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ORIGINAL RESEARCH

Relationship between the *iceA* gene of *Helicobacter pylori* and clinical outcomes

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Background: The complex pathogenesis of *Helicobacter pylori* (*H. pylori*) and the features of the host influence the diverse clinical outcomes. A mass of studies about virulence genes have accelerated the exploration of pathogenesis of *H. pylori* infection. Induced by contact with epithelium gene A (*iceA*) is one of the biggest concerned virulence genes. In this study, we explored the relationship between *iceA* and the magnitude of the risk for clinical outcomes and the prevalence of *iceA*-positive *H. pylori* in People's Republic of China and other countries.

Methods: We searched the electronic databases of PubMed, Embase, CNKI, VIP, and Wanfang by literature search strategy. The studies conforming to the inclusion criteria were assessed. With these data, we systematically analyzed the relationship between the *iceA* gene of *H. pylori* and clinical outcomes.

Results: Nineteen articles with 22 studies, a total of 2,657 cases, were involved in the study. The *iceA1* gene was significantly associated with peptic ulcer disease (odds ratio =1.28, 95% confidence interval =1.03–1.60; *P*=0.03), especially in People's Republic of China (odds ratio =1.40, 95% confidence interval =1.07–1.83; *P*=0.01). Moreover, the prevalence of *iceA1* was significantly higher than *iceA2* in People's Republic of China (*P*<0.0001). The prevalence of both *iceA1* and *iceA2* was significantly different (*P*<0.0001) in People's Republic of China and in other countries.

Conclusion: The system analysis showed that infection with the *iceA1*-positive *H. pylori* significantly increased the overall risk for peptic ulcer disease, especially in People's Republic of China. The *iceA2* gene status and clinical outcome of *H. pylori* infection have no significant correlation. *H. pylori iceA1* genotype is the major epidemic strain in People's Republic of China.

Keywords: *Helicobacter pylori*, *iceA*, gastritis, peptic ulcer disease, gastric carcinoma

Introduction

Helicobacter pylori (*H. pylori*) is a gram-negative microaerophilic spiral bacterium tenaciously colonizing the gastric mucosa of approximately half the human population in the world. A minority of the infected population will suffer from chronic gastritis and peptic ulcer disease (PUD), and some even progress to gastric carcinoma (GC) and gastric mucosa-associated lymphoid tissue lymphoma. The International Agency for Research on Cancer confirmed that *H. pylori* infection was the most significant risk factor for gastric cancer and that the eradication of *H. pylori* can reduce the risk of gastric cancer in 2012.[1] *H. pylori*, the host, and environment factors influence the diverse clinical outcomes. In particular, many virulence genes of *H. pylori* play an important role.[2–5] Different genotypes of *H. pylori* produce different virulence factors. Urea enzymes, adhesins, *cagA*,[6] and *vacA*[7] are conclusively associated with severe gastroduodenal diseases. Some other virulence genes have been found, one of which is induced by contact with epithelium gene A (*iceA*), which is independent of *cagA* and *vacA*.[4,8–10]

The *iceA* gene was identified in the *H. pylori* isolated from PUD and gastritis patients. There are at least two alleles of *iceA*, *iceA1*, and *iceA2*.[11] The relationship between *H. pylori iceA* and clinical outcomes is controversial. Some studies have suggested that *iceA* (*iceA1/iceA2*) may be significantly associated with diseases of digestive system,[10,12,13] whereas others showed contrary findings.[14–17] In this study, the relationship between the genetic status of *iceA* (*iceA1* and *iceA2*) and gastritis, nonulcer dyspepsia (NUD), PUD, and GC were systematically assessed. We also evaluated the distribution difference of *iceA* status in People's Republic of China and other countries.

Materials and methods
Literature search strategy
A literature search was performed using PubMed, Embase, CNKI, VIP, and Wanfang databases for articles assessing the relationship between *iceA* gene and clinical outcomes in *H. pylori*-infected populations. All included studies were retrieved using the search terms: 1) "*iceA*" or "*iceA1*"or "*iceA2*" and 2) "*Helicobacter pylori*" or "*H. pylori*" or "*Hp*". Papers written in English or Chinese and published before October 2014 were selected for this study.

Inclusion criteria
The criteria applied to select papers were as follows: 1) studies exploring the relationship between *iceA* gene (*iceA1* or *iceA2*) status and clinical outcomes; 2) fully published case–control studies; 3) studies with case groups including gastritis or NUD, PUD (gastric ulcer or duodenal ulcer), and GC defined by upper gastric endoscopy and histological examination; 4) studies in which genomic DNA was extracted using isolated *H. pylori* colony; 5) studies in which the presence/absence of *iceA* gene was examined by polymerase chain reaction; and 6) studies written in English or Chinese.

Exclusion criteria
Studies were excluded if they were reviews or conference proceedings, did not present integrated raw data, included only children or adults and if DNA was extracted from mucosal biopsy specimens of the antrum/corpus. When overlapping data existed, only the largest and latest study was selected.

Quality evaluation and data extraction
Assessment of all the included articles and extraction of raw data were performed by two investigators independently. The following information was extracted from each study: first author's name, year of publication, country of the study population, *iceA1* and *iceA2* status according to clinical outcomes

(gastritis/NUD, PUD, and GC), and the total number of cases and controls, respectively. Disagreements were resolved by discussion, and all the entered data were consistent finally.

Statistical analysis
Statistical analysis was carried out using RevMan software (Version 5.3.0, The Cochrane Collaboration, Copenhagen, Denmark). The strength of association between the presence/absence of *iceA* gene and gastritis/NUD, PUD, or GC was evaluated by odds ratios (ORs) and corresponding 95% confidence intervals (CIs). Heterogeneity among the included studies was measured by χ^2-based Q and I^2 statistics. When the heterogeneity was not considered significant ($P \geq 0.1$ for Q test and $I^2 < 50\%$), a fixed-effects model was applied to calculate the pooled OR. Otherwise, a random-effects model was used. To exclude any possible influence of a single study, a sensitivity analysis was performed to evaluate the substantially altered or statistical results of the summary estimate. In addition, publication bias was evaluated qualitatively by funnel plots. Two-sided P-values were assessed in the meta-analysis, and $P < 0.05$ was considered as statistically significant.

Results
Characteristics of selected studies
According to the literature search strategy, a total of 123 potentially relevant records were retrieved, and 37 studies were excluded after duplicate checking. Sixty-seven studies were further excluded because they were irrelevant, abstracts, or reviews, or because they did not conform to the inclusion criteria. Finally, only 19 articles met the inclusion criteria (Figure 1). One article reported by Yamaoka et al[14] investigated the correlation of *iceA* gene with clinical outcomes in four countries, so the data were treated separately. Finally, a total of 22 independent studies were considered for the systematic analyses. Twenty-two studies (with 2,657 patients) assessed the association between *iceA1* and infection outcomes, but eight studies did not involve GC patients. Nineteen articles with 2,281 patients showing the relationship between *iceA2* and infection outcomes were included, but seven studies did not involve GC patients. Thirteen of the 22 studies were related to the Chinese population. The main characteristics of the studies included in the systematic analyses are summarized in Table 1.

Association between the *iceA1* status and clinical outcomes
There were 22 studies, comprising 1,005 cases and 1,247 controls, that assessed the distribution difference of *iceA1* status between patients with PUD and gastritis or NUD controls.
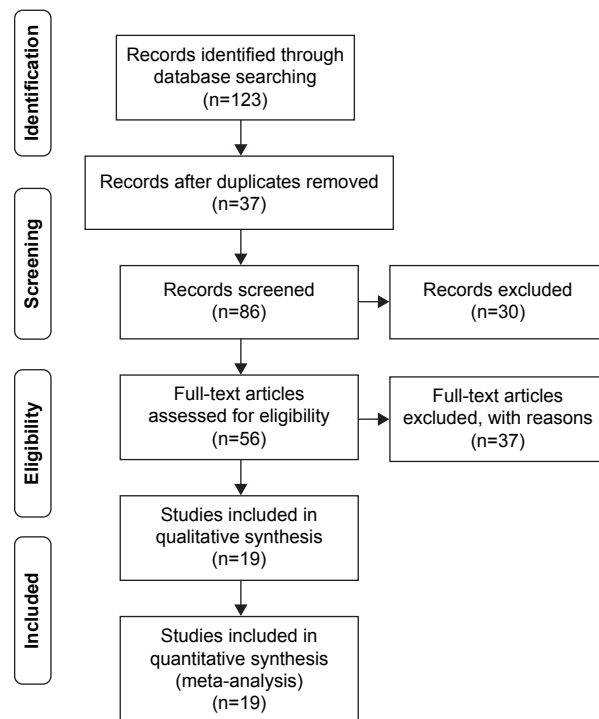
Figure 1 Flowchart of literature inclusion and exclusion.

The prevalence of *iceA1* ranged from 10.64% to 96.97% in PUD patients and from 25.42% to 90.91% in gastritis or NUD patients. The general prevalence of *iceA1* was 67.66% (680/1,005) in PUD patients and 62.95% (785/1,247) in

controls. In the pooled estimate for PUD, the summary OR in the fixed-effects model was 1.23 (95% CI =1.01–1.50, *P*=0.04), but moderate heterogeneity existed (I^2=35%, *P*=0.06). Sensitivity analysis was conducted, and four studies,[10–13] that showed significant differences in the prevalence of *iceA1* compared with other studies were removed. The summary OR was 1.28 (95% CI =1.03–1.60, *P*=0.03), and significant heterogeneity no longer existed among the studies (I^2=0%, *P*=0.92). Subgroup analysis was also performed to explore the correlations with *iceA1* status in People's Republic of China and other countries. The overall prevalence of *iceA1* in People's Republic of China was 70.74% (394/557) in PUD cases and 64.21% (461/718) in gastritis or NUD controls, while in other countries it was 69.93% (200/286) and 62.68% (179/286), respectively. A statistical relationship between *iceA1* and PUD was observed in People's Republic of China subgroup (OR =1.40, 95% CI =1.07–1.83; *P*=0.01), but no significant association was found in the subgroup of other countries (OR =1.07, 95% CI =0.73–1.57; *P*=0.73). There was no significant heterogeneity among the subgroups (I^2=23.7%, *P*=0.25) (Figure 2; Table 2).
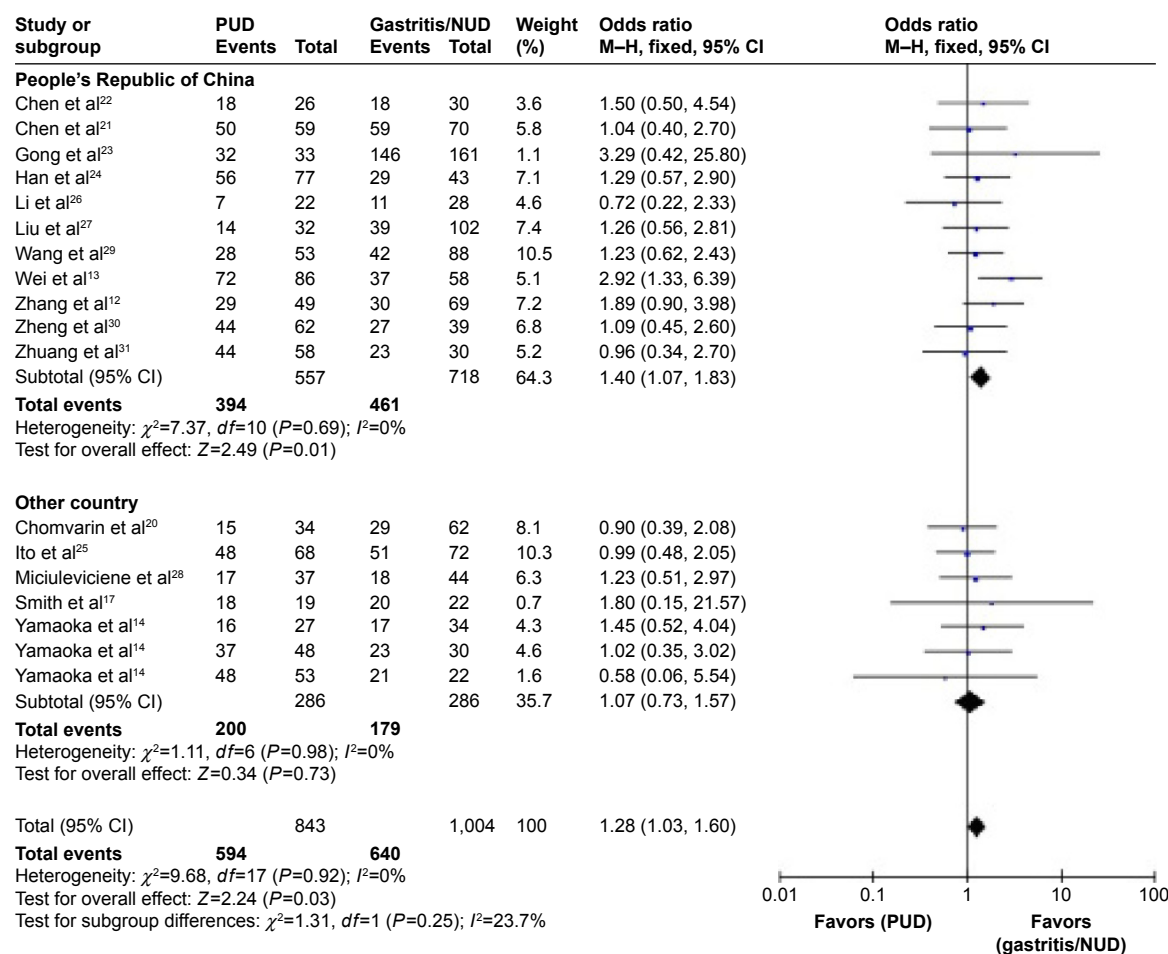
There were 14 studies, comprising 405 cases and 823 controls, that studied the distribution difference of *iceA1* status between patients with GC and gastritis or NUD controls. The overall prevalence of *iceA1* was 61.98% (251/405) in

Table 1 Characteristics of studies included in the systematic analyses

Authors (references)	Countries	Gastritis/NUD *iceA1*+/total (%)	PUD *iceA1*+/total (%)	GC *iceA1*+/total (%)	Gastritis/NUD *iceA2*+/total (%)	PUD *iceA2*+/total (%)	GC *iceA2*+/total (%)
Ashour et al[15]	Brazil	15/59 (25.42)	5/47 (10.64)	4/36 (11.11)	48/59 (81.36)	46/47 (97.87)	35/36 (97.22)
Chomvarin et al[20]	Thailand	29/62 (46.77)	15/34 (44.12)	7/16 (43.75)	19/62 (30.65)	12/34 (35.29)	6/16 (37.5)
Chen et al[22]	People's Republic of China	59/70 (84.29)	50/59 (84.75)	–	28/70 (40.00)	32/59 (54.24)	–
Chen et al[21]	People's Republic of China	18/30 (60.00)	18/26 (69.23)	12/14 (85.71)	–	–	–
Gong et al[23]	People's Republic of China	146/161 (90.68)	32/33 (96.97)	23/28 (82.14)	136/161 (84.47)	31/33 (93.94)	25/28 (89.29)
Han et al[24]	People's Republic of China	29/43 (67.44)	56/77 (72.73)	18/21 (85.71)	8/43 (18.60)	10/77 (12.99)	2/21 (9.52)
Ito et al[25]	Japan	51/72 (70.83)	48/68 (70.59)	–	21/72 (29.17)	20/68 (29.41)	–
Li et al[26]	People's Republic of China	11/28 (39.29)	7/22 (31.82)	–	25/28 (89.29)	22/22 (100)	–
Liu et al[27]	People's Republic of China	39/102 (38.24)	14/32 (43.75)	20/30 (66.67)	29/102 (28.43)	10/32 (31.25)	11/30 (36.67)
Miciuleviciene et al[28]	Lithuania	18/44 (40.91)	17/37 (45.95)	–	24/44 (54.55)	16/37 (43.24)	–
Smith et al[17]	Nigeria	20/22 (90.91)	18/19 (94.74)	–	3/22 (13.64)	1/19 (5.26)	–
Wang et al[29]	People's Republic of China	42/88 (47.73)	28/53 (52.83)	7/11 (63.64)	41/88 (46.59)	26/53 (49.06)	6/11 (54.55)
Wang et al[18]	People's Republic of China	77/108 (71.30)	70/77 (90.91)	–	–	–	–
Wei et al[13]	People's Republic of China	37/58 (63.79)	72/86 (83.72)	42/53 (79.25)	24/58 (41.38)	23/86 (26.74)	12/53 (22.64)
Yamaoka et al[14]	Colombia	17/34 (50.00)	16/27 (59.26)	24/46 (52.17)	25/34 (73.53)	15/27 (55.56)	32/46 (69.57)
Yamaoka et al[14]	Korea	21/22 (95.45)	49/53 (92.45)	51/60 (85.00)	5/22 (22.73)	16/53 (30.19)	20/60 (33.33)
Yamaoka et al[14]	Japan	23/30 (76.67)	37/48 (77.08)	28/34 (82.35)	13/30 (43.33)	18/48 (37.50)	12/34 (35.29)
Yamaoka et al[14]	USA	8/26 (30.77)	3/28 (10.71)	3/16 (18.75)	18/26 (69.23)	28/28 (100)	12/16 (75.00)
You et al[16]	People's Republic of China	45/50 (90.00)	6/10 (60.00)	–	6/50 (12.00)	3/10 (30.00)	–
Zhang et al[12]	People's Republic of China	30/69 (43.48)	29/49 (59.18)	6/20 (30.00)	32/69 (46.38)	32/49 (65.31)	2/20 (10.00)
Zheng et al[30]	People's Republic of China	27/39 (69.23)	44/62 (70.97)	14/20 (70.00)	–	–	–
Zhuang et al[31]	People's Republic of China	23/30 (76.67)	44/58 (75.86)	–	8/30 (26.67)	11/58 (18.97)	2/20 (10.00)

Note: "–" indicated data not provided in study.
Abbreviations: PUD, peptic ulcer disease; NUD, nonulcer dyspepsia; GC, gastric carcinoma.

Study or subgroup	PUD Events	Total	Gastritis/NUD Events	Total	Weight (%)	Odds ratio M–H, fixed, 95% CI	Odds ratio M–H, fixed, 95% CI
People's Republic of China							
Chen et al[22]	18	26	18	30	3.6	1.50 (0.50, 4.54)	
Chen et al[21]	50	59	59	70	5.8	1.04 (0.40, 2.70)	
Gong et al[23]	32	33	146	161	1.1	3.29 (0.42, 25.80)	
Han et al[24]	56	77	29	43	7.1	1.29 (0.57, 2.90)	
Li et al[26]	7	22	11	28	4.6	0.72 (0.22, 2.33)	
Liu et al[27]	14	32	39	102	7.4	1.26 (0.56, 2.81)	
Wang et al[29]	28	53	42	88	10.5	1.23 (0.62, 2.43)	
Wei et al[13]	72	86	37	58	5.1	2.92 (1.33, 6.39)	
Zhang et al[12]	29	49	30	69	7.2	1.89 (0.90, 3.98)	
Zheng et al[30]	44	62	27	39	6.8	1.09 (0.45, 2.60)	
Zhuang et al[31]	44	58	23	30	5.2	0.96 (0.34, 2.70)	
Subtotal (95% CI)		557		718	64.3	1.40 (1.07, 1.83)	
Total events	**394**		**461**				
Heterogeneity: χ^2=7.37, df=10 (P=0.69); I^2=0%							
Test for overall effect: Z=2.49 (P=0.01)							
Other country							
Chomvarin et al[20]	15	34	29	62	8.1	0.90 (0.39, 2.08)	
Ito et al[25]	48	68	51	72	10.3	0.99 (0.48, 2.05)	
Miciuleviciene et al[28]	17	37	18	44	6.3	1.23 (0.51, 2.97)	
Smith et al[17]	18	19	20	22	0.7	1.80 (0.15, 21.57)	
Yamaoka et al[14]	16	27	17	34	4.3	1.45 (0.52, 4.04)	
Yamaoka et al[14]	37	48	23	30	4.6	1.02 (0.35, 3.02)	
Yamaoka et al[14]	48	53	21	22	1.6	0.58 (0.06, 5.54)	
Subtotal (95% CI)		286		286	35.7	1.07 (0.73, 1.57)	
Total events	**200**		**179**				
Heterogeneity: χ^2=1.11, df=6 (P=0.98); I^2=0%							
Test for overall effect: Z=0.34 (P=0.73)							
Total (95% CI)		843		1,004	100	1.28 (1.03, 1.60)	
Total events	**594**		**640**				
Heterogeneity: χ^2=9.68, df=17 (P=0.92); I^2=0%							
Test for overall effect: Z=2.24 (P=0.03)							
Test for subgroup differences: χ^2=1.31, df=1 (P=0.25); I^2=23.7%							

Figure 2 Forest plot for the risk of *iceA1* gene and peptic ulcer disease compared with gastritis or nonulcer dyspepsia.
Abbreviations: CI, confidence interval; PUD, peptic ulcer disease; NUD, nonulcer dyspepsia; M–H, Mantel–Haenszel Test.

Table 2 Association between the *iceA1* status and clinical outcomes

Group/ subgroup	OR (95% CI)	P-value	I^2 (%)	P_{het}	Analysis model
Total					
PUD vs gastritis/ NUD	1.28 (1.03, 1.60)	0.03	0	0.92	FE
GC vs gastritis/ NUD	1.05 (0.78, 1.40)	0.75	25	0.19	FE
GC vs PUD	0.88 (0.64, 1.19)	0.40	3	0.42	FE
People's Republic of China					
PUD vs gastritis/ NUD	1.40 (1.07, 1.83)	0.01	0	0.69	FE
GC vs gastritis/ NUD	1.30 (0.89, 1.89)	0.17	35	0.15	FE
GC vs PUD	0.85 (0.57, 1.26)	0.42	34	0.15	FE
Other countries					
PUD vs gastritis/ NUD	1.07 (0.73, 1.57)	0.73	0	0.98	FE
GC vs gastritis/ NUD	0.74 (0.46, 1.20)	0.22	0	0.50	FE
GC vs PUD	0.92 (0.56, 1.49)	0.73	0	0.34	FE

Abbreviations: PUD, peptic ulcer disease; NUD, nonulcer dyspepsia; GC, gastric carcinoma; OR, odds ratio; I^2, I-squared; P_{het}, P-value for heterogeneity test; FE, fixed-effect model; CI, confidence interval.

GC cases and 58.44% (481/823) in controls. In the pooled estimate for GC, the summary OR in the fixed-effects model was 1.05 (95% CI =0.78–1.40, P=0.75), and there was no significant heterogeneity among the studies (I^2=25%, P=0.19). Subgroup analysis was also performed to explore the correlations between *iceA1* status in People's Republic of China and other countries. The overall prevalence of *iceA1* in People's Republic of China was 68.02% (134/197) in GC cases and 62.37% (368/590) in gastritis or NUD controls, while in other countries it was 56.25% (117/208) and 48.50% (113/233), respectively. No significant association was found in People's Republic of China (OR =1.30, 95% CI =0.89–1.89; P=0.17) and other countries (OR =0.74, 95% CI =0.46–1.20; P=0.22) (Table 2, Figure S1).

We also analyzed the distribution difference of *iceA1* status between patients with GC and PUD controls. In the pooled estimate for GC, the summary OR in the fixed-effects model was 0.88 (95% CI =0.64–1.19, P=0.40), and there was no significant heterogeneity among the studies (I^2=3%, P=0.42). Subgroup analysis was also performed to explore

the correlations with *iceA1* status in People's Republic of China and other countries. The overall prevalence of *iceA1* in People's Republic of China was 68.02 % (134/197) in GC cases and 70.10% (293/418) in PUD controls, while in other countries it was 56.25% (117/208) and 52.74% (125/237), respectively. No significant association was found in People's Republic of China (OR =0.85, 95% CI =0.57–1.26; P=0.42) and other countries (OR =0.92, 95% CI =0.56–1.49; P=0.73). There was no significant heterogeneity among the subgroups (I^2=0%, P=0.80) (Table 2, Figure S2).

Association between the *iceA2* status and clinical outcomes

There were 19 studies, comprising 840 cases and 1,070 controls, that examined the distribution difference of *iceA2* status between patients with PUD and gastritis or NUD controls. In the pooled estimate for PUD, the summary OR in the fixed-effects model was 1.16 (95% CI =0.94–1.44, P=0.17), and significant heterogeneity existed among these studies (I^2=42%, P=0.03). Exploring the sources of heterogeneity showed that Yamaoka et al[14] (study in the USA) and Ashour et al[15] reported quite a high prevalence of *iceA2* in PUD patients (100% and 97.87%, respectively). In addition, the study by Smith et al[17] demonstrated an extremely low prevalence of *iceA2* in PUD patients (5.26%). After omitting these studies with abnormal results, the heterogeneity was not significant any more (I^2=27%, P=0.15), but the analysis still showed there was no significant association between *iceA2* and PUD compared with gastritis or NUD controls (OR =1.07, 95% CI =0.85–1.33; P=0.58). Subgroup analysis was also performed to explore the correlations with *iceA2* status between People's Republic of China and other countries. No significant association was found in People's Republic of China (OR =1.19, 95% CI =0.90–1.58; P=0.21) and other countries (OR =0.87, 95% CI =0.60–1.26; P=0.48). There was no significant heterogeneity among the subgroups (I^2=42.6%, P=0.19) (Table 3, Figure S3).

There were 12 studies, comprising 371 cases and 754 controls, that examined the distribution difference of *iceA2* status between patients with GC and gastritis or NUD. In the pooled estimate for GC, the summary OR in the fixed-effects model was 0.91 (95% CI =0.67–1.24, P=0.57), and moderate heterogeneity existed among these studies (I^2=42%, P=0.06). Exploring the sources of heterogeneity showed that Zhang et al[12] and Wei et al[13] reported quite a low prevalence of *iceA2* in GC patients (10% and 22.64%, respectively). After omitting these studies with abnormal results, the heterogeneity was no longer significant (I^2=0%, P=0.65), but the analysis still showed that

Table 3 Association between the *iceA2* status and clinical outcomes

Group/subgroup	OR (95% CI)	P-value	I^2 (%)	P_{het}	Analysis model
Total					
PUD vs gastritis/NUD	1.07 (0.85, 1.33)	0.58	27	0.15	FE
GC vs gastritis/NUD	1.27 (0.89, 1.82)	0.19	0	0.65	FE
GC vs PUD	0.97 (0.69, 1.36)	0.86	0	0.74	FE
People's Republic of China					
PUD vs gastritis/NUD	1.19 (0.90, 1.58)	0.21	43	0.07	RE
GC vs gastritis/NUD	1.24 (0.70, 2.18)	0.46	0	0.65	FE
GC vs PUD	0.92 (0.55, 1.53)	0.74	0	0.89	FE
Other countries					
PUD vs gastritis/NUD	0.87 (0.60, 1.26)	0.48	0	0.62	FE
GC vs gastritis/NUD	1.29 (0.81, 2.05)	0.28	0	0.65	FE
GC vs PUD	1.01 (0.65, 1.59)	0.95	10	0.35	FE

Abbreviations: PUD, peptic ulcer disease; NUD, nonulcer dyspepsia; GC, gastric carcinoma; OR, odds ratio; I^2, I-squared; P_{het}, P-value for heterogeneity test; FE, fixed-effect model; RE, random-effect model; CI, confidence interval.

there was no significant association between *iceA2* and GC compared with gastritis or NUD controls (OR =1.27, 95% CI =0.89–1.82; P=0.19). Subgroup analysis was also performed to explore the correlations with *iceA2* status between People's Republic of China and other countries. No significant association was found in People's Republic of China (OR =1.24, 95% CI =0.70–2.18; P=0.46) and other countries (OR =1.29, 95% CI =0.81–2.05; P=0.28). There was no significant heterogeneity among the subgroups (I^2=0%, P=0.91) (Table 3, Figure S4).

In the pooled estimate for GC compared with PUD, the summary OR in the fixed-effects model was 0.79 (95% CI =0.58–1.09, P=0.15), and moderate heterogeneity was observed (I^2=43%, P=0.06). Exploring the sources of heterogeneity showed that Zhang et al[12] reported quite a low prevalence of *iceA2* in GC patients (10%) compared with controls (65.31%). After omitting these data, the heterogeneity was no longer significant (I^2=0%, P=0.74), but the analysis still showed there was no significant association between *iceA2* and GC compared with PUD controls (OR =0.97, 95% CI =0.69–1.36; P=0.86). Subgroup analysis was also performed to explore the correlations with *iceA2* between People's Republic of China and other countries. No significant association was found in People's Republic of China (OR =0.92, 95% CI =0.55–1.53; P=0.74) and other countries (OR =1.01, 95% CI =0.65–1.59; P=0.95). There was no significant heterogeneity among the subgroups (I^2=0%, P=0.77) (Table 3, Figure S5).

Difference in the prevalence of *iceA* in People's Republic of China and other countries

The overall prevalence of *iceA* was 51.95% (1,784/3,434) in People's Republic of China and 52.77% (992/1,880) in other countries. There was no significant difference in the prevalence of *iceA* in People's Republic of China and other countries (*P*=0.57). The overall prevalence of *iceA1* was 69.25% (1,189/1,717) in People's Republic of China and 56.06% (527/940) in other countries. The *iceA1* status was significantly different in People's Republic of China than in other countries (*P*<0.01). Meanwhile, the prevalence of *iceA2* was 34.65% (595/1,717) in People's Republic of China and 49.47% (465/940) in other countries, and such a difference was significant (*P*<0.01). The *iceA1* and *iceA2* status in People's Republic of China was also significantly different (*P*<0.01), as in other countries (*P*=0.04).

Publication bias analysis

Publication bias was qualitatively estimated by funnel plots. No significant publication bias was observed for the meta-analyses of association between *iceA* status and the clinical outcome of *H. pylori* infection (Figures S6–S11).

Discussion

This meta-analysis included 19 articles, 22 studies, and 2,657 cases and systematically analyzed the association between the *iceA* gene status and clinical outcomes. The analysis showed that the prevalence of *iceA1* significantly increased the risk of PUD compared with gastritis or NUD controls. This significant correlation is particularly significant in Chinese population but not in patients from other countries, suggesting the effect of geographical difference on the relationship between *iceA1* and PUD. No significant risk association between *iceA1* status and GC was observed in any country population, possibly due to the relatively small sample size of GC cases compared to PUD or gastritis. *H. pylori iceA1* affects the immune response of gastric mucosa epithelial cells, and *iceA1*-positive strains produce more inflammatory cytokines (IL-6, IL-8, etc) than *iceA1*-negative strains. Meanwhile, it facilitates neutrophil infiltration in gastric mucosa, aggravating mucosal inflammation and PUD.[7,11,18,19] Moreover, *iceA1*-postive strains perhaps disrupt the microecological balance in gastrointestinal tract and accelerate the development of disease. These processes are the potential pathogenic mechanisms of diseases. The finding that there was no significant association between *iceA2* and clinical outcomes in People's Republic of China and other countries is consistent with nearly all the original studies.[14–18,20–31] *iceA2* gene expression may be more influenced by the gene structure, which has a repeated protein structure but has no homology to known genes, and the function of the *iceA2* product remains unclear.[20,32]

The analysis results showed that the overall prevalence of *iceA1* is higher than *iceA2* (64.58% and 39.89%, respectively), which is significantly different (*P*<0.01), and agrees with previous studies.[13,16,17,20,27,31] Interestingly, the analysis showed that the prevalence of *iceA1* and *iceA2* is significantly different in People's Republic of China (*P*<0.01), as in other countries (*P*=0.04). Also, the prevalence of both *iceA1* and *iceA2* is significantly different between People's Republic of China and other countries (*P*<0.01). In People's Republic of China, *H. pylori iceA1* is the major epidemic strain, which is consistent with previous studies showing that *H. pylori iceA1* subtype infection is more frequently found in People's Republic of China, Japan, and Korea.[2,4,12] The information about geographical difference in *iceA1* gene suggests that it could be used as a potential biomarker for distinguishing PUD from other digestive diseases in People's Republic of China and reveals a phylogenetic difference of *H. pylori* strains between People's Republic of China and other countries. Presumably, damage to the *iceA1* gene should reduce the incidence of PUD, which could be a testable hypothesis for future studies.

The pathogenicity of microorganism is not based on one single factor. Virulence genes have many factors that influence the diverse clinical outcomes of bacterial infection, and factors such as the invasion of the bacteria, protein activity, host susceptibility and immune response, age, sex, ethnicity, region, diet habits, concomitant microenvironment, treatment of the host, and collaborative pathogenic mixed infection should not be ignored.[33–36]

Urea enzymes, *cagA*, and *vacA* are conclusively associated with severe gastroduodenal diseases, but the relationship of *iceA* with clinical outcome is ambiguous. Multiple genes in the *H. pylori* strain, other unclear subtypes of *iceA*, detection methods of *iceA* status, and sample size may influence the analysis results. To confirm the significance of *iceA*, it is better to carry out a multivariate analysis involving *iceA* status and other factors. However, it is difficult to obtain the raw data from sufficient studies, which is a caveat of the current analysis. Moreover, children suffer from peptic ulcer or gastric cancer to a lesser extent. So, when a system analysis is performed, this difference between children and adults should be taken into consideration.

The study also had some potential limitations. First, the original literature was insufficient. We only screened papers written in English or Chinese from the electronic databases of PubMed,

Embase, CNKI, VIP, and Wanfang. Second, the included studies involved only a few countries. The number of GC patient was relatively small as compared to PUD or gastritis. Third, many factors influence the diverse clinical outcomes of *H. pylori* infection, such as the geographical distribution of *H. pylori* strains, host susceptibility and age, sex, ethnicity, region, diet habits, treatment of the host, and collaborative pathogenic mixed infection. These factors may have influenced the results.

Conclusion

In conclusion, the results of this study show that infection with *iceA1*-positive *H. pylori* significantly increases the overall risk for PUD, especially in People's Republic of China. The correlation between *iceA2* and clinical outcome of *H. pylori* infection is not significant. *H. pylori iceA1* genotype is the major epidemic strain in People's Republic of China.

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Disclosure

The authors report no conflicts of interest in this work.

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