution concerns to the *Helicobacter pylori* infection as cause of chronic superficial gastritis which, in some cases, will progress to peptic ulceration, and gastric carcinoma. This bacteria shows a very important genetic diversity. Nevertheless the genotypes involved, it has been demonstrated that successful treatment of *H. pylori* infection results in the cure of peptic ulcer, and the prevention of more severe diseases. Recently, it has been also demonstrated that the emergence of resistant strains to the antimicrobial agents of common clinical use are not only due to pinpoint mutations, but also to deletion of nucleotidic sequences, and to insertion of transposons¹⁻⁶.

Several authors have show the prevalence of the virulence genes (*iceA*, *cagA*, *babA2*, *vacA*) in the *Helicobacter pylori* strains isolated from patients with clinic and histologically proved diagnosis of gastric and duodenal ulcer⁵⁻⁹. The same authors have elucidated the presence or absence of IL-1 genes. Also, have studied the *H. pylori vacA s1/m2 iceA1 cagA+* and *vacA s1/m2 iceA1 cagA+ babA2* genotypes, and the different isolated of clinical simples.

They also have found that the combination of the IL-1B-511*?/IL-1B+3954*C/IL-1RN*2 alleles is prevalent among the patients with gastric and duodenal ulcer. But any the correlation between the *H. pylori* genotypes, the IL-1 genes and the ulcer particularity was not found⁷⁻¹⁰. Finally, in basis of above consideration; I recommend the publication of the article.

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The role of the proinflammatory cytokine interleukin-1 (IL-1) in host susceptibility to *Helicobacter pylori*-associated gastric pathophysiology, inflammation and carcinogenesis is well-established. Recent data suggest that this susceptibility may be under genetic control. The presence of highly prevalent genetic polymorphisms provided for an ideal opportunity to design the appropriate epidemiologic studies to test for the role of potential candidate loci.

Since *H. pylori* achieves most of its damage through induction of chronic inflammation, it is worth considering candidate interleukin genes that control this process. Thus, functional polymorphism of IL-1 gene have been related to various risk factors of gastric cancer and duodenal ulcer. However, their importance in gastric ulcer remains elusive. To clarify the possible association between gastric and duodenal ulcer and the polymorphism in the IL-1, the authors studied the genotypes of *H. pylori* isolates from 21 patients in Kazan, Russia. The biopsy specimens were followed with PCR assay and electron microscopy. Genotyping revealed the prevalence of IL-1B-511*T/IL-1B+3954*C/IL-1RN*2 allele combination. Since different loci are reported by others in a similar studies, it appears that the variations are due to ethnogeographic aspects.

Therefore, the presented study reporting genetic data from Tatarstan, Russia can be regarded as an interesting contribution to previous epidemiological studies. The lack of correlation between the reported genotypes and disease parameters is also supported by the findings of other groups, implying that apparently the relationships among IL-1 gene polymorphism, the presence of *H. pylori* infection, and disease outcome are more complex than initially proposed. The present work undoubtedly is a valuable addition to more detailed studies of the IL-1 gene cluster needed, as well as to its role in *H. pylori* determined gastric pathogenesis.

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