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The Geographic Origin of *Helicobacter pylori* Influences the Association of the *homB* Gene with Gastric Cancer

Jieun Kang,a Kathleen R. Jones,b Sungil Jang,a Cara H. Olsen,c Yun-Jung Yoo,a D. Scott Merrell,b and Jeong-Heon Cha,a,d

Department of Oral Biology, Oral Science Research Center, BK21 Project, Research Center for Orofacial Hard Tissue Regeneration, Yonsei University College of Dentistry, Seoul, South Korea,a Department of Microbiology and Immunology, Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA,b Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA,c and Oral Cancer Research Institute, Yonsei University College of Dentistry, Seoul, South Korea,d

We found that South Korean *Helicobacter pylori* isolates predominantly carry *homB* at locus B and that there is no association between the *homB* allele and the *cagA* allele or the development of gastric cancer within this population. Uniquely, several East Asian strains carried multiple copies of the *hom* genes.

*Helicobacter pylori* colonizes the gastric mucosas of over 50% of the world’s population (6, 13) and is the etiological agent of gastritis, duodenal ulcers, gastric ulcers, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma (4, 5, 19, 20, 22). Due to this bacterium’s association with gastric cancer, which is the second most common cause of cancer-associated death (14), the World Health Organization has classified *H. pylori* as a class I carcinogen (8). Gastric cancer mortality rates vary geographically; the highest rates are in East Asian countries like China, Japan, and South Korea, which also display high rates of *H. pylori* infection (7, 8, 21, 23). Clearly, gastric diseases are due, at least in part, to infection by *H. pylori*, and the ultimate disease developed appears to be affected by variability in *H. pylori* virulence factors. Recently, we presented detailed epidemiological studies of *cagA* and *vacA* from a collection of 260 isolates from South Korea (9, 10, 11). Our studies showed that there is a significant association between infection with *H. pylori* strains carrying the EPIYA-ABD *cagA* genotype and the development of gastric cancer (11). Moreover, the majority of *H. pylori* isolates encoded the most virulent CagA (EPIYA-ABD) and VacA (s1/i1/m1) proteins (9, 11). The polymorphisms in *cagA* and *vacA*, alone and in concert, impact the progression to severe gastric disease, but the impact of these two virulence factors alone is not sufficient to explain the vast discrepancy in gastric cancer rates between East Asian and Western populations. Thus, it is important to examine the impacts of different virulence factors among both Western and East Asian populations (9, 10, 11, 16).

In vitro, *Helicobacter* outer membrane B (HomB) promotes the secretion of the proinflammatory cytokine interleukin-8 (IL-8) and increases *H. pylori*’s ability to adhere to host cells (15). More importantly, *homB* presence is significantly associated with development of peptic ulcer disease in Portuguese children and young adults (15, 18) and with gastric cancer development and the presence of *cagA* in U.S. and Colombian populations (12). These findings suggest that the outer membrane protein HomB is a novel virulence factor. Thus, it and other members of the small paralogous family of hom adhesion molecules are currently being investigated. The two best-studied *hom* genes, *homA* and *homB*, are 90% identical at the nucleotide level (2, 3, 18). These *homA* and *homB* genes can be present at two different loci within the *H. pylori* genome: locus A and locus B. Strains can carry a single copy of one of the *hom* genes, a double copy of a single gene, a single copy of each gene, or neither gene (15, 16). Previous studies suggest geographic differences, either in distribution, location, or copy number, of the *hom* genes in the genome and suggest that these differences influence any association with disease outcome (15, 16, 17).

In the present study, our collection of 260 South Korean isolates was assessed for any associations between the distribution of the *homA* or *homB* genes and disease state, as well as any associations between the *hom* genes and the different *cagA* and *vacA* alleles. The South Korean isolates include 115 isolates from patients diagnosed with gastritis, 60 isolates from patients diagnosed with duodenal ulcers, 55 isolates from patients diagnosed with gastric ulcers, 55 isolates from patients diagnosed with duodenal ulcers, and 30 isolates from patients diagnosed with gastric cancer (9, 10, 11). A complete description of all strains can be found in Table S1 in the supplemental material.

To analyze the *hom* genotype of the South Korean *H. pylori* strains, the presence of the *homA* and/or *homB* gene(s) was identified by a single PCR with the *hom* primers hf and hr (Table 1 and Fig. 1). We successfully genotyped 225 samples for which we had complete epidemiological data for the *hom* genes (Fig. 2; see also Table S2 in the supplemental material). Of note, two strains showed an amplicon with a length that was intermediate (approximately 146 bp) of what was expected for either the *homA* PCR product (~128 bp) or the *homB* PCR product (~161 bp). This intermediate-length *homA* or *homB* genotype has been previously described and shown to be due to random deletions and/or insertions within the *hom* genes (12).

Once the strains were genotyped for the presence of the two *hom* genes, we next sought to define the copy numbers and locations of the genes. This was accomplished through two additional PCRs (Fig. 1). The distribution of *homA/homB* is shown in Fig. 2. Within this pop-
and K-Af/K-Ar primers or K-Bf/K-Br primers (Table 1) was performed. These K-Af/K-Ar and K-Bf/K-Br primers were designed according to the genome as previously described (18). If an indeterminate result was obtained from the PCR using the Af and Ar primers or Bf and Br primers, another PCR with the K-Af and locus B. The annealing positions (arrows) and names of the primers used in this study are shown. The presence of a homA allele (first lane). Next, a PCR using the Af and Ar primers or the K-Af and K-Ar primers yielded a 2,000-bp amplicon, which denotes an occupied locus B, and this PCR product was purified and used as the template in a PCR using the hf and hr primers. This PCR yielded a 128-bp amplicon produced from the PCR using the Bf and Br primers indicates that locus B is occupied (fourth lane). These results indicate that K3-CA has a genotype of homA/homB. Finally, four strains failed to amplify either hom gene at either locus A or locus B and therefore were considered hom negative.

### TABLE 1 Primer sequences

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Primer sequence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>hf (F1-jhp0870/jhp0849)</td>
<td>AGAGGGTGTGATACCCGCTAATA</td>
<td>18</td>
</tr>
<tr>
<td>hr (R1-jhp0870/jhp0849)</td>
<td>GGTGAGTTGTCTCCGGTTTG</td>
<td>18</td>
</tr>
<tr>
<td>Af (F1-jhp0648/HP0709)</td>
<td>TAAATTTCCGCAAAAACATC</td>
<td>18</td>
</tr>
<tr>
<td>Ar (R1-jhp0650/HP0711)</td>
<td>ATCCAGGGCTAATGAGC</td>
<td>18</td>
</tr>
<tr>
<td>Bf (F1-jhp0869/HP0935)</td>
<td>AAGGAGTGGTCGTTGAGTGT</td>
<td>18</td>
</tr>
<tr>
<td>Br (R1-jhp0871/HP0936)</td>
<td>GGGTGCCCTTGGGCTGGA</td>
<td>18</td>
</tr>
<tr>
<td>K-Af</td>
<td>GGGTTGGTCCCTTTGGGCTTGGA</td>
<td>18</td>
</tr>
<tr>
<td>K-Ar</td>
<td>GGTTTTGTGCAATGAAAATC</td>
<td>This study</td>
</tr>
<tr>
<td>K-Bf</td>
<td>GATTCTTTCCACCTTCTTTTATG</td>
<td>This study</td>
</tr>
<tr>
<td>K-Br</td>
<td>GGTGTTGGTCAATGACAATC</td>
<td>This study</td>
</tr>
</tbody>
</table>

FIG 1 Genotyping of the hom genes at the respective loci. (Top) Schematic representation of the two loci where the hom genes are traditionally found, locus A and locus B. The annealing positions (arrows) and names of the primers used in this study are shown. The presence of a hom gene in a particular locus is depicted by the presence of a dashed box. (Bottom) The strains were genotyped for the hom gene by a single PCR with the hf and hr primers. A PCR amplicon of 128 bp indicates the presence of the homA gene, and an amplicon of 161 bp denotes the presence of the homB gene. In order to determine the location (locus A or B) of the hom gene, two additional PCRs were performed. To amplify locus A, primers Af and Ar were used, and to amplify locus B, the Bf and Br primers were used, as previously described (18). If an indeterminate result was obtained from the PCR using the Af and Ar primers or Bf and Br primers, another PCR with the K-Af and K-Ar primers or K-Bf and K-Br primers (Table 1) was performed. These K-Af/K-Ar and K-Bf/K-Br primers were designed according to the genome sequences of the Korean *H. pylori* strains HP51 and HP52 (GenBank accession numbers CP000012 and CP001680, respectively). For locus A, a resulting amplicon of 300 to 900 bp indicates that locus A is empty, whereas the presence of a 2,000- to 2,500-bp amplicon confirms that locus A is occupied by a hom gene. In the case of locus B, a 1,300- to 1,800-bp amplicon denotes that B is empty and the presence of a 2,500- to 4,000-bp amplicon indicates that locus B is occupied by a hom gene. (Left) For the K3-CA strain, the PCR with the hf and hr primers yielded a single amplicon of 161 bp (second lane), denoting the presence of the homB gene. The PCR with the Af and Ar primers (amplifying locus A) yielded a 600-bp product, indicating that locus A is empty (third lane), whereas a 3,000-bp amplicon produced from the PCR using the Bf and Br primers indicates that locus B is occupied (fourth lane). These results indicate that K3-CA has a genotype of homB. (Right) For K57-G and any strains that carried both homA and homB, an additional set of nested PCRs was also performed. First, PCRs with the hf and hr primers yielded two differently sized amplicons, a 128-bp amplicon, indicating the presence of a homB allele (first lane). Next, a PCR using the Af and Ar primers or the K-Af and K-Ar primers yielded a 2,000-bp amplicon, which denotes an occupied locus. In the case of K57-G (right, third lane), the K-Af and K-Ar primers were used to amplify locus A because it showed no band for PCR using the Af/Ar primers. This PCR product was then purified and used as the template in a PCR using the hf and hr primers (fourth lane). This PCR yielded a 128-bp amplicon (sixth lane), which indicates that homB is located at locus B. Thereby, these results indicate that K57-G has a genotype of homA/homB.
For these four −/− strains, PCR amplification of locus A and locus B yielded products that were indicative of an empty locus. However, each of the four −/− strains indicated the presence of a hom gene through the hom PCR amplification: two had homA, one had homB, and one had homA and homB. This suggests that for these strains, homA or homB is presumably located at an alternate unknown location within the genome. It is interesting to speculate that perhaps homA or homB may be carried in the normal locus for the virtually unstudied homC or homD gene. Further study is clearly necessary to elucidate whether the location of the homA/homB genes corresponds to a functional difference and whether homA/homB can be located at other locations besides locus A and locus B. Of note, no strains carrying homA/−, homB/−, or homA/homB alleles were found.

We next assessed whether there was an association between the distribution of the individual hom genes and the disease state. A complete breakdown of the relationship between the hom alleles and the disease state is provided in Tables S1 and S2 in the supplemental material. We first assessed if there was any association between the distribution of the hom alleles and the different vacA alleles (see Table S3 in the supplemental material) and found that the distribution of vacA alleles among the two hom genes was statistically significant (P = 0.0142). This association was dependent only on the homB allele (P = 0.0275), since there was no association between the distribution of the homA allele and that of the vacA allele (P = 0.3955). The overall association between the vacA alleles and the hom alleles was also influenced by the distribution of cagA alleles; the association was present in the non-EPIYA-ABD population (P = 0.0319) but not in the EPIYA-ABD population (P = 0.1014). Due to this difference, we used logistic modeling to determine if there was a three-way association between the cagA, vacA, and hom alleles. However, no association between these three virulence factors was identified (P = 0.681). Another interesting aspect of this vacA/hom association was that it appeared to be influenced by the age of the patient; the association only became evident in the population above 60 years of age (P = 0.0076). A higher-order association between the cagA, vacA, and hom alleles and disease states was also assessed, but no significant associations existed between the virulence factors and the disease state.

Recently, it has become clear that individual virulence factors interact in order to impact Helicobacter pylori pathogenesis (1, 9, 10, 24). Since homB is associated with cagA within Western populations (12, 15), we next assessed the distribution of hom genes in combination with the cagA alleles, the vacA alleles, and the disease state. A complete breakdown of the strains based on these factors is provided in Table S3 in the supplemental material. We first assessed if there was any association between the distribution of the hom alleles and the different cagA alleles (a canonical EPIYA-ABD versus all other EPIYA motifs). We found that there was no association between the distribution of the hom alleles and the different cagA alleles (P = 0.0872). Furthermore, when each gene was considered separately, there was no association between the distribution of homA (P = 0.6139) or that of homB (P = 0.2217) across the different cagA alleles. We next analyzed the association between the distribution of the hom alleles and the different vacA alleles (see Table S3 in the supplemental material) and found that the distribution of vacA alleles among the two hom genes was statistically significant (P = 0.0142). This association was dependent only on the homB allele (P = 0.0275), since there was no association between the distribution of the homA allele and that of the vacA allele (P = 0.3955). The overall association between the vacA alleles and the hom alleles was also influenced by the distribution of cagA alleles; the association was present in the non-EPIYA-ABD population (P = 0.0319) but not in the EPIYA-ABD population (P = 0.1014). Due to this difference, we used logistic modeling to determine if there was a three-way association between the cagA, vacA, and hom alleles. However, no association between these three virulence factors was identified (P = 0.681). Another interesting aspect of this vacA/hom association was that it appeared to be influenced by the age of the patient; the association only became evident in the population above 60 years of age (P = 0.0076). A higher-order association between the cagA, vacA, and hom alleles and disease states was also assessed, but no significant associations existed between the virulence factors and the disease state.

### Table 2: Significance of the associations between the distributions of homA/homB and the disease states

<table>
<thead>
<tr>
<th>Disease comparison</th>
<th>P value for distribution of:</th>
</tr>
</thead>
<tbody>
<tr>
<td>homA vs homB</td>
<td>homA vs homB</td>
</tr>
<tr>
<td>Crosses all diseases</td>
<td>0.9978 0.9982 0.9335</td>
</tr>
<tr>
<td>Gastritis vs all other diseases</td>
<td>0.8953 0.7215 0.7311</td>
</tr>
<tr>
<td>Duodenal ulcers vs all other diseases</td>
<td>1.0000 1.0000 1.0000</td>
</tr>
<tr>
<td>Gastric ulcers vs all other diseases</td>
<td>0.9219 1.0000 1.0000</td>
</tr>
<tr>
<td>Gastric cancer vs all other gastric diseases</td>
<td>0.8805 0.7931 0.6121</td>
</tr>
<tr>
<td>Peptic ulcers (both duodenal and gastric ulcers) vs gastritis and gastric cancer</td>
<td>1.0000 1.0000 1.0000</td>
</tr>
<tr>
<td>More severe diseases (gastric ulcer and gastric cancer) vs less severe diseases (gastritis and duodenal ulcer)</td>
<td>0.9379 0.8481 0.7146</td>
</tr>
<tr>
<td>Gastritis vs peptic ulcers vs gastric cancer</td>
<td>0.9803 0.9084 0.7894</td>
</tr>
</tbody>
</table>

*hom* represents the distribution of the homA, homB, and hom-negative strains for the different disease states listed.
In conclusion, this is the first study to assess the association between the presence of the homB gene and gastric cancer in a population of predominantly East Asian strains; we found that the impact of the homB allele on disease is geographically dependent. In Western strains, there is a more even distribution of the homA and homB genes, while in East Asian strains, homB is more common (Fig. 2). Furthermore, Western strains carry a single hom gene at locus A, whereas East Asian strains carry a single hom gene at locus B. This study was the first to identify homB to develop geographically tailored treatment regimens. The need for information about the presence and function of different virulence factors within different populations and the need to develop geographically tailored treatment regimens.

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