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

Olga A. Chernova¹, Elmira R. Nasybullina¹, Vladislav M. Chernov¹, Oleg V. Gorshkov¹, Gulnara F. Shaimordanova¹, Rustem A. Abdulhakov², Maxim V. Trushin¹*

¹Kazan Institute of Biochemistry and Biophysics, Kazan, Russia
²Kazan State Medical University, Kazan, Russia

mtrushin @ mail.ru


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 *H. pylori* 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 *ureI, iceA, cagA, babA2* as well as *vacA* genes². Distribution of the *H. pylori* genotypes depends on the ethnogeographic peculiarities³. Moreover, polypathia (*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 host.

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

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-workers. 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 multiformal 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-workers. The amplified regions were treated with Aval (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 Aval. 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 HitachiHU-125 transmissible device (Hitachi, Japan).

The data were analyzed using the $\chi^2$ 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 pathogenicity. 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.

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 Russia. High frequency variability of the gene as well as alterability in genetically determined susceptibility of the host organism to various H. pylori strains 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 persistence.

All the cagA+ H. pylori strains contained vacA s1 allele. It should be noted that distribution of the vacA alleles is different in various ethnopolulations. 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 not. 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 stomach. 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 people. However, there is no a well-defined link between development of ulcer disease and presence of the iceA1 gene. We revealed iceA1 gene in 73.7% of the investigated patients while the iceA2 gene was not determined at all.

K. Momynaliev and co-workers 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 location\textsuperscript{17}.

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\textsuperscript{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: \textit{IL-1B-511*T/IL-1B+3954*C/IL-1RN*2}.

According to the data of Garcia-Ganzales et al., combination of \textit{IL-1B-31*T/IL-1B-511*T/IL-1B+3954*C/IL-1RN*2} is important for duodenal ulcer progression in Europeans\textsuperscript{8}. The data differences concerning the \textit{IL-1B-511} might be connected with ethnogeographic aspects. It is likely that other factors including polyphathia (in particular, *H. pylori*+*U. urealyticum* infection) may promote to ulcer progression in people with other combination of IL-1 alleles (Table 1, Fig 1).
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.

REFERENCES


5. Roitt IM, Brostoff J, Male DK Immunology, Moscow: Mir, 2000. In Russian


Comment of the reviewer Angel San Miguel, MD. PhD Servicio de Análisis Clínicos. Hospital Universitario Rio Hortega. Valladolid. España

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 same 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.

References:

http://biomed.uninet.edu/2006/n1/chernova.html


Comment of the reviewer Erhan Süleymanoglu PhD. G.U.E.F., Department of Pharmaceutical Chemistry, Gazi University.
Gazi Mahallesi, Ankara. Turkey

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 undoubtly 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.

* Corresponding author: Dr. Maxim V Trushin, Kazan Institute of Biochemistry and Biophysics, PO BOX 30, Kazan 420111, Russia
Mail: mtrushin@mail.ru

Received, January 12, 2006.
Published, January 30, 2006.