Chinese-German J Clin Oncol
DOI 10.1007/s10330-014-0029-9

The vacA i1 genotype of Helicobacter pylori is associated with peptic ulcer and gastric cancer: A meta-analysis*

Juping Rui, Guochang Chen, Boneng Mao, Qi Pan

Department of Gastroenterology, Yixing Hospital, Jiangsu University, Yixing 214200, China

Received: 4 July 2014 / Revised: 20 July 2014 / Accepted: 16 August 2014

Abstract *Objective:* There are two genotypes of the vacA intermediate region, i1 and i2; however, the association between the genotypes and gastroduodenal disease remains to be elucidated. The aim of this article was to investigate the interaction between the genotypes and *H. pylori*-associated diseases such as chronic gastritis, peptic ulcer disease (PUD) and gastric cancer. *Methods:* The meta-analysis was performed in Review Manager 4.2.2. *Results:* Eleven (ten articles and one abstract) met the inclusion criteria and were included. The i1 genotype increased the risk of PUD (OR = 1.70, 95% CI: 1.24–2.33, $P < 0.001$) and gastric cancer (OR = 3.90, 95% CI: 2.64–5.78, $P < 0.001$). Sub-analysis showed that the i1 genotype was significantly associated with gastric ulcers (OR = 2.59, 95% CI: 1.05–6.35, $P = 0.040$), but not with duodenal ulcers (OR = 1.04, 95% CI: 0.61–1.76, $P = 0.90$). In addition, the association between the i1 genotype and PUD and GC existed in studies not only from Europe but also Asia, except for the association between the i1 genotype and PUD in Asian population. *Conclusion:* The vacA i1 genotype is associated with an increased risk of the development of peptic ulcer disease (mainly gastric ulcer) and gastric cancer. In geographical distribution, the association between the i1 genotype and PUD and GC existed in studies not only from Europe but also Asia, except for the association between the i1 genotype and PUD in Asian population.

Key words vacA gene; intermediate region; *H. pylori*-associated diseases

Helicobacter pylori infects almost half of the adult population worldwide [1], but the consequences of this infection such as chronic gastritis, peptic ulcers and gastric cancer vary among different individuals, different geographic regions and different ethnical populations [2]. Although genetic and environmental factors may play substantial roles in the variation of the consequences, bacterial factors, especially virulence factors, have been implicated in the development of the consequences. One of the major virulence factors of *H. pylori* is vacA. VacA has many biological functions, including vacuolization of cultured epithelial cells, induction of apoptosis, increase in the permeability of the epithelial monolayer, formation of hexameric pores in cells, and suppression of immune cell functions [3–4]. It has been demonstrated that vacA was associated with peptic ulcer disease and the development of gastric cancer [5–9]. The vacA gene has polymorphy within its signal, intermediate, and middle regions, and each has two genotypes (s1 and s2, m1 and m2, and i1 and i2). It has been described that the signal region affects the vacuolating activity of vacA, and the middle region determines the cell specificity of the toxin [10–13]; the strains with s1 have greater cytotoxin activity than those with s2; similarly, those with m1 have greater cytotoxin activity than those with m2 [14–17]. Rhead *et al* reported the i-region, for the first time, in 2007, and showed that s1/m1 and s2/m2 strains exclusively have i1 and i2, respectively and only the s1/m2 strains vary in their i types [10, 18]. This region determines the vacuolating activity among the s1/m2 strains [10, 13, 19]. Most importantly, a close correlation was found between the i1 genotype of vacA and gastric cancer in Iran [10]. Subsequent studies showed that infection with the vacA i1 strains was associated with more severe inflammatory cell infiltration in the gastric mucosa than that with vacA i2 strains [20]. Moreover, studies from Western countries showed that the vacA i1 strains are significantly associated with the development of peptic ulcer disease (PUD) and gastric cancer [21]. However, a few studies from East Asian and Southeast Asian countries, where strains of H. pylori almost always belong to the i1 genotype, did not confirm such an association [22–23]. Thus, the association of the i1

Correspondence to: Juping Rui. Email: staff1056@yxph.com
* Supported by grants from the National Natural Science Foundation of China (No. 81072032, 30770992).

genotype of *H. pylori* with consequences of *H. pylori* infection remains unclear.

Therefore, the present meta-analysis was carried out to determine whether i1 genotype of *H. pylori* is associated with the development of PUD and gastric cancer in patients with *H. pylori* infection.

Materials and methods

Study sources and literature search

We searched PubMed, MEDLINE, EMBASE, Google Scholar, CNKI (Chinese) and Wanfang (Chinese) digital database for relevant articles and abstracts published in English and Chinese from January 2007 to April 2013. The search was limited to human studies, but was otherwise unrestricted. MeSH terms and keywords used to identify articles were as follow: "vacA gene", "i-region", "intermediate region", "vacA i1 genotype", "*Helicobacter pylori*", "*H. pylori*" and "genotypes". Boolean operators (NOT, AND, OR) were used in succession to narrow and widen the search.

Study selection

Abstracts or full articles that passed the primary screening were further reviewed. The inclusion criteria of the articles for the meta-analysis were: (a) genotyping the vacA s-, m- and i-regions in *H. pylori* strains isolated in any countries worldwide; (b) providing detailed genotype information; and (c) determining the association of the vacA s-, m- and i-region genotypes with the risk of developing gastrointestinal diseases. However, review articles or meta-analyses were excluded. In addition, in the eligible studies, patients in whom the vacA gene was not fully genotyped, or those who appeared to be infected with multiple strains (both s1 and s2 or both m1 and m2 or both i1 and i2) were excluded from the meta-analysis.

Data extraction

Two independent reviewers identified articles meeting the inclusion criteria during the literature search and then extracted the data from the articles. Data on study design, study population, genotypes, the number of patients in the study and in each disease and genotype group and the number and frequency of i1 genotype in each group. There was greater than 95% agreement in data extraction between the two investigators; all disagreed extracted data were resolved with a face-to-face conference discussion.

Data analysis

The analysis was performed using Review Manager 4.2.2. The association of vacA i-region genotypes with the risk of development PUD and gastric cancer were expressed as odds ratios (ORs) with 95% confidence intervals (CIs) with reference to that with non-ulcer dyspepsia (NUD) or chronic gastritis, since there were no *H. pylori* positive "healthy" controls in any of the studies, and thus the enrolled studies used either "NUD" or "chronic gastritis" as the control reference group. NUD was generally defined as dyspeptic symptoms without a history or endoscopic evidence of gastroesophageal reflux disease, PUD or gastric cancer. In the present meta-analysis, "chronic gastritis" was accordingly designated into the "NUD" category. Sub-group analyses on the association between vacA i-region genotypes and type of PUD (i.e. duodenal ulcer and gastric ulcer) were also performed. For each analysis, we assessed the heterogeneity in the recruited studies. The critical P values were set to be 0.05, meaning if there was a significant heterogeneity ($P < 0.05$), a random-effects model would be selected to pool the data; if not, a fixed-effects model would be selected. All P values were two-sided and P values less than 0.05 were considered to be statistically significant.

Results

Included studies

Initially, a total of 19 articles, 16 full articles and three abstracts, were identified according to the MeSH terms and keywords for literature search. Of these articles, eight articles were excluded; two articles and two abstracts [25–28] did not provide detailed genotype formation, three [18, 19, 29] did not determine the association between the i-region and gastrointestinal diseases and one article [30] was a meta-analysis. Thus, a total of eleven articles, ten full articles [10, 21–24, 31–35] and one abstract [36] were identified to meet the inclusion criteria, and thus further included in this meta-analysis. Of these eleven articles, ten were published in English [10, 21–23, 31–36], and one in Chinese [24]

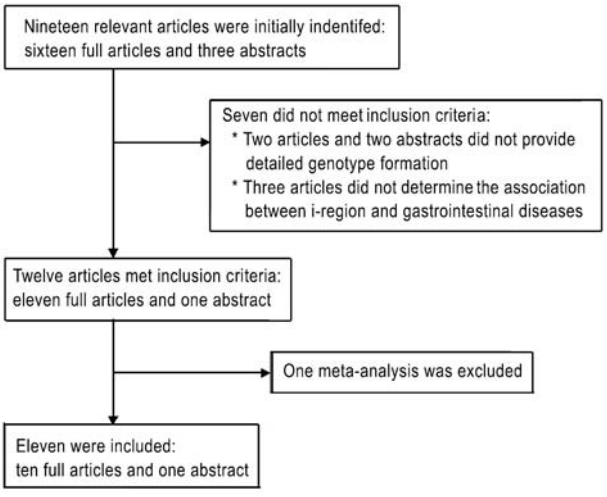

Fig. 1 The flow chart illustrating the process of identification of articles eligible for the meta-analysis

Table 1 The genotypes of vacA i regions in studies included in the meta-analysis

Authors [ref.]	Year of publication	Country/ region	Patients (*n*)	Diseases	Genetypes of strains [*n* (%)]		
					i1	i2	Mixed*
Rhead *et al* [10]	2007	Iran	73	NU[illegible]C	30 (41.1)	28 (38.4)	15 (20.5)
		USA/UK	42	NA	22 (52.4)	18 (42.9)	2 (4.8)
Ogiwara *et al* [22]	2008	East Asia	313	NUD, DU, GU, GC	300 (96)	4 (1.3)	9 (2.9)
Hussein *et al* [31]	2008	Iran, Iraq	108	NUD, DU, GU	29 (26.9)	58 (53.7)	21 (19.4)
Basso *et al* [21]	2008	Italy	170	CG, DU, GU, GC	96 (56.5)	64 (37.6)	10 (5.9)
Yoshiyuki *et al* [36]	2008	Japan	114	CG, DU, GU, GC	96 (84.2)	16 (14.0)	2 (1.8)
Douraghi *et al* [32]	2009	Iran	212	NUD, PUD, GC	111 (52.4)	96 (45.3)	5 (2.4)
Sheu *et al* [23]	2009	China	107	CG, DU, GU, GC	107 (100)	0	7 (6.5)
Qian *et al* [24]	2010	China	124	CG, DU, GU, GC	116 (93.5)	6 (4.8)	4 (3.2)
Effrosini *et al* [33]	2010	Greece	155	NUD, PUD	91 (58.7)	53 (34.2)	11 (7.1)
Daniel *et al* [34]	2012	Bulgaria	230	NUD, PUD	79 (34.3)	137 (59.6)	14 (6.1)
Rui *et al* [35]	2012	Portugal	192	NUD, GC	78 (40.6)	73 (38.0)	41 (21.4)

NUD, non-ulcer dyspepsia; GC, gastric cancer; NA, ; GU, gastric ulcer; DU, duodenal ulcer; PUD, peptic ulcer disease. * s1/s2, m1/m2, or i1/i2 strains

Table 2 Correlations of vacA i genotype with vacA s/m genotypes of H. pylori strains included in the meta-analysis [*n* (%)]

Authors [ref.]	s1/m1 (*n* = 584)		s1/m2 (*n* = 419)		s2/m1 (*n* = 2)	
	i1	i2	i1	i2	i1	i2
Rhead *et al* [10]	35 (35.7)	1 (1.0)	17 (17.3)	26 (26.5)	1 (1.0)	0
Ogiwara *et al* [22]	254 (83.6)	0	46 (15.1)	4 (1.3)	0	0
Hussein *et al* [31]	23 (26.4)	2 (2.3)	6 (6.9)	36 (41.4)	0	0
Basso *et al* [21]	52 (42.3)	0	14 (11.4)	5 (4.1)	0	1 (0.8)
Douraghi *et al* [32]	64 (30.9)	5 (2.4)	47 (22.7)	43 (20.8)	0	0
Sheu *et al* [23]	19 (19)	0	81 (81)	0	0	0
Yoshiyuki *et al* [36]	91 (81.3)	0	5 (4.5)	9 (8.0)	0	0
Qian *et al* [24]	37 (30.8)	1 (0.8)	76 (63.3)	4 (3.3)	0	0
Effrosini *et al* [33]	54 (37.5)	0	36 (25.0)	15 (10.4)	1 (0.7)	0
Daniel *et al* [34]	85 (39.4)	4 (1.9)	49 (22.7)	50 (23.1)		3 (1.4)
Rui *et al* [35]	51 (42.9)	2 (1.5)	9 (7.2)	2 (1.7)	1 (0.8)	1 (0.7)
Total	765 (46.9)	15 (1.0)	386 (23.7)	194 (11.9)	3 (0.2)	5 (0.3)

Authors [ref.]	s2/m2 (*n* = 146)		Total (*n* = 1151)	
	i1	i2	i1	i2
Rhead *et al* [10]	0	18 (18.4)	53 (54.1)	45 (45.9)
Ogiwara *et al* [22]	0	0	300 (98.7)	4 (1.3)
Hussein *et al* [31]	0	20 (23.0)	29 (33.3)	58 (66.7)
Basso *et al* [21]	0	51 (41.5)	66 (53.7)	57 (46.3)
Douraghi *et al* [32]	0	48 (23.2)	111 (53.6)	96 (46.4)
Sheu *et al* [23]	0	0	100 (100)	0 (0)
Yoshiyuki *et al* [36]	0	7 (6.3)	96 (85.7)	16 (14.3)
Qian *et al* [24]	0	2 (1.7)	113 (94.2)	7 (5.8)
Effrosini *et al* [33]	0	38 (26.6)	91 (63.2)	53 (36.8)
Daniel *et al* [34]	0	25 (11.6)	137 (63.4)	79 (36.6)
Rui *et al* [35]	0	53 (44.5)	62 (52.1)	57 (47.9)
Total	0	262 (12.7)	1158 (71.0)	472 (29.0)

(Fig. 1). Characteristics of the eleven studies used in the meta-analysis were summarized in Table 1.

The frequencies of vacA s-, m- and i-genotypes

In the included studies, 1781 *H. pylori* strains were isolated and genotyped from a total of 1814 patients with *H. pylori* infection. Among these patients, vacA was not completely genotyped in 57 patients, and infection of more than one strain appeared in 94 patients; thus, strains from these patients were excluded from the further analysis. Therefore, 1630 strains from 1630 patients with clear genotyping of vacA and diagnosis of gastrointestinal diseases were included in the meta-analysis. Of these 1630 strains, 780 were typed as s1/m1 genotype [765 (98.1%) were i1 type and 15 (1.9%) were i2 type], 262 as s2/m2 genotype (all 262 were i2 type), 580 as s1/m2 genotype

Table 3 Association between vacA i genotypes and gastrointestinal diseases with non-ulcer dyspepsia or chronic gastritis as a reference [*n* (%)]

Authors [ref.]	Country / regions	NUD†	DU	GU	PUD	GC
Rhead *et al* [10]	Iran	14 (36.8)	NA	NA	NA	16 (80)*
Ogiwara *et al* [22]	East and Southeast Asian	114 (96.6)	50 (92.6)	58 (96.7)	108 (94.7)	79 (97.5)
Hussein *et al* [31]	Iraq	4 (13.8)	2 (13.3)	4 (80)*	6 (30)	NA
Basso *et al* [21]	Italy	31 (43.7)	27 (69.2)*	3 (50)	30 (66.7)	35 (79.5)*
Douraghi *et al* [32]	Iran	61 (43.9)	NA	NA	20 (58.8)	30 (88.2)*
Sheu *et al* [23]	Taiwan	30 (100)	30 (100)	30 (100)	60 (100)	17 (100)
Yoshiyuki *et al* [33]	Japan	45 (63.3)	13 (59.1)	18 (100)*	31 (77.5)	20 (100)*
Qian *et al* [24]	China	85 (94.4)	5 (83.3)	8 (100)	13 (92.9)	18 (90.0)
Effrosini *et al* [33]	Greece	45 (57.0)	NA	NA	46 (73.0)	NA
Daniel *et al* [34]	Bulgaria	16 (25.0)	NA	NA	63 (41.4)	NA
Rui *et al* [35]	Portugal	36 (34.6)	NA	NA	NA	42 (89.4)
Total		481 (58.9)	127 (76.5)	121 (95.3)*	377 (69.6)**	257 (90.8)*

NUD, non-ulcer dyspepsia; GU, gastric ulcer; DU, duodenal ulcer; PUD, peptic ulcer disease; GC, gastric cancer; NA, . † chronic gastritis is included in the NUD group; * $P < 0.01$, compared with NUD; ** $P < 0.05$, compared with NU

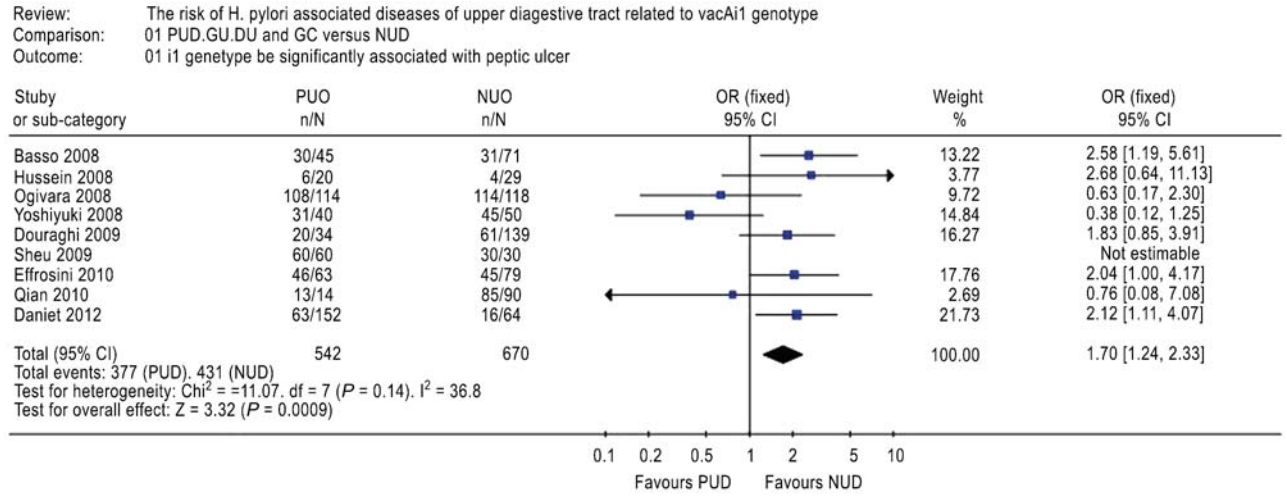

Fig. 2 Analyses of association between vacA i1 genotype and peptic ulcer disease (PUD), with non-ulcer dyspepsia (NUD) as a reference. Chronic gastritis is included in the NUD group

[386 (66.6%) were i1 type and 194 (33.4%) were i2 type], and eight strain as a rare s2/i2/m1 genotype (Table 2).

Association between infection of H. pylori strains of vacA i1 genotype and peptic ulcer disease

Analysis of nine articles [21–24, 31–34, 36] showed that there was a significant association (OR = 1.70, 95% CI: 1.24–2.33, $P < 0.001$) between the vacA i1 genotype and the development of PUD (Table 3, Fig. 2). The test for heterogeneity in Fig. 2 showed no significant difference among articles ($P = 0.14$).

Then, sub-analysis, in terms of type of PUD (i.e. duodenal ulcer and gastric ulcer) was performed. The study by Douraghi *et al*, Effrosini *et al* and Daniel *et al* [32–34] did not provide the formation on the subgroups, and thus was excluded from the sub-analysis. Analysis of the remaining six studies' [21–24, 31, 36] results indicated that the vacA i1 genotype was associated with the risk of gastric ulcer, compared to NUD (OR = 2.59, 95% CI: 1.05–6.35, $P = 0.040$; Fig. 3a); no heterogeneity was found among the studies ($P = 0.200$). However, there was no significant association between the i1 genotype and duodenal ulcers (OR = 1.04, 95% CI: 0.61–1.76, $P = 0.90$; Fig. 3b); however, significant heterogeneity among studies was observed ($P = 0.009$).

Sub-analysis in relation to the different regions worldwide showed that the association between the i1 genotype and PUD existed in studies from Europe, but not in studies from Asia (Fig. 4).

Association between infection of H. pylori strains of vacA i1 genotype and gastric cancer

Analysis of eight articles showed that vacA i1 genotype was associated with an increased risk of gastric cancer (OR = 3.90, 95% CI: 2.64–5.78, $P < 0.001$, Fig. 5); there

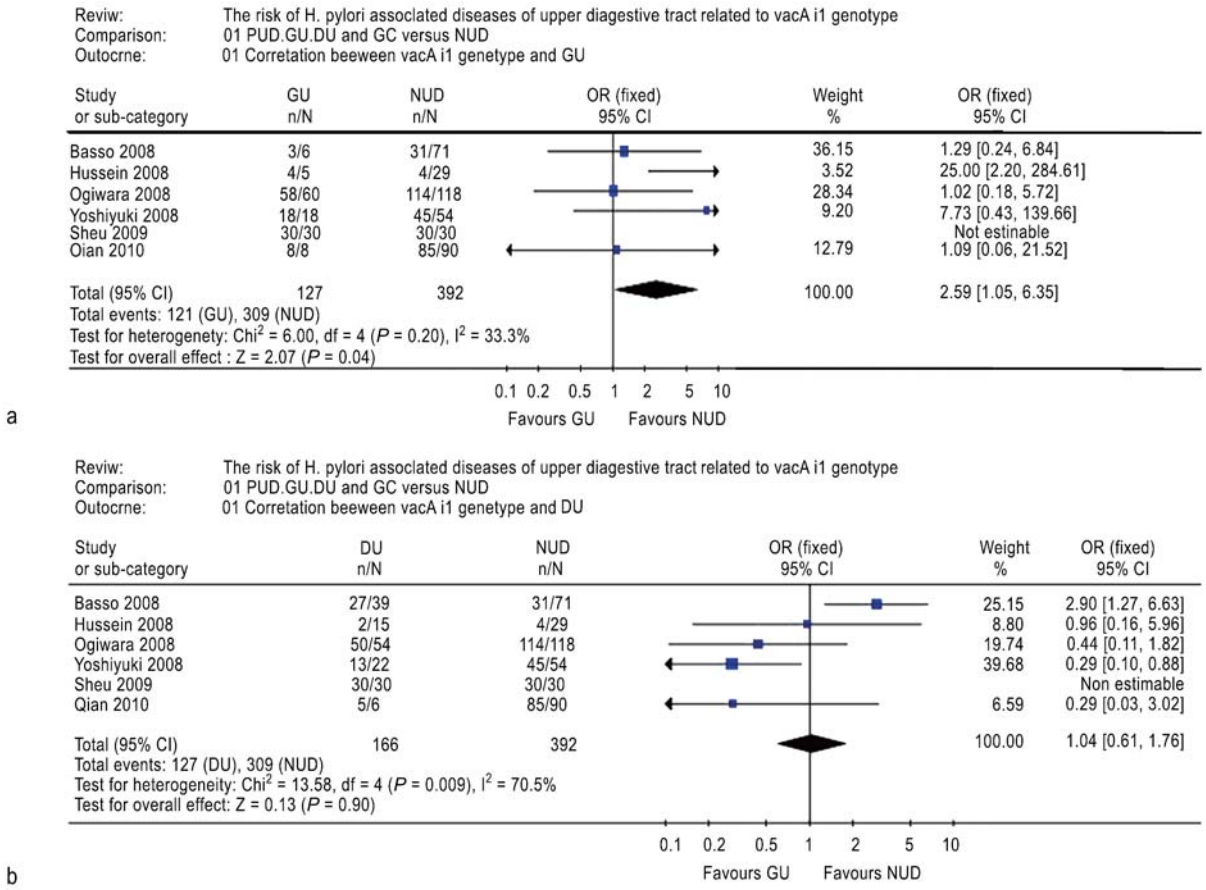

Fig. 3 Sub-analyses of associations of the vacA i1 genotype with gastric ulcer (a) and duodenal ulcer (b), with non-ulcer dyspepsia as a reference. Chronic gastritis is included in the NUD group

was no heterogeneity among the studies ($P < 0.001$).

In contrast to PUD, sub-analysis in relation to the different regions worldwide showed that the association between the i1 genotype and gastric cancer existed in studies from Europe and Asia (Fig. 6).

Discussion

In the present meta-analysis, we found that 98.1% of s1/m1 vacA genotype strains of *H. pylori* were the i1 type, 100.0% of s2/m2 genotype strains were the i2 type, and 66.6% of s1/m2 genotype strains were the i1 type. We confirmed that the i1 genotype was associated with PUD and gastric cancer; however, the association varied among different geographic regions.

The vcaA amino-terminal fragment of 422 residues, consisting of p37 and the first 111 residues of p58, appears to be essential for the induction of intracellular vacuolation [37]. This fragment includes the i-region, but not the m-region. Three clusters of the i1 region, clusters A, B, and C, have been defined [10]. The N-terminal region of vacA has a strong tendency to interact with the membrane, and some residues are protected from proteolysis when vacA interacts with lipid membranes [38]. One such "protected" region is located just 6 residues downstream of the i-region cluster B and included the cluster C, and this could explain why cluster C had a greater effect on vacuolating activity [10]. Therefore, the vacA i-region is suggested to be a more important determinant of H pylori toxicity than vacA s- or m-type and the best independent marker of vacA-associated pathogenicity [10].

This hypothesis was further supported by Basso [21] and Douraghi [32], who demonstrated that the vacA i1 genotype was a risk factor for duodenal ulcer in Italian population and for gastric cancer in both Italian and Iranian population. Hussein [31] and Yoshiyuki [33] also showed that the i1 genotype was closely associated with an increase risk of gastric ulcer in Middle Eastern and Japanese populations. However, such as associations were not confirmed by Oqiwara [22], Sheu [23] and Qian [24], in the South, Southeast and Eastern Asian populations.

As reported in most previous studies [10, 21–24, 31–33], this meta-analysis confirmed that nearly all s1/m1 were the i1 type, and all s2/m2 were the i2 type, whereas and s1/m2 could be either the i1 or i2 type. In the present meta-anal-

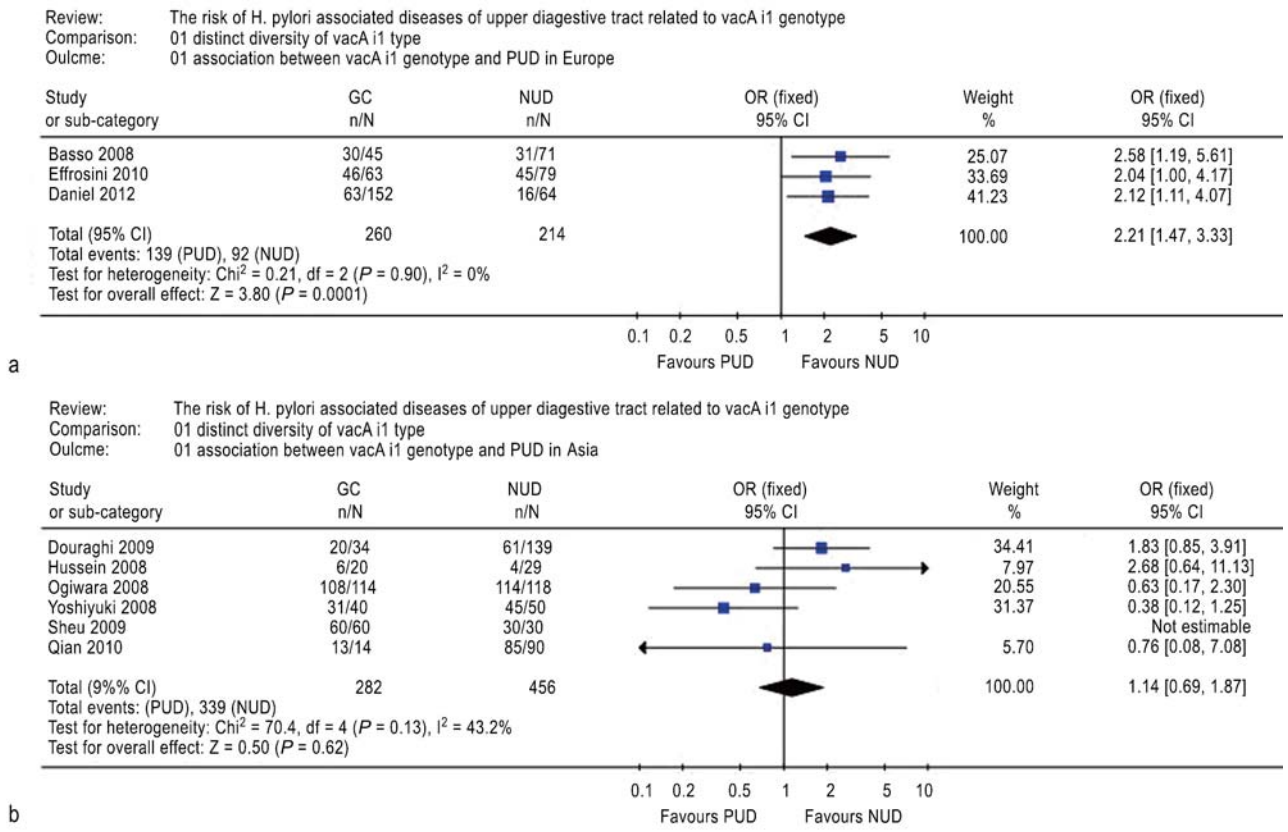

Fig. 4 Analyses of association between the i1 genotype and peptic ulcer disease (PUD) in studies from Europe (a) and Asia (b), respectively. Chronic gastritis is included in the NUD group

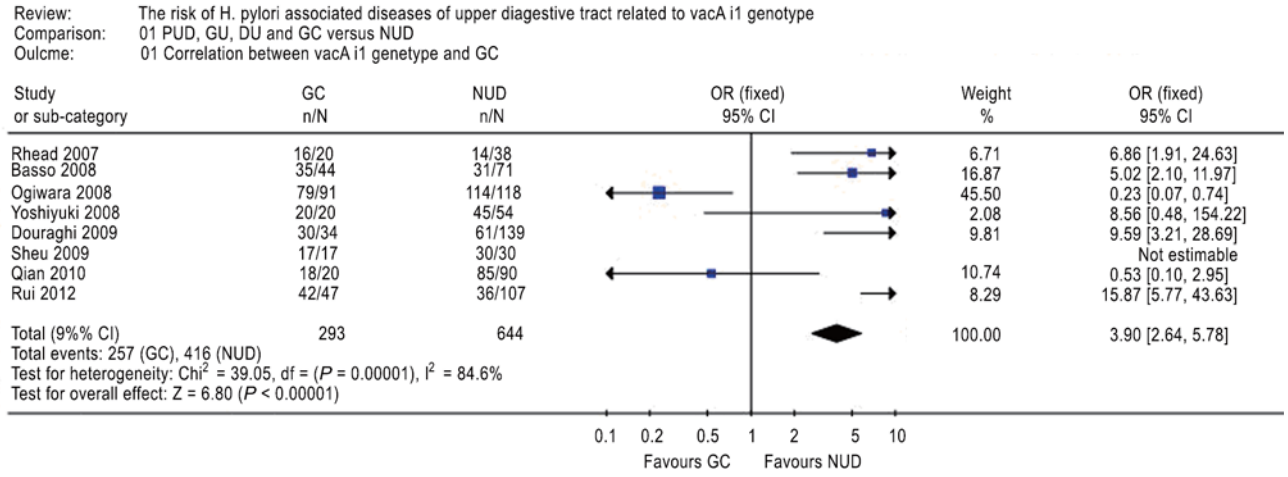

Fig. 5 Analyses of association between vacA i1 genotype and gastric cancer, with non-ulcer dyspepsia as a reference. Chronic gastritis is included in the NUD group

ysis, we integrated all of above genotype information, and found that the vacA i1 genotype was significantly associated with PUD and gastric cancer. However, sub-analysis revealed an association of the i1 genotype with gastric ulcer, but not with duodenal ulcer.

In the sub-analyses according to studies from different regions, it was found that the i1 genotype was only associated with any H. pylori-associated diseases such as PUD and gastric cancer in a Europe population (i.e. Italian) and Middle Eastern populations (i.e. Iranian and Irapi), but not in populations in the other regions of Asia. The discrepancies among the studies may be largely explained

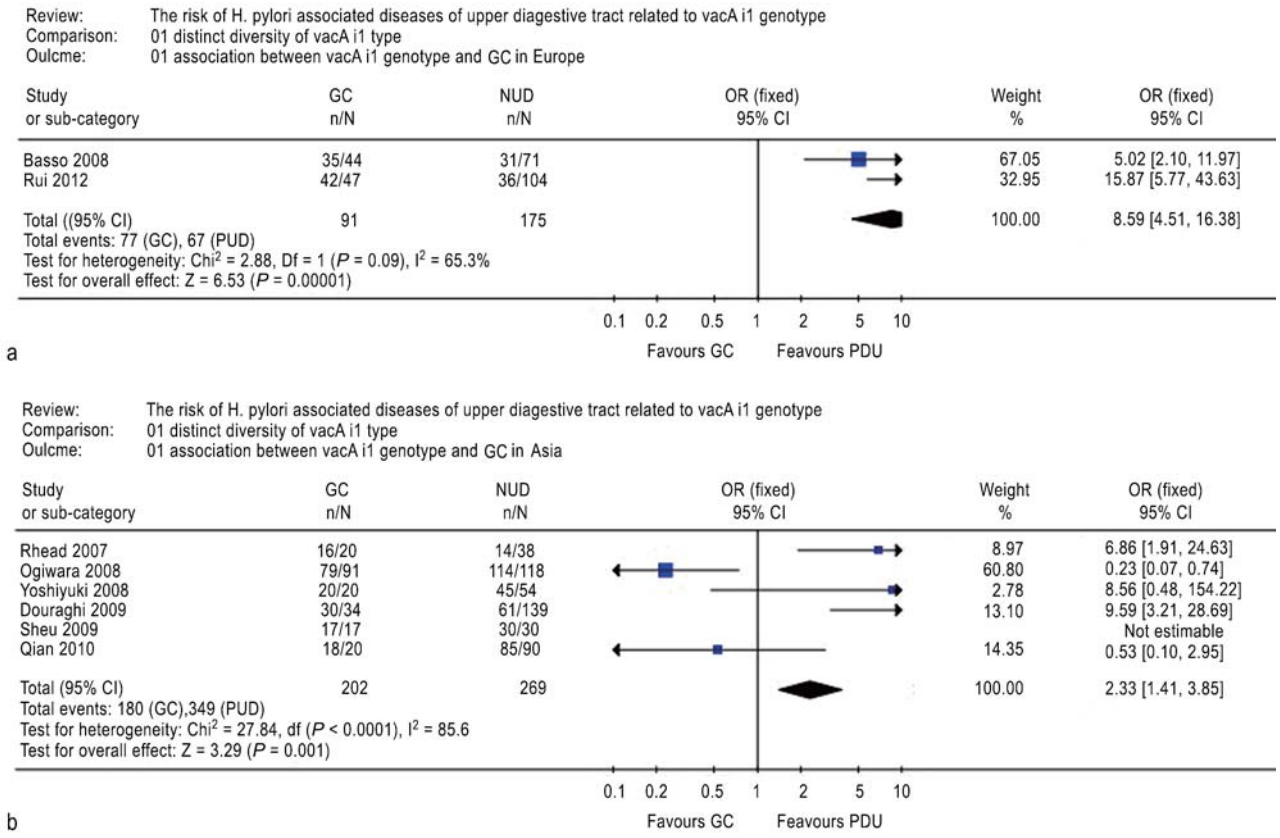

Fig. 6 Analyses of association between the i1 genotype and gastric cancer (GC) in studies from Europe (a) and Asia (b), respectively

by the fact that almost all strains in the other regions of Asian populations are the i1 genotype whereas only approximately 50% of strains in European and Middle Eastern populations are the i1 genotype as shown in Table 1 [10, 21–24, 32]. Statistically, a significant association between the i1 genotype and gastrointestinal diseases (if any) would be more likely to be detected when the frequency of the i1 genotype approaches to 50%, and a large sample size would be required when the frequency is over 90%. Unfortunately, the sample sizes in the included studies were all relevantly small or very small (ranging form 73 to 313). Therefore, studies with larger sample sizes are required to further confirm the findings in the present meta-analysis.

A few limitations existed in the present meta-analysis. First, as described earlier, the number of enrolled studies, the numbers of cases included in the studies, and the numbers of patients with each of different gastrointestinal diseases were small, which may affect the results. However, even with the small number of studies with relatively small number of cases, the present meta-analysis did demonstrated positive associations of the i1 genotype with PUD (mainly gastric ulcer) and gastric cancer, especially in populations in which the strains of i1 genotype are less predominant. It is expected that the associations would be revealed populations in which the i1 genotype strains are predominant if the sample sizes in the studies are increased, as described above. Second, only one study [21] was from Italy, a Western country, which reported positive associations of the i1 genotype with PUD and gastric cancer. It is noted that this was the only study showing a positive association between the i1 genotype and duodenal ulcer, whereas another study from Japan showed a negative association [33]. Third, there lacked well-designed epidemiological studies that specifically determine the associations of the i1 genotype with PUD or gastric cancer with reference to general populations or healthy volunteers. Finally, a family history of gastrointestinal diseases, especially gastric cancer, was not reported in all the included studies, and thus, the impact of a family history on the results is unknown. Thus, a family history should be taken into account in the future studies.

In conclusion, the vacA i1 genotype is associated with an increased risk of the development of peptic ulcer disease (mainly gastric ulcer) and gastric cancer, especially in a European population and Middle Eastern populations. However, further studies with large sample sizes from other countries or regions in the world are required to elucidate the associations between vacA i-region and

H. pylori-associated disease of the upper digestive tract.

Acknowledgements

We thank Medjaden Bioscience Limited for assisting in the preparation of the manuscript. Li Shang conducted the data extraction, data analysis and wrote the first draft of the manuscript. Guoxin Zhang contributed to the design of the study and performed the statistical analysis. Xueying Tian and Juan Qian performed the literature search and study selection, and Chunli Tang conducted the data extraction.

Conflicts of interest

The authors indicated no potential conflicts of interest.

References

1. Everhart JE. Recent developments in the epidemiology of Helicobacter pylori. Gastroenterol Clin North Am, 2000, 29: 559–579.
2. McColl KE. Clinical practice. Helicobacter pylori Infection. N Engl J Med, 2010, 362: 1597–1604.
3. Atherton JC. The pathogenesis of Helicobacter pylori-induced gastroduodenal diseases. Annu Rev Pathol, 2006, 1: 63–96.
4. Iwamoto H, Czajkowsky DM, Cover TL, et al. VacA from Helicobacter pylori: a hexameric chloride channel. FEBS Lett, 1999, 450: 101–104.
5. Atherton JC, Peek RM Jr, Tham KT, *et al.* Clinical and pathological importance of heterogeneity in vacA, the vacuolating cytotoxin gene of Helicobacter pylori. Gastroenterology, 1997, 112: 92–99.
6. Kidd M, Lastovica AJ, Atherton JC, *et al*. Heterogeneity in the Helicobacter pylori vacA and cagA genes: association with gastroduodenal disease in South Africa? Gut, 1999, 45: 499–502.
7. Miehlke S, Kirsch C, Agha-Amiri K, *et al*. The Helicobacter pylori vacA s1, m1 genotype and cagA is associated with gastric carcinoma in Germany. Int J Cancer, 2000, 87: 322–327.
8. Sugimoto M, Yamaoka Y. The association of vacA genotype and Helicobacter pylori-related disease in Latin American and African populations. Clin Microbiol Infect, 2009, 15: 835–842.
9. Hussein Ezzat AH, Ali MH, El-Seidi EA, *et al.* Genotypic characterization of Helicobacter pylori isolates among Egyptian patients with upper gastrointestinal diseases. Chinese-German J Clin Oncol, 2012, 11: 15–23.
10. Rhead JL, Letley DP, Mohammadi M, *et al*. A new Helicobacter pylori vacuolating cytotoxin determinant, the intermediate region, is associated with gastric cancer. Gastroenterology, 2007, 133: 926–936.
11. Letley DP, Rhead JL, Twells RJ, *et al.* Determinants of nontoxicity in the gastric pathogen Helicobacter pylori. J Biol Chem, 2003, 278: 26734–26741.
12. Ji X, Fernandez T, Burroni D, *et al.* Cell specificity of the Helicobacter pylori cytotoxin is determined by a short region in the polymorphic midregion. Infect Immun, 2000, 68: 3754–3757.
13. Pagliaccia C, de Bernard M, Lupetti P, *et al*. The m2 form of the Helicobacter pylori cytotoxin has cell type-specific vacuolating activity. Proc Natl Acad Sci U S A, 1998, 95: 10212–10217.
14. Atherton JC, Cao P, Peek RM Jr, *et al*. Mosaicism in vacuolating cytotoxin allele of Helicobacter pylori-Association of specific vacA types with cytotox in production and peptic ulceration. J Biol Chem, 1995, 270: 17771–17777.
15. McClain, MS, Cao P, Iwamoto H, *et al*. A 12-amino-acid segment, present in type s2 but not type s1 Helicobacter pylori VacA proteins, abolishes cytotoxin activity and Alters membrane channel formation. J Bacteriol, 2001, 183: 6499–6508.
16. Van Doorn LJ, Figueiredo C, Mégraud F, *et al*. Geographic distribution of vacA allelic types of Helicobacter pylori. Gastroenterology, 1999, 116: 823–830.
17. Van Doorn LJ, Figueiredo C, Sanna R, *et al.* Expanding allelic diversity of Helicobacter pylori vacA. J Clin Microbiol, 1998, 36: 2597–2603.
18. Chung C, Olivares A, Torres E, *et al*. Diversity of VacA intermediate region among Helicobacter pylori strains from several regions of the world. J Clin Microbiol, 2010, 48: 690–696.
19. Douraghi M, Shokrgozar M, Saberi S, *et al*. The intermediate allele of vacA and the vacuolation induced by s1m2 genotypes of H. pylori strains. Helicobacter, 2009, 14: 373.
20. Ogiwara H, Sugimoto M, Ohno T, *et al.* Structure-function and clinical relationships of Helicobacter pylori vacA. Gastroenterology, 2009, 136: 5 (A548).
21. Basso D, Zambon CF, Letley DP, *et al*. Clinical relevance of Helicobacter pylori cagA and vacA gene polymorphisms. Gastroenterology, 2008, 135: 91–99.
22. Ogiwara H, Graham DY, Yamaoka Y. vacA i-region subtyping. Gastroenterology, 2008, 134: 1267–1276.
23. Sheu SM, Hung KH, Sheu BS, *et al*. Association of nonsynonymous substitutions in the intermediate region of the vacA gene of helicobacter pylori with gastric diseases in Taiwan. J Clin Microbiol, 2009, 47: 249–251.
24. Qian B, Shang Li, Xiaolin Pan, *et al*. The assciations between H.pylori vacA i-region genotypes and peptic ulcer and gastric cancer in Jiangsu population. Chin J Digest (Chinese), 2010, 30: 402–404.
25. Ogiwara H, Sugimoto M, Ohno T, *et al*. Role of deletion located between the intermediate and middle regions of the Helicobacter pylori vacA gene in cases of gastroduodenal diseases. J Clin Microbiol, 2009, 47: 3493–3500.
26. Ferreira RM, Letley D, Carneiro F, *et al*. H. pylori vacA intermediate region i1 strains are associated with more severe histologic features of chronic gastritis and increased gastric carcinoma risk in Portugal. Helicobacter, 2009, 14: 4(377).
27. Tuncel IE, Hussein NR, Bolek BK, *et al*. Helicobacter pylori virulence factors and their role in peptic ulcer diseases in Turkey. Acta Gastroenterol Belg, 2010, 73: 235–238.
28. Sgouras DN, Panayotopoulou EG, Papadakos K, *et al*. CagA and vacA polymorphisms do not correlate with severity of histopathological lesions in Helicobacter pylori-infected greek children. J Clin Microbiol, 2009, 47: 2426–2434.
29. Kim YS, Kim N, Kim JM, *et al*. Helicobacter pylori genotyping findings from multiple cultured isolates and mucosal biopsy specimens: strain diversities of Helicobacter pylori isolates in individual hosts. Eur J Gastroenterol Hepatol, 2009, 21: 522–528.
30. Sugimoto M, Zali MR, Yamaoka Y. The association of vacA genotypes and Helicobacter pylori-related gastroduodenal diseases in the Middle East. Eur J Clin Microbiol Infect Dis, 2009, 28: 1227–1236.
31. Hussein NR, Mohammadi M, Talebkhan Y, *et al*. Differences in virulence markers between Helicobacter pylori strains from Iraq and those from Iran: potential importance of regional differences in H.pylori-associated disease. J Clin Microbiol, 2008, 46: 1774–1779.
32. Douraghi M, Talebkhan Y, Zeraati H, *et al.* Multiple gene status in Helicobacter pylori strains and risk of gastric cancer development. Digestion, 2009, 80: 200–207.
33. Panayotopoulou EG, Sgouras DN, Papadakos KS, *et al*. CagA and

VacA polymorphisms are associated with distinct pathological features in Helicobacter pylori-infected adults with peptic ulcer and non-peptic ulcer disease, Greece. J Clin Microbiol, 2010, 48: 2237–2239.
34. Yordanov D, Boyanova L, Markovska R, *et al*. Significance of Helicobacter pylori vacA region genotyping-a Bulgarian study, Bulgaria. Diagn Microbiol Infect Dis, 2012, 74: 253–257.
35. Ferreira RM, Machado JC, Letley D, *et al.* A novel method for genotyping the Helicobacter pylori vacA intermediate region directly in gastric biopsy specimens, Portugal. J Clin Microbiol, 2012, 50: 3983–3989.
36. Ito Y, Inagaki T, Yamakawa A, *et al.* Helicobacter pylori vacA I-region genotype is associated with an increased risk of gastric cancer, vacuolating cytotoxin activity, and CagA status in Okinawa, Japan. Gastroenterology, 2008, 134, S1: A565.
37. Ye D, Willhite DC, Blanke SR. Identification of the minimal intracellular vacuolating domain of the Helicobacter pylori vacuolating toxin. J Biol Chem, 1999, 274: 9277–9282.
38. Wang X, Wattiez R, Paggliacia C, *et al.* Membrane topology of VacA cytotoxin from H. Pylori. FEBS Lett, 2000, 481: 96–100.

DOI 10.1007/s10330-014-0029-9
Cite this article as: Rui JP, Chen GC, Mao BN, *et al*. The vacA i1 genotype of Helicobacter pylori is associated with peptic ulcer and gastric cancer: A meta-analysis. Chinese-German J Clin Oncol, 2014, : .