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cagA and *vacA* Status of Spanish *Helicobacter pylori* Clinical Isolates

Helicobacter pylori is a gram-negative rod associated with gastroduodenal pathologies, such as gastritis, peptic ulcer, and gastric adenocarcinoma. Although most *H. pylori* infections are clinically silent, the organism is associated with substantial morbidity and mortality. It is unknown why the bacteria are able to produce severe disease in some hosts and be innocuous in others.

Different virulence factors have been described in *H. pylori* infection, including urease, lipopolysaccharide, adhesins, and vacuolating cytotoxin (2). The formation of vacuoles is associated with a higher degree of virulence, and two genes, *cagA* and *vacA*, have been described to be involved in vacuole production. It is assumed that *cagA*$^+$ strains and those with the *s1* allele of *vacA* are more virulent and produce more severe disease than do strains lacking *cagA* and *vacA s2* strains. Recently, Rudi et al. (5) published a study on *cagA* and *vacA* genes and their relationship to the production of several gastroduodenal diseases. They studied 65 strains by PCR (43 from gastritis, 19 from peptic ulcer, and 3 from cancer patients) and found a prevalence of the *cagA* gene of 84.2% in peptic ulcer and 67.4% in gastritis strains. When the *vacA* gene was studied, the *s1* allele was found to be more prevalent in strains from patients with peptic ulcers (100% showed this allele) and the *s2* allele was found only in strains from patients with gastritis. They also found a strong association between the presence of the *cagA* gene and the *s1* allele of *vacA*.

Recently, we isolated 104 Spanish *H. pylori* strains, 69 from peptic ulcer patients and 35 from gastritis patients, and aimed to study *cagA* and *vacA* status and the relationship with specific pathologies. Antral biopsy specimens were cultured onto blood agar plates (Columbia agar plus 5% horse blood) and incubated for 10 days under microaerobic conditions at 37°C. *H. pylori* was identified by colony morphology, Gram strain, and positive urease, catalase, and oxidase test results. We performed two PCRs in order to detect the *cagA* gene and the *s1* or *s2* allele of the *vacA* gene. DNA was extracted by the CTAB method (6). *cagA* was detected by applying a protocol described by Covacci and Rappuoli (3) using primers D008 (5′-ATAATGCTAAATTAGACAACTTGAGCGA-3′) and R008 (5′-TTAGAATAATCAACAAACATCACGCCAT-3′ to detect a 297-bp amplified fragment. For the study of *vacA*, the protocol of Atherton (1) was performed by using two primers (VA1-F, 5′-ATGGAAATACAACAAACACAC-3′, and VA1-R, 5′-CTGCTTGAATGCGCCAAAC-3′) and two fragments of 259 and 286 bp corresponding to the *s1* and *s2* alleles were detected. Results were studied by 2% agarose gel electrophoresis and visualized in a UV transilluminator. *cagA* and *vacA* detections are shown in Figs. 1 and 2.

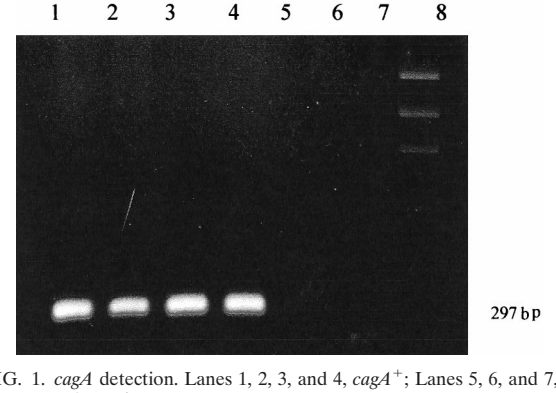

FIG. 1. *cagA* detection. Lanes 1, 2, 3, and 4, *cagA*$^+$; Lanes 5, 6, and 7, *cagA* absent; Lane 8, marker.

We agree with Rudi et al. about the prevalence of *s1* among *cagA*$^+$ strains; however, we found a smaller percentage (64.1 versus 87.0%) and we detected four strains that lacked *cagA* and possessed the *s1* allele of *vacA*, a genotype which was not found by those authors. When we studied the relationship between *cagA* and pathology, we also found no statistically significant differences among strains obtained from patients with ulcers (91% *cagA*$^+$) and gastritis (83.3% *cagA*$^+$). Moreover, in our study we found similar percentages of *s1* alleles in strains from ulcer patients (62%) and gastritis patients (57%) and we detected the *s2* allele in 38% of strains obtained from patients with peptic ulcer disease. These data are not in agreement with those published by Rudi et al., who detected *s1* in all ulcer strains and *s2* only in gastritis strains. When *cagA* and *vacA* genes were combined and were related to the pathologies, *cagA*$^+$ strains with the *vacA s1* allele were found to be more prevalent in ulcer isolates, although differences were not statistically significant.

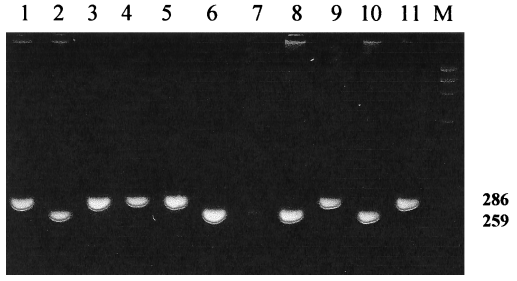

FIG. 2. Detection of *s1* and *s2* alleles of the *vacA* gene. Lanes, 2, 6, 7, 8, and 10, *s1* allele; lanes 1, 3, 4, 5, 9, and 11, *s2* allele; lane M, DNA marker. Numbers on the right indicate molecular size (in base pairs).

From our experience, we conclude that, as in other populations (4), *cagA* and *vacA* genes cannot be used as predictive markers in *H. pylori* clinical isolates to identify a particular strain as a gastritis or ulcer producer. Perhaps new virulence factors should be described with more power to discriminate among *H. pylori* strains.

REFERENCES

1. **Atherton, J. C., P. Cao, R. M. Peek, Jr., M. K. R. Tummuru, M. J. Blaser, and T. L. Cover.** 1995. Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori.*. Association of specific *vacA* types with cytotoxin production and peptic ulceration. J. Biol. Chem. **270:**17771–17777.
2. **Blaser, M. J.** 1996. Role of *vacA* and the *cagA* locus of *Helicobacter pylori* in human disease. Aliment. Pharmacol. Ther. **10**(Suppl. 1)**:**73–77.
3. **Covacci, A., and R. Rappuoli.** 1996. PCR amplifications of *H. pylori* gene sequences, p. 94–111. *In* A. Lee and F. Megraud (ed.), *Helicobacter pylori*: techniques for clinical diagnosis. W. B. Saunders Company Ltd., London, United Kingdom.
4. **Maeda, S., K. Ogura, H. Yoshida, F. Kanai, T. Ikenoue, N. Kato, Y. Shiratori, and M. Omata.** 1998. Major virulence factors, *vacA* and *cagA*, are commonly

positive in *Helicobacter pylori* isolates in Japan. Gut **42:**338–343.
5. **Rudi, R., C. Kolb, M. Maiwald, D. Kuck, A. Sieg, P. R. Galle, and W. Stremmel.** 1998. Diversity of *Helicobacter pylori vacA* and *cagA* genes and relationship to VacA and CagA protein expression, cytotoxin production, and associated diseases. J. Clin. Microbiol. **36:**944–948.
6. **Wilson, K.** 1987. Preparation of genomic DNA from bacteria, p. 2.4–2.4. *In* R. Asubel, R. Brent, D. D. Kingston, J. G. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.), Current protocols in molecular biology. Greene Publishing and Wiley Interscience, New York, N.Y.

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Authors' Reply

Domingo et al. have studied the *vacA* signal sequence genotypes and the presence of the *cagA* gene in Spanish *Helicobacter pylori* isolates. In contrast to our results, they could not find significant differences in *vacA s1-s2* genotypes or the presence of the *cagA* gene between isolates from patients with peptic ulcer disease (PUD) and those with chronic gastritis (2). In our study, all patients with PUD had no previous medication with nonsteroidal anti-inflammatory drugs (NSAID). Thus, we could conclude that ulcers in these patients had, indeed, been caused by *H. pylori.* Our results are in ageement with several other studies from the United States, the United Kingdom, The Netherlands, and Portugal, which demonstrated the *vacA s1* genotype and the *cagA* gene as predictors of PUD (1, 4, 5). In addition to the previously studied *H. pylori* strains, we have now evaluated a further 142 *H. pylori* strains, yielding similiar results (3). In the latter study, only one patient with PUD harbored a *vacA s2-m2* type and *cagA*-negative strain. This patient had taken NSAID. Concomitant treatment with NSAID might lead to PUD, even if a *vacA s2* type strain without the *cagA* gene is present. Therefore, the use of NSAID should be evaluated for patients with PUD, especially those with *vacA s2* type strains. Nevertheless, the possibility that regional differences in the genomic pattern of *H. pylori* might exist cannot be excluded.

The finding of a *cagA*-negative strain with the *vacA s1* allele is very interesting. However, due to the enormous heterogeneity of *H. pylori*, mutations in the *cagA* gene could lead to false-negative PCR results. Therefore, we have used two PCRs detecting different sites of the *cagA* gene. As mentioned in the study, in one of 48 strains the presence of the *cagA* gene could be confirmed only by one of two PCRs (2). We agree with Domingo et al. that virulence markers other than the *vacA* and *cagA* genes might be useful to discriminate between more and less pathogenic *H. pylori* strains. These factors, however, have yet to be found.

REFERENCES

1. **Atherton, J. C., P. Cao, R. M. Peek, M. K. R. Tummuru, M. J. Blaser, and T. L. Cover.** 1995. Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori.* J. Biol. Chem. **270:**17771–17777.
2. **Rudi, J., C. Kolb, M. Maiwald, D. Kuck, A. Sieg, P. R. Galle, and W. Stremmel.** 1998. Diversity of *Helicobacter pylori vacA* and *cagA* genes and relationship to VacA and CagA protein expression, cytotoxin production, and associated diseases. J. Clin. Microbiol. **36:**944–948.
3. **Rudi, J., A. Rudy, M. Maiwald, D. Kuck, A. Sieg, and W. Stremmel.** Direct determination of *Helicobacter pylori vacA* genotypes and *cagA* gene in gastric biopsies and relationship to gastrointestinal diseases. Am. J. Gastroenterol., in press.
4. **Stephens, J. C., J. A. D. Stewart, A. M. Folwell, and B. J. Rathbone.** 1998. *Helicobacter pylori cagA* status, *vacA* genotypes and ulcer disease. Eur. J. Gastroenterol. Hepatol. **10:**381–384.
5. **van Doorn, L. J., C. Figueiredo, R. Rossau, G. Jannes, M. van Asbroeck, J. C. Sousa, F. Carneiro, and W. G. Quint.** 1998. Typing of *Helicobacter pylori vacA* gene and detection of *cagA* gene by PCR and reverse hybridization. J. Clin. Microbiol. **36:**1271–1276.

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