H. pylori isolates with amino acid sequence polymorphisms as presence of both HtrA-L171 & CagL-Y58/E59 increase the risk of gastric cancer

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Abstract

Background: H. pylori CagL-Y58/E59 increase gastric cancer risk by stronger binding with integrin to facilitate type IV secretory system (T4SS). H. pylori can secrete high temperature requirement A (HtrA) to mediate E-Cadherin cleavage for gastric epithelial junction disruption, so H. pylori CagL can adhere to integrin located on basolateral side of epithelium. The study test whether H. pylori HtrA amino acid polymorphisms can increase gastric cancer risk synergistically with CagL-Y58/E59.

Methods: One-hundred and sixty-four H. pylori-positive patients, including 71 with non-ulcer dyspepsia (NUD), 63 with peptic ulcers (PU), and 30 with gastric cancers (GC), were enrolled to receive upper gastrointestinal endoscopy to obtain gastric biopsies for H. pylori culture and histology by the updated Sydney system. Each isolate was screened for htrA & cagL genotype by polymerase chain reaction and HtrA & CagL-Y58/E59 amino acid sequence polymorphisms by sequencing.

Results: The prevalence rates of htrA & cagL gene were both 100%. The HtrA amino acid sequence polymorphisms were not different between NUD and PU. The H. pylori isolates of GC had higher rates of HtrA residue 171 as leucine than those of NUD (73.3% vs. 50.7%, P = 0.036, OR[95%CI] = 2.7[1.1–6.8]). The risk of the H. pylori-infected subjects to get gastric cancer was increased up to 15.4-fold, if the infected isolates had presence of both HtrA-L171 and CagL-Y58/E59 (P < 0.001).

Conclusions: The H. pylori isolates of gastric cancer subjects had a higher rate of HtrA-L171. H. pylori isolates with presence of both HtrA-171 & CagL-Y58/E59 can synergistically increase the risk of gastric cancer.

Keywords: Gastric cancer, H. pylori, HtrA, CagL, Type IV secretory system

Introduction

Helicobacter pylori infection leads to chronic gastritis, peptic ulcers and gastric adenocarcinoma [1, 2]. H. pylori isolates can display extensive diversities in polymorphisms of virulence factor genes, which may determine an increased risk to have gastrointestinal disorders [3–5]. To search the virulence factors of the infected H. pylori isolates can thus identify the risky groups for earlier treatment to control the adverse outcome, particularly gastric cancer.

Triple positive cagA-vacA-babA2 H. pylori infection increases the risks of peptic ulcer and gastric cancer in the Western countries [6]. However, in Taiwan, the incidence of triple positive infection is near 100%, so such genomic polymorphisms are not related to the different clinical outcomes [7–10]. CagL, as a component of type VI secretion system (T4SS), binds to integrins α5β1 at
the basolateral side of host gastric epitheliums to facilitate CagA translocation for carcinogenesis [11]. The *H. pylori* isolates with CagL amino acid polymorphisms as Y58/E59 exploit higher integrin α5β1 to carry 4.6-fold risk increase of gastric cancer [4]. Studies analyzed Asian and non-Asian subject cohorts were consistent with our finding to show that CagL-E59 was associated with gastric cancer [12–14]. Moreover, we previously reported *H. pylori* CagL-Y58/E59 can prime higher integrin α5β1 in adverse pH condition to enhance hypochlorhydria vicious cycle for gastric carcinogenesis [15]. However, some results contrasted with those finding in 26,695 and P12 strains [16, 17]. In addition, it is still nearly 50% of gastric cancer *H. pylori* isolates without CagL-Y58/E59, and additional virulence factors to increase risk of gastric cancers are in need of further validation.

High-temperature requirement A (HtrA) protein is a chaperone and serine protease. *H. pylori* HtrA consists of signal domain, serine protease domain, PDZ-1 and PDZ-2 domain [18, 19]. The secreted HtrA of *H. pylori* opened tight junctions and adherence junctions via cleaving occludin, claudin-8, and the extracellular domain of E-cadherin, and consequently *H. pylori* can cross epithelial monolayer to the basolateral membranes to interact with integrin α5β1 for T4SS injection of CagA [18, 20]. The residue S221 has been shown as the active site [18, 21]. Moreover, a second hot-spot site is around Q81 according to iPred interface prediction and is confirmed by catalytic activity of Q81A mutation. In addition, some charged residues at the HtrA surface, such as D165, D168 and D260, also display important roles for HtrA activity [22]. Additionally, S164, S166, N208 and K328 are the presumed binding sites of HtrA inhibitor, and their mutations lose proteolytic activity against E-cadherin [19].

The *H. pylori* isolates from worldwide are highly variable in nucleotide sequence of htrA gene, and had strain-specific difference in the HtrA cleavage E-cadherin [23]. This study thus validated whether htrA genopositivity or any specific polymorphisms of HtrA amino acid sequence can determine the risk with clinical outcomes after *H. pylori* infection, especially in non CagL-Y58/E59 status. In addition, we validated whether any specific HtrA amino acid sequences synergistically increase the risk of gastric cancer with CagL-Y58/E59. Our data shall be original to suggest HtrA amino acid polymorphisms of *H. pylori* isolates as virulence factor of gastric cancer. The screening strategy for specific HtrA sequences of *H. pylori* isolates will be promising to select risky group for early *H. pylori* eradication to improve gastric cancer control.

**Materials & methods**

**Patients and collection of *H. pylori* isolates**

*H. pylori* strains were obtained from the gastric biopsy of *H. pylori*-infected patients who underwent upper gastrointestinal endoscopy at National Cheng Kung University Medical Center, Tainan, Taiwan. We proposed the rate of HtrA amino acid polymorphisms in non-cancer strains was 50%. The number of *H. pylori* strains from patients with gastric cancers, non-ulcer dyspepsia, and peptic ulcers was at a 1:2:2 ratio. The total number of strains required was 123 to detect a 30% difference in the polymorphism rates between cancer strains and non-cancer strains with a two-sided α value of 0.05 and a power of 80% (β = 0.20). Assuming a screening failure rate of 20%, 148 strains at least were needed. A total of 164 isolates were obtained from 71 non-ulcer dysplasia (NUD), 63 peptic ulcer (PU), and 30 gastric adenocarcinoma (GC) patients.

In each patient, the endoscopic diagnosis and topographic gastric biopsy for *H. pylori* related pathology were reviewed. Biopsies were stained with haematoxylin and eosin, as well as with modified Giemsa stains, to evaluate the *H. pylori*-related histological features and to grade severity according to the updated Sydney system [24]. The acute inflammatory score (AIS, range 0–3), chronic inflammation score (CIS, range 0–3), the *H. pylori* density (HPD) for each specimen was scored as our previous studies: in range of 0–5 for each biopsy specimen.

None of the cases have used with antibiotics or proton pump inhibitor before endoscopy. Each patient has provided blood sampling before endoscopy to obtain serum for pepsinogen and gastrin assay.

**htrA & cagL-gnosequencing for htrA & cagL gene**

Genomic DNA of *H. pylori* was extracted by using the Genomic DNA Purification Kit (ThermoFisher Scientific). The extracted DNA of each isolate was subjected to PCR to amplify the htrA genes using paired primers: htrA_F (5′- GCA TCG GGA TGA TTT TAA CG-3′) and htrA_R (5′-AAA CAA CGC TCG TTT GTT TG-3′) (Genomics, Taiwan). The PCR mixtures were made in a volume of 50 μl containing 200 ng of DNA, 0.2 mM of primers and 25 μl of GoTaq ® Green Master Mix (Promega, Madison, WI). The PCR reaction was performed with a thermal cycler (2720 thermal cycler, Applied Biosystems, Foster City, CA) under 94 °C for 5 min, 30 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min, and then followed by a final elongation step at 72 °C for 10 min. The mixture was stored at 4 °C. The PCR products were separated by 2% agarose gel electrophoresis and examined under UV illumination [4, 25]. The genotype of cagL was applied as the method as used in our previous literature [4].

**htrA & cagL-gnosequencing for translating into amino acid sequences**

The amplified products of the htrA were then subjected to the direct sequencing (Genomics, Taiwan). The nucleotide sequence identities were compared with sequences
Serum pepsinogen and gastrin by enzyme-linked immuno-sorbent assay (ELISA)

Blood samples were collected and centrifuged at 3000 rpm for 15 min at 4°C. Sera were obtained and frozen immediately at -80°C until analysis. These collected sera were then measured for the levels of pepsinogen I and II (Biohit Oyj, Helsinki, Finland), and gastrin using ELISA kit (BlueGene Biotech, Shanghai, China) according to the manufacturer's specifications [15, 26].

Statistics

The statistical analysis was performed with the SPSS software (SPSS 12, Chicago, IL, USA). The χ² test was used to validate the correlation among gender, htrA prevalence rates, and amino acid sequence polymorphisms. The t test was used to validate age and serum levels of pepsinogen I, pepsinogen II and gastrin. The Mann-Whitney U test was applied to analyze the differences in histological severity. P value < 0.05 was considered significant with two-tailed analysis.

Results

The association of HtrA amino acid polymorphism with the clinical diagnosis

Of the 164 H. pylori-positive dyspeptic patients, endoscopic diagnoses included 71 with NUD, 63 with PU, and 30 with GC. The enrolled subjects with GC had higher mean age than those with NUD or PU (59.2 ± 13.2 vs. 47.3 ± 12.3 vs. 49.5 ± 13.2, respectively, P < 0.001). Males had higher rates of PU than females as compared to NUD subjects (61.9% vs. 31%, P < 0.001).

The prevalence of htrA gene of 164 collected isolates was 100%. The HtrA amino acid sequence of our isolates had more than 95% homology. Based on these predicted amino acid sequences, there were 7 residues of HtrA with more than 10% variation in these isolates, including residue 6F/L, 25 N/S, 68S/N, 171 L/S, 303 V/I, 312A/V, and 382 T/A or V (Table 1).

Among these 7 variant residues, 6F/L was located at signal domain, 171 L/S was located at protease domain, as well as 303 V/I and 312A/V were located at PDZ-1 domain (Fig. 1). We analyzed the correlation between HtrA amino acid polymorphisms and clinical diagnosis showed in Table 1. The active sits S211 and functional regulation residues at Q81, S164, D165, S166, D168, N208, D260 and K328 were all conserved among all isolates (Fig. 1).

There were no difference of HtrA amino acid sequence polymorphisms between NUD and PU subjects (P > 0.05). However, isolates from GC had significantly higher rates of amino acid sequence 171 as leucine than the isolates from the NUD subjects (P = 0.036, OR[95% CI] = 2.7[1.1–6.8]) (Table 1). There were no difference in the present rate of F6, N25, S68, V303, A312, and T382 between NUD and GC subjects (P > 0.05).

Combined HtrA-L171 and CagL-Y58/E59 between gastric cancer and NUD subjects

Our previous study showed about 50% gastric cancer isolates with CagL-Y58/E59 sequence, and subjects infected with H. pylori with this sequence had increased risk of gastric cancer [4]. However, there were nearly no difference of HtrA-L171 and CagL-Y58/E59 between NUD and PU subjects (P > 0.05). However, isolates from GC had significantly higher rate of HtrA-L171 as leucine than the isolates from the NUD subjects (P = 0.014, OR[95% CI] = 2.5[1.1–6.1]) (Table 1).

Table 1 The polymorphisms of HtrA amino acid sequence among isolate from patients with different clinical diagnosis

| Residue n (%) | NUD (n = 71) | PU (n = 63) | GC (n = 30) | P value " OR (95% CI) | P value " OR (95% CI) |
|---------------|--------------|-------------|-------------|-----------------------|-----------------------|
| 6 F (56.3)    | 40 (61.9)    | 19 (63.3)   | 0.513       | 0.13 (0.6–2.5)        | 0.515 (0.6–3.2)       |
| 25 N (28.2)   | 20 (25.4)    | 11 (36.7)   | 0.718       | 0.9 (0.4–1.9)         | 0.397 (0.6–3.6)       |
| 68 S (15.5)   | 11 (14.3)    | 8 (26.7)    | 0.845       | 0.9 (0.4–2.4)         | 0.189 (0.7–5.6)       |
| 171 L (50.7)  | 36 (60.3)    | 22 (73.3)   | 0.264       | 1.5 (0.7–2.9)         | 0.036 (1.1–6.8)       |
| 303 V (73.2)  | 52 (76.2)    | 24 (80.0)   | 0.695       | 1.2 (0.5–2.6)         | 0.472 (0.5–4.1)       |
| 312 A (62.0)  | 44 (61.9)    | 19 (63.3)   | 0.994       | 1.0 (0.5–2.0)         | 0.897 (0.4–2.6)       |
| 382 T (22.5)  | 16 (22.5)    | 9 (30.0)    | 0.465       | 0.7 (0.3–1.7)         | 0.427 (0.6–3.8)       |

Abbreviations: NUD non-ulcer dyspepsia, PU peptic ulcer, GC gastric cancer, OR odds ratio, 95% CI 95% confidence interval. The P value was determined by χ² test. * indicated significance with P < 0.05 of such parameter between NUD and PU; * indicated significance with P > 0.05 of such parameter between NUD and GC.
50% gastric cancer isolates without bearing CagL-Y58/E59. We thus further examined whether \textit{H. pylori} infection carrying with HtrA-L171 correlates the risk of gastric cancer if subjects infected with a CagL-Y58/E59 absent \textit{H. pylori}.

In Table 2 showed as CagL-Y58/E59 absence, the prevalence of HtrA-L171 was 86.7% in GC isolates, and significantly higher than in NUD isolates (50.9% \(P = 0.013; \text{OR}[95\%\text{CI}] = 6.3[1.3–30.4]\)). We further analyzed whether combined HtrA-L171 and CagL-Y58/E59 have synergistic effect on the risk of gastric cancer development. In Table 2, the odds ratio was higher for those infected by \textit{H. pylori} bearing combined HtrA-L171 and CagL-Y58/E59, and the risk could be even higher than as \textit{H. pylori} with HtrA-L171 alone or CagL-Y58/E59 alone.
Table 2 The comparison of the GC risk as H. pylori bearing with different HtrA-L171 and CagL-Y58/E59 status

| HtrA-L171 | CagL-Y58/E59 | NUD n (%) | GC n (%) | P value | OR (95% CI) |
|-----------|-------------|-----------|----------|---------|-------------|
| -         | -           | 27 (49.1) | 2 (13.3) | 0.013   | 6.3 (1.3–30.4) |
| -         | +           | 28 (50.9) | 13 (86.7) |         |             |
| -         | -           | 27 (81.8) | 2 (25.0) | 0.002   | 13.5 (2.2–84.0) |
| +         | -           | 6 (18.2)  | 6 (75.0) |         |             |
| -         | -           | 27 (79.4) | 2 (20.0) | <0.001  | 15.4 (2.7–89.5) |
| +         | +           | 7 (20.6)  | 8 (80.0) |         |             |

Abbreviations: NUD non-ulcer dyspepsia, GC gastric cancer, OR odds ratio, 95% CI 95% confidence interval. The P value was determined by χ² test

Table 3 The comparison of histopathology among NUD subjects with different status of HtrA-L171 & CagL-Y58/E59 of H. pylori infection

| Histology score, mean (SD) | HtrA-L171 & CagL-Y58/E59 status | Both absence (n = 27) | HtrA-L171 L alone (n = 28) | CagL-Y58/E59 alone (n = 6) | Both presence (n = 7) | P value |
|---------------------------|----------------------------------|-----------------------|-----------------------------|-----------------------------|-----------------------|---------|
| Antrum                    |                                  |                       |                             |                             |                       |         |
| AIS                       | 1.44 (0.97)                      | 1.64 (0.78)           | 1.33 (1.03)                 | 1.29 (0.95)                 | NS                    |         |
| CIS                       | 2.74 (0.53)                      | 2.96 (0.19)           | 2.67 (0.82)                 | 2.71 (0.49)                 | 0.039<sup>a</sup>, 0.037<sup>b</sup> |         |
| HPD                       | 3.26 (1.26)                      | 3.36 (1.19)           | 3.17 (1.72)                 | 3.86 (1.07)                 | NS                    |         |
| Corpus                    |                                  |                       |                             |                             |                       |         |
| AIS                       | 1.00 (0.96)                      | 0.93 (0.98)           | 1.17 (0.98)                 | 0.57 (0.98)                 | NS                    |         |
| CIS                       | 2.37 (0.79)                      | 2.32 (0.82)           | 2.83 (0.41)                 | 2.14 (0.07)                 | NS                    |         |
| HPD                       | 3.41 (1.37)                      | 3.46 (1.17)           | 3.67 (1.03)                 | 2.43 (1.62)                 | NS                    |         |

Abbreviations: AIS acute inflammation score (range 0–3), CIS chronic inflammation score (range 1–3); HPD, H. pylori density (range 0–5). Statistical analysis was performed by Mann-Whitney U test. <sup>a</sup> indicated significance with P < 0.05 of such parameter between both absence and HtrA-L171 alone H. pylori infection; <sup>b</sup> between HtrA-L171 alone and both presence. NS: no significant difference
CagL-Y58/E59 [4]. We thus further validate whether HtrA amino acid sequence can be helpful to identify the gastric cancer risk, especially when the H. pylori isolates lack CagL-Y58/E59. In this study, we disclosed H. pylori carrying with HtrA-L171 indeed exhibited an increased GC risk as CagL-Y58/E59 absence (Table 2). It reveals that HtrA-L171 has potential as a marker of GC development.

Residue 171 is located at the protease domain of HtrA. Base on the report by Perna and colleagues showing that HtrA mutant S164A, S166A, N208A, and K328A lost their ability to cleave E-cadherin. This observation supports that these residues played relevant roles for the functional regulation of HtrA [19]. L171 is also closed to S164 and S166 (according to NCBI database, S164 and S166 should be S165 and S167 respectively, Fig. 1). Accordingly, it deserves future study to test whether L171 can be a relevant site for the HtrA regulation or whether amino acid change at 171 alters the function of L171 can be a relevant site for the HtrA regulation or whether amino acid change at 171 alters the function of HtrA-L171 and stronger integrin a5β1 activates the interaction of CagL and integrin a5β1 at basolateral membrane and then to inject effector protein CagA into host epithelium cells [18, 21]. The study thus checked whether combined HtrA-L171 and CagL-Y58/E59 have synergistic risk on GC. In Table 2, the risk of GC as H. pylori carrying with combined HtrA-L171 and CagL-Y58/E59 was higher than as those isolates carrying with HtrA-L171 only or with CagL-Y58/E59 only. The combined effect from stronger E-cadherin cleavage by HtrA-171 and stronger integrin expression for T4SS by CagL-Y58/E59 may explain the risk increment in part.

We previously elaborated the CagL-Y58/E59 infection with corpus shift of integrin a5β1 can cause more severe gastric corpus-predominant injury [4]. Corpus-predominant chronic inflammation reduced acid secretion by parietal cell loss, and facilitates carcinogenic progression [27]. H. pylori with CagL-Y58/E59 can prime more integrin a5β1 to translocate CagA under hypochlorhydria for gastric carcinogenesis [15]. Unlike HtrA-L171 & CagL-Y58/E59 status

| Parameters, mean (SD) | HtrA-L171 & CagL-Y58/E59 status | Both absence (n = 11) | HtrA-L171 L alone (n = 7) | CagL-Y58/E59 alone (n = 6) | Both presence (n = 5) | P value |
|-----------------------|----------------------------------|----------------------|--------------------------|--------------------------|---------------------|---------|
| Gastrin (pg/ml)       | 340 (26.9)                       | 96.6 (36.8)          | 60.8 (48.0)              | 54.6 (20.7)              | 0.003*              |
| PG I (ng/ml)          | 105.3 (59.2)                     | 112.3 (27.7)         | 116.8 (29.0)             | 99.6 (26.1)              | NS                  |
| PG II (ng/ml)         | 12.2 (6.1)                       | 11.5 (5.0)           | 17.4 (8.6)               | 11.7 (3.9)               | NS                  |
| PG I/II               | 9.0 (3.2)                        | 10.6 (3.2)           | 7.4 (1.8)                | 9.4 (4.2)                | NS                  |

Abbreviations: PG pepsinogen. Statistical analysis was performed by t test. * indicated significance with P < 0.05 of such parameter between both absence of HtrA-L171 & CagL-Y58/E59 and HtrA-L171 only. H. pylori infection. NS: no significant difference.
The authors declare that they have no competing interests.

All the individuals had signed consent forms for participating in this research. Ethical approval and consent to participate was obtained from the Ethics Committee of Cheng Kung University Hospital, Tainan, Taiwan. All data and materials are available.

Authors’ contributions
YCY conducted the HtrA analysis and draft composition. HCC and HYK conducted the HtrA analysis and draft composition. HCC, HYK, and WLC contributed to the study design and progress with all the individuals. MSW and BSS coordinated the study design and progress with all the individuals. We thanks Ms. Hui-Wen Wu and Ching-Chun Chuang for the clinical assistance to collect samples.

Availability of data and materials
All data and materials are available.

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Ethics approval and consent to participate
This study was reviewed by the Ethics Review Board (IRB) of National Cheng Kung University Hospital, Tainan, Taiwan.

Consent for publication
All the individuals had signed consent forms for participating in this research project and use the obtained data in relevant publications.

Competing interests
The authors declare that they have no competing interests.

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Abbreviations
AIS: Acute inflammatory score; CIS: Chronic inflammation score; ELISA: Enzyme-linked immuno-sorbent assay; GC: Gastric cancers; H. pylori: Helicobacter pylori; HPD: H. pylori density; HtrA: High temperature requirement A; NUD: Non-ulcer dyspepsia; PCR: Polymerase chain reaction; PU: Peptic ulcers; TPS5: Type IV secretory system

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Additional files

Additional file 1: Accession numbers of htrA gene analyzed in this study. (DOCX 37 kb)

Additional file 2: Accession numbers of cagL gene analyzed in this study. (DOCX 28 kb)

References
1. Labigne A, de Reuse H. Determinants of helicobacter pylori pathogenicity. Infect Agents Dis. 1996;5(4):191–202.
2. Makola D, Peura DA, Crowe SE. Helicobacter pylori infection and related gastrointestinal diseases. J Clin Gastroenterol. 2007;41(6):S48–58.
3. Sheu BS, Yang HB, Yeh YC, Wu JJ. Helicobacter pylori colonization of the human gastric epithelium: a bug’s first step is a novel target for us. J Gastroenterol Hepatol. 2010;25(1):26–32.
4. Yeh YC, Chang WL, Yang HB, Cheng HC, Wu JJ, Sheu BS. H. pylori cagA amino acid sequence polymorphism Y58E59 induces a corpus shift of gastric integrin alpha5beta1 related with gastric carcinogenesis. Mol Carcinog. 2011;50(10):751–9.
5. Cover TL. Helicobacter pylori diversity and gastric cancer risk. Mbio. 2016;7(1):e01869–15.
6. Prinz C, Schoniger M, Rad R, Becker L, Keiditsch E, Wagenpfel S, Classen M, Rosch T, Schepp W, Gerhard M. Key importance of the helicobacter pylori adherence factor blood group antigen binding adhesin during chronic gastric inflammation. Cancer Res. 2001;61(3):1903–9.
7. Lai CH, Kuo CH, Chen YC, Chao FY, Poon SK, Chang CS, Wang WC. High prevalence of cagA- and babA2-positive helicobacter pylori clinical isolates in Taiwan. J Clin Microbiol. 2002;40(10):3860–2.
8. Chen TS, Chang FY, Lee SD. Smoking and male gender rather than CagA protein are associated with increased risk for duodenal ulcer in helicobacter pylori-infected patients in Taiwan. Dig Dis Sci. 1999;44(10):2076–80.
9. Sheu BS, Sheu SM, Yang HB, Huang AH, Wu JJ. Host gastric Lewis expression determines the bacterial density of helicobacter pylori in babA2 genopositive infection. Gut. 2003;52(7):927–32.
10. Sheu SM, Sheu BS, Yang HB, Li C, Chu TC, Wu JJ. Presence of iceA1 but not cagA, cagC, cagE, cagF, cagN, cagT, or orf13 genes of helicobacter pylori is associated with more severe gastric inflammation in Taiwanese. J Formos Med Assoc. 2002;101(1):18–23.
11. Kwok T, Zabler D, Urman S, Rohde M, Hartig R, Wessler S, Misselwitz R, Berger J, Sewald N, Konig W, et al. Helicobacter exploits integrin for type IV secretion and kinase activation. Nature. 2007;449(7164):862–6.
12. Ogawa H, Iwamoto A, Tanahashi T, Okada R, Yamamoto K, Nishiumi S, Yoshida M, Azuma T. Genetic variants of helicobacter pylori type IV secretion system components CagL and CagJ and their association with clinical outcomes. Gut Pathog. 2017;9:21.
13. Gorrell RJ, Zwickel N, Reynolds J, Bulach D, Kwok T. Helicobacter pylori CagL hypervariable motif: a global analysis of geographical diversity and association with gastric cancer. J Infect Dis. 2016;213(12):1927–31.
14. Cherati MR, Shokri-Shinvan J, Karkhah A, Rajabnia R, Nouri HR. Helicobacter pylori cagA amino acid polymorphism D58E59 pave the way toward peptic ulcer disease while NS58E59 is associated with gastric cancer in north of Iran. Microb Pathog. 2017;107:413–8.
15. Yeh YC, Cheng HC, Yang HB, Chang WL, Sheu BS, H. pylori CagL-Y58/E59 prime higher integrin alpha5beta1 in adverse pH condition to enhance hypochlorhydria vicious cycle for gastric carcinogenesis. PLoS One. 2013;8(8):e72735.
16. Tafreshi M, Zwickel N, Gorrell RJ, Kwok T. Preservation of helicobacter pylori CagA translocation and host cell proinflammatory responses in the face of CagL Hypervariability at amino acid residues 58/59. PLoS One. 2015;10(7):e0133531.
17. Tegtmeyer N, Lind J, Schmid B, Backert S. Helicobacter pylori CagL Y58/E59 mutation turns-off type IV secretion-dependent delivery of CagA into host cells. PLoS One. 2014;9(6):e97782.
18. Hoy B, Lower M, Weydig C, Carra G, Tegtmeyer N, Geppert T, Schroder P, Sevald N, Backert S, Schneider G, et al. Helicobacter pylori HtrA is a new secreted virulence factor that cleaves E-cadherin to disrupt intercellular adhesion. EMBO Rep. 2010;11(10):798–804.
19. Perma AM, Reisen F, Schmidt TP, Geppert T, Pillogn M, Weisell M, Hoy B, Simister PC, Feller SM, Wessler S, et al. Inhibiting helicobacter pylori HtrA protease by addressing a computationally predicted allosteric ligand binding site. Chem Sci. 2014;5:3583–90.
20. Tegtmeyer N, Wessler S, Necchi T, Rohde M, Harer A, Rau TT, Asche CJ, Boehm M, Loesener H, Figueiredo C, et al. Helicobacter pylori employs a new basolateral type IV secretion mechanism for CagA delivery. Cell Host Microbe. 2017;22(4):552–60 e555.
21. Lower M, Weydig C, Metzler D, Reuter A, Starzincki-Powitz A, Wessler S, Schneider G. Prediction of extracellular proteases of the human pathogen helicobacter pylori reveals proteolytic activity of the Hp1018/19 protein HtrA. PLoS One. 2008;3(10):e3510.
22. Geppert T, Hoy B, Wessler S, Schneider G. Context-based identification of protein-protein interfaces and “hot-spot” residues. Chem Biol. 2011;18(3):344–53.
23. Tegtmeyer N, Moodley Y, Yamaoka Y, Pernitzsch SR, Schmidt V, Traverso FR, Schmidt TP, Rad R, Yeoh KI, Bow H, et al. Characterisation of worldwide
helicobacter pylori strains reveals genetic conservation and essentiality of serine protease HtrA. Mol Microbiol. 2016;99(5):925–44.

24. Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney system. International workshop on the histopathology of gastritis, Houston 1994. Am J Surg Pathol. 1996;20(10):1161–81.

25. Yeh YC, Cheng HC, Chang WL, Yang HB, Sheu BS. Matrix metalloproteinase-3 promoter polymorphisms but not dupA-H. pylori correlate to duodenal ulcers in H. pylori-infected females. BMC Microbiol. 2010;10:218.

26. Cheng HC, Tsai YC, Yang HB, Yeh YC, Chang WL, Kuo HY, Lu CC, Sheu BS. The corpus-predominant gastritis index can be an early and reversible marker to identify the gastric cancer risk of helicobacter pylori-infected nonulcer dyspepsia. Helicobacter. 2017;22(4):e12385.

27. Rieder G, Merchant JL, Haas R. Helicobacter pylori cag-type IV secretion system facilitates corpus colonization to induce precancerous conditions in Mongolian gerbils. Gastroenterology. 2005;128(5):1229–42.

28. Smith JP, Nadella S, Osborne N. Gastrin and gastric cancer. Cell Mol Gastroenterol Hepatol. 2017;4(1):75–83.

29. Lamberti R, Creutzfeldt W, Struber HG, Brunner G, Solcia E. Long-term omeprazole therapy in peptic ulcer disease: gastrin, endocrine cell growth, and gastritis. Gastroenterology. 1993;104(5):1356–70.

30. Chuang CH, Sheu BS, Yang HB, Kao AW, Cheng HC, Yao WJ. Hypergastrinemia after helicobacter pylori infection is associated with bacterial load and related inflammation of the oxyntic corpus mucosa. J Gastroenterol Hepatol. 2004;19(9):988–93.

31. Wiedemann T, Hofbaur S, Tegtmeyer N, Huber S, Sewald N, Wessler S, Backert S, Rieder G. Helicobacter pylori CagL dependent induction of gastrin expression via a novel alphavbeta5-integrin-integrin linked kinase signalling complex. Gut. 2012;61(7):986–96.

32. Inge LJ, Barwe SP, D’Ambrosio J, Gopal J, Lu K, Ryazantsev S, Rajasekaran SA, Rajasekaran AK. Soluble E-cadherin promotes cell survival by activating epidermal growth factor receptor. Exp Cell Res. 2011;317(6):838–48.

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