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# Association of cytotoxic T lymphocyte-associated protein 4 gene -1772T/C polymorphism with gastric cancer risk

## A prisma-compliant meta-analysis

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**Abstract**

**Background:** We performed a meta-analysis to more precisely evaluate the association between the cytotoxic T lymphocyte-associated protein 4 (CTLA-4) -1772T/C polymorphism and overall gastric cancer (GC) risk and the influence of ethnicity and the source of controls on that association.

**Methods:** A systematic literature search was performed in PubMed, EMBASE, the Cochrane Library, Web of Science (WOS) Database, Chinese National Knowledge Infrastructure (CNKI), China biomedical literature database (CBM), Wanfang database, and VIP. Two investigators independently reviewed the articles, and disagreements were resolved by discussion and consensus. The odds ratio (OR) with 95% confidence intervals (CIs) was used to assess the strength of the association between the CTLA-4 -1722T/C polymorphism and GC risk, based on the genotype frequencies in cases and controls. The meta-analyses were performed with Stata 12.0, using two-sided *P* values. Trial sequential analysis (TSA) was calculated by TSA Software.

**Results:** Overall, we identified 5 studies including 1039 GC cases and 2136 controls that evaluated the association of the CTLA-4 -1722T/C polymorphism and GC risk. Overall, there was no significant association between the CTLA-4-1722T/C polymorphism and the risk of GC. In the subgroup analysis based on ethnicity, the results showed that the relationship between the CTLA-4 -1722T/C polymorphism and GC susceptibility was strongest in the Chinese population rather than in the Iranian population (TC vs CC: OR = 1.405, 95% CI: 1.100–1.796, *P* = .007; TC+TT vs CC: OR = 1.329, 95% CI: 1.052–1.680, *P* = .017). Then, there was a significant association between the CTLA-4 -1722T/C polymorphism and the risk of GC in studies with HB controls. However, the above correlation can only be reflected in specific populations and gene models. Therefore, we believe that the evidence of this correlation is insufficient.

**Conclusion:** Our meta-analysis showed that the CTLA-4 -1722T/C polymorphism may be associated with the susceptibility to GC. However, the slight correlation can only be reflected in specific populations and gene models. Therefore, we believe that this association is negligible. The large and well-designed case-control studies are needed to validate our findings.

**Abbreviations:** CIs = confidence intervals, CTLA-4 = cytotoxic T lymphocyte-associated protein 4, FDR = False Discovery Rate, GC = gastric cancer, HB = hospital-based, HWE = Hardy - Weinberg equilibrium, OR = odds ratio, PB = population-based, SNPs = single-nucleotide polymorphisms.

**Keywords:** cytotoxic T lymphocyte-associated protein 4, gastric cancer, meta-analysis

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*The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.*

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## 1. Introduction

Gastric cancer (GC) is the fourth most commonly diagnosed cancer and the second most common cause of cancer-related death worldwide,[1,2] especially in some Asian countries such as China, Japan, Malaysia, and the Philippines. Even with the development of surgical treatment and endoscopy, the mortality rate of patients with advanced GC is still very high, and early detection and surgery treatment constitute the only means of reducing mortality. Therefore, finding and confirming more risk factors associated with gastric carcinogenesis is important for identifying high-risk groups.[3]

Cytotoxic T lymphocyte-associated protein 4 (CTLA-4) (GeneBank Accession No. GI: 224809308) is expressed on T cells and prevents the activation of cytotoxic T cells.[4] Its main mechanism by which it inhibits T cell activation and proliferation and cytokine production is by transmitting a negative feedback signal to T cells via the B7/CTLA-4 pathway, which is involved in the development of a variety of tumors.[5,6] CTLA-4 can also induce Fas cell surface death receptor independent apoptosis of activated T lymphocytes, and then further restrain T cells.[7] Therefore, CTLA-4 may attenuate the T-cell activation threshold, thereby decreasing the antitumor response and conferring susceptibility to cancer.

Previous studies showed CTLA-4 gene polymorphisms influenced the expression of CTLA-4 on cell surface, which was linked to some autoimmune diseases, such as Grave's disease, Hashimoto's thyroiditis, and atopic dermatitis.[8–11] More independent case–control studies validated that CTLA-4 gene polymorphisms are associated with the risk of multiple cancers.[12–14] CTLA-4 is polymorphic and contains more than 100 single-nucleotide polymorphisms (SNPs). Among them, some SNPs (such as rs3087243, rs16840252, and rs4553808) in CTLA-4 gene were extensively studied and were reported to be correlated with risk of human malignancy.

Considering the relatively small sample size in most studies and controversial results in some studies, it is necessary to perform a rigorous quantitative synthesis of the evidence. Here, we performed a meta-analysis to more precisely evaluate the association between the CTLA-4 -1772T/C polymorphism and the overall GC risk; we also evaluated the influence of ethnicity and the source of controls.

## 2. Materials and methods

### 2.1. Literature search

A systematic literature search was performed in PubMed, EMBASE, the Cochrane Library, Web of Science (WOS) Database, Chinese National Knowledge Infrastructure (CNKI), China biomedical literature database (CBM), Wanfang database, and VIP to identify all relevant articles published up to March 2020. We used the following terms: "cytotoxic T-lymphocyte antigen-4", "CTLA-4", "-1722T/C", "rs733618 ", "gastric cancer", "Stomach Neoplasms", "polymorphism" and so on. The detailed strategy for the English databases was shown in Table 1. References of the retrieved publications were also screened. Only published studies with full text articles were included. When overlapping articles were found, we only included the publications that reported the most extensive information.

### 2.2. Inclusion criteria

The inclusion criteria were as follows:

1. published in English or Chinese;
2. case-control studies of GC patients with the CTLA-4 -1722T/C polymorphism; and
3. available genotype frequencies for both cancer cases and controls.

The major reasons for the exclusion of studies were

1. they were reviews and letters,
2. they lacked detailed genotype frequencies,
3. there was a lack of conformation to Hardy-Weinberg equilibrium (HWE).

### 2.3. Data extraction

Two investigators independently reviewed the articles, and disagreements were resolved by discussion and consensus. We extracted the following information from each study: first authors surname, publication year, ethnicity of participants, source of controls, and the number of cases and controls with each genotype. The ethnicities were categorized as either Chinese or Iranian. All eligible studies were defined as hospital-based (HB) or population-based (PB) according to the source of the controls. HWE was calculated by the Chi-Squared test ($P < .01$ was considered significant disequilibrium) based on the genotype distributions of the 2 polymorphisms in the controls.

### 2.4. Statistical analysis

The odds ratio (OR) with 95% confidence intervals (CIs) was used to assess the strength of the association between the CTLA-4 -1722T/C polymorphism and GC risk, based on the genotype frequencies in cases and controls. The pooled ORs were calculated for 5 models: an allelic genetic model (T vs C); a homozygous genetic model (TT vs CC); a heterozygous genetic model (TC vs CC); a recessive genetic model (TT vs TC+CC) and a dominant genetic model (CT+TT vs CC). The between-study heterogeneity was calculated with the Chi-Squared-based Q statistic test. If the between-study heterogeneity was considered not significant ($P > .1$), the fixed effects model was used; otherwise, the random effects model was applied. Subgroup analyses were conducted among variables, such as ethnicity and the source of controls. A sensitivity analysis was conducted by removing one data set at a time to identify individual study effects on the pooled results, and to test the reliability of the results. Funnel plots were used to assess the potential publication bias with Eggers linear regression test. The meta-analyses were performed with Stata 12.0, using two-sided $P$ values. Trial sequential analysis (TSA) was calculated by TSA Software. The False Discovery Rate (FDR, Benjamini-Hochberg) method and Bonferroni method were applied for multiple comparisons.[15,16]

## 3. Results

### 3.1. Characteristics of studies

Overall, we identified 5 studies[5,17–20] including 1039 GC cases and 2136 controls that evaluated the association of the CTLA-4 -1722T/C polymorphism with the risk of GC. The characteristics of these studies are listed in Table 2. In the subgroup analysis based on ethnicity and the source of controls. Four studies were

**Table 1**

**Databases searching terms.**

| Search NO. | Search criterion of PubMed (from inception to 27 Mar 2020) (n = 19) | Items found |
|---|---|---|
| #4 | Search ((((((cytotoxic T lymphocyte-associated protein 4[Title/Abstract]) OR CTLA-4 [Title/Abstract]) OR rs733618 [Title/Abstract]) OR -1722T/ C[Title/Abstract])) AND ((((((((((((((((((((((((((((("Polymorphism, Single Nucleotide" [Mesh]) OR "Genotype" [Mesh]) OR "Alleles" [Mesh]) OR polymorphism [Title/Abstract]) OR genetic variant [Title/Abstract]) OR genetic variants [Title/Abstract]) OR genetic polymorphism [Title/Abstract]) OR genetic [Title/Abstract]) OR genetic variant) OR genetic variants) OR "Genetic Variation" [Mesh])))))))) OR (((((SNP/) OR mutation/) OR variation/) OR single nucleotide polymorphism) OR variant/))))))))) AND ((((((((((((((("Stomach Neoplasms"[Mesh]) OR Stomach Neoplasms) OR Gastric Neoplasms) OR Gastric Neoplasm) OR Neoplasm, Gastric) OR Neoplasms, Gastric) OR Cancer of Stomach) OR Stomach Cancers) OR Gastric Cancer) OR Cancer, Gastric) OR Cancers, Gastric) OR Gastric Cancers) OR Stomach Cancer) OR Cancer, Stomach) OR Cancers, Stomach) | 19 |
| #3 | Search (((cytotoxic T lymphocyte-associated protein 4[Title/Abstract]) OR CTLA-4[Title/Abstract]) OR rs733618[Title/Abstract]) OR -1722T/ C[Title/Abstract] | 7131 |
| #2 | Search ((((((((((((((((((((((("Polymorphism, Single Nucleotide" [Mesh]) OR "Genotype" [Mesh]) OR "Alleles" [Mesh]) OR polymorphism [Title/Abstract]) OR genetic variant [Title/Abstract]) OR genetic variants [Title/Abstract]) OR genetic polymorphism[Title/Abstract]) OR genetic [Title/Abstract]) OR genetic variant) OR genetic variants) OR "Genetic Variation" [Mesh])))))))) OR (((((SNP/) OR mutation/) OR variation/) OR single nucleotide polymorphism) OR variant/)))))) | 2358730 |
| #1 | Search ((((((((((((("Stomach Neoplasms"[Mesh]) OR Stomach Neoplasms) OR Gastric Neoplasms) OR Gastric Neoplasm) OR Neoplasm, Gastric) OR Neoplasms, Gastric) OR Cancer of Stomach) OR Stomach Cancers) OR Gastric Cancer) OR Cancer, Gastric) OR Cancers, Gastric) OR Gastric Cancers) OR Stomach Cancer) OR Cancer, Stomach) OR Cancers, Stomach | 146171 |
| Search NO. | Search criterion of Embase (from 1966 to 27 Mar 2020) (n = 37) | Items found |
| #4 | #1 AND #2 AND #3 | 37 |
| #3 | 'cytotoxic t lymphocyte-associated protein 4' OR 'ctla 4'/exp OR rs733618:ab,ti OR '-1722t/ c':ab, ti | 19873 |
| #2 | 'polymorphism, single nucleotide'/exp OR 'polymorphism, single nucleotide' OR 'genotype'/exp OR 'genotype' OR 'alleles'/exp OR 'alleles' OR 'polymorphism': ab, ti OR 'genetic variant': ab, ti OR 'genetic variants': ab, ti OR 'genetic polymorphism': ab, ti AND 'genetic': ab, ti OR 'genetic variant': ab, ti OR 'genetic variants': ab, ti OR 'genetic variation'/exp OR 'genetic variation' OR 'snp' OR 'mutation'/exp OR 'mutation' OR 'variation' OR 'single nucleotide polymorphism'/exp OR 'single nucleotide polymorphism' OR 'variant' | 2295298 |
| #1 | 'stomach neoplasms': ab, ti OR' gastric neoplasms': ab, ti OR' gastric neoplasm': ab, ti OR' cancer of stomach': ab, ti OR' stomach cancers': ab, ti OR' gastric cancer': ab, ti OR' gastric cancers': ab, ti OR' stomach cancer': ab, ti | 93352 |
| Search NO. | Search criterion of Cochrane Library (27 Mar 2020) (n = 0) | Items found |
| #1 | MeSH descriptor: [Polymorphism, Single Nucleotide] explode all trees | 1191 |
| #2 | MeSH descriptor: [Alleles] explode all trees | 676 |
| #3 | MeSH descriptor: [Genotype] explode all trees | 4376 |
| #4 | MeSH descriptor: [Genetic Variation] explode all trees | 5034 |
| #5 | polymorphism: ti, ab, kw or genetic variant: ti, ab, kw or genetic variants: ti,ab,kw or genetic polymorphism: ti, ab, kw or genetic: ti, ab, kw or genetic variant: ti, ab, kw or genetic variants: ti, ab, kw | 20129 |
| #6 | #1 OR #2 OR #3 OR #4 OR #5 | 23151 |
| #7 | MeSH descriptor: [Stomach Neoplasms] explode all trees | 2487 |
| #8 | MeSH descriptor: [Stomach Neoplasms] explode all trees | 2487 |
| #9 | cancer of stomach:ti,ab,kw or stomach cancers:ti,ab,kw or gastric cancer:ti,ab,kw or gastric cancers:ti,ab,kw or stomach cancer:ti, ab,kw | 8552 |
| #10 | #7 OR #8 OR #9 | 8792 |
| #11 | cytotoxic T lymphocyte-associated protein 4:ti,ab,kw | 34 |
| #12 | CTLA-4:ti,ab,kw | 423 |
| #13 | #11 OR #12 | 434 |
| #14 | #6 and #10 and #13 | 0 |
| Search NO. | Search criterion of Web of Science (WOS) Database (from inception to 27 Mar 2020) (n = 21) | Items found |
| #1 | TS = ("stomach neoplasms" OR "gastric neoplasms" OR "gastric neoplasm" OR "cancer of stomach" OR "stomach cancers" OR "gastric cancer" OR "gastric cancers" OR "stomach cancer") | 82,797 |
| #2 | TS = ((((((((((((((((((((("Polymorphism, Single Nucleotide") OR "Genotype") OR "Alleles") OR polymorphism) OR genetic variant) OR genetic variants) OR genetic polymorphism) OR genetic) OR genetic variant) OR genetic variants) OR "Genetic Variation"))))))) OR (((((SNP) OR mutation) OR variation) OR single nucleotide polymorphism) OR variant))))) | 3,869,519 |
| #3 | TS = ("cytotoxic T lymphocyte-associated protein 4" OR "CTLA-4" OR "rs733618" OR "-1722T/C") | 10,362 |
| #4 | #1 AND #2 AND #3 | 21 |
| Search NO. | Search criterion of Chinese National Knowledge Infrastructure (CNKI) Database (from inception to 27 Mar 2020) (n = 9) | Items found |
| #1 | TI = ("gastric cancer" OR "stomach neoplasm") | - |
| #2 | US = ("genetic variant" OR "single nucleotide polymorphism") | - |
| #3 | US = ("cytotoxic T lymphocyte-associated protein 4" OR "CTLA-4" OR "rs733618" OR "-1722T/C") | - |
| #4 | #1 AND #2 AND #3 | 9 |
| Search NO. | Search criterion of China biomedical literature database (CBM) Database (from inception to 2020) (n = 10) | Items found |
| #1 | Mesh = ("gastric cancer" AND "single nucleotide polymorphism") | 72 |
| #2 | Keywords = "cytotoxic T lymphocyte-associated protein 4" OR "CTLA-4" OR "rs733618" OR "-1722T/C" | 9714 |
| #3 | #1 AND #2 | 10 |
| Search NO. | Search criterion of Wanfang Database (from inception to 2020) (n = 33) | Items found |
| #1 | Title or Keywords ("gastric cancer" OR "stomach neoplasm")*Title or Keywords ("genetic variant" OR "single nucleotide polymorphism")*Title or Keywords ("cytotoxic T lymphocyte-associated protein 4" OR "CTLA-4" OR "rs733618" OR "-1722T/C")) | 33 |
| Search NO. | Search criterion of VIP Database (from inception to 2020) (n = 15) | Items found |
| #1 | M = ("gastric cancer" OR "stomach neoplasm") | 82,187 |
| #2 | M = ("genetic variant" OR "single nucleotide polymorphism") | 35,827 |
| #3 | U = ("cytotoxic T lymphocyte-associated protein 4" OR "CTLA-4" OR "rs733618" OR "-1722T/C")) | 59 |
| #4 | #1 AND #2 AND #3 | 15 |

**Table 2**

**Characteristics of published studies included in this meta-analysis.**

| First author | Year | Ethnicity | Control | Cases | | | Control | | | WHE | Matched |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | TT | TC | CC | TT | TC | CC | | |
| Hou | 2010 | Chinese | PB | 75 | 111 | 19 | 93 | 139 | 30 | 0.04 | Matched (age, sex and ethnicity) |
| QI | 2012 | Chinese | HB | 40 | 69 | 9 | 37 | 45 | 14 | 0.96 | Unclear |
| Hadinia | 2007 | Iranian | PB | 42 | 4 | 0 | 165 | 24 | 1 | 0.90 | Matched (age, sex) |
| Song | 2006 | Chinese | HB | 62 | 113 | 8 | 45 | 54 | 17 | 0.90 | Matched (age, sex) |
| Liu | 2019 | Chinese | HB | 168 | 242 | 77 | 525 | 685 | 262 | 0.14 | Matched (age, sex, smoking status, alcohol use, height, and weight) |

HB = hospital-based, HWE = Hardy–Weinberg equilibrium, PB = population-based.

carried out in Chinese populations, and 1 was performed with an Iranian population; 3 were carried out with PB controls, and 2 were conducted with HB controls. The distribution of genotypes in the controls conformed to HWE.

### 3.2. Main results

The association between the CTLA-4 -1722T/C polymorphism and GC risk is presented in Table 3. Overall, there was no significant association between the CTLA-4 -1722T/C polymorphism and the risk of GC, although there are weak links in some gene models (TC vs CC: OR = 1.399, 95% CI: 1.096–1.787, $P = .007$; TC+TT vs CC: OR = 1.325, 95% CI: 1.049–1.674, $P = .018$). In the subgroup analysis based on ethnicity, the results showed that the relationship between the CTLA-4 -1722T/C gene polymorphism and GC susceptibility existed in the Chinese population (TC vs CC: OR = 1.405, 95% CI: 1.100–1.796, $P = .007$; TC+TT vs CC: OR = 1.329, 95% CI: 1.052–1.680, $P = .017$) but not in the Iranian population. In the subgroup analysis according to the source of the control group, there was a significant association between the CTLA-4 -1722T/C polymorphism and the risk of GC in studies with HB controls (TC vs CC: OR = 1.432, 95% CI: 1.097–1.870, $P < .001$; TC+TT vs CC: OR = 1.340, 95% CI: 1.040–1.728, $P = .018$). And there were

**Table 3**

**Total and stratified analyses of association of the CTLA-4 -1722T/C polymorphism with the GC risk.**

| Overall and subgroup | No. of study | OR (95% CI) | *P* value for heterogeneity | *P* for OR | Bonferroni | FDR |
|---|---|---|---|---|---|---|
| Total | | | | | | |
| T vs. C | 5 | 1.048 (0.934–1.175) | .915 | .425 | 1.000 | 0.531 |
| TT vs. CC | | 1.244 (0.964–1.606) | .343 | .093 | 0.465 | 0.155 |
| TC vs CC | | 1.399 (1.096–1.787) | .060 | .007 | 0.035 | 0.035 |
| TT vs TC +CC | | 0.950 (0.806–1.120) | .774 | .540 | 1.000 | 0.540 |
| TC+TT vs CC | | 1.325 (1.049–1.674) | .111 | .018 | 0.09 | 0.045 |
| Ethnicity | | | | | | |
| Chinese | 4 | | | | | |
| T vs. C | | 1.042 (0.928–1.169) | .954 | .487 | 1.000 | 0.487 |
| TT vs. CC | | 1.248 (0.966–1.612) | .220 | .090 | 0.45 | 0.150 |
| TC vs CC | | 1.405 (1.100–1.796) | .033 | .007 | 0.035 | 0.035 |
| TT vs TC +CC | | 0.937 (0.793–1.107) | .817 | .447 | 1.000 | 0.487 |
| TC+TT vs CC | | 1.329 (1.052–1.680) | .060 | .017 | 0.085 | 0.043 |
| Iranian | | | | | | |
| T vs. C | 1 | 1.616 (0.550–4.750) | | .383 | 1.000 | 0.874 |
| TT vs. CC | | 0.770 (0.031–19.249) | - | .874 | 1.000 | 0.874 |
| TC vs CC | | 0.551 (0.019–15.780) | - | .728 | 1.000 | 0.874 |
| TT vs TC +CC | | 1.591 (0.525–4.820) | - | .412 | 1.000 | 0.874 |
| TC+TT vs CC | | 0.736 (0.030–18.363) | - | .852 | 1.000 | 0.874 |
| Control | | | | | | |
| PB | 2 | | | | | |
| T vs. C | | 1.102 (0.851–1.428) | .469 | .461 | 1.000 | 0.597 |
| TT vs. CC | | 1.250 (0.660–2.367) | .764 | .493 | 1.000 | 0.597 |
| TC vs CC | | 1.230 (0.664–2.280) | .634 | .510 | 1.000 | 0.597 |
| TT vs TC +CC | | 1.101 (0.770–1.157) | .485 | .597 | 1.000 | 0.597 |
| TC+TT vs CC | | 1.244 (0.685–2.259) | .745 | .472 | 1.000 | 0.597 |
| HB | | | | | | |
| T vs. C | 3 | 1.035 (0.910–1.176) | .870 | .600 | 1.000 | 0.600 |
| TT vs. CC | | 1.243 (0.941–1.643) | .111 | .125 | 0.625 | 0.208 |
| TC vs CC | | 1.432 (1.097–1.870) | .013 | .008 | 0.040 | 0.040 |
| TT vs TC +CC | | 0.913 (0.758–1.099) | .770 | .335 | 1.000 | 0.419 |
| TC+TT vs CC | | 1.340 (1.040–1.728) | .025 | 0.018 | 0.090 | 0.045 |

FDR = false discovery rate, GC = gastric cancer, HB = hospital-based, No. = number, OR = odds ratio, PB = population-based.

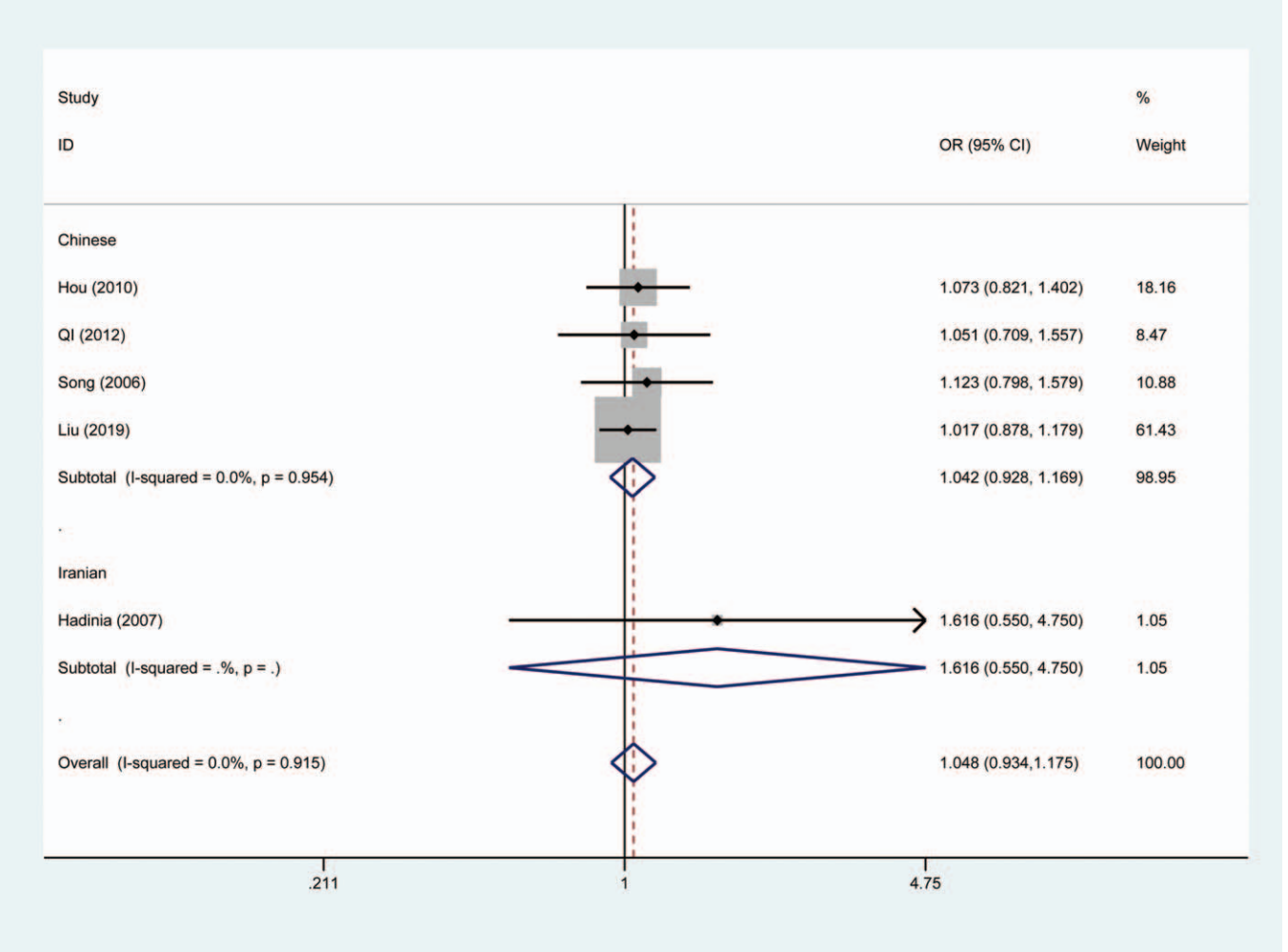


**Figure 1.** Meta-analysis with a fixed effects model for the ORs for GC risk in different ethnicities associated with CTLA-4 -1722T/C (T vs C).

also significant statistical differences after Bonferroni correction. (Table 3, Figs. 1 and 2). However, the above correlation can only be reflected in specific populations and gene models. Therefore, we believe that the evidence of this correlation is insufficient.

### 3.3. Evaluation of heterogeneity

There was not significant heterogeneity in any gene model (T vs C: $I^2=0\%$, $P=.915$; TT vs CC: $I^2=11.0\%$, $P=.343$; TC vs CC: $I^2=55.9\%$, $P=.060$; TT vs TC+CC: $I^2=0\%$, $P=.774$; TT+TC vs CC: $I^2=46.8\%$, $P=.111$;), so a fixed effects model was applied, except for heterozygous genetic model (TC vs CC) (Table 3)

### 3.4. Sensitivity analysis

We used a sensitivity analysis to estimate the influence of individual studies on the pooled ORs, and the results of the sensitivity analysis showed that no single study qualitatively influenced the summary OR, suggesting the stability of the meta-analysis. (Fig. 3)

### 3.5. Publication bias

Funnel plots are shown in Figure 4 for the allelic genetic model (T vs C). The arrangement of data points did not reveal any evidence of obvious asymmetry. Formal evaluation using Eggers regression asymmetry tests for the allelic genetic model still did not show any evidence of publication bias ($t=-1.74$, $P=.180$).

### 3.6. Trial sequential analysis

Trial sequential analysis (TSA) was carried out to diminish random errors and fortify the robustness of our findings under the allelic genetic model (T vs C). Although the cumulative z-curve has not exceeded the required information size (RIS), which suggested that the sample size may be insufficient (Fig. 5). Furthermore, we also found there is no statistical difference between the case group and the control group under the allelic genetic model (T vs C).

## 4. Discussion

The present meta-analysis, including 1039 GC cases and 2136 controls from 5 published case-control studies, showed that the

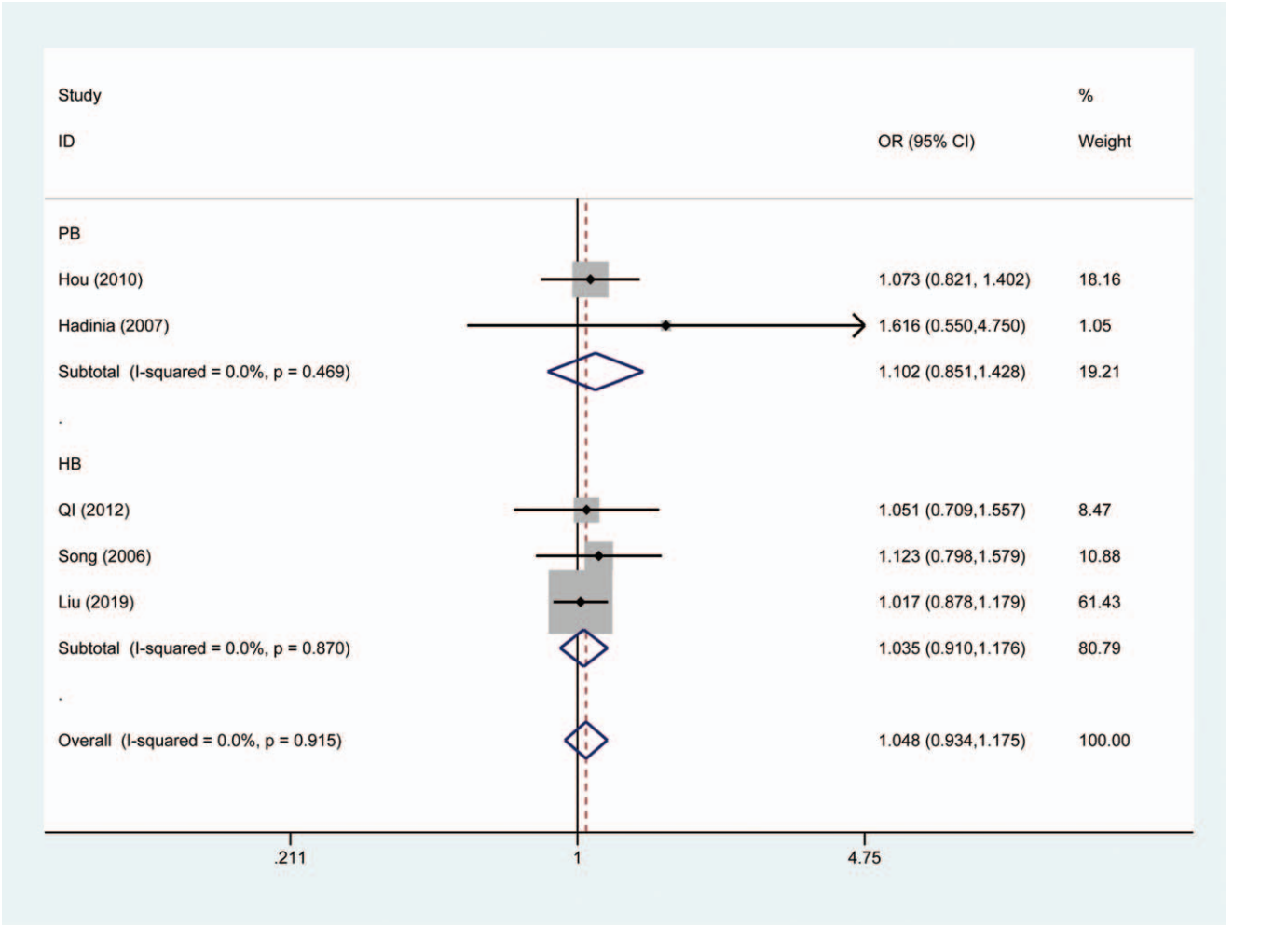


**Figure 2.** Meta-analysis with a fixed effects model for the ORs for GC risk based on different sources of controls associated with CTLA-4 -1722T/C (T vs C).

CTLA-4 -1772T/C polymorphism was no significantly associated with GC risk. When stratified by ethnicity, we found an association between the CTLA-4 -1772T/C polymorphism and GC risk in the Chinese population but not the Iranian population. Then, the source of the control group was further analyzed, and there was a significant association between the CTLA-4 -1722T/C polymorphism and the risk of GC in studies that had HB controls, which reflected the influence of the control group on the results. Given the important role of CTLA-4 in the risk of GC, it is biologically possible that a CTLA-4 polymorphism could be associated with the risk of GC by acting as a potent inhibitor in the T-cell response, which facilitates the malignant transformation of cancer. Studies on the functionality of the CTLA-4 -1722T/C polymorphism might contribute to a better understanding of tumor biology and behavior and help us predict genetic susceptibility to GC.

CTLA-4 -1722T/C polymorphism was not found to be associated with GC susceptibility in the Iranian population. Different ethnicities may have different genetic backgrounds. In addition, it is likely that the small sample size may have resulted in insufficient statistical power to detect a real effect. Therefore, in the future, more studies, especially those based on a large population and with additional ethnicities, should be conducted to further examine this association.

As a crucial immune-checkpoint receptor regulating T-cell activation, CTLA4 has been evaluated for its function in several cancers, including GC,[21] colorectal cancers,[5] and cervical cancer.[22] Tumors are variably infiltrated by cytotoxic T lymphocytes, but a dense infiltration portends a better prognosis.[23–25] Blocking CTLA-4-mediated inhibition of the T-cell effector response has been an attractive therapeutic target. Monoclonal antibodies (mAb) that block CTLA-4 are effective in mouse models of a variety of tumors.[26] Furthermore, the CTLA-4 -1722T/C polymorphism has also been shown to be related to immune system diseases.[27,28] These phenomena may be the result of the involvement of CTLA-4 -1722T/C in tumors and immunity. However, a case-control study and a further meta-analysis failed to identify an association between the CTLA-4 -1722T/C polymorphism and the risk of esophageal cancer.[29] Other CTLA-4 polymorphism sites were frequently investigated in previous studies to evaluate their associations with cancers in diverse populations. Some other SNPs of CTLA-4, such as the −60G/A and −1661A/G polymorphisms, were found to be associated with cancers, especially gastric cancer,[29] and the −49 A/G polymorphism contributed to genetic susceptibility to hepatocellular carcinoma and cervical cancer.[30]

Some other limitations in our meta-analysis should be acknowledged. First, the sample size is not large enough. Second,

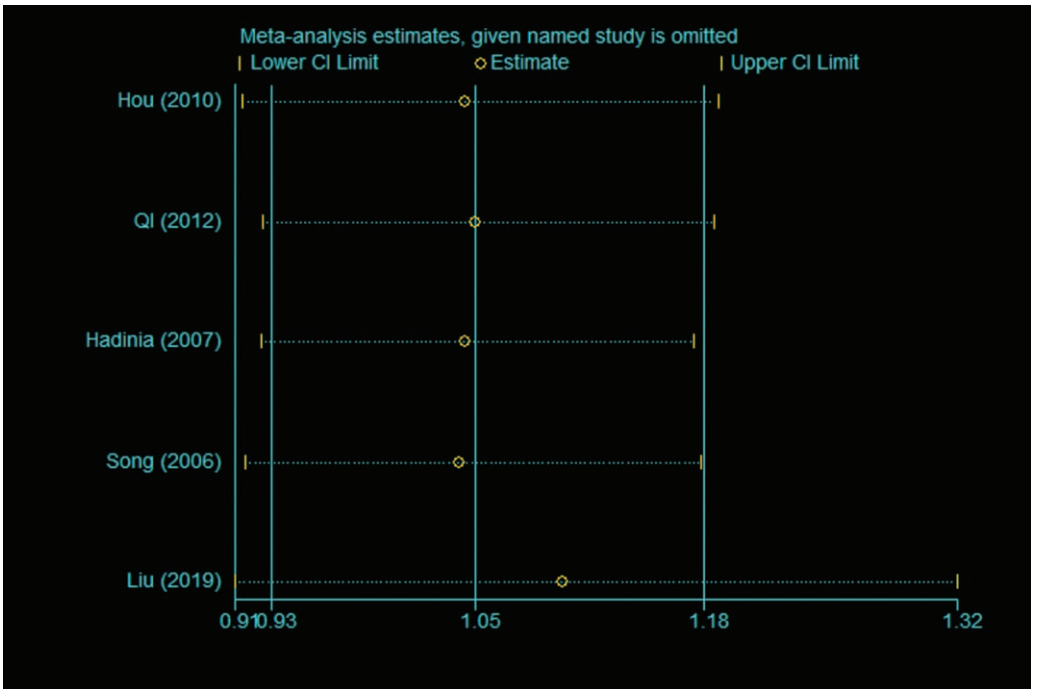


**Figure 3.** Sensitivity analysis of the meta-analysis of GC risk and the CTLA-4-1722T/C polymorphism (C vs T).

only English and Chinese language studies were included, which might have led to publication bias. Heterogeneity is a potential problem that needs to be considered. To reduce heterogeneity as much as possible, we performed a careful search for published studies based on strict criteria for study inclusion and an accurate statistical analysis. As a result, we controlled for heterogeneity within a smaller range, which made our results more reliable.

In conclusion, our meta-analysis suggests that the CTLA-4-1722T/C polymorphism may be related to GC risk. However, the slight correlation can only be reflected in specific populations and gene models. Therefore, we believe that this association is negligible. Larger sample sizes of different populations should be included in future studies, which should lead to a better and more comprehensive understanding of the association between the CTLA-4-1722T/C polymorphism and GC.

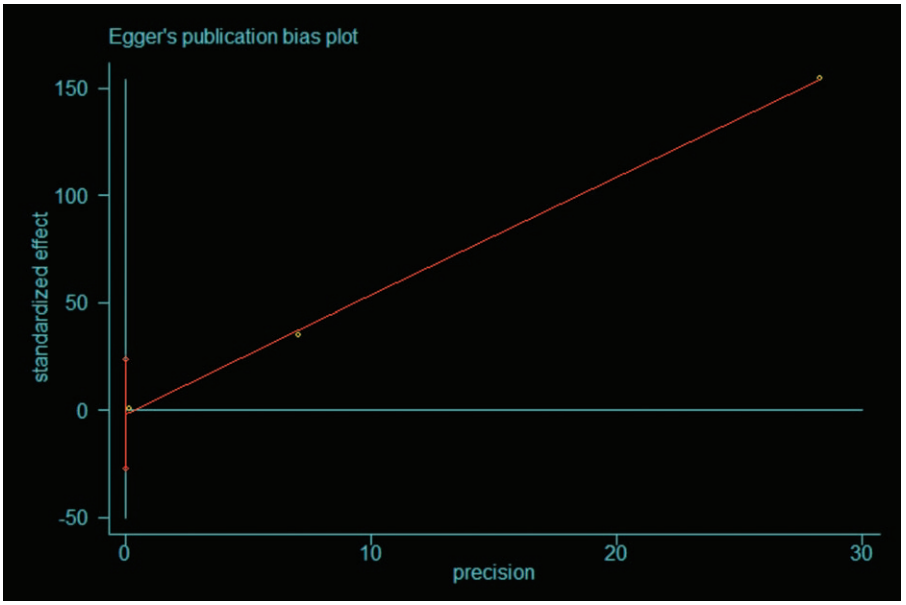


**Figure 4.** Funnel plot for publication bias in the meta-analysis of GC risk and the CTLA-4-1722T/C polymorphism (C vs T).

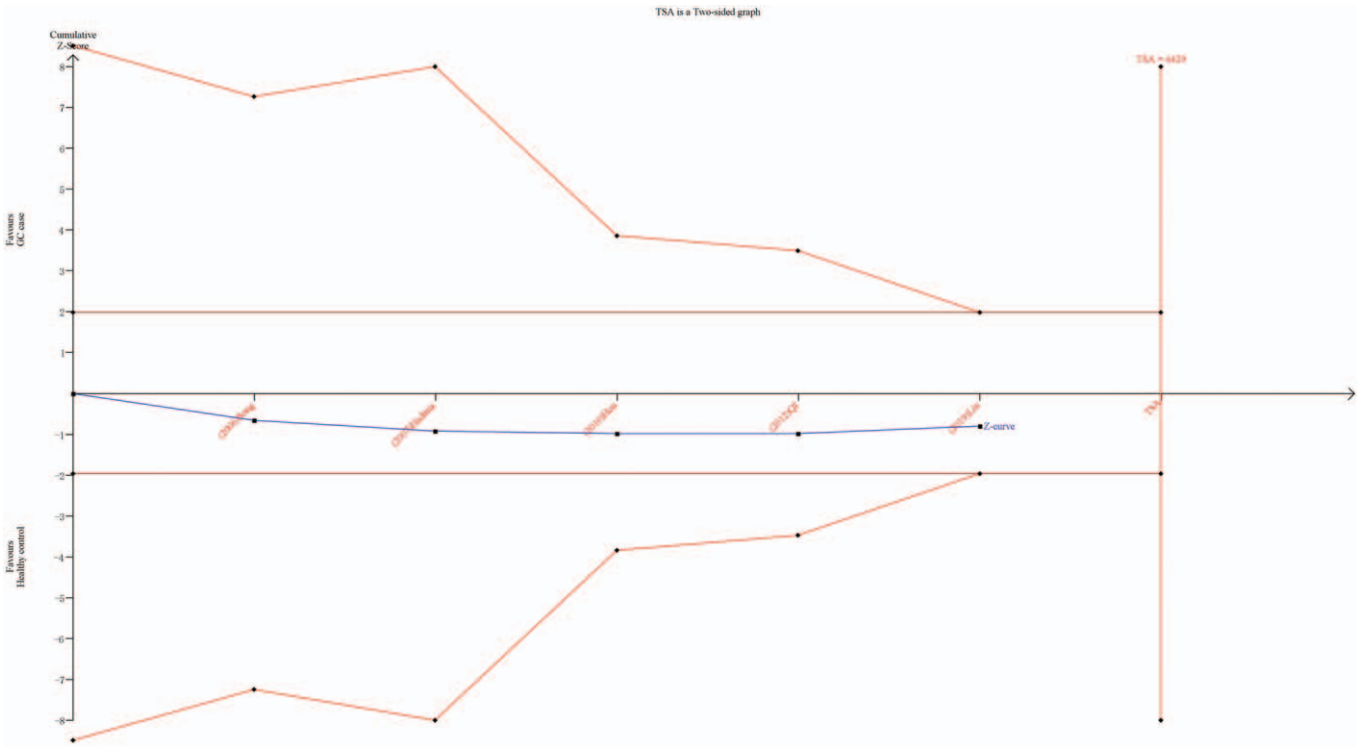


**Figure 5.** Trial sequential analysis (TSA) for CTLA-4 -1722T/C polymorphism under the allele contrast model (C vs T).

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## References

[1] Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: Cancer J Clin 2018;68:394–424.
[2] Jemal A, Center MM, DeSantis C, et al. Global patterns of cancer incidence and mortality rates and trends. Cancer Epidemiol Biomarkers Prev 2010;19:1893–907.
[3] Leung WK, Wu MS, Kakugawa Y, et al. Screening for gastric cancer in Asia: current evidence and practice. Lancet Oncol 2008; 9:279–87.
[4] Zielinski C, Knapp S, Mascaux C, et al. Rationale for targeting the immune system through checkpoint molecule blockade in the treatment of non-small-cell lung cancer. Ann Oncol 2013;24:1170–9.
[5] Hadinia A, Hossieni SV, Erfani N, et al. CTLA-4 gene promoter and exon 1 polymorphisms in Iranian patients with gastric and colorectal cancers. J Gastroenterol Hepatol 2007;22:2283–7.
[6] Wong YK, Chang KW, Cheng CY, et al. Association of CTLA-4 gene polymorphism with oral squamous cell carcinoma. J Oral Pathol Med 2006;35:51–4.
[7] Scheipers P, Reiser H. Fas-independent death of activated CD4 (+) T lymphocytes induced by CTLA-4 crosslinking. Proc Natl Acad Sci U S A 1998;95:10083–8.
[8] Ueda H, Howson JM, Esposito L, et al. Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. Nature 2003;423:506–11.
[9] Cozar JM, Romero JM, Aptsiauri N, et al. High incidence of CTLA-4 AA (CT60) polymorphism in renal cell cancer. Hum Immunol 2007;68:698–704.
[10] Kristiansen OP, Larsen ZM, Pociot F. CTLA-4 in autoimmune diseases–a general susceptibility gene to autoimmunity. Genes Immun 2000; 1:170–84.
[11] Jones G, Wu S, Jang N, et al. Polymorphisms within the CTLA4 gene are associated with infant atopic dermatitis. Br J Dermatol 2006;154:467–71.
[12] Hu S, Pu D, Xia X, et al. CTLA-4 rs5742909 polymorphism and cervical cancer risk: a meta-analysis. Medicine 2020;99:e19433.
[13] Zou C, Qiu H, Tang W, et al. CTLA4 tagging polymorphisms and risk of colorectal cancer: a case-control study involving 2306 subjects. Onco Targets Therapy 2018;11:4609–19.
[14] Liu X, Swen JJ, Diekstra MHM, et al. A genetic polymorphism in CTLA-4 is associated with overall survival in sunitinib-treated patients with clear cell metastatic renal cell carcinoma. Clin Cancer Res 2018; 24:2350–6.
[15] Benjamini Y, Drai D, Elmer G, et al. Controlling the false discovery rate in behavior genetics research. Behav Brain Res 2001;125:279–84.
[16] Armstrong RA. When to use the Bonferroni correction. Ophthalmic Physiol Opt 2014;34:502–8.
[17] Hou R, Cao B, Chen Z, et al. Association of cytotoxic T lymphocyte-associated antigen-4 gene haplotype with the susceptibility to gastric cancer. Mol Biol Rep 2010;37:515–20.
[18] Yu-Qin QI, Jun-Li SI, Cui JY. Polymorphism of CTLA-4 gene promoter region and extron region in gastric cancer. Chin Clin Oncol 2012; 17:828–31.
[19] Jiquan S, Bing X, Rui L, et al. Association of the CTLA4 promoter region polymorphism with gastric cancer. Chin J Exper Surg 2006;1373–4.
[20] Liu J, Tang W, Lin W, et al. Lack of association between CTLA-4 genetic polymorphisms and noncardiac gastricc in a Chinese Population. DNA Cell Biol 2019;38:443–8.
[21] Kordi-Tamandani DM, Davani SK, Baranzehi T, et al. Analysis of promoter methylation, polymorphism and expression profile of cytotoxic T-lymphocyte-associated antigen-4 in patients with gastric cancer. Journal of gastrointestinal and liver diseases: JGLD 2014; 23:249–53.

[22] Xu HB, Yang H, Liu T, et al. Association of CTLA4 gene polymorphism (rs5742909) with cervical cancer: a meta-analysis. Tumour Biol 2014;35:1605–8.
[23] Galon J, Costes A, Sanchez-Cabo F, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science (New York, NY) 2006;313:1960–4.
[24] Taube JM. Emerging immunologic biomarkers: setting the (TNM-immune) stage. Clin Cancer Res 2014;20:2023–5.
[25] Pages F, Berger A, Camus M, et al. Effector memory T cells, early metastasis, and survival in colorectal cancer. N Engl J Med 2005; 353:2654–66.
[26] Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. Science (New York, NY) 1996; 271:1734–6.
[27] Takeuchi F, Nakaue N, Kawasugi K, et al. The CTLA-4 -1661A/G and -1772T/C dimorphisms in Japanese patients with systemic sclerosis. Rheumatol Int 2007;27:785–7.
[28] Takeuchi F, Kuwata S, Mori M. CTLA-4 -1661A/G and -1772T/C dimorphisms in Japanese patients with systemic lupus erythematosus. J Rheumatol 2005;32:2062.
[29] Tang W, Qiu H, Jiang H, et al. Lack of association between cytotoxic T-lymphocyte antigen 4 (CTLA-4) -1722T/C (rs733618) polymorphism and cancer risk: from a case-control study to a meta-analysis. PloS One 2014;9:e94039.
[30] Hu L, Liu J, Chen X, et al. CTLA-4 gene polymorphism +49 A/G contributes to genetic susceptibility to two infection-related cancers-hepatocellular carcinoma and cervical cancer. Human Immunol 2010; 71:888–91.