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Original Article

Association between *CTLA-4* rs231775 polymorphism and risk of colorectal cancer: a meta analysis

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Abstract: Objective: Many previous studies that examined the association between *CTLA-4* rs231775 polymorphism and CRC risk have produced inconsistent results. In this study, a meta-analysis was performed to systematically summarize the possible association. Methods: We identified relevant studies using PubMed, Embase, and China National Knowledge Infrastructure literature databases. Eligible studies were selected using specific criteria. Data were extracted independently by two authors. The pooled OR with 95% CI was estimated by applying the fixed-effects model to examine the association of interest. Results: Eight studies were identified for the meta-analysis. In overall analysis, we observed an significantly increased CRC risk attributed to the AG genotype as compared to the AA genotype (OR: 1.17, 95% CI: 1.02-1.35 for AG vs AA). Stratified analysis according to ethnicity also showed a significant association in Caucasians under the AG vs AA model (OR: 1.22, 95% CI: 1.03-1.46). No significant heterogeneity or publication bias was tested in our meta-analysis. Conclusion: In conclusion, the meta-analysis suggests that rs231775 in the *CTLA-4* gene may be a risk factor for CRC, especially in Caucasians.

Keywords: CTLA-4, rs231775, polymorphism, CRC, risk

Introduction

Colorectal cancer (CRC) is one of the most frequently diagnosed cancers and is estimated to have affected more than one million people in 2008 [1]. Factors known to contribute to CRC incidence include different frequency of exposure to possible risk factors such as tobacco use, physical inactivity, and obesity [2]. Inherited genetic factors also play a significant role in individual susceptibility to this cancer [3-5]. A variety of genes responsible for CRC cases have already been established. Such discoveries have been introduced into clinical practice and have improved risk evaluation [6]. Despite the favorable advances due to use of genetic testing, the genes involved in the regulation of CRC risk still remain to be characterized.

Cytotoxic T-lymphocyte antigen-4 (*CTLA-4*) is a fundamental immunomodulatory gene and regulates immune response to many antigens. T cells are molecules important for antitumor immunity and for the mediation of T-cell activation that is correlated with cancer initiation. *CTLA-4*, also known as *CD152*, and its homolog CD28 in conjunction with their common ligands (CD80 and CD86), comprise the CD28-CTLA-4 costimulatory pathway of T-cell activation. However, CTLA-4 and CD28 have opposite effects on T-cell activation: CTLA4-ligand interaction maintains peripheral tolerance through negatively regulating adaptive immune responses [7] and inhibits T-cell activation by downregulating immune response [8-10]; in contrast, CD28/ligand interaction engages in T-cell antigen receptor and thus sustains T-cell response [11, 12]. Molecular studies showed that the *CTLA-4* gene combined with CD80 or CD86 ligands can trigger apoptosis of CTLA-4-expressed tumor cells [13]. CTLA-4 deficiency is prone to many lethal diseases, including cancer [14, 15], suggesting the importance of *CTLA-4* in the etiology of human cancers.

Several single nucleotide polymorphisms (SNPs, rs5742909, rs4553808) in the promoter region of *CTLA-4* gene may modulate the gene expression [16]. Genotype-phenotype stu-

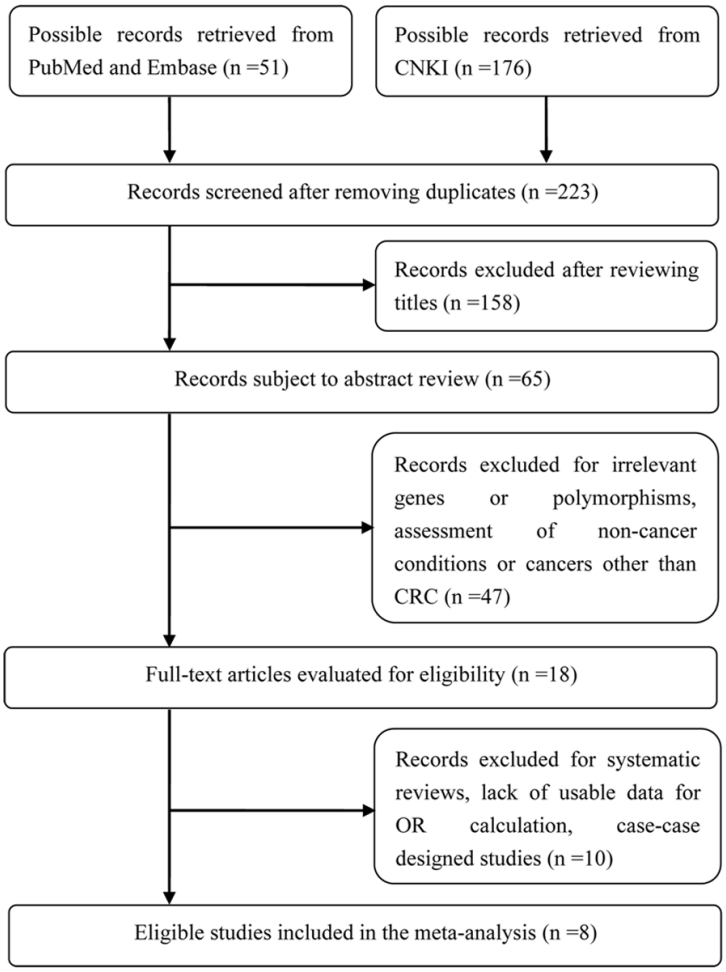

Figure 1. Flow chart of study selection.

dies of the rs231775 polymorphism (at position +49 in exon 1 with an A to G change) showed that the G allele is associated with altered expression of CTLA-4, and hence increases the chance of developing common diseases [17]. Independent case-control studies have extensively investigated the association between rs231775 and CRC [18-25]. But the reported genetic effects varied across the published studies, and a clear association is limited by the insufficient detection power of these small-sized studies. In addition, several meta-analyses of rs231775 and cancer susceptibility may have suggested biased results on CRC risk, as these studies either failed to summarize all available data or did not consider Chinese publications [26-28]. Therefore, we performed a meta-analysis of all early-released studies to precisely assess the effects of rs231775 on CRC risk.

Materials and methods

Search strategy

We searched the PubMed, Embase, and China National Knowledge Infrastructure literature databases up to January 31, 2014 without language limitation. The search strategy to identify all possible studies involved combination of cytotoxic T-lymphocyte antigen-4, *CTLA-4*, polymorphism, polymorphisms, colorectal cancer, rectal cancer, and colon cancer. We also hand searched reference lists of all retrieved articles. When a group of case samples was analyzed in more than one article concerning the same topic, only the study with the largest size was included.

Eligible studies and data abstraction

We selected all case-control studies without consideration of sample size, but these studies must investigate the relationship between *CTLA-4* rs231775 polymorphism and CRC and had to publish adequate genotype information. We excluded systematic reviews, and the studies that had a case-case design or contained unavailable genotype data. Data extraction was done by two independent investigators. The characteristics collected from each of the eligible case-control studies were authors, publication year, location where the cases were recruited, study country, ethnicity, allele and genotype frequencies in cases and controls. Disagreements, if any, were resolved by discussion.

Statistical analysis

Deviation from Hardy-Weinberg equilibrium (HWE) was tested for all studies in controls using the goodness-of-fit X^2 test. The association between rs231775 and CRC risk was examined by estimating odds ratio (OR) and its corresponding 95% confidence interval (CI). The OR was first calculated for the GG vs AA and AG vs AA comparisons, followed by G vs A,

Table 1. Characteristics of the studies included

No.	Study (Reference no.)	Year	Country	Ethnicity	Case	Control	Case		Control	
							A allele	G allele	A allele	G allele
1	Solerio [18]	2005	Italy	Caucasian	132	238	195	69	347	129
2	Cozar [19]	2007	Spain	Caucasian	96	176	136	56	233	119
3	Hadinia [20]	2007	Iran	Caucasian	105	190	151	59	293	87
4	Dilmec [21]	2008	Turkey	Caucasian	56	162	91	21	259	65
5	Qi [22]	2010	China	Asian	124	407	68	180	269	545
6	Li [23]	2011	China	Asian	248	380	136	360	251	509
7	Fan [24]	2012	China	Asian	291	352	392	190	478	226
8	Cui [25]	2013	China	Asian	128	205	64	192	174	236

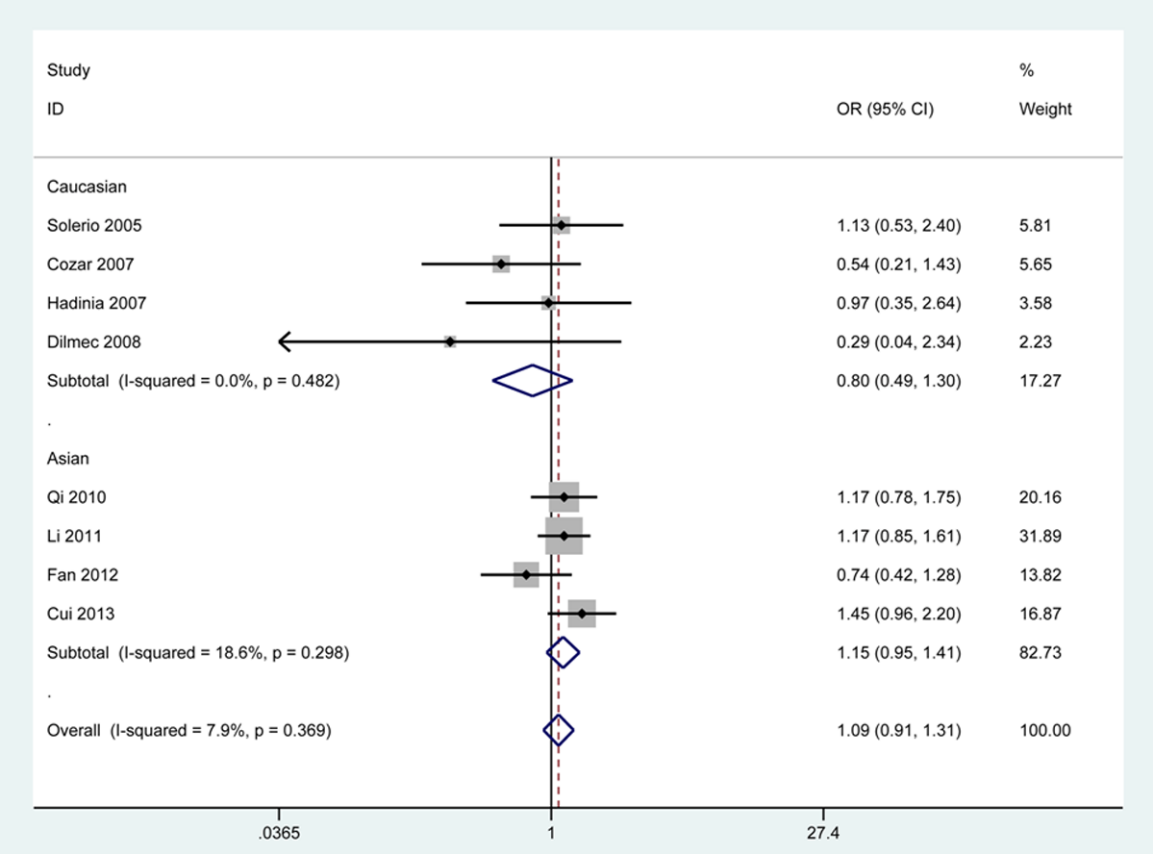

Figure 2. Meta-analysis of *CTLA-4* rs231775 polymorphism and CRC risk under the GG vs AA model.

GG + AG vs AA, and GG vs AG + AA models. Overall meta-analysis as well as stratified analysis according to ethnicity was carried out in this study. Heterogeneity assumption was determined by the Chi square based Q test. We considered the heterogeneity significant when *P*-value < 0.10. The fixed-effects model [29] was applied to estimate the pooled ORs on condition that the Q test revealed no significant heterogeneity ($P > 0.10$). Otherwise, the random-effects model [30] was more appropriate to pool the genetic data from each study. Potential publication bias was examined using the funnel plots and Egger's liner regression test [31], a method that uses a weighted regression approach to examine the association of outcome effects (log odds ratio) with its standard error for each study. A probability (*P*) value of < 0.10 was considered statistically significant. The statistical software STATA v. 12.0

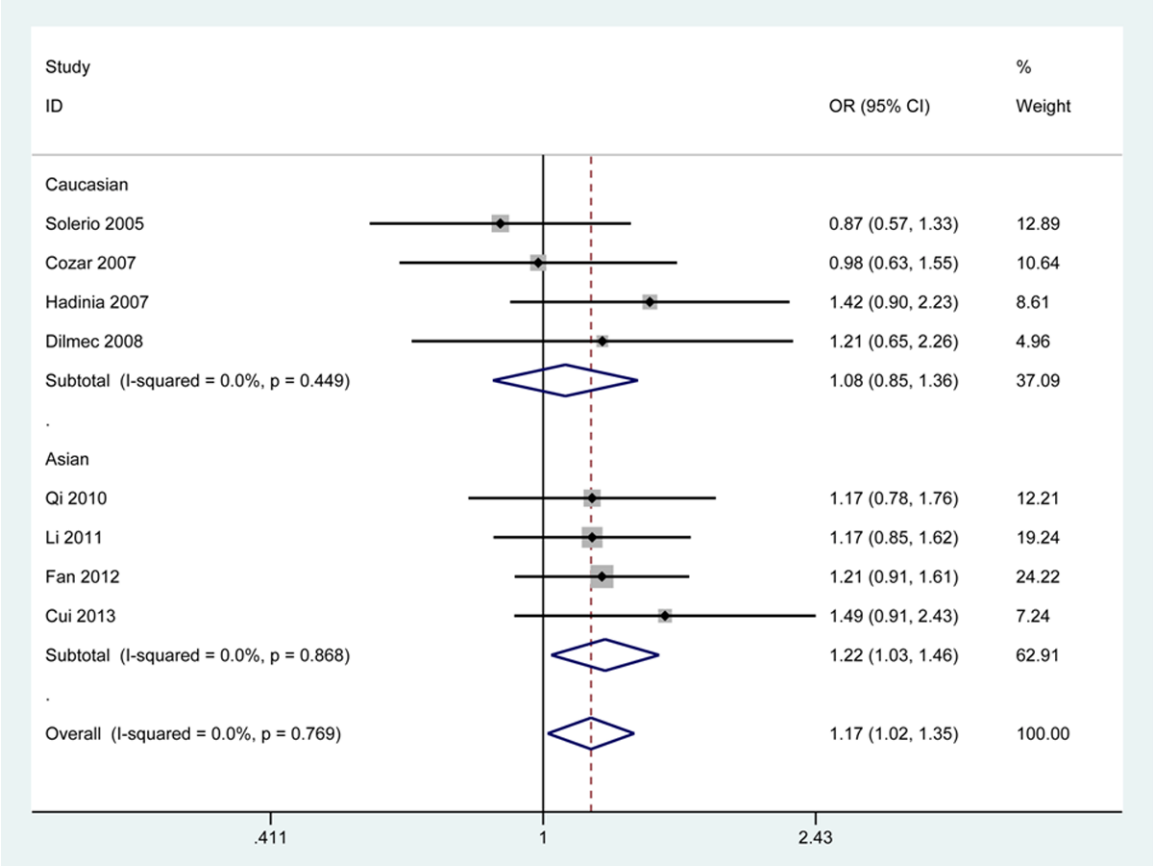

Figure 3. Meta-analysis of *CTLA-4* rs231775 polymorphism and CRC risk under the AG vs AA model.

(Stata Corporation, College Station, TX, USA) was used for meta-analyses.

Results

Through database search, we yielded a total of 227 possibly eligible studies. We reviewed all titles or abstracts, and selected 18 studies for detailed evaluation. Of these, ten were further excluded after full-text review because of the reasons listed below: systematic reviews, data reported in original articles were not sufficient enough to calculate ORs and case-case designs. Therefore, eight publications [18-25] were considered eligible for this analysis. The search flow is detailed in **Figure 1**.

As shown in **Table 1**, the studies carried out in Italy, Spain, Iran and Turkey were categorized into Caucasians. The remaining studies were all done in China and merged into the Asian group. The distribution of allele frequency was distinct between Caucasians and Asians. Overall, the G allele was minor allele for the former population and major allele for the latter. All studies included in our analysis had a case-control design. Significant departure from HWE was revealed in two studies ($P < 0.10$) [21, 25].

Quantitative analysis

The eight eligible studies included in the meta-analysis provided 1,180 CRC cases and 2,110 controls. The first analysis was performed by comparing the GG genotype with the AA genotype. The results showed that carriage of the GG genotype was not significantly associated with CRC risk (OR: 1.09, 95% CI: 0.91-1.31 for GG vs AA) (illustrated in **Figure 2**). However, the AG genotype increased CRC risk when compared to the AA genotype (OR: 1.17, 95% CI: 1.02-1.35 for AG vs AA) (illustrated in **Figure 3**). No obvious heterogeneity was tested across studies (**Table 2**).

We then carried out stratified analysis according to ethnicity. Among the genetic models adopted, an significant association was obs-

Table 2. Meta-analysis of CTLA-4 rs231775 polymorphism with CRC susceptibility

Subtypes		GG vs AA		AG vs AA		G vs A		GG + AG vs AA		GG vs AG + AA	
	N	OR (95% CI)	P_h	OR (95% CI)	P_h	OR (95% CI)	P_h	OR (95% CI)	P_h	OR (95% CI)	P_h
Total	8	1.09 (0.91, 1.31)	0.369	1.17 (1.02, 1.35)	0.769	1.08 (0.98, 1.18)	0.639	1.10 (0.98, 1.23)	0.909	1.02 (0.86, 1.20)	0.160
Ethnicity											
Caucasian	4	0.80 (0.49, 1.30)	0.482	1.08 (0.85, 1.36)	0.449	0.99 (0.82, 1.20)	0.592	1.04 (0.83, 1.29)	0.613	0.77 (0.48, 1.23)	0.353
Asian	4	1.15 (0.95, 1.41)	0.298	1.22 (1.03, 1.46)	0.868	1.11 (0.99, 1.23)	0.506	1.12 (0.98, 1.28)	0.906	1.06 (0.89, 1.27)	0.103

P_h: *P* value of Q-test for heterogeneity test. Fixed-effects model was used.

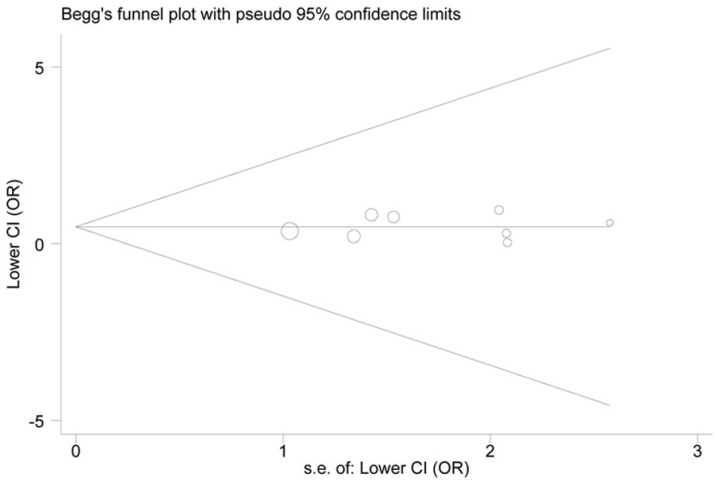

Figure 4. Funnel plot analysis to detect publication bias.

erved among Caucasians only in the AG vs AA model (OR: 1.22, 95% CI: 1.03-1.46 for AG vs AA) (illustrated in **Figure 3**), suggesting AG genotype carriers of Caucasian ancestry are at higher risk of developing CRC as compared to the AA genotype carriers. We observed no association among Asians. There was no significant between-study heterogeneity in the two subgroups (**Table 2**).

Publication bias

As shown in **Figure 4**, no obvious asymmetry was revealed in the funnel plot. We proceeded to perform the Egger's test to further examine the asymmetry. Statistical evidence suggested that there was no significant publication bias among studies selected for the meta-analysis (t = 0.41, P = 0.699 for GG vs. AG + AA).

Discussion

In the current study, we performed a meta-analysis and examined the association between *CTLA-4* rs231775 polymorphism and risk of CRC. For overall analysis, we observed an obviously increased CRC risk associated with the AG genotype as compared with the AA genotype. In stratified analysis, Caucasians carrying the AG genotype were suggested to have higher risk to develop CRC when compared to the AA genotype carriers. However, no significant association was indicated in Asians. The obtained data of our meta-analyses showed evidence of ethnicity being an important component for cancer susceptibility attributed to rs231775. The significant association observed among Caucasians rather than Asians suggested that Caucasian ancestry may be more susceptible to CRC in relation to rs231775. Ethnicity engaged in the modulation of CRC cancer risk has been reported in a previous study, where the incidence rate was found to remain the highest in Caucasian ancestry and the lowest in African or Asian ancestry [1], because there is substantial difference in dietary patterns and exposure to carcinogens such as smoking, and excessive alcohol consumption [32].

China has witnessed a rapid increase of mortality rate related to CRC over the past years [33]. Established etiological factors include immunodeficiency and failure of immune surveillance [34]. The *CTLA-4* gene is located at chromosome 2q33 and has different effects on immune response. Altered expression or malfunction of CTLA-4 may lead to breakdown of the immune system [22]. A series of studies have concentrated on rs231775, as the G allele of this polymorphism influences CTLA-4 expression [17]. Nevertheless, published data on the association between rs231775 and CRC risk implicated inconclusive results [19, 24]. A reliable assessment of the association, to a large extent, may be prevented due to the relatively small-sized studies.

Substantially different results were also implicated in meta-analyses addressing the connection between cancer risk and rs231775. Two analyses with a relatively larger sample size (thirty-two and forty-three case-control studies, respectively) showed evidence of a significantly increased risk of cancer in Caucasians and Asians [26, 28]. In contrast, the analysis included twenty-eight case-control studies indicated a protective association for cancer and Caucasians [27]. In this study, we performed a tissue-specific meta-analysis and the results showed significantly elevated CRC risk in Caucasians. These analyses suggest that rs231775 may play a major role in cancer susceptibility not only in Caucasians, also in Asians. This hypothesis in turn confirms a previous discovery that rs231775 increases expression of CTLA-4, affects immune function and

hence may promote cancer development [35]. The role of rs231775 in cancer progression nevertheless remains to be elucidated in a future larger study.

In this analysis, to identify all possible studies, we did not use language restraints and put equal emphasis on positive and negative publications, which helped to minimize potential publication bias, maximize detection power, and achieve robust and less biased results. Although we carried out a systematical literature search in the databases mentioned above, we had restricted access to unpublished studies or publications included in other databases we did not search. So publication bias may occur in this study. Moreover, cancer susceptibility can be modified by the combination of genes and environmental carcinogens. Since no available data can be extracted from the included studies, we failed to evaluate the interactions. Finally, the numbers of published studies were still not sufficiently large for the analysis of the effect of rs231775 on CRC risk and for subgroups.

In conclusion, results of the present meta-analysis suggested that rs231775 was associated with significantly increased risk of CRC. Significant association was also indicated in the subgroup of Caucasians. Well-designed studies with a sufficient sample size in various populations are required to further evaluate the association between rs231775 and CRC risk.

Disclosure of conflict of interest

None.

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