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Original Article

Association between cytotoxic T lymphocyte antigen-4 gene polymorphisms and gastric cancer risk: a meta-analysis of case-control studies

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Abstract: Many previous studies have reported the existence of CTLA-4 polymorphisms in various cancers. However, the effects of CTLA-4 polymorphisms on the risk of gastric cancer (GC) remain conflicting and have not been studied in detail. This meta-analysis was performed to clarify the association between CTLA-4+49A/G, -1661A/G and -1722T/C polymorphisms and GC risk. A systematic literature search for eligible studies published before October 10, 2015. We assessed the possible association by pooled odds ratio (OR) and its 95% confidence interval (95% CI) using the fixed or random effect model. A total of 9 independent case-control studies were enrolled in the final meta-analysis. Overall, no significant association between +49A/G polymorphism and GC risk was identified in all genetic models. However, increased GC risk was found in the subgroups of Caucasian populations and Hospital-Based (HB). For the -1661A/G polymorphism, pooled estimates showed that the -1661A/G polymorphism was significantly associated with an increased GC risk under allele comparison, heterozygote comparison and dominant models. Similar results were also found in subgroup analyses according to ethnicity, source of control and genotyping method. For the -1772T/C polymorphism, a significantly decreased risk of GC was observed under homozygote comparison and recessive model, especially in Asian populations and HB subgroups. The results suggest that both -1661A/G and -1722T/C polymorphisms in CTLA-4 are risk factors for GC. While no significant association was detected in the overall results of +49A/G polymorphism, an increased GC risk was found in Caucasian populations and HB subgroups.

Keywords: Cytotoxic T lymphocyte antigen-4, gastric cancer, polymorphism, meta-analysis

Introduction

Gastric cancer (GC) is the fourth most common form of cancer and the second most common cancer cause of death worldwide [1]. More than 70% of deaths have been observed in developing countries, seriously threatened the human health and place a heavy burden on patients. To date, the precise aetiology of GC has not been completely elucidated. Environmental and multiple genetic risk factors possibly play a vital role in etiology of the disease [2, 3]. However, these risk factors cannot absolutely explain the development of GC, since only a small percentage of exposed population finally suffered from GC, indicating possible interaction between risk factors and personal background including genetic susceptibility [4]. Numerous studies have begun the search for the association between genetic variants and GC risk, and Cytotoxic T-lymphocyte antigen-4 (CTLA-4) gene has been extensively analyzed.

CTLA-4, also known as CD152 belonging to immunoglobulin superfamily that is expressed transiently by activated T cells, plays a critical role in the negative regulation of T-cell proliferation and activation [5]. The CTLA-4 molecule is homolog to CD28, and both of them and their common co-stimulatory molecules ligands (B7-1 and B7-2) constitute the B7/CD28-CTLA-4 costimulatory pathway of T-cell activation. CTLA-4/ligand interaction maintains peripheral tolerance through negatively regulating adaptive immune responses [6] and inhibits T-cell activation by down regulating immune response [7-9], In contrast, CD28/ligand plays a important role in increasing and maintaining the T-cell

response initiated through T-cell antigen receptor [10, 11]. Since CTLA-4 performs as a potent inhibitor in T-cell response, it has been considered to facilitate the malignant transformation of cancer. A series of studies reported that genetic variations could induce the functional changes of CTLA-4 protein. The CTLA-4 protein is encoded by *CTLA-4* gene, which is located in the human chromosome 2q33, and it harbors four exons, three introns, and an upstream regulatory sequence. More than 100 single nucleotide polymorphisms (SNPs) have been identified in the CLTA-4 gene.

In recent years, *CTLA-4* gene has been extensively researched. Many studies have found that CTLA-4 gene polymorphisms possibly associated with various cancers, including breast cancer, cervical cancer, lung cancer, gastric cancer and so on. Several polymorphisms in the CTLA-4 gene have been widely studied and expected to be involved in the etiology of gastric cancer, such as +49A/G (rs231775), -1661A/G (rs4553808), -1722T/C (rs733618). A number of papers investigated the association between the three SNPs and GC risk [12-20], but the results were mixed and remained inconclusive. For example, Hou et al. found that CTLA-4-1661A/G is associated with significantly increased risk of GC [18], but Hadinia et al. reported that no significant association was found between CTLA-4-1661A/G polymorphism and GC [14].

Therefore, the association between *CTLA-4* gene polymorphisms and GC risk requires further investigation. Meta-analysis uses a statistical method to combine the results from multiple studies then provide more reliable results than a single case-control study. We performed this meta-analysis to explore an accurate estimation of the relationship between CTLA-4 gene variants and GC risk.

Materials and methods

Search strategy

The EndNote software version X7 (Thomson Reuters Corporation, Toronto, Ontario, Canada) was used throughout the searching process. We carried out a comprehensive literature search in PubMed, Web of Science, WanFang database and Chinese National Knowledge Infrastructure (CNKI). The last search was performed on October 10, 2015. The key words included: "gastric cancer OR GC OR stomach cancer OR gastric neoplasm" AND "polymorphism OR variation OR mutation" AND "cytotoxic T-lymphocyte antigen-4 OR CTLA-4 OR CD152". There was no restriction on publication years but the languages were limited to English and Chinese in our search. Reference lists of review articles and primary studies were manually searched to identify additional eligible studies. If data were published in more than one article, only the study with the largest sample size was included.

Inclusion and exclusion criteria

All studies included in this meta-analysis were selected based on the following criteria: (1) evaluating the associations between the CTLA-4+49A/G (rs231775) and/or -1661A/G (rs4553808) and/or -1772 T/C (rs733618) and gastric cancer; (2) case-control design for human beings; (3) supplying useful genotype frequencies of cases and controls or could be calculated from the article text; and (4) diagnosis of GC was objectively confirmed.

Exclusion criteria included: (1) case-only study; (2) studies with no detailed genotype frequencies; (3) reviews, comments or animal studies; and (4) duplicate publications.

Data extraction

The following data were independently and carefully extracted by two researchers (L. Lu, W. Wang) from all eligible studies: last name of first author, year of publication, original country, ethnicity, genotyping method, source of control, Hardy-Weinberg equilibrium in controls, numbers of cases and controls, and genotype frequencies for cases and controls. If disagreements existed, the original data were rechecked and consensus was reached through discussion.

Study validity assessment

The quality of the studies that were included in this review was independently evaluated by two researchers (L. Lu, W. Wang) according to the Newcastle Ottawa Scale (NOS) [21]. In this scale, three main items were assessed: selection, comparability and exposure. Studies with scores equal to or higher than five were consid-

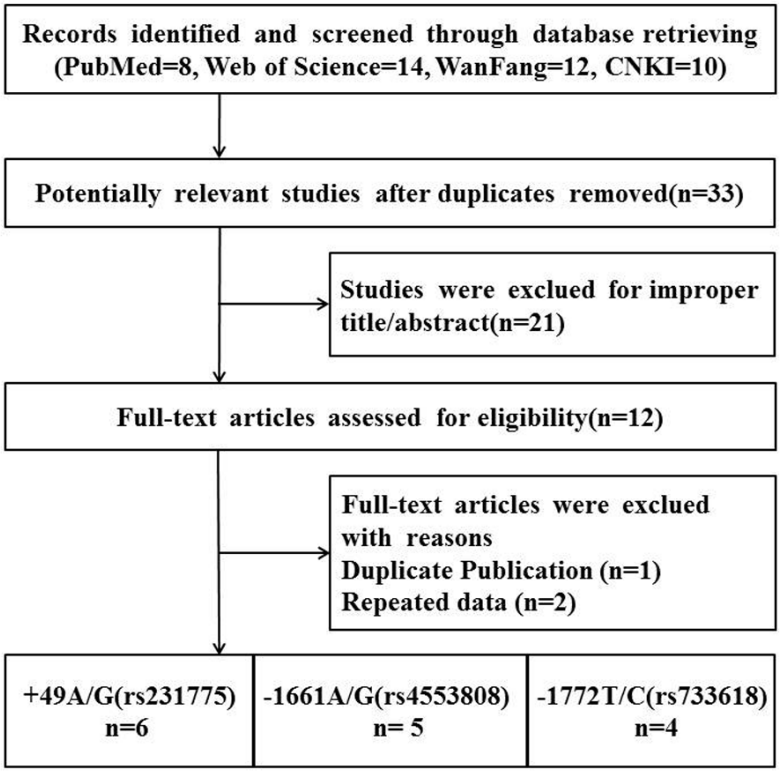

Figure 1. Flowchart of the selection process for including articles.

ered to be of high quality. Discrepancies were settled by discussion between the two researchers.

Statistical analysis

All statistical analyses were performed using the software STATA version 12.0 (Stata Corp, College Station, Texas). The strength of the association between the three SNPs and gastric cancer risk were estimated by the odds ratio (OR) and its 95% confidence interval (95% CI). The pooled ORs were estimated for five genetic models: allele comparison (+49A/G, -1661A/G: G vs. A; -1722T/C: C vs. T), heterozygote comparison (+49A/G, -1661A/G: AG vs. AA; -1722T/C: TC vs. TT), homozygote comparison (+49A/G, -1661A/G: GG vs. AA; -1722T/C: CC vs. TT), dominant model (+49A/G, -1661A/G: AG+GG vs. AA; -1722T/C: TC+CC vs. TT) and recessive model (+49A/G, -1661A/G: GG vs. AG+AA; -1722T/C: CC vs. TC+TT). The significance of the pooled ORs was examined by the Z-test. Heterogeneity between studies was tested by Chi-square-based Q test and I^2 statistics; $P<0.10$ or $I^2>50\%$ indicated evidence of heterogeneity [22]. The fixed-effects model (Mantel-Haenszel method) was used to estimate the summary ORs when there was no significant heterogeneity [23]; otherwise, the random-effects model (DerSimonian and Laird method) was used [24]. We performed subgroup analyses to avoid the potential impact of confounding factors. Subgroup analyses were conducted according to ethnicity, type of disease, source of control and genotyping method. Meta-regression was conducted for further exploration of heterogeneity. The study characteristics included as covariates in the meta-regression were publication year and NOS score. Furthermore, sensitivity analysis was used to assess the stability of the results by deleting one single study at a time to examine the influence of single data points. Potential publication bias was evaluated using the Begg test [25] and Egger regression test [26]; an asymmetric plot and $P<0.05$ were considered as statistically significant publication bias. All statistical tests were 2-sided, and *P* values <.05 were considered statistically significant.

Results

Study selection and characteristics

As shown in **Figure 1**, a total of 44 relevant studies were initially identified after a systematic search; 11 studies were duplicated and 21 studies were excluded after title and abstract screening according to the inclusion and exclusion criteria. After reviewing full text articles, 3 studies were excluded with reason of duplicate publication and repeated data. Thus, a total of 9 independent case-control studies including 4 Chinese articles [13, 17, 19, 20] and 5 English articles [12, 14-16, 18] were used in the meta-analysis. These studies involved 2547 cases and 3301 controls. The main baseline characteristics of eligible studies are summarized in **Table 1**. Among the 9 eligible studies, 6 studies [12, 14-16, 18, 20] investigated the association between +49A/G and gastric cancer in a total of 1259 cases and 1739 controls, 5 stud-

Table 1. Baseline characteristics of studies included in the meta-analysis

Author	Year	Country	Ethnicity	Control source	Genotyping methods	SNP	Sample size (men)		Genotype distribution (Case/Control)			HWE	NOS
							Case	Control	Wildtype	Heterozygous	Mutant	Y/N (P)	
Song	2006	China	Asian	HB	PCR-RFLP	-1722T/C	183	116	62/45	113/54	8/17	Y (0.903)	5
Cheng	2006	China	Asian	HB	PCR	+49A/G	62	250	2/29	26/102	34/119	Y (0.323)	7
					PCR	-1661A/G	183	116	120/98	57/17	6/1	Y (0.784)	
Hadinia	2007	Iran	Caucasian	PB	PCR-RFLP	+49A/G	43	190	24/117	13/59	6/14	Y (0.097)	8
					PCR-RFLP	-1661A/G	109	188	74/145	33/36	2/7	N (0.020)	
					PCR-RFLP	-1722T/C	46	190	42/165	4/24	0/1	Y (0.900)	
Sun	2008	China	Asian	PB	PCR-RFLP	+49A/G	530	530	60/39	235/209	235/282	Y (0.974)	6
Mahajan	2008	Poland	Caucasian	PB	TaqMan	+49A/G	301	411	89/152	153/189	59/70	Y (0.393)	7
Li	2009	China	Asian	HB	PCR-RFLP	-1661A/G	121	236	89/198	28/34	4/4	Y (0.087)	5
Hou	2010	China	Asian	PB	PCR-RFLP	+49A/G	205	262	41/100	70/55	94/107	N (0.000)	6
					PCR-RFLP	-1661A/G	205	262	112/163	71/54	22/45	N (0.000)	
					PCR-ARMS	-1722T/C	205	262	75/93	111/139	19/30	N (0.041)	
Cui	2011	China	Asian	HB	PCR-RFLP	-1661A/G	118	96	88/85	26/11	4/0	Y (0.552)	5
Qi	2012	China	Asian	HB	PCR-RFLP	+49A/G	118	96	8/21	45/45	65/30	Y (0.595)	5
					PCR-RFLP	-1722T/C	118	96	40/37	69/45	9/14	Y (0.958)	

HB, hospital-based; PB, population-based; PCR, polymerase chain reaction; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; PCR-ARMS, polymerase chain reaction-amplification refractory mutation system; SNP, single nucleotide polymorphism; HWE, Hardy-Weinberg equilibrium; NOS, Newcastle-Ottawa scale.

Table 2. Results of meta-analysis for CTLA-4+49A/G polymorphism and GC risk

Category		G vs A			AG vs AA			GG vs AA			AG+GG vs AA			GG vs AG+AA		
	N	OR (95% CI)	I^2 (%)	P_Q	OR (95% CI)	I^2 (%)	P_Q	OR (95% CI)	I^2 (%)	P_Q	OR (95% CI)	I^2 (%)	P_Q	OR (95% CI)	I^2 (%)	P_Q
Overall	6	1.36 (0.95, 1.93)	88.2	0.000	1.61 (0.97, 2.69)	76.9	0.001	1.87 (0.95, 3.67)	84.8	0.000	1.65 (0.97, 2.82)	82.3	0.000	1.30 (0.87, 1.95)	79.9	0.000
Ethnicity																
Caucasian	2	1.25 (1.02, 1.52)	0.0	0.231	1.32 (0.97, 1.80)	0.0	0.545	1.52 (1.02, 2.27)	0.0	0.521	1.37 (1.03, 1.83)	0.0	0.798	1.27 (0.89, 1.82)	0.0	0.331
Asian	4	1.41 (0.81, 2.45)	92.7	0.000	2.00 (0.80, 5.05)	85.6	0.000	2.09 (0.70, 6.26)	90.7	0.000	2.02 (0.76, 5.35)	89.4	0.000	1.28 (0.74, 2.22)	88.6	0.000
Source of control																
HB	2	1.89 (1.19, 3.02)	57.8	0.124	2.88 (1.32, 6.28)	0.0	0.699	5.21 (2.38, 11.39)	0.0	0.717	3.87 (1.84, 8.15)	0.0	0.979	1.90 (0.95, 3.78)	66.8	0.083
PB	4	1.17 (0.79, 1.73)	88.9	0.000	1.36 (0.75, 2.45)	83.4	0.000	1.31 (0.66, 2.62)	85.4	0.000	1.29 (0.73, 2.28)	85.5	0.000	1.07 (0.72, 1.58)	73.3	0.010
Genotyping method																
PCR-RFLP	4	1.39 (0.79, 2.44)	92.5	0.000	1.56 (0.71, 3.45)	85.0	0.000	1.84 (0.67, 5.06)	90.2	0.000	1.60 (0.70, 3.63)	88.6	0.000	1.38 (0.74, 2.58)	87.1	0.000

OR, odds ratio; CI, confidence interval; P_Q, *p*-value for heterogeneity test; HB, hospital-based; PB, population-based; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism.

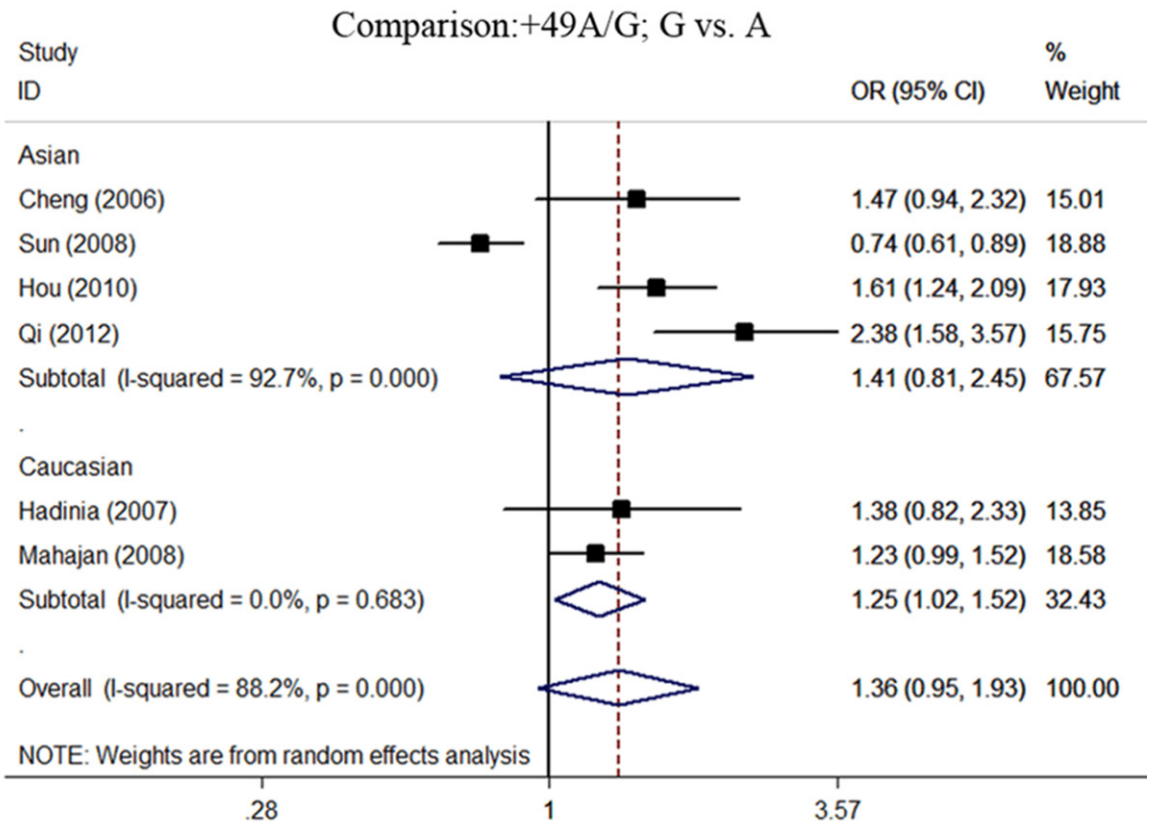

Figure 2. Forest plot for the subgroup analysis of ethnicity (G vs. A). The CTLA-4+49A/G polymorphism was found to be associated with increased risk of Caucasian.

ies [12, 14, 17-19] involving 736 cases and 898 controls for -1661A/G and 4 studies [13, 14, 18, 20] involving 552 cases and 664 controls for -1772T/C. There were only 2 studies carried out in Caucasians, while the other studies were conducted in Asians. Detailed genotype distribution and the results of the HWE test in control population are summarized in **Table 1**; all studies were consistent with HWE except for 2 studies [14, 18]. NOS quality scores for each study ranged from 6 to 8, with all studies being classified as high quality.

Association between +49A/G (rs231775) polymorphism and gastric cancer risk

The association between +49A/G polymorphism and GC risk was analyzed in 6 independent studies with a total of 1259 cases and 1739 controls. Results of the meta-analysis are shown in **Table 2**. There was a significant heterogeneity within studies of all models, thus the random-effects model was used. No significant association between +49A/G polymorphism and GC risk was identified in any of the genetic models (G vs. A: OR=1.36, 95% CI 0.95-1.93, P=0.090; AG vs. AA: OR=1.61, 95% CI 0.97-2.69, P=0.068; GG vs. AA: OR=1.87, 95% CI 0.95-3.67, P=0.069; AG+GG vs. AA: OR=1.65, 95% CI 0.97-2.82, P=0.066; GG vs. AG+AA: OR=1.30, 95% CI 0.87-1.95, P=0.198).

Subsequently, we performed subgroup analysis according to ethnicity. In Caucasian populations, we found a significant increased GC risk in allele comparison, homozygote comparison and dominant model (G vs. A: OR=1.25, 95% CI 1.02-1.52, P=0.028, **Figure 2**; GG vs. AA: OR=1.52, 95% CI 1.02-2.27, P=0.041; AG+GG vs. AA: OR=1.37, 95% CI 1.03-1.83, P=0.031). In Asian populations, no evidence of association was observed in any genetic model (**Table 2**). When stratifying by source of control, we found the +49A/G polymorphism was significantly associated with an increased GC risk of Hospital-Based (HB) in all genetic models except the recessive model (GG vs. AG+AA: OR=1.897, 95% CI 0.95-3.78, P=0.068). No evi-

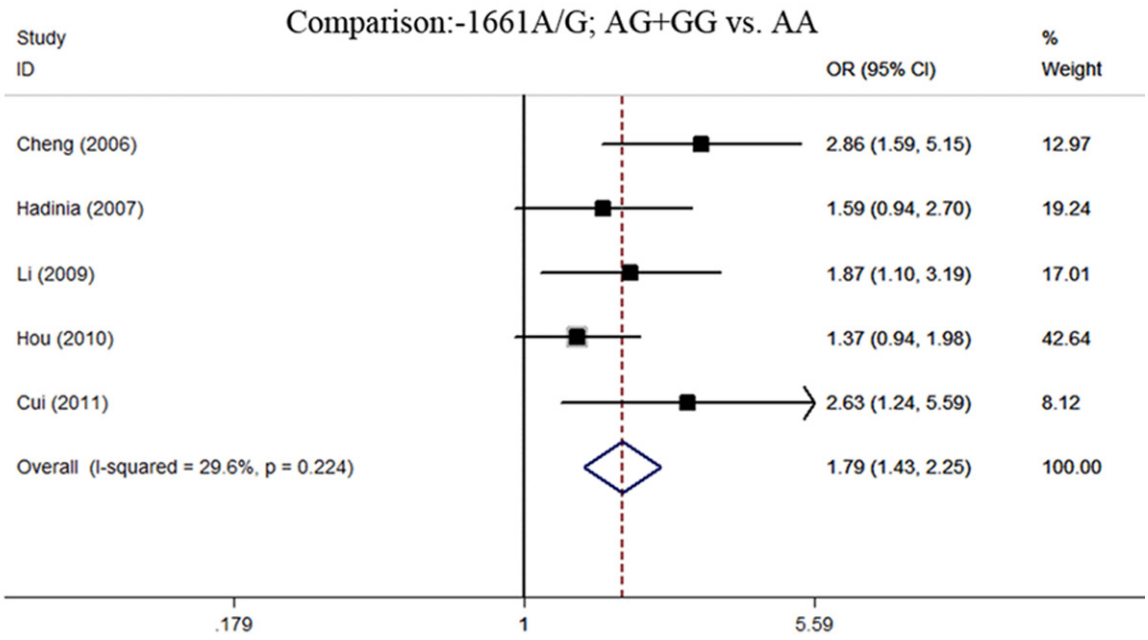

Figure 3. Forest plots of meta-analysis for association between CTLA-4-1661A/G polymorphism and GC risk (AG+GG vs. AA). A significant increased susceptibility was observed between the -1661A/G polymorphism and risk of GC.

dence of association was found in any genetic model between +49A/G polymorphism and GC risk in the Population-Based (PB) group (**Table 2**). Four out of the six included studies were conducted by the genotyping method of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). In genotyping method subgroup analysis, no association was found to be statistical significant in PCR-RFLP group in any genetic model (**Table 2**). No publication bias was detected with either the Begg's test or Egger's test (P_B=0.707, P_E=0.449 for AG+GG vs. AA).

The heterogeneity was partly decreased in HB group (G vs. A: I^2=57.8%, P=0.124; AG vs. AA: I^2=0.0%, P=0.699; GG vs. AA: I^2=0.0%, P=0.23; AG+GG vs. AA: I^2=0.0%, P=0.979; GG vs. AG+AA: I^2=66.8%, P=0.083) or removed in Caucasian population (G vs. A: I^2=0.0%, P=0.683; AG vs. AA: I^2=0.0%, P=0.545; GG vs. AA: I^2=0.0%, P=0.521; AG+GG vs. AA: I^2=0.0%, P=0.798; GG vs. AG+AA: I^2=0.0%, P=0.331). However, there was still significant heterogeneity among Asian population, PB and PCR-RFLP group. A meta-regression was used next, but it failed to confirm that the publication year and NOS score were the sources of heterogeneity.

Association between -1661A/G (rs4553808) polymorphism and gastric cancer risk

5 studies involving 736 cases and 898 controls were assessed for the association between -1661A/G polymorphism and GC risk. Q-test showed no significant heterogeneity in all genetic models except the allele comparison (I^2=71.6%, P=0.007), so that random-effects model was used. We found a significantly increased GC risk in allele comparison, heterozygote comparison and dominant model (G *vs.* A: OR=1.68, 95% CI 1.13-2.49, P=0.010; AG vs. AA: OR=2.04, 95% CI 1.60-2.61, P<0.001; AG+GG *vs.* AA: OR=1.79, 95% CI 1.43-2.25, P<0.001 **Figure 3**). No significant association was found in homozygote comparison and recessive model (GG vs. AA: OR=1.01, 95% CI 0.65-1.58, P=0.962; GG vs. AG+AA: OR=1.15, 95% CI 0.46-2.85, P=0.768).

Four out of the five included studies were conducted in Asian population. As shown in **Table 3**, similar result was found in ethnicity subgroup analysis (G vs. A: OR=1.82, 95% CI 1.09-3.04, P=0.023; AG *vs.* AA: OR=2.11, 95% CI 1.60-2.78, P<0.001; AG+GG *vs.* AA: OR=1.84, 95% CI 1.43-2.37, P<0.001). Regarding the source of control, we found significantly increased GC risk in the HB group under all genetic models

Table 3. Results of meta-analysis for CTLA-4-1661A/G polymorphism and GC risk

Category		G vs A			AG vs AA			GG vs AA			AG+GG vs AA			GG vs AG+AA		
	N	OR (95% CI)	I^2 (%)	P_Q	OR (95% CI)	I^2 (%)	P_Q	OR (95% CI)	I^2 (%)	P_Q	OR (95% CI)	I^2 (%)	P_Q	OR (95% CI)	I^2 (%)	P_Q
Overall	5	1.68 (1.13, 2.49)	71.6	0.007	2.04 (1.60, 2.61)	0.0	0.839	1.01 (0.65, 1.58)	45.9	0.117	1.79 (1.43, 2.25)	29.6	0.224	0.82 (0.53, 1.27)	48.5	0.101
Ethnicity																
Asian	4	1.82 (1.09, 3.04)	78.5	0.003	2.11 (1.60, 2.77)	0.0	0.753	1.07 (0.67, 1.72)	56.9	0.073	1.84 (1.43, 2.37)	45.4	0.139	0.87 (0.55, 1.37)	59.6	0.059
Source of control																
HB	3	2.23 (1.62, 3.06)	0.0	0.469	2.25 (1.57, 3.22)	0.0	0.630	3.75 (1.31, 10.77)	0.0	0.637	2.37 (1.68, 3.35)	0.0	0.545	3.19 (1.11, 9.16)	0.0	0.667
PB	2	1.11 (0.87, 1.41)	0.0	0.352	1.87 (1.33, 2.62)	0.0	0.859	0.69 (0.41, 1.18)	0.0	0.781	1.44 (1.06, 1.95)	0.0	0.639	0.57 (0.34, 0.95)	0.0	0.832
Genotyping method																
PCR-RFLP	4	1.49 (1.01, 2.20)	65.3	0.034	1.91 (1.46, 2.50)	0.0	0.964	0.90 (0.56, 1.44)	38.6	0.181	1.64 (1.28, 2.10)	0.0	0.441	0.74 (0.47, 1.17)	43.9	0.148

OR, odds ratio; CI, confidence interval; P_Q, *p*-value for heterogeneity test; HB, hospital-based; PB, population-based; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism.

Table 4. Results of meta-analysis for CTLA-4-1772T/C polymorphism and GC risk

Category		C vs T			TC vs TT			CC vs TT			TC+CC vs TT			CC vs CT+TT		
	N	OR (95% CI)	I^2 (%)	P_Q	OR (95% CI)	I^2 (%)	P_Q	OR (95% CI)	I^2 (%)	P_Q	OR (95% CI)	I^2 (%)	P_Q	OR (95% CI)	I^2 (%)	P_Q
Overall	4	0.91 (0.76, 1.09)	0.0	0.899	1.17 (0.90, 1.52)	0.0	0.363	0.60 (0.38, 0.95)	0.0	0.511	1.05 (0.82, 1.36)	0.0	0.617	0.54 (0.35, 0.83)	31.7	0.222
Ethnicity																
Asian	3	0.92 (0.77, 1.11)	0.0	0.965	1.22 (0.93, 1.60)	3.2	0.356	0.59 (0.37, 0.94)	4.2	0.352	1.09 (0.84, 1.42)	0.0	0.638	0.54 (0.35, 0.82)	51.0	0.130
Source of control																
HB	2	0.92 (0.71, 1.19)	0.0	0.461	1.48 (1.01, 2.16)	0.0	0.862	0.45 (0.23, 0.86)	0.0	0.412	1.22 (0.85, 1.78)	0.0	0.976	0.36 (0.19, 0.66)	0.0	0.347
PB	2	0.91 (0.70, 1.18)	0.0	0.469	0.94 (0.65, 1.36)	0.0	0.491	0.80 (0.42, 1.51)	0.0	0.764	0.91 (0.64, 1.30)	0.0	0.485	0.80 (0.44, 1.46)	0.0	0.745

OR, odds ratio; CI, confidence interval; P_Q, *p*-value for heterogeneity test; HB, hospital-based; PB, population-based.

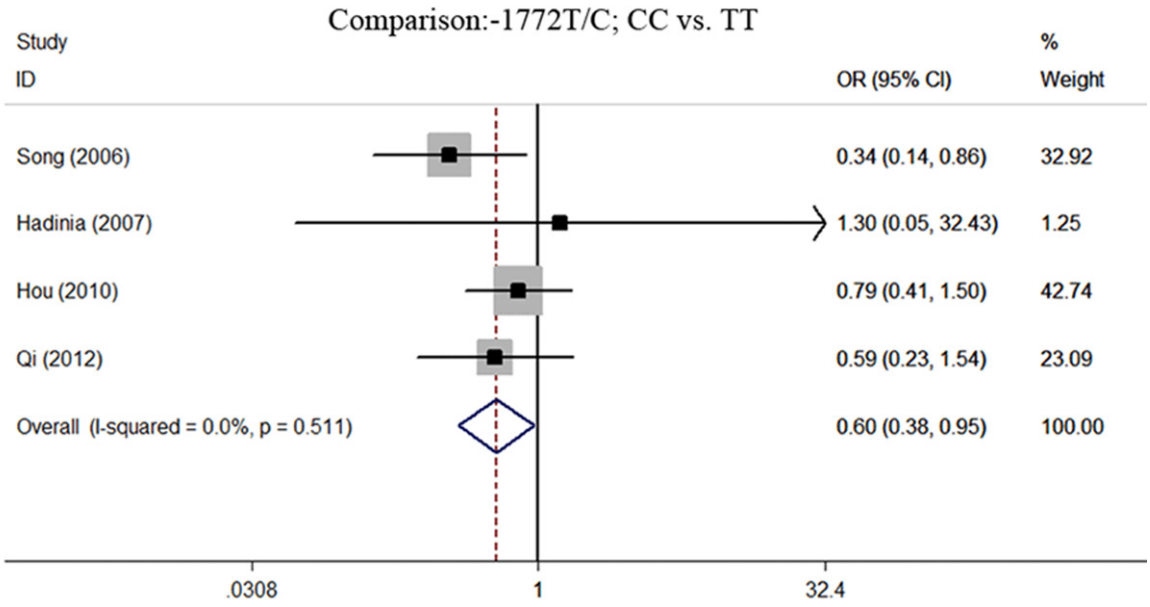

Figure 4. Forest plots of meta-analysis for association between CTLA-4-1772T/C polymorphism and GC risk (CC vs. TT). A significant reduction susceptibility was observed between the -1772T/C variant and risk of GC.

(**Table 3**) and significant association between -1661A/G polymorphism and GC risk in the PB group under heterozygote comparison, dominant model and recessive model. It is worth noting that decreased GC risk was found in the recessive model (GG vs. AG+AA: OR=0.57, 95% CI 0.34-0.95, P=0.032) in the PB group. Moreover, in the stratified analysis based on genotyping method, a significantly decreased risk of GC was observed under the allele comparison, heterozygote comparison and dominant model in the PCR-RFLP group (G vs. A: OR=1.49, 95% CI 1.01-2.20, P=0.043; AG vs. AA: OR=1.91, 95% CI 1.46-2.50, P<0.001; AG+GG vs. AA: OR=1.64, 95% CI 1.28-2.10, P<0.001). No publication bias was detected with either the Begg's test or Egger's test (P_B=0.086, P_E=0.059 for AG+GG vs. AA).

Heterogeneity vanished in the source of control subgroup (HB: I^2=0.0%, P=0.469; PB: I^2=0.0%, P=0.352), suggesting that source of control might contribute mainly to the heterogeneity.

Association between -1772T/C (rs733618) polymorphism and gastric cancer risk

The association between -1772T/C polymorphism and risk to GC was analyzed in 4 independent studies with 552 cases and 664 controls. The main results of this meta-analysis and the heterogeneity test are shown in **Table 4**. No significant statistical heterogeneity was identified in all of the models so that fixed-effects model was used. We observed a significantly decreased risk of GC in homozygote comparison and recessive model (CC vs. TT: OR=0.60, 95% CI 0.38-0.95, P=0.029, **Figure 4**; CC vs. TC+TT: OR=0.54, 95% CI 0.35-0.83, P=0.005). No significant association was found in allele comparison, heterozygote comparison and dominant model (C vs. T: OR=0.91, 95% CI 0.76-1.09, P=0.322; TC vs. TT: OR=1.17, 95% CI 0.90-1.52, P=0.248; TC+CC vs. TT: OR=1.05, 95% CI 0.82-1.36, P=0.692).

In the subgroup analysis based on ethnicity, the similar results were obtained in Asian population (**Table 4**). When stratifying by source of control, a significantly decreased risk of GC was observed in the HB group under the homozygote comparison, and recessive model (CC vs. TT: OR=0.45, 95% CI 0.23-0.86, P=0.016; CC vs. TC+TT: OR=0.36, 95% CI 0.19-0.66, P=0.001), while a significantly increased risk of GC was observed under heterozygote comparison (TC vs. TT: OR=1.48, 95% CI 1.01-2.16, P=0.045). None of the genetic models produced a significant association in the PB group (**Table 4**). No publication bias was detected with either the Begg's test or Egger's test (P_B=0.734, P_E=0.643 for TC+CC vs. TT).

Sensitivity analysis

When excluding the studies which deviated from HWE, the overall results were not materially affected. Then we analyzed the three SNPs by deleting each study from the total dataset respectively, we did not notice any significant difference in the pooled estimates. The results indicated that this meta-analysis provided reliable evidence (data not shown).

Discussion

Tumor progression is a multistep process that depends on the tumor behavior and genetic constituents of the host. However, we found that genetic variations have become more prominent in tumor development when people with the same behavior patterns and living environment. As a negative regulator of T-cell proliferation and activation, recent studies show that CTLA-4 plays an important role in cancer immunosurveillance and may be involved in cancer development and progression [27]. The accurate control of CTLA-4 expression is complicated, and more studies are needed to confirm the mechanisms of the polymorphisms that regulate CTLA-4 expression in T cells. It has been suggested that CTLA-4 may elevate the T-cell activation threshold during early stages of tumorigenesis, reducing the antitumor response and increasing cancer susceptibility [28]. However, studies focusing on the association of the CTLA-4 gene polymorphism with cancer susceptibility had controversial conclusions. Meta-analysis is a powerful tool which can summarize the results from different eligible studies thereby achieve more credible results than a single case-control study.

In this meta-analysis, 9 independent case-control studies including 2547 cases and 3301 controls were involved. We investigated the association between CTLA-4+49A/G (rs231775), -1661A/G (rs4553808) and -1772T/C (rs733618) and GC risk. The subgroup analysis stratified by ethnicity, source of control and genotyping method was also performed. As for CTLA-4+49A/G polymorphism, our results suggested no significant increased GC risk in any genetic comparison model. The results were robust, which did not vary materially after we excluded the study with controls not in HWE. When we performed subgroup analysis by ethnicity, we found the CTLA-4+49A/G polymorphism is correlated to significant increased GC in Caucasian populations. However, we did not found any significant increased GC risk in Asian population, suggesting that ethnicity may be an essential biological factor which influences CTLA-4+49A/G polymorphism through gene to gene interaction. Moreover, in the subgroup analysis of source of control, hospital-based group showed significant increased risk of GC. The remaining pooled estimates from this analysis were insignificant (all $P>0.05$). In addition, Geng's meta-analysis reported that individuals with the CTLA-4+49A allele had increased cancer risk [29], Zhang et al. also obtained consistent results that the +49A/G polymorphism in CTLA-4 could be an important single nucleotide polymorphism that promotes the tumorigenesis [30].

For CTLA-4-1661A/G polymorphism, the association between this polymorphisms and cancer risk has been estimated by many previous studies. Yan's meta-analysis found that the CTLA-4-1661A/G polymorphism was significantly associated with an increased cancer risk, especially in gastric cancer, breast cancer and in Asians population subgroups [31]. In our meta-analysis, we found significant association between CTLA-4-1661A/G polymorphism and increased GC risk in allele comparison, heterozygote comparison and dominant model. The results suggested that G allele and AG/GG genotype carriers were significantly associated with an increased risk of GC. In the subgroup analysis, similar result was found whereas opposite results were seen under the recessive model in PB group. As we know, the SNP-1661A/G is located in the promoter region of CTLA-4. Allelic variants located in the promoter region may change the motif of functional DNA binding sites and then affect the affinities for the relevant transcription factors [32]. Mao et al. indicated that the transcription factor c/EBP/β, which is a highly conserved family of leucine zipper could bind to the -1661 sites in the presence of G allele, thereby regulate the function of CTLA-4 [33].

Previous researchers [34, 35] found that the -1722C allele can increase breast cancer risk and oral cancer risk compared with the T allele for CTLA-4-1722T/C polymorphism. By contrast, the -1722T/C polymorphism of CTLA-4 showed a significantly decreased risk of GC

under homozygote comparison and recessive model in our meta-analysis. The results indicate that CC carriers had a lower risk of GC compared with the TT/TC carriers. In the subgroup analysis by ethnicity and source of control, we also found protective effects on GC in overall studies. The possible mechanism is that the -1722C allele to T allele would produce a transcription factor binding site for nuclear factor 1 (NF-1) [33], a cellular DNA-binding protein that could serve as a transcription selectivity factor for RNA polymerase II and as an initiation factor for DNA replication [36]. Thus, the promoter enhances the level of gene transcription, which could promote the expression of cell surface CTLA-4 by binding NF-1.

Heterogeneity is hard to avoid in a meta-analysis and the key is determining the sources of heterogeneity. Significant heterogeneity was found for the association of +49A/G polymorphism and VT in all genetic models. By conducting stratified analyses, the heterogeneity was partly decreased in HB group or removed in Caucasian population. However, there was still significant heterogeneity among Asian population, PB and PCR-RFLP group. Meta-regression also failed to confirm that the publication year and NOS score were the sources of heterogeneity. For -1661A/G polymorphism, heterogeneity vanished in the source of control subgroup, suggesting that source of control might contribute mainly to the heterogeneity.

To some extent, our meta-analysis has several advantages. Firs, this is the first meta-analysis to investigate the association between the CTLA-4 gene polymorphisms and GC risk in detail. Second, all included studies were of high quality according to the quality assessment and demonstrated no publication bias. Third, our research included better stratified analyses to avoid more potential confounding factors. In addition, the results may have crucial public health implications for improving the prediction of VT risk. Several underlying limitations of our study should also be noted. Firstly, the original articles' lack of relevant data limited the evaluation of potential gene-gene and gene-environment interactions. Secondly, the included studies were mainly from China, so our results only applicable to limited population such as Asians. Thirdly, two studies did not conform to HWE; fortunately, when we limited the studies to those complying with HWE, the results were not altered. Fourthly, the number of original articles included in our research might not be sufficiently large; Therefore, the results should be extrapolated very cautiously.

In conclusion, the results of our meta-analysis suggest that the CTLA-4-1661A/G polymorphism contribute to increased risk of GC, similar results was found in subgroup analyses except the recessive model in PB group; and the CTLA-4-1772T/C polymorphism is significantly associated with decreased GC risk while the TC genotype plays a dangerous role in GC development in HB group. In addition there was insufficient evidence to fully confirm that +49A/G had any influence on the susceptibility to GC in overall results, but increased GC risk was found in the subgroups of Caucasian populations and HB group. Large-scale well-designed studies should be conducted to validate the findings of this study.

Disclosure of conflict of interest

None.

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