

Analysis of *vacA*, *cagA*, and IS605 Genotypes and Those Determined by PCR Amplification of DNA between Repetitive Sequences of *Helicobacter pylori* Strains Isolated from Patients with Nonulcer Dyspepsia or Mucosa-Associated Lymphoid Tissue Lymphoma

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The *vacA* s and m genotypes and the presence of *cagA* and IS605 were determined in *Helicobacter pylori* strains from patients with mono- and multiple infections. Surprisingly, these genetic markers were not associated with nonulcer dyspepsia or mucosa-associated lymphoid tissue lymphoma. The presence of *cagA* correlated with the presence of the *vacA* s1 allele ($P < 0.05$), whereas the presence of IS605 was associated with the presence of the s2 allele ($P < 0.05$).

Helicobacter pylori infection is associated with the presence of gastritis, peptic ulcers, and gastric carcinoma (4, 8, 9). In a small number of patients, the mucosa-associated lymphoid tissue (MALT) that is triggered by *H. pylori* may undergo malignant changes and develop into MALT lymphoma. Several bacterial virulence factors, like *cagA* and *vacA* subtype s1, show a strong association with the development of duodenal ulcer disease and carcinoma (1–3, 9, 12, 14, 15). The association of these virulence genes with MALT lymphoma seems to be less clear (6, 7, 10, 11, 18), and the status of other virulence factors is unknown in this group of *H. pylori* strains.

In the present study we used PCR amplification of DNA between repetitive sequences (REP-PCR) to investigate whether patients with nonulcer dyspepsia (NUD) or MALT lymphoma were infected with multiple *H. pylori* strains. For these strains we determined the *vacA* s and m types and the presence of *cagA* and IS605. The association of these genotypes with disease was investigated.

Twenty-four patients (10 men and 14 women; age range, 19 to 67 years) had clinical symptoms of NUD, but biopsy specimens showed no history of previous peptic ulcer or antral gastritis. In 12 patients (5 men and 7 women; age range, 22 to 82 years) MALT lymphoma was diagnosed by histology and immunohistochemistry as described previously (6). The *H. pylori* strains from nine patients in the MALT lymphoma group were a kind gift of W. van Dijk, Department of Microbiology, Slotervaartziekenhuis, Amsterdam, The Netherlands. *H. pylori* was cultured on Columbia agar plates containing 7% horse blood and Dent supplement (Oxoid, Basingstoke, United Kingdom) for 3 to 5 days at 37°C under microaerobic conditions.

REP-PCR was used to determine whether patients were infected with one or more *H. pylori* strains. Five single colonies of the primary *H. pylori* isolate from each patient were expanded, and genomic DNA was isolated by a standard procedure (13). The conditions and primers used for REP-PCR were described previously (17).

Amplification of parts of the *cagA* gene and the s and m regions of the *vacA* gene was performed by PCR with biotin-labeled primers. The primer sequences and PCR conditions have been described previously (1, 14–16), and PCR products were analyzed by reverse hybridization by a line probe assay (LiPA) (14–16).

The presence of IS605 was detected by PCR with primers IS-1 (5'-GGATGAATGCTTTAGTTCCGC-3') and IS-5 (5'-TTTGAATTTAGGGTATCTCGC-3') (5). Each 25- μ l PCR mixture contained 5 pmol of primers IS-5 and IS-1 (Perkin-Elmer Applied Biosystems UK, Warrington, United Kingdom), 0.625 U of AmpliTaq DNA polymerase (Perkin-Elmer Applied Biosystems UK), 200 μ M deoxynucleoside triphosphates (Boehringer Mannheim UK Ltd., Lewes, United Kingdom), PCR buffer II with 2.5 mM MgCl₂ (Perkin-Elmer Applied Biosystems UK), and approximately 200 ng of genomic DNA. A touchdown PCR was performed in a thermal cycler (Cyclogene, Techne Ltd., Cambridge, United Kingdom). Two cycles of 30 s at 94°C, 30 s at 52°C, and 1 min at 72°C were performed, and the annealing temperature was lowered to 44°C in three steps of two cycles. Then, 25 cycles of 30 s at 94°C, 30 s at 42°C, and 1 min at 72°C were performed. PCR products (850 bp) were visualized by electrophoresis on 2% agarose gels by a standard procedure (13).

A single *H. pylori* strain was isolated from 31 of 36 patients (86%). All five colonies obtained from the primary *H. pylori* culture for these patients had identical genotypes as determined by REP-PCR. The colonies of strains from five patients with NUD (14%) had two different REP-PCR patterns, indicating an infection with two different *H. pylori* strains (results not shown). All 41 *H. pylori* strains had unique genotypes as determined by REP-PCR.

The *vacA* s and m regions of all *H. pylori* strains were detected by LiPA. The combinations s1-m1, s1-m2, and s2-m2 were present in 42, 26, and 32% of the strains, respectively (Table 1). The combination of the s1 a and m1 alleles was not present in *H. pylori* strains isolated from patients with MALT lymphoma, and the combination of the s1b and m2a alleles was

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TABLE 1. *vacA*, *cagA*, and IS605 genotypes of *H. pylori* strains obtained from patients with NUD and lymphoma

Genotype	No. of <i>H. pylori</i> strains isolated from patients with:	
	NUD (<i>n</i> = 24)	Lymphoma (<i>n</i> = 12)
<i>vacA</i>		
s1a m1	6	0
s1a m2a	4	4
s1b m1	4	3
s2 m2a	5	5
Multiple ^a	5	0
<i>cagA</i>		
Positive	16 (11) ^b	5
Negative	8 (8)	7
IS605		
Positive	9 (7)	6
Negative	15 (12)	6

^a More than one *vacA* s and/or m type was detected by LiPA.

^b Values in parentheses indicate the number of strains with *cagA* or IS605 in patients with mono-infections.

not observed in either patient group. The distribution of the s and m alleles in patients with mono-infections did not show an association with clinical disease ($P > 0.05$). The presence of multiple infections in five patients was confirmed by determination of *vacA* genotypes. The *H. pylori* strains from these patients contained different s and/or m alleles.

Fifteen of 31 strains from patients (52%) infected with a single *H. pylori* strain were *cagA* positive. In patients infected with multiple strains, one or both *H. pylori* strains were *cagA* positive. Although *cagA*-positive *H. pylori* strains were more frequently present in patients with NUD than in patients with MALT lymphoma (67 versus 42%, respectively; Table 1), this difference was not significant ($P > 0.05$). However, the presence of *cagA* showed a strong association with the presence of the *vacA* s1 allele ($P < 0.05$).

The majority of the patients with mono-infections (18 of 31; 58%) contained *H. pylori* strains which were IS605 negative (Table 1). IS605 was present in significantly fewer *H. pylori* strains with the *vacA* s1 allele (6 of 21; 29%) than in strains with the s2 allele (7 of 10; 70%) ($P < 0.05$). The presence of IS605 was not associated either with disease or with the presence of *cagA*.

Three s1 allele subtypes (s1a, s1b, and s1c) and the geographic distribution of the s1 allele have recently been described (1, 16). Two studies have shown that *vacA* s1-positive *H. pylori* strains from Dutch patients more frequently contain subtype s1a than subtype s1b or s1c (14, 15), which is in agreement with the data from the present study. The *vacA* s1 genotype of *H. pylori* shows a strong correlation with peptic ulcer disease and carcinoma (1, 2, 12, 14, 15). Surprisingly, an association with one of the s genotypes was not observed for the strains obtained from patients with MALT lymphoma, which has also been shown by Miehke et al. (10). However, the complete absence of the s1a m1 genotype in *H. pylori* strains from patients with MALT lymphoma was remarkable and needs further investigation.

The *cagA* gene can also be used as a marker for strains with enhanced virulence. However, the association between *cagA*

and MALT lymphoma seems to be less clear and shows variation in different geographic regions (6, 7, 11, 18).

In some *H. pylori* strains IS605 flanks the 40-kb pathogenicity island of *H. pylori* (5), but the presence of IS605 does not seem to be associated with *cagA*, as shown in this study. Remarkably, IS605 was more frequently associated with the *vacA* s2 allele than with the s1 allele.

In conclusion, the results of this study suggest that *H. pylori* strains obtained from patients with MALT lymphoma are more heterogeneous than *H. pylori* strains isolated from patients with peptic ulcer disease or gastric carcinoma.

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