

# Association of CagA EPIYA-D or EPIYA-C phosphorylation sites with peptic ulcer and gastric cancer risks

## A meta-analysis

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### Abstract

**Background:** Increasingly, studies have focused on the relationship between *Helicobacter pylori* (*H pylori*) cytotoxin associated gene A protein (CagA) Glu-Pro-Ile-Tyr-Ala (EPIYA)-D motifs or multiple EPIYA-C phosphorylation sites and peptic ulcer disease (PUD) or gastric cancer (GC) risk. However, the conclusions have been inconsistent. The aim of this meta-analysis was to evaluate whether 1 CagA EPIYA-D motif or multiple EPIYA-C phosphorylation sites were associated with PUD or GC risk.

**Materials and methods:** A literature search was performed in PubMed, Web of Science, Wanfang Data, Excerpt Medica Database, and the Chinese National Knowledge Infrastructure database to identify eligible research. We analyzed the odds ratios (OR) and 95% confidence intervals (CI) to assess the strength of association.

**Results:** Compared with 1 EPIYA-C motif in Asian populations, 1 EPIYA-D site was associated with an increased GC risk (OR=1.91, 95% CI=1.19–3.07,  $P=.008$ ). However, 1 EPIYA-D motif was not significantly associated with PUD (OR=0.90, 95% CI=0.46–1.76,  $P=.764$ ), gastric ulcer (GU) (OR=0.85, 95% CI=0.27–2.63,  $P=.771$ ), or duodenal ulcer (DU) (OR=0.89, 95% CI=0.25–3.16,  $P=.859$ ) risk. Compared with no more than 1 EPIYA-C motif, multiple motifs were associated with increased PUD (OR=2.33, 95% CI=1.29–4.20,  $P=.005$ ) and DU (OR=2.32, 95% CI=1.08–5.00,  $P=.031$ ) risk in Asia and GC risk in the United States and Europe (OR=3.28, 95% CI=2.32–4.64,  $P<.001$ ). Multiple EPIYA-C sites were not associated with GU risk (OR=4.54, 95% CI=0.95–21.83,  $P=.059$ ). There was no publication bias identified in these comparisons.

**Conclusions:** In Asia, 1 EPIYA-D motif was significantly associated with increased GC risk. Multiple EPIYA-C motifs were associated with increased PUD and DU risk, particularly in Asia. In the United States and Europe, multiple EPIYA-C motifs were associated with increased GC risk. Therefore, detection of polymorphic CagA EPIYA motifs may improve clinical prediction of disease risk.

**Abbreviations:** CagA = cytotoxin associated gene A protein, CI = confidence intervals, DU = duodenal ulcer, EPIYA = Glu-Pro-Ile-Tyr-Ala, F = fixed effects model, FD = functional dyspepsia, GC = gastric cancer, GU = gastric ulcer, *H pylori* = *Helicobacter pylori*, NOS = Newcastle–Ottawa scale, OR = odds ratios, PCR = Polymerase Chain Reaction, PUD = peptic ulcer disease, R = random effects model, S = sequencing.

**Keywords:** EPIYA, gastric diseases, *Helicobacter pylori*

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## 1. Introduction

*Helicobacter pylori* (*H pylori*) is a Gram-negative bacteria that colonizes the human gastric mucosa of different races and from different regions. More than half of the world's population is infected.<sup>[1]</sup> Persistent *H pylori* infection in the stomach may cause chronic gastritis, peptic ulcer disease (PUD) and gastric cancer (GC).<sup>[2,3]</sup> However, the clinical outcomes following infection vary in severity in different populations. This is likely to be a combined result of host genetic susceptibility, environmental factors and differing *H pylori* pathogenicity.<sup>[4–6]</sup>

Many *H pylori* virulence factors, including CagA, lipopolysaccharide, peptidoglycan, vacuolating cytotoxin, and gamma glutamyl transpeptidase, have been associated with gastric disease. Among them, CagA is a virulence factor encoded by the terminal *cagA* gene of the type IV secretion system.<sup>[7,8]</sup> CagA can be translocated into the host cells by this secretion system, where the C-terminal repeated EPIYA tyrosine can be rapidly phosphorylated by Src Family Kinases (SFKs). This may eventually lead to abnormal gastric epithelial cell proliferation, cytoskeletal abnormalities<sup>[9–15]</sup> or even activation of cellular oncogenes.<sup>[16–21]</sup> However, not all studies have indicated that the CagA protein performs the aforementioned functions. Polymorphisms in the EPIYA sequence determine differences in CagA

protein function. Based on the EPIYA motifs, *H pylori* was subcategorized as Western or East Asian strains. Western *H pylori* strains contain EPIYA-A, EPIYA-B, and EPIYA-C segments, and the EPIYA-C site is often repeated. The common EPIYA polymorphic types are primarily ABC, ABCC, and ABCCC. In contrast, East Asian *H pylori* strains contain EPIYA-A, EPIYA-B, and EPIYA-D motifs. The most common EPIYA polymorphic type is ABD.<sup>[22,23]</sup> It has been previously demonstrated that phosphorylation of the CagA protein EPIYA motif is closely associated with the existence and number of C or D, but not A or B, sites.

EPIYA-C or -D site phosphorylation plays an important role in activating downstream molecular events. For example, Vianna et al<sup>[24]</sup> analyzed the association between *H pylori* strains with multiple EPIYA-C sites and PUD and compared that to *H pylori* strains with no more than 1 EPIYA-C motif. The authors observed that multiple C sites were associated with increased PUD risk. In contrast, Torres et al<sup>[25]</sup> determined that multiple EPIYA-C sites did not increase PUD risk. Ferreira et al<sup>[26]</sup> analyzed the relationship between *H pylori* strains carrying multiple EPIYA-C sites and GC and concluded that multiple C sites were associated with increased GC risk. However, Chomvarin<sup>[27]</sup> determined that multiple EPIYA-C sites were not associated with GC risk. Similarly, several studies have contradictory conclusions regarding the relationship between EPIYA-D and GC. For example, Li et al.<sup>[28]</sup> found that *H pylori* strains carrying 1 EPIYA-D site are associated with increased GC risk. However, Xia et al.<sup>[29]</sup> determined that 1 D site did not increase the risk of GC. Taken together, the conclusions regarding the association of EPIYA-C or -D phosphorylation sites with gastric diseases are inconsistent. Therefore, to further clarify the association between CagA EPIYA polymorphisms and gastric disease, we proposed a systematic review to explore the association of the existence and number of EPIYA-C or -D phosphorylation sites with gastric disease. Our meta-analysis will help clarify the diverse clinical outcomes in patients with *H pylori* and detect *H pylori* infection based on EPIYA classification. Furthermore, this study will allow for earlier eradication of *H pylori* carrying risky EPIYA types and ultimately reduce the development of *H pylori*-associated gastric diseases.

## 2. Methods

### 2.1. Ethics statement: N/A

**2.1.1. Identification and eligibility of relevant studies.** Literature in electronic databases, including PubMed, Embase, Web of Knowledge, Wanfang Data, and CNKI, were systematically searched using the terms, “cytotoxin associated gene A/CagA,” “EPIYA,” and “*H pylori*.” The corresponding Chinese terms were used when searching Chinese databases. Furthermore, references that were cited in each included study were also searched manually to identify potential additional relevant studies. When the information provided in the article was unclear, we contacted the author for detailed raw data. If data were overlapping, we adopted the most recent and comprehensive research for this meta-analysis. The last search date was March 28, 2016.

**2.1.2. Inclusion and exclusion criteria.** The inclusion criteria were as follows: studies investigating the association of CagA EPIYA-D or EPIYA-C phosphorylation sites with PUD and GC risk; studies with sufficient raw data to estimate ORs and 95% CIs; and studies with gastritis or functional dyspepsia (FD) as a

control group. Exclusion criteria included: reviews or meta-analyses; animal or cytology experiments; duplicate publications; studies not involving PUD or GC; and studies published neither in English nor Chinese.

**2.1.3. Data extraction.** From the included studies, data, including first author, year of publication, population ethnicity, population age, number of cases and controls, detection methods of CagA EPIYA-D, and EPIYA-C phosphorylation sites and *H pylori* isolate source, were carefully extracted by 2 authors (Qiuping Li and Yuehua Gong) independently. Inconsistencies were resolved following discussion, and a consensus was reached for all extracted data.

**2.1.4. Evaluation of the validity of the included studies.** The Newcastle–Ottawa scale (NOS) with 8 items was used to estimate the validity of the included studies.<sup>[30]</sup> We evaluated the studies on a 9 star scale based on selection (4 stars maximum), comparability (2 stars maximum) and exposure (3 stars maximum). NOS scores of 1–3, 4–6, and 7–9 were considered low, medium, and high quality, respectively (Table S1, <http://links.lww.com/MD/B662>).

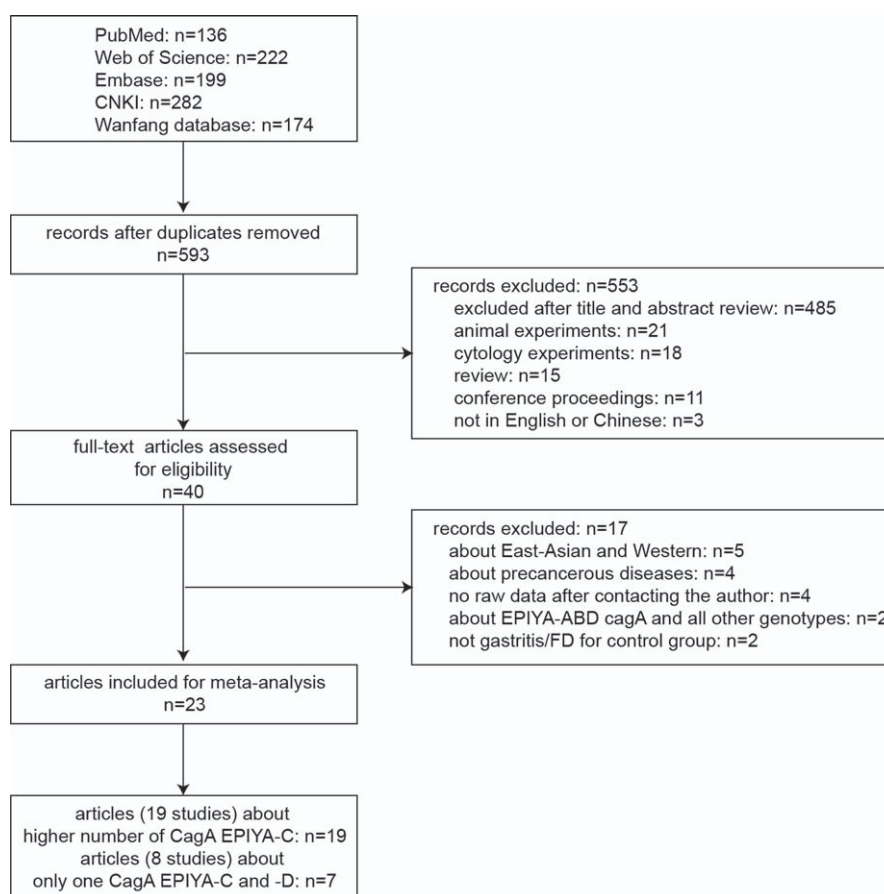
## 2.2. Statistical analysis

We analyzed the association of 1 CagA EPIYA-D or multiple EPIYA-C phosphorylation sites with PUD and GC risk using Stata software (version 11.0; StataCorp, College Station, TX). Cumulative ORs and the corresponding 95% CIs were used to measure the strength of associations. All *P* values were 2 sided, and *P* < .05 was considered statistically significant. Heterogeneity across the studies was assessed using a *Q* statistic (considered significant heterogeneity if *P* < .10) and an *I*-squared (*I*<sup>2</sup>) value.<sup>[31]</sup> When significant heterogeneity was detected, a random-effects model based on the DerSimonian and Laird method<sup>[32]</sup> was used to perform the meta-analysis. Otherwise, a fixed-effects model using the Mantel–Haenszel method was performed.<sup>[33]</sup> A sensitivity analysis was performed to explore heterogeneity when significant heterogeneity was indicated. Subgroup analysis was used to explore the effect of geographical region and GU or DU. Moreover, publication bias was evaluated quantitatively using Begg's<sup>[34]</sup> and Egger's tests.<sup>[35]</sup> Significant publication bias was indicated if *P* value < .05.

## 3. Results

### 3.1. Characteristics of the included studies

The flow chart of included studies is summarized in Fig. 1. A systematic search through electronic databases yielded 593 citations after duplicate removal. After reviewing the titles, abstracts and full texts, articles that were not relevant to this analysis, animal experiments and reviews or cytology experiments were removed, resulting in the exclusion of 553 records. The remaining 40 full-text articles were further assessed for eligibility. Finally, 23 full-text articles that met the inclusion criteria were included in this meta-analysis.<sup>[24–29,36–53]</sup> Among these articles, 7 (8 studies) compared 1 EPIYA-C with 1 EPIYA-D site (6 studies: PUD vs gastritis/FD; 3 studies: GU vs gastritis/FD; 3 studies: DU vs gastritis/FD; 7 studies: GC vs gastritis/FD). Additionally, 19 articles (19 studies) compared no more than 1 EPIYA-C with multiple EPIYA-C sites (15 studies: PUD vs gastritis/FD; 5 studies: GU vs gastritis/FD; 9 studies: DU vs gastritis/FD; 14 studies: GC vs gastritis/FD).



**Figure 1.** Flow chart of literature search and study selection. DU = duodenal ulcer, FD = functional dyspepsia, GC = gastric cancer, GU = gastric ulcer, PUD = peptic ulcer disease.

The primary characteristics of all studies included in this meta-analysis are summarized in Table 1. For the meta-analysis comparing 1 EPIYA-D with 1 EPIYA-C site, all the populations were from Asia. EPIYA motif types were all detected by PCR-based sequencing. Only 1 article<sup>[28]</sup> divided the population into adults and children. The detailed analysis of 1 EPIYA-D and 1 EPIYA-C motif and the total number of subjects is displayed in Table 1. For the meta-analysis comparing multiple EPIYA-C sites with no more than 1 site, data were compiled from 4 geographical regions, including Asia, South America, North America, and Europe. The number of EPIYA-C motifs was detected by PCR-based sequencing, except for the study by Salih et al<sup>[36]</sup> that used PCR. The details of this analysis and the total number of subjects are also displayed in Table 1. Analysis of 1 EPIYA-D and 1 EPIYA-C motif revealed that the EPIYA polymorphisms included D, ABD, and ABABD types when 1 D site was present and AC, BC, ABC, AABC, and ABBC types when 1 C site was present. Comparison of multiple EPIYA-C sites with no more than 1 EPIYA-C site revealed that the EPIYA polymorphisms included ABCC, ABCCC, ABCCCC, and ABCCCCC types when multiple C sites were present and AB, AC, BC, ABC, AABC, and ABBC types when no more than 1 C site was present.

### 3.2. Association between 1 EPIYA-D or EPIYA-C site and PUD and GC

First, we analyzed the relationship between 1 EPIYA-D or EPIYA-C phosphorylation site and PUD. With gastritis and FD as

the control, compared with 1 EPIYA-C site, 1 EPIYA-D site was not associated with increased PUD risk (OR = 0.90, 95% CI: 0.46–1.76,  $P = .764$ ; Table 2 and Fig. 2). However, there was heterogeneity in these studies ( $I^2 = 46.80\%$ ,  $P = .094$ ). To further investigate the sources of heterogeneity, we conducted a sensitivity analysis (Fig. S1, <http://links.lww.com/MD/B662>). After removing the most obvious outlying study by Chomvarin<sup>[27]</sup> (OR = 0.35), no significant heterogeneity remained ( $I^2 = 0.00\%$ ,  $P = .586$ ). In the remaining studies, 1 EPIYA-D site was still not associated with increased PUD risk (OR = 1.32, 95% CI: 0.86–2.03,  $P = .201$ ). Similarly, with gastritis and FD as controls, 1 EPIYA-D motif was not associated with increased risk of GU (OR = 0.85, 95% CI: 0.27–2.63,  $P = .771$ ; Table 2) or DU (OR = 0.89, 95% CI: 0.25–3.16,  $P = .859$ ; Table 2).

Next, we evaluated the relationship between 1 EPIYA-D or EPIYA-C phosphorylation site and GC. Compared with 1 EPIYA-C site, 1 EPIYA-D site was significantly associated with increased GC risk (controls: gastritis and FD; OR = 1.91, 95% CI: 1.19–3.07,  $P = .008$ ; Table 2 and Fig. 3). There was no significant heterogeneity in these studies ( $I^2 = 27.20\%$ ,  $P = .221$ ).

### 3.3. Association between multiple EPIYA-C sites or no more than 1 EPIYA-C site and PUD and GC

First, we evaluated the association between multiple EPIYA-C phosphorylation sites or no more than 1 EPIYA-C phosphorylation site and PUD. Multiple EPIYA-C motifs were significantly

**Table 1**

**Characteristics of selected studies for cagA EPIYA-D or EPIYA-C phosphorylation sites analysis.**

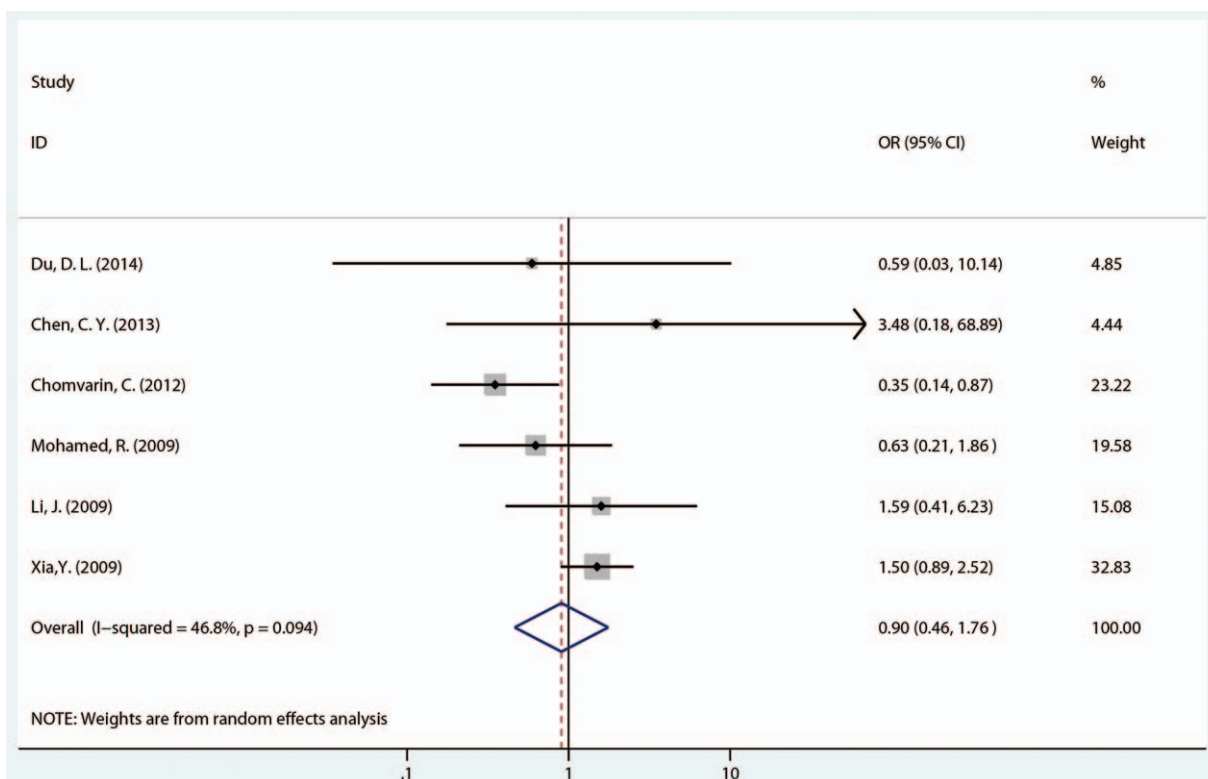
Author	Ethnicity	Year	Region	Age	Method	Gastritis or FD cagA EPIYA* / Total (%)	PUD cagA EPIYA* / Total (%)	GU cagA EPIYA* / Total (%)	DU cagA EPIYA* / Total (%)	GC cagA EPIYA* / Total (%)
For 1 EPIYA-D or 1 EPIYA-C studies										
Du, D. L.	Chinese	2014	Asia	Adult	PCR+S	27/28 (96.4)	16/17 (94.1)	11/11 (100.0)	5/6 (83.3)	16/18 (88.9)
Chen, C. Y.	Chinese	2013	Asia	NA	PCR+S	87/90 (96.7)	43/43 (100.0)	17/17 (100.0)	26/26 (100.0)	20/20 (100.0)
Chomvarin, C.	Thai	2012	Asia	Mix	PCR+S	23/48 (47.9)	10/41 (24.4)	—	—	7–15 (46.7)
Mohamed, R.	Chinese/ Malay/Indian	2009	Asia	NA	PCR+S	36/46 (78.3)	18/26 (69.2)	10/14 (71.4)	7/9 (77.8)	—
Li, J.	Chinese	2009	Asia	Children	PCR+S	11/12 (91.7)	—	—	—	1/1 (100.0)
Li, J.	Chinese	2009	Asia	Adult	PCR+S	19/30 (63.3)	11/15 (73.3)	—	—	5/5 (100.0)
Xia, Y.	Mix	2009	Mix	NA	NA	66/131 (50.4)	64/106 (60.4)	—	—	38/60 (63.3)
Schmidt, H. M.	Chinese/ Malay/Indian	2009	Asia	Adult	PCR+S	53/89 (59.6)	—	—	—	18/18 (100.0)
For multiple or no more than 1 EPIYA-C studies										
Honarmand, J. S.	Iranian	2015	Asia	Adult	PCR+S	1/81 (1.2)	9/86 (10.5)	8/49 (16.3)	1/37 (2.7)	—
Rocha, G. A.	Brazilian	2015	South America	Adult	PCR+S	33/174 (19.0)	—	—	—	89/213 (41.8)
Vianna, J. S.	Brazilian	2015	South America	NA	PCR+S	8/38 (21.1)	9/17 (52.9)	—	—	—
Kocazeybek, B.	Turkish	2015	Asia	Adult	PCR+S	4/72 (5.6)	8/22 (36.4)	—	8/22 (36.4)	22/38 (57.9)
Beltrán-Anaya, F.	Mexican	2014	North America	Mix	PCR+S	34/164 (20.7)	25/39 (64.1)	—	—	6/11 (54.5)
Salih, B. A.	Turkish	2014	Asia	Adult	PCR	3/13 (23.1)	9/16 (56.3)	2/6 (33.3)	7/10 (70.0)	—
Vilar, E. Silva A.	Brazilian	2014	South America	Adult	PCR+S	28/74 (37.8)	—	—	—	53/76 (69.7)
Kalaf, E. A.	Iraqi	2013	Asia	Adult	PCR+S	0/6 (0.0)	5/32 (15.6)	5/15 (33.3)	0/17 (0.0)	2/4 (50.0)
Ferreira, R. M.	Portuguese	2012	Europe	Adult	PCR+S	11/55 (20.0)	—	—	—	21/48 (43.8)
Chomvarin, C.	Thai	2012	Asia	Mix	PCR+S	4/65 (6.2)	4/52 (7.7)	—	—	1/17 (5.9)
Torres, L. E.	Cuban	2012	North America	NA	PCR+S	8/36 (22.2)	13/59 (22.0)	3/19 (15.8)	10/40 (25.0)	—
Battista, S.	Brazilian	2011	South America	Adult	PCR+S	25/136 (18.4)	15/112 (13.4)	—	15/112 (13.4)	78/188 (41.5)
Acosta, N.	Colombian	2010	South America	NA	PCR+S	12/40 (30.0)	8/22 (36.4)	—	8/22 (36.4)	6/18 (33.3)
Shokrzadeh, L.	Iranian	2010	Asia	Adult	PCR+S	1/77 (1.3)	1/11 (9.1)	—	—	1/4 (25.0)
Quiroga, A. J.	Colombian	2010	South America	Adult	PCR+S	21/52 (40.4)	12/24 (50.0)	—	12/24 (50.0)	12/17 (70.6)
Salih, B. A.	Turkish	2010	Asia	Adult	PCR+S	5/40 (12.5)	22/37 (59.5)	7/9 (77.8)	15/28 (53.6)	—
Xia, Y.	Mix	2009	Mix	NA	NA	18/149 (12.1)	8/114 (7.0)	—	—	17/77 (22.1)
Schmidt, H. M.	Chinese/ Malay/Indian	2009	Asia	Adult	PCR+S	16/105 (15.2)	—	—	—	3/21 (14.3)
Basso, D.	Italian	2008	Europe	Mix	PCR+S	12/42 (28.6)	5/18 (27.8)	—	—	30/47 (63.8)

For 1 EPIYA-D or 1 EPIYA-C studies, CagA EPIYA\* represents the cases number of 1 EPIYA-D site. For multiple or no more than 1 EPIYA-D site, CagA EPIYA\* represents the cases number of multiple EPIYA-C sites. CagA = cytotoxin associated gene A protein, DU = duodenal ulcer, EPIYA = Glu-Pro-Ile-Tyr-Ala, FD = functional dyspepsia, GC = gastric cancer, GU = gastric ulcer, PCR = polymerase chain reaction, PUD = peptic ulcer disease, S = sequencing.

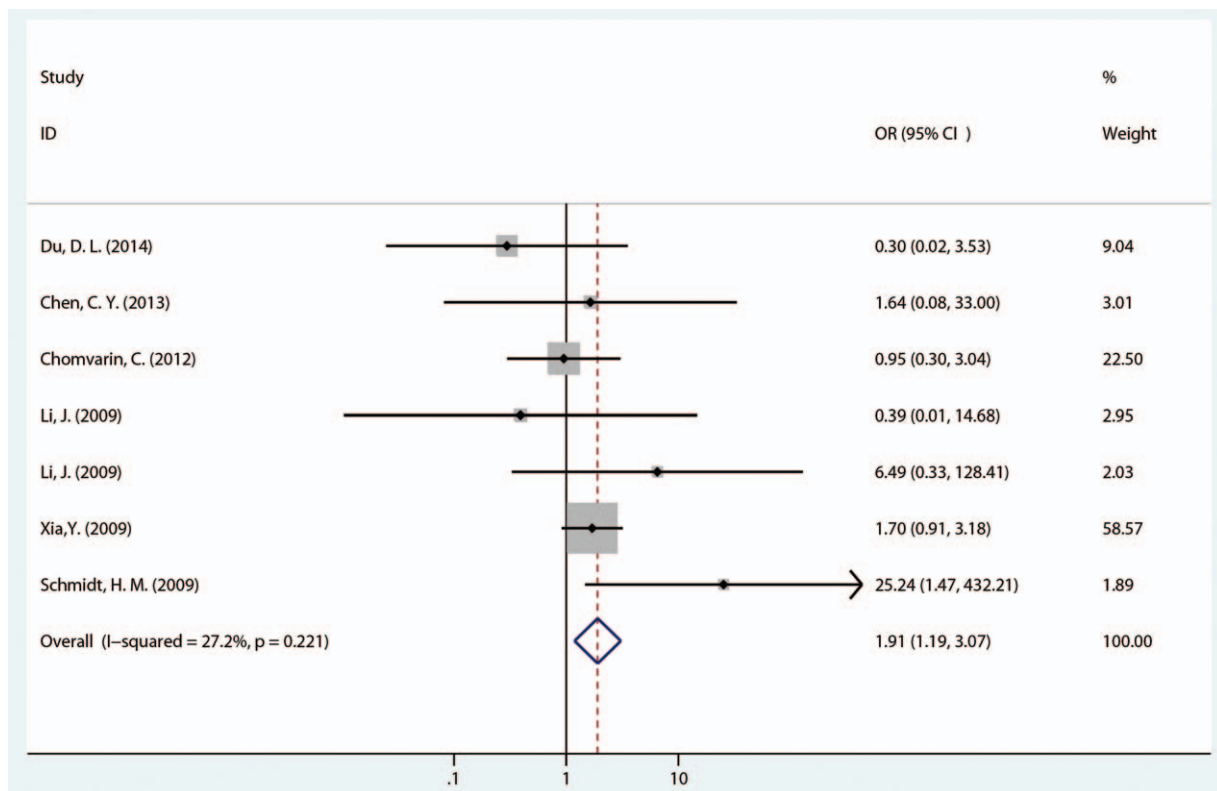
**Table 2**  
**Meta-analysis results for association between cagA EPIYA-D or EPIYA-C phosphorylation sites and PUD and GC.**

Variables	No. of studies	Heterogeneity Test		Statistical model	Test for overall effect	
		I <sup>2</sup> (%)	Phet		OR (95%CI)	P
For 1 EPIYA-D or 1 EPIYA-C studies						
PUD vs gastritis/FD	6	46.80%	0.094	R	0.90 (0.46–1.76)	.764
GU vs gastritis/FD	3	0.00%	0.885	F	0.85 (0.27–2.63)	.771
DU vs gastritis/FD	3	0.00%	0.488	F	0.89 (0.25–3.16)	.859
GC vs gastritis/FD	7	27.20%	0.221	F	1.91 (1.19–3.07)	.008
For multiple or no more than 1 EPIYA-C studies						
PUD vs gastritis/FD						
Overall	15	72.40%	<0.001	R	2.33 (1.29–4.20)	.005
Region						
Asia	7	7.10%	0.374	F	5.57 (3.05–10.20)	<.001
South America	4	55.20%	0.082	R	1.39 (0.67–2.88)	.379
North America	2	89.10%	0.002	R	2.67 (0.40–17.81)	.309
Europe	1	–	–	R	0.96 (0.28–3.29)	.950
GU vs gastritis/FD						
Overall	5	66.60%	0.018	R	4.54 (0.95–21.83)	.059
Region						
Asia	4	22.00%	0.279	F	9.08 (3.23–25.57)	<.001
North America	1	–	–	R	0.66 (0.15–2.83)	.572
DU vs gastritis/FD						
Overall	9	69.50%	0.002	R	2.32 (1.08–5.00)	.031
Region						
Asia	5	0.00%	0.830	F	7.66 (3.56–16.51)	<.001
South America	3	0.00%	0.371	F	0.96 (0.59–1.59)	.883
North America	1	–	–	R	1.17 (0.40–3.38)	.776
GC vs gastritis/FD						
Overall	14	43.00%	0.044	R	3.28 (2.32–4.64)	<.001
Region						
Asia	5	75.00%	0.003	R	5.20 (0.90–30.11)	.066
South America	5	0.00%	0.563	F	3.06 (2.29–4.08)	<.001
North America	1	–	–	R	4.59 (1.32–15.94)	.017
Europe	2	0.00%	0.584	F	3.69 (1.98–6.88)	<.001

CagA = cytotoxin associated gene A protein, CI = confidence intervals, DU = duodenal ulcer, EPIYA = Glu-Pro-Ile-Tyr-Ala, F = fixed effects model, FD = functional dyspepsia, GC = gastric cancer, GU = gastric ulcer, OR = odds ratios, PUD = peptic ulcer disease, R = random effects model.



**Figure 2.** Forest plot of the association between 1 EPIYA-D phosphorylation site and PUD risk. EPIYA = Glu-Pro-Ile-Tyr-Ala, PUD = peptic ulcer disease.



**Figure 3.** Forest plot of the association between 1 EPIYA-D phosphorylation site and GC risk. EPIYA = Glu-Pro-Ile-Tyr-Ala, GC=gastric cancer.

associated with increased PUD risk (control: gastritis and FD; OR=2.33, 95% CI: 1.29–4.20,  $P=.005$ ; Table 2 and Fig. 4). However, significant heterogeneity existed between these studies ( $I^2=72.40\%$ ,  $P<.001$ ). Thus, we conducted a sensitivity analysis to further identify the sources of heterogeneity (Fig. S2, <http://links.lww.com/MD/B662>). After omitting the most obvious outlying study by Xia et al<sup>[29]</sup> (OR=0.55), heterogeneity still remained ( $I^2=68.80\%$ ,  $P<.001$ ). In the remaining studies, the conclusion was still significant (OR=2.64, 95% CI: 1.46–4.76,  $P=.001$ ). Subgroup differences and study design or quality could also not explain the heterogeneity source. In the subgroup analysis according to geographical region, multiple EPIYA-C phosphorylation sites were significantly associated with increased PUD risk in Asia (OR=5.57, 95% CI: 3.05–10.20,  $P<.001$ ; Table 2) but not in South America, North America, or Europe (South America: OR=1.39, 95% CI: 0.67–2.88,  $P=.379$ ; North America: OR=2.67, 95% CI: 0.40–17.81,  $P=.309$ ; Europe: OR=0.96, 95% CI: 0.28–3.29,  $P=.95$ ; Table 2). Compared with no more than 1 EPIYA-C site, multiple EPIYA-C phosphorylation sites were not associated with GU risk (OR=4.54, 95% CI: 0.95–21.83,  $P=.059$ ; Table 2). Furthermore, the subgroup analysis revealed that multiple EPIYA-C phosphorylation sites were significantly associated with increased GU risk in Asian (OR=9.08, 95% CI: 3.23–25.57,  $P<.001$ ; Table 2) but not North American populations (OR=0.66, 95% CI: 0.15–2.83,  $P=.572$ ; Table 2). Multiple EPIYA-C phosphorylation sites were significantly associated with DU risk (OR=2.32, 95% CI: 1.08–5.00,  $P=.031$ ; Table 2). Subgroup analysis revealed that multiple EPIYA-C phosphorylation sites were associated with increased DU risk in Asia (OR=7.66, 95% CI: 3.56–16.51,  $P<.001$ ; Table 2) but not in South America or North America (South America: OR=0.96, 95% CI: 0.59–1.59,

$P=.883$ ; North America: OR=1.17, 95% CI: 0.40–3.38,  $P=.776$ ; Table 2).

Next, we evaluated the correlation between EPIYA-C phosphorylation sites and GC. Compared with no more than 1 EPIYA-C site, multiple C phosphorylation sites were significantly associated with increased GC risk (controls: gastritis and FD; OR=3.28, 95% CI: 2.32–4.64,  $P<.001$ ; Table 2 and Fig. 5). Because there was significant heterogeneity among these studies ( $I^2=43.00\%$ ,  $P=.044$ ), a sensitivity analysis was performed (Fig. S3, <http://links.lww.com/MD/B662>). After omission of the most obvious outlying study by Kocazeybe et al<sup>[37]</sup> (OR=23.38), the heterogeneity was no longer significant ( $I^2=0.00\%$ ,  $P=.462$ ). However, multiple EPIYA-C phosphorylation sites were still associated with increased GC risk in the remaining studies (OR=2.95, 95% CI: 2.34–3.72,  $P<.001$ ). A subgroup analysis of different regions was performed. In the Asian subgroup, we observed no significant association of multiple EPIYA-C phosphorylation sites with GC (controls: gastritis and FD; OR=5.20, 95% CI: 0.90–30.11,  $P=.066$ ; Table 2). However, there was a statistically significant increased GC risk identified in South American, North American and European subgroups (South America: OR=3.06, 95% CI: 2.29–4.08,  $P<.001$ ; North America: OR=4.59, 95% CI: 1.32–15.94,  $P=.017$ ; Europe: OR=3.69, 95% CI: 1.98–6.88,  $P<.001$ , Table 2).

### 3.4. Publication bias

We performed Begg's and Egger's tests to quantitatively evaluate the publication bias of the association of 1 CagA EPIYA-D or multiple CagA EPIYA-C phosphorylation sites with PUD and GC risk. Publication bias observed in this meta-analysis was not

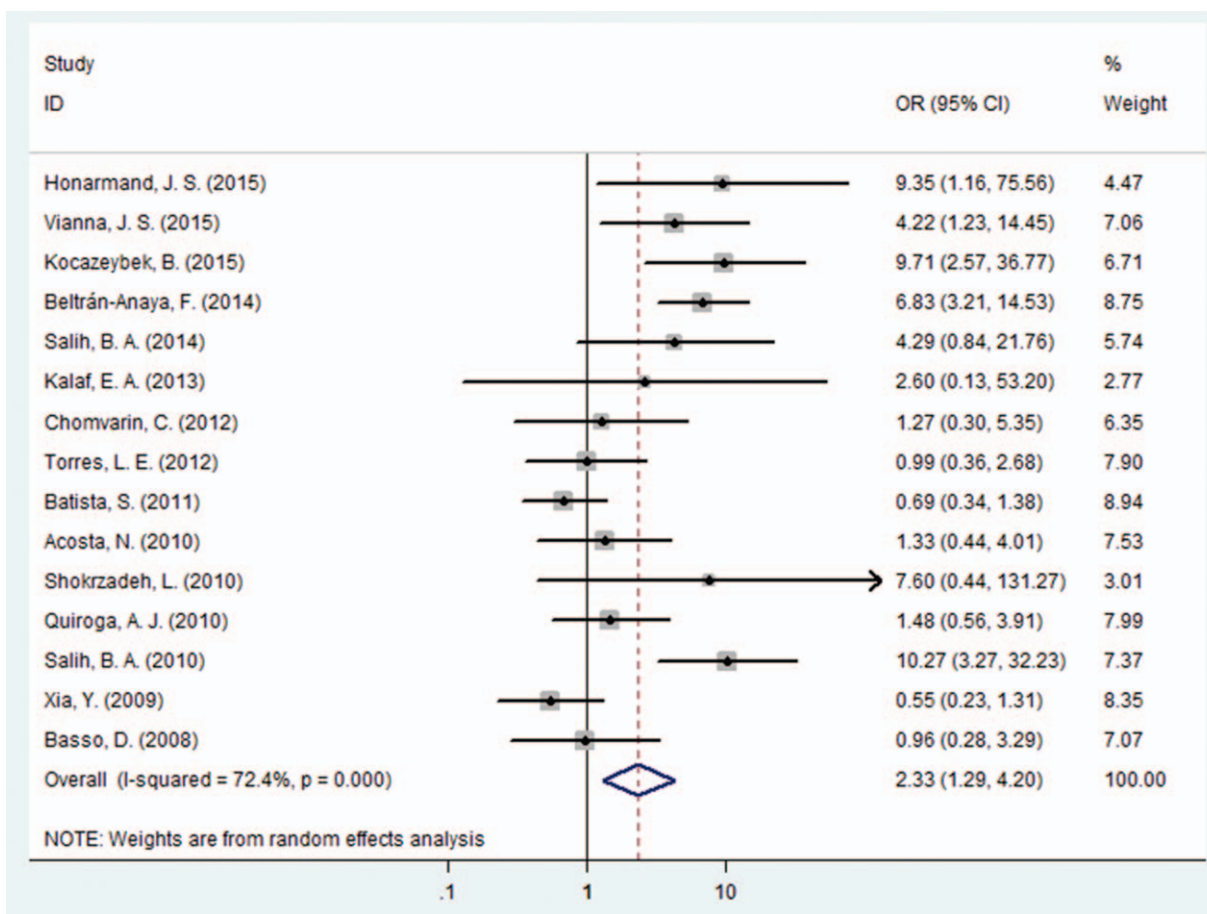


Figure 4. Forest plot of the association between multiple EPIYA-C phosphorylation sites and PUD risk. EPIYA = Glu-Pro-Ile-Tyr-Ala, PUD=peptic ulcer disease.

significant (Table 3, Figs. S4 and S5, <http://links.lww.com/MD/B662>).

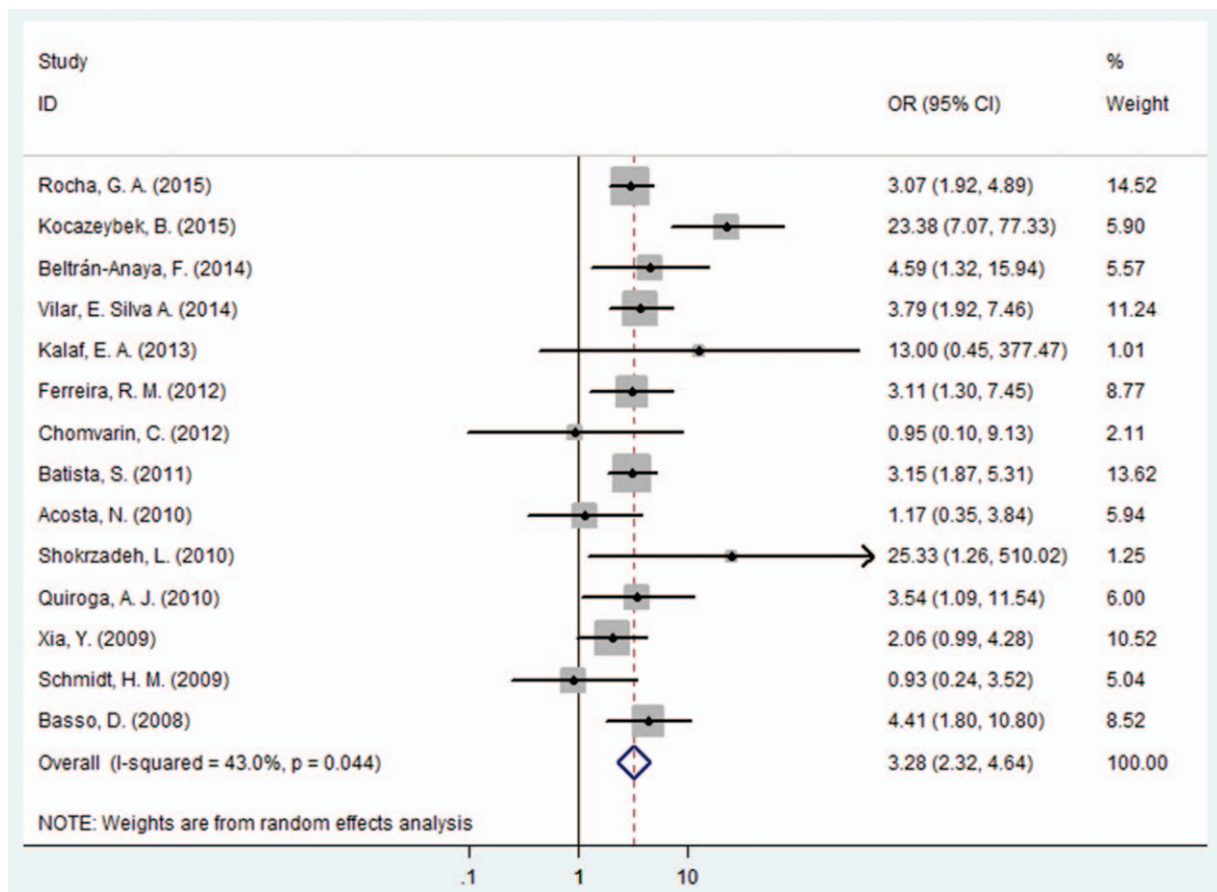
#### 4. Discussion

The type and number of CagA C-terminal EPIYA motifs impact protein size, function and polymorphisms, resulting in bacterial virulence (pathogenicity) differences.<sup>[21,54]</sup> Therefore, the correlation of CagA EPIYA motifs with clinical results has become of great interest in *H pylori* research. However, research investigating whether *H pylori* CagA EPIYA-D or -C phosphorylation sites are related to increased PUD and GC risk has been inconsistent. In this meta-analysis, we determined that 1 EPIYA-D site, compared with 1 EPIYA-C segment, was significantly associated with an increased GC risk in Asia. Moreover, we observed that, compared with no more than 1EPIYA-C site, multiple EPIYA-C sites were associated with increased PUD risk (controls: gastritis and FD). Furthermore, in Asia, multiple EPIYA-C sites were not only associated with increased PUD risk but also with increased risk of GU and DU. Additionally, multiple EPIYA-C segments were also associated with increased GC risk, particularly in South America, North America, and Europe. To the best of our knowledge, this is the first meta-analysis investigating the relationship between EPIYA-D or -C phosphorylation sites and PUD and GC.

For the comparison between 1 EPIYA-D and 1 EPIYA-C site, the entire study population was from Asia. For the pooled

analysis, 1 EPIYA-D segment was not significantly associated with PUD risk (controls: gastritis and FD). Similarly, 1 EPIYA-D segment was not related to an increased risk of GU or DU. However, we did identify a significant association between 1 EPIYA-D segment and increased GC risk. These results suggest that the presence of only 1 EPIYA-D segment in Asian populations was closely associated with increased risk of GC but not PUD. Phosphorylated CagA binds the Src homology 2 (SH2) domain of Src homology 2 phosphatase (SHP-2), altering SHP-2 conformation and interfering with host cell signaling pathways, ultimately resulting in epithelial structure disorder.<sup>[55,56]</sup> In addition, the phosphorylated CagA and SHP-2 complex can activate the ERK signaling pathway in a Ras-dependent or -independent manner. De Souza et al.<sup>[57]</sup> demonstrated that SHP-2 domains recognize phosphopeptide motifs composed of phosphotyrosine (pY) followed by several CagA C-terminal residues. Amino acid differences at the C-terminal residues within the CagA EPIYA-D and -C segments can result in different SHP-2 affinities. As a result, SHP-2 binds EPIYA-D more tightly than EPIYA-C. Furthermore, Higashi et al.<sup>[23]</sup> determined in vitro that the East Asian EPIYA-D strain bound SHP-2 more tightly, compared with the Western EPIYA-C strain, and induced epithelial cell morphology transformation. This may explain why the EPIYA-D site has a greater pathogenicity than the EPIYA-C site.

The studies comparing multiple EPIYA-C sites with no more than 1 EPIYA-C site included populations from Asia, North and



**Figure 5.** Forest plot of the association between multiple EPIYA-C phosphorylation sites and GC risk. EPIYA = Glu-Pro-Ile-Tyr-Ala, GC=gastric cancer.

South America, and Europe. For the pooled analysis of the EPIYA-C phosphorylation site, we determined that multiple EPIYA-C motifs were associated with increased PUD risk. However, significant heterogeneity among studies was identified, and we, therefore, conducted further sensitivity analyses. Though the most obvious outliers were eliminated, heterogeneity was still significant, and subgroup analysis could not explain the heterogeneity source. The geographical stratification analysis demonstrated that, in Asia, multiple EPIYA-C phosphorylation sites were associated with increased PUD risk. However, this association was not identified in North America, South America, or Europe. Additional disease and regional subgroup analyses revealed that multiple EPIYA-C phosphorylation sites were

significantly associated with increased GU and DU risk in Asia. The pooled estimate also demonstrated that multiple EPIYA-C phosphorylation sites were associated with increased GC risk in Europe and America. Some mechanistic studies have partially explained the association between multiple EPIYA-C phosphorylation sites and PUD and GC. Higashi et al<sup>[23]</sup> found that when strains carrying multiple EPIYA-C sites were co-cultured with human gastric adenocarcinoma cell (AGS), each EPIYA-C site could be equally phosphorylated. Thus, phosphorylation was associated with the number of EPIYA-C sites. Naito et al<sup>[58]</sup> also confirmed that CagA with multiple EPIYA-C or EPIYA-D motifs bound SHP-2 more robustly than CagA with single EPIYA-C or EPIYA-D motif, and the former had a stronger ability to activate

**Table 3**

**Publication bias.**

Variables	Begg's test		Egger's test	
	z value	P value	t value	P value
For 1 EPIYA-D or 1 EPIYA-C studies				
PUD vs gastritis/FD	0.38	.707	-0.38	.724
GC vs gastritis/FD	0.00	1.000	0.15	.889
For multiple or no more than 1 EPIYA-C studies				
PUD vs gastritis/FD	1.19	.235	1.30	.216
GC vs gastritis/FD	0.99	.324	0.53	.603

P value < .05 was considered as significant publication bias.

EPIYA = Glu-Pro-Ile-Tyr-Ala, FD = functional dyspepsia, GC = gastric cancer, PUD = peptic ulcer disease.



SHP-2. Higashi et al<sup>[23]</sup> suggested that the ability of CagA to bind SHP-2 was dependent on the number and sequence of tyrosine phosphorylation sites. Additionally, EPIYA-C sites increased CagA protein phosphorylation, which significantly increased CagA-SHP-2 complex formation and enhanced the ability of CagA to induce cellular phenotypic changes. Therefore, the number of CagA EPIYA-C sites is the key factor affecting the ability of CagA to interfere with intracellular signal transduction. In addition, CagA with EPIYA-ABCCC and CagA with EPIYA-D have the same carcinogenic potential.<sup>[18]</sup> Hence, strains with multiple EPIYA-C sites are associated with PUD and GC risk. It is possible that regional differences in the association between EPIYA-C and PUD and GC may be attributed to fewer studies investigating multiple EPIYA-C sites and PUD in Europe and America. Thus, it is necessary to further expand the sample size to confirm the above conclusions. Alternatively, these findings may be due to a different distribution of Eastern and Western strains. Most Eastern strains carry EPIYA-D, while Western strains tend to harbor the EPIYA-C site. Therefore, multiple EPIYA-C motifs were more relevant to GC risk in Europe and America but not in Asia.

This meta-analysis had some limitations. First, we only included studies written in English or Chinese. Thus, selection bias might exist. Second, the sample size of the studies included was relatively small, resulting in an even smaller sample size for the stratified analyses. This may be related to the stringent requirements for *H pylori* culture technology or the high cost of EPIYA sequencing; both limit the acquisition of large sample sizes. Third, in Asian populations, because there were fewer articles investigating the relationship between multiple EPIYA-D or single EPIYA-C motifs (or multiple EPIYA-C motifs) and PUD and GC, we only compared the association of a single EPIYA-D or C motif with PUD and GC. We concluded that a single D motif was related to increased GC risk, which still needs further verification. Fourth, there was significant heterogeneity in the analysis between multiple EPIYA-C motifs and PUD. Moreover, subgroup and sensitivity analyses could not explain the source of heterogeneity. Furthermore, the limited number of relevant studies also prevented us from performing meta-regression analysis to further explore the sources of heterogeneity.

## 5. Conclusions

This meta-analysis demonstrated that a single EPIYA-D phosphorylation site, compared with a single EPIYA-C phosphorylation site, was associated with increased GC risk in Asia. Multiple EPIYA-C phosphorylation sites, compared with no more than 1 EPIYA-C site, are associated with increased PUD, GU and DU risk in Asia and are related to increased GC risk in Europe and America. *H pylori* carrying a single EPIYA-D motif or multiple EPIYA-C motifs may be a potential marker for predicting PUD or GC risk. In addition, it is necessary to expand the sample size of studies investigating the association between CagA EPIYA polymorphisms and PUD and GC to confirm our meta-analysis conclusions.

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