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The prevalence of *Helicobacter pylori vacA* alleles in patients with chronic gastritis

Wystepowanie alleli genu *vacA* u osób chorych na przewlekłe zapalenie żołądka zakażonych *Helicobacter pylori*

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Abstract

Background. *Helicobacter pylori* is involved in the pathogenesis of peptic ulcer disease and associated with gastric carcinoma. Several virulence factors of *H. pylori*, including urease (Ure), vacuolating cytotoxin (VacA), and cytotoxin-associated antigen (CagA), have been identified.
Objectives. This study focused on the detection of the *H. pylori* cytotoxin-associated gene A (*cagA*) and genotyping of the vacuolating cytotoxin gene (*vacA*).
Material and Methods. The presence of the *cagA* gene and the diversity of the gene encoding the vacuolating cytotoxin were analyzed using PCR. The material consisted of 47 human gastric mucosa antrum biopsies derived from *H. pylori*-infected individuals with chronic gastritis.
Results. The *cagA* gene was present in 65.96% of the *H. pylori* strains. The *VacA* s1a/m2 (36.17%) and s2/m2 (36.17%) types were the most common. The *VacA* s1b/m1 strain was found only in one case (2.13%). The prevalence of s1a/m1 *H. pylori* strains was 19.15% and of s1b/m2 6.38%.
Conclusions. Chronic *H. pylori*-induced gastritis seems to be most often associated with the s1a/m2 and s2/m2 *vacA* gene allele combinations (**Adv Clin Exp Med 2007, 16, 1, 29–33**).

Key words: *Helicobacter pylori, cagA* gene, *vacA* gene alleles, PCR

Streszczenie

Wprowadzenie. *Helicobacter pylori* jest czynnikiem etiologicznym choroby wrzodowej żołądka, a jego obecność wiąże się z występowaniem raka żołądka. Zidentyfikowano kilka czynników wirulencji *H. pylori*, w tym: ureazę (Ure), cytotoksynę wakuolizującą (VacA) oraz białko CagA.
Cel pracy. Detekcja genu *cagA* oraz określenie genotypu genu *vacA H. pylori.*
Materiał i metody. Obecność genu *cagA* i zróżnicowanie genu kodującego cytotoksynę VacA analizowano metodą PCR. Materiałem badanym było 47 wycinków błony śluzowej okolicy odźwiernikowej żołądka pochodzących od osób z przewlekłym zapaleniem żołądka zakażonych *H. pylori*.
Wyniki. Obecność genu *cagA* stwierdzono w 65,96% szczepów *H. pylori*. Najczęściej występującymi odmianami genu *vacA* były subtypy: s1a/m2 (36,17%) i s2/m2 (36,17%). Szczep s1b/m1 zidentyfikowano tylko w jednym przypadku (2,13%). Częstość występowania szczepów *H. pylori* s1a/m1 wynosiła 19,15%, a s1b/m2 – 6,38%.
Wnioski. Przewlekłe zakażenie *H. pylori* powodujące zapalenie błony śluzowej żołądka wydaje się najczęściej powiązane z kombinacją s1a/m2 oraz s2/m2 alleli genu *vacA* (**Adv Clin Exp Med 2007, 16, 1, 29–33**).

Słowa kluczowe: *Helicobacter pylori,* gen *cagA,* allele genu *vacA*, PCR.

Helicobacter pylori (*H. pylori*) infection affects more than half of the world's population. The clinical consequences range from asymptomatic gastritis to peptic ulceration and gastric malignancy [1, 2]. The outcome of this infection may be related to differences in virulence among the bacterial strains or dependent on host factors. Several virulence factors of *H. pylori*, including urease (Ure), vacuolating cytotoxin (VacA), and cytotoxin-associated antigen (CagA), have been identified [2, 3].

An important characteristic of *H. pylori* is its substantial urease activity, which appears to be essential for the survival and pathogenesis of the bacterium. Urease is present in all *H. pylori* isolates [4]. Molecular diagnosis of *H. pylori* infection is based on urease gene fragment detection.

The *vacA* gene, encoding the VacA protein, is present in all *H. pylori* strains, but the corresponding vacuolating cytotoxin is produced by approximately 50–60% of strains [5, 6]. A heterogeneity in the level of *vacA* transcription is observed [7]. The VacA protein induces epithelial cell vacuolization [1]. The *vacA* gene contains both conserve and variable regions. The gene segment encoding the C-terminus of the protoxin and the segment encoding the region near the N-terminus appear to be conserved in all isolates. However, there is sufficient diversity in the mid-region of the gene to define at least three allelic types, designated m1, m2, and m3 [7]. The s region, encoding the signal peptide, exists as s1 (including s1a, s1b, and s1c) or s2 allelic types [1]. Subtype s1c has been observed exclusively in isolates from East Asia and appears to be the major s1 allele in that part of the world [8]. *H. pylori* s1/m1 type strains produce *in vitro* the highest level of cytotoxin activity, type s1/m2 produces a moderate amount of toxin, whereas s2/m2 strains produce little or no toxin [9, 10, 11].

The cytotoxin-associated gene A (*cagA*) is a molecular marker for the *cag* island, whose presence is associated with a more severe clinical outcome [1, 12]. Several studies have shown that infection with CagA-positive strains is highly associated with peptic ulcer disease, atrophic gastritis, and gastric cancer. The *cag* island genes encode proteins that enhance the strain's virulence [1, 3]. According to Atherton et al. [13] the presence of *cagA* gene is connected with the coexistence of the s1a and s1b types of signal sequences of the *vacA* gene. Most *vacA* s2 strains are *cagA* negative. Specific *vacA*/*cagA* genotypes correlate significantly with cytotoxin activity and peptic ulceration. Thus, the typing of *H. pylori* strains may become useful in the molecular diagnosis of gastric *H. pylori* infection [14].

Material and Methods

The study was conducted on gastric mucosa samples taken during gastroendoscopy from *H. pylori*-infected individuals treated at the Chair and Clinic of Internal Diseases, Angiology, and Physical Medicine in Bytom of the Medical University of Silesia in Katowice in the years 2004–2005. Patients with malignancy, immunosuppression, and metabolic disorders were excluded from the study. During gastroendoscopy, three gastric antrum mucosa specimens were obtained for rapid urease testing, histopathology examination, and biomolecular tests. Forty-seven specimens with positive rapid urease test result which were *ureA* gene positive and diagnosed as chronic gastritis were further analyzed. The study protocol was approved by The Local Ethics Committee of the Medical University of Silesia (NN-043-33/94).

DNA was isolated from 10 mg of frozen gastric biopsy samples using Genomic DNA Prep Plus (A&A Biotechnology, Poland). The quality and quantity of DNA were spectrophotometrically determined by measuring the absorbency at 260 nm and 280 nm and by electrophoresis of the DNA samples on 2% agarose gel.

The presence of the *ureA* and *cagA* genes and the *vacA* allele combinations were analyzed in isolated DNA using PCR. For detection of the 411-bp ureA gene fragment, HPU1 and HPU2 primers were used [15]. PCR was carried out in a volume of 25 µl. The mixture contained 12.5 µl of PCR Master Mix 2X (50 U/ml Taq polymerase, 400 µM dNTPs, 3 mM $MgCl_2$; Promega, USA), 2.5 µl of HPU1 primer and 2.5 µl of HPU2 primer (10 µM each), 5 µl of DNA, and 2.5 µl of molecular grade water (Eppendorf, Germany). Denaturation (94˚C, 5 min) followed by 39 PCR cycles consisting of denaturation (94˚C, 1 min), annealing (45˚C, 1 min), extension (72˚C, 1 min), and a final extension (72˚C, 5 min). Amplification of the DNA isolated from an *H. pylori ureA* (+) strain (DNA Gdańsk, Poland) and water instead of the DNA target as positive and negative controls, respectively, were used.

PCR amplification of *cagA* gene used the primers D008 and R008, which determine the 298-bp fragment [15]. PCR was carried out in volume of 25 µl. The mixture contained 12.5 of µl PCR Master Mix, 2X (50 U/ml Taq polymerase, 400 µM dNTPs, 3 mM $MgCl_2$; Promega, USA), 2.5 µl of D008 primer, and 2.5 µl of R008 primer (10 µM each), 5 µl of DNA, and 2.5 µl of molecular grade water (Eppendorf, Germany). The PCR steps were: denaturation (94˚C, 5 min) followed by 39 cycles consisting of denaturation (94˚C, 1 min), annealing (60˚C, 1 min), extension (72˚C, 1 min),

and a final extension (72˚C, 5 min). Amplification of the DNA isolated from an *H. pylori cagA* (+) strain (Department of Clinical Microbiology, The Children's Memorial Health Institute, Warsaw, Poland) and water instead of the DNA target as positive and negative controls, respectively, were used.

For the *vacA* gene allele analysis, the primers specific to the particular s/m regions were used, as previously described [13]. Reactions were performed under the following conditions: denaturation (94˚C, 4 min) followed by 35 cycles of denaturation (94˚C, 1 min), annealing (57˚C, 1 min), extension (72˚C, 1 min), and final extension (72˚C, 5 min). The reaction mixture of 25 µl volume contained 12,5 µl of PCR Master Mix 2X (Promega, USA), 2.5 µl of F primer, 2.5 µl of R primer (10 µM each), and 2.5 µl of molecular grade water (Eppendorf, Germany). Examples of the detected amplified products of *vacA* gene are shown in Fig. 1.

PCR was performed in a Mastercycler Personal (Eppendorf, Germany). The final amplification products were analyzed by electrophoresis in 2% agarose gel stained with ethidium bromide (0.5 mg/ml) under UV illumination. To minimize the risk of contamination, all procedures (isolation, PCR, and the electrophoresis suite) were performed in separate rooms.

Results

The prevalence of the *cagA* gene and the *vacA* s/m types is shown in Table 1. The *cagA* gene was present in 65.96% of the *H. pylori* strains (31/47). In 34.04% (16/47) of the strains the *cagA* gene was not detected. The *VacA* s1a/m2 (36.17%) and s2/m2 (36.17%) types were the most common. The *VacA* s1b/m1 strain was found in only one case (2.13%), that strain being *cag*A(+). All the s1a/m2 and s1b/m2 strains were *cagA* positive as well. Among the *vacA* s1a/m1 strains, 88.99% (8/9) were *cagA*(+) and 11.11% (1/9) were *cagA*(–). 11.76% (2/17) of the s2/m2 *H. pylori* strains contained *cagA* gene and 88.24% (15/17) were *cagA*(–). There were no mixed-infection cases among the examined samples.

Discussion

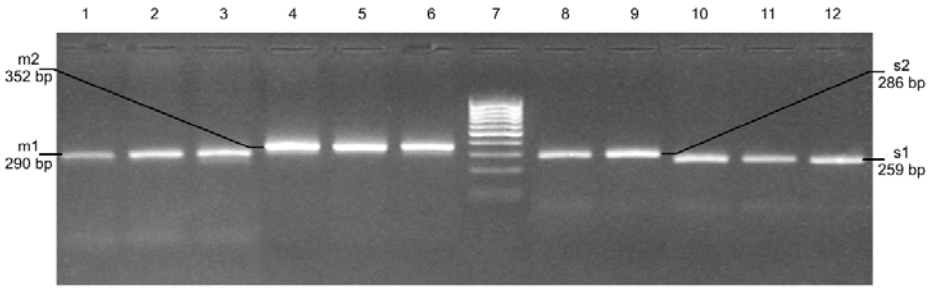

Fig. 1. Detection of the *vacA* gene alleles: s1, s2, m1, and m2 on 2% agarose gel. Lane 7: molecular marker GeneRuler™ 100bp DNA Ladder (MBI Fermentas, Lithuania); lanes: 1, 2, 3: m1 allele (290 bp); lanes: 4, 5, 6: m2 allele (352 bp); lanes: 8, 9: s2 allele (286 bp); lanes: 10, 11, 12: s1 allele (259 bp)

Ryc. 1. Rozdział w 2% żelu agarozowym alleli genu *vacA*: s1, s2, m1 i m2. Ścieżka 7 – wzorzec wielkości GeneRuler™ 100bp DNA Ladder (MBI Fermentas, Litwa); ścieżki: 1, 2, 3 – allel m1 (290 bp); ścieżki: 4, 5, 6 – allel m2 (352 bp); ścieżki: 8, 9 – allel s2 (286 bp); ścieżki: 10, 11, 12 – allel s1 (259 bp)

Table 1. The prevalence of *Helicobacter pylori* s/m alleles of the *vacA* gene in correlation with the presence of the *cagA* gene

Tabela 1. Częstość występowania alleli s/m genu *vacA Helicobacter pylori* w powiązaniu z obecnością genu *cagA*

cagA gene (Gen *cagA*)	*vacA* allele combination (Układ alleli *vacA*)					
	s1a/m1	s1a/m2	s1b/m1	s1b/m2	s2/m2	total
Present (Obecny)	8 (17.02%)	17 (36.17%)	1 (2.13%)	3 (6.38%)	2 (4.26%)	31 (65.96%)
Absent (Nieobecny)	1 (2.13%)	0 (0%)	0 (0%)	0 (0%)	15 (31.91%)	16 (34.04%)
Total (Razem)	9 (19.15%)	17 (36.17%)	1 (2.13%)	3 (6.38%)	17 (36.17%)	47 (100.00%)

Although *H. pylori* is cosmopolitan, with prevalence ranging from approximately 30% in developed countries to more than 80% in the developing world, little is known about the geographic distribution of specific *H. pylori* strains [8, 10, 16]. Knowledge of the existence of different *H. pylori* genotypes may become clinically important since strains containing *cagA* gene are more likely to cause more severe disease than strains that lack *cagA*. Type s1 *vacA* strains are more often associated with gastric disease than type s2 strains [8]. The possession of certain genotypes (*cagA*-positive, *vacA* type s1) is significantly associated with different responses to anti-*H. pylori* therapy [17, 18]. Particular genotypes are geographically related. *VacA* and *cagA* sequence motifs in strains from the United States and Europe differ from those predominating in East Asia. In Western populations, *cagA*(+) *vacA* s1/m1 *H. pylori* strains are more highly associated with disease than *cagA*(–) *vacA* s2/m2 strains [19].

Van Doorn et al. [20], in their multicentre study, investigated the *cagA* and *vacA* status of a large collection of 735 *H. pylori* cultures of patients from 24 diverse geographic regions. The prevalence of *cagA*(+) *H. pylori* strains in Northern and Eastern Europe was 72.1%, in France and Italy 74%, and in Portugal and Spain 86.7% [10]. According to the same authors the frequency of *cagA*(+) *H. pylori* infection in the Netherlands was 67% [11]. The frequency of *cagA*(+) *H. pylori* infection in our study was 65.96%. Gzyl et al. [20] report that the prevalence of *cagA*(+) *H. pylori* strains in Poland is 72.4%, while in the study of Dzierżanowska et al. [9] the frequency of *cagA* gene was 60.00%.

Several studies have demonstrated that gastric infection with *H. pylori* strains containing type s1 *vacA* alleles is associated with a higher risk for the development of peptic ulcer disease than infection with strains containing type s2 *vacA* alleles [10, 13, 21]. This association seems to be less apparent in many Asian countries than in Europe and the Americas [22, 23]. Because most *vacA* s1 strains are *cagA* positive, the two markers are closely related [10], even though these two genetic elements do not have any physical linkage on the *H. pylori* chromosome [1]. It is unclear whether one or both of these is important [24]. In the present study it was found that 93.55% (29/31) of the *cagA*-positive *H. pylori* strains were associated with the *vacA* s1 genotype. Of the 16 *cagA*-negative *H. pylori* strains, 15 (93.75%) were associated with the non-toxin-producing *vacA* s2 genotype [25]. The s2 allele, known to be associated with less cytotoxic activity, was found in 36.17% (17/47) of strains.

Among the type s1 strains, subtypes s1a, s1b, and s1c have been identified. In Europe, a distribution gradient of s1 genotypes was observed. Van Doorn et al. reported that in Northern and Eastern Europe, 89% of *H. pylori* strains are s1a. Types s1a and s1b were equally present in France and Italy, whereas in Spain and Portugal 89% of strains were of subtype s1b. s1a and s1b were approximately equally prevalent in North America [10]. According to Ando et al., most *H. pylori vacA* s1 strains in Western countries are s1a or s1b, while about 80% of the s1 strains from East Asia are s1c [1]. In all parts of the world, *vacA* s1/*cagA*(+) genotypes have been associated with peptic ulcer disease [10]. In the present study, 55.32% (26/47) strains were s1a and 8.51% (4/47) were s1b. Among 26 *vacA* s1a strains, 25 (96.15%) were *cagA*(+). All s1b strains were *cagA*(+).

H. pylori m1 strains are associated with increased gastric epithelial damage and s1a strains are associated with increased mucosal neutrophil and lymphocyte infiltration *in vivo*. These results suggest that s1a/m1 strains are the most virulent allelic type [24].

In the present investigation, 19.15% (9/47) of the examined *H. pylori* strains were s1a/m1, but the most common combinations were *vacA* s1a/m2 and s2/m2 alleles (36.17% each). In conclusion, chronic *H. pylori*-induced gastritis seems to be associated with the s1a/m2 and s2/m2 *vacA* gene allele combinations.

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