Current Evidence on the Association between Cytotoxic T-Lymphocyte Antigen 4 +49G > A Polymorphism and Digestive System Cancer Risks: a Meta-analysis Involving 11,923 Subjects

Biao Jiang, Meng Ji, Ailiang Wang, Wei Zhang, Zhixin Zhang and Qiang Li*
Tianjin Cancer Institute and Hospital, Tianjin, China

Abstract

Cytotoxic T-lymphocyte antigen 4 (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 5,176 cancer patients and 6,747 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 higherrisks 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 = 1.098-2.054; G vs. A, OR=1.394, 95%CI = 1.098-1.770). We also observed increased susceptibility of hepatocellular 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 = 1.129-1.397). 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 not associated with an elevated digestive system cancer risks.

Keywords: CTLA-4; Polymorphisms; Cancer; Meta-analysis

Introduction

CTLA-4, a member of the immunoglobulin super-family, is a co-stimulatory molecule expressed by activated T cells and has the function of down-regulating T-cell activation [1]. 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 kinases production [2]. In tumor-transplanted mice, injection with antibodies that block CTLA-4 function enhanced T cell activation [3], rejected a variety of different tumors, and had long-lasting anti-tumor immunity [4], suggesting that the CTLA-4 may play 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 [5-7]. This gene is polymorphic and more than 100 single nucleotide polymorphisms have been identified [8]. An common polymorphism 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 [9]. Recent studies indicated that this polymorphism may influence the ability of CTLA-4 to bind with B7.1 and affect T-cell activation subsequently [10,11].

Previous studies have identified that this polymorphism is associated with different cancers including lung cancer, breast cancer, and cervical cancer [10,12]. However, the results of studies on the association between the +49 A > G polymorphism and the risk of digestive system cancers remain inconsistent [10,13-26]. 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.

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 November, 2012) 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 was 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.

Data Extraction

Data extraction was carried out independently by two investigators after the concealment of authors, journals, supporting organizations references of original studies or reviewed articles on this topic to identify additional studies. The following criteria was 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.

Received March 22, 2013; Accepted April 27, 2013; Published April 30, 2013

Citation: Jiang B, Ji M, Wang A, Zhang W, Zhang Z, et al. (2013) Current Evidence on the Association between Cytotoxic T-Lymphocyte Antigen 4 +49G > A Polymorphism and Digestive System Cancer Risks: a Meta-analysis Involving 11,923 Subjects. J Bioanal Biomed S9: 002. doi:10.4172/1948-593X.S9-002

Copyright: © 2013 Jiang B, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
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.

**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 Manage (v.5; Oxford, England).

**Results**

**Eligible studies**

By the inclusion and exclusion criteria, 17 relevant studies involving 5,176 cases and 6,747 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 and controls of the 17 studies are shown in table 2. All studies were case–control studies, including five colorectal cancer studies, four gastric cancer studies, two esophageal cancer studies, two hepatocellular cell carcinoma studies, two oral cancer studies and two pancreatic cancer studies. There were 12 studies of Asian descent and five studies of Caucasian descent. Hospital based controls were carried out in 12 studies, while population based controls were carried out in 5 studies. The genotyping method contains the classic polymerase chain reaction–restriction fragment length polymorphism assay (PCR-RFLP), RFLP and Taqman. The distribution of genotypes in the controls was all in agreement with HWE.

**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=1.161, 95% CI = 0.991–1.360; GG/GA vs. AA, OR=1.165, 95% CI = 0.932–1.456; GG vs. GA/AA, OR=1.114, 95% CI = 0.948–1.312; 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 higherrisks 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 = 1.098-2.054; G vs. A, OR=1.394, 95% (CI = 1.098-1.770). We alsoobserved increased susceptibility of hepatocellular 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-

---

**Table 1:** Main characteristics of included studies in the meta-analysis.
Table 2: Distribution of CTLA-4 + 49G/A polymorphism among cancer cases and controls in this meta-analysis.

| Study groups | N* | GG vs. AA | OR (95% CI) | GA vs. AA | OR (95% CI) | GG/GA vs. AA | OR (95% CI) | G vs. A | OR (95% CI) |
|--------------|----|-----------|-------------|-----------|-------------|--------------|-------------|---------|-------------|
|              |    | OR         | P           | OR         | P           | OR           | P           | OR      | P           |
| Total        | 17 | 1.217 (0.923-1.605) ‡ | <0.001 | 1.161 (0.991-1.360)‡ | <0.001 | 1.165 (0.932-1.456)‡ | <0.001 | 1.114 (0.948-1.312)‡ | <0.001 | 0.966 (0.829-1.126)‡ | <0.001 |
| Cancer type  |    |            |             |           |             |              |             |         |             |
| Hepatocellular | 2 | 1.433 (1.100-1.866) | 0.851 | 1.291 (0.992-1.681) | 0.771 | 1.360 (1.059-1.746) | 0.796 | 1.168 (0.996-1.367) | 0.920 | 0.857 (0.761-0.964) | 0.983 |
| Gastric      | 4 | 1.160 (0.601-2.237)‡ | <0.001 | 1.300 (0.670-2.252)‡ | <0.001 | 1.235 (0.662-2.302)‡ | <0.001 | 1.077 (0.814-1.350) ‡ | 0.042 | 1.033 (0.696-1.532)‡ | <0.001 |
| Colorectal   | 5 | 1.028 (0.479-2.207)‡ | 0.020 | 0.805 (0.498-1.301)† | 0.006 | 0.858 (0.543-1.354)‡ | 0.006 | 1.079 (0.804-1.447)† | 0.215 | 0.929 (0.727-1.188)‡ | 0.060 |
| Esophagus    | 2 | 1.004 (0.235-4.295)‡ | <0.001 | 1.454 (1.110-1.906) | 0.146 | 1.194 (0.482-2.957)‡ | 0.002 | 0.809 (0.273-2.398) ‡ | <0.001 | 0.708 (0.627-0.799)‡ | 0.368 |
| Oral         | 2 | 0.725 (0.379-1.385) | 0.312 | 1.086 (0.259-4.554) | 0.013 | 1.017 (0.300-3.449)‡ | 0.026 | 0.876 (0.563-1.364) | 0.478 | 1.058 (0.786-1.424) | 0.240 |
| Pancreatic   | 2 | 1.976 (1.496-2.611) | 0.173 | 1.433 (1.093-1.879) | 0.766 | 1.668 (1.286-2.164) | 0.347 | 1.502 (1.098-2.054)‡ | 0.033 | 1.394 (1.098-1.770)‡ | 0.049 |
| Ethnicity    |    |            |             |           |             |              |             |         |             |
| Asian        | 12 | 1.240 (0.908-1.695)‡ | <0.001 | 1.164 (0.895-1.514)‡ | <0.001 | 1.179 (0.896-1.551)‡ | <0.001 | 1.139 (0.956-1.358)‡ | <0.001 | 0.974 (0.807-1.175)‡ | <0.001 |
| European     | 5  | 1.143 (0.660-1.977) | 0.043 | 0.988 (0.698-1.397) | 0.021 | 1.101 (0.776-1.562) | 0.029 | 1.015 (0.763-1.351) | 0.154 | 0.951 (0.745-1.213) | 0.053 |
| Source of Control Population-based | 5  | 1.169 (0.694-1.970)‡ | <0.001 | 1.156 (0.873-1.530) | 0.029 | 1.170 (0.800-1.712)‡ | <0.001 | 0.965 (0.678-1.373)‡ | <0.001 | 1.063 (0.802-1.468)‡ | <0.001 |
| Hospital-based | 12 | 1.255 (0.901-1.749) | 0.001 | 1.125 (0.828-1.530)‡ | <0.001 | 1.154 (0.864-1.541)‡ | <0.001 | 1.257 (1.129-1.399) | 0.150 | 0.919 (0.778-1.086)‡ | <0.001 |

Abbreviations: CI, confidence interval; OR, odds ratio.
* Studies of comparison, ‡ P-value of Q-test for heterogeneity test, † Random model was used.
In our analysis, we first reported that there was no statistically significant association observed in all models. In the stratified analysis by ethnicity, no significantly increased risks were found in either Asian or Caucasian.

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 (Figure 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.

Discussion

The result of this meta-analysis suggested that CTLA-4 +49G/A polymorphism was not overall significantly associated with digestive system cancer risk. In stratified analysis by ethnicity, we also failed to detect any significant association in either Asian or Caucasian. However, in subgroup analysis, this polymorphism was significantly linked to higher risks for pancreatic cancer. Besides, when stratified according to study design, positive associations were observed in hospital-based studies.

The CTLA-4 49G>A SNP has been linked to elevated risk of breast cancer in an Iranian population [6], and non–Hodgkin’s lymphoma in an European Caucasian population [26]. In addition, two more studies suggested that this polymorphism is associated with different cancers including lung cancer and cervical cancer [10,21]. A meta-analysis conducted by Zheng et al. suggested that the CTLA-4 +49G/A polymorphism was significantly linked to higher risks for pancreatic cancer. In addition, all of these results should be interpreted with caution. On one hand, 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 be not associated with an elevated digestive system cancer risks. Large well-designed epidemiological studies are needed to validate our findings.

References

1. Hodi FS, Mihm MC, Soffer RJ, Haluska FG, Butler M, et al. (2003) Biologic activity of cytotoxic T lymphocyte-associated antigen 4 antibody blockade in previously vaccinated metastatic melanoma and ovarian carcinoma patients. Proc Natl Acad Sci USA 100: 4712-4717.
2. Greenwald RJ, Oosterwegel MA, van der Woude D, Kubal A, Mandelbrot DA, et al. (2002) CTLA-4 regulates cell cycle progression during a primary immune response. Eur J Immunol 32: 366-373.
3. Vandenborre K, Van Gool SW, Kasran A, Ceuppens JL, Boogaerts MA, et al. (1999) Interaction of CTLA-4 (CD152) with CD80 or CD86 inhibits human T-cell activation. Immunology 96: 413-421.
4. Leach DR, Krummel MF, Allison JP (1996) Enhancement of anti-tumor immunity by CTLA-4 blockade. Science 271: 1734-1736.
5. Qi P, Ruan CP, Wang H, Zhou GF, Xu XY, et al. (2010) CTLA-4 +49A>G polymorphism is associated with the risk but not with the progression of colorectal cancer in Chinese. Cancer Gene Ther 17: 1-7.
6. Ghaderi A, Yeganeh F, Kalantari T, Talei AR, Peszheshi AM, et al. (2004) Cytotoxic T lymphocyte antigen-4 gene in breast cancer. Breast Cancer Res Treat 86: 1-7.
7. Ligers A, Teleshova N, Masterman T, Huang WX, Hillert J (2001) CTLA-4 gene expression is influenced by promoter and exon 1 polymorphisms. Genes Immune 2: 145-152.
8. Ueda H, Howson JM, Esposito L, Sheward J, Snook H, et al. (2003) Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. Nature 423: 506-511.
9. Harper K, Balzano C, Rouvier E, Mattéi MG, Luciani MF, et al. (1991) CTLA-4 and CD28 activated lymphocyte molecules are closely related in both mouse and human as to sequence, message expression, gene structure, and chromosomal location. J Immunol 147: 1037-1044.
10. Sun T, Zhou Y, Yang M, Hu Z, Tan W, et al. (2008) Functional genetic variations in cytokotic T-lymphocyte antigen 4 and susceptibility to multiple types of cancer. Cancer Res 68: 7025-7034.
11. Wang L, Li D, Fu Z, Li H, Jiang W, et al. (2007) Association of CTLA-4 gene polymorphisms with sporadic breast cancer in Chinese Han population. BMC Cancer 7: 173.
12. Erfani N, Razmikhah M, Talei AR, Peszheshri AM, Douroudchi M, et al. (2006)
Cytotoxic T lymphocyte antigen-4 promoter variants in breast cancer. Cancer Genet Cytogenet 165: 114-120.

13. Hadinia A, Hossieni SV, Erfani N, Saberi-Firozi M, Fattahi MJ, et al. (2007) CTLA-4 gene promoter and exon 1 polymorphisms in Iranian patients with gastric and colorectal cancers. J Gastroenterol Hepatol 22: 2283-2287.

14. Solerio E, Tappero G, Iannace L, Matullo G, Ayoubi M, et al. (2005) CTLA4 gene polymorphism in Italian patients with colorectal adenoma and cancer. Dig Liver Dis 37: 170-175.

15. Dilmec F, Ozgonul A, Uzunkoy A, Akkafa F (2008) Investigation of CTLA-4 and CD28 gene polymorphisms in a group of Turkish patients with colorectal cancer. Int J Immunogenet 35: 317-321.

16. Cheng TY, Lin JT, Chen LT, Shun CT, Wang HP, et al. (2006) Association of T-cell regulatory gene polymorphisms with susceptibility to gastric mucosa-associated lymphoid tissue lymphoma. J Clin Oncol 24: 3483-3489.

17. Wong YK, Chang KW, Cheng CY, Liu CJ (2006) Association of CTLA-4 gene polymorphism with oral squamous cell carcinoma. J Oral Pathol Med 35: 51-54.

18. Gu X, Qi P, Zhou F, Ji Q, Wang H, et al. (2010) +49G > A polymorphism in the cytotoxic T-lymphocyte antigen-4 gene increases susceptibility to hepatitis B-related hepatocellular carcinoma in a male Chinese population. Hum Immunol 71: 83-87.

19. Hou R, Cao B, Chen Z, Li Y, Ning T, et al. (2010) Association of cytotoxic T lymphocyte-associated antigen-4 gene haplotype with the susceptibility to gastric cancer. Mol Biol Rep 37: 515-520.

20. Cozar JM, Romero JM, Aptitsauri N, Vazquez F, Vilchez JR, et al. (2007) High incidence of CTLA-4 AA (CT60) polymorphism in renal cell cancer. Hum Immunol 68: 698-704.

21. Hu L, Liu J, Chen X, Zhang Y, Liu L, et al. (2010) CTLA-4 gene polymorphism +49A/G contributes to genetic susceptibility to two infection-related cancers-hepatocellular carcinoma and cervical cancer. Hum Immunol 71: 888-891.

22. Kämmerer PW, Toyoshima T, Schöder F, Kämmerer P, Kuhr K, et al. (2010) Association of T-cell regulatory gene polymorphisms with oral squamous cell carcinoma. Oral Oncol 46: 543-548.

23. Yang M, Sun T, Zhou Y, Wang L, Liu L, et al. (2012) The functional cytotoxic T lymphocyte-associated Protein 4 49G-to-A genetic variant and risk of pancreatic cancer. Cancer 118: 4681-4686.

24. Lang C, Chen L, Li S (2012) Cytotoxic T-lymphocyte antigen-4 +49G/A polymorphism and susceptibility to pancreatic cancer. DNA Cell Biol 31: 683-687.

25. Mahajan R, El-Omar EM, Lisowska J, Grillo P, Rabkin CS, et al. (2008) Genetic variants in T helper cell type 1, 2 and 3 pathways and gastric cancer risk in a Polish population. Jpn J Clin Oncol 38: 626-633.

26. Zheng C, Huang D, Liu L, Bjorkholm M, Holm G, et al. (2001) Cytotoxic T-lymphocyte antigen-4 microsatellite polymorphism is associated with multiple myeloma. Br J Haematol 112: 216-218.

27. Zheng J, Yu X, Jiang L, Xiao M, Bai B, et al. (2010) Association between the Cytotoxic T-lymphocyte antigen 4 +49G >A polymorphism and cancer risk: a meta-analysis. BMC Cancer 10: 522.

28. Zhang Y, Zhang J, Deng Y, Tian C, Li X, et al. (2011) Polymorphisms in the cytotoxic T-lymphocyte antigen 4 gene and cancer risk: a meta-analysis. Cancer 117: 4312-4324.

Submit your next manuscript and get advantages of OMICS
Group submissions

Unique features:
- User-friendly/feasible website-translation of your paper to 50 world’s leading languages
- Audio Version of published paper
- Digital articles to share and explore

Special features:
- 250 Open Access Journals
- 20,000 editorial team
- 21 days rapid review process
- Quality and quick editorial, review and publication processing
- Indexing of PubMed (partial), Scopus, EBSCO, Index Copernicus and Google Scholar etc
- Steering Option: Social Networking Enabled
- Authors, Reviewers and Editors rewarded with online Scientific Credits
- Better discount for your subsequent articles

Submit your manuscript at: www.editorialmanager.com/jbabm

Citation: Jiang B, Ji M, Wang A, Zhang W, Zhang Z, et al. (2013) Current Evidence on the Association between Cytotoxic T-Lymphocyte Antigen 4 +49G > A Polymorphism and Digestive System Cancer Risks: a Meta-analysis Involving 11,923 Subjects. J Bioanal Biomed 59: 002. doi:10.4172/1948-593X.S9-002

This article was originally published in a special issue, Cancer Stem Cells handled by Editor(s). Dr. DaoTai Nie, Southern Illinois University, USA