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High Prevalence of *cagA*- and *babA2*-Positive *Helicobacter pylori* Clinical Isolates in Taiwan

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Two virulence markers, *cagA* and *babA2*, were characterized by PCR in 101 *Helicobacter pylori* isolates from a population in Taiwan. *cagA* was detected in 99% of the isolates, while *babA2* was present in all of the isolates. Base deletions and substitutions at the forward *babA2* primer annealing sites were found. Given their high prevalence, *cagA* and *babA2* cannot be useful markers for predicting the high-risk patients of *H. pylori* infection in Taiwan.

Helicobacter pylori is a gram-negative spiral bacterium that inhabits the gastric mucosa of the human stomach in approximately half of the world's population for a lifetime (9). Infection of this unique ecological niche by *H. pylori* induces gastric mucosal inflammation, which may progress into peptic ulcers. Persistent infection also increases an individual's risk for development of gastric adenocarcinoma and gastric mucosa-associated lymphoid tissue lymphoma (17). In 1994, *H. pylori* was declared a group I carcinogen (28) for gastric cancer, the fourth leading cause of cancer death in Taiwan.

Different degrees of bacterial virulence, environmental influences, and host factors are believed to contribute to the differential clinical sequelae of the infection. For the bacterium to succeed in its long-term colonization in the human stomach, a set of bacterial virulence determinants was developed for initial adhesion, for maintenance, and for the altering of gastric physiology. Of these factors, vacuolating toxin (VacA), cytotoxin-associated antigen (CagA), and the blood group antigen-binding adhesion molecule (BabA) have been shown to be related to more severe clinical outcomes (13). VacA is an 87-kDa exotoxin that can induce intracellular vacuolation in epithelial cells (18), leading to swelling and cell death. Alleles of the *vacA* gene vary among strains, particularly in the region encoding the signal sequence (typed s1 or s2) and in the 300-amino-acid midregion (typed m1 or m2) (2). Strains producing VacA or the *vacAs1* genotype have been associated with increased gastric damage and peptic ulceration (2, 3). CagA is a 120-kDa immunodominant antigen and can be translocated by the type IV secretion system into the epithelial cells, where it is tyrosine phosphorylated, possibly for host-cell signaling (6, 7, 24, 25). The CagA-positive phenotype has been detected in a higher proportion of patients with peptic ulcer disease (7, 8), atrophic gastritis (17), and gastric adenocarcinoma (4). BabA is a 75-kDa adhesion molecule that mediates the attachment of *H. pylori* to Lewis b (α-1,3/4-difucosylated) blood group antigens on human gastric epithelial cells (5, 11, 12). Three *bab* alleles have been identified: *babA1*, *babA2*, and *babB* (14). *babA1* and *babA2* are identical alleles except that *babA1* has a 10-bp deletion of the signal peptide sequence that leads to elimination of the translational initiation codon. The *babA2* and *babB* alleles, which encode homologous proteins, have polymorphic midregion sequences but rather conserved sequences in the 5′ and 3′ regions (1, 14). Only the *babA2* gene product is necessary for Lewis b binding activity (14). By use of a mismatch PCR method to characterize the presence of *babA2*, about 70% of *H. pylori* strains in Western countries were typed as *babA2*, which was associated with increased virulence (13). Moreover, the triple-positive phenotype (*babA2*, *cagA*, and *vacAs1*) was detected at a higher frequency in isolates from patients with ulcers and adenocarcinomas, which might serve as useful markers of high-risk patients in Western countries (13).

There is, on the other hand, a high prevalence of *cagA*- and *vacAs1*-positive bacteria in Asian countries (15, 19, 20, 22, 27, 29). In some regions, nearly all isolates are *vacAs1* and *cagA* positive. In a recent report from Japan, a higher proportion (85%) of strains belonged to the *babA2* genotype (21). Furthermore, no significant difference was found between the *babA2* genotype and different clinical diseases, in contrast with that in found in Western countries. The aim of this study was to characterize the presence of *babA2* and *cagA* in clinical isolates from a central Taiwan population. The correlation with various clinical outcomes was also investigated.

A total of 101 patients (54 male and 47 female) undergoing upper digestive endoscopy for the evaluation of dyspeptic symptoms at Taichung Veterans General Hospital, Taichung, Taiwan, between June 1996 and April 2001 were enrolled in this study. The subjects ranged in age from 32 to 83 years (mean ± standard deviation, 55.9 ± 13.0). Patients were classified at the time of endoscopy as having gastritis (n = 41), duodenal ulcers (n = 31), gastric ulcers (n = 15), or gastric cancer (n = 14). All these patients were *H. pylori* positive on the basis of bacterial culture performed on biopsy samples as previously described (16). *H. pylori* strains from patients were isolated, identified, stored, and recovered as previously described (23). For PCR genotyping, the oligonucleotide primer

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sequences were 5′-AATCCAAAAAGGAGAAAAAGTATG AAA-3′ (*babA2-F*), 5′-AATCGAAAAAGGAGAAAACATG AAAAA-3′ (nthu_*babA2-F*), 5′-TGTTAGTGATTTCGGTG TAGGACA-3′ (*babA2-R*), 5′-GATAACAGGCAAGCTTTT GAGG-3′ (Cf1), and 5′-CTGCAAAAGATTGTTTGCGAG A-3′ (Cr1). The *babA2* genotype was determined by using a PCR method developed by Gerhard et al. (13) with *babA2-F* and *babA2-R* or with nthu_*babA2-F* and *babA2-R*. The *babA2* gene was amplified directly from the genomic DNA of v254 by *babA2-F* and *babA*-stop (5′-TTAGTAAGCGAACACATAAT TC-3′, nucleotide positions 2205 to 2226 of GenBank strain AF033654) and cloned into pGEM-T (Promega, Madison, Wis.) to generate pGEM-babA2. A *babA1* fragment that had a 10-bp deletion in the signal sequence region (13) was prepared by PCR with pGEM-babA2 as a template and with two primers, *babA1-F* (5′-AATCGAAAAAGGAGAAAACACATCCT TTCATTAGC-3′, corresponding to positions −21 to 26 of AF033654) and *babA*-stop. This PCR product was cloned into pGEM-T to produce pGEM-babA1 as a negative control in *babA2* typing. The presence of the *cagA* gene was detected by a PCR method with primers Cf1 and Cr1 to produce a 0.35-kb fragment (26, 29). A PCR-amplified 522-bp 16S rRNA fragment by Rf1 and Rr1 (10, 29) was used as an internal control in each assay. PCR amplification was carried out in a total volume of 50 μl containing 2.0 U of VioTaq polymerase (Viogene, Taipei, Taiwan), 0.1 ng of *H. pylori* genomic DNA, 0.1 μM concentrations of each primer, and PCR buffer (200 μM concentrations of each deoxynucleotide, 10 mM Tris-HCl [pH 8.3], 50 mM KCl, 2.5 mM $MgCl_2$, 0.01% gelatin). PCR was performed by using a thermal cycler (MJ Research, Waltham, Mass.) under the following conditions: an initial denaturation step at 95°C for 5 min; 35 cycles at 95°C for 1 min, 55 to 62°C for 1 min, and 72°C for 1 min; and a final extension at 72°C for 20 min. PCR products were analyzed on 1.5% agarose gels. For sequence analysis of the *babA2* signal sequence region, a 0.4-kb fragment was amplified by PCR with oligonucleotide primers ss*babA-F* (5′-ATGACAAAATTTTTAAGAAAATG-3′, corresponding to bp 138 to 160 of AF033654) and c1*babA-R* (5′-CGTTAATCGCACTCGGATCAGCG-3′, corresponding to bp 509 to 532 of AF033654), followed by cloning into the pGEM-T plasmid. Nucleotide sequences were determined on both strands by the dideoxy chain termination procedure with an ABI Prism dye terminator cycle sequencing ready reaction kit (PerkinElmer, Boston, Mass.) in an automated DNA sequencer (model 377-96; PerkinElmer). Sequence analysis was done with the University of Wisconsin Genetics Computer Group (Madison, Wis.) package. The relationship between *H. pylori* genotypes and various diseases was analyzed by the Chi-square test with Yates's correction or by Fisher's exact test. A P of <0.05 was considered statistically significant.

Of the 101 isolates from a central Taiwan population, 100 tested positive for the presence of *cagA*. The only *cagA*-negative strain was found in a patient with gastric cancer. Given the high prevalence, no clinical relevance could be drawn between *cagA* and various diseases, which was consistent with that found in a region in northern Taiwan (29). For *babA2* typing, weak amplification was obtained for a number of the strains tested. To examine whether there were mutations in the primer region, the *babA2* signal region sequences were determined for eight strains. Sequence analysis of the aligned signal sequence region showed that there were two base deletions near the 3′ end of the forward primer. In addition, there were base substitutions at the *babA2-F* annealing sites, thus resulting in ambiguous PCR results (Fig. 1). We also determined the region spanning the *babA2-R* fragment; the *babA2-R* annealing sites, on the other hand, were much more conservative (data not shown). By use of the modified forward primer (nthu-_*babA2-F*) that contained conserved sequences among Taiwanese isolates, all 101 strains yielded positive PCR amplification. No correlation was found between the *babA2* and *cagA* genotypes. No clinical relevance could be drawn between *cagA* and various diseases due primarily to the predominant prevalence of *cagA*-positive and *babA2*-positive strains. Despite the fact that such a high proportion of *babA2* isolates has not been reported elsewhere, the high prevalence of another virulence marker *cagA* was found in a northern Taiwan population (29). Moreover, the 101 isolates in this study were typed as *vacAs1* (data not shown) in accordance with a previous finding (27). No clinical association could be found for *babA2* because of its predominance, as opposed to the frequent association of *babA2* with severe diseases in other reports (13, 14). Our results thus suggest that *babA2*, like the *cagA* and *vacAs1*-positive phenotype, cannot be a useful virulence predictor in Taiwan (27, 29), similar to the findings from a recent study in Japan (21). These data thus collectively suggest that *H. pylori* populations vary among different geographic regions and that the relationship of virulence determinants with diseases needs to be assessed for a given region. The triple-positive virulence marker seen in Western countries cannot be feasible for the prediction of high-risk patients in Japan and in Taiwan.

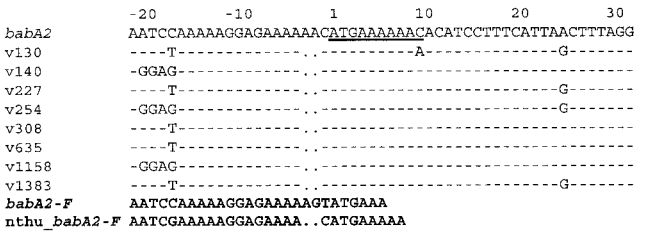

FIG. 1. Aligned nucleotide sequences of the *babA2* signal sequence region. The published sequence of *babA2* (GenBank accession number AF033654) and those of eight Taiwanese strains (v130, v140, v227, v254, v308, v635, v1158, and v1383) were aligned and compared. Dashes and dots indicate the nucleotide identity and deletion, respectively, of the *babA2* sequences. Underlining indicates the deletion of 10 nucleotides in the *babA1* signal sequence. *babA2-F*, the forward primer used for a mismatch PCR (15); nthu_*babA2-F*, the modified forward primer containing conserved sequences among Taiwanese isolates.

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