Cytotoxic T-Lymphocyte Associated Antigen 4 Polymorphisms and Asthma Risk: A Meta-Analysis

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Abstract

Background: A number of studies assessed the association of cytotoxic T-lymphocyte associated antigen 4 (CTLA-4) gene polymorphisms with asthma in different populations. However, the results were contradictory. We performed a meta-analysis to examine the association between CTLA-4 polymorphisms and asthma susceptibility.

Methods: Pubmed, EMBASE, HuGE Navigator, and Wanfang Database were searched. Data were extracted independently by two reviewers. Odds ratios (ORs) with 95% confidence intervals (CIs) were used to assess the strength of associations.

Results: Seventeen studies involving 6378 cases and 8674 controls were included. Significant association between +49 A/G polymorphism and asthma was observed for AA vs. AG=GG (OR = 1.18, 95% CI 1.01–1.37, P = 0.04). There were no significant associations between –318 C/T, –1147 C/T, CT60 A/G, –1722 C/T, or rs926169 polymorphisms and asthma risk.

Conclusions: This meta-analysis suggested that the +49 A/G polymorphism in CTLA-4 was a risk factor for asthma.

Citation: Nie W, Chen J, Xiu Q (2012) Cytotoxic T-Lymphocyte Associated Antigen 4 Polymorphisms and Asthma Risk: A Meta-Analysis. PLoS ONE 7(7): e42062. doi:10.1371/journal.pone.0042062

Editor: Susanne Krauss-Etschmann, Ludwig-Maximilians-University Munich, Germany

Received December 25, 2011; Accepted July 2, 2012; Published July 26, 2012

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Funding: This study was supported by grants NO. 81170025 from National Natural Science Foundation of China and projects of “Major New Drugs Innovation and Development” from the National Ministry of Science and Technology (NO. 2011ZX09302-003-001). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Asthma is a major public health problem worldwide. The disease affects over 300 million people [1]. In developed countries, the prevalence of asthma has increased considerably over the past three decades [2]. Asthma is a complex inflammatory disorder that results from interactions between more than 100 susceptibility genes and multiple environmental factors [3,4]. It is, therefore, important to identify the gene variants contributing to asthma pathogenesis. Numerous studies have focused on this field, and the cytotoxic T-lymphocyte associated antigen 4 (CTLA-4) gene has been extensively studied.

CTLA-4, a B7-hinding protein, was initially described as a classical type I glycoprotein on the surface of activated T cells [5]. Cumulative evidence suggested that CTLA-4 may play an important role in the pathogenesis of asthma. CTLA-4 is a powerful negative regulator of T cell activation and is associated with Th cell differentiation. Oosterwegel et al. [6] demonstrated that CTLA-4 is a potent and critical inhibitor of Th2 cell differentiation. Expression of CTLA-4 in Th2 cells was much higher than in Th1 cells [7]. CTLA-4 was also demonstrated to suppress the production of cytokines produced by Th2 cells [7]. A number of studies showed that administration of CTLA-4-Ig significantly ameliorated airway hyperresponsiveness (AHR), reduced the level of eosinophils in average bronchoalveolar lavage fluid and serum IgE, as well as cytokine production in murine asthma model [8–11]. Recently, Choi et al. [12] reported that intranasal administration of Hph-1-ctCTLA-4 could significantly reduce infiltration of inflammatory cells, secretion of Th2 cytokines, serum IgE levels and AHR in a mouse model of allergic airway inflammation. Lin and co-workers demonstrated that decreased allergic inflammation by surfactant protein D was mediated by an increased expression of CTLA-4 in T cells [13].

The human CTLA-4 gene is located on chromosome 2q33.2 [14]. Several single nucleotide polymorphisms (SNPs) of the CTLA-4 gene have been identified. Some of these studies have demonstrated a significant association of CTLA-4 polymorphisms with atopy or asthma [15–17]. However, the results were not consistent in other studies [18,19].

Considering a single study may lack the power of providing a reliable conclusion, we performed a meta-analysis to investigate the relationship between CTLA-4 gene variants and asthma. To our knowledge, this is the first meta-analysis of the association between CTLA-4 polymorphisms and asthma susceptibility.

Methods

Publication search

Pubmed, EMBASE, HuGE Navigator, and Wanfang Database were searched (Last search was updated on March, 2012). The following MeSH terms were used in Pubmed: “asthma” and “polymorphism, genetic” and “CTLA 4 antigen”. The search terms used in EMBASE and Wanfang Database were as follows:
Inclusion and exclusion criteria

Studies fulfilled the following criteria were included in this meta-analysis: (1) asthma diagnosed by a physician or according to asthma guidelines, (2) evaluation of the polymorphisms in CTLA-4 gene and asthma risk performed, (3) using a case-control design, (4) genotype distributions in both asthma cases and controls should be available for estimating an odds ratio (OR) with 95% confidence interval (CI). Studies were excluded if one of the following existed: (1) not clinical studies, and (2) reviews and abstracts. For overlapping studies, only the one with the largest sample size was included. There was no language restriction.

Qualitative assessment

Two authors independently assessed the quality of each study. Any disagreement was resolved by consensus. Quality assessment scores of molecular association studies of asthma were used to assess the quality of selected articles [20]. This quality scoring system was based on both traditional epidemiologic considerations and genetic issues. Total scores ranged from 0 (worst) to 15 (best). Studies with quality scores ≤ 4 were defined as low quality studies [21].

Data extraction

Two investigators (Nie and Chen) independently reviewed full manuscripts of eligible studies, and the relevant data were extracted into predesigned data collection forms. We verified accuracy of data by comparing collection forms from each investigator. Any discrepancy was resolved by discussion or a third author (Xiu) would assess these articles. The following data were collected from each study: first author’s name, year of publication, original country, ethnicity, age, atopic status, sample size, asthma and atopy definition, genotyping method, the polymorphisms in CTLA-4 gene, and genotype number in cases and controls. Authors of the included studies were contacted via E-mail when additional study data were needed.

Statistical analysis

When the data from at least 2 similar studies were available, meta-analysis was performed. ORs and 95% CIs were employed to assess the strength of association between SNPs in +49 A/G, −318 C/T, −1147 C/T, CT60 A/G, −1722 C/T, rs926169 and asthma risk. OR1, OR2, and OR3 were calculated for the genotypes 1) AA vs. GG (OR1), AG vs. GG (OR2), and AA vs. AG (OR3) for the +49 A/G and CT60 A/G polymorphisms, 2) CC vs. TT (OR1), CT vs. TT (OR2), and CC vs. CT (OR3) for the −318 C/T, −1147 C/T, and −1722 C/T polymorphisms, and 3) AA vs. CC (OR1), AC vs. CC (OR2), and AA vs. AC (OR3) for the rs926169, respectively. These pairwise differences were used to indicate the most appropriate genetic model as following: if OR1 = OR3 ≠ 1 and OR2 = 1, then a recessive model was suggested; if OR1 = OR2 ≠ 1 and OR3 = 1, then a dominant model was suggested; if OR2 = 1/OR3 ≠ 1 and OR1 = 1, then a complete overdominant model was suggested; if OR1 > OR2 > 1 and OR1 > OR3 > 1 (or OR1 < OR2 < 1 and OR1 < OR3 < 1), then a codominant model was suggested [22]. Once the best genetic model was identified, this model was used to collapse the three genotypes into two groups (except in the case of a codominant model) and to pool the results again. A random-effects model, using the Mantel-Haenszel method, was used to calculate the

Figure 1. Flow of study identification, inclusion, and exclusion.

doi:10.1371/journal.pone.0042062.g001
Table 1. Characteristics of the case-control studies included in meta-analysis.

| First authors/Year | Country | Ethnicity | Age group | Atopic status | Case (n) | Control (n) | Asthma definition | Atopy definition | Quality score | Genotyping method | CTLA-4 polymorphisms |
|---------------------|---------|-----------|-----------|--------------|----------|-------------|------------------|-----------------|--------------|-------------------|----------------------|
| Nakao [27] 2000     | Japan   | Asian     | Children  | Atopic       | 120      | 200         | Physician’s diagnosed | Total IgE, RAST | 5            | PCR-RFLP          | +49 A/G, −318 C/T    |
| Hizawa [15] 2001    | Japan   | Asian     | Adults    | NA           | 339      | 305         | ATS diagnosis criteria | RAST            | 12           | PCR-RFLP          | +49 A/G, −318 C/T    |
| Howard [16] 2002    | Netherlands | Caucasian | Adults   | NA           | 200      | 201         | A algorithm based on SPT, total IgE | −1147 C/T, −658 C/T, ATS diagnosis criteria | −318 C/T, +49 A/G |
| Lee [17] 2002      | Korea   | Asian     | Adults    | Mixed*       | 88       | 86          | ATS diagnosis criteria | SPT, total IgE  | 11           | PCR-RFLP          | +49 A/G, −318 C/T    |
| Schubert [31] 2006 | Germany | Caucasian | Children  | NA           | 235      | 270         | Physician’s diagnosed  | NA              | 9            | PCR-RFLP          | +49 A/G, −318 C/T    |
| Jasek [28] 2006     | Poland  | Caucasian | Adults/   | Atopic/juveniles | 219      | 102         | NHLBI/WHO guideline SPT, total IgE | −1147 C/T, +49 A/G, 318 C/T | 11           | PCR-RFLP          | −318 C/T            |
| Qian [32] 2007      | China   | Asian     | Children  | NA           | 90       | 100         | Chinese asthma diagnosis | Total IgE        | 7            | PCR-RFLP          | −318 C/T            |
| Sohn [25] 2007      | Korea   | Asian     | Children  | Mixed*       | 326      | 254         | Physician’s diagnosed | SPT, total IgE  | 11           | PCR-RFLP          | +49 A/G, −318 C/T    |
| Chan [29] 2008      | China   | Asian     | Adults    | NA           | 298      | 175         | ATS diagnosis criteria | Allergen-specific IgE | 9            | PCR-RFLP          | −1147 C/T, +49 A/G, 301147 C/T, +49 A/G, 318 C/T |
| Daley [33] 2009     | Australia | Caucasian | Adults/   | NA           | 644      | 751         | A positive answer to the question: “Has a doctor ever told you that you had asthma?” | SPT             | 9            | Illumina          | +49 A/G, −318 C/T    |
| Berce [30] 2010     | Slovenia | Caucasian | Children  | Mixed*       | 102      | 84          | ATS diagnosis criteria | Allergen-specific IgE, SPT | 7            | PCR-RFLP          | −1147 C/T, CT60 A/G  |
| Oh [26] 2010        | Korea   | Asian     | Children  | Mixed*       | 742      | 238         | ATS diagnosis criteria | Allergen-specific IgE, SPT | 11           | PCR-RFLP          | +49 A/G            |
| Undarmaa 1 [34] 2010| Japan   | Asian     | Children  | Atopic       | 325      | 336         | NIH criteria SPT       | TaqMan-ASA      | 9            | TaqMan-ASA        | +49 A/G, −318 C/T    |
| Undarmaa 2 [34] 2010| Japan   | Asian     | Adults    | Atopic       | 367      | 676         | ATS diagnosis criteria | SPT             | 9            | TaqMan-ASA        | +49 A/G, −318 C/T    |
| DeWan [35] 2010     | USA     | Mixed     | Children  | Atopic       | 66       | 42          | Physician’s diagnosed  | NA              | 11           | Affymetrix        | −318 C/T, −1147 C/T, CT60 A/G |
| Sleiman [36] 2010   | USA     | Caucasian | Children  | NA           | 793      | 1998        | Physician’s diagnosed  | NA              | 12           | Illumina HH550 BeadChip | −1722 C/T, rs926169, rs231731 |
| Noguchi [37] 2011   | Japan   | Asian     | Children  | Mixed        | 938      | 2376        | Physician’s diagnosed on the basis of the NIH criteria | Allergen-specific IgE | 12           | Illumina HumanHap550v3 | −1722 C/T, rs926169 |
| Anantharaman [38] 2011| Singapore | Asian     | Adults    | Atopic       | 490      | 490         | A positive answer to the question: “Have you ever had asthma?” and a doctor’s diagnosis | SPT             | 12           | Illumina BeadXpress platform and Sequenom platform | −318 C/T, −1147 C/T, CT60 A/G |

*Data for atopic or non-atopic asthma patients could be separately extracted.

ATS, American Thoracic Society; NHLBI, The National Heart, Lung, and Blood Institute; WHO, The World Health Organization; NIH, National Institutes of Health; SPT, skin prick test to common aeroallergens; RAST, radioallergosorbent test; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; TaqMan-ASA, TaqMan allele-specific amplification method; NA, not available.

doi:10.1371/journal.pone.0042062.t001
Table 2. Distribution of CTLA-4 genotype among patients and controls included in the meta-analysis.

| Studies         | Asthma       | Control     | HWE (P value) |
|-----------------|--------------|-------------|---------------|
|                 | 11<sup>a</sup> | 12<sup>b</sup> | 22<sup>c</sup> | 11 | 12 | 22 |
| +49 A/G         |              |             |               |    |    |    |
| Nakao           | 27           | 52          | 41            | 32 | 107 | 61 | 0.189 |
| Hizawa          | 40           | 178         | 121           | 40 | 140 | 125 | 0.935 |
| Howard          | 76           | 82          | 19            | 39 | 72  | 23  | 0.297 |
| Lee             | 15           | 24          | 49            | 8  | 29  | 49  | 0.238 |
| Schubert        | 98           | 105         | 28            | 105| 127 | 38  | 0.968 |
| Jasek           | 66           | 101         | 52            | 33 | 48  | 21  | 0.645 |
| Sohn            | 45           | 125         | 156           | 19 | 103 | 132 | 0.859 |
| Chan            | 40           | 119         | 113           | 21 | 75  | 75  | 0.737 |
| Daley           | 238          | 290         | 88            | 291| 338 | 98  | 0.992 |
| Oh              | 61           | 312         | 369           | 16 | 107 | 115 | 0.178 |
| Undarmaa 1      | 49           | 153         | 123           | 43 | 155 | 138 | 0.959 |
| Undarmaa 2      | 58           | 175         | 134           | 106| 323 | 247 | 0.981 |
|                   |              |             |               |    |    |    |
| -318 C/T        |              |             |               |    |    |    |
| Nakao           | 97           | 19          | 4             | 157| 43  | 0   | 0.088 |
| Hizawa          | 265          | 71          | 3             | 238| 65  | 2   | 0.278 |
| Howard          | 144          | 30          | 2             | 115| 14  | 2   | 0.059 |
| Lee             | 70           | 16          | 2             | 67 | 15  | 4   | 0.022 |
| Jasek           | 172          | 44          | 3             | 79 | 22  | 1   | 0.694 |
| Schubert        | 181          | 47          | 3             | 214| 53  | 3   | 0.889 |
| Qian            | 75           | 13          | 2             | 84 | 15  | 1   | 0.721 |
| Sohn            | 247          | 77          | 2             | 199| 54  | 1   | 0.182 |
| Daley           | 537          | 100         | 5             | 616| 128 | 7   | 0.902 |
| Undarmaa 1      | 253          | 67          | 5             | 263| 68  | 5   | 0.801 |
| Undarmaa 2      | 284          | 78          | 5             | 512| 153 | 11  | 0.911 |
| DeWan           | 58           | 7           | 1             | 35 | 6   | 1   | 0.267 |
| Anantharaman    | 350          | 128         | 12            | 343| 134 | 13  | 0.799 |
|                   |              |             |               |    |    |    |
| -1147 C/T       |              |             |               |    |    |    |
| Howard          | 108          | 46          | 2             | 97 | 18  | 2   | 0.295 |
| Jasek           | 146          | 65          | 8             | 66 | 31  | 5   | 0.587 |
| Chan            | 216          | 68          | 7             | 129| 43  | 3   | 0.787 |
| Daley           | 445          | 181         | 18            | 500| 226 | 25  | 0.931 |
| DeWan           | 52           | 13          | 1             | 29 | 12  | 1   | 0.853 |
| Anantharaman    | 335          | 140         | 15            | 307| 162 | 21  | 0.949 |
|                   |              |             |               |    |    |    |
| CT60 A/G        |              |             |               |    |    |    |
| Chan            | 13           | 97          | 183           | 6  | 58  | 109 | 0.611 |
| Berce           | 14           | 62          | 26            | 21 | 34  | 29  | 0.093 |
| Daley           | 191          | 317         | 132           | 229| 369 | 148 | 0.977 |
| DeWan           | 8            | 31          | 27            | 4  | 19  | 19  | 0.810 |
| Anantharaman    | 36           | 193         | 261           | 21 | 161 | 308 | 0.994 |
|                   |              |             |               |    |    |    |
| -1722 C/T       |              |             |               |    |    |    |
| Daley           | 551          | 89          | 4             | 644| 102 | 4   | 0.986 |
| Sleiman         | 663          | 127         | 6             | 1666|308 | 14  | 0.954 |
| Noguchi         | 340          | 463         | 135           | 862| 1131| 391 | 0.537 |
| rs926169 A/C    |              |             |               |    |    |    |
| Daley           | 236          | 307         | 100           | 282| 356 | 111 | 0.937 |
| Sleiman         | 284          | 382         | 127           | 743| 945 | 300 | 0.987 |
pooled ORs. The statistical significance of OR was determined with $Z$ test.

Departure from Hardy-Weinberg equilibrium (HWE) in controls was tested by the chi-square test. The $Q$ statistic and the $I^2$ statistic were used to test for heterogeneity among the studies included in the meta-analysis. Sensitivity analyses were performed by including studies not in HWE. In addition, sensitivity analyses were also done by ethnicity and atopic status. Graphic exploration with funnel plots was used to evaluate the publication bias visually. The Begg's test and the Egger's test were used to assess publication bias statistically [23,24].

All statistical tests were performed by using the Revman 5.1 software (Nordic Cochrane Center, Copenhagen, Denmark), STATA 11.0 software (Stata Corporation, College Station, TX), and SPSS 18.0 software (Chicago, IL, USA). A $P$ value of 0.05 was considered statistically significant, except for tests of heterogeneity where a level of 0.10 was used.

**Results**

**Literature search and study characteristics**

Figure 1 outlines our selection process. Briefly, a total of 93 articles were identified after an initial search. After removing duplications, 32 articles were excluded. After reviewing the titles and abstracts, 35 articles were excluded because of abstracts, reviews, not clinical studies, or irrelevance of asthma risk. After reviewing full texts of the remaining 26 articles, 9 articles were further excluded. One article reported two cohorts [34], and each cohort was considered as a separate case-control study. Finally, a total of 18 case-control studies in 17 articles were identified [15-17,25-38], including 6378 cases and 8674 controls. There were 11 studies on $+49\ A/G$, 12 studies on $-318\ C/T$, 5 studies on $CT60 A/G$, 3 studies on $-1722\ C/T$ and rs926169. There were 10 studies of Asians [15,17,25-27,29,32,34,37,38] and 6 studies of Caucasians [16,28,30,31,33,36]. Five studies were performed in adults [15-17,25,26,38], 11 studies in children [25-27,29-34,37]. Two studies included both adults and juveniles [28,33]. Five studies included only atopic asthma patients [27,28,34,35,38]. Four studies included both atopic and non-atopic asthma patients but the data for these patients could be separately extracted [17,25,26,30]. Seven studies did not offer detailed information [15,16,29,31-33,36]. Asthma was defined with different criteria (physician’s diagnosis, ATS diagnosis criteria, NHLBI/WHO guideline, NIH criteria, and Chinese asthma diagnosis criteria for children). Atopy was defined based on total IgE in 6 studies [16,17,25,27,28,32], radioallergosorbent test (RAST) in 2 studies [15,27], skin prick test to common aeroallergens (SPT) in 9 studies [16,17,25,26,28,30,33,34,38], and allergen-specific IgE in 4 studies [26,29,30,37]. The quality scores ranged from 5 to 12, suggesting high quality. The characteristics of each study included in this meta-analysis are presented in Table 1. Genotype frequencies and HWE examination results are listed in Table 2.

**Table 2.** Cont.

| Studies   | Asthma | Control | HWE |
|-----------|--------|---------|-----|
|           | $11^a$ | $12^b$  | $22^c$ | $11$ | $12$ | $22$ | ($P$ value) |
| Noguchi   | 135    | 455     | 342   | 336  | 1110 | 938  | 0.793       |

$^a$AA or CC; $^b$AG, CT, or AC; $^c$GG, TT or CC. HWE, Hardy-Weinberg equilibrium. doi:10.1371/journal.pone.0042062.t002

Figure 2. Meta-analysis with a random-effects model for the association between asthma risk and the $CTLA-4\ +49\ A/G$ polymorphism (AA vs. AG+GG). doi:10.1371/journal.pone.0042062.g002
Quantitative data synthesis

The \textit{CTLA-4} \texttt{+49 A/G} polymorphism. Eleven studies determined the association between \texttt{+49 A/G} polymorphism and asthma [15–17,25–29,31,33,34]. Total sample sizes for asthma and control groups were 3822 and 3499, respectively. All studies in HWE were included in pooling. The estimated OR1, OR2 and OR3 were 1.18, 1.02, and 1.16, respectively (Table 3). These estimates suggested a recessive genetic model, therefore AG and GG were combined and compared with AA. The pooled OR was 1.18 (95% CI 1.01–1.37, \( P = 0.04 \)) (Figure 2). The exclusion of studies with Asians altered the significance of the result (OR = 1.12, 95% CI 0.85–1.48, \( P = 0.41 \)). However, the exclusion of studies with Caucasians did not change the result (OR = 1.21, 95% CI 1.01–1.46, \( P = 0.04 \)). Sensitivity analyses were also performed by atopic status. Borderline yet significant increase of asthma risk was found among the AA carriers of atopic asthma patients (OR 1.26, 95% CI 1.00–1.59, \( P = 0.05 \)). The funnel plot was slightly asymmetrical (Figure 3). Begg’s test and Egger’s test indicated significant publication bias (\( P = 0.011 \) and \( P = 0.049 \), respectively).

The \textit{CTLA-4} \texttt{2318 C/T} polymorphism. Twelve case-control studies identified an association between \texttt{2318 C/T} polymorphism and asthma risk [15–17,25,27,28,31–35,38]. Total sample sizes for asthma and control groups were 3391 and 3657, respectively. All studies in HWE except one study [17] were included in pooling. The estimated OR1, OR2 and OR3 were 0.99, 0.99, and 1.03, respectively (Table 3). These estimates suggested a dominant genetic model, therefore CT and CC were combined and compared with TT. The pooled OR was 0.96 (95% CI 0.63–1.47, \( P = 0.86 \)) (Figure 4). Sensitivity analysis was performed by including the study [17] that did not observe HWE. The results were similar in showing no genetic effect (OR = 1.01, 95% CI 0.67–1.52, \( P = 0.98 \)). Furthermore, no statistically significant results were found in sensitivity analyses conducted by ethnicity and atopic status (Table 3). The funnel plot was slightly asymmetrical (Figure 5). Begg’s test and Egger’s test indicated significant publication bias (\( P = 0.011 \) and \( P = 0.049 \), respectively).

The \textit{CTLA-4} \texttt{21147 C/T}, \texttt{CT60 A/G}, \texttt{21722 C/T}, and rs926169 polymorphisms. Six studies studied the association between \texttt{21147 C/T} polymorphism and asthma risk [16,28,29,33,35,38]. Total sample sizes for asthma and control groups were 1866 and 1677, respectively. The estimated OR1, OR2 and OR3 were 1.29, 1.16 and 1.04, respectively (Table 3). These estimates suggested a codominant genetic model. The pooled OR was 1.29 (95% CI 0.87–1.91, \( P = 0.21 \)) and 1.16 (95% CI 0.77–1.73, \( P = 0.48 \)). Only 5 studies and 3 studies were eligible for meta-analysis on \texttt{CT60 A/G}, \texttt{21722 C/T}, and rs926169 polymorphisms. Dominant genetic models were chosen based on the estimated OR1, OR2 and OR3 of these three polymorphisms. Results from our meta-analysis demonstrated that CT60 A/G, \texttt{21722 C/T}, and rs926169 polymorphisms were not risk factors for asthma. Summary of comparisons are listed in Table 3.

Discussion

This meta-analysis of 17 case-control studies including 6378 cases and 8674 controls systematically evaluated the association between \texttt{+49 A/G}, \texttt{2318 C/T}, \texttt{21147 C/T}, CT60 A/G, \texttt{21722 C/T}, and rs926169 polymorphisms in the \textit{CTLA-4} gene and asthma risk. We found that \texttt{+49 A/G} polymorphism was a modest risk factor for developing asthma in the overall study population. The results revealed that carriers of the AA homozygote had 18% increased asthma risk compared to those individuals with the G allele carriers (AG+GG). In the sensitivity analysis, we found that individuals carrying AA homozygote had increased asthma risk in Asians, but not in Caucasians. These results suggested that interactions between different ethnicities and genetic variants may contribute to asthma risk. However, there were only 4 studies on Caucasians for this polymorphism [16,28,31,33]. It is therefore possible that the observed ethnic difference was due to chance. More studies with Caucasian population are required to validate the effect of ethnic differences on asthma risk through the \texttt{+49 A/G} polymorphism.

Figure 3. Funnel plot for publication bias in selection of studies on the \textit{CTLA-4} \texttt{+49 A/G} polymorphism (AA vs. AG+GG).
doi:10.1371/journal.pone.0042062.g003
G polymorphism. In addition, significant heterogeneity was observed in the Caucasians subgroup ($I^2 = 56\%$) but not in the Asians subgroup ($I^2 = 7\%$). Furthermore, asthma is a complex disease. Both genetic and environmental factors affect the risk of asthma in different populations. It is possible that different asthma risks in Asians and Caucasians were due to exposure to various environmental factors. However, no reported article was performed to assess the effect of environment-CTLA-4 interactions in different ethnicities. In the future, more studies should be designed to analyze these associations. We also carried out sensitivity analysis for atopic status. We found that atopic patients had increased asthma risk, suggesting a possibility of atopic status differences in asthma pathogenesis.

Ligers and co-workers showed that CTLA-4 cell-surface expression was significantly increased in individuals carrying the AA genotype, compared to levels in carriers of the AG and GG genotypes [39]. CTLA-4$^{+49 G}$ caused 17Ala$\rightarrow$Thr substitution in the leading peptide of CTLA-4 [40]. 17Thr substitution increased binding of CTLA-4 to B7.1, causing stronger inhibition on T cell activation then CTLA-417Ala [41]. In addition, T cells with $+49 GG$ genotype had higher activation and proliferation rates compared to those with $+49 AA$ genotype [41]. Recently, the G allele of the $+49 A/G$ polymorphism was reported to have a

| Polymorphisms | Study | Sample size | No. of studies | Test of association | Model | Heterogeneity |
|---------------|-------|-------------|----------------|--------------------|-------|---------------|
| +49 A/G       | AA vs. GG Overall 2108 1875 12 | 1.18 (1.00–1.40) | 1.98 0.05 R 12.78 0.31 14.0 |
|               | AG vs. GG Overall 3008 2746 12 | 1.02 (0.91–1.14) | 0.34 0.74 R 6.58 0.83 0.0 |
|               | AA vs. AG Overall 2529 2377 12 | 1.16 (0.99–1.36) | 1.89 0.06 R 14.76 0.19 25.0 |
|               | AA vs. AG+GG Overall 3822 3499 12 | 1.18 (1.01–1.37) | 2.07 0.04 R 15.42 0.16 29.0 |
|               | AA vs. AG+GG Asian 2579 2266 8 | 1.21 (1.01–1.46) | 2.01 0.04 R 7.52 0.38 7.0 |
|               | AA vs. AG+GG Caucasian 1243 1233 4 | 1.12 (0.85–1.48) | 0.83 0.41 R 6.77 0.08 56.0 |
|               | AA vs. AG+GG Atopic 1954 1892 7 | 1.26 (1.00–1.59) | 1.94 0.05 R 8.30 0.22 28.0 |
| -318 C/T      | CC vs. TT Overall 2710 2903 12 | 0.99 (0.65–1.51) | 0.04 0.97 R 4.62 0.95 0.0 |
|               | CT vs. TT Overall 699 798 12 | 0.99 (0.64–1.52) | 0.04 0.97 R 5.90 0.88 0.0 |
|               | CC vs. CT Overall 3344 3610 12 | 1.03 (0.92–1.16) | 0.52 0.61 R 5.38 0.91 0.0 |
|               | CC+CT vs. TT Overall 3391 3657 12 | 0.96 (0.63–1.47) | 0.18 0.86 R 4.64 0.95 0.0 |
|               | CC+CT vs. TT HWE 3479 3743 13 | 1.01 (0.67–1.52) | 0.03 0.98 R 5.38 0.94 0.0 |
|               | CC+CT vs. TT Asian 2057 2361 7 | 0.91 (0.55–1.51) | 0.36 0.72 R 4.16 0.66 0.0 |
|               | CC+CT vs. TT Caucasian 1268 1254 4 | 1.06 (0.48–2.34) | 0.14 0.89 R 0.29 0.96 0.0 |
|               | CC+CT vs. TT Atopic 1793 2058 6 | 0.97 (0.57–1.65) | 0.11 0.91 R 3.70 0.59 0.0 |
| -1147 C/T     | CC vs. TT Overall 1353 1185 6 | 1.29 (0.87–1.91) | 1.26 0.21 R 1.05 0.96 0.0 |
|               | CT vs. TT Overall 564 549 6 | 1.16 (0.77–1.73) | 0.70 0.48 R 1.21 0.94 0.0 |
|               | CC vs. CT Overall 1815 1620 6 | 1.04 (0.81–1.34) | 0.31 0.75 R 10.67 0.06 53.0 |
| CT60 A/G      | AA vs. GG Overall 891 894 5 | 1.18 (0.80–1.74) | 0.82 0.41 R 6.63 0.16 40.0 |
|               | AG vs. GG Overall 1329 1254 5 | 1.20 (0.94–1.52) | 1.45 0.15 R 7.01 0.14 43.0 |
|               | AA vs. AG Overall 962 922 5 | 0.95 (0.63–1.45) | 0.23 0.82 R 7.95 0.09 50.0 |
|               | AA+AG vs. GG Overall 1591 1535 5 | 1.19 (0.94–1.49) | 1.47 0.14 R 6.86 0.14 42.0 |
| -1722 C/T     | CC vs. TT Overall 1699 3581 3 | 1.12 (0.90–1.40) | 1.01 0.31 R 0.32 0.85 0.0 |
|               | CT vs. TT Overall 824 1950 3 | 1.17 (0.94–1.45) | 1.39 0.16 R 0.33 0.85 0.0 |
|               | CC vs. CT Overall 2233 4713 3 | 0.97 (0.86–1.09) | 0.54 0.59 R 0.01 0.99 0.0 |
|               | CC+CT vs. TT Overall 2378 5122 3 | 1.15 (0.93–1.41) | 1.32 0.19 R 0.37 0.83 0.0 |
| rs926169      | AA vs. CC Overall 1244 2710 3 | 0.99 (0.85–1.15) | 0.18 0.86 R 1.48 0.48 0.0 |
|               | AC vs. CC Overall 1713 3760 3 | 1.05 (0.93–1.19) | 0.74 0.46 R 1.61 0.45 0.0 |
|               | AA vs. AC Overall 1799 3772 3 | 0.96 (0.85–1.09) | 0.63 0.53 R 0.07 0.97 0.0 |
|               | AA+AC vs. CC Overall 2368 5121 3 | 1.03 (0.91–1.17) | 0.50 0.61 R 2.15 0.34 7.0 |

vs., versus; R, random-effects model.

doi:10.1371/journal.pone.0042062.t003
strong association with autoimmune diseases [42–44]. Considering the inverse relationship between allergic diseases (Th2 dominant) and autoimmune diseases (Th1 dominant), and the role of CTLA-4 polymorphisms in determining the Th1/Th2 balance [45], it is biologically plausible that the A allele of the +49 A/G polymorphism could increase the susceptibility of asthma. Our findings and a previous study by Yang et al. [46] supported this speculation. Furthermore, Jones et al. [47] indicated +49 A allele was associated with infant atopic dermatitis. However, how A allele of the +49 A/G polymorphism influences asthma risk is unclear. Park et al. [48] reported significantly lower serum sCTLA-4 levels in Behcet’s disease patients with the CTLA-4 +49 G allele than those in healthy controls. Serum sCTLA-4 concentrations also increased in patients with allergic asthma and after allergen inhalation in sensitized asthmatic subjects [49–52]. These data suggested that +49 A/G polymorphism could influence asthma susceptibility through affecting serum sCTLA-4 level.

Results from our meta-analysis showed the lack of associations between the −318 C/T, −1147 C/T, CT60 A/G, −1722 C/T, or rs926169 polymorphisms and asthma risk. However, these results should be interpreted with caution. Because −318 C/T was shown to be associated with asthma severity and may serve as a clinically useful marker of severe asthma [17]. More studies are required to assess the associations between −1147 C/T, CT60 A/G, −1722 C/T, or rs926169 polymorphisms and asthma risk.
since less than 6 case-control studies were included in this meta-analysis. A positive association between these polymorphisms and asthma could not be ruled out because studies with small sample size may have insufficient statistical power to detect a slight effect.

Publication bias and heterogeneity may influence the results of meta-analyses. In our meta-analysis, only studies indexed by the selected databases were included. Negative studies were less likely to be published in journals and be available in computerized database [33], resulting in potential overestimation of effect sizes. In this meta-analysis, Beggs’s test and Egger’s test showed significant publication bias, thus the current results should be interpreted cautiously. In addition, there was no significant heterogeneity in most of the overall comparisons for all 4 polymorphisms. Therefore, heterogeneity did not seem to have influenced the results, suggesting the reliability of our results.

Some limitations of this meta-analysis should be considered. First, the number of available studies that could be included was moderate. Therefore, the results could be influenced by factors like random error. Second, only 7 of the 17 studies were conducted in non-Asian population. Third, the overall outcomes were based on individual unadjusted ORs, while a more precise evaluation should be adjusted by other potentially suspected factors including age, sex and lifestyle. Finally, this study could not address gene and gene-environment interactions, due to insufficient information from the primary publication.

In conclusion, our meta-analysis suggested that the +49 A/G polymorphism in CTLA-4, but not the −318 C/T, −1147 C/T, CT60 A/G, −1722 C/T, or rs926169 polymorphisms, represented a risk factor for asthma. Future large-scale studies are still needed to validate our findings. Moreover, gene-gene and gene-environment interactions should also be considered in future studies.

Acknowledgments

We would like to acknowledge the helpful comments on this paper received from reviewers and Dr Susanne Kraus-Etchman. We thank Dr Yoichi Suzuki (Department of Public Health, Chiba University Graduate School of Medicine), Dr Andrew T. DeWan (Department of Epidemiology and Public Health, Yale University School of Public Health), Dr Hakon Hakonarson (Center for Applied Genomics, 1216E, Abramson Research Center), Dr Emiko Noguchi (Department of Medical Genetics, Graduate School of Comprehensive Human Sciences, University of Tsukuba), and Dr Fook Tim Chew (Department of Biological Sciences, National University of Singapore) for providing relevant information.

Author Contributions

Conceived and designed the experiments: WN JQC. Performed the experiments: WN JQC QYX. Analyzed the data: WN JQC QYX. Contributed reagents/materials/analysis tools: WN JQC. Wrote the paper: WN JQC QYX. Financial support: QYX. Final approval of manuscript: WN JQC QYX.

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