Association of *cagA* and *vacA* Genotypes of *Helicobacter pylori* with Gastric Diseases in Estonia

Helena Andreson,[1] Krista Lõivukene,[1] Toomas Sillakivi,[2] Heidi-Ingrid Maaroos,[3]
Mart Ustav,[4] Ants Peetsalu,[2] and Marika Mikelsaar[1]*

*Department of Microbiology,[1] Department of Surgery,[2] Department of Polyclinic and Family Medicine,[3] and Institute of
Molecular and Cell Biology,[4] University of Tartu, Tartu 50411, Estonia*

Gastric biopsy specimens from 156 adult patients from southern Estonia suffering from chronic gastritis, peptic ulcer disease, and perforated peptic ulcer were analyzed by PCR. The *cagA* gene was evenly distributed throughout 87% of the specimens from the patients with the different gastric diseases. The presence of the *cagA* gene correlated with that of *vacA* signal sequence type s1a (99%). However, no clear differences were found in the distribution of *cagA* and *vacA* genotypes among patients in Estonia with severe perforated peptic ulcer, uncomplicated peptic ulcer, or chronic gastritis.

Helicobacter pylori colonizes approximately half of the world's human population (6). It is considered the etiological agent of chronic gastritis (CG) and peptic ulcer and their complications (3, 14, 15, 21). In Estonia, antibodies against *H. pylori* have been detected in 80% of the inhabitants (12, 13, 30). Moreover, peptic ulcer disease (PUD) (both gastric and duodenal) is widespread in Estonia (13), and the incidence of perforated peptic ulcer (PPU) in particular is very high (21 of 100,000 cases) (19).

According to the literature, virulent cytotoxin VacA- and CagA-producing strains are more common among patients with peptic ulcer and gastric cancer (2, 4). Yet in many studies, patients with a variety of clinical symptoms of gastritis, gastric ulcer, duodenal ulcer, and reflux esophagitis have been examined for similar virulence markers of *H. pylori* (26, 27). It has not been resolved whether differences in the pathogenesis of this spectrum of diseases may be related to particular virulence markers differentially expressed by strains of *H. pylori*.

The aim of the present study was to compare the distributions of well-known virulence markers (*cagA* and *vacA*) in *H. pylori* strains isolated from southern Estonian patients suffering from CG, PUD, and PPU.

This study was carried out at Tartu University Clinic (Tartu, Estonia) between 1995 and 2000. Samples were collected from 156 adult patients from southern Estonia with CG, PUD, and PPU. Initially, 105 patients were investigated from 1 May 1995 to 30 April 2000, following referral to the Tartu University outpatient clinic for upper gastrointestinal endoscopy. In addition, samples were collected from 51 patients who were operated on for PPUs during the period from 1 January 1997 to 31 December 1999.

For the detection of CG and PUD, *H. pylori* strains were isolated from biopsy samples obtained by endoscopy. Upper endoscopy was performed with an Olympus GIF 21 gastroscope. Endoscopic diagnoses of PUD were made for 69 pa-

tients (duodenal ulcer in 61 patients and gastric ulcer in 8 patients), and diagnoses of no PUD were made for 36 patients, in whom CG was diagnosed histologically according to the Sydney classification method (16).

For *H. pylori* isolation, the biopsy samples from 93 patients with CG and PUD were placed into Stuart transport medium and taken to the laboratory within 2 h. *H. pylori* was isolated on a Columbia agar base supplemented with 7% horse blood and 1% IsoVitaleX. The plates were incubated for 3 to 4 days at 37°C under microaerophilic conditions (CampyGen; Oxoid). *H. pylori* was identified by Gram staining and by oxidase, catalase, and urease reactions. The *H. pylori* suspension in brucella broth was stored at −70°C until DNA extraction. For DNA isolation from *H. pylori* cells, the suspension was incubated at 100°C for 30 min and the lysates were stored at −20°C prior to PCR analysis. For patient samples ($n = 12$) for which attempts to cultivate *H. pylori* were unsuccessful, biopsy samples stored at −20°C in lysis buffer (200 mM Tris-HCl [pH 8.0], 25 mM EDTA, 300 mM NaCl, 1.2% sodium dodecyl sulfate) were used for PCR analysis.

From the 51 PPU patients, gastric specimens of the antral mucosa were obtained for molecular analysis intraoperatively through the perforation or postoperatively with panendoscopy. These biopsy samples were placed directly into the aforementioned lysis buffer. For DNA extraction from the frozen gastric biopsy specimens, we used a previously described procedure (20).

The presence in each strain of *cagA* and *vacA* was determined by PCR using primers, reaction mixtures, and thermal cycling (20). PCR products were identified by electrophoresis on 2% agarose gels.

Statistical analysis was performed by the two-tailed χ^2 test or by Fisher's exact test. Significance was set at a P value of <0.05.

Among the 156 patients infected with *H. pylori* strains, no s1b strains were found. Multiple *H. pylori* strains were detected in 5 (3.2%) of the 156 patients studied; these included 3 patients with duodenal ulcer disease, 1 patient with CG, and 1 patient with PPU. The results from those patients were not included in the following analysis.

Among the 151 remaining investigated samples, the *cagA*

* Corresponding author. Mailing address: Department of Microbiology, University of Tartu, Ravila 19, Tartu 50411, Estonia. Phone: 372 7 374 171. Fax: 372 7 374 172. E-mail: marikam@ut.ee.

TABLE 1. Relationships between *vacA* signal alleles and *cagA* status of 151 *H. pylori* clinical isolates ($P < 0.001$)

vacA subtype	No. (%) of isolates	
	cagA$^+$	*cagA* negative
s1a	131 (99)	4 (21)
s2	1 (1)	15 (79)
Total	132 (100)	19 (100)

gene was detected in 132 (87%) of them. Concerning *vacA* subtypes, the s1a/m1 allelic combination was the most prevalent (65%), whereas combinations of s1a/m2 and s2/m2 were found in 24 and 11% of the cases, respectively.

The presence of the *cagA* gene correlated with that of *vacA* signal sequence type s1a, whereas type s2 was predominantly found in *cagA*-negative strains ($P < 0.001$) (Table 1). The relationships between *cagA* status, *vacA* subtypes, and disease in patients are shown in Table 2. In CG and PUD patients, all *cagA*-negative isolates were associated with the s2/m2 genotype. In contrast, for the PPU group, four *cagA*-negative isolates exhibited the *vacA* genotype s1a and conversely, one *cagA*-positive isolate exhibited the *vacA* genotype s2. However, statistically the PPU group results do not differ from those of the CG and PUD groups ($P > 0.05$), with the results of all three demonstrating the same correlation between *cagA* positivity and *vacA* s1a type.

This paper provides a comparison of the distributions of *H. pylori* *cagA* status and *vacA* genotypes in selected Estonian patients with confirmed diagnoses of CG, PUD, and PPU. Different consensus criteria for the role of *H. pylori* have been set for populations with high and low prevalences of *H. pylori* infections (11). We attempted to determine whether the same set of criteria was valid for a population with a high prevalence of *H. pylori*. In Estonia, according to serological studies, *H. pylori* prevalences are 87% in adults and 56% in children (28, 30).

In a study by Vorobjova et al. (29), high prevalences of the CagA protein in sample populations of Estonian adults (63%) and children (46%) have been demonstrated. In the present study, we found that the prevalence of the *cagA* gene among the large sample of patients with various clinical diagnoses was very high (87%). This corresponds to the findings of a previous study, which described a high (82%) prevalence of the *cagA* gene in cases of clinically complicated PUD (20). In addition, the present study hints that the *cagA* gene of *H. pylori* and its defined protein are important predictors of several different gastric diseases, even in populations with a high prevalence of infection.

In countries with a low prevalence of *cagA* positivity (1, 2, 18), a significant correlation between *vacA* subtypes and *cagA* status has been demonstrated. In our study of Estonian patients, a significant association between *vacA* subtypes and *cagA* status was also found. In particular, 87% of the *cagA*-positive strains had the *vacA* s1a/m1/m2 subtype and only 1 strain out of the 131 *cagA*-positive strains carried the *vacA* s2/m2 subtype.

According to a large number of studies (1, 2, 4, 7, 24), the *vacA* s1a/m1 subtype is considered more cytotoxic than the s1a/m2 and s2/m2 *vacA* genotypes and is therefore related to the development of PUD. The s2 strains are rarely associated with PUD and are more common in cases of CG or nonulcer dyspepsia (5, 7, 18, 22, 25). In our study, there was no difference in colonization by *H. pylori* cytotoxic strains between patients with peptic ulcer and those with the more severe PPU. Furthermore, few *H. pylori* strains were of the s2 subtype (6% in PUD patients and 10% in PPU patients). It could be speculated that, in these patients, the patchy distribution of gastric mucosa obscures the s1a strain, whereas the s2 strains are cocolonizers of the mucosa. Such colonization of the gastric mucosa with several different *H. pylori* strains has been described previously in numerous reports (8, 10, 17, 23). The *cagA*-positive strains from the CG patient samples exclusively carried the s1a subtype of the *vacA* gene. Within this group, however, the proportion of *cagA*-negative and *vacA* s2 alleles was the highest (20%) of all the disease groups. Therefore, the unusual result may depend on the severity of gastritis in these patients.

One important finding regarding the PPU group is that a small number of patients (8%) were apparently colonized by a *cagA*-negative strain that simultaneously expressed the *vacA* s1a subtype. Consequently, the possibility cannot be excluded that additional virulence markers of *H. pylori* contribute to the development of PPU in some patients with PUD, indicating a role for microbial as well as host genetic diversity.

The present study has also confirmed the findings of a previous investigation on geographic differences in the distribution of *H. pylori* strains (20). In the extended sample of 151 patients examined in the present investigation, no s1b subtype was detected. Our findings are similar to those for a population in Asia (*Helicobacter pylori* Study Group, Abstr. 9th Int. Workshop Gastroduodenal Pathol. Helicobacter, GUT **39**(Suppl. 2):A73–A74, 1996) and one in Poland, for which Gošciniak et al. (9) found only 1 of 72 strains with the s1b type. Findings

TABLE 2. Relationship between *H. pylori* *cagA* status, *vacA* subtypes, and disease of patient group ($P > 0.05$)

vacA subtype	No. (%) of isolates in patients with:					
	CG ($n = 35$)		PUD ($n = 66$)		PPU ($n = 50$)	
	cagA$^+$ ($n = 29$)	*cagA* negative ($n = 6$)	*cagA*$^+$ ($n = 62$)	*cagA* negative ($n = 4$)	*cagA*$^+$ ($n = 41$)	*cagA* negative ($n = 9$)
s1a/m1	23 (79)	0	43 (69)	0	29 (71)	3 (33)
s1a/m2	6 (21)	0	19 (31)	0	11 (27)	1 (11)
s2/m2	0	6 (100)	0	4 (100)	1 (2)	5 (56)
Total	29 (100)	6 (100)	62 (100)	4 (100)	41 (100)	9 (100)

very different from ours have been established in several studies conducted in The Netherlands, Portugal, Brazil, Mexico, and the United States (1, 5, 17, 24). Our findings relate to the widely disparate evolutions of circulating *H. pylori* strains in a particular geographic area and the subsequent necessity for area-specific diagnostics for *H. pylori* virulence markers.

In conclusion, we found a high prevalence of the *cagA* gene of *H. pylori* within the population of strains studied. However, no clear differences existed in the distributions of *cagA* and *vacA* genotypes in Estonia between patients with severe PPU, uncomplicated PUD, or CG.

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