Cytotoxic T-lymphocyte-associated antigen-4 polymorphisms and susceptibility to cervical cancer: A meta-analysis

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Abstract. Cytotoxic T-lymphocyte associated antigen-4 (*CTLA-4*) polymorphisms have been examined for associations with cervical cancer in various countries. The results, however, are inconclusive. The present study aimed to explore whether *CTLA-4* +49 A/G, -318 C/T and CT60 G/A polymorphisms confer susceptibility to cervical cancer. A meta-analysis was performed with 7,794 subjects included in 15 case-control studies that were published up to January 1, 2013. The results from the meta-analysis indicated that there were no significant associations between the risk of cervical cancer and the three studied polymorphisms [+49 A/G: Odds ratio (OR), 0.94 and 95% confidence interval (CI), 0.82-1.07 for GG+AG vs. AA; -318 C/T: OR, 1.33 and 95% CI, 0.82-2.16 for TT+TC vs. CC; and CT60: OR, 0.98 and 95% CI, 0.72-1.33 for AA+AG vs. GG]. Stratified analyses by ethnicity for the +49 A/G and -318 C/T polymorphisms suggested that Asian populations had a decreased risk of cervical cancer for the +49 A/G polymorphism (OR, 0.75 and 95% CI, 0.58-0.97 for GG+AG vs. AA), but an increased risk for the -318 C/T polymorphism (OR, 2.02 and 95% CI, 1.36-3.00 for TC vs. CC). In summary, the current meta-analysis showed that the +49 A/G and -318 C/T polymorphisms in *CTLA-4* constitute risk factors for cervical cancer.

Introduction

Cervical cancer is the third most common type of cancer diagnosed and the fourth leading cause of female cancer mortality worldwide, accounting for ~529,000 new cancer cases and 275,000 cancer deaths in 2008, >85% of which occurred in developing countries (1). It is widely accepted that infection by an oncogenic type of human papillomavirus (HPV) is a necessary but not sufficient risk factor for cervical carcinogenesis (2). Various genetic and environmental factors may also play roles in the pathogenesis of or predisposition to cervical cancer, as only a small percentage of infected females develop the cancer (3). Evidence that first-degree relatives of females with cervical cancer have a doubled risk of tumor development distinctly indicates a strong genetic predisposition to the disease (4). The first known cervical cancer susceptibility gene was human leukocyte antigen (*HLA)-DQw3* (5). With the development of new technology and invention of new genomic tools, a number of genes outside the *HLA* region have been examined in patients with cervical cancer across populations of various ethnicities. These include cytotoxic T-lymphocyte-associated antigen-4 (*CTLA-4*) (6), interleukin-10 (7) and cyclin D1 (8), among other genes.

CTLA-4, also known as cluster of differentiation (CD)152, is a member of the immunoglobulin super family and plays a crucial role in the negative regulation of T-lymphocyte activation and proliferation, indirectly controlling effector T cells (9). The *CTLA-4* gene is situated on chromosome 2q33, close to genes of other regulatory molecules, including CD28 and inducible costimulator (10). It consists of four exons that encode separate functional domains: Leader sequence, and extracellular, transmembrane and cytoplasmic domains (11). In the early stages of tumorigenesis, *CTLA-4* may elevate the activation threshold of T-cells, thereby weakening the antitumor response and increasing susceptibility to cancer (12). Studies have shown that *CTLA-4* blockade results in enhancement of the immune response (13), rejection (14) or in certain cases, cure of tumors in mice treated with a combination of tumor vaccines (15). Moreover, recent studies have demonstrated that *CTLA-4* polymorphisms were associated with susceptibility to cancer (16-19). Thus, it is extremely likely that *CTLA-4* polymorphisms are involved in the pathogenesis of cervical cancer. The most frequently studied *CTLA-4* polymorphisms are +49 A/G (rs231775), -318 C/T (rs5742909) and CT60 G/A (rs3087243) (6,20-22). A number of studies have been previously performed to determine whether these polymorphisms confer susceptibility to cervical cancer in various populations

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Abbreviations: CI, confidence interval; OR, odds ratio; CTLA-4, cytotoxic T-lymphocyte associated antigen-4; HWE, Hardy-Weinberg equilibrium; HPV, human papillomavirus

Key words: cervical cancer, gene polymorphism, cytotoxic T-lymphocyte associated antigen-4, meta-analysis

(6,23-29), however, results have been inconclusive. The discrepancy may result from limitations of individual studies, publication bias (30) or yet-unknown effects of the *CTLA-4* molecule.

Therefore, with the aim of deriving a more precise assessment of the correlation between *CTLA-4* +49 A/G, -318 C/T and CT60 G/A polymorphisms and cervical cancer, a meta-analysis of 15 published case-control studies was performed. To the best of our knowledge, this is the most comprehensive evaluation method with regards to the associations between *CTLA-4* polymorphisms and cervical cancer risk.

Subjects and methods

Study identification and selection. To identify all the published studies that have examined the association of *CTLA-4* gene polymorphisms with cervical cancer, the electronic databases of PubMed, Embase and the Chinese Biomedical Database were searched, with the last search update being performed on January 1, 2013. Combinations of keywords, including 'cytotoxic T-lymphocyte associated antigen-4', 'CTLA-4', 'CD152', 'polymorphism' and 'uterine cervical neoplasms' were entered as Medical Subject Headings and text words. No language restriction was applied. References of retrieved articles were also screened. All of the eligible studies were case-control in design, based on unrelated individuals and had available data (distribution of alleles and genotypes for cases and controls) to estimate the odds ratios (ORs) with the 95% confidence intervals (CIs). Furthermore, genotype distributions in the control groups were required to be in Hardy-Weinberg equilibrium (HWE), according to an exact test.

Data extraction. Data were extracted by two reviewers independently. A consensus was reached following discussion if there was disagreement. The following information was extracted from each eligible study: Author, year of publication, ethnicity of the study population, sample size, genotyping method and genotype number in cases and controls.

Statistical analysis. HWE was tested by an internet-based HWE calculator (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl; accessed March 5, 2013). The strength of associations between the *CTLA-4* polymorphisms and cervical cancer risk were evaluated by ORs with the corresponding 95% CIs. The genetic models that were assessed for pooled ORs of these polymorphisms were dominant models (GG+GA vs. AA for +49 A/G, TT+TC vs. CC for -318 C/T and AA+AG vs. GG for CT60 G/A). For each *CTLA-4* polymorphism, other genetic models (+49 A/G, GG vs. GA+AA, GG vs. AA, GA vs. AA and G vs. A; -318 C/T, TT vs. TC+CC, TT vs. CC, TC vs. CC and T vs. C; and CT60 G/A, AA vs. AG+GG, AA vs. GG, AG vs. GG and A vs. G) were used to evaluate the association with cervical cancer risk.

Statistical heterogeneity among studies was tested with Cochran's Q-statistic, where $P<0.10$ was considered to indicate a statistically significant difference. The random-effects model (DerSimonian-Laird method) or the fixed-effects model (Mantel-Haenszel method) was used to summarize the combined OR according to the heterogeneity. When $P\geq0.10$, the fixed-effects model was used to calculate pooled OR, whereas the random-effects model was used if $P<0.10$. Significance of the pooled OR was estimated using a Z-test. $P<0.05$ was considered to indicate a statistically significant difference. Subgroup analyses were performed by ethnic group for the +49 A/G and -318 C/T polymorphisms.

Publication bias was checked by the Begg's funnel plot and Egger's test (31). If publication bias existed, the trim and fill method was applied to adjust the results. Statistical manipulations were carried out using Review Manager 5.0 (Cochrane Collaboration, 2008; www.cc-ims.net/RevMan; accessed March 20, 2013) and Stata 12.0 software (StataCorp LP, College Station, TX, USA).

Results

Study selection and subject characteristics. A total of eight relevant articles investigating *CTLA-4* polymorphisms (+49 A/G, -318 C/T and CT60 G/A) and cervical cancer risk met the study inclusion criteria (6,23-29). Fig. 1 shows the detailed procedure for selecting eligible articles. The studies included in the meta-analysis contained 3,684 cervical cancer cases and 4,110 controls. Among the eight articles, seven focused on the +49 A/G polymorphism (6,23-25,27-29), six on the -318 C/T polymorphism (6,24,26-29) and two focused on the CT60 G/A polymorphism (27,29). Furthermore, four articles were of Caucasian origin (6,26-28) and four were from an Asian population (23-25,29). In the eight articles, genomic DNA was extracted from peripheral blood samples. For genotyping, various methods were used, including restriction fragment length polymorphism, TaqMan, amplification-refractory mutation system and multiplex polymerase chain reaction with hybridization and Sequenom MassArray. Characteristics of each article included in this meta-analysis are summarized in Table I and the genotype numbers are listed in Table II.

Quantitative synthesis

+49 A/G polymorphism. In total, 2,398 cervical cancer cases and 3,546 controls from 7 case-control studies were included in the meta-analysis of the association between *CTLA-4* +49 A/G polymorphism and cervical cancer (6,23-25,27-29). Of these, 4 case-control studies were from an Asian population (23-25,29) and 3 from a Caucasian population (6,27,28). As shown in Fig. 2, the heterogeneity of GG+AG vs. AA was tested for the 7 case-control studies. Data from the meta-analysis were as follows: χ^2, 9.41, degrees of freedom (df), 6 and $P=0.152$ in a fixed-effects model. I^2 value, an additional heterogeneity indicator, was 36.2%, indicating low heterogeneity. Therefore, the fixed-effects model was used for the synthesis of data. The results obtained showed no association between overall cervical cancer risk and the *CTLA-4* +49 A/G polymorphism (OR, 0.94, 95% CI, 0.82-1.07 and $P=0.349$ for GG+AG vs. AA; Fig. 2). In subgroup analysis, significantly decreased cervical cancer risks were found in Asians (OR, 0.75, 95% CI, 0.58-0.97 and $P=0.028$ for GG+AG vs. AA), but not in Caucasians (OR, 1.02, 95% CI, 0.87-1.20 and $P=0.775$ for GG+AG vs. AA; Fig. 3). Other comparison results are listed in Table III. No publication bias was detected by Begg's funnel plot or Egger's test (P>|t|=0.809; Fig. 8).

Table I. Characteristics of the individual studies included in the meta-analysis.

Study	Year	Country (ethnicity)	Sample size, case/control	Genotype method	Polymorphisms
Li *et al* (23)	2011	China (Asians)	314/320	RFLP	+49 A/G
Jiang *et al* (24)	2011	China (Asians)	100/100	Sequenom MassArray	+49 A/G, -318 C/T
Rahimifar *et al* (6)	2010	Iran (Caucasians)	55/110	RFLP, PCR-ARMS	+49 A/G, -318 C/T
Hu *et al* (25)	2010	China (Asians)	696/709	TaqMan	+49 A/G
Ivansson *et al* (26)	2010	Sweden (Caucasians)	1,281/554	TaqMan	-318 C/T
Pawlak *et al* (27)	2010	Poland (Caucasians)	141/224	RFLP	+49 A/G, -318 C/T, CT60 G/A
Castro *et al* (28)	2009	Sweden (Caucasians)	953/1,715	Multiplex PCR with hybridization	+49 A/G, -318 C/T
Su *et al* (29)	2007	Taiwan (Asians)	144/378	RFLP	+49 A/G, -318 C/T, CT60 G/A

RFLP, restriction fragment length polymorphism; PCR, polymerase chain reaction; ARMS, amplification-refractory mutation system.

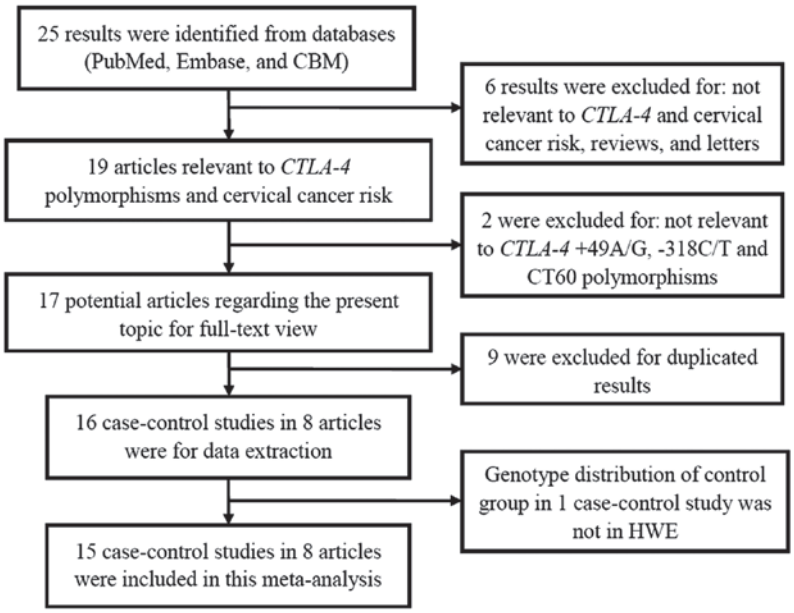

Figure 1. Flow diagram of the article selection process for *CTLA-4* gene polymorphisms and cervical cancer risk meta-analysis. *CTLA-4*, cytotoxic T-lymphocyte-associated antigen-4.

-318 C/T polymorphism. A total of 2,667 cases and 3,058 controls from 6 case-control studies were included in the meta-analysis of the correlation between the -318 C/T polymorphism and cervical cancer (6,24,26-29). Two case-control studies were from an Asian population (24,29) and 4 from a Caucasian population (6,26-28). As shown in Fig. 4, the heterogeneity of TT+TC vs. CC was evaluated for the 6 case-control studies. Data from the meta-analysis were as follows: χ^2, 19.82, df, 5 and P=0.001 in a random-effects model. Additionally, I^2 value was 74.8%, indicating moderated heterogeneity. Thus, the random-effects model was used for the synthesis of data. No statistical evidence of an association between the -318 C/T polymorphism and cervical cancer risks (OR, 1.33, 95% CI, 0.82-2.16 and P=0.249 for TT+TC vs. CC; Fig. 4) was observed. In the subgroup analysis, significantly increased cervical cancer risks were observed in the Asian population (OR, 2.02, 95% CI, 1.36-3.00 and P=0.000 for TC vs. CC; Fig. 5), but not in the Caucasian population (OR, 0.98, 95% CI, 0.53-1.81 and P=0.950 for TC vs. CC; Fig. 6). Other comparison results are listed in Table III. No publication bias was detected by Begg's funnel plot or Egger's test (P>|t|=0.989; Fig. 9).

CT60 G/A polymorphism. A total of 253 cases and 602 controls from 2 case-control studies were included in the meta-analysis of the correlation between the CT60 G/A polymorphism and cervical cancer (27,29). One case-control

Table II. Distribution of *CTLA-4* polymorphism genotypes and alleles among cervical cancer patients and controls.

A, Polymorphism +49 A/G

	Case			Control			Case		Control		
Study	AA	AG	GG	AA	AG	GG	G	A	G	A	HWE
Li *et al* (23)	30	144	140	18	129	173	424	204	475	165	Yes
Jiang *et al* (24)	13	42	45	19	39	42	132	68	123	77	Yes
Rahimifar *et al* (6)	28	27	0	58	45	7	27	83	59	161	Yes
Hu *et al* (25)	80	290	326	56	300	353	942	450	1,006	412	Yes
Pawlak *et al* (27)	43	72	26	71	103	43	124	158	189	245	Yes
Castro *et al* (28)	252	449	252	456	825	434	953	953	1,693	1,737	Yes
Su *et al* (29)	17	62	60	42	155	178	182	96	511	239	Yes

B, Polymorphism -318 C/T

	Case			Control			Case		Control		
Study	CC	TC	TT	CC	TC	TT	C	T	C	T	HWE
Jiang *et al* (24)	75	24	1	92	8	0	174	26	192	8	Yes
Rahimifar *et al* (6)	51	3	0	89	20	1	105	3	198	22	Yes
Ivansson *et al* (26)	1,044	228	9	458	92	4	2,316	246	1,008	100	Yes
Pawlak *et al* (27)	99	38	3	180	35	1	236	44	395	37	Yes
Castro *et al* (28)	5	124	819	6	223	1,471	134	1,762	235	3,165	Yes
Su *et al* (29)	105	38	1	306	67	5	248	40	679	77	Yes

C, Polymorphism CT60 G/A

	Case			Control			Case		Control		
Study	GG	GA	AA	GG	GA	AA	G	A	G	A	HWE
Pawlak *et al* (27)	41	58	15	77	104	43	140	88	258	190	Yes
Su *et al* (29)	87	45	7	238	123	17	219	59	599	157	Yes

HWE, Hardy-Weinberg equilibrium. *CTLA-4*, cytotoxic T-lymphocyte-associated antigen-4.

study was from a Caucasian population (27) and the other from an Asian population (29). The results showed no statistical evidence of an association between the CT60 G/A polymorphism and the overall cervical cancer risk (OR, 0.98, 95% CI, 0.72-1.33 and P=0.898 for AA+GA vs. GG; Fig. 7). Further subgroup analysis was not performed due to the limited data for this polymorphism. Other comparison results are listed in Table III.

Discussion

Cervical cancer is a complex malignant tumor that affects female reproductive organs and has a number of genetic and environmental determinants. Specific oncogenic HPV is an important etiologic agent in cervical cancer. However, HPV infection alone is insufficient in inducing malignant changes (2). Host genetic factors may be important in cervical cancer susceptibility. *CTLA-4*, one of the key mediators for inhibiting activated T-lymphocytes, plays a pivotal role in cancer immunosurveillance (13). Considering the importance of *CTLA-4*, variations in this gene may affect the risk of developing cervical cancer. In addition, the effect of gene polymorphisms involved in tumorigenesis or susceptibility to cervical cancer has gained increasing interest in previous years. Specific studies have reported an association between genetic risk factors and cervical cancer (6-8,23,24,32). Rahimifar *et al* (6) observed that at the -318 locus in *CTLA-4*, higher C allele frequency, as well as increased frequency of -318 CC genotype, was found in patients with cervical cancer when compared with controls. However, this association was from a study with a small sample size and was restricted to females in Iran. Li *et al* (23) showed that Chinese females with the +49 AA genotype have a 2.06-fold higher risk of developing cervical cancer compared with GG carriers. In addition, Jiang *et al* (24) observed that a

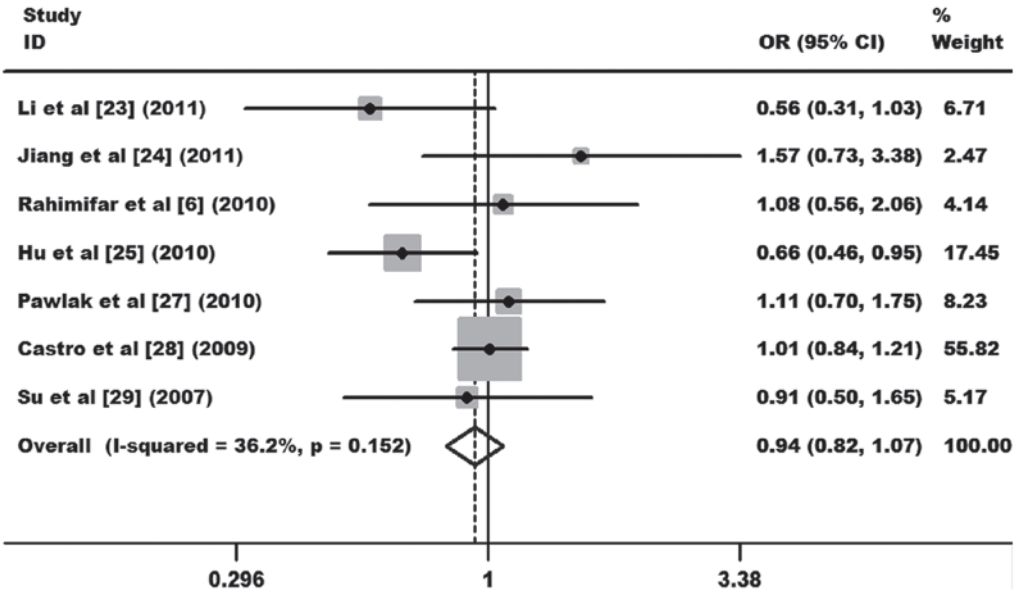

Figure 2. Meta-analysis with a fixed-effects model for the association between the risk of developing cervical cancer and the *CTLA-4* +49 A/G polymorphism (GG+AG vs. AA). *CTLA-4*, cytotoxic T-lymphocyte-associated antigen-4.

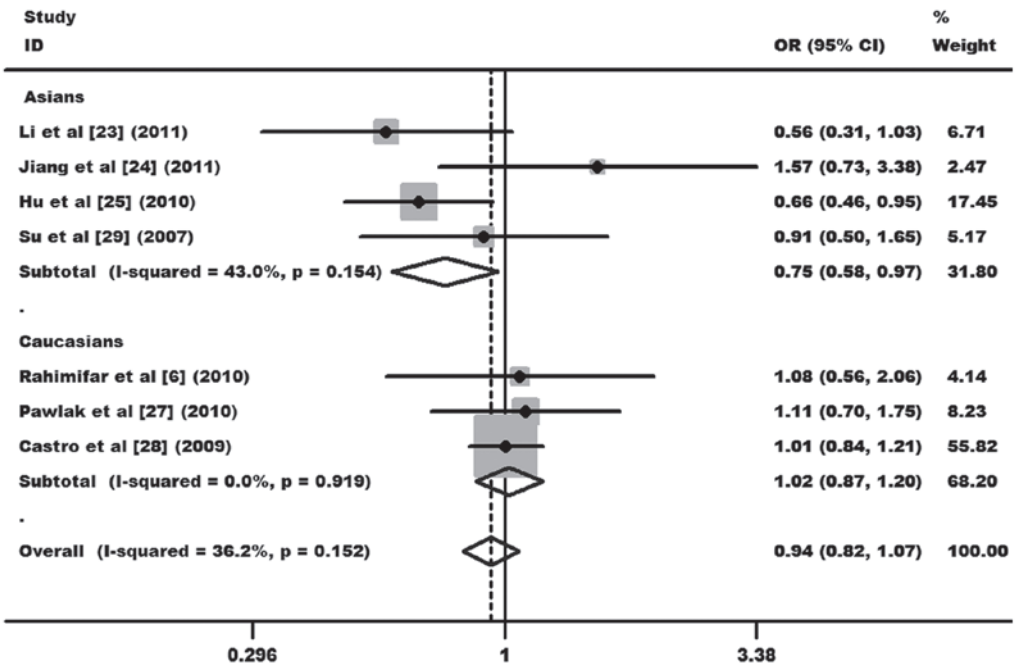

Figure 3. Meta-analysis with a fixed-effects model for the association between the risk of developing cervical cancer and the *CTLA-4* +49 A/G polymorphism (GG+AG vs. AA). Subgroup analysis by ethnicity. *CTLA-4*, cytotoxic T-lymphocyte-associated antigen-4.

single-nucleotide polymorphism in the promoter region of the *CTLA-4* gene may increase susceptibility to cervical cancer. However, negative results were also obtained in certain studies, due to conflicting observations and the limited sample size of individual studies. Alternatively, meta-analysis is a strategy to reduce the limitations of individual studies and is often applied in genetic association studies. Thus, meta-analysis was used in the present study to assess whether an association exists between the most commonly studied polymorphisms of the *CTLA-4* gene, +49 A/G, -318 C/T and CT60 G/A and the risk of developing cervical cancer.

A total of 15 case-control studies from 8 articles, comprising 3,684 cervical cancer cases and 4,110 controls, were included in the meta-analysis. The results indicated no association of *CTLA-4* +49 A/G, -318 C/T and CT60 G/A polymorphisms with overall cervical cancer risk. Furthermore, stratification by ethnicity showed that Asian individuals with GG/AG genotypes had a significantly decreased cervical cancer risk compared with AA carriers for the +49 A/G polymorphism. By contrast, for the -318 C/T polymorphism, an increased cervical cancer risk was observed for the TC genotype, compared with CC carriers. However, the results should be interpreted with

Table III. Summary of results of the meta-analysis from various comparative genetic models.

A, Polymorphism +49 A/G

Genetic comparison	Population	OR (95% CI); P-value	Test of heterogeneity P-value; I^2 (%)	Model
GG+AG vs. AA	All	0.94 (0.82-1.07); 0.349	0.152; 36.2	F
	Asian	0.75 (0.58-0.97); 0.028	0.154; 43.0	F
	Caucasian	1.02 (0.87-1.20); 0.775	0.919; 0.0	F
GG vs. AA+AG	All	0.92 (0.82-1.03); 0.154	0.201; 29.8	F
	Asian	0.84 (0.72-0.98); 0.028	0.389; 0.6	F
	Caucasian	1.03 (0.87-1.22); 0.754	0.307; 15.3	F
GG vs. AA	All	0.84 (0.62-1.12); 0.234	0.057; 51.0	R
	Asian	0.71 (0.54-0.92); 0.011	0.141; 45.1	F
	Caucasian	1.02 (0.83-1.25); 0.833	0.384; 0.0	F
AG vs. AA	All	0.95 (0.82-1.10); 0.482	0.310; 15.7	F
	Asian	0.79 (0.61-1.04); 0.093	0.257; 25.7	F
	Caucasian	1.02 (0.86-1.21); 0.812	0.695; 0.0	F
G vs. A	All	0.94 (0.87-1.02); 0.133	0.120; 40.6	F
	Asian	0.85 (0.76-0.96); 0.007	0.190; 37.0	F
	Caucasian	1.02 (0.92-1.13); 0.716	0.870; 0.0	F

B, Polymorphism -318 C/T

Genetic comparison	Population	OR (95% CI); P-value	Test of heterogeneity P-value; I^2 (%)	Model
TT+TC vs. CC	All	1.33 (0.82-2.16); 0.249	0.001; 74.8	R
	Asian	2.28 (0.97-5.38); 0.060	0.070; 69.5	R
	Caucasian	0.97 (0.51-1.85); 0.930	0.008; 74.6	R
TT vs. TC+CC	All	1.00 (0.80-1.25); 0.982	0.752; 0.0	F
	Asian	0.90 (0.18-4.45); 0.901	0.373; 0.0	F
	Caucasian	1.00 (0.80-1.26); 0.967	0.603; 0.0	F
TT vs. CC	All	1.04 (0.53-2.03); 0.917	0.613; 0.0	F
	Asian	1.05 (0.21-5.11); 0.955	0.351; 0.0	F
	Caucasian	1.03 (0.49-2.18); 0.929	0.441; 0.0	F
TC vs. CC	All	1.34 (0.83-2.15); 0.232	0.003; 72.7	R
	Asian	2.02 (1.36-3.00); 0.000	0.105; 62.0	F
	Caucasian	0.98 (0.53-1.81); 0.950	0.015; 71.2	R
T vs. C	All	1.29 (0.90-1.83); 0.161	0.001; 77.2	R
	Asian	2.11 (0.86-5.18); 0.105	0.047; 74.7	R
	Caucasian	1.08 (0.74-1.59); 0.687	0.005; 76.3	R

C, Polymorphism CT60 G/A

Genetic comparison	Population	OR (95% CI); P-value	Test of heterogeneity P-value; I^2 (%)	Model
AA+AG vs. GG	All	0.98 (0.72-1.33); 0.898	0.786; 0.0	F
AA vs. AG+GG	All	0.76 (0.45-1.28); 0.308	0.313; 1.9	F
AA vs. GG	All	0.80 (0.46-1.39); 0.420	0.356; 0.0	F
AG vs. GG	All	1.02 (0.74-1.41); 0.904	0.891; 0.0	F
A vs. G	All	0.93 (0.74-1.18); 0.565	0.437; 0.0	F

OR, odds ratio; CI, confidence interval; F, fixed model; R, random model.

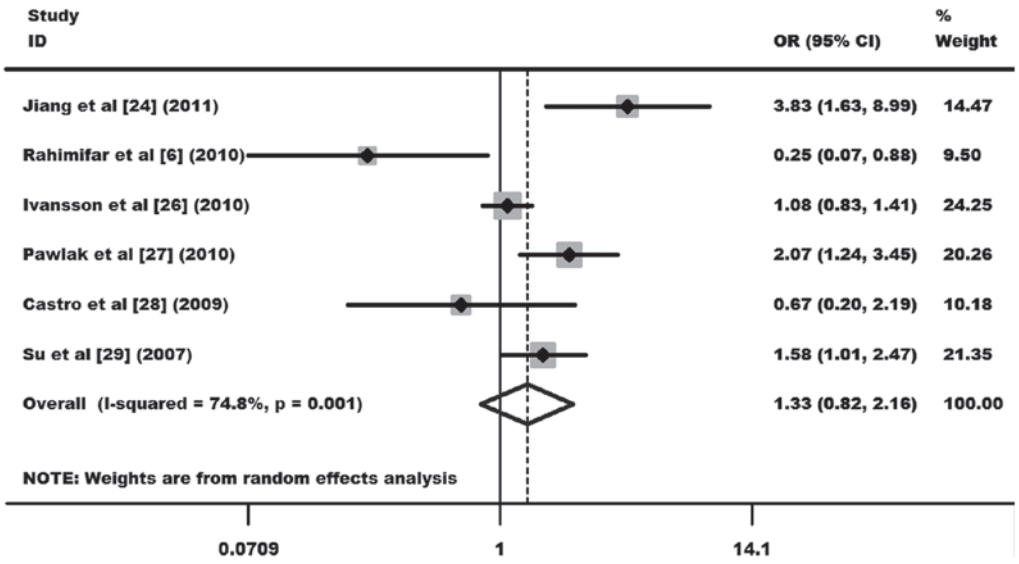

Figure 4. Meta-analysis with a random-effects model for the association between the risk of developing cervical cancer and the *CTLA-4* -318 C/T polymorphism (TT+TC vs. CC). *CTLA-4*, cytotoxic T-lymphocyte-associated antigen-4.

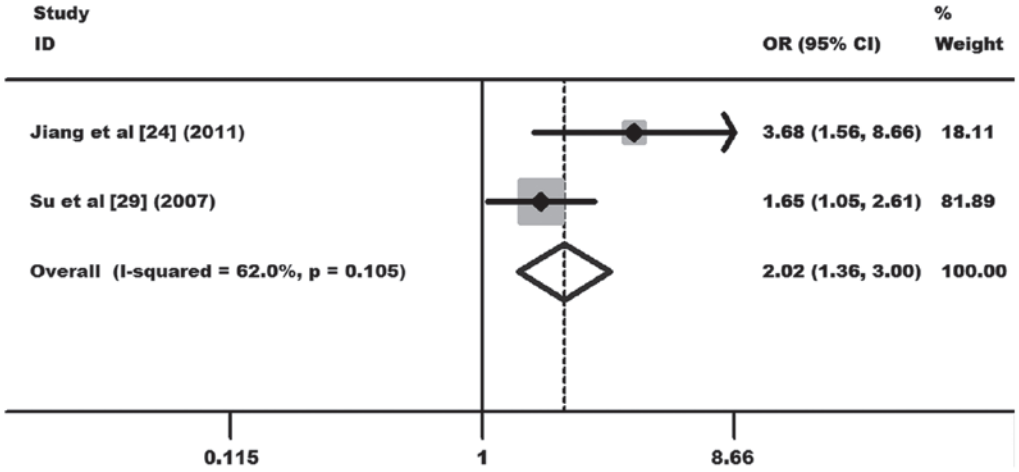

Figure 5. Meta-analysis with a fixed-effects model for the association between the risk of developing cervical cancer and the *CTLA-4* -318 C/T polymorphism (TC vs. CC) in an Asian population. *CTLA-4*, cytotoxic T-lymphocyte-associated antigen-4.

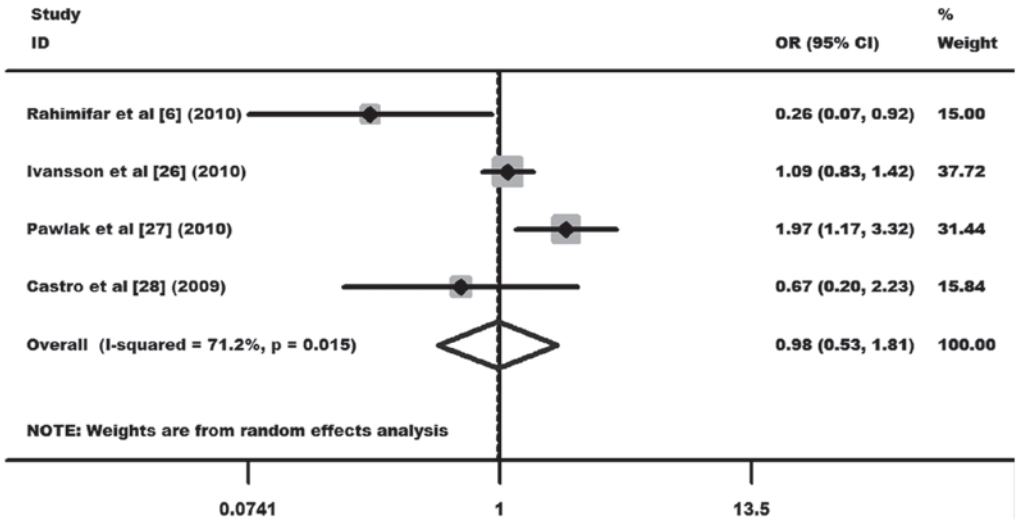

Figure 6. Meta-analysis with a random-effects model for the association between the risk of developing cervical cancer and the *CTLA-4* -318 C/T polymorphism (TC vs. CC) in a Caucasian population. *CTLA-4*, cytotoxic T-lymphocyte-associated antigen-4.

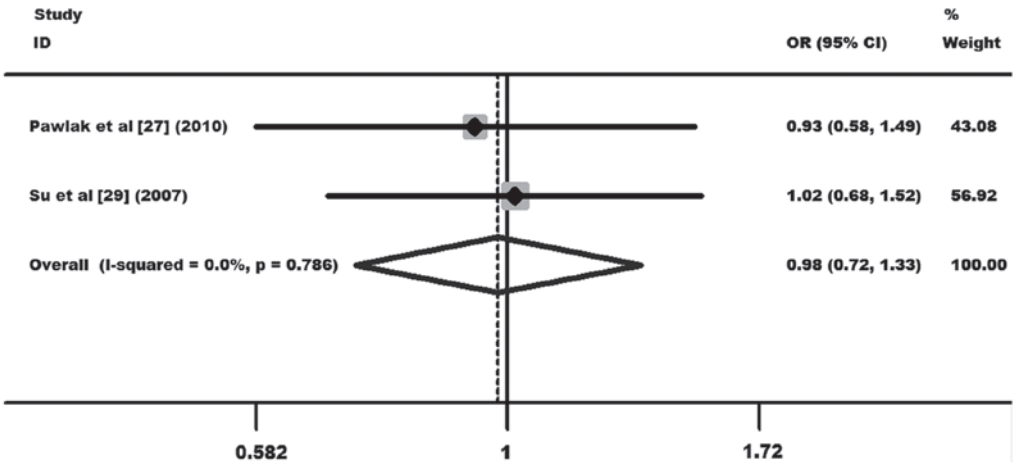

Figure 7. Meta-analysis with a fixed-effects model for the association between the risk of developing cervical cancer and the *CTLA-4* CT60 G/A polymorphism (AA+AG vs. GG). *CTLA-4*, cytotoxic T-lymphocyte-associated antigen-4.

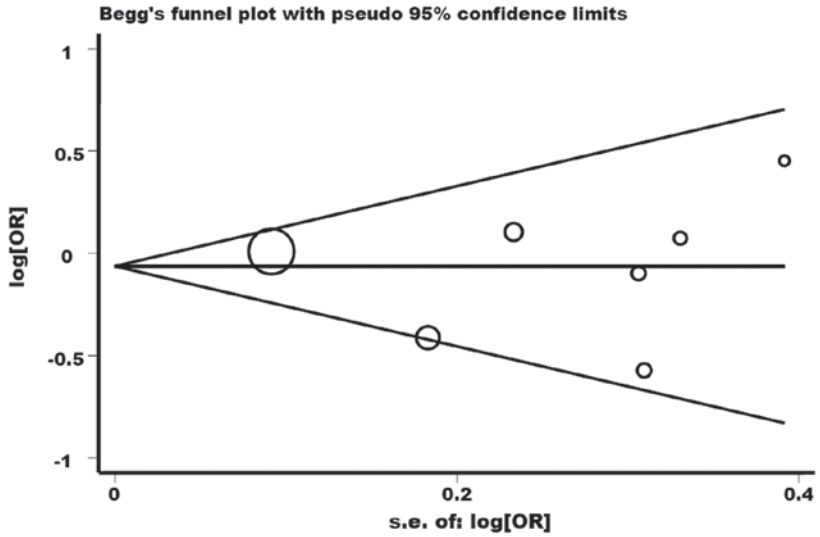

Figure 8. Begg's funnel plot of studies examining the association between the *CTLA-4* +49 A/G polymorphism and cervical cancer for overall studies in the dominant model. *CTLA-4*, cytotoxic T-lymphocyte-associated antigen-4.

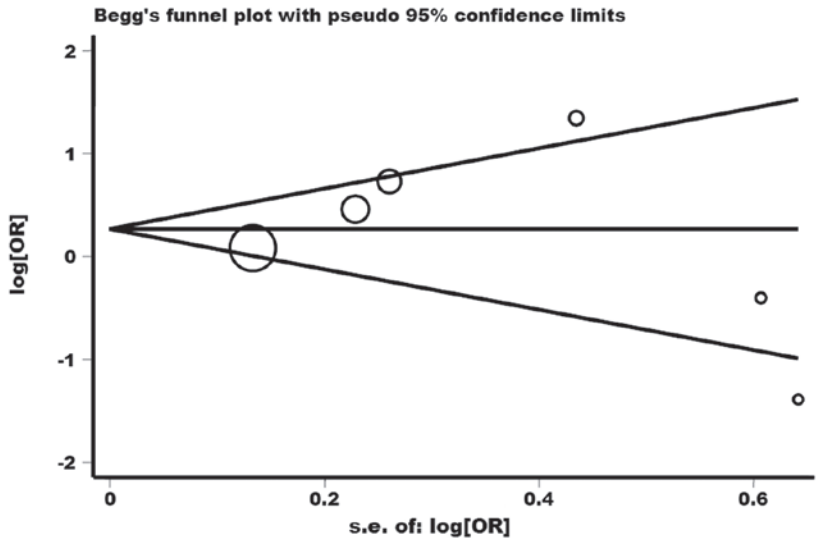

Figure 9. Begg's funnel plot of studies examining the association between the *CTLA-4* -318 C/T polymorphism and cervical cancer for overall studies in the dominant model. *CTLA-4*, cytotoxic T-lymphocyte-associated antigen-4.

caution, as only two case-control studies were included in an Asian population for the -318 C/T polymorphism, which may have limited the statistical power to reveal a reliable association. Therefore, future studies are required to validate the association. The present meta-analysis found that the +49 A/G polymorphism correlated with a decreased risk for cervical cancer among Asian but not Caucasian individuals, while the -318 C/T polymorphism correlated with an increased risk among Asian but not Caucasian individuals. These observations suggest that interactions between genetic diversity in various ethnicities and genetic variants may contribute to various risks of cervical cancer.

Since Su *et al* (29) reported that the -318 C/T variant in the promoter region of the *CTLA-4* gene was associated with HPV-16-associated cervical squamous cell carcinoma in Taiwanese females in 2007, more studies have focused on the association between *CTLA-4* polymorphisms and cervical cancer. However, certain results have been conflicting. In the current meta-analysis, eight eligible articles published up to January 1, 2013 were considered, comprising a total of 7,794 subjects. Thus, the statistical analysis of the present study may provide more powerful evidence of an association. Moreover, it was found that the *CTLA-4* +49 A/G and -318 C/T polymorphisms may play various roles in cervical cancer susceptibility across various populations, indicating that the associations may be ethnicity-specific. In the future, a large number of studies are required to analyze these associations in diverse ethnicities.

Heterogeneity and publication bias are two important issues that should be addressed, as they may have affected the results of the meta-analysis. Heterogeneity was observed between studies for the +49 A/G and -318 C/T polymorphisms, in overall comparisons in the dominant model. However, when stratification by ethnicity was employed, heterogeneity decreased or was removed in specific subgroups, indicating various roles for genetic backgrounds, even for the same polymorphism. Significant publication bias was not detected for the three polymorphisms, indicating the reliability of the results from this meta-analysis.

To a certain extent, several limitations may have affected the results of the present study and should be considered when interpreting the results. Firstly, the limited study sample size of certain participants may have weakened the statistical power to evaluate the association between *CTLA-4* polymorphisms and cervical cancer. Secondly, the number of studies included in the meta-analysis was relatively small, which prevented further subgroup analysis for the CT60 G/A polymorphism. Furthermore, on account of the small amount of data available for each included study, it was not possible to conduct a subgroup analysis by other covariates, including HPV subtype and status, grade of differentiation of cervical cancer, lifestyle and environmental factors. Some of these variables are documented as important risk factors for cervical cancer (33). Thirdly, since all studies for data analysis were from Asian and Caucasian populations, the results may only be applicable to these two ethnic groups. Additionally, eligible articles were identified from the selected databases; thus, specific published articles concerning the current topic or unpublished articles which had negative observations were missed, which may have distorted the analysis.

In conclusion, in spite of the several aforementioned limitations, results from the meta-analysis suggest that the *CTLA-4* +49 A/G and -318 C/T polymorphisms, but not the CT60 G/A polymorphism, may be risk factors for cervical cancer. In the future, more intensive studies based on various ethnicities are required to further the understanding of gene-gene and gene-environment interactions between *CTLA-4* polymorphisms and cervical cancer risk.

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