Prevalence of Helicobacter pylori vacA, cagA and iceA genotypes and correlation with clinical outcome

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Abstract. The aim of this study was to assess the genetic status of cagA, vacA subtype and iceA genotypes of Helicobacter pylori and the relationship with upper gastrointestinal diseases in Northeast China. Gastric biopsies were obtained from 378 patients with upper gastrointestinal diseases and 197 samples were used. The cagA, vacA alleles and iceA genotypes were determined by polymerase chain reaction. CagA was present in 176 (89.3%) of 197 patients. Of the 197 cases, 186 (94.4%) had vacA signal sequence s1c allele, 6 (3%) had s1a and 5 (2.5%) had s1b. The vacA s2 genotype was not detected in our study. VacA middle region sequences, m1 and m2, were found in 20 (10.2%) and 150 (76.1%), respectively. The allelic variant iceA1 (70.1%) was more prevalent than iceA2 (23.4%). The vacA allele slm2 had a significant relationship with the presence of gastric cancer (p<0.05) and the iceA1 genotype was also associated with gastric cancer (p<0.05). These may be useful risk factors for upper gastrointestinal diseases.

Introduction

Helicobacter pylori (H. pylori) is a gram-negative microaerophilic bacterium which is one of the most common pathogens in humans and has a worldwide distribution. It is associated with the development of chronic gastritis, peptic ulcer and even gastric cancer (1). On the basis of abundant epidemiological research, H. pylori was classified as a class I carcinogen in humans by the World Health Organization International Agency for Research on Cancer (2).

Several H. pylori virulence genes that may be associated with the risk of developing diseases have been identified. The cagA is a marker of genomic pathogenicity island (cagPAI) encoding the gene product which causes upregulation of interleukin-8 (IL-8) (3). It is considered that H. pylori strains possessing cagA are related to a more severe clinical outcome such as atrophic gastritis or gastric cancer (4,5). The vacA exists in all H. pylori strains and encodes vaculating cell toxins which cause vacuole degeneration of epithelial cells. It includes two different parts: the signal (s) region encoding the signal peptide and the middle (m) region. The s-region is situated at the 5’ end of the gene and exists as s1 and s2 alleles. The s1 exists as an s1a, s1b and s1c. The m-region occurs as m1 or m2 alleles (6). The mosaic combination of s- and m-region allelic types produces cytotoxin and is associated with the pathogenicity of the bacterium. In general, type slm1 and slm2 strains produce high and moderate levels of toxin, respectively, whereas s2m2 strains produce little or no toxin. (7). VacA m1 strains are associated with greater gastric epithelial damage than m2 strains (8). Another virulence gene designated iceA has two main allelic variants iceA1 and iceA2 but the function of these variants is uncertain. IceA1 is upregulated upon contact of H. pylori with the gastric epithelium and has been considered as a marker for peptic ulcer disease (9).

In Northeast China, there are no data regarding the pattern of H. pylori genotypes in patients. This study aimed to investigate the prevalence of the vacA, cagA and iceA genotypes of H. pylori from patients with upper gastrointestinal diseases and the relationship with clinical outcome in Northeast China.

Materials and methods

Study subjects. We evaluated 378 patients with upper gastrointestinal diseases referred for endoscopy at the Second Affiliated Hospital of Harbin Medical University in 2007 and 2008. Gastric mucosal biopsy specimens were obtained from each patient: one for pathological diagnosis, another for histological detection of H. pylori and the last for genomic DNA extraction and polymerase chain reaction (PCR).

The study was approved by the Ethics Committee of Harbin Medical University. Written informed consent was obtained from each patient prior to enrolling in the study.
Histological assessment. The biopsy samples were fixated in 10% formalin, then sliced into 4- to 6-mm pieces, dehydrated in ethanol, embedded in paraffin wax, sectioned (5-µm thick), and stained with hematoxylin and eosin (H&E). The presence of *H. pylori* in the sections was determined using a modified Gram staining protocol and taking into consideration its morphological characteristics which included a curved and spiral form and intense purple coloring (10). Pathological diagnoses were evaluated in a blinded manner by two independent pathologists and were defined as gastritis (active chronic gastritis or closed-type atrophic gastritis), gastric ulcer and gastric cancer.

Genomic DNA extraction. DNA was extracted from the biopsy specimens using the Genomic DNA purification system (Promega, USA) according to the manufacturer’s instructions and stored at -20ºC until analysis.

Diagnosis of *H. pylori* infection. *H. pylori*-positive status was defined as positive histology and positive 16S-rRNA PCR. A 500-bp region of 16S-rRNA was amplified by PCR using primers CP-1/CP-2 (Table I). Five microlitres of DNA was added to 50 µl of reaction mixture containing 1X PCR buffer, 0.2 mM dNTPs and 0.3 µM primers as well as 1.25U Taq polymerase (Takara Bio, Inc., Japan). The incubation conditions were as follows: a 5-min preincubation at 95ºC, followed by 30 cycles of 1 min at 94ºC, 1 min at 58ºC, 1 min at 72ºC, and a final 5-min incubation at 72ºC. Positive results were indicative of a diagnosis of *H. pylori* infection.

Genotyping of *H. pylori*. The systems of PCR were the same as mentioned above except for the primers. The amplification cycles consisted of an initial denaturation at 94ºC for 5 min and then denaturation at 94ºC for 30 sec, primer annealing at 60, 56, 58 and 48ºC for *cagA*, *vacA* (s1a, s1b, s1c and s2), *vacA* (m1, m2) and *iceA*, respectively, for one-half minute and extension at 72ºC for 45 sec. All reactions were performed through 35 cycles. The final cycle included an extension step for 5 min. Primers used for genotyping *cagA*, *vacA* and *iceA* genes are listed in Table I. PCR products were analyzed on 1.5% agarose gel electrophoresis with ethidium bromide. Images were quantified via the Gene Genius system (Syngene, England, UK). For strains that were *cagA*-negative as determined by PCR, Southern blotting was performed according to the method described by Pan et al (11).

**Table I. Primer sequences for human HP 16S rRNA, cagA, vacA and iceA.**

| Gene       | Primer   | Primer sequence (5’→3’)     | Product size (bp) | Reference |
|------------|----------|-----------------------------|-------------------|-----------|
| 16S rRNA   | cp-1     | GCGCAATCAGCGTCAGGTAATG       | 500               | (37)      |
|            | cp-2     | GCTAAGAGATCAGGCTATG          |                   |           |
| cagA       | cagA-F   | GATAAACGGCAAGCTTTTGAGG       | 349               | (18)      |
|            | cagA-R   | CTGAAAAAGATTGTGGGCAGA        |                   |           |
| s1a        | S1a-F    | TCTYGGCTTTAGTGAGG            | 212               | (18)      |
|            | VA1-R    | CTGCTTTGAATGCGGCAAC         |                   |           |
| s1b        | SS3-R    | AGGCCCATACCGCAGAGG           | 187               | (18)      |
|            | VA1-R    | CTGCTTTGAATGCGGCAAC         |                   |           |
| s1c        | S1c-F    | CTYGCTTTAAGTGGGYTA          | 213               | (18)      |
|            | VA1-R    | CTGCTTTGAATGCGGCAAC         |                   |           |
| s2         | SS2-F    | GCTAACACGGCAAAATATGCC       | 199               | (8)       |
|            | VA1-R    | CTGCTTTGAATGCGGCAAC         |                   |           |
| m1         | VA3-F    | GTTCAAAATTGCGGTCA          | 290               | (38)      |
|            | VA3-R    | CCATTGTAACCTGTAAGA         |                   |           |
| m2         | VA4-F    | GGAGCCCCAGGAACACCTG         | 352               | (38)      |
|            | VA4-R    | CATAACGTGCGCTTGC            |                   |           |
| iceA1      | iceA1-F  | GTGTATTTACCAAAATGCA         | 247               | (35)      |
|            | iceA1-R  | CTATAGCACTTTCCTTGC          |                   |           |
| iceA2      | iceA2-F  | GTTGGGTATATCCACAATTAT       | 229/334           | (35)      |
|            | iceA2-R  | TTRCCCTATTTTCTAGGT          |                   |           |

*Y is C or T, R is A or G and S is C or G.*

**Statistical analyses.** Statistical tests were performed with SPSS software version 11.5 (SPSS Inc., Chicago, IL, USA). A Chi-square test and Fisher’s exact test were used to assess the association amongst the genotypes and between specific genotypes and upper gastrointestinal diseases. P-values <0.05 were considered to indicate a statistically significant result.
Results

DNA was successfully extracted from 378 gastric mucosa tissues of patients with gastrointestinal diseases and 197 were confirmed as \textit{H. pylori} infection-positive by histology and PCR amplification. \textit{H. pylori}-infected patients were evaluated for the relationship of age and gender with disease as shown in Table II.

Detection of \textit{H. pylori} genotypes. Overall, the presence of the \textit{cagA} gene was detected in 176 cases (89.3%). A negative status for the other 21 (10.7%) cases was confirmed by Southern blotting, and the results were negative as before. All of the samples were positive for \textit{vacA} (both the s-region and the m-region). Of the 197 cases, 186 (94.4%) had \textit{vacA} signal sequence s1c allele, 6 (3%) had s1a and 5 (2.5%) had s1b. The \textit{vacA} s2 genotype was

Table II. Distribution of 197 patients with different clinical outcomes, according to age and gender.

| Classification | GU\textsuperscript{a} n=86 (%) | GS\textsuperscript{b} n=58 (%) | GC\textsuperscript{c} n=53 (%) | Total n=197 (%) |
|----------------|-------------------------------|-------------------------------|-------------------------------|-----------------|
| Age (years)    |                               |                               |                               |                 |
| 21-30          | 7 (8.1)                       | 2 (3.5)                       | 0 (0.0)                       | 9 (4.6)         |
| 31-40          | 12 (14.0)                     | 11 (19.0)                     | 4 (7.5)                       | 27 (13.7)       |
| 41-50          | 33 (38.4)                     | 17 (29.3)                     | 17 (32.1)                     | 67 (34.0)       |
| 51-60          | 24 (27.9)                     | 17 (29.3)                     | 16 (30.2)                     | 57 (29.0)       |
| >60            | 10 (11.6)                     | 11 (18.9)                     | 16 (30.2)                     | 37 (18.7)       |
| Gender     |                               |                               |                               |                 |
| Male (M)      | 52 (60.5)                     | 37 (63.8)                     | 34 (64.2)                     | 123 (62.4)      |
| Female (F)    | 34 (39.5)                     | 21 (36.2)                     | 19 (35.8)                     | 74 (37.6)       |
| M:F           | 1:0.7                         | 1:0.6                         | 1:0.6                         | 1:0.6           |

\textsuperscript{a}Gastric ulcer; \textsuperscript{b}gastritis; \textsuperscript{c}gastric cancer.

Table III. Association of \textit{vacA} with \textit{cagA} and \textit{iceA} genotypes.

| vacA   | \textit{cagA}\textsuperscript{+} n (%) | \textit{cagA}\textsuperscript{-} n (%) | \textit{iceA1} n (%) | \textit{iceA2} n (%) | \textit{iceA1/iceA2} n (%) |
|--------|-----------------------------------------|---------------------------------------|----------------------|----------------------|--------------------------|
| s-region |                                          |                                       |                      |                      |                          |
| s1a    | 4 (66.7)                                 | 2 (33.3)                              | 5 (83.3)             | 1 (16.7)             | 0 (0.0)                  |
| s1b    | 3 (60.0)                                 | 2 (40.0)                              | 5 (100.0)            | 0 (0.0)              | 0 (0.0)                  |
| s1c    | 169 (90.9)                               | 17 (9.1)                              | 128 (68.8)           | 45 (24.2)            | 13 (7.0)                 |
| m-region |                                          |                                       |                      |                      |                          |
| m1     | 18 (90.0)                                | 2 (10.0)                              | 14 (70.0)            | 6 (30.0)             | 0 (0.0)                  |
| m2     | 139 (93.0)                               | 11 (7.0)                              | 110 (73.4)           | 38 (25.3)            | 2 (1.3)                  |
| m1m2   | 19 (84.0)                                | 8 (16.0)                              | 14 (70.4)            | 2 (22.2)             | 11 (7.4)                 |
| s/m region |                                          |                                       |                      |                      |                          |
| s1am2  | 4 (66.7)                                 | 2 (33.3)                              | 5 (83.3)             | 1 (16.7)             | 0 (0.0)                  |
| s1bm2  | 3 (60.0)                                 | 2 (40.0)                              | 5 (100.0)            | 0 (0.0)              | 0 (0.0)                  |
| s1cm1  | 18 (90.0)                                | 2 (10.0)                              | 14 (70.0)            | 6 (30.0)             | 0 (0.0)                  |
| s1cm2  | 132 (95.0)                               | 7 (5.0)                               | 100 (71.9)           | 37 (26.6)            | 2 (1.5)                  |
| s1cm1m2 | 19 (70.4)                                | 8 (29.6)                              | 14 (51.9)            | 2 (7.4)              | 11 (40.7)                |

Figure 1. Distribution of \textit{cagA}, \textit{vacA} and \textit{iceA} alleles of \textit{H. pylori} from 197 patients with upper gastrointestinal diseases. M1m2, multiple \textit{vacA} genotypes with m1 and m2. IceA1/iceA2, mixed \textit{iceA} genotypes with iceA1 and iceA2.

Results

DNA was successfully extracted from 378 gastric mucosa tissues of patients with gastrointestinal diseases and 197 were confirmed as \textit{H. pylori} infection-positive by histology and PCR amplification. \textit{H. pylori}-infected patients were evaluated for the relationship of age and gender with disease as shown in Table II.

Detection of \textit{H. pylori} genotypes. Overall, the presence of the \textit{cagA} gene was detected in 176 cases (89.3%). A negative status for the other 21 (10.7%) cases was confirmed by Southern blotting, and the results were negative as before. All of the samples were positive for \textit{vacA} (both the s-region and the m-region). Of the 197 cases, 186 (94.4%) had \textit{vacA} signal sequence s1c allele, 6 (3%) had s1a and 5 (2.5%) had s1b. The \textit{vacA} s2 genotype was
not detected in our study. In the m-region, 27 cases contained both m1 and m2. In these cases the m1 allele was found in 20 (10.2%) isolates and m2 (76.1%) in 150 cases, which indicating the presence of mixed infection. The \textit{vacA}s1am2 genotype was identified in 6 (3.0%) participants, the \textit{vacA}s1bm2 was identified in 5 (2.5%) participants, \textit{s1cm}1 was identified in 20 participants, and \textit{s1cm}2 gene was identified in 139 ones. \textit{IceA}1 was found in 138 (70.1%) and \textit{iceA}2 was detected in 46 (23.4%) cases. The \textit{iceA}2 amplification yielded both the 229- and 334-bp bands due to the presence of a 105-bp in-frame amplicon present in the 334-bp band that was absent in the 229-bp band. Mixed \textit{iceA} (\textit{iceA}1 + \textit{iceA}2) genotypes were found in 13 (6.7%) of our isolates (Fig. 1).

\textbf{Association among the genotypes.} CagA was present in 124 out of 138 \textit{iceA}1 cases (91.1%) and 44 out of 46 \textit{iceA}2 cases (95.5%) (p>0.05) where 29 patients with mixed infection were excluded. Due to the lack of \textit{vacA}s2, we could not analyse the association between \textit{cagA} status and \textit{vacA} genotypes and between \textit{iceA} and \textit{vacA} genotypes (Table III).

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
\textbf{Genotype status} & \textbf{GU} a & \textbf{GS} b & \textbf{GC} c & \textbf{Total} \\
\hline
\textit{vacA}s1am2 & 4 (4.6) & 2 (3.4) & 0 (0.0) & 6 (3.0) \\
\textit{vacA}s1bm2 & 0 (0.0) & 0 (0.0) & 5 (9.4) & 5 (2.5) \\
\textit{vacA}s1cm1 & 16 (18.6) & 4 (6.9) & 0 (0.0) & 20 (10.2) \\
\textit{vacA}s1cm2 & 51 (59.4) & 43 (74.2) & 45 (84.9) & 139 (70.6) \\
\textit{vacA}s1cm1m2 & 15 (17.4) & 9 (15.5) & 3 (5.7) & 27 (13.7) \\
\hline
\textit{cagA} & & & & \\
\textit{cagA}+ & 78 (90.7) & 53 (91.4) & 45 (84.9) & 176 (89.3) \\
\textit{cagA}− & 8 (9.3) & 5 (8.6) & 8 (15.1) & 21 (10.7) \\
\hline
\textit{iceA} & & & & \\
\textit{iceA}1 & 63 (73.3) & 34 (58.6) & 41 (77.4) & 138 (70.0) \\
\textit{iceA}2 & 14 (16.3) & 21 (36.2) & 11 (20.8) & 46 (23.4) \\
\textit{iceA}1/\textit{iceA}2 & 9 (10.4) & 3 (5.2) & 1 (1.9) & 13 (6.6) \\
\hline
\end{tabular}
\caption{\textit{vacA}, \textit{cagA} and \textit{iceA} status of \textit{H. pylori} from 197 patients.}
\end{table}

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
\textbf{Combination} & \textbf{GU} a & \textbf{GS} b & \textbf{GC} c & \textbf{Total} \\
\hline
\textit{cagA}+/\textit{iceA}1 & 1 (1.5) & 2 (4.1) & 0 (0.0) & 3 (1.8) \\
\textit{cagA}−/\textit{iceA}1 & 2 (2.9) & 0 (0.0) & 0 (0.0) & 2 (1.2) \\
\textit{cagA}+/\textit{iceA}2 & 1 (1.5) & 0 (0.0) & 0 (0.0) & 1 (0.6) \\
\textit{cagA}−/\textit{iceA}2 & 0 (0.0) & 0 (0.0) & 3 (6.0) & 3 (1.8) \\
\textit{cagA}+/\textit{iceA}1 & 0 (0.0) & 0 (0.0) & 2 (4.0) & 2 (1.2) \\
\textit{cagA}+/\textit{iceA}2 & 10 (14.3) & 2 (4.1) & 0 (0.0) & 12 (7.1) \\
\textit{cagA}−/\textit{iceA}2 & 2 (2.9) & 0 (0.0) & 0 (0.0) & 2 (1.2) \\
\textit{cagA}+/\textit{iceA}1 & 4 (5.8) & 2 (4.1) & 0 (0.0) & 6 (3.6) \\
\textit{cagA}−/\textit{iceA}1 & 40 (58.0) & 23 (47.0) & 32 (64.0) & 95 (56.5) \\
\textit{cagA}+/\textit{iceA}2 & 2 (2.9) & 1 (2.0) & 2 (4.0) & 5 (3.0) \\
\textit{cagA}−/\textit{iceA}2 & 6 (8.7) & 18 (36.7) & 11 (22.0) & 35 (20.8) \\
\textit{cagA}+/\textit{iceA}1 & 1 (1.5) & 1 (2.0) & 0 (0.0) & 2 (1.2) \\
\hline
\textbf{Total} & 69 (100) & 49 (100) & 50 (100) & 168 (100) \\
\hline
\end{tabular}
\caption{Combined \textit{vacA}, \textit{cagA}, \textit{iceA} genotypes.}
\end{table}

\textsuperscript{a}Gastric ulcer; \textsuperscript{b}gastritis; \textsuperscript{c}gastric cancer. Twenty-nine patients with mixed infection were excluded.
Relationship between genotypes and gastric diseases. Of the 197 strains studied, 86 were diagnosed with gastric ulcer, 58 with gastritis and 53 with gastric cancer. VacA s1c m2 was detected in all the disease conditions, and it was more significantly associated with the presence of gastric cancer (p<0.05). S1a m2 and s1c m1 were detected in all the disease except gastric cancer, while s1b m2 was found in gastric cancer alone (Table IV). Surprisingly, iceA1 had a statistically significant association with gastric cancer (p<0.05). Neither cagA nor iceA2 was associated with various diseases. The most prevalent combination cagA/s1c m2/iceA1 was present in 56.6% (95 of 168) including 58.0% (40 of 69) of gastric ulcer, 47.0% (23 of 49) of gastritis and 64.0% (32 of 50) of gastric cancer (Table V). However, no significant association was found between the combination genotypes and diseases (p>0.05).

Discussion

This study was designed to characterize the genotype of H. pylori from gastric biopsy specimens from patients with upper gastrointestinal diseases and the relationship with clinical outcome in Northeast China. H. pylori was analysed for the presence of the genes for cagA, vacA and iceA. To our knowledge, this was the first study to analyse the different proposed virulence genes characterized in H. pylori and the relationship between the genes and upper gastrointestinal diseases in Northeast China.

CagA gene, as a major H. pylori virulence factor, was reported to be strongly associated with atrophic gastritis and gastric cancer as previously described. This is probably the main cause of a high incidence of gastric cancer in the region of East Asia, where the percentage of cagA-positive strains is above 90% (12). Worldwide, the presence of the cagA gene varies from 50% in some Middle Eastern countries to 99% in East Asian countries (13-15). In this study, cagA was found in 89.3% of H. pylori-infected patients. The result is similar to data reported from other districts of China (11,16). However, we did not find an association between cagA and clinical results. Notably, of the 29 mixed infection cases, 8 had cagA-negative and strains with an absence of cagA appeared to be associated with mixed infection (p=0.004).

The present study demonstrated that all strains of H. pylori carried the vacA s1 allele. Previous studies noted that s1c was present exclusively in isolates from East Asia (16-18). Our report also demonstrated a high prevalence of type s1c strains in this region, up to 94.4%. The result was similar to the report of Wang et al (19) and slightly higher than the prevalence in Beijing and Shanghai, which may result from the fact that more foreigners from America and Europe live in the two cities above, as either the sla or sib subtype was present in almost all strains in Central and South America, and in the majority of strains in Spain and Portugal (20,21), nevertheless rarely in East Asia (12,16). The vacA s2 genotype was prominently prevalent in Africa (9), and consistent to the outcome reported from China and Korea, s2 failed to be detected in this study (19,22).

Worldwide prevalence of vacA strains varies geographically. S1m1 strains were predominant in Japan and Korea (18,23) while s1m2 was found in Turkey and Northern and Eastern Europe (20,24). In Alaskans, H. pylori had either the vacA s1m1 (44.6%) or s2m2 (38.3%) (15). In China, prevalence of strains documented a greatly distinct pattern, with s1m1 and s1m2 sharing the same proportion in the Province of Xi'an (25) and s1m2 strains in Beijing, Taiwan and Hong Kong (16,19,26). The latter condition was similar to our study.

Generally, s1m2 forms of vacA bind to and vacuolate a narrower range of cells than s1m1 forms and induce less damage, yet they also act as efficient membrane pores and increase paracellular (27) permeability. The alleles of s1m1 and s1m2 encode to produce toxin which are common in patients with gastritis (27). In Latin America and Germany, s1m1 was found to have a high correlation with gastric ulcer and gastric carcinoma (21,28). The strains of vacA s2m1 and s2m2 engender low toxic toxin which rarely correlates with gastric ulcer and gastric carcinoma (29). In our study, the vacA gene encoding the s1c m2 was associated with gastric cancer. Therefore, the s-region should be responsible for gastrosis other than the m-region.

Another virulent factor is the iceA gene, with two allelic variants iceA1 and iceA2 having been identified. The prevalence of the iceA1 genotype is 70.1% in this study, basically consistent with data reported from China, Thailand, Korea and Tunisia (9,23,30,31). Meanwhile, iceA2 is predominant in Brazil, the US, Europe and South Africa (6,18,32,33). It was demonstrated that iceA1 was significantly associated with peptic ulcer disease in Holland (34) and the US (35). However, studies from other countries such as in Korea, Colombia and India could not confirm the result (18,36). Some researchers found that the iceA2 genotype was most frequently found in patients with duodenal ulcer or gastric carcinoma (18,36). However, it is difficult to admit that iceA2, a gene that is considered as a protective factor in some regions and that is associated with more severe diseases in other places, could be considered a molecular marker of more virulent H. pylori strains (33). It was well worth mentioning that the iceA1 strains, based on this study, have a significant association with gastric cancer.

In common with other studies, there exists a strong indication that the presence of multiple H. pylori strains are detectable in clinical samples. Some studies have claimed multiple genotypes have a link with duodenal ulcers (9). It may be speculated that multiple strains contribute to increasing the potential chances of infecting pathogen. By colonizing a variety of receptors expressed on gastric epithelial cells, m1 and m2 strains probably tend to bring about pathological changes. Multi-colonization arising from the co-existence of more than one strain exert burden to patients under eradication treatment and furthermore dramatically enhance the risk of malignant tumors of the digestive tract among adult patients. However, our data did not indicate that multiple strain infection increases the risk of developing diseases (p>0.05).

In conclusion, the present study identified the prevalence of main virulence factor genes cagA, s1c m2 and iceA1 in Northeast China. The vacA gene encoding s1c m2 was found to predominate in gastric cancer patients, and the iceA1 genotype was also associated with gastric cancer. It may be insufficient to analyse gastrointestinal diseases simply by genotyping H. pylori, and therefore, we must evaluate the pathogenesis of diseases by a combination of the analysis of bacterial factors, genetic factors of the host and environmental factors.
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