Current evidence on the cytotoxic T-lymphocyte antigen 4 + 49G>A polymorphism and digestive system cancer risks: a meta-analysis involving 11,923 subjects

Liu Xiaolei a,1, Yang Baohong a, Ren Haipeng a, Liu Shuzhen a, Gao Jianfeng a, Pan Xiangpo a,1, Liu Haiyu a,b, Yu Yuan a, Zheng Dejie b, Yang Jinhong a, Wang Huanxin a, Wang Wenhui a, Yu Guohua a,b

a Wei Fang People’s Hospital, Yu He Road 151#, Kui Wen District, Weifang, Shandong, China
b Ping Du People’s Hospital, Hong Qi Road 15#., Ping Du, Shandong, China

A B S T R A C T

Cytotoxic T-lymphocyte antigen (CTLA-4) plays an important role in downregulating T cell activation and proliferation. The CTLA-4 + 49G>A polymorphism is one of the most commonly studied polymorphisms in this gene due to its association with many cancer types, but the association between CTLA-4 + 49G>A polymorphism and digestive system cancer risks remain inconclusive. An updated meta-analysis based on 17 independent case-control studies consisting of 5176 cancer patients and 6747 controls was performed to address this association. Overall, there was no statistically increased risk of digestive system cancers in every genetic comparison. In subgroup analysis, this polymorphism was significantly linked to higher risks for pancreatic cancer (GG vs. AA, OR = 1.976, 95% CI = 1.496–2.611; GA vs. AA, OR = 1.433, 95% CI = 1.093–1.879; GG/GA vs. AA, OR = 1.668, 95% CI = 1.286–2.164; GG vs. GA/AA, OR = 1.502, 95% CI = 1.088–2.054; G vs. A, OR = 1.394, 95% CI = 1.098–1.770). We also observed increased susceptibility of hepatocellular cell carcinoma in homozygote comparison (OR = 1.433, 95% CI = 1.100–1.866) and dominant model (OR = 1.360, 95% CI = 1.059–1.746). According to the source of controls, significant effects were only observed in hospital-based studies (GA/AA vs. GG, OR = 1.257, 95% CI = 1.129–1.399). In the stratified analysis by ethnicity, no significantly increased risks were found in either Asian or Caucasian. Our findings suggest that the CTLA-4 + 49G>A polymorphism may be associated with the risk of pancreatic cancer and hepatocellular cell carcinoma.

© 2015 Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

CTLA-4, a member of the immunoglobulin (Ig) super-family, is a co-stimulatory molecule expressed by activated T cells and has the function of down-regulating T-cell activation (Hodi et al., 2003). CTLA-4 can also induce FAS-independent apoptosis of activated T cells, which may further inhibit immune function of T lymphocytes. A list of mechanisms of CTLA-4 function have been indicated, such as ligand competition with the positive T-cell co-stimulatory CD28 molecule, interference of TCR signaling, and inhibition of cyclin D3 and cyclin-dependent kinase production (Greenwald et al., 2002). In tumor-transplanted mice, injection with antibodies that block CTLA-4 function enhanced T cell activation (Vandenborne et al., 1999), rejected a variety of different tumors, and had long-lasting anti-tumor immunity. (Leach et al., 1996) suggesting that the CTLA-4 plays an important role in carcinogenesis.

The CTLA-4 gene is located on chromosome 2q33, consisting 4 exons that encode separate functional domains: a leader sequence, an extracellular domain, a transmembrane domain, and a cytoplasmic domain (Qi et al., 2010; Ghaderi et al., 2004; Ligers et al., 2001). This gene is polymorphic, more than 100 single nucleotide polymorphisms have been identified (Ueda et al., 2003). An AG dimorphism at position 49 in CTLA-4 exon 1 (rs231775), which causes an amino acid change (threonine to alanine) in the peptide leader sequence of the CTLA-4 protein (Harper et al., 1991). Recent studies indicated that this polymorphism may influence the ability of CTLA-4 to bind with B7.1 and affect T-cell activation subsequently (Sun et al., 2008; Wang et al., 2007).

Previous studies have identified that this polymorphism is associated with different cancers including lung cancer, breast cancer, and cervical cancer (Sun et al., 2008; Erfani et al., 2006). However, the results of studies on the association between the +49A>G polymorphism and the risk of digestive system cancers remain inconsistent (Qi et al., 2010; Ghaderi et al., 2004; Sun et al., 2008; Hadinia et al., 2007; Solerio et al., 2005; Dilmeec et al., 2008; Cheng et al., 2006; Wong et al., 2006; Gu et al., 2010; Hou et al., 2010; Cozar et al., 2007; Hu et al., 2010; Kämmerer et al., 2010; Yang et al., 2012; Lang et al., 2012). To
improve the efficiency of meta-analysis on digestive cancers and reduce the potential between-study heterogeneity which might derive from various cancers in diverse systems, we focused on digestive system cancers only and added more recent studies in this meta-analysis.

2. Search strategy

In this meta-analysis, a comprehensive literature research of the US National Library of Medicine’s Pub Med database, ISI Web of Knowledge, Medline, Embase and Google Scholar Search (update to August, 2014) were conducted using the search terms including “CTLA-4”, “polymorphisms”, “cancer”, and the combined phrases in order to obtain all genetic studies on the relationship of CTLA-4 + 49G/A polymorphism and cancer. We also used a hand search of references of original studies or reviewed articles on this topic to identify additional studies. The following criteria were used to select the eligible studies: (1) a case-control study on the association between CTLA-4 + 49G/A polymorphism and cancer, (2) detailed number of different genotypes for estimating an odds ratio (OR) with 95% confidence interval (3) when several publications reported on the same population data, the largest or most complete study was chosen.

3. Data extraction

Data extraction was carried out independently by two investigators after the concealment of authors, journals, supporting organizations and funds to avoid investigators’ bias. For each eligible study, the following information was recorded: the first author’s name, the year of publication, country of origin, cancer type, genotyping method, sources of controls, racial descent of the study population, number of cases and number of controls with different allele frequencies.

4. Statistical analysis

The strength of relationship between CTLA-4 + 49G/A polymorphism and cancer was assessed by using Crude OR with 95% CI. We examined the association between the CTLA-4 + 49G/A polymorphism and digestive cancer risks using the following genetic contrasts: homozygote comparison (GG vs. AA), heterozygote comparison (GA vs. AA), dominant genetic model (GG + GA vs. AA), recessive genetic model (GG vs. GA + AA) and allelic comparison (G vs. A). Between-study heterogeneity was evaluated by Q-test. Fixed effects model was used to pool the data when the P-value of Q-test ≥ 0.05, otherwise, random-effects model was selected. Both funnel plot and Egger’s test were used to assess the publication bias (P < 0.05 was considered representative of statistical significance). All statistical analyses were performed using STATA11.0 software and Review Manager (v.5; Oxford, England).

5. Results

5.1. Eligible studies

By the inclusion and exclusion criteria, 17 relevant studies involving 5176 cases and 6747 controls were selected in this meta-analysis. The main characteristics of these studies are shown in Table 1. Genotype distribution of the CTLA-4 + 49G/A polymorphism among cancer cases

---

**Table 1**

Main characteristics of included studies in the meta-analysis.

| Author    | Year | Type           | Country | Genotype assay | Source of control | Cases | Controls |
|-----------|------|----------------|---------|----------------|-------------------|-------|----------|
| Yang      | 2012 | Pancreatic     | China   | PCR-RFLP       | Population        | 926   | 368      |
| Lang      | 2012 | Pancreatic     | China   | PCR-RFLP       | Population        | 651   | 602      |
| Cheng     | 2011 | Esophageus     | China   | PCR-RFLP       | Population        | 205   | 205      |
| Cozar     | 2007 | Colon          | Spain   | TaqMan          | Hospital          | 176   | 221      |
| Dilmec    | 2008 | Colorectal     | Turkey  | RFLP            | Hospital          | 162   | 56       |
| Gu        | 2010 | Hepatocellular | China   | PCR-LDR         | Hospital          | 376   | 407      |
| Hadinia   | 2007 | Colorectal     | Iran    | RFLP, PCR-ARMS  | Hospital          | 190   | 105      |
| Hadinia   | 2007 | Gastric        | Iran    | RFLP, PCR-ARMS  | Hospital          | 190   | 43       |
| Hu        | 2010 | Hepatocellular | China   | TaqMan          | Population        | 854   | 853      |
| Hou       | 2010 | Gastric        | China   | PCR-ARMS        | NA                | 205   | 262      |
| Kammerer  | 2010 | Oral           | German  | RT-PCR          | Hospital          | 40    | 83       |
| Mahajan   | 2008 | Gastric        | Poland  | TaqMan          | Population        | 411   | 301      |
| Qi        | 2010 | Colorectal     | China   | PCR-LDR         | NA                | 124   | 407      |
| Solerio   | 2005 | Colorectal     | Italy   | RFLP            | Hospital          | 238   | 132      |
| Sun       | 2008 | Esophageus     | China   | RFLP            | Hospital          | 1008  | 1010     |
| Sun       | 2008 | Gastric        | China   | RFLP            | Hospital          | 530   | 530      |
| Wong      | 2006 | Oral           | China   | RFLP            | Hospital          | 147   | 118      |

---

**Table 2**

Distribution of CTLA-4 + 49G/A polymorphism among cancer cases and controls in this meta-analysis.

| Author     | Year | Type           | AA (control) | AG (control) | GG (control) | AA (case) | AG (case) | GG (case) | G (control) | A (control) | G (case) | A (case) | HWE |
|------------|------|----------------|--------------|--------------|--------------|-----------|-----------|-----------|-------------|-------------|----------|----------|-----|
| Gu         | 2010 | Hepatocellular | 51           | 166          | 150          | 45        | 179       | 183       | 268         | 466         | 269      | 545      | Yes |
| Hu         | 2010 | Hepatocellular | 106          | 380          | 367          | 79        | 376       | 399       | 592         | 1114        | 534      | 1174     | Yes |
| Hadinia    | 2007 | Gastric        | 24           | 13           | 6            | 117       | 59        | 14        | 25          | 61          | 87       | 293      | Yes |
| Mahajan    | 2008 | Gastric        | 89           | 153          | 59           | 152       | 189       | 70        | 94          | 269         | 255      | 258      | 152  |
| Hou        | 2010 | Gastric        | 100          | 55           | 107          | 41        | 70        | 94        | 269         | 255         | 258      | 152      | Yes |
| Sun        | 2008 | Gastric        | 60           | 235          | 235          | 39        | 209       | 282       | 355         | 705         | 287      | 773      | Yes  |
| Qi         | 2010 | Colorectal     | 4            | 60           | 40           | 45        | 179       | 183       | 68          | 269         | 545      | Yes      |      |
| Solerio    | 2005 | Colorectal     | 76           | 43           | 13           | 128       | 91        | 19        | 195         | 69          | 347      | 129      | Yes  |
| Hadinia    | 2007 | Colorectal     | 52           | 47           | 6            | 117       | 59        | 14        | 59          | 151         | 87       | 293      | Yes  |
| Cozar      | 2007 | Colorectal     | 119          | 87           | 15           | 76        | 77        | 21        | 325         | 233         | 213      | 119      | Yes  |
| Dilmec     | 2008 | Colorectal     | 36           | 19           | 1            | 108       | 43        | 11        | 21          | 91          | 65       | 259      | Yes  |
| Cheng      | 2011 | Esophageus     | 36           | 79           | 90           | 46        | 105       | 54        | 259         | 151         | 213      | 197      | Yes  |
| Sun        | 2008 | Esophageus     | 128          | 434          | 448          | 73        | 406       | 529       | 690         | 1330        | 552      | 1464     | Yes  |
| Kammerer   | 2010 | Oral           | 35           | 32           | 16           | 11        | 23        | 6         | 102         | 64          | 45       | 35       | Yes  |
| Wong       | 2006 | Oral           | 12           | 58           | 48           | 25        | 64        | 38        | 82          | 154         | 114      | 180      | Yes  |
| Yang       | 2012 | Pancreatic     | 50           | 178          | 140          | 70        | 374       | 482       | 458         | 278         | 1338     | 514      | Yes  |
| Lang       | 2012 | Pancreatic     | 82           | 312          | 208          | 62        | 326       | 263       | 728         | 476         | 852      | 450      | Yes  |
Table 3

| Study groups          | N  | OR (95% CI)       | P       |
|-----------------------|----|-------------------|---------|
| GG vs. AA             | 17 | 1.217 (0.923–1.62) | <0.001  |
| GA vs. AA             | 12 | 1.197 (0.713–1.51) | <0.001  |
| GG/GA vs. AA          | 27 | 1.668 (1.286–2.16) | <0.001  |
| GG vs. GA/AA          | 23 | 1.496 (1.103–1.97) | 0.020   |
| GA vs. AA             | 5  | 1.510 (0.973–2.34) | 0.075   |
| GG vs. AA             | 12 | 1.55 (0.96–2.47)   | 0.084   |

Cancer type

- All population: 17 cases, 12 controls
- Hepatocellular: 2 cases, 1 control
- Gastric: 4 cases, 3 controls
- Colorectal: 5 cases, 5 controls
- Gastric: 2 cases, 1 control
- Esophageal: 2 cases, 1 control
- Pancreatic: 2 cases, 2 controls
- Ethnicity
- Asian: 12 cases, 7 controls
- European: 5 cases, 5 controls
- Source of control
- Population-based: 12 cases, 12 controls
- Hospital-based: 12 cases, 12 controls

The funnel plot of overall GG vs. AA.

5.2. Meta-analysis

The association strength between CTLA-4 + 49G/A polymorphism and the susceptibility for digestive system cancers are shown in Table 3. Overall, there was no statistically increased risk of digestive system cancers in every genetic comparison (GG vs. AA, OR = 1.217, 95% CI = 0.923–1.605; GA vs. AA, OR = 1.160, 95% CI = 0.991–1.362; GG/GA vs. AA, OR = 1.165, 95% CI = 0.932–1.456; GG vs. AA, OR = 0.897, 95% CI = 0.762–1.054; G vs. A, OR = 0.966, 95% CI = 0.829–1.126).

We then evaluated the effects of CTLA-4 + 49G/A polymorphism according to specific cancer types, different ethnicities and different sources of control. As shown in Table 3, we demonstrated that this locus polymorphism was significantly linked to higher risks for pancreatic cancer (GG vs. AA, OR = 1.976, 95% CI = 1.496–2.611; GA vs. AA, OR = 1.433, 95% CI = 1.093–1.879; GG/GA vs. AA, OR = 1.668, 95% CI = 1.286–2.164; GG vs. AA, OR = 1.502, 95% CI = 1.098–2.054; G vs. A, OR = 1.394, 95% CI = 1.098–1.770). We also observed increased susceptibility of hepatocellular cell carcinoma in homozygote comparison (OR = 1.433, 95% CI = 1.100–1.866) and dominant model (OR = 1.360, 95% CI = 1.059–1.746). Furthermore, we observed increased susceptibility of esophageal cancer only in heterozygote comparison (OR = 1.454, 95% CI = 1.110–1.906). No significant associations were found in colorectal cancer, gastric cancer and oral cancer.

According to the source of controls, significant effects were observed in hospital-based studies (GA/AA vs. GG, OR = 1.257, 95% CI = 1.129–1.399), but in population-based studies, no significant association was observed in all models. In the stratified analysis by ethnicity, no significantly increased risks were found in either Asian or Caucasian.

6. Publication bias

Both Begg’s funnel plot and Egger’s test were performed to assess the publication bias of the literature. The shape of the funnel plots did not reveal any evidence of obvious asymmetry in the overall meta-
analysis (Fig. 1 shows the funnel plot of overall GG vs. AA). Then, Egger’s test was used to provide statistical evidence of funnel plot symmetry. The results still did not present any obvious evidence of publication bias in the subgroup analyses.

7. Discussion

The result of this meta-analysis suggested that CTLA-4 + 49G/A polymorphism was significantly linked to higher risks for pancreatic cancer. Besides, the polymorphism was associated with an increased risk of developing hepatocellular cell carcinoma. The CTLA-4 49G→A SNP has been linked to elevated risk of breast cancer in an Iranian population (Ghaderi et al., 2004), and non-Hodgkin’s lymphoma in a European Caucasian population (Lang et al., 2012). In addition, two more studies suggested that this polymorphism is associated with different cancers including lung cancer and cervical cancer (Sun et al., 2008; Kämmerer et al., 2010). A meta-analysis conducted by Zheng et al. suggested that the CTLA-4 + 49G/A polymorphism was associated with an increased risk of developing solid tumors (including lung cancer, breast cancer, colorectal cancer, gastric cancer, skin cancer, thymoma, nasopharyngeal carcinoma, cervical squamous cell carcinoma, esophageal cancer, oral squamous cell carcinoma, HBV-related hepatocellular carcinoma, and renal cell cancer) (Mahajan et al., 2008). Interestingly, Yonggang Zhang et al. conducted a meta-analysis and the results indicated that the polymorphism is associated with a decreased risk of lung cancer and breast cancer but not of cervical cancer, colorectal cancer, or gastric cancer (Zhang et al., 2011).

In our analysis, we first reported that there was no statistically increased risk between the CTLA-4 + 49G/A polymorphism and digestive system cancers. In subgroup analysis, we observed that this polymorphism was significantly linked to higher risks for pancreatic cancer. We also observed the CTLA-4 + 49G/A polymorphism was associated with an increased risk of developing hepatocellular cell carcinoma but not gastric cancer, colorectal cancer and oral cancer. However, all of these results should be interpreted with caution. On condition that, for some cancer types, only two case–control studies were included, which may have limited power to reveal a reliable association. Furthermore, we observed inconsistent results between hospital-based studies and population-based studies, which may be explained by the biases brought by hospital-based studies. Controls in hospital-based studies may be less representative of general population than controls from population-based studies.

There were some limitations in our meta-analysis. Firstly, sample size in any given cancer was not sufficiently large. It might be difficult to get a concrete conclusion if the number of included studies in subgroup was few. Secondly, due to the original data of the eligible studies were unavailable, it is difficult for us to evaluate the roles of some special environmental factors and lifestyles such as diet, alcohol consumption, and smoking status in developing cancer. And thirdly, language bias might derive from the screened references of English documents only.

In conclusion, our meta-analysis suggested that the CTLA-4 + 49G/A polymorphism may not be associated with an elevated digestive system cancer risks. Large well-designed epidemiological studies are needed to validate our findings.

Conflict of interest

None.

Acknowledgment

The authors will thank Dr. Pan Xiangpo for his great help in revising this article.