Association of peptic ulcer with increased expression of Lewis antigens but not \textit{cagA}, \textit{iceA}, and \textit{vacA} in \textit{Helicobacter pylori} isolates in an Asian population

P Y Zheng, J Hua, K G Yeoh, B Ho

Abstract

\textbf{Background}—Studies in Western populations suggest that \textit{cagA}, \textit{iceA}, and \textit{vacA} gene status in \textit{Helicobacter pylori} isolates is associated with increased virulence and peptic ulcer disease.

\textbf{Aim}—To investigate the relationship between peptic ulcer and expression of Lewis (Le) antigens as well as \textit{cagA}, \textit{iceA}, and \textit{vacA} in \textit{H pylori} isolates in Singapore.

\textbf{Methods}—Expression of Le antigens in \textit{H pylori} isolates obtained from patients with dyspepsia was measured by enzyme linked immunosorbent assay. The \textit{cagA}, \textit{iceA}, and \textit{vacA} status was determined by polymerase chain reaction.

\textbf{Results}—Of 108 \textit{H pylori} isolates, 103 (95.4\%) expressed Le\(^a\) and/or Le\(^b\), while Le\(^c\) and Le\(^d\) were expressed in 23 (21.3\%) and 47 (43.5\%) isolates, respectively. Expression of two or more Le antigens (Le\(^b\), Le\(^c\), Le\(^d\), or Le\(^e\)) was significantly higher in \textit{H pylori} isolated from ulcer patients than in non-ulcer patients (89.6\% vs 73.2\%, \(p=0.035\)). There were no significant differences in the prevalence of \textit{cagA} or \textit{iceA} in \textit{H pylori} isolates from peptic ulcer and non-ulcer patients (86.6\% vs 90.2\% for \textit{cagA}; 70.1\% vs 68.3\% for \textit{iceA}), and no association of peptic ulcer with any specific \textit{vacA} genotype.

\textbf{Conclusions}—The present study indicates that peptic ulcer disease is associated with increased expression of Lewis antigens but not \textit{cagA}, \textit{iceA}, or \textit{vacA} genotype in \textit{H pylori} isolates in our population. This suggests that \textit{cagA}, \textit{iceA}, and \textit{vacA} are not universal virulence markers, and that host-pathogen interactions are important in determining clinical outcome.

\textbf{Keywords:} Lewis blood group antigens; \textit{cagA}; \textit{iceA}; \textit{vacA}; \textit{Helicobacter pylori}; peptic ulcer

\textit{Helicobacter pylori} is the major aetiological agent of chronic active gastritis and is generally accepted as having a causative role in the pathogenesis of peptic ulcer (PU) disease. \textit{H pylori} infection has also been aetiologically linked to the development of gastric carcinoma.\(^1\)\(^2\) It is estimated that more than 50\% of the world’s population are infected with \textit{H pylori}. However, only a minority of \textit{H pylori} infected subjects develop PU or gastric cancer. The reasons for this are not well understood.

Vaccinating cytoxin gene (\textit{vacA}) s1a genotype and the cytoxin associated gene (\textit{cagA}) have been demonstrated to be related to the virulence of \textit{H pylori} infection and the development of peptic ulcer.\(^1\)\(^4\) However, there are also reports to the contrary.\(^5\)\(^7\) A novel gene \textit{iceA} (induced by contact with epithelium gene) has been reported and two allelic variants of the gene (\textit{iceA}1 and \textit{iceA}2) described.\(^3\) Studies based on Western populations suggested that \textit{iceA}1 is associated with PU.\(^10\) Recent studies showed that the lipopolysaccharides (LPS) of most \textit{H pylori} isolates express Lewis\(^b\) (Le\(^b\)) and/or Le\(^e\) blood group antigens,\(^11\) and these antigens are also expressed on human gastric mucosa.\(^12\) It is postulated that this molecular mimicry may play a role in the pathogenesis of \textit{H pylori} infections.\(^13\) Peptic ulcer disease has been suggested to be associated with \textit{H pylori} expression of Le\(^b\)/Le\(^c\), and an association between \textit{cagA} gene and expression of Le\(^c\)/Le\(^e\) has also been reported.\(^11\) Expression of Le antigens and the prevalence of \textit{iceA} have not been fully investigated in Asian countries where the prevalence of the \textit{cagA} gene is high regardless of the presence of the disease.\(^15\) \textit{H pylori} strains may differ in various geographical regions\(^7\) and studies in different populations may clarify the importance and universality of putative virulence factors. In the present study expression of Le antigens and the prevalence of \textit{cagA} as well as \textit{iceA} and \textit{vacA} were investigated in 108 \textit{H pylori} isolates in Singapore.

\textbf{Materials and methods}

\textbf{PATIENTS AND H PYLORI ISOLATES}

\textit{H pylori} strains were isolated from the gastric biopsies of 108 patients undergoing upper gastrointestinal endoscopy for dyspepsia at the National University Hospital, Singapore. Informed consent was obtained from all patients for gastroscopy and biopsies. All patients included in the study were \textit{H pylori} positive as assessed using the rapid urease test and culture. The patient population comprised 88 Chinese, 13 Indians, and seven Malays. Of these, 67 were males and 41 females. Mean age

\textbf{Abbreviations used in this paper:} \textit{cagA}, cytoxin associated gene; \textit{iceA}, induced by contact with epithelium gene; \textit{vacA}, vaculating cytoxin gene; Le, Lewis blood group antigen; PCR, polymerase chain reaction; PU, peptic ulcer; LPS, lipopolysaccharides; NUD, non-ulcer dyspepsia; OD, optical density.
Table 1  Polymerase chain reaction (PCR) for amplification of H pylori cagA, iceA, and vacA genes

<table>
<thead>
<tr>
<th>Region</th>
<th>Primer</th>
<th>Nucleotide sequence (5’→3’)</th>
<th>Size of PCR product (bp)</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>cagA</td>
<td>cagA-F</td>
<td>ATATACACACACGCGCTCCCAAG</td>
<td>400</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>cagA-R</td>
<td>ATACACACACGCGCTCCCAAG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>iceA1</td>
<td>iceA1-F</td>
<td>TTGTTGCGCGTTTGCCTCTC</td>
<td>246</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>iceA1-R</td>
<td>TTGTTGCGCGTTTGCCTCTC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>iceA2</td>
<td>iceA2-F</td>
<td>TTTGCGTTTGCCTCTCTCGT</td>
<td>229 or 334</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>iceA2-R</td>
<td>TTTGCGTTTGCCTCTCTCGT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>m1</td>
<td>m1-F</td>
<td>GATGCGCCATTAGGCTAAATG</td>
<td>290</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>m1-R</td>
<td>GATGCGCCATTAGGCTAAATG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>m2</td>
<td>m2-F</td>
<td>AGGCCCAAGGAAACATGTG</td>
<td>352</td>
<td>23</td>
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<tr>
<td></td>
<td>m2-R</td>
<td>AGGCCCAAGGAAACATGTG</td>
<td></td>
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</tr>
<tr>
<td>m1T</td>
<td>m1TF</td>
<td>GGGCCAAATAGGATGCTTTGCG</td>
<td>290</td>
<td>6</td>
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<tr>
<td></td>
<td>m1-R</td>
<td>GGGCCAAATAGGATGCTTTGCG</td>
<td></td>
<td></td>
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<tr>
<td>m1Tm2</td>
<td>m1TF</td>
<td>GGGCCAAATAGGATGCTTTGCG</td>
<td>300</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>m1Tm2-R</td>
<td>GGGCCAAATAGGATGCTTTGCG</td>
<td></td>
<td></td>
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<tr>
<td>s1a</td>
<td>s1a-F</td>
<td>GATCCACATACACGCGCAAC</td>
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<td>s1a-R</td>
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<tr>
<td>s1b</td>
<td>s1b-F</td>
<td>AGGCCCAATAGGCAAGAG</td>
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<td>s1b-R</td>
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<tr>
<td>s2</td>
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<td>GCTCTGCGGAAATGCGCAAC</td>
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<td></td>
<td>s2-R</td>
<td>GCTCTGCGGAAATGCGCAAC</td>
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</table>

Table 2  Relationship between H pylori expression of Le antigens, cagA, iceA, and vacA genes

<table>
<thead>
<tr>
<th>Lesion</th>
<th>IsoType</th>
<th>Leα</th>
<th>Leβ</th>
<th>Leα and/or Leβ</th>
<th>Leγ</th>
<th>Leδ</th>
<th>&gt;2 Le antigens*</th>
<th>cagA</th>
<th>iceA1</th>
<th>iceA2</th>
<th>vacA s1a/m1T</th>
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<tbody>
<tr>
<td>Peptic ulcer</td>
<td></td>
<td>67</td>
<td>61</td>
<td>(91.0)</td>
<td>58</td>
<td>86.6</td>
<td>64 (95.5)</td>
<td>18</td>
<td>26.9</td>
<td>34</td>
<td>50.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>64</td>
<td>(95.5)</td>
<td>58</td>
<td>86.6</td>
<td>64 (95.5)</td>
<td>34</td>
<td>50.7</td>
<td>60</td>
<td>89.6</td>
</tr>
<tr>
<td>Non-ulcer</td>
<td></td>
<td>41</td>
<td>36</td>
<td>(87.8)</td>
<td>34</td>
<td>82.9</td>
<td>39 (95.1)</td>
<td>5</td>
<td>12.2</td>
<td>13</td>
<td>31.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>39</td>
<td>(95.1)</td>
<td>37</td>
<td>90.2</td>
<td>39 (95.1)</td>
<td>28</td>
<td>68.3</td>
<td>37</td>
<td>90.2</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>108</td>
<td>97</td>
<td>92</td>
<td>103</td>
<td>92</td>
<td>90</td>
<td>23</td>
<td>47</td>
<td>90</td>
<td>95</td>
</tr>
</tbody>
</table>

Values are number (percentage).

*Lewis antigens (Leα, Leβ, Leγ, and Leδ).

†p< 0.05 compared with non-ulcer group.
Levels of expression of Le\(^{-}\) and Le\(^{+}\) were not significantly different in the H pylori isolates from patients with peptic ulcer compared with those without ulcers (26.9% \(v\) 12.2% (p=0.091) for Le\(^{-}\) and 50.7% \(v\) 31.7% (p=0.072) for Le\(^{+}\), respectively). The results also showed that Le\(^{+}\) coexpressed with Le\(^{-}\).

**RELATIONSHIP BETWEEN cagA STATUS AND CLINICAL OUTCOME**

The cagA gene was positive in 95 (88%) of 108 H pylori isolates. As shown in table 2, the cagA gene was found in 58 (86.6%) of 67 PU isolates compared with 37 (90.2%) of 41 non-ulcer isolates (p=0.035). Furthermore, 32/67 (47.8%) isolates from PU patients expressed three or more Le antigens compared with 11/41 (26.8%) isolates from NUD patients (p=0.043).

Of the 95 cagA positive isolates, 91 (95.8%) expressed Le\(^{-}\) and/or Le\(^{+}\) antigens compared with 12 (92.3%) of 13 cagA negative isolates (p=0.480). Furthermore, the mean OD level of Le\(^{-}\) or Le\(^{+}\) expression was not significantly different between cagA positive and cagA negative isolates (1.0490 (0.6125) \(v\) 0.9862 (0.8928) for Le\(^{-}\) (p=0.745) and 1.2860 (1.0970) \(v\) 1.8615 (1.3687) for Le\(^{+}\) (p=0.088).

**RELATIONSHIP BETWEEN iceA STATUS AND CLINICAL OUTCOME**

Of 108 isolates, iceA1 was positive in 75 isolates and iceA2 was detected in 26 isolates. Four isolates were positive for both iceA1 and iceA2, while three isolates did not yield either iceA1 or iceA2 fragments. There was no significant difference in the presence of iceA1 in H pylori isolates from PU and NUD patients (70.1% \(v\) 68.3%; p=0.833). iceA1 was not associated with cagA status (p=0.531). Similarly, there was no significant difference in the presence of iceA2 in H pylori isolates from PU and NUD patients (23.9% \(v\) 24.4%; p=1.000).

Of 75 iceA1 positive isolates, 72 (96.0%) expressed Le\(^{-}\) and/or Le\(^{+}\) antigens compared with 31 (93.9%) of 33 iceA1 negative isolates (p=0.640). The mean OD level of Le\(^{-}\) or Le\(^{+}\) expression was not significantly different between iceA1 positive and iceA1 negative isolates (1.0106 (0.6242) \(v\) 1.0699 (0.7026) for Le\(^{-}\) (p=0.663); 1.2570 (1.0831) \(v\) 1.4669 (1.2140) for Le\(^{+}\) (p=0.373)).

**RELATIONSHIP BETWEEN vacA GENOTYPE AND CLINICAL OUTCOME**

Of 108 isolates, 107 were typed as s1a of the vacA genotype and the other isolate was typed as s2. Four vacA genotypes (s1a/m1T, s1a/ m1Tm2, s1a/m2, and s2/m2) of H pylori isolates were identified (table 3), with the distribution as follows: 39 s1a/m1T, 4 s1a/ m1Tm2, 64 s1a/m2, and 1 s2/m2. There was no significant difference between PU and NUD patients for infection by s1a/m1T genotype H pylori isolates (37.3% \(v\) 34.1%; p=0.837).

**Discussion**

In this study we observed the occurrence of Le\(^{-}\), Le\(^{+}\), Le\(^{+}\), Le\(^{-}\), and blood group A antigen in H pylori isolates. Of 108 isolates, 106 (98.1%) were typeable with Mabs specific for Lewis and other blood group antigens. The two isolates which were non-typeable showed O side chain (data not shown), indicating the existence of other serotypes that were not reactive with the Mabs used as described by Simoons-Smit and colleagues.15 One strain of H pylori expressed blood group A antigen which has so far been reported in one other Helicobacter species, H mustelae.24 However, the low prevalence of this antigen in H pylori isolates suggests that it does not have an important role in the pathogenesis of gastric diseases. The chemical structures of Le\(^{-}\), Le\(^{+}\), and Le\(^{+}\) of H pylori were elucidated earlier,25 while the chemical structure of Le\(^{-}\) has recently been determined.26

Le\(^{-}\) and Le\(^{+}\) were frequently encountered in our local H pylori isolates. Expression of Le\(^{-}\) and Le\(^{+}\) was similar to the finding in Canada27 but was higher than the findings in the USA14 and Europe.18 Expression of Le\(^{-}\) and Le\(^{+}\) in H pylori isolates in our study was much higher than that found in the USA,14 Netherlands,17 and Canada.19 Broadhurst and Lin-Chu reported that the Le(a+b+) phenotype is frequent in Chinese patients but rare or absent in Caucasians.28 The relationship between Le antigen expression by H pylori and host phenotype is not clear.27 29 Our observation of higher expression of Le\(^{-}\) and Le\(^{+}\) in our population of predominantly Chinese patients lends support to the suggestion by Wirth and colleagues24 that H pylori Le antigen expression is related to the host phenotype. However, Taylor and colleagues did not find such a correlation in their study.27

Wirth and colleagues24 suggested that the risk of peptic ulcer increases with expression of Le antigens in H pylori isolates. They found that expression of Le\(^{-}\) or Le\(^{+}\) in H pylori isolates was significantly higher in PU than in NUD patients. It is important to note that only three of 96 subjects in their multicentre study were of Chinese origin. In contrast, in our predominantly Chinese patients (88/108 (81.5%)), we found equally high expression of Le\(^{-}\) and Le\(^{+}\) in H pylori isolates from both PU and NUD patients. Furthermore, the mean OD level of Le\(^{-}\) or Le\(^{+}\) expression was not significantly different between the two groups.

In this study we showed that the presence of two or more Le antigens (Le\(^{-}\), Le\(^{+}\), Le\(^{+}\), or Le\(^{-}\))

<table>
<thead>
<tr>
<th>Patient Type</th>
<th>Peptic Ulcer</th>
<th>Non-Ulcer</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Le(^{-})</td>
<td>25 (37.3)</td>
<td>14 (34.1)</td>
<td>39 (36.3)</td>
</tr>
<tr>
<td>Le(^{+})</td>
<td>38 (56.7)</td>
<td>26 (63.4)</td>
<td>64 (58.3)</td>
</tr>
</tbody>
</table>

Values are number (percentage).

<table>
<thead>
<tr>
<th>vacA Genotype</th>
<th>s1a/m1T</th>
<th>s1a/m1Tm2</th>
<th>s1a/m2</th>
<th>s2/m2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptic Ulcer</td>
<td>39</td>
<td>4</td>
<td>64</td>
<td>1</td>
</tr>
<tr>
<td>Non-Ulcer</td>
<td>38</td>
<td>26</td>
<td>64</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 3  Relationship between H pylori vacA genotype and peptic ulcer
was significantly higher in *H pylori* isolates from PU than from NUD patients. Expression of Le<sup>a</sup> and Le<sup>o</sup> antigen in *H pylori* isolates from patients with PU was not significantly different from NUD patients.

As this was an exploratory study on the expression of Le antigens in *H pylori* strains and indeed the first such study in our population, statistical analyses were performed on multiple parameters. We cannot exclude the possibility that some findings may be due to chance. However, the finding of higher expression of Le antigens in peptic ulcer associated *H pylori* strains is novel in our population because it holds true on testing for ≥2 Le antigens and ≥3 Le antigens.

Several studies in Caucasian populations suggest an association between infection by *cagA* positive *H pylori* and PU disease. Our study indicates that *cagA* status is not predictive of gastroduodenal disease in the Singapore population, and adds to the growing evidence that the *cagA* gene should not be regarded as a universal virulence marker of peptic ulcer disease.

An association of peptic ulcer and infection by icaA positive *H pylori* isolates was described in two previous studies of Western patients. In accordance with van Doorn and colleagues, we found that the presence of icaA was independent of the *cagA* gene. Similar to *cagA* status, the absence of icaA in *H pylori* was not associated with PU risk in our population. This constitutes further evidence that distinct *H pylori* genotypes circulate in Western and Asian countries.

Four vacA genotypes (s1a/m1T, s1a/m1Tm2, s1a/m2, and s2/m2) of *H pylori* isolates were found in the Singapore population. In the present study almost all of the *H pylori* isolates were typed as s1a, which is similar to reports from China and Taiwan. The majority (95.4%) of *H pylori* isolates in Singapore were typed as s1a/m1T or s1a/m2, suggesting that there is less mosaicism in vacA alleles of *H pylori* in Singapore where the population is of mainly Chinese origin. The s1a/m1 genotype *H pylori* that was reported to be associated with PU was not detected in our population. In contrast with the finding by Wang and colleagues, there was no association between infection of s1a/m1T *H pylori* isolates and peptic ulcer in the present study.

The present study showed that peptic ulcer disease was not associated with host genotypes or icaA or vacA genotypes but there was an association with increased expression of a combination of Le antigens. This suggests that the pathogenesis of *H pylori* induced gastric diseases may be due to host-pathogen interactions rather than *H pylori* itself. Expression of Le antigens may favour development of disease in the host by two mechanisms. Firstly, *H pylori* strains expressing Le antigens may adapt more readily to a host possessing similar antigens. Expression of host related antigens on the surface of bacteria could allow bacteria to elude elimination by the host immune response and thus facilitate persistence of infection in the host. Chronicity of infection may lead to the development of disease by increasing inflammation and promoting mucosa atrophy. Secondly, expression of host related antigens on *H pylori* may increase the pathogenic potential of the bacterium via induction of autoantibodies that cross react with gastric mucosa. However, it is noted that expression of Le antigens in *H pylori* was also high in NUD patients, which indicates that additional unidentified factors also contribute to the pathogenesis of *H pylori* associated peptic ulcer in our population.

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22. Lage AP, Goddroid E, Pauconnier A, et al. Diagnosis of *Helicobacter pylori* infection by PCR: comparison with


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