Beta-2 Adrenergic Receptor (ADRB2) Gene Polymorphisms and the Risk of Asthma: A Meta-Analysis of Case-Control Studies

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Abstract

Background and Objective: A number of studies have assessed the relationship between beta-2 adrenergic receptor (ADRB2) gene polymorphisms and asthma risk. However, the results are inconsistent. A meta-analysis that focused on the association between asthma and all ADRB2 polymorphisms with at least three case-control studies was thus performed.

Methods: A literature search of the PubMed, Embase, Web of Science, CNKI, and Wangfang databases was conducted. Odds ratios with 95% confidence intervals were used to assess the strength of associations.

Results: Arg16Gly, Gln27Glu, Thr164Ile, and Arg19Cys single nucleotide polymorphisms (SNPs) were identified in 46 case-control studies. The results showed that not all of the SNPs were associated with asthma in the overall population. Significant associations were found for the Arg16Gly polymorphism in the South American population via dominant model comparison (OR = 1.754, 95% CI = 1.179–2.609, I² = 16.9%, studies = 2, case = 314, control = 237) in an analysis stratified by ethnicity. For the Gln27Glu polymorphism, a protective association was found in children via recessive model comparison (OR = 0.566, 95% CI = 0.417–0.769, I² = 0.0%, studies = 11, case = 1693, control = 502) and homozygote genotype comparison (OR = 0.610, 95% CI = 0.434–0.856, I² = 0.0%, studies = 11, case = 1693, control = 1502), and in adults via dominant model comparison (OR = 0.864, 95% CI = 0.768–0.971, I² = 46.9%, n = 18, case = 3160, control = 3433).

Conclusions: None of the ADRB2 gene polymorphisms were reproducibly associated with a risk of asthma across ethnic groups in the general population.

Introduction

Asthma, which is characterized by variable airway obstruction caused by bronchial hyper-reactivity and airway inflammation, is one of the most common chronic respiratory diseases worldwide. The prevalence of asthma varies worldwide, ranging from 0.2% in China to 21.0% in Australia [1]. Recent studies show that asthma is a genetically related disease, with heritability estimates varying between 48% and 79% [2]. An increasing number of studies are focusing on asthma genetics research. Therefore, the identification of asthma susceptibility genes contributing to asthma pathogenesis is important. Candidate-gene linkage studies, positional cloning, and genome-wide association studies (GWAS) have already identified a large number of asthma susceptibility genes, and one of these, the beta-2 adrenergic receptor (ADRB2, also known as β2-AR) gene, has been extensively studied.

The β2-AR (ADRB2), a member of the G protein-coupled receptor (GPCR) family, is abundantly expressed on bronchial smooth muscle cells, and specifically binds and is activated by a class of ligands known as catecholamines, and epinephrine in particular [3]. The activation of β2-AR can result in the expansion of the small airways, and thus β2-AR agonists are used in first-line bronchodilator therapy in asthma [4]. The β2-AR, which can directly influence the effect of beta-2 adrenergic bronchodilator, is encoded by an intronless gene located on chromosome 5q31–32 [5]. It has been reported that ADRB2 variants are associated with airway hypersensitivity, asthma severity, and the response to medications [6,7]. Several single nucleotide polymorphisms (SNPs), including Arg16Gly (A46G, rs1042713), Gln27Glu (C79G, rs1042714), and Thr164Ile (C491T, rs1800888) have been identified in the coding region of the ADRB2 gene [8]. Replacement of the base may not only alter the gene expression and function of the β2-AR, it may also alter the response to β2-AR agonist therapies and even increase the risk of asthma.

To date, various case-control studies have been conducted to investigate the relationship between ADRB2 gene polymorphisms and asthma risk in different population groups [9–13], but the results have been conflicting and inconclusive. One reason for this inconsistency may be the typically small sample size of the individual studies, which may mean that there was insufficient
ADRB2 Polymorphisms and Asthma: A Meta-Analysis

Inclusion and exclusion criteria

Studies that fulfilled the following criteria were incorporated into the meta-analysis: (1) case-control studies that evaluated the association between ADRB2 gene polymorphisms and risk of asthma; (2) the genotype distributions or allele frequency of each study was available or sufficient data could be extracted for calculating the odds ratio (OR) with 95% confidence interval (CI). For overlapping studies, the one with the most suitable data was selected. Studies were only excluded if they did not meet these inclusion criteria.

Data extraction

The basic information extracted for each study was as follows: name of first author, publication year, country and ethnicity of case control, age of case, asthma definition, sample size, and genotype frequencies in cases and controls.

Statistical analysis

Pearson’s chi-square test was performed to evaluate whether the genotype distribution deviated from Hardy-Weinberg equilibrium (HWE) in the control group. Significantly deviating samples were re-assessed by 1000 time Montecarlo permutation analysis using the freely available software at http://krunch.med.yale.edu/hwsim. The OR with 95% CI was used to assess the strength of the association between ADRB2 polymorphism and asthma risk. The pooled OR for ADRB2 polymorphisms and asthma risk was performed for four genetic model comparisons (dominant model comparison [AA+Aa vs. aa], recessive model comparison [AA vs. Aa+aa], homozygote genotype comparison [AA vs. aa] and allele comparison [A vs. a]) to estimate the risk. In the current study, the aa genotype was a wild-type, while the AA genotype was a mutant. The Q-test and F² test were used to assess the effect of heterogeneity. Heterogeneity was considered statistically significant when Q-test (P<0.10) or F²>50%. If heterogeneity was indicated, data were combined according to the random-effects model; when the Q-test (P>0.10) or F²<50%, the fixed-effect model was used. Stratified analysis was performed by 1000 time permutation HWE P-value, ethnicity and case age to further explore HWE-specific, ethnicity-specific and age-specific effects. Sensitivity analysis was conducted by sequentially excluding one study at a time to examine the effect of each study on the combined result. Potential publication bias was investigated through the funnel plot and further assessed using Egger’s test. A cumulative analysis was conducted after sorting by publication date. All statistical analyses of this meta-analysis were performed using the computer software STATA 11.0 (State Corp., College Station, TX, USA).

Results

Characteristics of included studies

After a comprehensive search of the PubMed, Embase, Web of Science, Wanfang, and CNKI databases, 1154 articles were identified, 948 of which were subsequently excluded because they were not relevant to ADRB2 polymorphisms and asthma risk. Thus, 206 relevant records were identified. Of these, 121 were excluded due to the lack of a case-control design. Of the remaining 85 articles, 26 were excluded due to overlapping data. Therefore, 59 articles were identified for further study. Of these 59 articles, four [17–20] were excluded as they were conference abstracts, seven [12,21–26] did not report useable data, and one [27] was excluded because the full text was not available. In addition, one article [28] was excluded as it was in Polish. Ultimately, 46 articles [8–11,13,29–69] met the inclusion criteria (Figure 1). The

Materials and Methods

Literature search

A literature search of the PubMed, Embase, Web of Science, Chinese National Knowledge Infrastructure (CNKI), and Wangfang databases (the last search was conducted on April 15, 2013) was conducted. The search strategy was as follows: “asthma” or “asthmatic” and “β2-adrenergic receptor” or “ADRB2” or “β2-AR” in combination with “polymorphism,” “mutation,” or “variant”. The searches were performed without restrictions with regard to publication date and language. Articles that were not published in English or Chinese were subsequently excluded.

Figure 1. Flow diagram of included/excluded studies.
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### Table 1. Detailed information of each article in the meta-analysis.

| First author | Year | Country | Ethnicity | Age group | Case age (year) | Control age (year) | Source of controls | Genotyping method | Cases  | Control | Asthma definition |
|--------------|------|---------|-----------|-----------|-----------------|-------------------|--------------------|-------------------|--------|---------|------------------|
| Cui LY        | 2007 | China   | Asia      | Adult     | 21–69           | 22–69             | Population         | AS-PCR/PCR-CTPP   | 72     | 60      | Guidelines of prevention and treatment of bronchial asthma (Chinese Medical Association) |
| Ye WX         | 2011 | China   | Asia      | Adult     | 18–57           | 22–60             | Population         | AS-PCR            | 31     | 37      | Guidelines of prevention and treatment of bronchial asthma (Chinese Medical Association) |
| Zhang XY      | 2008 | China   | Asia      | Children  | 1–17            | 2–13              | Population         | PCR-RFLP          | 217    | 50      | The guidelines of treatment for bronchial asthma in children |
| Wang W        | 2004 | China   | Asia      | Adult     | 17–72           | 18–71             | Hospital           | SSP-PCR           | 123    | 89      | Guidelines of prevention and treatment of bronchial asthma (Chinese Medical Association) |
| Yang Z        | 2012 | China   | Asia      | Children  | 7.7±2.6         | 7.69±2.55         | Hospital           | Sequencing        | 212    | 52      | Guidelines of prevention and treatment of bronchial asthma in children (China) |
| Feng DX       | 2004 | China   | Asia      | Adult     | 25–63           | 28–63             | Population         | AS-PCR            | 74     | 39      | Guidelines of prevention and treatment of bronchial asthma (Chinese Medical Association) |
| He XQ         | 2012 | China   | Asia      | Adult     | 42.5±16.2       | 43.39±20.7        | Hospital           | Sequenom MassARRAY | 171    | 148     | Guidelines of prevention and treatment of bronchial asthma (Chinese Medical Association) |
| Xie Y         | 2008 | China   | Asia      | Children  | 5.0±2.8         | 5.30±3.40         | Hospital           | SSP-PCR           | 57     | 62      | The guidelines of treatment for bronchial asthma in children |
| Xing J        | 2001 | China   | Asia      | Adult     | 20–66           | 25–46             | Population         | AS-PCR            | 55     | 38      | Guidelines of prevention and treatment of bronchial asthma (Chinese Medical Association) |
| Liu L         | 2009 | China   | Asia      | Adult     | 39.7±5.7        | 40.9±6.0          | Population         | Sequencing        | 120    | 120     | Guidelines of prevention and treatment of bronchial asthma |
| Dai LM        | 2002 | China   | Asia      | Adult     | 42±7            | 46±8              | Hospital           | Sequencing        | 87     | 94      | - |
| Shi XH        | 2008 | China   | Asia      | Both      | 14–66           | 18–56             | Hospital           | PCR-RFLP          | 48     | 48      | Guidelines of prevention and treatment of bronchial asthma (Chinese Medical Association) |
| Liao W        | 2001 | China   | Asia      | Children  | 1.2–11.7        | 2.5–13.2          | Population         | PCR-RFLP          | 50     | 50      | The Chinese Medical Association Respiratory Diseases Asthma Study Group |
| Tuerxun KLBN  | 2007 | China   | Asia      | Adult     | 38.35±9.17      | 18–71             | Population         | SSP-PCR           | 76     | 89      | Guidelines of prevention and treatment of bronchial asthma (Chinese Medical Association) |
| Zheng BQ      | 2012 | China   | Asia      | Children  | 0–14            | 0–14              | Population         | PCR-RFLP          | 198    | 110     | Guidelines of prevention and treatment of bronchial asthma (Chinese Medical Association) |
| Birbian N     | 2012 | Indian  | Asia      | Adult     | 38.1±16.2       | 41.9±166          | Population         | PCR-RFLP          | 410    | 414     | GINA (Global Initiative for Asthma) guidelines |
| Isaza C       | 2012 | Colombia| South America | Children | 11.6±5.4      | 11.8±5.2          | Students           | Mini-sequencing   | 109    | 137     | Standardised questionnaires with detailed questions on the occurrence and severity of symptoms of asthma |
| First author | Year | Country (Ethnicity) | Age group | Case age (year) | Control age (year) | Source of controls | Genotyping method | Control method | Asthma definition |
|--------------|------|---------------------|-----------|----------------|-------------------|-------------------|------------------|----------------|------------------|
| Kohyama K. | 2011 | Japan (Asia) | Adult | 50.4 | 47.1 | Hospital | Sequence-specific thermal-elution chromatography | 238 | Global Initiative for Asthma guidelines for diagnosis of asthma and long-term control of asthma. |
| Fu WP | 2011 | China (Asia) | Adult | 50.4 | 48.7 | Hospital | Sequencing | 135 | The American Thoracic Society guidelines for the diagnosis of asthma. |
| Qiu YY | 2011 | China (Asia) | Adult | 50.4 | 48.7 | Hospital | Sequencing | 135 | The American Thoracic Society guidelines for the diagnosis of asthma. |
| Szczepankiewicz A | 2009 | Poland (Europe) | Children | 6–18 | 10–5 | Population | PCR-RFLP | 113 | The American Thoracic Society guidelines for the diagnosis of asthma. |
| Llanes E | 2009 | Spain (Europe) | Adult | 22.9 | 23–58 | Population | PCR-RFLP | 113 | The American Thoracic Society guidelines for the diagnosis of asthma. |
| Munakata M | 2006 | Japan (Asia) | Not available | Not available | Not available | Population | PCR-RFLP | 113 | The American Thoracic Society guidelines for the diagnosis of asthma. |
| Tsai HJ | 2006 | Taiwan (Asia) | Adult | 22.9 | 23–58 | Population | PCR-RFLP | 113 | The American Thoracic Society guidelines for the diagnosis of asthma. |
| Santillan AA | 2003 | Mexico (North America) | Adult | 22.9 | 23–58 | Population | PCR-RFLP | 113 | The American Thoracic Society guidelines for the diagnosis of asthma. |
| Gao JM | 2000 | China (Asia) | Adult | 22.9 | 23–58 | Population | PCR-RFLP | 113 | The American Thoracic Society guidelines for the diagnosis of asthma. |
| Holloway JW | 2000 | New Zealand (Oceania) | Adult | 22.9 | 23–58 | Population | PCR-RFLP | 113 | The American Thoracic Society guidelines for the diagnosis of asthma. |
| Reihsaus E | 1993 | USA (Europe) | Adult | 22.9 | 23–58 | Population | PCR-RFLP | 113 | The American Thoracic Society guidelines for the diagnosis of asthma. |
| Chiang CH | 2012 | China (Asia) | Adult | 22.9 | 23–58 | Population | PCR-RFLP | 113 | The American Thoracic Society guidelines for the diagnosis of asthma. |
| Lanocca N | 2012 | Venezuela (South America) | Adult | 22.9 | 23–58 | Population | PCR-RFLP | 113 | The American Thoracic Society guidelines for the diagnosis of asthma. |
| Wang JY | 2009 | China (Asia) | Children | 22.9 | 23–58 | Population | PCR-RFLP | 113 | The American Thoracic Society guidelines for the diagnosis of asthma. |
Table 1. Cont.

| First author | Year | Country | Ethnicity | Age group | Case age (year) | Control age (year) | Source of controls | Genotyping method | Asthma definition |
|--------------|------|---------|-----------|-----------|----------------|-------------------|-------------------|-------------------|------------------|
| Lv J         | 69   | China   | Asia      | Children  | 3–12           | 18–22             | Students          | PCR-RFLP          | 2006 Global Initiative for Asthma guideline |
| Binaei S     | 62   | USA     | Europe    | Children  | Not available  | Not available     | Not available     | PCR-RFLP          | Not available     |
| Kotani Y     | 63   | Japan   | Asia      | Adult     | 48.4           | 16.8              | Not available     | PCR               | Not available     |
| Weir T R     | 64   | UK      | Europe    | Adult     | 34.3           | 13.8              | Not available     | PCR               | Not available     |
| Hakonarson H | 66   | Iceland | Europe    | Both      | 12–59          | 13.8              | Not available     | PCR               | Not available     |
| Leung TF     | 67   | China   | Asia      | Children  | 5–15           | Not available     | Both              | PCR               | Not available     |
| Lin YC       | 68   | China   | Asia      | Children  | 7–13           | Not available     | Both              | PCR               | Not available     |
| Chen X       | 69   | Taiwan  | Asia      | Both      | Not available  | Not available     | Not available     | PCR               | Not available     |
| Leung TF     | 70   | Canada  | North America | Children | 5–15          | Not available     | Both              | PCR               | Not available     |
| Chen X       | 71   | China   | Asia      | Both      | Not available  | Not available     | Not available     | PCR               | Not available     |

AS-PCR: Allele-specific polymerase chain reaction; PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism; SSP-PCR: sequence-specific primers-polymerase chain reaction.

For **Meta-analysis of ADRB2 polymorphisms and asthma**

For ADRB2, there was no significant association in any of the genetic model comparisons in the overall population (Figures 2 to 5). In the analysis stratified by ethnicity, a significant association was found in the South American population in the dominant model comparison ($OR = 1.754$, $95\% CI = 1.179–2.609$, $I^2 = 16.9\%$, studies $= 2$, case $= 314$, control $= 237$), but not in the other genetic comparisons or other ethnic groups. In the Chinese population, there was no significant association in any of the genetic model comparisons. The results are shown in Table 6.

**Meta-analysis of Gln27Glu variants and asthma.** For Gln27Glu, no evidence of an association with asthma risk was found in the overall population in any of the genetic model comparisons (Figures 6 to 9). In the analysis stratified by case age, a protective association was found in children only in the recessive model comparison ($OR = 0.566$, $95\% CI = 0.417–0.769$, $I^2 = 0.0\%$, studies $= 11$, case $= 1693$, control $= 1502$) and in adults only in the dominant model comparison ($OR = 0.610$, $95\% CI = 0.434–0.856$, $I^2 = 0.0\%$, studies $= 11$, case $= 1693$, control $= 1502$), and in adults only in the dominant model comparison ($OR = 0.864$, $95\% CI = 0.768–0.971$, $I^2 = 46.9\%$ $n = 18$, case $= 3116$, control $= 3433$). In the Chinese population, there was no significant association in any of the genetic model comparisons. The results are shown in Table 6.

**Meta-analysis of Thr164Ile variants and asthma.** For Thr164Ile, only four case-control studies were included, so no stratified analysis was performed. There was no evidence of an association with asthma risk in any of the genetic models in the overall population. The results are shown in Table 6.

**Meta-analysis of Arg19Cys variants and asthma.** For Arg19Cys, only three case-control studies provided genotype distribution data, therefore no stratified analysis was conducted. No significant association was found in the overall population in any of the genetic models. The results are shown in Table 6.

**Cumulative meta-analysis**

Cumulative analysis of the association between Arg16Gly and Gln27Glu polymorphisms and the risk of asthma was performed...
| First author  | Year | Country | Ethnicity | Age group | Case AA | Case AG | Case GG | Control AA | Control AG | Control GG | HWE(P) | HWE(P)1000 permutations |
|---------------|------|---------|-----------|-----------|---------|---------|---------|-----------|-----------|-----------|--------|--------------------------|
| Cui LY        | 2007 | China   | Asia      | Adult     | 9       | 55      | 8       | 12        | 39        | 9         | 73     | 71                       | 0.019  | 0.038                    |
| Ye WX         | 2011 | China   | Asia      | Adult     | 5       | 19      | 7       | 5         | 26        | 6         | 29     | 33                       | 0.013  | 0.030                    |
| Zhang XY      | 2008 | China   | Asia      | Children  | 81      | 111     | 25      | 19        | 23        | 8         | 273    | 161                      | 0.814  | 1.000                    |
| Wang W        | 2004 | China   | Asia      | Adult     | 48      | 59      | 16      | 26        | 54        | 9         | 155    | 91                       | 0.014  | 0.027                    |
| Yang Z       | 2012 | China   | Asia      | Children  | 78      | 104     | 30      | 24        | 23        | 5         | 260    | 164                      | 0.725  | 1.000                    |
| Feng DX       | 2004 | China   | Asia      | Adult     | 13      | 35      | 26      | 6         | 28        | 5         | 61     | 87                       | 0.006  | 0.016                    |
| He XQ         | 2012 | China   | Asia      | Adult     | 32      | 130     | 9       | 50        | 66        | 32        | 194    | 148                      | 0.249  | 1.000                    |
| Xie Y        | 2008 | China   | Asia      | Children  | 14      | 37      | 6       | 21        | 34        | 7         | 65     | 49                       | 0.220  | 0.337                    |
| Xing J       | 2001 | China   | Asia      | Adult     | 9       | 62      | 29      | 29        | 55        | 16        | 80     | 120                      | 0.234  | 0.385                    |
| Liu L        | 2009 | China   | Asia      | Adult     | 27      | 59      | 34      | 23        | 71        | 26        | 113    | 127                      | 0.044  | 0.082                    |
| Dai LM        | 2002 | China   | Asia      | Adult     | 33      | 33      | 21      | 36        | 33        | 25        | 99     | 75                       | 0.005  | 0.027                    |
| Shi XH       | 2008 | China   | Asia      | Both      | 22      | 19      | 7       | 10        | 25        | 13        | 63     | 33                       | 0.751  | 0.774                    |
| Liao W        | 2001 | China   | Asia      | Children  | 12      | 27      | 11      | 35        | 46        | 19        | 51     | 49                       | 0.577  | 0.721                    |
| Tuexun KLBN  | 2007 | China   | Asia      | Adult     | 13      | 36      | 27      | 26        | 54        | 9         | 62     | 90                       | 0.014  | 0.024                    |
| Zheng BQ     | 2012 | China   | Asia      | Children  | 77      | 99      | 28      | 31        | 55        | 24        | 253    | 155                      | 0.966  | 1.000                    |
| Birbhan N    | 2012 | India   | Asia      | Adult     | 62      | 199     | 149     | 48        | 188       | 178       | 323    | 497                      | 0.878  | 0.933                    |
| Isaza C      | 2012 | Colombia | South America | Children | 30      | 39      | 40      | 48        | 42        | 47        | 99     | 119                      | 0.000  | 0.000                    |
| Kohyama K    | 2011 | Japan   | Asia      | Adult     | 40      | 160     | 100     | 15        | 50        | 35        | 240    | 360                      | 0.677  | 0.856                    |
| Fu WP        | 2011 | China   | Asia      | Adult     | 85      | 88      | 65      | 106       | 92        | 67        | 258    | 218                      | 0.000  | 0.000                    |
| Qiu YY       | 2010 | China   | Asia      | Adult     | 77      | 85      | 39      | 88        | 135       | 53        | 239    | 163                      | 0.924  | 1.000                    |
| Szczepankiewicz AM  | 2009 | Polish | Europe    | Children  | 16      | 48      | 49      | 26        | 54        | 41        | 80     | 146                      | 0.304  | 0.449                    |
| Llanes E    | 2009 | Spain   | Europe    | Adult     | 17      | 54      | 37      | 8         | 25        | 17        | 88     | 128                      | 0.813  | 1.000                    |
| Munakata M  | 2006 | Japan   | Asia      | Not available | 14      | 21      | 11      | 23        | 47        | 30        | 49     | 43                       | 0.580  | 0.771                    |
| Tsai HJ      | 2006 | -       | African American | Both | -      | -      | -      | -       | -       | 285       | 243       | 162   | 190                      | -      | -                        |
| Telleria JF  | 2005 | Spain   | Europe    | Both      | 13      | 43      | 24      | 17        | 29        | 18        | 69     | 91                       | 0.454  | 0.674                    |
| Bhatnagar P  | 2005 | India   | Asia      | Adult     | 19      | 54      | 28      | 12        | 30        | 13        | 92     | 110                      | 0.499  | 0.624                    |
| Gao JM       | 2004 | China   | Asia      | Adult     | 38      | 59      | 28      | 35        | 53        | 8         | 135    | 115                      | 0.051  | 0.108                    |
| Santillan AA | 2003 | Mexico  | North America | Adult | 56      | 163     | 84      | 101       | 318       | 185       | 275    | 331                      | 0.070  | 0.170                    |
| Gao GK       | 2000 | China   | Asia      | Both      | 14      | 26      | 18      | 12        | 68        | 9        | 54     | 62                       | 0.000  | 0.000                    |
| Wang Z       | 2001 | China   | Asia      | Adult     | 25      | 54      | 22      | 38        | 64        | 34        | 104    | 98                       | 0.499  | 0.676                    |
| Holloway JW  | 2000 | New Zealand | Oceania | Adult | 78      | 47      | 29      | 35        | 39        | 17        | 203    | 105                      | 0.303  | 0.469                    |
| Reinhuaus ES | 1993 | USA     | Europe    | Adult     | 5       | 19      | 27      | 7         | 16        | 33        | 29     | 73                       | 0.042  | 0.174                    |
| Neslihan Aygun Kocabas  | 2007 | Turkish | West Asia and Southern Europe | Not available | -      | -      | -       | -       | -       | 91        | 167       | 108   | 146                      | -      | -                        |
| First author | Year | Country | Ethnicity | Age group | Case | Control | Case | Control | HWE(\(P\)) | HWE(\(P\)) 1000 permutations |
|--------------|------|---------|-----------|-----------|------|---------|------|---------|------------|---------------------------------|
| Larocca N\(^6\) | 2012 | Venezuela | South America | Adult | 30 | 17 | 58 | 47 | 18 | 35 | 77 | 133 | 112 | 88 | 0.000 | 0.000 |
| Chan IH\(^9\) | 2008 | China | Asia | Children | 101 | 135 | 59 | 51 | 89 | 33 | 337 | 253 | 191 | 155 | 0.597 | 0.700 |
| Wang JY\(^1\) | 2009 | China | Asia | Children | 138 | 207 | 97 | 173 | 250 | 87 | 483 | 401 | 596 | 424 | 0.837 | 0.674 |
| Lv J\(^2\) | 2009 | China | Asia | Children | 30 | 76 | 86 | 46 | 100 | 46 | 136 | 248 | 192 | 192 | 0.564 | 0.725 |
| Binaei S\(^3\) | 2003 | USA | Europe | Children | 7 | 24 | 7 | 34 | 67 | 54 | 38 | 38 | 135 | 175 | 0.132 | 0.243 |
| Kotani Y\(^4\) | 1999 | Japan | Asia | Adult | 30 | 52 | 35 | 28 | 45 | 30 | 112 | 122 | 101 | 105 | 0.201 | 0.342 |
| Weir TD\(^5\) | 1998 | Europe | Adult | - | - | - | - | - | - | - | - | 195 | 125 | 102 | 66 | - | - |
| Weir TD\(^6\) | 1998 | Asia | Adult | - | - | - | - | - | - | - | - | 13 | 19 | 62 | 62 | - | - |
| Dewar JC\(^7\) | 1998 | UK | Europe | Adult | 14 | 50 | 53 | 74 | 263 | 180 | 78 | 156 | 411 | 623 | 0.158 | 0.251 |
| Hakonarson H\(^8\) | 2001 | Iceland | Europe | Both | 45 | 151 | 127 | 21 | 85 | 75 | 241 | 405 | 127 | 235 | 0.677 | 0.874 |
| Leung TF\(^9\) | 2002 | China | Asia | Children | 25 | 38 | 13 | 22 | 37 | 11 | 88 | 64 | 81 | 59 | 0.483 | 0.675 |
| Lin YC\(^10\) | 2003 | China | Asia | Children | 34 | 35 | 11 | 27 | 25 | 17 | 103 | 57 | 79 | 59 | 0.031 | 0.104 |
| Shachor J\(^11\) | 2003 | Israel | Asia | Both | 11 | 38 | 17 | 26 | 52 | 35 | 60 | 72 | 104 | 122 | 0.433 | 0.531 |

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Table 3. Genotype and allele distributions in the meta-analysis for Gln27Glu (rs1042714).

| First author | Year | Country | Ethnicity | Age group | Case | Control | HWE(\(P\)) | HWE(\(P\)) 1000 permutations |
|--------------|------|---------|-----------|-----------|------|---------|-----------|---------------------------------|
| Cui LY       | 2007 | China   | Asia      | Adult     | 52   | 11      | 9         | 52 4 115 29 108 12 0.000 0.024   |
| Ye WX        | 2011 | China   | Asia      | Adult     | 10   | 17      | 4         | 14 19 4 37 25 47 27 0.511 0.763 |
| Zhang XY     | 2008 | China   | Asia      | Children  | 54   | 119     | 44        | 8 24 18 227 207 40 60 1.000 1.000 |
| Wang W       | 2004 | China   | Asia      | Adult     | 73   | 33      | 17        | 52 27 10 179 67 131 47 0.038 0.153 |
| Yang Z       | 2012 | China   | Asia      | Children  | 183  | 28      | 1         | 52 0 394 30 104 0 - -            |
| Feng DX      | 2004 | China   | Asia      | Adult     | 25   | 39      | 10        | 15 20 4 89 59 50 28 0.475 0.510 |
| Xie Y        | 2008 | China   | Asia      | Children  | 49   | 5       | 3         | 51 4 103 11 106 18 0.000 0.000   |
| Xing J       | 2001 | China   | Asia      | Adult     | 35   | 58      | 7         | 23 74 3 128 72 120 80 0.000 0.000 |
| Dai LM       | 2002 | China   | Asia      | Adult     | 71   | 13      | 3         | 76 14 4 155 19 166 22 0.007 0.015 |
| Liao W       | 2001 | China   | Asia      | Children  | 26   | 20      | 4         | 52 36 12 72 28 140 60 0.153 0.327 |
| Tuexun KLBN  | 2007 | China   | Asia      | Adult     | 44   | 29      | 3         | 52 34 3 117 35 138 40 0.363 0.646 |
| Birbian N    | 2012 | Indian  | Asia      | Adult     | 224  | 146     | 40        | 203 168 43 594 226 574 254 0.350 0.465 |
| Isaza C      | 2012 | Colombia| South America | Children  | 76   | 29      | 4         | 103 29 5 181 37 235 39 0.120 0.322 |
| Fu WP        | 2011 | China   | Asia      | Adult     | 179  | 38      | 21        | 209 37 19 396 80 455 75 0.000 0.001 |
| Qiu YY       | 2010 | China   | Asia      | Adult     | 166  | 32      | 3         | 226 45 5 364 38 497 55 0.129 0.386 |
| Szczepankiewicz A | 2009 | Polish | Europe | Children | 31   | 58      | 24        | 39 48 36 120 106 126 120 0.015 0.540 |
| Llanes E     | 2009 | Spain   | Europe    | Adult     | 49   | 40      | 18        | 24 22 4 138 76 70 30 0.736 0.783 |
| Munakata M   | 2006 | Japan   | Asia      | Not available | 39  | 6       | 1         | 86 14 0 84 8 186 14 0.452 1.000 |
| Tsai HF      | 2005 | Spain   | Europe    | Both      | 27   | 39      | 14        | 30 20 14 93 67 80 48 0.008 0.420 |
| Gao JM       | 2004 | China   | Asia      | Adult     | 46   | 76      | 3         | 39 56 1 168 82 134 58 0.000 0.002 |
| Santillan AA | 2003 | Mexican | North America | Adult     | 241  | 53      | 9         | 385 202 17 535 71 972 236 0.117 0.248 |
| Gao G       | 2000 | China   | Asia      | Both      | 20   | 32      | 6         | 32 49 8 72 44 113 65 0.077 0.171 |
| Wang Z       | 2001 | China   | Asia      | Adult     | 108  | 19      | 1         | 113 22 1 235 21 248 24 0.950 0.303 |
| Holloway JW  | 2000 | New Zealand | Oceania | Adult     | 28   | 76      | 49        | 19 37 35 132 174 75 107 0.125 0.235 |
| Reihus F     | 1993 | USA     | Europe    | Adult     | 13   | 26      | 12        | 17 23 16 52 50 57 55 0.182 0.384 |
| Chiang CH    | 2012 | China   | Asia      | Adult     | 400  | 66      | 10        | 85 26 1 866 86 196 28 0.517 0.743 |
| Larocca N    | 2012 | Venezuela| South America | Adult     | 37   | 57      | 11        | 30 60 10 131 79 120 80 0.012 0.060 |
| Chan IH      | 2008 | China   | Asia      | Children  | 232  | 43      | 19        | 133 19 21 507 81 285 61 0.000 0.000 |
| Wang JY      | 2009 | China   | Asia      | Children  | 359  | 84      | 5         | 425 77 9 802 94 927 95 0.016 0.201 |
| Binaei S     | 2003 | USA     | Europe    | Children  | 23   | 12      | 2         | 107 36 12 58 16 250 60 0.001 0.039 |
| Kotani Y     | 1999 | Japan   | Asia      | Adult     | 94   | 23      | 0         | 89 14 0 211 23 192 14 0.459 1.000 |
| Weir TD      | 1998 | -       | Europe    | Adult     | -    | -       | -         | - 174 136 101 67 - -           |
| Weir TD      | 1998 | -       | Asia      | Adult     | -    | -       | -         | - 26 6 91 33 - -              |
| Dewar JC     | 1998 | UK      | Europe    | Adult     | 33   | 51      | 35        | 134 271 106 117 121 559 483 0.149 0.225 |
after sorting by publication date. As shown in Figures 10 to 13, for Arg16Gly, there was a stable trend in the estimated risk effect in the dominant model comparison from 2009 to 2012 and in the allelic comparison from 1993 to 2012. As shown in Figures 14 to 17, for Gln27Glu, there was a trend toward no significant association over time in all genetic model comparisons.

Sensitivity analysis

Sensitivity analysis was conducted by sequentially excluding individual studies to estimate the stability of the results. After sequentially excluding each study, statistically similar results were found.

Publication bias

Potential publication bias was investigated using the funnel plot and was further assessed using Egger’s test. Significant publication bias was detected for the Gln27Glu polymorphism in the dominant model comparison ($t = 2.69$, $P = 0.011$). No evidence of publication bias was found for the Arg16Gly, Thr164Ile, or Arg19Cys polymorphism in any of the genetic model comparisons. The results are shown in Table 7.

Discussion

Asthma is a well-known disease of the respiratory system that is characterized by cramps and obstruction of the small bronchus. $B_2$-AR binds specifically to a class of ligands that can lead to the expansion of the small airways. In the present study, the relationship between all related $ADRB2$ gene polymorphisms and the overall risk of asthma was examined. The purpose of this meta-analysis was to provide more information for asthma candidate gene research, based on the hypothesis that genetic effects vary across different ethnic cohorts.

Four $ADRB2$ polymorphisms that had been investigated in at least three case-control studies were included in the study. The results indicated that Arg16Gly, Gln27Glu, Thr164Ile, and Arg19Cys were not associated with risk of asthma in the overall population. The findings of the current study are consistent with those of Migita [14] and Contopoulos-Ioannidis [6]. Migita and his colleagues performed a meta-analysis by a random-effects model that showed a non-significant odds ratio for the Arg16Gly and the Gln27Glu polymorphism. Contopoulos-Ioannidis found that polymorphisms of $ADRB2$ are not major risk factors for the development of asthma. Cumulative analysis further confirmed that there was no significant association between the Arg16Gly polymorphism or the Gln27Glu polymorphism and the risk of asthma, showing that the variants had no effect with the accumulation of more data over time.

In the analysis stratified by case age, a protective effect for the Gln27Glu polymorphism was observed in adults in the dominant model comparison and in children in the recessive model comparison and the homozygote genotype comparison. This finding corroborates the ideas of Ammarin Thakkinstian, who suggested that the Gln/Glu and Glu/Glu genotypes could reduce the risk of asthma [15]. Besides, the pathogenesis of asthma in adults and children may differ, but the exact mechanism remains unknown and needs further detailed research.

In the analysis stratified by ethnicity, an increased risk of asthma was only seen with the Arg16Gly polymorphism in the South American population, and a protective effect was only found with the Gln27Glu polymorphism in the North American population and only in the dominant model comparison. The discrepancies in linkage disequilibrium (LD) structure in Chinese and Europeans may explain these differences; the minor allele of the $ADRB2$
Table 4. Genotype and allele distributions in the meta-analysis for Thr164Ile (rs1800888).

| First author | Year | Country | Ethnicity | Age group | Case CC | Case TT | Control CC | Control TT | HWE(\(P\)) | HWE(\(P\)) 1000 permutations |
|--------------|------|---------|-----------|-----------|---------|---------|-----------|------------|------------|-------------------------------|
| Yang Z       | 2012 | China   | Asia      | Children  | 211     | 1       | 0         | 52         | 0          | 0                | -               |
| Gao JM\(^{33}\) | 2004 | China   | Asia      | Adult     | 56      | 67      | 2         | 48         | 48         | 0.001            | 0.021           |
| Gao GK\(^{35}\) | 2000 | China   | Asia      | Both      | 6       | 48      | 4         | 27         | 47         | 0.475            | 0.546           |
| Reihsaus E\(^{36}\) | 1993 | USA     | Europe    | Adult     | 51      | 0       | 0         | 53         | 3          | 0.837            | 1.000           |

Table 5. Genotype and allele distributions in the meta-analysis for Arg19Cys (rs1042711).

| First author | Year | Country | Ethnicity | Age group | Case TT | Case CT | Case CC | Control TT | Control CT | Control CC | HWE(\(P\)) | HWE(\(P\)) 1000 permutations |
|--------------|------|---------|-----------|-----------|---------|---------|---------|------------|------------|------------|------------|-------------------------------|
| Fu WP\(^{46}\) | 2011 | China   | Asia      | Adult     | 162     | 69      | 7       | 199        | 61         | 5          | 393        | 459 71 0.897 1.000           |
| Qiu YY\(^{47}\) | 2010 | China   | Asia      | Adult     | 166     | 32      | 3       | 226        | 45         | 5          | 364        | 497 55 0.129 0.384           |
| Szczepankiewicz A\(^{48}\) | 2009 | Polish | Europe    | Children  | 51      | 41      | 21      | 57         | 49         | 17         | 143        | 163 83 0.227 0.407           |
| Tsai HJ\(^{51}\) | 2006 | -       | African American | Both | -      | -      | -       | -         | -         | -          | 454        | 74 289 63 - -               |
| SNP | Groups | Dominant model comparison | Recessive model comparison | Homozygote genotype comparison | Allelic comparison |
|-----|--------|---------------------------|---------------------------|-----------------------------|------------------|
|     | OR (95%CI) | P | OR (95%CI) | P | OR (95%CI) | P | OR (95%CI) | P |
| Arg16Gly Total | 1.069 (0.978–1.167) | 0.142 | 1.110 (0.994–1.233) | 0.08 | 1.274 (1.097–1.477) | 0.008 | 1.344 (1.275–1.419) | 0.000 |
| Adult | 1.077 (0.956–1.213) | 0.225 | 1.170 (0.942–1.454) | 0.155 | 1.230 (0.965–1.569) | 0.094 | 1.100 (0.939–1.260) | 0.262 |
| Children | 1.055 (0.925–1.203) | 0.121 | 1.061 (0.798–1.410) | 0.685 | 1.158 (0.851–1.575) | 0.350 | 1.092 (0.930–1.282) | 0.282 |
| Both | 0.846 (0.607–1.181) | 0.326 | 1.064 (0.617–1.833) | 0.824 | 0.946 (0.526–1.702) | 0.853 | 0.896 (0.704–1.203) | 0.398 |
| Asia | 1.055 (0.954–1.168) | 0.297 | 1.122 (0.913–1.380) | 0.275 | 1.139 (0.914–1.420) | 0.247 | 1.074 (0.970–1.189) | 0.167 |
| Europe | 1.205 (0.910–1.596) | 0.192 | 1.055 (0.793–1.404) | 0.713 | 1.202 (0.881–1.640) | 0.245 | 1.079 (0.929–1.252) | 0.069 |
| North America | 0.886 (0.618–1.270) | 0.509 | 0.869 (0.640–1.179) | 0.366 | 0.819 (0.540–1.241) | - | 0.910 (0.748–1.107) | - |
| Oceania | 0.609 (0.359–1.032) | 0.065 | 1.010 (0.520–1.962) | 0.977 | 0.765 (0.373–1.572) | 0.466 | 0.772 (0.352–1.710) | 0.181 |
| China | 1.093 (0.914–1.365) | 0.30 | 1.199 (0.923–1.548) | 0.62 | 1.290 (0.928–1.773) | 0.159 | 1.106 (0.980–1.252) | 0.015 |
| HWE (P < 0.05) | 1.058 (0.943–1.214) | 0.30 | 1.030 (0.819–1.283) | 0.76 | 1.149 (0.892–1.490) | 0.26 | 1.057 (0.853–1.309) | 0.611 |
| HWE (P > 0.05) | 1.007 (0.943–1.073) | 0.83 | 0.995 (0.932–1.061) | 0.78 | 1.013 (0.943–1.085) | 0.92 | 1.023 (0.954–1.096) | 0.877 |
| Thr164Ile Total | 1.460 (0.544–3.916) | 0.451 | 0.772 (0.089–6.684) | 0.814 | 1.502 (0.416–5.419) | 0.535 | 1.173 (0.383–3.573) | 0.818 |
| Asia | 1.460 (0.544–3.916) | 0.451 | 0.772 (0.089–6.684) | 0.814 | 1.502 (0.416–5.419) | 0.535 | 1.173 (0.383–3.573) | 0.818 |
| HWE (P < 0.05) | 1.058 (0.943–1.214) | 0.30 | 1.030 (0.819–1.283) | 0.76 | 1.149 (0.892–1.490) | 0.26 | 1.057 (0.853–1.309) | 0.611 |
| HWE (P > 0.05) | 1.007 (0.943–1.073) | 0.83 | 0.995 (0.932–1.061) | 0.78 | 1.013 (0.943–1.085) | 0.92 | 1.023 (0.954–1.096) | 0.877 |
Figure 2. Forest plots of the association between the Arg16Gly (rs1042713) polymorphism and risk of asthma in dominant model comparison.
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Figure 3. Forest plots of the association between the Arg16Gly (rs1042713) polymorphism and risk of asthma in recessive model comparison.
doi:10.1371/journal.pone.0104488.g003
Arg16Gly (A46G, rs1042713) in the population of northern and western European ancestry (CEU) was A with a frequency of 0.358, whereas it was G with a frequency of 0.439 among the Han Chinese in Beijing (HCB). The minor allele of the ADRB2 Gln27Glu (C79G, rs1042714) was 0.467, whereas it was 0.122 in HCB. Another reason for these differences is that sample size was

Figure 4. Forest plots of the association between the Arg16Gly (rs1042713) polymorphism and risk of asthma in homozygote genotype comparison.
doi:10.1371/journal.pone.0104488.g004

Figure 5. Forest plots of the association between the Arg16Gly (rs1042713) polymorphism and risk of asthma in allele comparison.
doi:10.1371/journal.pone.0104488.g005

ADRB2 Polymorphisms and Asthma: A Meta-Analysis
PLOS ONE | www.plosone.org 13 August 2014 | Volume 9 | Issue 8 | e104488
Figure 6. Forest plots of the association between the Gln27Glu (rs1042714) polymorphism and risk of asthma in dominant model comparison.
doi:10.1371/journal.pone.0104488.g006

Figure 7. Forest plots of the association between the Gln27Glu (rs1042714) polymorphism and risk of asthma in recessive model comparison.
doi:10.1371/journal.pone.0104488.g007
small for the South American and North American populations, and therefore the current boundary result may have been unable to demonstrate that the Arg16Gly and Gln27Glu polymorphisms are associated with the risk of asthma in these populations. More studies with a larger sample size are needed. In the Chinese population, the results of the current meta-analysis showed that

Figure 8. Forest plots of the association between the Gln27Glu (rs1042714) polymorphism and risk of asthma in homozygote genotype comparison.
doi:10.1371/journal.pone.0104488.g008

Figure 9. Forest plots of the association between the Gln27Glu (rs1042714) polymorphism and risk of asthma in allele comparison.
doi:10.1371/journal.pone.0104488.g009
Figure 10. Forest plots of cumulative meta-analysis of Arg16Gly (rs1042713) in association with asthma by published year under dominant model comparison.

doi:10.1371/journal.pone.0104488.g010

Figure 11. Forest plots of cumulative meta-analysis of Arg16Gly (rs1042713) in association with asthma by published year under recessive model comparison.

doi:10.1371/journal.pone.0104488.g011
Figure 12. Forest plots of cumulative meta-analysis of Arg16Gly (rs1042713) in association with asthma by published year under homozygote genotype comparison.
doi:10.1371/journal.pone.0104488.g012

Figure 13. Forest plots of cumulative meta-analysis of Arg16Gly (rs1042713) in association with asthma by published year under allele comparison.
doi:10.1371/journal.pone.0104488.g013
ADRB2 Polymorphisms and Asthma: A Meta-Analysis

Figure 14. Forest plots of cumulative meta-analysis of Gln27Glu (rs1042714) in association with asthma by published year dominant model comparison.
doi:10.1371/journal.pone.0104488.g014

Figure 15. Forest plots of cumulative meta-analysis of Gln27Glu (rs1042714) in association with asthma by published year recessive model comparison.
doi:10.1371/journal.pone.0104488.g015
| Study ID   | OR (95% CI)  |
|-----------|--------------|
| Nollhaus E (1993) | 1.00 (0.86, 1.17) |
| Weis T (1988) | 1.17 (0.86, 1.59) |
| Weis T (1990) | 1.18 (0.84, 1.63) |
| Chen C (1990) | 1.13 (0.80, 1.59) |
| Kolcan T (1995) | 1.20 (0.87, 1.62) |
| Gas G (2000) | 1.12 (0.84, 1.51) |
| Holloway NR (2002) | 1.00 (0.82, 1.22) |
| Li C (2000) | 1.04 (0.83, 1.32) |
| Liu L (2001) | 1.03 (0.80, 1.33) |
| Wang J (2001) | 1.03 (0.80, 1.35) |
| Rijkeboom H (2001) | 1.03 (0.80, 1.35) |
| Dai LM (2002) | 1.05 (0.80, 1.35) |
| Lee YG (2002) | 1.03 (0.80, 1.35) |
| Skrinar J (2000) | 1.03 (0.80, 1.35) |
| Wang J (2004) | 1.05 (0.80, 1.35) |
| Feng DG (2004) | 1.03 (0.80, 1.35) |
| Gao JN (2004) | 1.02 (0.80, 1.35) |
| Teller Ya J (2000) | 1.02 (0.80, 1.35) |
| Murakami M (2004) | 1.02 (0.80, 1.35) |
| Chen H (2008) | 1.02 (0.80, 1.35) |
| Siccioppi A (2008) | 1.02 (0.80, 1.35) |
| Xie F (2008) | 1.02 (0.80, 1.35) |
| Zhang YX (2008) | 1.02 (0.80, 1.35) |
| Zhu X (2008) | 1.02 (0.80, 1.35) |
| Zhang CH (2011) | 1.02 (0.80, 1.35) |
| Larnaca N (2012) | 1.02 (0.80, 1.35) |

Figure 16. Forest plots of cumulative meta-analysis of Gln27Glu (rs1042714) in association with asthma by published year under homozygote genotype comparison.
doi:10.1371/journal.pone.0104488.g016

| Study ID   | OR (95% CI)  |
|-----------|--------------|
| Nollhaus E (1993) | 0.98 (0.75, 1.31) |
| Weis T (1988) | 1.35 (0.78, 2.30) |
| Weis T (1990) | 1.02 (0.68, 1.52) |
| Chen C (1990) | 1.20 (0.70, 2.02) |
| Kolcan T (1995) | 1.18 (0.70, 1.92) |
| Gas G (2000) | 1.12 (0.70, 1.79) |
| Holloway NR (2002) | 1.12 (0.70, 1.79) |
| Li C (2000) | 1.00 (0.75, 1.34) |
| Liu L (2001) | 0.98 (0.75, 1.37) |
| Wang J (2001) | 1.00 (0.75, 1.34) |
| Rijkeboom H (2001) | 1.00 (0.75, 1.34) |
| Dai LM (2002) | 1.00 (0.75, 1.34) |
| Lee YG (2002) | 1.00 (0.75, 1.34) |
| Skrinar J (2000) | 1.00 (0.75, 1.34) |
| Wang J (2004) | 1.00 (0.75, 1.34) |
| Feng DG (2004) | 1.00 (0.75, 1.34) |
| Gao JN (2004) | 1.00 (0.75, 1.34) |
| Teller Ya J (2000) | 1.00 (0.75, 1.34) |
| Murakami M (2004) | 1.00 (0.75, 1.34) |
| Chen H (2008) | 1.00 (0.75, 1.34) |
| Siccioppi A (2008) | 1.00 (0.75, 1.34) |
| Xie F (2008) | 1.00 (0.75, 1.34) |
| Zhang YX (2008) | 1.00 (0.75, 1.34) |
| Zhu X (2008) | 1.00 (0.75, 1.34) |
| Zhang CH (2011) | 1.00 (0.75, 1.34) |
| Larnaca N (2012) | 1.00 (0.75, 1.34) |

Figure 17. Forest plots of cumulative meta-analysis of Gln27Glu (rs1042714) in association with asthma by published year under allele comparison.
doi:10.1371/journal.pone.0104488.g017
there was no significant association with the risk of asthma with either the Arg16Gly polymorphism or the Gln27Glu polymorphism in any of the genetic model comparisons, supporting Ni Suiqin’s [16] conclusion.

In the analysis stratified by HWE according to the P-value for the Arg16Gly and Gln27Glu polymorphisms, a significant association was found in the recessive model comparison and the homozygote genotype comparison for Arg16Gly in the group with $P<0.05$, but not in the group with $P>0.05$. For Gln27Glu, a significant association was found in the dominant model comparison in the group with $P>0.05$. These results therefore need to be interpreted with caution. There are several possible explanations as to why the control group population was not in HWE. First, the population was not characterized by random mating. Second, the locus under consideration exhibited an inconstant fluctuating mutation rate. Third, there was selection for a particular phenotype. Fourth, the population was not sufficiently large or non-random. Fifth, there had been a change in the population structure during the period of study due to migration.

No significant association with the risk of asthma was found for the Thr164Ile and Arg19Cys polymorphisms. Thus, the Thr164Ile and Arg19Cys polymorphisms may not be involved in the pathogenesis of asthma. Further research is needed because, as only four case-controls were included in the study, there might not be sufficient statistical evidence to clarify the association between the Thr164Ile and Arg19Cys polymorphisms and the risk of asthma.

$ADRB2$ is located on chromosome 5q31–32, encodes 413 amino acids, and is an intronless gene [5]. According to the SNPper database, there are more than 100 SNPs in the promoter region, five SNPs in the 5’UTR region and 18 SNPs in the coding region of the gene. The mutation of the two most important SNPs, Arg16Gly and Gln27Glu, which are located at nucleotide positions 46 and 79 of the coding region of the $ADRB2$ gene, respectively, can cause changes in the amino acid sequence. The altered amino acid sequence can lead to down-regulation of the β2-AR and may cause the desensitization of related reactions [70]. Thr164Ile is also located in the coding region of the $ADRB2$ gene; a base change from C to T can lead to a change in amino acid from threonine (Thr) to isoleucine (Ile). The missense polymorphisms of Arg16Gly, Gln27Glu, and Thr164Ile may lead to functional changes in $ADRB2$. Most of the studies relating to $ADRB2$ and asthma risk have focused on coding region polymorphisms. In recent years, studies on $ADRB2$ have not been confined to coding region polymorphisms alone, as more and more studies have begun to pay attention to promoter region polymorphisms. Arg19Cys is located in the 5’ leader region that harbors an open reading frame (ORF) in the promoter region of the $ADRB2$ gene; a base change from T to C leads to a change in amino acid from arginine (Arg) to cysteine (Cys). Recent in vivo and in vitro research has demonstrated that this change can impede the translation of $ADRB2$ mRNA, and thus can regulate cellular expression of the receptor [71]. Further studies are therefore required to assess whether the SNPs in $ADRB2$ alter signal regulation, gene expression, or the function of its product or not.

There are certain inevitable limitations to the current meta-analysis. First, all available literature should be included in the meta-analysis, but we only included literature published in English and Chinese, thus neglecting studies published in other languages. In addition, most of the included studies just focus on Chinese and Asian, which may result in an inability to detect modest association due to lack of power because of underreporting/lower incidence of asthma in these populations. Second, most original literature only

| SNP            | Study number (n) | Dominant model comparison | Recessive model comparison | Allele comparison |
|----------------|-----------------|---------------------------|---------------------------|------------------|
|                |                 | t  | P              | t  | P              | t  | P              |
| Arg16Gly (rs1042713) | 45 | 1.02 | 0.315 | 1.12 | 0.475 | 0.72 | 0.475 |
| Gln27Glu (rs1042714)  | 37 | 2.69 | 0.011 | 0.71 | 0.496 | 0.79 | 0.579 |
| Thr164Ile (rs1800888) | 4  | -0.37 | 0.71 | -0.78 | 0.596 | -0.78 | 0.596 |
| Arg19Cys (rs1042711) | 4  | 2.01 | 0.294 | 2.01 | 0.294 | 2.01 | 0.294 |

| Table 7. Publication bias results of Egger’s test |
|------------------------------------------------|
| SNP            | Study number (n) | Allele comparison |
|----------------|-----------------|------------------|
|                |                 | t  | P            |
| Arg16Gly (rs1042713) | 45 | 1.02 | 0.315 |
| Gln27Glu (rs1042714)  | 37 | 2.69 | 0.011 |
| Thr164Ile (rs1800888) | 4  | -0.37 | 0.71 |
| Arg19Cys (rs1042711) | 4  | 2.01 | 0.294 |

doi:10.1371/journal.pone.0104488.t007
provides a generic asthma definition, and does not describe asthma phenotype(s) and environmental factors in detail, so we cannot supply this information. Third, several studies were not included because they did not provide sufficient data for statistical analysis, which may have biased the result. Fourth, publication bias was only detected for the Gln27Glu polymorphism in the dominant model comparison (t = 2.69, P = 0.011), but not in the other three genetic model comparisons. In fact, positive results or results with “expected” findings are more likely to be published. Publication bias may lead to a false positive result. We detected significant publication bias for the Gln27Glu polymorphism in the dominant model, so the results need to be interpreted with caution. Fifth, moderate heterogeneity was found in some genetic models for the Arg16Gly polymorphism. Because no information was available other than the factors we performed a stratified analysis, and thus we were unable to use meta-regression to explore other possible sources of between-group heterogeneity. Furthermore, the result of the sensitivity analysis was stable. Therefore, the heterogeneity seemed to have no effect on the results, suggesting their reliability.

In conclusion, the current meta-analysis suggests that the Arg16Gly, Gln27Glu, Thr164Ile, and Arg19Cys polymorphisms may not be involved in the risk of asthma in the overall population of the Chinese population. Well-designed, high-quality studies with a larger sample size and various ethnicities should be conducted to confirm these results.

Supporting Information
Checklist S1 PRISMA checklist. (DOC)

Author Contributions
Conceived and designed the experiments: SQ. Performed the experiments: CG. Analyzed the data: ZW. Contributed reagents/materials/analysis tools: SQ. Wrote the paper: SQ. XLC.
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