A Meta-Analysis of Interleukin-10-1082 Promoter Polymorphism Associated with Gastric Cancer Risk

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We aimed to explore the role of allele A/G single nucleotide polymorphism (SNP) of gene *Interleukin 10* (IL-10) promoter-1082 in the susceptibility to gastric cancer through a systematic review and meta-analysis. Each initially included article was scored for quality appraisal. Desirable data were extracted and registered into databases. Twenty studies were ultimately eligible for the meta-analysis of IL-10-1082 A/G SNP. We adopted the most probably appropriate genetic model (dominant model), with the combined group of GG-plus-GA genotypes compared with the AA genotype. Potential sources of heterogeneity were sought out via subgroup analyses and sensitivity analyses, and publication biases were estimated. Between IL-10-1082 GG-plus-GA genotypes with the risk of developing gastric cancer, statistically significant association could be noted with overall gastric cancer, being mainly in Asian subgroup, large sample subgroup, high quality subgroup, intestinal-type subgroup, cardia-type subgroup, and some genotyping method subgroups. Our meta-analysis indicates that IL-10-1082 GG-plus-GA genotypes are associated with the overall risk of developing gastric cancer and seem to be more susceptible to overall gastric cancer in Asian populations. IL-10-1082 GG-plus-GA genotypes are more associated with the pathologically intestinal-type gastric cancer or anatomically cardia-type gastric cancer.

Introduction

GASTRIC CANCER RANKS fourth in incidence and second in mortality in the world (Parkin *et al.*, 2005) and is one of the most deadly malignancies in the midwestern China (Yang *et al.*, 2006). It is known that infectious, dietary, environmental, and genetic factors are involved in gastric carcinogenesis, but the proportion of persons who finally develop gastric cancer after being exposed to risk factors like *Helicobacter pylori* infection is very small (Suerbaum and Michetti, 2002), suggesting that host genetic susceptibility like single nucleotide polymorphisms (SNPs) of susceptible genes could play an important role in gastric carcinogenesis.

Lately, the promoter SNPs of IL-1 β , a key proinflammatory interleukin (Hwang *et al.*, 2002), associated with the gastric cancer risk have been extensively explored among different ethnicities. A recently published meta-analysis (Xue *et al.*, 2010) found that both IL-1B –511 T allele and IL-1 RN *2 VNTR are significantly associated with an increased risk

of developing gastric cancer and even more significantly with noncardia gastric cancer or with intestinal-type gastric cancer and that both are significantly associated with an increased risk of developing gastric carcinoma among Caucasians but not Asians or Hispanics, suggesting the discrepant genetic backgrounds among different ethnic populations.

Likewise, potential associations of SNPs of other interleukins with the risk of gastric cancer should also be quantitatively conducted via meta-analysis. IL-10 is a potent pleiotropic cytokine that has the dual ability to immunosuppress or immunostimulate anticancer properties (Mocellin *et al.*, 2005). The human IL-10 gene, located on chromosome 1q31-32, consists of five exons and four introns. There are three frequently investigated SNPs of IL-10 gene, that is, at position –1082 A/G and –819 C/T SNPs in its proximal promoter region and at position –592 A/C SNP in its 5'-flanking region, but IL-10-1082 was mostly widely reported and –10-1082 G allele was found to be associated with increased IL-10 production (Turner *et al.*, 1997).

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Correlated with altered IL-10 levels, functional IL-10-1082 polymorphism was suspected to influence cancer susceptibility by altered inflammatory responses (Seifart *et al.*, 2005). Interestingly, the IL-10-1082 AA genotype was also reported to be significantly increased in prostate cancer patients and its action, together with vascular endothelial growth factor and IL-8, was suspected to possibly influence cancer angiogenesis (McCarron *et al.*, 2002). Elevated levels of serum IL-10 were also reported in gastrointestinal carcinoma patients compared with healthy controls, with those patients with metastatic disease showing significantly higher levels than patients with undisminated disease (De Vita *et al.*, 1999). Thus, it could be presumed that specific IL-10-1082 SNP may predispose to gastric cancer through the elevated IL-10 expression.

Since the year 2002, a lot of studies have focused on the IL-10-1082 G allele association with the susceptibility to gastric cancer, but with mixed or conflicting results (Wu *et al.*, 2002; El-Omar *et al.*, 2003; Wu *et al.*, 2003; Savage *et al.*, 2004; Alpízar-Alpízar *et al.*, 2005; Guo *et al.*, 2005; Lee *et al.*, 2005; Lu *et al.*, 2005; Zambon *et al.*, 2005; Kamangar *et al.*, 2006; Morgan *et al.*, 2006; García-González *et al.*, 2007; Sugimoto *et al.*, 2007; Bai *et al.*, 2008; Crusius *et al.*, 2008; Deng *et al.*, 2008; Zhou, *et al.*, 2008a; Kang *et al.*, 2009; Ko *et al.*, 2009; Xiao *et al.*, 2009; Liu *et al.*, 2011; Zhou *et al.*, 2011). Up to now, there has been just one relevant published meta-analysis regarding IL-10-1082 A/G SNP (Zhou *et al.*, 2008b). Unlike that meta-analysis, our meta-analysis adopted the most appropriate genetic model in an attempt to accurately illuminate the role of IL-10-1082 G allele in the risk of developing gastric cancer among the eligible studies.

Materials and Methods

Search strategy

A comprehensive literature review was conducted for the relationship between IL-10-1082 A/G SNP and gastric cancer risk. The MEDLINE, EMBASE databases, and Chinese National Knowledge Infrastructure were used simultaneously with English and/or Chinese key terms “Interleukin 10,” “IL-10,” “interleukin,” or “cytokine”; “gene”; “polymorphism,” “variant,” or “SNP”; and “gastric cancer,” “gastric carcinoma,” “diffuse gastric cancer,” or “stomach cancer” from 2000 to 2011. The scope of computerized literature search was expanded according to the lists of retrieved articles. The relevant original articles were also sought manually.

Study selection

Studies concerning the association of IL-10-1082 A/G SNP with the risk of developing gastric cancer were included if the following conditions were met: (i) articles in English or Chinese; (ii) study described the association of IL-10-1082 A/G SNP with gastric cancer; (iii) study reported the numbers of both controls and gastric cancer cases; (iv) results expressed as odds ratio (OR) with 95% confidence intervals (CIs); and (v) studies as case-control or nested case-control ones.

Methodological quality appraisal

To identify high quality studies, we mainly adopted pre-defined criteria for Quality Appraisal (Thakkinian *et al.*, 2005a; Camargo *et al.*, 2006; Xue *et al.*, 2010; Cui *et al.*, 2011; Xue

et al., 2011). The criteria (seen in Supplementary Table 1; Supplementary Data are available online at www.liebertonline.com/dna) cover credibility of controls, representativeness of cases, consolidation of gastric cancer, genotyping examination, and association assessment. Methodological quality was independently assessed by two investigators (P. N. and H. X.). Disagreements were resolved through discussion. Scores ranged from the lowest zero to the highest 10. Articles with the score lower than 6.5 were considered as “low or moderate quality” ones, whereas the others as “high quality” ones.

Data extraction

The following data from each article were extracted: authors, year of publication, country, ethnicity of participants (categorized as Caucasians, Asians, Latinos, etc.), study design, source of controls, number of controls and of cases, genotyping method, distribution of age and gender, Lauren's classification (intestinal, diffuse, or mixed), and anatomic classification (cardia or noncardia cancer).

The extracted data registered into two databases independently were blind to two investigators (P. N. and H. X.) for journal names, institutions, or fund grants. Any discrepancy between the two investigators was resolved by the third one (H. Xue), who participated in the discussion with them and made an ultimate decision.

Statistical analysis

All statistical analyses were performed using STATA statistical software (Version 10.1; STATA Corp., College Station, TX). Two-sided *p*-values <0.05 were considered statistically significant.

Hardy-Weinberg equilibrium (HWE) in controls was calculated again in our meta-analysis. The chi-square goodness-of-fit was used to test deviation from HWE (significant at the 0.05 level).

ORs and 95% CIs were employed to assess the strength of associations between IL-10-1082 A/G SNP and gastric cancer risk. OR₁, OR₂, and OR₃ regarding IL-10-1082 A/G SNP were calculated for genotypes GG versus AA, GA versus AA, and GG versus GA, respectively.

The above pairwise differences were used to determine the most appropriate genetic model. If OR₁=OR₂≠1 and OR₃=1, a dominant model is implied. If OR₁=OR₃≠1 and OR₂=1, a recessive model is suggested. If OR₂=1/OR₃≠1 and OR₁=1, a complete overdominant model is suggested. If OR₁>OR₂>1 and OR₁>OR₃>1, or OR₁<OR₂<1 and OR₁<OR₃<1, a codominant model is indicated (Thakkinian *et al.*, 2005b). If a dominant model was indicated, the original grouping was collapsed and the combined group of GG-plus-GA genotypes was compared with the AA genotype; if a recessive model was suggested, the GG genotype was compared with the combined group of GA-plus-AA genotypes; if a complete overdominant model was implied, the combined group of GG-plus-AA genotypes was compared with the GA genotype; or if a codominant model was insinuated, the GG genotype was compared with the GA genotype and with the AA genotype, respectively.

The Q statistic was used to test for heterogeneity among the studies included in the meta-analysis. A fixed-effects model, using Mantel-Haenszel (M-H) method, was used to calculate the pooled ORs when homogeneity existed on the

TABLE 1. STUDY CHARACTERISTICS OF GENOTYPES IN GASTRIC CANCER CASES AND CONTROLS
IN THE ANALYSIS OF IL-10-1082 GENETIC POLYMORPHISMS

First author	Year of publication	Quality assessment scores	Genotyping method	Total sample size	Number of controls	Number of cases	Study location	Ethnic group	p-Values for HWE	Controls, genotypes (n)			All cases, genotypes (n)		
										GG	GA	AA	GG	GA	AA
Wu <i>et al.</i>	2002	4.5	Direct sequencing	340	220	120	China	Asians	0.0574583	1	11	208	1	13	106
Wu <i>et al.</i>	2003	5	Direct sequencing	450	230	220	China	Asians	0.0002432	2	11	217	2	23	195
El-Omar <i>et al.</i> ^{a,b}	2003	7	TaqMan	524	210	314	United States	Caucasians	0.8127225	48	103	59	61	133	120
Savage <i>et al.</i> ^a	2004	6	Snapshot	469	385	84	China	Asians	0.0000540	284	81	20	60	20	4
Lee <i>et al.</i>	2005	5	RFLP	242	120	122	Korea	Asians	0.8421283	1	18	101	1	17	104
Guo <i>et al.</i> ^a	2005	7.5	RFLP	622	443	179	China	Asians	0.0239239	12	164	267	30	56	93
Lu <i>et al.</i>	2005	8	DHPLC	550	300	250	China	Asians	0.0375060	3	29	268	6	43	201
Alpizar-Alpizar <i>et al.</i>	2005	5.5	Pyrosequencing	89	44	45	Costa Rica	Latinos	0.9392248	0	1	43	0	0	45
Zambon <i>et al.</i> ^b	2005	5.75	TaqMan	773	644	129	Italy	Caucasians	0.0877679	86	326	232	25	56	48
Morgan <i>et al.</i> ^{b,c}	2006	8	TaqMan	331	161	170	Honduras	Latinos	0.1457748	11	49	101	7	42	121
Kamangar <i>et al.</i> ^{b,c}	2006	6	MGBEclipse/ TaqMan	317	205	112	Finland	Caucasians	0.6133237	37	96	72	27	47	38
Sugimoto <i>et al.</i> ^d	2007	5	ASP	272	168	104	Japan	Asians	0.1444992	0	34	134	0	26	78
Garcia-Gonzalez <i>et al.</i> ^{a-c,e}	2007	9	TaqMan/MGB	808	404	404	Spain	Caucasians	0.3226628	82	189	133	77	204	123
Deng <i>et al.</i> ^b	2008	4	Direct sequencing	235	110	125	China	Asians	0.0000000	37	24	49	22	62	41
Crusius <i>et al.</i> ^{a,b}	2008	8.5	Real-time PCR	1369	1134	235	European	Caucasians	0.0209657	268	526	340	50	131	54
Xiao <i>et al.</i>	2009	7.5	RFLP	844	624	220	China	Asians	0.5245795	0	31	593	3	41	176
Kang <i>et al.</i> ^{c-e}	2009	8	RFLP	658	324	334	Korea	Asians	0.3040760	0	35	289	4	49	281
Ko <i>et al.</i>	2009	7	Snapshot	392	312	80	Korea	Asians	0.9216096	1	35	276	1	12	67
Zhou <i>et al.</i> ^{a-c,e}	2011	7.5	RFLP	300	150	150	China	Asians	0.0003520	45	53	52	59	62	29
Liu <i>et al.</i>	2011	6.5	RFLP	477	243	234	China	Asians	0.0146605	3	23	217	6	39	189

^aData of cardia-type gastric cancer were accessible.

^bData of noncardia-type gastric cancer were accessible.

^cData of intestinal-type gastric cancer were accessible.

^dData of the status of *Helicobacter pylori* of gastric cancer were accessible.

^eData of sporadic diffuse-type gastric cancer were accessible.

RFLP, restriction fragment length polymorphism; PCR, polymerase chain reaction; DHPLC, PCR-based denaturing high-performance liquid chromatography; TaqMan, TaqMan PCR method; Direct sequencing, method of methylation analysis using bisulfite-treated DNA utilized PCR and standard dideoxynucleotide DNA sequencing to directly determine the nucleotide resistant to bisulfite conversion; MGBEclipse, MGBEclipse Assay PCR method; Snapshot, the Snapshot assay that provides detection of certain SNPs; Pyrosequencing, a method of DNA sequencing (determining the order of nucleotides in DNA) based on the "sequencing by synthesis" principle. It differs from Sanger sequencing, in that it relies on the detection of pyrophosphate release on nucleotide incorporation, rather than chain termination with dideoxynucleotides; ASP, the allele-specific primer PCR (ASP-PCR) method; SNP, single nucleotide polymorphism.

basis of Q -test p -value no less than 0.1. By contrast, a random-effects model, using DerSimonian and Laird method (D+L), was utilized if there was heterogeneity based on Q -test p -value less than 0.1. The significance of pooled ORs was tested by Z -test ($p < 0.05$ was considered significant).

Sensitivity analysis was performed, in which the meta-analysis estimates were computed after every one study being omitted in each turn. Included studies, if deviated from HWE, should be tested for the influence on results when those deviated-from-HWE studies were all removed from the meta-analysis.

Finally, publication bias was assessed by performing funnel plots qualitatively, and estimated by Begg's and Egger's tests quantitatively.

Results

Literature search and study selection

After comprehensive searching, a total of 235 articles in English and 8 in Chinese were retrieved. Twenty-two studies (Wu *et al.*, 2002; Wu *et al.*, 2003; El-Omar *et al.*, 2003; Savage *et al.*, 2004; Alpízar-Alpízar *et al.*, 2005; Guo *et al.*, 2005; Lee *et al.*, 2005; Lu *et al.*, 2005; Zambon *et al.*, 2005; Kamangar *et al.*, 2006; Morgan *et al.*, 2006; García-González *et al.*, 2007; Sugimoto *et al.*, 2007; Bai *et al.*, 2008; Crusius *et al.*, 2008; Deng

et al., 2008; Zhou *et al.*, 2008a; Kang *et al.*, 2009; Ko *et al.*, 2009; Xiao *et al.*, 2009; Liu *et al.*, 2011; Zhou *et al.*, 2011) were initially included in our meta-analysis. Those 22 studies were preliminarily appropriate to the meta-analysis of the associations with gastric cancer regarding IL-10-1082 A/G SNP. Two studies (Bai *et al.*, 2008; Zhou *et al.*, 2008a) were eliminated finally because they both only described the number of combined group GG-plus-GA rather than the number of genotype GG and the number of genotype GA, respectively; and no responses could be gotten after having sought the relevant data via email contacts. Besides, eight studies (Wu *et al.*, 2003; Savage *et al.*, 2004; Guo *et al.*, 2005; Lu *et al.*, 2005; Crusius *et al.*, 2008; Deng *et al.*, 2008; Liu *et al.*, 2011; Zhou *et al.*, 2011) were deviated from HWE. Generally speaking, any study that deviated from HWE through our calculation should have been removed; however, considering that the numbers of participants in those studies were large and given that sensitivity analyses would be conducted, we remained those studies in our meta-analysis. Minelli *et al.* stated that studies appearing to deviate from HWE should be included and examined further rather than just excluded unless there are other convincing reasons for doubting the quality of those studies (Minelli *et al.*, 2008). Thus, 20 studies (Wu *et al.*, 2002; El-Omar *et al.*, 2003; Wu *et al.*, 2003; Savage *et al.*, 2004; Alpízar-Alpízar *et al.*, 2005; Guo *et al.*, 2005; Lee *et al.*, 2005; Lu *et al.*, 2005; Zambon *et al.*, 2005; Kamangar *et al.*, 2006; Morgan *et al.*, 2006; García-González *et al.*, 2007; Sugimoto *et al.*, 2007; Crusius *et al.*, 2008; Deng *et al.*, 2008; Kang *et al.*, 2009; Ko *et al.*, 2009; Xiao *et al.*, 2009; Liu *et al.*, 2011; Zhou *et al.*, 2011) with a total of 6431 controls and 3631 cases were ultimately eligible for the meta-analysis of IL-10-1082 A/G SNP. The corresponding characteristics were seen in Table 1. The flow chart of literature search and study selection was illuminated in Figure 1.

Overall meta-analysis and subgroup analyses

OR₁ (p -value), OR₂ (p -value), and OR₃ (p -value) of IL-10-1082 A/G SNP were 1.45 ($p = 0.042$), 1.41 ($p = 0.004$), and 1.12 ($p = 0.544$), respectively, suggesting a dominant model effect of putative susceptible G allele (OR₁ = OR₂ ≠ 1 and OR₃ = 1). We divided the included studies into Caucasian study, Asian study, and Latino study according to the ethnicity of participants to further confirm our preliminary choice of the most possible genetic model. For Asian study, OR₁ (p -value), OR₂ (p -value), and OR₃ (p -value) of IL-10-1082 A/G SNP were 2.27 ($p = 0.009$), 1.79 ($p = 0.000$), and 1.29 ($p = 0.509$), respectively, highly indicating a dominant effect of putative susceptible G allele. Thus, the dominant genetic model of susceptible G allele was finally chosen in all our meta-analyses. Thus, the combined group of GG-plus-GA genotypes was compared with the AA genotype. As in Figure 2, for overall gastric cancer a statistically significant finding could be observed from the fact that the pooled OR (95% CI, p -value) was 1.41 (1.13–1.76, $p = 0.002$). Statistically significant findings were apparently found in Asians but not in Caucasians. The pooled ORs (95% CIs, p -value) in Asians and in Caucasians were 1.81 (1.44–2.27, $p = 0.000$) and 1.01 (0.77–1.33, $p = 0.926$), respectively. The pooled OR in Latinos (Alpízar-Alpízar *et al.*, 2005; Morgan *et al.*, 2006) was 0.67 (0.43–1.06, $p = 0.087$), seeming a reverse association trend but the difference was not significant.

FIG. 1. The flow chart of literature search and study selection.

FIG. 2. Odds ratios (ORs) for associations between IL-10-1082 A/G SNP and gastric cancer risk (the combined group of GG-plus-GA genotypes was compared with the AA genotype) in all ethnicities and among different ethnicities, in order of increasing publication year, 2002–2011. Studies were entered into the meta-analysis sequentially by year of publication. The sizes of the squares indicate the relative weight of each study. Weights were derived from random-effects analysis. Bars, 95% CI. SNP, single nucleotide polymorphism; CI, confidence interval.

As shown in Table 2, on the basis of sample size, the included studies were divided into two subgroups: large sample (the total number of controls and cases not less than 400) and small-and-moderate sample (the total number of controls and cases less than 400) subgroups. Statistically

significant finding was noted in large sample subgroup but not in small-and-moderate sample counterpart, given that the pooled ORs (95% CIs, *p*-value) were 1.50 (1.10–2.03, *p*=0.010) for the former and 1.29 (0.95–1.75, *p*=0.098) for the latter.

TABLE 2. SUBGROUP ANALYSES FOR THE TEST OF HETEROGENEITY ON IL-10-1082 GG-PLUS-GA VERSUS AA BASED ON A DOMINANT GENETIC MODEL

	Q-test			OR (95% CI)	p-Value
	Chi-squared	df	p		
Overall	70.16	19	0.000	1.41 (1.13–1.76)	0.002
Large sample	53.07	10	0.000	1.50 (1.10–2.03)	0.010
Small-and-moderate sample	16.55	8	0.035	1.29 (0.95–1.75)	0.098
High quality	60.05	10	0.000	1.49 (1.09–2.05)	0.013
Low-and-moderate quality	9.34	8	0.314	1.26 (1.00–1.58)	0.049
Publication before or in 2005	24.14	8	0.002	1.25 (0.88–1.75)	0.209
Publication after 2005	42.02	10	0.000	1.53 (1.15–2.04)	0.004
Caucasians	10.83	4	0.029	1.01 (0.77–1.33)	0.926
Asians	25.06	12	0.015	1.81 (1.44–2.27)	0.000
Latinos	0.21	1	0.647	0.67 (0.43–1.06)	0.087
Noncardia type	24.89	6	0.000	1.12 (0.80–1.58)	0.514
Cardia type	6.40	5	0.269	1.38 (1.11–1.72) ^a	0.004 ^b
Intestinal type	2.53	3	0.470	1.33 (1.04–1.71) ^a	0.024 ^b
Diffuse type	9.25	2	0.010	1.53 (0.73–3.21)	0.260
Hp (+) cases versus Hp (–) controls	3.83	1	0.050	2.82 (0.38–20.68)	0.309
PCR-RFLP genotyping	21.76	5	0.001	1.89 (1.25–2.88)	0.003
TaqMan/MGBEclipse	7.36	4	0.118	0.88 (0.69–1.11)	0.276
Direct	0.60	2	0.741	1.91 (1.31–2.77)	0.001
Snapshot	0.21	1	0.644	1.37 (0.76–2.45)	0.296

^aM-H ORs (95% CIs), otherwise D+L ORs (95% CIs).

^b*p*-Values of M-H estimates, otherwise *p*-values of D+L estimates.
OR, odds ratio; CI, confidence interval.

FIG. 3. Influence analysis of the summary odds ratio coefficients on the association for the IL-10-1082 A/G SNP with gastric cancer risk (the combined group of GG-plus-GA genotypes was compared with the AA genotype). Results were computed by omitting each study (on the bottom) in turn. Bars, 95% confidence interval. Meta-analysis random-effects estimates (exponential form) were used.

The included studies were also divided, in accordance with the quality appraisal scores, into high quality (scores no less than 6.5) and low-and-moderate quality (scores less than 6.5) subgroups. Statistically significant findings were witnessed in both high quality subgroup and in low-and-moderate quality counterpart, given that the pooled ORs (95% CIs, p -value) were 1.49 (1.09–2.05, $p=0.013$) for the former and 1.26 (1.00–1.58, $p=0.049$) for the latter.

The included studies were further divided, in line with publication time, into the earlier publication subgroup (articles published prior to or in 2005) and the later publication subgroup (articles published after 2005). A statistically significant finding was observed in the later publication subgroup but not in the earlier on the grounds that the pooled ORs (95% CIs, p -value) were 1.53 (1.15–2.04, $p=0.004$) in the former and 1.25 (0.88–1.75, $p=0.209$) in the latter.

When gastric cancers were classified into noncardia (or distal) and cardia subtypes, a statistically significant finding was noted among cardia subtype but not among noncardia subtype on the grounds that the pooled ORs (95% CIs, p -value) were 1.38 (1.11–1.72, $p=0.004$) among cardia type and 1.12 (0.80–1.58, $p=0.514$) among noncardia subtype.

In terms of pathology, gastric cancer could be classified into intestinal, diffuse, or mixed subtypes, and a statistically significant finding was observed in intestinal-type cancer but not in diffuse-type cancer, for the pooled ORs (95% CIs, p -value) were 1.33 (1.04–1.71, $p=0.024$) in the former and 1.53 (0.73–3.21, $p=0.260$) in the latter.

In terms of *H. pylori* infection status, two studies provided the detailed data on positive *H. pylori* infection cases versus negative *H. pylori* infection controls and the pooled OR (95% CI, p -value) was 2.82 (0.38–20.68, $p=0.309$).

And when genotyping techniques were considered, no statistically significant finding was noted in TaqMan/MGBEclipse subgroup or Snapshot subgroup, whereas statistically significant findings were observed in PCR-RFLP and Direct genotyping technique subgroups, respectively. Pooled ORs (95% CIs, p -value) were 0.88 (0.69–1.11, $p=0.276$)

in TaqMan/MGBEclipse subgroup, 1.89 (1.25–2.88, $p=0.003$) in PCR-RFLP subgroup, 1.91 (1.31–2.77, $p=0.001$) in Direct subgroup, and 1.37 (0.76–2.45, $p=0.296$) in Snapshot subgroup.

Sensitivity analysis

Meta-analyses were conducted repeatedly when each particular study had been removed. The results indicated that fixed-effects estimates and/or random-effects estimates before and after the deletion of each study were similar at large, suggesting high stability of the meta-analysis results. As shown in Figure 3, the most influencing single study on the overall pooled estimates seemed to be the study conducted by Xiao *et al.* (2009); the sensitivity analysis, however, indicated high stability of the results from the facts that the ORs (95% CIs, p -value) were 1.41 (1.13–1.76, $p=0.002$) before the removal of that study and 1.30 (1.09–1.56, $p=0.004$) after the removal of that study. When studies deviated from HWE were all removed, the overall OR (95% CI, p -value) became 1.23 (0.88–1.72, $p=0.217$), indicating low stability of the results for overall ethnicity; however, for Asians, the ORs (95% CIs, p -value) were 1.81 (1.44–2.27, $p=0.000$) before the removal of those studies and 1.80 (1.09–2.96, $p=0.021$) after the removal of those studies, still indicating high stability of the results for Asians.

Cumulative meta-analysis

Cumulative meta-analyses of IL-10-1082 A/G SNP association were also conducted among Asians (Fig. 4A) and among Caucasians (Fig. 4B) via the assortment of total number of sample size. As shown in Figure 4A, the inclination, though slightly undulated, toward significant associations with overall gastric cancer was obviously seen among Asians, whereas in Figure 4B, the tendency toward insignificant associations with overall gastric cancer was apparently observed among Caucasians.

FIG. 4. Cumulative meta-analysis of associations between the IL-10-1082 A/G SNP and gastric cancer risk (the combined group of GG-plus-GA genotypes was compared with the AA genotype) among different ethnicities sorted by total number of sample size. Horizontal line: the accumulation of estimates as each study was added rather than the estimate of a single study. **(A)** Among Asians; **(B)** among Caucasians.

Publication bias analysis

Publication bias was preliminarily examined by funnel plots qualitatively and estimated by Begg's and Egger's tests quantitatively. Its funnel plot (Fig. 5) showed that dots were nearly symmetrically distributed, predominantly within pseudo 95% confidence limits. *p*-Values were 0.327 in Begg's test and were 0.249 in Egger's test, also insinuating no publication bias.

Discussion

In our meta-analysis, a statistically significant finding could be noted with the overall risk of developing gastric cancer and it was more apparently noted in Asians but not in Caucasians (GG-plus-GA vs. AA). Based on the findings of cumulative meta-analyses, the inclinations toward significant associations in Asians could be obviously seen when sorted by total sample size (GG-plus-GA vs. AA). The IL-10-

1082 GG-plus-GA genotypes may seem to be more susceptible to gastric cancer in Asians. The differential influence of the IL-10-1082 polymorphisms on the risk of gastric cancer in Asians and Caucasians should be further investigated and reconfirmed in the future (Cui *et al.*, 2011).

Our subgroup analyses also indicate that significant associations could be found in the large sample subgroup but not in the small-and-moderate sample counterpart (GG-plus-GA vs. AA). In large sample subgroup the influences of ORs in the studies conducted by Xiao *et al.* (2009), Wu *et al.* (2003), and Lu *et al.* (2005) are all strong enough (4.78, 2.14, and 2.04, respectively) to make the overall OR at a significant value (1.50), whereas in small-and-moderate sample subgroup the ORs are averagely distributed around 1, and the influence of OR in the study conducted by Wu *et al.* (2002) is not so strong (2.29), thus the overall OR being at the insignificant value (1.29). A statistically significant finding was also witnessed in high quality subgroup but not in low-and-moderate quality

FIG. 5. Funnel plot of publication bias for IL-10-1082 A/G SNP with gastric cancer risk (the combined group of GG-plus-GA genotypes was compared with the AA genotype). Note: Funnel plot with pseudo 95% confidence limits was used.

counterpart (GG-plus-GA vs. AA). It is natural that high quality studies should be designed in the future in order to accurately explore the real associations between IL-10-1082 SNP and gastric cancer.

Additionally, 7 (El-Omar *et al.*, 2003; Zambon *et al.*, 2005; Kamangar *et al.*, 2006; García-González *et al.*, 2007; Crusius *et al.*, 2008; Deng *et al.*, 2008; Zhou *et al.*, 2011) out of 18 eligible studies were dealt with anatomically noncardia gastric cancer and 6 (El-Omar *et al.*, 2003; Savage *et al.*, 2004; Guo *et al.*, 2005; García-González *et al.*, 2007; Crusius *et al.*, 2008; Zhou *et al.*, 2011) with cardia gastric cancer. A statistically significant finding could be noted in cardia gastric cancer. Likewise, four studies (Kamangar *et al.*, 2006; García-González *et al.*, 2007; Kang *et al.*, 2009; Zhou *et al.*, 2011) in our meta-analysis were dealt with pathologically intestinal-type gastric cancer and 3 (García-González *et al.*, 2007; Kang *et al.*, 2009; Zhou *et al.*, 2011) out of 18 studies were dealt with pathologically sporadic diffuse-type gastric cancer. A statistically significant finding could be noted in intestinal-type but not in sporadic diffuse-type cancer (GG-plus-GA vs. AA). As is known, cardia-type gastric cancer differs from noncardia-type gastric cancer in etiology, pathology, carcinogenesis, and/or prognosis (Heidl *et al.*, 1993; Kim *et al.*, 2005; Saito *et al.*, 2006), so is intestinal-type cancer versus sporadic diffuse-type cancer. It could be said that the indiscriminate combination of cardia-type and noncardia-type cases or intestinal-type and diffuse-type cases in the majority of eligible studies may mask or at least underestimate the strength of the real associations (Xue *et al.*, 2010, 2011; Cui *et al.*, 2011).

With the coming of sophisticated genotyping technologies like seminested polymerase chain reaction, TaqMan allelic discrimination test, or real-time PCR, we may witness an upsurge of genetic association studies in the future (Xue *et al.*, 2010, 2011; Cui *et al.*, 2011). In our meta-analysis, no statistically significant finding was noted in TaqMan/MGBEclipse subgroup or Snapshot subgroup, whereas statistically significant findings were observed in PCR-RFLP

and Direct genotyping subgroups, respectively. The difference should be concerned with great caution. We propose that the sensitivity and specificity of those genotyping techniques need to be further explored in order to seek out the optimal approaches that could minimize the genotyping errors.

Finally, the strength of our meta-analysis could be summarized as follows. We sought to find as many publications as we could by means of various searching approaches. We laid more emphasis on assessing biases across studies and pinpointing the potential sources of heterogeneity via stratification, and sensitivity analyses. We comprehensively assessed the publication biases using several means like Begg's and Egger's tests as well as funnel plot tests. In view of this, we convince that the results of our meta-analysis, in essence, are sound and reliable.

In conclusion, IL-10-1082 G allele is associated with the overall risk of developing gastric cancer and may seem to be more susceptible to overall gastric cancer in Asian populations. IL-10-1082 G allele is more associated with the intestinal-type gastric cancer or cardia gastric cancer.

Acknowledgments

This work was supported by the National Science Foundation of China (Grant No. 30873057) and the Key Basic Project of Shanghai Municipal Science and Technology Commission (No. 08JC1413600).

Disclosure Statement

No competing financial interests exist.

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Received for publication September 11, 2011; received in revised form December 28, 2011; accepted December 28, 2011.