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Association between vacA genotypes and the risk of duodenal ulcer: a meta-analysis

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Abstract Epidemiological studies have reported the relationship between vacuolating cytotoxin A (vacA) s-/m-region genotypes and duodenal ulcer (DU), but the results remained inconclusive. We performed the present meta-analysis to investigate a more authentic association between vacA s-/m- region genotypes and DU. Literature search was performed by searching Embase, PubMed and ISI Web of Science databases as well as checking references from identified articles, reviews and the abstracts presented at related scientific societies meetings. The association was assessed by combined odds ratio (OR) with 95 % confidence interval (CI). A total of 42 studies were included in our final meta-analysis. The combined ORs (95 % CIs) showed that vacA s1 (OR = 2.96, 95 % CI = 2.34–3.75), m1 (OR = 1.46, 95 % CI = 1.05–2.04) and s1m1 (OR = 1.89, 95 % CI = 1.47–2.42) were associated with increased DU risk significantly in the overall studied population. Subgroup analyses by ethnicity showed that vacA s1 increased the risk of DU in Asian countries (OR = 1.92, 95 % CI = 1.30–2.83), European countries (OR = 3.58, 95 % CI = 2.13–6.03) and Latin American countries (OR = 4.20, 95 % CI = 2.21–7.98); vacA m1 increased the risk of DU in Latin American countries (OR = 2.98, 95 % CI = 1.59–5.56); vacA s1m1 increased the risk of DU in Asian countries (OR = 2.04, 95 % CI = 1.12–3.73) and Latin American countries (OR = 2.05, 95 % CI = 1.20–3.48); vacA s2m1 increased the risk of DU in Latin American countries (OR = 2.30, 95 % CI = 1.17–4.50). The data suggest that genotype testing of vacA s- and m- region will be useful in screening susceptible individuals for DU development.

Keywords Duodenal ulcer · Genotype · Helicobacter pylori · Meta-analysis · Vacuolating cytotoxin A

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Introduction

Duodenal ulcer (DU) is one type of peptic ulcer in duodenum, which may result from infection of Helicobacter pylori (*H. pylori*) bacteria, overuse of alcohol and medications (aspirin and non-steroidal anti-inflammatory drugs) [1, 2]. Among these etiological factors, *H. pylori* infection was the most common one [3], which could lead to increased gastric acid, degradation of mucus barrier and eventual ulceration. However, it was reported that >20 % of patients with *H. pylori* infection would develop DU [4].

The reason why the *H. pylori*-induced pathologies are different is unclear. The vacuolating cytotoxin A (vacA), one of major virulence factors, has been reported to be involved. The vacA protein was encoded by the polymorphic *H. pylori* vacA gene. It is a secreted cytotoxin being capable to form vacuoles in gastric epithelial cells [5]. The vacA gene is polymorphic, distributed in three principal regions: the signal (s), intermediate (i) and middle (m) regions. Each type can be further divided into two main subtypes, numbered 1 and 2 [6, 7]. The two subtypes of the s-region (s1 and s2) and m-region (m1 and m2) were mostly studied. Different s or m genotypes of *H. pylori*

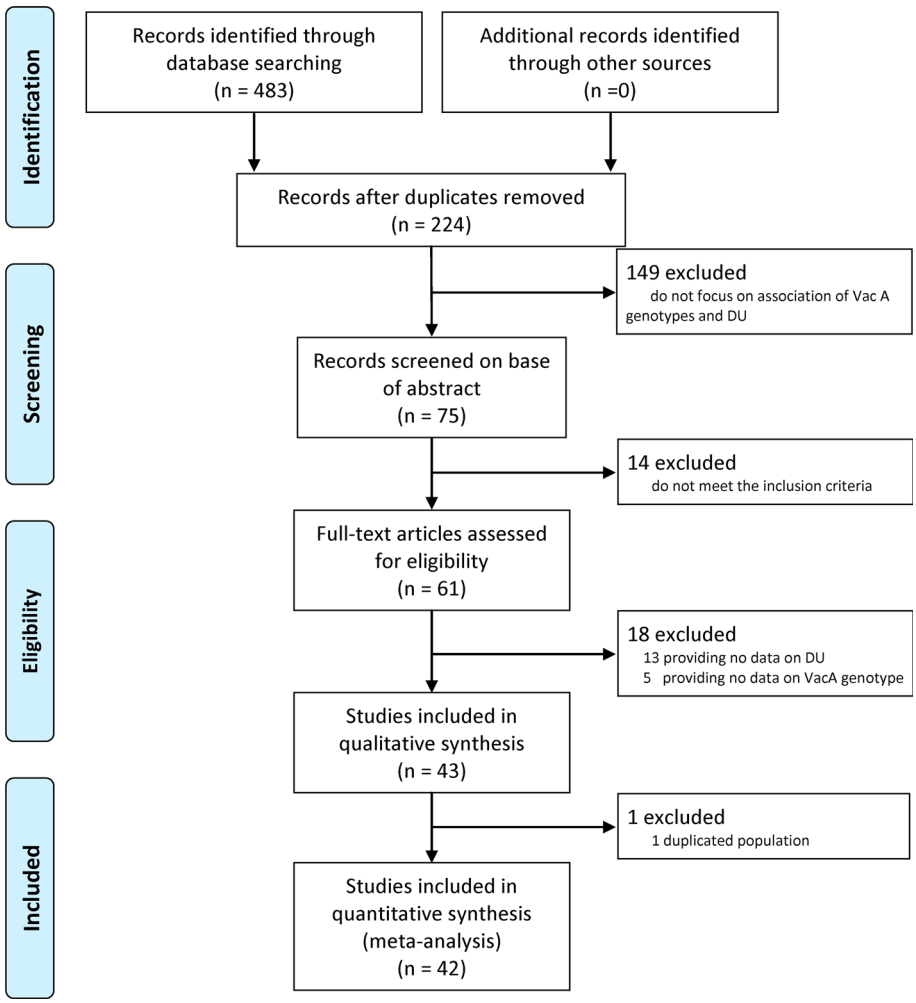

Fig. 1 Flow diagram of the study selection process

varied in the vacuolating activities, which might contribute to different clinical outcomes [6]. It was reported that vacA s1 genotype produced a large amount of cytotoxin, whereas s2 secreted few or no cytotoxin at all. In addition, both s1m1 and s1m2 subtypes were able to produce high or moderate levels of vacA, whereas s2m2 subtype was not [6]. Until now, numerous studies have reported the association between DU and vacA gene s- and m- region genotypes [8–50], but the conclusions were inconsistent. In addition, the relatively small sample size in each single published study may limit the credibility of the conclusions. Therefore, we designed this meta-analysis to evaluate the association between vacA s-/m-region genotypes and DU risk.

Materials and methods

Literature search

The meta-analysis followed the preferred reporting items for systematic reviews and meta-analyses (PRISMA) criteria [51]. We collected all published studies on humans up to Aug. 15, 2013 by systematically searching Embase (1966 to Aug. 2013), PubMed (up to Aug. 2013) and ISI Web of Science (2003 to Aug. 2013) with search strategy: "duodenal ulcer" AND "vacA OR vacuolating cytotoxin A" AND "variation OR variant OR mutation OR polymorphism OR genotype". The language was limited to English and Chinese. In addition, references from identified articles, reviews and the abstracts presented at related scientific societies meetings were also checking.

Inclusion criteria

The inclusion criteria were as follows: (1) studies of the association between vacA s- or m-region genotypes and DU; (2) studies being published; (3) studies with case–control design; (4) studies with sufficient data for estimating odds ratio (OR) and 95 % confidence interval (CI). Two investigators (Zhang BB and Yang B) screened the title, abstract and full text of each study to determine inclusion independently. If they could not reach a consensus, a third author (Li Y) was consulted.

Table 1 Characteristics of included studies

Author	Country	Year	Patients N	Sex ratio male/female	Age (mean)	s1N, (%)	s2N, (%)	m1N, (%)	m2N, (%)	s1m1N, (%)	s1m2N, (%)	s2m1N, (%)	s2m2N, (%)	Control N
Basso	Italy	1998	29	18/11	51	24 (82.8)	5 (17.2)	16 (55.2)	13 (44.8)					25
Go	USA	1998	38			32 (84.2)	6 (15.8)	16 (53.3)	14 (46.7)	16 (53.3)	8 (26.7)	0 (0.0)	6 (20.0)	39
Strobel	Germany	1998	106		51	101 (96.2)	4 (3.8)	30 (97.6)	52 (2.4)					52
Warburton	UK	1998	50		52[a]	49 (98.0)	1 (2.0)							99
Yamaoka	Japan	1998	78							74 (98.7)	1 (1.3)	0 (0.0)	0 (0.0)	243
Henning	Poland	1999	80			111 (95.7)	5 (4.3)	58 (50.0)	58 (50.0)					49
Sadakane	Japan	1999	23		45	22 (95.7)	1 (4.3)							15
Audibert	France	2000	46			34 (77.3)	10 (22.7)	18 (40.9)	26 (59.1)					74
De Gusmão	Brazil	2000	24	16/8	11.7	19 (100.0)	0 (0.0)	16 (84.2)	3 (15.8)					41
Figueiredo	Netherlands	2001	73		43.2	39 (86.7)	6 (13.3)	21 (47.7)	23 (52.3)					171
Miehlke	Germany	2001	49	29/20	47[a]	44 (89.8)	5 (10.2)	17 (37.0)	29 (63.0)	15 (32.6)	26 (56.5)	0 (0.0)	5 (10.9)	32
Park	Korea	2001	57			53 (93.0)	4 (7.0)	45 (78.9)	12 (21.1)	45 (78.9)	8 (14.0)	0 (0.0)	4 (7.0)	26
Wong	China	2001	34			30 (100.0)	0 (0.0)	9 (30.0)	21 (70.0)	9 (30.0)	21 (70.0)	0 (0.0)	0 (0.0)	38
Ashour	Brazil	2002	25	15/10	40.6	18 (90.0)	2 (10.0)	18 (90.0)	2 (10.0)	18 (90.0)	0 (0.0)		2 (10.0)	21
Chattopadhyay	India	2002	52			44 (97.8)	1 (2.2)	29 (64.4)	16 (35.6)					48
Choe	Korea	2002	31							25 (89.3)	3 (10.7)	0 (0.0)	0 (0.0)	42
Smith	Germany	2002	19			19 (100.0)	0 (0.0)	5 (26.3)	14 (73.7)	5 (26.3)	14 (73.7)		0 (0.0)	22
Brito	Brazil	2003	21			17 (81.0)	4 (19.0)							40
Leodolter	Germany	2003	35	21/14		28 (80.0)	7 (20.0)	18 (51.4)	17 (48.6)					35
Perng	China	2003	52		60.8	52 (100.0)	0 (0.0)	11 (32.4)	23 (67.6)					24
Qiao	China	2003	20			19 (95.0)	1 (5.0)	12 (60.0)	8 (40.0)					100
Ribeiro	Brazil	2003	62			48 (85.7)	8 (14.3)	22 (39.3)	34 (60.7)	22(39.3)	26 (46.4)		8 (14.3)	30
Yin	China	2003	34							9 (30.0)	21 (70.0)			38
Ando	UK	2004	10			10 (100.0)	0 (0.0)	10 (100.0)	0 (0.0)					20
Lin	China	2004	66		58.9	64 (97.0)	2 (3.0)	16 (37.2)	27 (62.8)					25
Salih	Turkey	2005	11			11 (100.0)	0 (0.0)	11 (100.0)	0 (0.0)					8
Siavoshi	Iran	2005	58	32/26	42[a]	46 (79.3)	12 (20.7)			4 (26.7)	9 (60.1)	1 (6.6)	1 (6.6)	61
Erzin	Turkey	2006	30			28 (100.0)	0 (0.0)	15 (53.6)	13 (46.4)	15 (53.6)	13 (46.4)	0 (0.0)	0 (0.0)	30
Bolek	Turkey	2007	35							21 (70.0)	8 (26.7)	1 (3.3)	0 (0.0)	10
Caner	Turkey	2007	16			15 (93.8)	1 (6.2)	5 (31.3)	11 (68.7)	5 (31.3)	10 (62.5)		1 (6.2)	30
Linpisarn	Thailand	2007	45			41 (91.1)	4 (8.9)	27 (65.9)	14 (34.1)					58
Proenca Modena	Brazil	2007	25			19 (86.4)	3 (13.6)	17 (77.3)	5 (22.7)	17 (77.3)	2 (9.1)		3 (13.6)	37
Tiwari	India	2007	28			18 (64.3)	10 (35.7)							24
Miciuleviciene	Lithuania	2008	37			28 (75.7)	9 (24.3)			6 (22.2)	12 (44.4)		9 (33.4)	44
Zhang	China	2008	101	59/42	41	96 (95.0)	5 (5.0)	18 (18.2)	81 (81.8)					133
Bindayna	Bahrain	2009	29							25 (86.2)	3 (10.4)		1 (3.4)	91
Nagiyev	Turkey	2009	73							67 (95.7)	3 (4.3)		0(0.0)	158
Salehi	Iran	2009	37			29(82.9)	6 (17.1)	23 (65.7)	12 (34.3)					32
Torres	Cuba	2009	46							30 (65.2)	5 (10.9)	0 (0.0)	11 (23.9)	51
Yakoob	Pakistan	2009	32			32 (100.0)	0 (0.0)							120
Dixit	India	2011	30			11 (78.6)	3 (21.4)							57

Table 1 continued

Author	Country	Year	Patients N	Sex ratio male/female	Age (mean)	s1N, (%)	s2N, (%)	m1N, (%)	m2N, (%)	s1m1N, (%)	s1m2N, (%)	s2m1N, (%)	s2m2N, (%)	Control N
Khan	Pakistan	2013	45							23 (60.5)	13 (34.2)	2 (5.3)		362
Author	Country	Year	Patients N	Sex ratio male/female	Age (mean)	s1N, (%)	s2N, (%)	m1N, (%)	m2N, (%)	s1m1N, (%)	s1m2N, (%)	s2m1N, (%)	s2m2N, (%)	Score
Basso	Italy	1998	29	11/14	52	15 (60.0)	10 (40.0)	8 (32.0)	17 (68.0)					6
Go	USA	1998	38			31 (81.6)	7 (18.4)	15 (51.7)	14 (48.3)	15(51.7)	7 (24.1)	0 (0.0)	7 (24.1)	5
Strobel	Germany	1998	106		47	36 (69.2)	16 (30.8)	19 (41.3)	27 (58.7)					5
Warburton	UK	1998	50		54[a]	84 (84.8)	15 (15.2)							5
Yamaoka	Japan	1998	78							228 (96.6)	7 (3.0)	0 (0.0)	1 (0.4)	6
Henning	Poland	1999	80			42 (71.2)	17 (28.8)	30 (40.0)	45 (60.0)					6
Sadakane	Japan	1999	23		47.8	14 (93.3)	1 (6.7)							6
Audibert	France	2000	46			54 (75.0)	18 (25.0)	35 (52.2)	32 (47.8)					6
De Gusmão	Brazil	2000	24	16/25	9.3	21 (58.3)	15 (41.7)	18 (51.4)	17 (48.6)					5
Figueiredo	Netherlands	2001	73		43.7	67 (54.5)	56 (45.5)	51 (37.2)	86 (62.8)					7
Miehlke	Germany	2001	49	16/16	47[a]	28 (87.5)	4 (12.5)	7 (21.9)	25 (78.1)	7 (21.9)	21 (65.6)	0 (0.0)	4 (12.5)	8
Park	Korea	2001	57			26 (100.0)	0 (0.0)	25 (96.2)	1 (3.8)	25 (96.2)	1 (3.8)	0 (0.0)	0 (0.0)	6
Wong	China	2001	34			36 (100.0)	0 (0.0)	13 (36.1)	23 (63.9)	13 (36.1)	23 (63.9)	0	0	6
Ashour	Brazil	2002	25	10/11	51.9	10 (55.6)	8 (44.4)	8 (44.4)	10 (55.6)	8 (44.4)	2 (11.2)		8 (44.4)	7
Chattopadhyay	India	2002	52			39 (97.5)	1 (2.5)	26 (65.0)	14 (35.0)					7
Choe	Korea	2002	31							34 (85.0)	6 (15.0)	0 (0.0)	0 (0.0)	6
Smith	Germany	2002	19			21 (95.5)	1 (4.5)	5 (22.7)	17 (77.3)	5 (22.7)	16 (72.7)		1 (4.6)	5
Brito	Brazil	2003	21			26 (74.3)	9 (25.7)							6
Leodolter	Germany	2003	35	21/14		17 (48.6)	18 (51.4)	9 (25.7)	26 (74.3)					6
Perng	China	2003	52		50.7	24 (100.0)	0 (0.0)	8 (42.1)	11 (57.9)					6
Qiao	China	2003	20			89 (89.0)	11 (11.0)	49 (49.0)	51 (51.0)					5
Ribeiro	Brazil	2003	62			13 (61.9)	8 (38.1)	7 (33.3)	14 (66.7)	7 (33.3)	6 (28.6)		8 (38.1)	6
Yin	China	2003	34							13 (36.1)	23 (63.9)			5
Ando	UK	2004	10			13 (100.0)	0 (0.0)	13 (100.0)	0 (0.0)					5
Lin	China	2004	66		51	25 (100.0)	0 (100.0)	8 (40.0)	12 (60.0)					6
Salih	Turkey	2005	11			7 (100.0)	0 (0.0)	1 (16.7)	5 (83.3)					5
Siavoshi	Iran	2005	58	27/34	45[a]	47 (77.0)	14 (23.0)			4 (12.1)	26 (78.8)	2 (6.1)	1 (3.0)	7
Erzin	Turkey	2006	30			23 (76.7)	7 (23.3)	10 (33.3)	20 (66.7)	10 (33.3)	13 (43.4)	0 (0.0)	7 (23.3)	6
Bolek	Turkey	2007	35							0 (0.0)	5 (71.4)	2 (28.6)	0 (0.0)	5
Caner	Turkey	2007	16			26 (86.7)	4 (13.3)	3 (10.0)	27 (90.0)	3 (10.0)	23 (76.7)		4 (13.3)	5
Linpisarn	Thailand	2007	45			49 (84.5)	9 (15.5)	29 (60.4)	19 (39.6)					5
Proenca Modena	Brazil	2007	25			18(62.1)	11 (37.9)	16 (89.7)	13 (10.3)	16 (55.2)	2 (6.9)		11 (37.9)	5
Tiwari	India	2007	28			12 (50.0)	12 (50.0)							5
Miciuleviciene	Lithuania	2008	37			18 (40.9)	26 (59.1)			5 (13.2)	8 (21.1)		25 (65.7)	5
Zhang	China	2008	101	80/53	59	121 (91.0)	12 (9.0)	45 (34.4)	86 (65.6)					5
Bindayna	Bahrain	2009	29							31 (34.0)	20 (22.0)		40 (44.0)	6
Nagiyev	Turkey	2009	73							115 (89.1)	9 (7.0)		5 (3.9)	5
Salehi	Iran	2009	37			16 (55.2)	13(44.8)	0 (0.0)	29 (100.0)					5

Table 1 continued

Author	Country	Year	Patients N	Sex ratio male/female	Age (mean)	s1N, (%)	s2N, (%)	m1N, (%)	m2N, (%)	s1m1N, (%)	s1m2N, (%)	s2m1N, (%)	s2m2N, (%)	Score
Torres	Cuba	2009	46							28 (56.0)	5 (10.0)	1 (2.0)	16 (32.0)	6
Yakoob	Pakistan	2009	32			109 (90.8)	11 (9.2)							6
Dixit	India	2011	30			13 (72.2)	5 (27.8)							5
Khan	Pakistan	2013	45							55 (45.8)	39 (32.5)	26 (21.7)		6

[a] Median age

Data extraction

The following data from the included studies were carefully extracted by the same two authors (Zhang BB and Yang B) independently: name of first author; publication year; country; total numbers, gender ratios and age of cases and controls; frequency of vacA s- or m-regions genotypes in cases and controls. Patients infected with strains of multiple vacA genotypes or undetected vacA genotypes were excluded from the meta-analysis.

Quality score assessment

The quality assessments of all the included studies were performed by the same two authors (Zhang BB and Yang B) independently using the Newcastle–Ottawa Scale (NOS) [52]. Disagreement was settled as described above. The NOS ranges from 0 (worst) to 9 points (best). Studies $\geq$7 points were assessed to be of high quality.

Statistical analysis

All the statistical analyses were performed using Stata 11.0. Pooled ORs with 95 % CIs were used to calculate the effects of vacA s- and m- region genotypes on the risk of DU respectively. Between-study heterogeneity was assessed by the I^2 statistic and Q-test [53, 54]. When there was significant between-study heterogeneity ($P < 0.10$ and $I^2 > 50$ %), the random effects model was used [55]. Otherwise, the fixed effects model was used [56]. Kappa statistic was used to evaluate the strength of agreement between reviewers regarding study selection. Subgroup analyses were performed according to ethnicity (Asia, Europe and Latin America). Galbraith plot was created to assess the source of heterogeneity graphically. Both Egger's test and Begg's funnel plot were applied to analyze publication bias [57].

Results

Study characteristics

The study selection process is detailed in Fig. 1. There were 483 potentially relevant articles after the searching. Based on the titles and abstracts, we included 61 studies for full-text assessment with a Kappa value of 0.87. After full-text assessment, a total of 43 studies were included in qualitative synthesis. As a same population was studied in two studies [32, 33], we included the more comprehensive one [32]. Hence, 42 articles [8–32, 34–50] were finally included in the meta-analysis with a Kappa value of 0.85. The main characteristics of the studies included are shown in Table 1. The

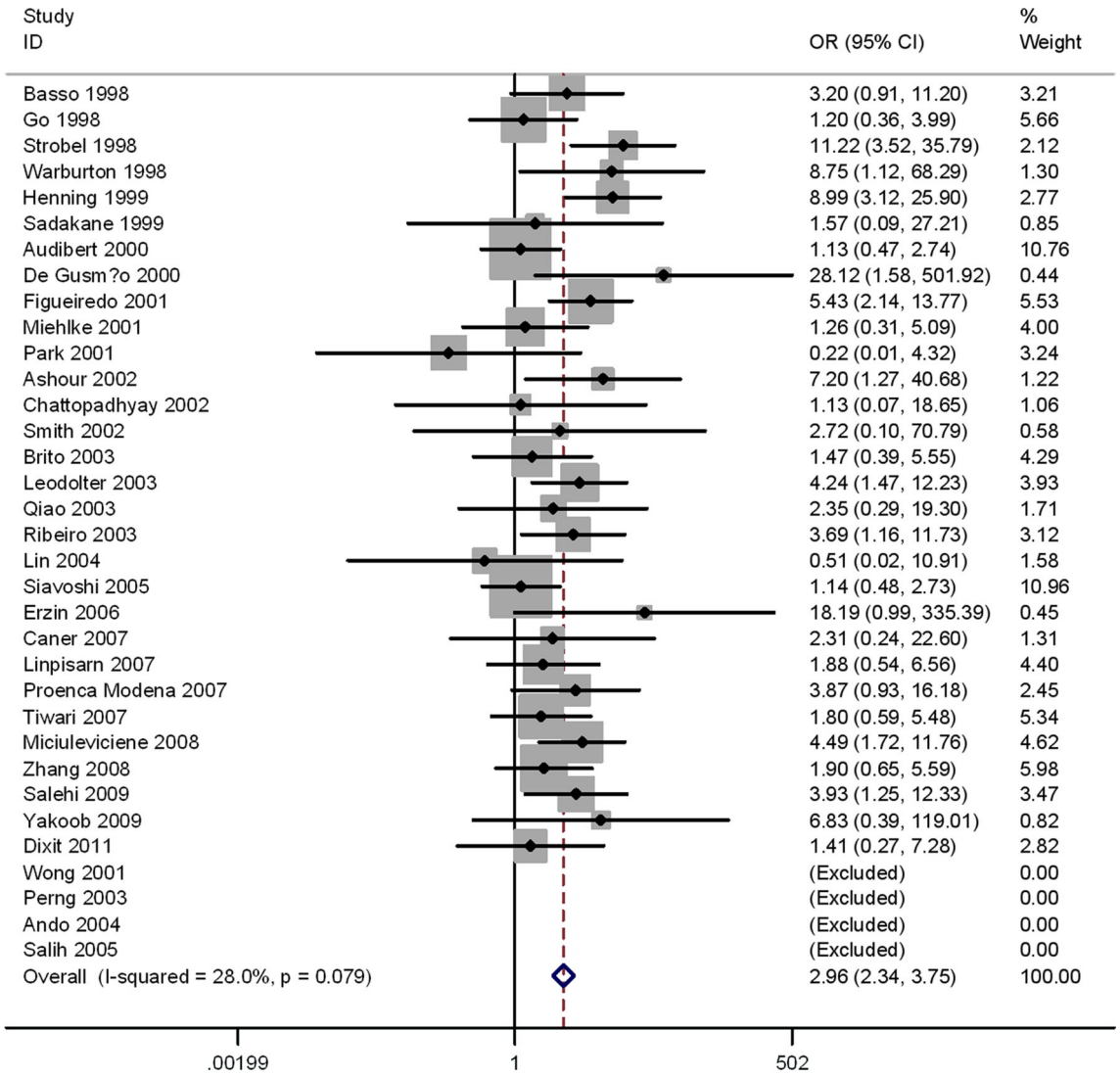

Fig. 2 Forest plot of the association between s1 and DU. The *white* diamonds indicate the summary pooled ORs and 95 % CIs

NOS results displayed that the average score was 5.69 (range 5–8). Studied countries included Cuba (one study), Bahrain (one study), Italy (one study), USA (one study), Poland (one study), France (one study), Netherlands (one study), Lithuania (one study), Thailand (one study), Iran (two studies), Korea (two studies), Japan (two studies), UK (two studies), Pakistan (two studies), India (three studies), Germany (four studies), Brazil (five studies), Turkey (five studies) and China (six studies). Six studies were from Latin America, 12 from Europe and 24 from Asia. One of the 42 articles was written in Chinese and the others in English.

Meta-analysis

There were 34 studies reporting the association between vacA s-region genotype and DU. The prevalence of vacA s1 was 90.9 % (1251/1376) in DU patients and 77.8 % (1190/1529) in controls. The combined OR and 95 %CI showed that vacA s1 increased the risk of DU significantly (OR = 2.96, 95 %CI = 2.34–3.75, Fig. 2). The prevalence of vacA s1 was 92.4 % (611/661) in DU patients and 87.1 % (676/776) in controls in Asian countries; 87.7 % (121/138) in DU patients and 63.3 % (88/139) in controls in European countries; 89.9 % (519/577) in DU patients and 69.4 % (426/614) in controls in Latin America countries. The combined ORs and 95 %CIs showed that vacA s1 increased the risk of DU in Asian countries (OR = 1.92, 95 %CI = 1.30–2.83), European countries (OR = 3.58, 95 %CI = 2.13–6.03) and Latin American countries (OR = 4.20, 95 %CI = 2.21–7.98).

There were 26 studies reporting the association between vacA m-region genotype and DU. The prevalence of vacA m1 is 48.8 % (503/1031) in DU patients and 41.7 % (458/1099) in controls. The combined OR and 95 %CI showed that vacA m1 increased the risk of DU significantly (OR = 1.46, 95 %CI = 1.05–2.04, Fig. 3). The prevalence of

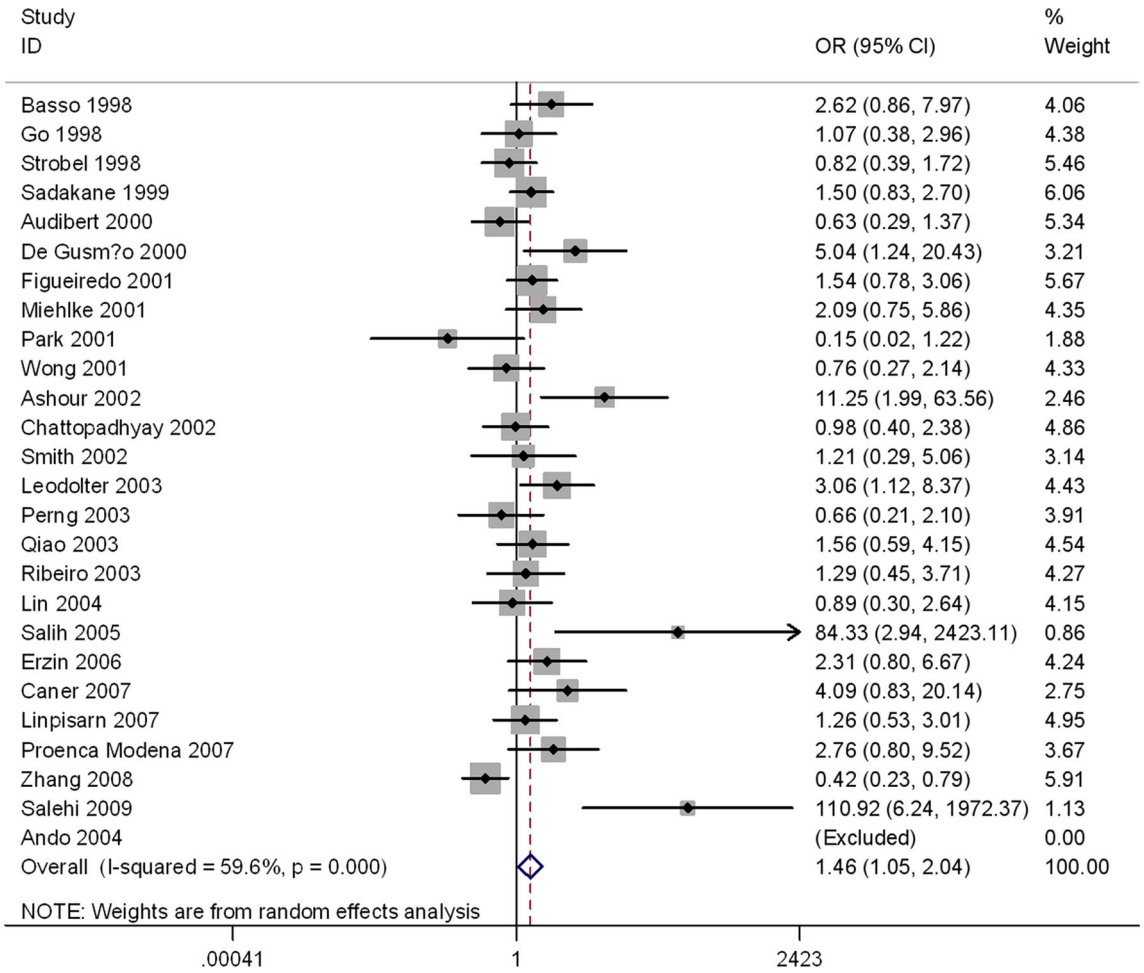

Fig. 3 Forest plot of the association between m1 and DU. The *white* diamonds indicate the summary pooled ORs and 95 % CIs

vacA m1 was 48.5 % (279/575) in DU patients and 41.9 % (247/590) in controls in Asian countries; 44.5 % (151/339) in DU patients and 40.3 % (162/402) in controls in European countries; 62.4 % (73/117) in DU patients and 47.6 % (49/103) in controls in Latin American countries. The combined ORs and 95 %CIs showed that vacA m1 increased the risk of DU in Latin American countries (OR = 2.98, 95 %CI = 1.59–5.56), whereas no significant associations were shown in Asian countries (OR = 1.27, 95 %CI = 0.75–2.16) and European countries (OR = 1.30, 95 %CI = 0.95–1.77).

There were 20 studies reporting the association between vacA s1m1 genotype and DU. The prevalence of vacA s1m1 is 63.3 % (451/712) in DU patients and 59.1 % (622/1053) in controls. The combined OR and 95 %CI showed that vacA s1m1 increased the risk of DU significantly (OR = 1.89, 95 %CI = 1.47–2.42, Fig. 4). The prevalence of vacA s1m1 was 72.2 % (322/446) in DU patients and 65.2 % (531/814) in controls in Asian countries; 34.4 % (42/122) in DU patients and 26.4 % (32/121) in controls in European countries; 60.4 % (87/144) in DU patients and 50.0 % (59/118) in controls in Latin America countries. The combined ORs and 95 %CIs showed that vacA s1m1 increased the risk of DU in Asian countries (OR = 2.04, 95 %CI = 1.12–3.73) and Latin American countries (OR = 2.05, 95 %CI = 1.20–3.48), whereas no significant association was shown in European countries (OR = 1.42, 95 %CI = 0.80–2.53).

There were 20 studies reporting the association between vacA s1m2 genotype and DU. The prevalence of vacA s1m2 was 28.9 % (206/712) in DU patients and 24.9 % (262/1053) in controls. The combined OR and 95 %CI showed no association between vacA s1m2 and DU (OR = 0.96, 95 %CI = 0.74–1.26, Fig. 5). The prevalence of vacA s1m2 was 25.3 % (113/446) in DU patients and 24.0 % (195/814) in controls in Asian countries; 49.2 % (60/122) in DU patients and 43.0 % (52/121) in controls in European countries; 22.9 % (33/144) in DU patients and 12.7 % (15/118) in controls in Latin American countries. The combined ORs and 95 %CIs showed no association between vacA s1m2 and DU in Asian countries (OR = 0.82, 95 %CI = 0.58–1.15), European countries

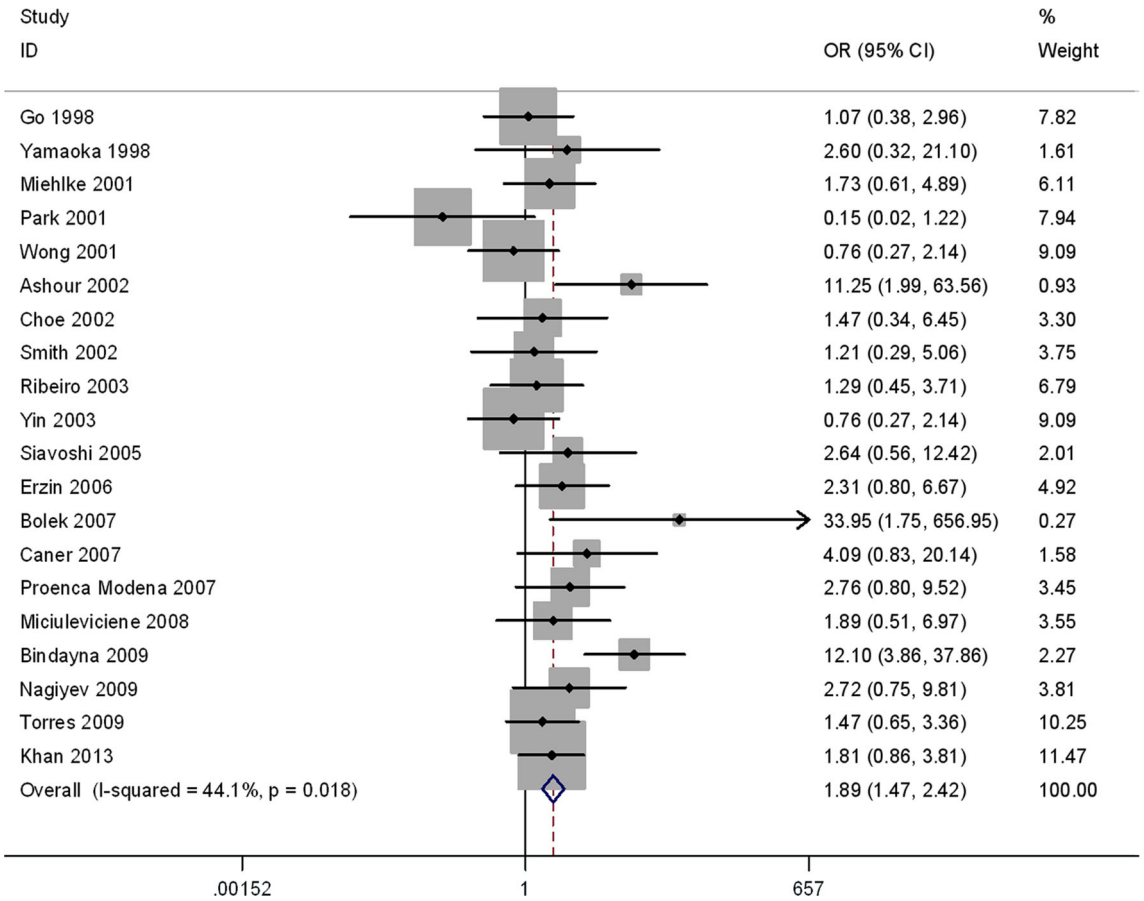

Fig. 4 Forest plot of the association between s1m1 and DU. The *white* diamonds indicate the summary pooled ORs and 95 % CIs

(OR = 1.21, 95 %CI = 0.70–2.08) and Latin America countries (OR = 1.33, 95 %CI = 0.65–2.71).

There were 20 studies reporting the association between vacA s2m1 genotype and DU. The prevalence of vacA s2m1 was 25.0 % (178/712) in DU patients and 22.2 % (234/1053) in controls. The combined OR and 95 % CI showed no association between vacA s2m1 and DU (OR = 1.64, 95 % CI = 0.81–3.30, Fig. 6). The prevalence of vacA s2m1 was 24.7 % (110/446) in DU patients and 23.6 % (192/814) in controls in Asian countries; 9.0 % (11/122) in DU patients and 8.3 % (10/121) in controls in European countries; 39.6 % (57/144) in DU patients and 27.1 % (32/118) in controls in Latin America countries. The combined ORs and 95 % CIs showed that vacA s2m1 increased the risk of DU in Latin American countries (OR = 2.30, 95 % CI = 1.17–4.50), whereas no associations were shown in Asian countries (OR = 1.27, 95 % CI = 0.37–4.39) and European countries (OR = 1.54, 95 % CI = 0.59–4.03).

Heterogeneity analysis

Significant heterogeneity existed in m1 genotype (I^2 = 60.0 %) and s2m1 genotype (I^2 = 65.7 %). To explore the source of heterogeneity graphically, we created Galbraith plots. Two studies [23, 43] were identified as the main source of heterogeneity for m1 genotype. Four studies [23, 37, 44, 50] were identified as the main contributors to heterogeneity for s2m1 genotype (Fig. 7).

Publication bias

No significant evidence of asymmetry was revealed by the funnel plots visually (Fig. 8). In addition, no statistical evidence of publication bias was found using Egger's regression: $P = 0.96$ for s1, $P = 0.70$ for m1, $P = 0.59$ for s1m1, $P = 0.28$ for s1m2 and $P = 0.54$ for s2m1, respectively.

Discussion

VacA is one of the most commonly studied virulence markers of *H. pylori*. To date, numerous studies have evaluated the association between vacA genotype and DU, but the conclusions remained inconsistent. In addition, the credibility of results from single case–control study is

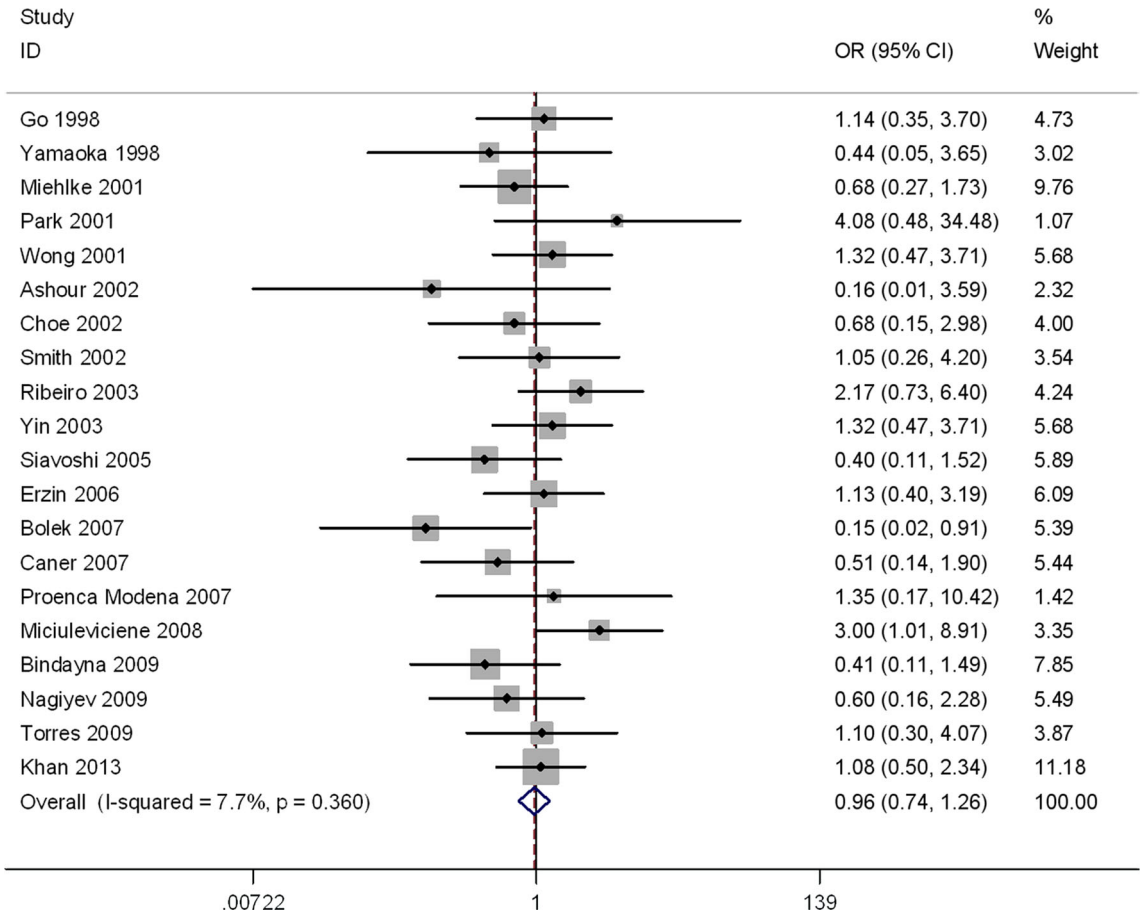

Fig. 5 Forest plot of the association between s1m2 and DU. The *white* diamonds indicate the summary pooled ORs and 95 % CIs

questionable due to their relatively small sample size. Meta-analysis is of benefit to increase the sample size generating more precise conclusions, which has been widely used in genetic association studies [58, 59]. To our knowledge, the present study is the first meta-analysis assessing the association between vacA s-/m- region genotype and DU. There were 1,876 patients and 2,704 controls in the present study. Results of our study showed that s1 genotype was associated with increased DU risk in overall studied population, and also Asian, European or Latin American population; m1 genotype increased the risk of developing DU in overall studied population and Latin American population; s1m1 genotype increased the risk of DU in overall studied population, Asian population and Latin American population; s2m1 genotype increased the risk of DU in Latin American population.

The s region encodes part of the cytotoxin's signal peptide and N-terminus, while the m region encodes part of the 55 kDa C-terminal subunit [6]. There were two types of s region: s1 and s2. The s2 genotype was reported to block the vacuolating activity since it encodes a shorter extension of the N-terminal peptide on the mature protein. On the contrary, the s1 genotype was reported to increase cytotoxin activity and thereby lead to gastric inflammation and duodenal ulceration [60]. Similar to s region, m region also has two subtypes: m1 and m2. Type m1 strains demonstrated more toxin activity than m2 strains [6, 61]. In this meta-analysis, we identified 34 articles focusing on the vacA s region genotype and 26 articles focusing on the vacA m region genotype. Among them, ten studies reported that the s1 genotype could increase the risk of DU; five studies reported that the m1 genotype was associated with increased DU risk; one study provided evidence that m1 was the protective factor for DU; the others revealed no significant difference between DU and control. Our study showed that vacA s1 increased the risk of DU by 2.96-folds and vacA m1 increased the risk of DU by 1.46-folds. Regarding the combination of s- and m- region, previous reports showed that the s1m1 genotype was closely tied to a large amount of toxin with high vacuolating activity in gastric epithelial cells, whereas the s1m2 genotype was associated with moderate amounts of toxin and s2m2 was associated with very little or no toxin [6, 60]. In this meta-analysis, we identified 20 articles reporting data on the

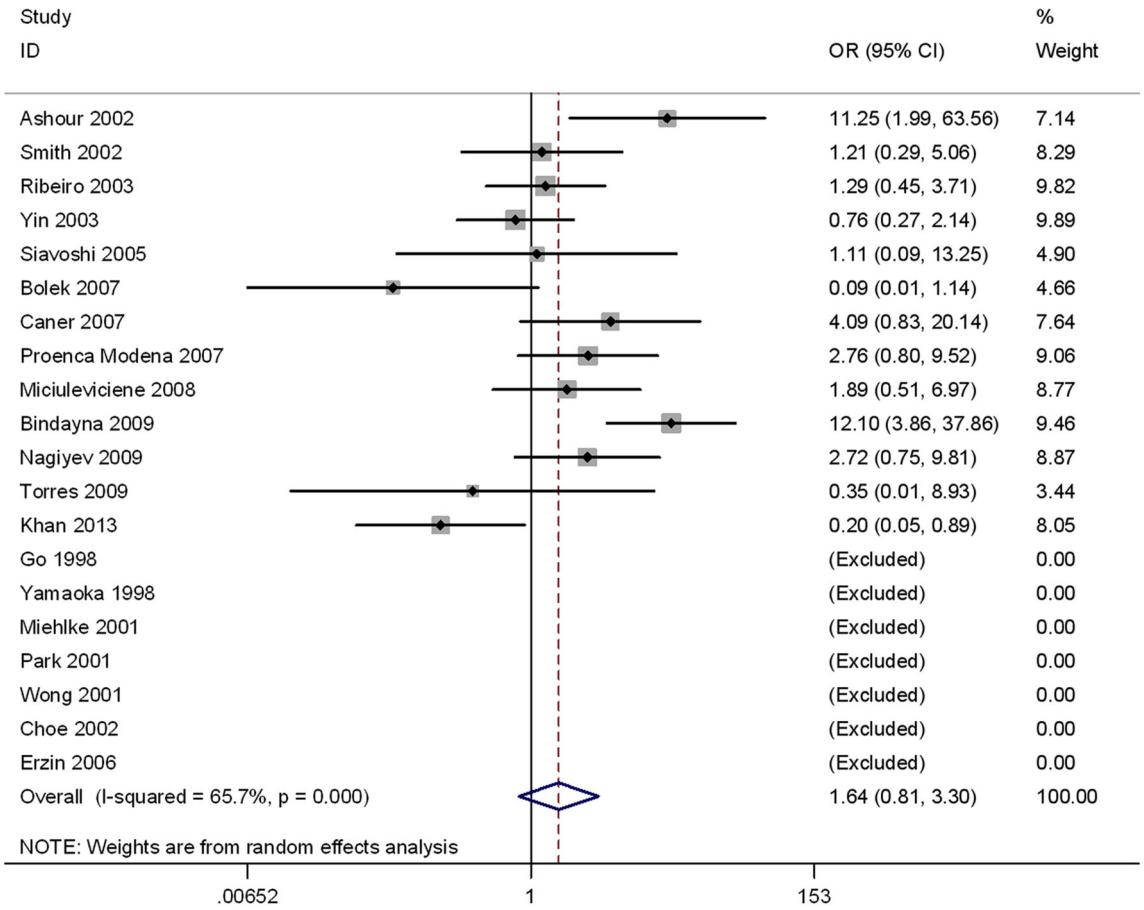

Fig. 6 Forest plot of the association between s2m1 and DU. The white diamonds indicate the summary pooled ORs and 95 % CIs

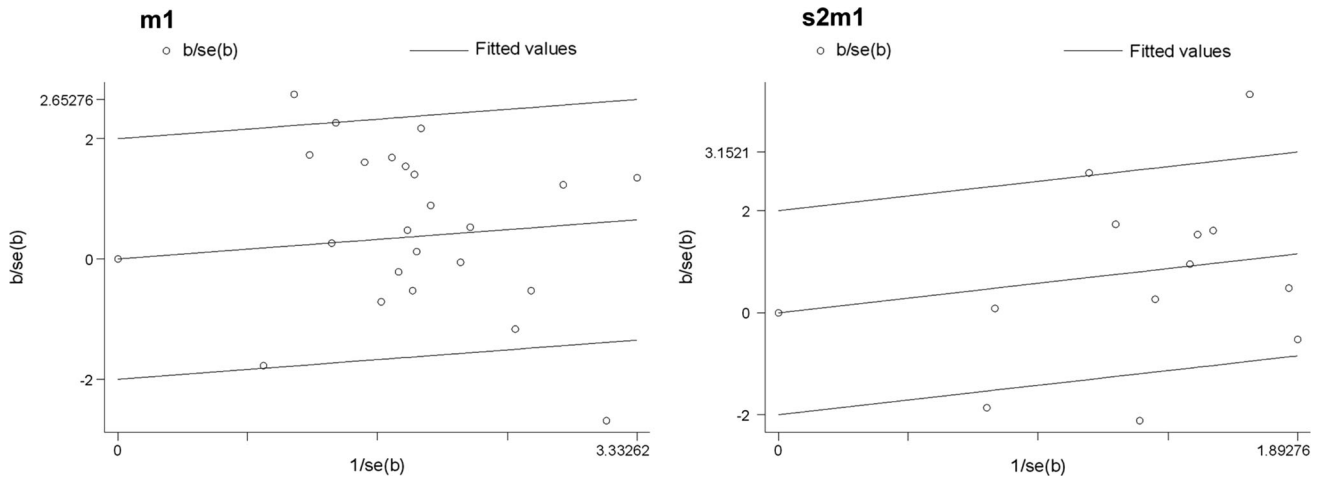

Fig. 7 Galbraith plots. The dots outlier indicate the study of Ashour et al. [23] and Zhang et al. [43]for m1; Ashour et al. [23], Bolek et al. [37], Bindayna et al. [44] and Khan et al. [50] for s2m1

combination of s- and m- genotypes. The combined results showed that only the vacA s1m1 could increase the risk of DU by 1.89-fold in the overall population. Taken the above results into consideration, it suggests that s1 and m1 are indeed the risk factors during the development of DU. Patients with s1 and m1 genotype would increase DU risk by 1.46–2.96-folds compared those without the same genotype.

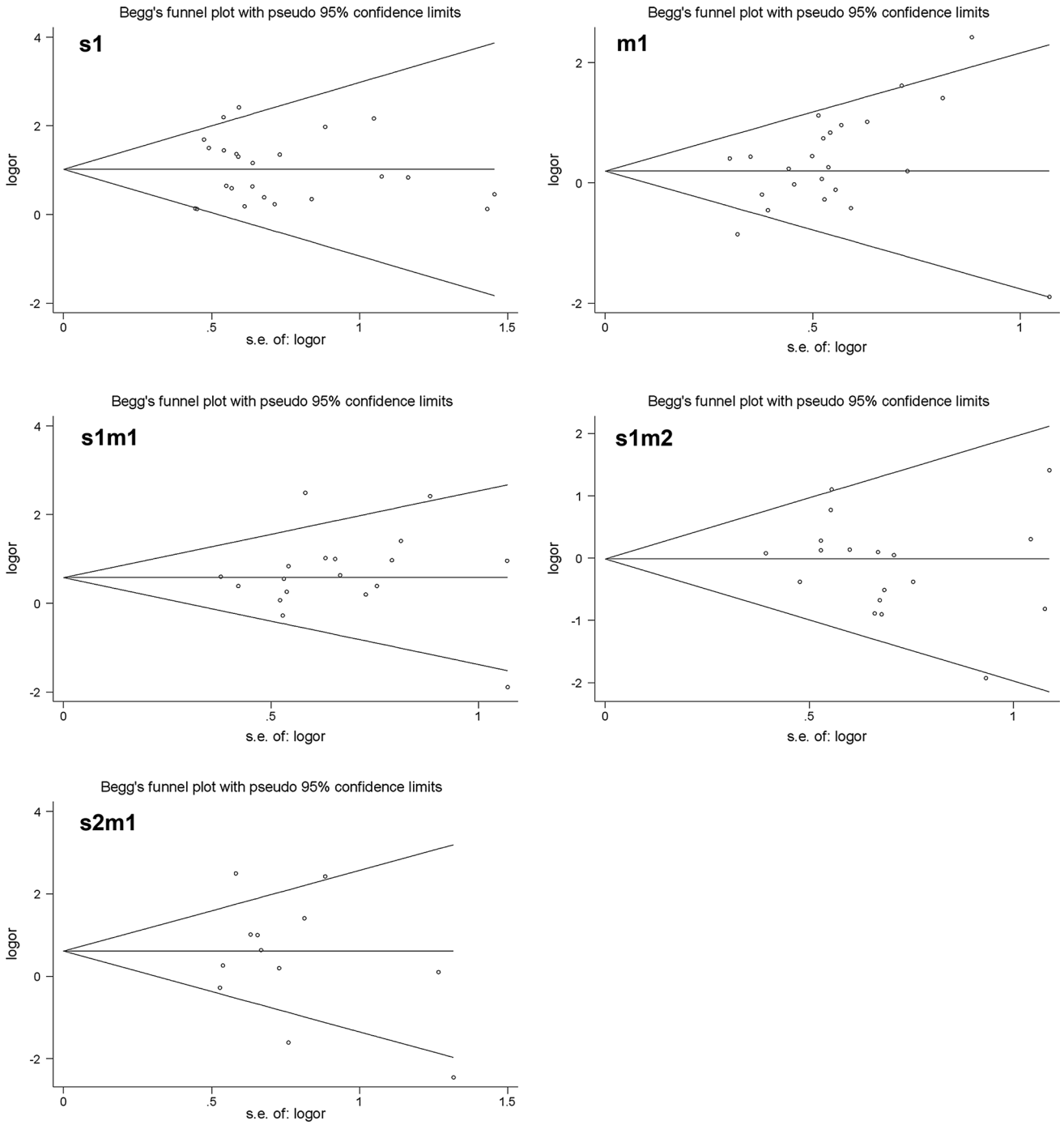

Fig. 8 Funnel plots. SE: standard error; OR: odds ratio

To explore a more precise relationship between vacA s-/m-region genotypes and DU, we performed subgroup analyses by ethnicity. Our results demonstrated that vacA s1 increased the risk of DU by 1.92–4.20-fold in all the three subgroups. VacA m1 increased the risk of DU only in Latin American population. In Latin American population, the s1m1 and s2m1 increased the risk of DU by 2.05 and 2.30-folds respectively. In Asian population, only s1m1 showed risk effect on DU. The above data suggest that there is region difference in the vacA m genotype distribution. In addition, gene-environment interaction may also influence the effect of vacA m1 on the development of DU.

Some limitations of this meta-analysis should be noted. Firstly, we could not obtain the original data, which may limit the further evaluation of potential interactions among gene–gene and gene–environment. Secondly, only studies

published in English or Chinese were included, which may lead to some inevitable bias, as eligible studies unpublished or reported in other languages would be missed.

Nonetheless, our meta-analysis with robust data and unbiased results demonstrated convincingly that VacA genotypes of *H. pylori* was well correlated with the risk of developing DU, and the correlation extent was various in different genotypes and also affected by region factors. Genotype testing of vacA s- and m- regions will be useful in screening susceptible individuals for DU development.

Acknowledgments

Conflict of Interest None.

References

1. Piper DW, Nasiry R, McIntosh J, Shy CM, Pierce J, Byth K (1984) Smoking, alcohol, analgesics, and chronic duodenal ulcer. A controlled study of habits before first symptoms and before diagnosis. Scand J Gastroenterol 19(8):1015–1021
2. Zapata-Colindres JC, Zepeda-Gomez S, Montano-Loza A, Vazquez-Ballesteros E, de Jesus Villalobos J, Valdovinos-Andraca F (2006) The association of Helicobacter pylori infection and nonsteroidal anti-inflammatory drugs in peptic ulcer disease. Canadian journal of gastroenterology = Journal canadien de gastroenterologie 20 (4):277-280
3. NIH Consensus Conference (1994) Helicobacter pylori in peptic ulcer disease. NIH Consensus Development Panel on Helicobacter pylori in Peptic Ulcer Disease. JAMA, J Am Med Assoc 272(1):65–69
4. Go MF (2002) Review article: natural history and epidemiology of Helicobacter pylori infection. Aliment Pharmacol Ther 16(Suppl 1):3–15
5. Cover TL (1996) The vacuolating cytotoxin of *Helicobacter pylori*. Mol Microbiol 20(2):241–246
6. Atherton JC, Cao P, Peek RM, Jr, Tummuru MK, Blaser MJ, Cover TL (1995) Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori*. Association of specific vacA types with cytotoxin production and peptic ulceration. J Biol Chem 270(30):17771–17777
7. Rhead JL, Letley DP, Mohammadi M, Hussein N, Mohagheghi MA, Eshagh Hosseini M, Atherton JC (2007) A new *Helicobacter pylori* vacuolating cytotoxin determinant, the intermediate region, is associated with gastric cancer. Gastroenterology 133(3):926–936. doi:10.1053/j.gastro.2007.06.056
8. Basso D, Navaglia F, Brigato L, Piva MG, Toma A, Greco E, Di Mario F, Galeotti F, Roveroni G, Corsini A, Plebani M (1998) Analysis of *Helicobacter pylori* vacA and cagA genotypes and serum antibody profile in benign and malignant gastroduodenal diseases. Gut 43(2):182–186
9. Go MF, Cissell L, Graham DY (1998) Failure to confirm association of vac A gene mosaicism with duodenal ulcer disease. Scand J Gastroenterol 33(2):132–136
10. Strobel S, Bereswill S, Balig P, Allgaier P, Sonntag HG, Kist M (1998) Identification and analysis of a new vacA genotype variant of *Helicobacter pylori* in different patient groups in Germany. J Clin Microbiol 36(5):1285–1289
11. Warburton VJ, Everett S, Mapstone NP, Axon AT, Hawkey P, Dixon MF (1998) Clinical and histological associations of cagA and vacA genotypes in Helicobacter pylori gastritis. J Clin Pathol 51(1):55–61
12. Yamaoka Y, Kodama T, Kita M, Imanishi J, Kashima K, Graham DY (1998) Relationship of vacA genotypes of Helicobacter pylori to cagA status, cytotoxin production, and clinical outcome. Helicobacter 3(4):241–253
13. Hennig EE, Trzeciak L, Regula J, Butruk E, Ostrowski J (1999) VacA genotyping directly from gastric biopsy specimens and estimation of mixed *Helicobacter pylori* infections in patients with duodenal ulcer and gastritis. Scand J Gastroenterol 34(8):743–749
14. Sadakane Y, Kusaba K, Nagasawa Z, Tanabe I, Kuroki S, Tadano J (1999) Prevalence and genetic diversity of cagD, cagE, and vacA in *Helicobacter pylori* strains isolated from Japanese patients. Scand J Gastroenterol 34(10):981–986
15. Audibert C, Janvier B, Grignon B, Salaun L, Burucoa C, Lecron JC, Fauchere JL (2000) Correlation between IL-8 induction, cagA status and vacA genotypes in 153 French *Helicobacter pylori* isolates. Res Microbiol 151(3):191–200
16. De Gusmao VR, Nogueira Mendes E, De Magalhaes Queiroz DM, Aguiar Rocha G, Camargos Rocha AM, Ramadan Ashour AA, Teles Carvalho AS (2000) vacA genotypes in *Helicobacter pylori* strains isolated from children with and without duodenal ulcer in Brazil. J Clin Microbiol 38(8):2853–2857
17. Figueiredo C, Van Doorn LJ, Nogueira C, Soares JM, Pinho C, Figueira P, Quint WG, Carneiro F (2001) *Helicobacter pylori* genotypes are associated with clinical outcome in Portuguese patients and show a high prevalence of infections with multiple strains. Scand J Gastroenterol 36(2):128–135
18. Miehlke S, Yu J, Schuppler M, Frings C, Kirsch C, Negraszus N, Morgner A, Stolte M, Ehninger G, Bayerdorffer E (2001) *Helicobacter pylori* vacA, iceA, and cagA status and pattern of gastritis in patients with malignant and benign gastroduodenal disease. Am J Gastroenterol 96(4):1008–1013. doi:10.1111/j.1572-0241.2001.03685.x
19. Park SM, Park J, Kim JG, Yoo BC (2001) Relevance of vacA genotypes of *Helicobacter pylori* to cagA status and its clinical outcome. Korean J Intern Med 16(1):8–13
20. Wong BC, Yin Y, Berg DE, Xia HH, Zhang JZ, Wang WH, Wong WM, Huang XR, Tang VS, Lam SK (2001) Distribution of distinct vacA, cagA and iceA alleles in *Helicobacter pylori* in Hong Kong. Helicobacter 6(4):317–324
21. Chattopadhyay S, Datta S, Chowdhury A, Chowdhury S, Mukhopadhyay AK, Rajendran K, Bhattacharya SK, Berg DE, Nair GB (2002) Virulence genes in *Helicobacter pylori* strains from West Bengal residents with overt *H. pylori*-associated disease and healthy volunteers. J Clin Microbiol 40(7):2622–2625
22. Choe YH, Kim PS, Lee DH, Kim HK, Kim YS, Shin YW, Hwang TS, Kim HJ, Song SU, Choi MS (2002) Diverse vacA allelic types of *Helicobacter pylori* in Korea and clinical correlation. Yonsei Med J 43(3):351–356
23. Ashour AA, Magalhaes PP, Mendes EN, Collares GB, de Gusmao VR, Queiroz DM, Nogueira AM, Rocha GA, de Oliveira CA (2002) Distribution of vacA genotypes in *Helicobacter pylori* strains isolated from Brazilian adult patients with gastritis, duodenal ulcer or gastric carcinoma. FEMS Immunol Med Microbiol 33(3):173–178
24. Smith SI, Kirsch C, Oyedeji KS, Arigbabu AO, Coker AO, Bayerdoffer E, Miehlke S (2002) Prevalence of *Helicobacter pylori* vacA, cagA and iceA genotypes in Nigerian patients with duodenal ulcer disease. J Med Microbiol 51(10):851–854
25. Brito CA, Silva LM, Juca N, Leal NC, de Souza W, Queiroz D, Cordeiro F, Silva NL (2003) Prevalence of cagA and vacA genes in isolates from patients with *Helicobacter pylori*-associated gastroduodenal diseases in Recife, Pernambuco Brazil. Mem Inst Oswaldo Cruz 98(6):817–821
26. Leodolter A, Wolle K, Peitz U, Ebert M, Gunther T, Kahl S, Malfertheiner P (2003) *Helicobacter pylori* genotypes and

expression of gastritis in erosive gastro-oesophageal reflux disease. Scand J Gastroenterol 38(5):498–502
27. Perng CL, Lin HJ, Sun IC, Tseng GY, Facg (2003) *Helicobacter pylori* cagA, iceA and vacA status in Taiwanese patients with peptic ulcer and gastritis. J Gastroenterol Hepatol 18(11):1244–1249
28. Qiao W, Hu JL, Xiao B, Wu KC, Peng DR, Atherton JC, Xue H (2003) cagA and vacA genotype of *Helicobacter pylori* associated with gastric diseases in Xi'an area. World J Gastroenterol 9(8):1762–1766
29. Ribeiro ML, Godoy AP, Benvengo YH, Mendonca S, Pedrazzoli J Jr (2003) Clinical relevance of the cagA, vacA and iceA genotypes of Helicobacter pylori in Brazilian clinical isolates. FEMS Immunol Med Microbiol 36(3):181–185
30. Yin Y, Zhang JZ, Wang ZY, Xia HX, Lin ZX (2003) Association between *Helicobacter pylori* virulence and duodenal ulcer disease in patients from Hong Kong in China. Zhonghua Liu Xing Bing Xue Za Zhi 24(2):123–126
31. Ando T, Tsuzuki T, Mizuno T, Minami M, Ina K, Kusugami K, Takamatsu J, Adachi K, El-Omar E, Ohta M, Goto H (2004) Characteristics of *Helicobacter pylori*-induced gastritis and the effect of *H. pylori* eradication in patients with chronic idiopathic thrombocytopenic purpura. Helicobacter 9(5):443–452. doi:10.1111/j.1083-4389.2004.00261.x
32. Lin HJ, Perng CL, Lo WC, Wu CW, Tseng GY, Li AF, Sun IC, Ou YH (2004) *Helicobacter pylori* cagA, iceA and vacA genotypes in patients with gastric cancer in Taiwan. World J Gastroenterol 10(17):2493–2497
33. Perng CL, Lin HJ, Lo WC, Tseng GY, Sun IC, Ou YH (2004) Genotypes of *Helicobacter pylori* in patients with peptic ulcer bleeding. World J Gastroenterol 10(4):602–605
34. Salih BA, Abasiyanik MF, Saribasak H, Huten O, Sander E (2005) A follow-up study on the effect of *Helicobacter pylori* eradication on the severity of gastric histology. Dig Dis Sci 50(8):1517–1522
35. Siavoshi F, Malekzadeh R, Daneshmand M, Ashktorab H (2005) *Helicobacter pylori* endemic and gastric disease. Dig Dis Sci 50(11):2075–2080. doi:10.1007/s10620-005-3010-1
36. Erzin Y, Koksal V, Altun S, Dobrucali A, Aslan M, Erdamar S, Dirican A, Kocazeybek B (2006) Prevalence of *Helicobacter pylori* vacA, cagA, cagE, iceA, babA2 genotypes and correlation with clinical outcome in Turkish patients with dyspepsia. Helicobacter 11(6):574–580. doi:10.1111/j.1523-5378.2006.00461.x
37. Bolek BK, Salih BA, Sander E (2007) Genotyping of *Helicobacter pylori* strains from gastric biopsies by multiplex polymerase chain reaction. How advantageous is it? Diagn Microbiol Infect Dis 58(1):67–70. doi:10.1016/j.diagmicrobio.2006.12.001
38. Caner V, Yilmaz M, Yonetci N, Zencir S, Karagenc N, Kaleli I, Bagci H (2007) *H. pylori* iceA alleles are disease-specific virulence factors. World J Gastroenterol 13(18):2581–2585
39. Linpisarn S, Suwan W, Lertprasertsuk N, Koosirirat C, Steger HF, Prommuangyong K, Phornphutkul K (2007) *Helicobacter pylori* cagA, vacA and iceA genotypes in northern Thai patients with gastric disease. Southeast Asian J Trop Med Public Health 38(2):356–362
40. Proenca Modena JL, Lopes Sales AI, Olszanski Acrani G, Russo R, Vilela Ribeiro MA, Fukuhara Y, da Silveira WD, Modena JL, de Oliveira RB, Brocchi M (2007) Association between *Helicobacter pylori* genotypes and gastric disorders in relation to the cag pathogenicity island. Diagn Microbiol Infect Dis 59(1):7–16. doi:10.1016/j.diagmicrobio.2007.03.019
41. Tiwari SK, Khan AA, Manoj G, Ahmed S, Abid Z, Habeeb A, Habibullah CM (2007) A simple multiplex PCR assay for diagnosing virulent *Helicobacter pylori* infection in human gastric biopsy specimens from subjects with gastric carcinoma and other gastro-duodenal diseases. J Appl Microbiol 103(6):2353–2360. doi:10.1111/j.1365-2672.2007.03478.x
42. Miciuleviciene J, Calkauskas H, Jonaitis L, Kiudelis G, Tamosiunas V, Praskevicius A, Kupcinskas L, Berg D (2008) *Helicobacter pylori* genotypes in Lithuanian patients with chronic gastritis and duodenal ulcer. Medicina 44(6):449–454
43. Zhang Z, Zheng Q, Chen X, Xiao S, Liu W, Lu H (2008) The *Helicobacter pylori* duodenal ulcer promoting gene, dupA in China. BMC Gastroenterol 8:49. doi:10.1186/1471-230X-8-49
44. Bindayna KM, Al Mahmeed A (2009) vacA genotypes in *Helicobacter pylori* strains isolated from patients with and without duodenal ulcer in Bahrain. Indian J Gastroenterol 28(5):175–179. doi:10.1007/s12664-009-0069-1
45. Nagiyev T, Yula E, Abayli B, Koksal F (2009) Prevalence and genotypes of *Helicobacter pylori* in gastric biopsy specimens from patients with gastroduodenal pathologies in the Cukurova Region of Turkey. J Clin Microbiol 47(12):4150–4153. doi:10.1128/JCM.00605-09
46. Salehi Z, Abadi AS, Ismail PB, Kqueen CY, Jelodar MH, Kamalidehghan B (2009) Evaluation of *Helicobacter pylori* vacA genotypes in Iranian patients with peptic ulcer disease. Dig Dis Sci 54(11):2399–2403. doi:10.1007/s10620-008-0633-z
47. Torres LE, Melian K, Moreno A, Alonso J, Sabatier CA, Hernandez M, Bermudez L, Rodriguez BL (2009) Prevalence of vacA, cagA and babA2 genes in Cuban *Helicobacter pylori* isolates. World J Gastroenterol 15(2):204–210
48. Yakoob J, Abid S, Abbas Z, Jafri W, Ahmad Z, Ahmed R, Islam M (2009) Distribution of *Helicobacter pylori* virulence markers in patients with gastroduodenal diseases in Pakistan. BMC Gastroenterol 9:87. doi:10.1186/1471-230X-9-87
49. Dixit P, Sharma AK, Sinha SK, Prasad KK, Singh K (2011) Prevalence of cagA and vacA genotypes of *H. pylori* as a putative virulence marker in dyspeptic patients in northern India. J Gastroenterol Hepatol 26:276
50. Khan A, Farooqui A, Raza Y, Rasheed F, Manzoor H, Akhtar SS, Quraishy MS, Rubino S, Kazmi SU, Paglietti B (2013) Prevalence, diversity and disease association of Helicobacter pylori in dyspeptic patients from Pakistan. J Infect Dev Ctries 7(3):220–228. doi:10.3855/jidc.2942
51. Moher D, Liberati A, Tetzlaff J, Altman DG (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med 6(7):e1000097. doi:10.1371/journal.pmed.1000097
52. Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, Tugwel lP (2011)The Newcastle–Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Ottawa Health Research Institute.www.ohri.ca/programs/clinical_epidemiology/oxford.asp Accessed Dec. 1 2011
53. Cochran WG (1954) The combination of estimates from different experiments. Biometrics 10:101–129
54. Higgins JP, Thompson SG (2002) Quantifying heterogeneity in a meta-analysis. Stat Med 21(11):1539–1558. doi:10.1002/sim.1186
55. DerSimonian R, Laird N (1986) Meta-analysis in clinical trials. Control Clin Trials 7(3):177–188 0197-2456(86)90046-2 [pii]
56. Mantel N, Haenszel W (1959) Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst 22(4):719–748
57. Egger M, Davey Smith G, Schneider M, Minder C (1997) Bias in meta-analysis detected by a simple, graphical test. BMJ 315(7109):629–634
58. Coppede F, Bosco P, Lorenzoni V, Migheli F, Barone C, Antonucci I, Stuppia L, Romano C, Migliore L (2013) The MTR 2756A>G polymorphism and maternal risk of birth of a child with Down syndrome: a case–control study and a meta-analysis. Mol Biol Rep. doi:10.1007/s11033-013-2810-1
59. Li YY, Wu XY, Xu J, Qian Y, Zhou CW, Wang B (2013) Apo A5–1131T/C, FgB -455G/A, -148C/T, and CETP TaqIB gene

polymorphisms and coronary artery disease in the Chinese population: a meta-analysis of 15,055 subjects. Mol Biol Rep 40(2):1997–2014. doi:10.1007/s11033-012-2257-9

60. Letley DP, Atherton JC (2000) Natural diversity in the N terminus of the mature vacuolating cytotoxin of *Helicobacter pylori* determines cytotoxin activity. J Bacteriol 182(11):3278–3280
61. Atherton JC, Peek RM Jr, Tham KT, Cover TL, Blaser MJ (1997) Clinical and pathological importance of heterogeneity in vacA, the vacuolating cytotoxin gene of *Helicobacter pylori*. Gastroenterology 112(1):92–99