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Review Article

Association between CTLA-4 exon-1 +49A/G polymorphism and asthma: an updated meta-analysis

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Abstract: The results of studies on association between CTLA-4 exon-1 +49A/G (rs231775) polymorphism and susceptibility to asthma are controversial. To derive a more precise estimation of the relationship between the CTLA-4 exon-1 +49A/G polymorphism and asthma, a meta-analysis of 15 published case-control studies was performed. 15 studies meeting our inclusion criteria comprising 4006 asthma cases and 3729 controls were included. The effect summary odds ratio (OR) and 95% confidence intervals were obtained. Publication bias was tested by funnel plot, Egger's test and heterogeneity was assessed. The combined results showed that there were significant differences in genotype distribution between asthma cases and control on the basis of all studies, GG + GA versus AA (OR = 0.76, 95% CI: 0.62-0.93; P = 0.008). When stratifying for the race, the phenomenon was found that asthma cases had a significantly higher frequency of GG/GA versus AA (OR = 0.71; 95% CI: 0.51-0.99; *P* = 0.04) than control in Caucasian. Stratifying subjects by age indicated an association between CTLA-4 +49 GG + GA genotype and asthma in children (OR = 0.75; 95% CI: 0.62-0.90; *P* = 0.002), but no association in adults (OR = 0.93; 95% CI: 0.76-1.14; *P* = 0.48). Furthermore, significant association was observed in atopic asthma under the fixed-effects model (GG + GA vs. AA: *P* = 0.03, *OR* = 0.81, 95% CI = 0.67-0.98, $P_{heterogeneity}$ = 0.22). Our meta-analysis results suggest that CTLA-4 exon-1 +49A/G polymorphism might be a risk factor for asthma susceptibility, at least in Caucasian, children, and patients with atopy status.

Keywords: Asthma, gene polymorphism, CTLA-4, meta-analysis

Introduction

Asthma is a chronic respiratory disease that is characterized by variable airway obstructions [1], which occurs in all countries regardless of the level of development [2]. The prevalence of asthma is increasing in most countries around the world in recent decades and poses a substantial global health burden to people of all ages and all ethnic backgrounds [2]. It was estimated that asthma affects as many as 300 million people worldwide and is expected to increase to 400 million by 2025 [3, 4]. The development of asthma is determined by the interaction between host genetic susceptibility and environment, including exposure to tobacco smoke, air pollution, allergens and infections, as well as genetic variation [5, 6]. Ober et al. reported that asthma had significant genetic contributions, with heritability estimates varying between 35% and 95% [2]. A complete understanding of the genetic risk factors for asthma is critical to design new treatments or prevention strategies that can reduce the disease burden. In this respect, findings of several studies support the notion that regulatory T cell genes play an important role in the pathogenesis of asthma.

Cytotoxic T lymphocyte associated antigen-4 (CTLA-4), a negative signaling molecule expressed on the T-cell surface, is involved in establishing and maintaining peripheral T-cell tolerance [7, 8]. CTLA-4 is important in the development of autoimmune diseases and its dysregulation has the potential to affect the pathogenesis of asthma. It was showed that the plasma sCTLA-4 concentration was significantly higher in all asthmatic patients, compared with control subjects [9], which indicated that

Table 1. Characteristics of studies included in a meta-analysis

First author	Year	Country (Ethnicity)	Age group	Atopic status	Case (n)	Control (n)
Wang D	2014	China (Asian)	Children	NA	80	80
Zhang KC	2012	China (Asian)	Children	NA	26	30
Zhang LH	2010	China (Asian)	Children	NA	118	160
Undarmaa S	2010a	Japan (Asian)	Children	Atopic	325	336
Undarmaa S	2010b	Japan (Asian)	Adults	Atopic	367	676
Oh KY	2010	Korean (Asian)	Children	Atopic, non-atopic	742	238
Daley D	2009	Australia (Caucasian)	Adults/Juveniles	NA	616	727
Chan IH	2008	China (Asian)	Children	NA	272	171
Sohn MH	2007	Korean (Asian)	Children	Atopic, non-atopic	326	254
Jasek M	2006	Poland (Caucasian)	NA	Atopic	219	102
Schubert K	2006	Germany (Caucasian)	Children	NA	231	270
Howard TD	2002	Netherlands (Caucasian)	Adults	NA	177	134
Lee YS	2002	Korean (Asian)	Adults	Atopic, non-atopic	88	86
Hizawa N	2001	Japan (Asian)	Adults	NA	339	305
Nakao F	2000	Japan (Asian)	Children	Atopic	120	200

NA, not available.

CTLA-4 played a fundamental role in the pathogenesis of asthma. The CTLA-4 gene is located on chromosome 2q33 whose polymorphism has been shown to affect the inhibitory function of CTLA-4. It has been found that the G allele at position 49 of the CTLA-4 gene is associated with the impaired control of T cell proliferation and it has been proposed that the G allele might contribute to the pathogenesis of autoimmune diseases [10].

Over the last two decades, the large number of study has been carried out to clarify the relation between CTLA-4 exon-1 +49A/G polymorphism (rs231775) and asthma in different ethnical populations. However, previous studies reported conflicting results. In 2012, Lee et al. performed a meta-analysis in order to clarify the effect of CTLA-4 genotype on the risk of asthma which only contained 8 studies. Therefore, we performed an updated meta-analysis of published studies to pool results between studies [11].

Materials and methods

Literature search strategy

Electronic databases-PubMed, Chinese biomedical database (CBM), and Chinese national knowledge infrastructure (CNKI) were searched up to December 2014 for all genetic association studies evaluating CTLA-4 exon-1 +49A/G polymorphism and asthma in humans. The following keywords and subjects terms were used: "asthma or asthmatic" and "CTLA-4 or Cytotoxic T lymphocyte associated antigen 4" and "polymorphism or variant". The language of publication was only in English and Chinese. In addition, all references cited were reviewed to identify additional studies.

Inclusion criteria and data extraction

We reviewed the titles and abstracts of all citations and retrieved literature. The following criteria were used to include published studies: (1) use a case-control design; (2) have available genotype frequency and present sufficient data to estimate an odds ratio (OR); and (3) the genotype distribution of control population must be in Hardy-Weinberg equilibrium (HWE).

The following information was extracted from study: first author, year of publication, original country, ethnicity, age, atopic status, the numbers of patients and controls for the study, and genotyping information and frequencies of alleles. Authors of the included studies were contacted via Email when additional study data were needed.

Statistical analysis

The pooled Odds ratios (ORs) and 95% confidence interval (CI) were used to assess the strength of association between the CTLA-4 exon-1 +49A/G polymorphism and asthma.

Table 2. The distribution of the CTLA-4 exon-1 +49A/G gene polymorphism for cases and controls

Author (reference)	Year	Sample size (Case/Con)	Distribution of genotype			HWE *P*
			GG	GA	AA	
			Case/Con	Case/Con	Case/Con	
Wang D [17]	2014	40/40	17/25	10/11	13/4	0.128
Zhang KC [15]	2012	26/30	12/7	10/13	4/10	0.495
Zhang LH [18]	2010	118/160	51/88	57/65	10/7	0.242
Undarmaa S [16]	2010a	325/336	123/138	153/155	49/43	0.959
Undarmaa S [16]	2010b	367/676	134/247	175/323	58/106	0.981
Oh KY [1]	2010	742/238	369/115	321/107	61/16	0.178
Daley D [19]	2009	616/727	88/98	290/338	238/291	0.992
Chan IH [20]	2008	272/171	113/75	119/75	40/21	0.737
Sohn MH [21]	2007	326/254	156/132	125/103	45/19	0.859
Jasek M [22]	2006	219/102	52/21	101/48	66/33	0.645
Schubert K [23]	2006	231/270	28/38	105/127	98/105	0.968
Howard TD [24]	2002	177/134	19/23	82/72	76/39	0.297
Lee YS [25]	2002	88/86	49/49	24/29	15/8	0.238
Hizawa N [26]	2001	339/305	121/125	178/140	40/40	0.935
Nakao F [27]	2000	120/200	41/61	52/107	27/32	0.189

Case: asthma; Con: Control.

Table 3. Summary ORs and 95% CI of CTLA-4 exon-1 +49A/G polymorphism and asthma

Populations (study no.)	OR (95% CI)	P_{OR}	I^2 (%)	P_H
Overall [15]	0.76 (0.62-0.93)[b]	*0.008*	60.1	0.001
Caucasian [4]	0.71 (0.51-0.99)[b]	*0.04*	68.6	0.02
Asian [11]	0.79 (0.62-1.01)[b]	*0.06*	48.9	0.03
Children [9]	0.75 (0.62-0.90)	0.002	27.5	0.20
Adult [4]	0.83 (0.56-1.24)[b]	0.36	68.0	0.02
Atopic [7]	0.81 (0.67-0.98)	0.03	27.7	0.22
Non-atopic [3]	0.69 (0.40-1.18)	0.18	0.0	0.95

I^2 (%) and P_H value for heterogeneity; [b]Estimates for random effects model. P_{OR}: The *P*-value of OR determined by the Z-test.

Statistical heterogeneity among studies was assessed with the Q and I^2 statistics [12] I^2 values of 25%, 50% and 75% were assigned as low, moderate, and high estimates, respectively. Heterogeneity was considered significant for $P < 0.10$. The random-effects model (if $P < 0.10$) or the fixed-effects model (if $P \geq 0.10$) was used to summarize the combined OR. Deviation from Hardy-Weinberg-equilibrium (HWE) ($P < 0.05$) among the control groups within each study was checked by exact test using an online HWE calculator (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). Publication bias was examined visually in a funnel plot of log OR against its standard error. Publication bias was checked by the Egger's regression test. *P* value < 0.1 indicated evidence of potential publication bias [13].

Analyses were performed using the software Stata version 10 (StataCorp LP, College Station, Texas, USA), Review Manager 4.2 (Cochrane Collaboration, http://www.cc-ims.net/RevMan/relnotes.htm). *P* value less than 0.05 was considered statistically significant.

Results

Study selection and subject characteristics

Through literature search and selection based on the inclusion criteria, a total of 15 relevant studies concerning CTLA-4 exon-1 +49A/G polymorphism and asthma were initially identified. Among the 15 eligible articles, an overlapping case–control study in two publications [14, 15], we selected the one study for the meta-analysis [15]. Additionally, one article provided data on two samples and each subpopulation in the article was treated as a separate study in our meta-analysis [16]. Finally, a total of 15 case-control studies in 14 articles were identified, including 4006 cases and 3729 controls [1, 15-27]. These 15 studies encompass 4 Caucasians and 11 Asians. 4 studies were performed in adults and 9 studies in children. One

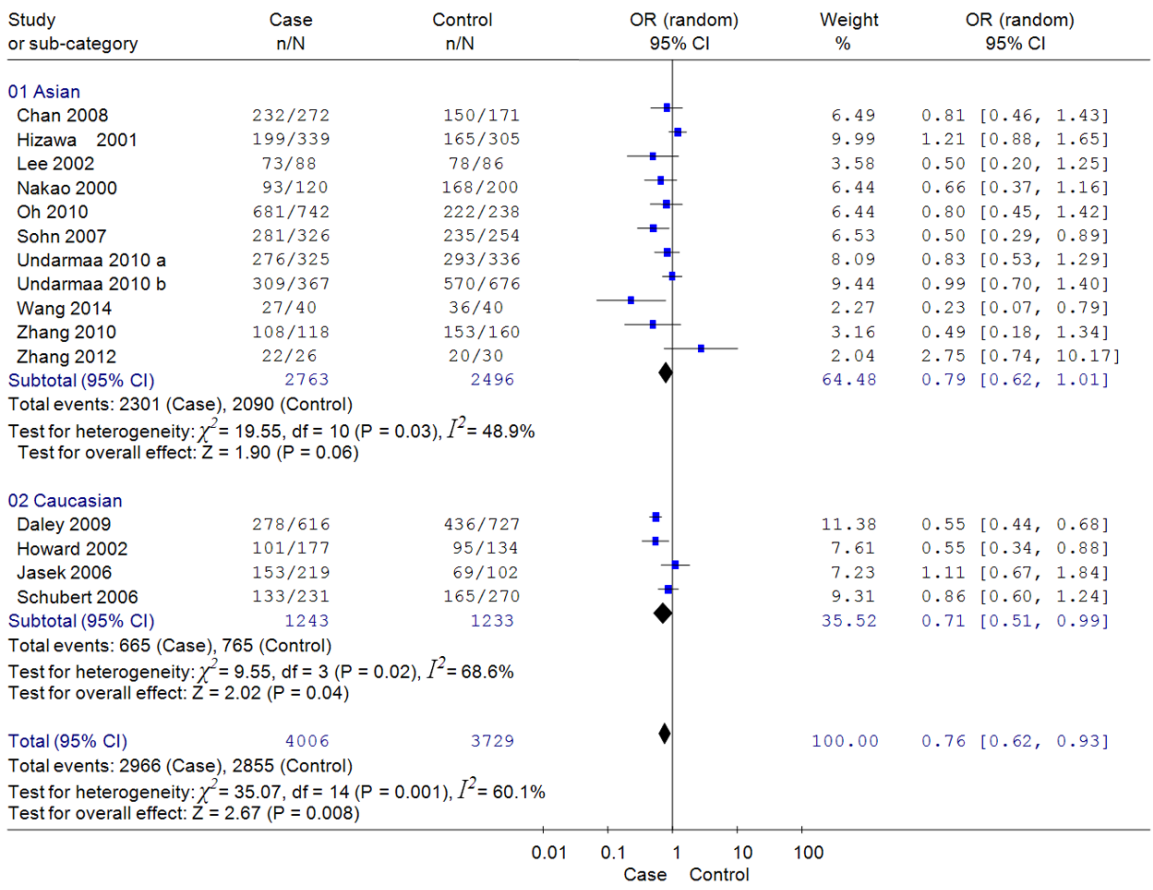

Figure 1. Meta-analysis with a random-effects model for the association between asthma risk and the CTLA-4 +49A/G polymorphism (GG + AG vs. AA). OR estimate for each study is marked with a solid black square. Size of the square represents the weight that the corresponding study exerts in the meta-analysis. CIs of the pooled estimates are displayed as a horizontal line through the diamond; this entire line may be inside the diamond if the CI is narrow.

study included both adults and juveniles. One study did not offer detailed information. 4 studies included only atopic asthma patients. 3 studies included both atopic and non-atopic asthma patients but the data for these patients could be separately extracted. 8 studies did not offer detailed information. Selected characteristics of the studies and the numbers of cases and controls genotypes in are shown in **Tables 1** and **2**. **Table 3** shows summary of ORs and 95% CI of CTLA-4 exon-1 +49A/G polymorphism and asthma.

Meta-analysis results and sensitivity analysis

Heterogeneity was observed among individual estimates of the ORs (I^2 = 60.1%; P = 0.001) and the original data were combined by means of random effects model. The main results of this meta-analysis were shown in the forest plot (**Figure 1**). The summary OR was 0.76 (95% CI: 0.62-0.93; P = 0.008) by the random effects model and 0.75 (95% CI: 0.67-0.84; P < 0.00001) by the fixed effects model. It was indicated that CTLA-4 +49A/G polymorphism resulted in an increased susceptibility to asthma on a worldwide population. In the subgroup analysis by ethnicity, significant risks were also found in Caucasian population for GG + GA versus AA (OR = 0.71; 95% CI: 0.51-0.99; P = 0.04) under the random effects model. Stratifying subjects by age indicated an association between CTLA-4 +49 GG + GA genotype and asthma in children (OR = 0.75; 95% CI: 0.62-0.90; P = 0.002), but no association in adults (OR = 0.93; 95% CI: 0.76-1.14; P = 0.48). Furthermore, significant association was observed in atopic asthma under the fixed-effects model (GG + GA vs. AA: P = 0.03, OR = 0.81, 95% CI = 0.67-0.98, $P_{\text{heterogeneity}}$ = 0.22).

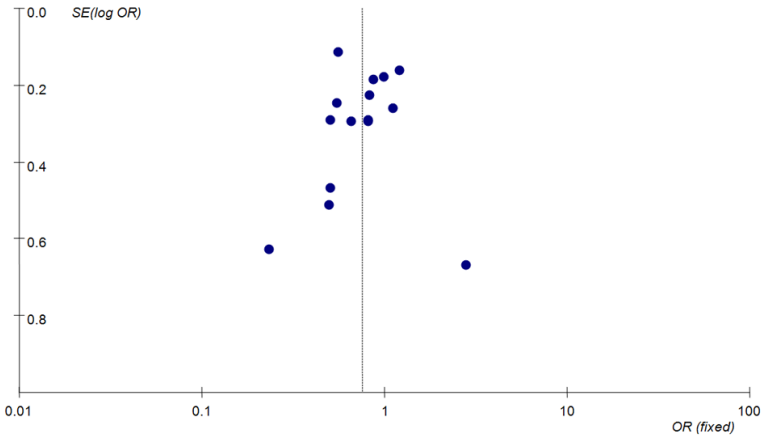

Figure 2. Funnel plot for publication bias in selection of studies on the CTLA-4 +49A/G polymorphism (GG + AG vs. AA).

A single study involved in the meta-analysis was deleted each time to reflect the influence of the individual data set on the pooled ORs, and the corresponding pooled ORs were not materially altered (data not shown).

Publication bias

As showed in **Figure 2**, the shape of the funnel plot did not reveal obvious asymmetry. Then, the Egger's test was used to provide statistical evidence of funnel plot symmetry. The results still did not suggest any evidence of publication bias (data not shown).

Discussion

In 2012, in order to clarify the effect of CTLA-4 exon-1 +49 polymorphism on the risk of developing asthma, Lee et al. and Nie et al. performed a meta-analysis respectively [11, 28]. And two studies reported conflicting results. After that, an expanding body of literatures in Caucasian population and Asian population provided conflict results. In this meta-analysis, a total of 4006 asthma cases and 3729 controls were used to assess the relationship between CTLA-4 exon-1 +49A/G polymorphism and asthma risk.

Based on the present meta-analysis, the overall data showed that CTLA-4 exon-1 +49 polymorphism may be an asthma susceptibility gene across populations. In the subgroup meta-analysis, there was an association between CTLA-4 exon-1 +49 polymorphism and asthma in Caucasian populations, but not in Asian populations, suggesting a possible influence among environmental exposures and different genetic backgrounds. It may arise from many aspects that the same polymorphism plays a different role in different ethnic populations or across different studies. Firstly, asthma is a complex disease and genetic heterogeneity exists in different populations. Secondly, the potential contribution of differences in patient populations (e.g. age and years from onset, disease severity, female proportion and so on) may lead to different results. Sometimes, we can only find association in stratification analysis according to the clinical character. Stratifying subjects by age indicated an association between CTLA-4 +49 GG + GA genotype and asthma in children (P = 0.002), but no association in adults (P = 0.48). Subgroup analysis was also performed among atopic status patients. Significant increased risk of asthma was found in patients with atopic status (P = 0.03, OR = 0.81, 95% CI = 0.67-0.98), suggesting a possible role of CTLA-4 exon-1 +49 polymorphism in the etiology of allergic asthma.

The benefit of meta-analysis is to combine comparable studies, to increase the sample size and statistical power and draw a more compelling result. To some extent, some limitations have affected the objectivity of the conclusions and should be considered when interpreting the results. First, given that only published studies were included in the meta-analysis, a publication bias may have occurred, even though it was not found when performing the statistical test. Second, a language bias may have occurred because this meta-analysis only contained Chinese and English language literature. However, some relevant articles in other languages were published in specific journals but were not found in the international journals.

In conclusion, our meta-analysis results suggest that CTLA-4 exon-1 +49A/G polymorphism might be a risk factor for asthma susceptibility, at least in Caucasian, children, and patients with atopy status.

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Disclosure of conflict of interest

None.

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