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Meta-Analysis

Association between polymorphisms of glucocorticoid receptor genes and asthma: A meta-analysis

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Abstract: Recent studies have evaluated the associations between polymorphisms of glucocorticoid receptor genes and asthma. However, the conclusions of these studies are conflicting. The objective of this meta-analysis was to clarify the association between all known polymorphisms of glucocorticoid receptor genetic loci and susceptibility to asthma, based on existing reports. We conducted a meta-analysis of the association between glucocorticoid receptor polymorphisms (NR3C1) and asthma risk. A systematical literature search was performed in PubMed, EMBASE, Web of Science, China National Knowledge Infrastructure (CNKI), and Cochrane Library until January 15, 2018. The odds ratio (OR), 95% confidence interval (CI), and P value were calculated using Mantel-Haenszel statistics under the allele, homozygote, heterozygote, dominant, or recessive models. *P* values of less than 0.05 were considered to represent statistically significant associations between glucocorticoid receptor gene polymorphisms and asthma. All statistical analyses were done using the "meta" package (version 4.9–0) of R version 3.4.3 and RStudio version 1.0.44. A total of fourteen studies, reported via ten articles from online databases were included in our meta-analysis. For *Bcl*I (from eight studies), a significant association was detected in the allele model, homozygote model, and recessive model (C versus G: OR (95% CI) = 0.63 (0.40–0.97), CC versus GG: OR (95% CI) = 0.41(0.17–0.97), CC versus GC + GG: OR (95% CI) = 0.54(0.34–0.88)), but not in the heterozygote model or the dominant model. For ER22/23EK (from four studies), TthIII1 (from two studies), no significant association was found for any genetic model. After subgroup analyses by age, significant associations were observed for the allele model, homozygote model, dominant model and recessive model for *Bcl*I in adults. The ER22/23EK and TthIII1 polymorphisms were not found to be associated with susceptibility to ASTHMA; however, the *Bcl*I polymorphisms were significantly associated with ASTHMA in adults.

Key words: Glucocorticoid receptor; Gene polymorphisms; Asthma.

Introduction

Asthma is a chronic airway inflammatory disease characterized by airway hyperresponsiveness and reversible limitation of airflow(1, 2). Airway hyperresponsiveness is an overreaction state due to airway inflammation(3-5). It shows a sensitive and excessive bronchial smooth muscle constriction reaction, causing airway stenosis and increased airway resistance, thereby leading to cough, chest distress, dyspnea and wheezing(6) .

Airway inflammation is a common feature of all types of asthma(3). It is a pathological basis for airway hyperresponsiveness and other clinical symptoms of asthma(7, 8). It is also a theoretical cornerstone for the application of glucocorticoids(8). Glucocorticoids is the most effective drug for clinical treatment of asthma, and its mechanism is mainly through the classic pathway of glucocorticoids/glucocorticoid receptor gene transcriptional regulation, which inhibits airway allergic inflammation from multiple links, thereby reducing airway hyperresponsiveness(9). As the mediator of the glucocorticoid effect, glucocorticoid receptor plays an important role in the physiological and pharmacological effects of glucocorticoids(1, 6, 10).

Although the supplement of exogenous GC is the preferred method for the treatment of asthma, but large doses of glucocorticoid drugs or long-term low-dose use of such drugs can cause the inhibition of adrenocortical function, resulting in decreased the content of endogenous COR or function of glucocorticoid receptor dysfunction, thus aggravate the inflammatory response and airway hyperresponsiveness in asthma (6, 9).

Glucocorticoid receptor (GR) is a ligand-activated endogenous transcription factor mainly located in the cytoplasm and belongs to the nuclear receptor family(11). The GR gene was officially named NR3C1 (nuclear receptor subfamily 3, group C, Member 1) in 1999(12). NR3C1 is located on chromosome 31 of the long arm of human chromosome 5 (5q31). A large number of genes were distributed on the long arm (5q) of chromosome 5 and played an important role in the regulation of IgE synthesis and the development of allergies and asthma-related inflammation(13). NR3C1 contains 10 exons. Exon 9 includes two parts, α and β, which can be transcribed and translated to form two protein isoforms, GRα and GRβ. GRα is a classical glucocorticoid ligand binding protein, which is mainly located in the cytoplasm in the resting state and binds to glucocorti-

coids to enter the nucleus and regulate the expression of glucocorticoid response genes. GRβ can not be combined with glucocorticoids, GRβ can not transcriptional activation like GRα, and it was mainly located in the nucleus at rest(14). Studies have shown that it has a negative regulation effect on GRα (15). Hurley(16) reported for the first time that mutations in the glucocorticoid susceptibility due to gene mutations were gradually discovered after mutations in the GR gene. Experimental studies (17, 18)found that more mutations and polymorphisms exist in NR3C1, and these changes can affect the level and activity of GR, affect the sensitivity of target tissues to GC, and then affect the therapeutic effect of GC. This article is based on the current research on GR gene polymorphisms, and reviews all literature on GR gene polymorphisms to understand genetic polymorphisms of GR gene polymorphisms in different sites of GR gene polymorphisms (BCL 1\ER22/ 23EK\N363S\ TthIII1) Relationship with Asthma.

To the best of our knowledge, there has been no systematic review or meta-analysis of the association between glucocorticoid receptor genetic variants and asthma risk to date. The present report is the first meta-analysis, based on existing reports in the literature, of the relationship between all known glucocorticoid receptor gene polymorphisms and susceptibility to asthma.

Materials and Methods

The present meta-analysis was conducted in accordance with the standards of the Systematic Reviews of Genetic Association Studies(19) and the meta-analysis of observational studies in epidemiology (MOOSE) for design, implementation, and reporting (20).

Literature search strategy

Relevant studies were identified by searching PubMed, EMBASE, Web of Science, China National Knowledge Infrastructure (CNKI), and Cochrane Library. The search terms were: "Glucocorticoid Receptors" and "Asthma". The full search strategy is available on Table 1. The last search was conducted on January 15, 2018.

Eligibility criteria

The following eligibility criteria were applied to select studies for inclusion in the meta-analysis: (1) articles evaluating the association of the glucocorticoid receptor gene polymorphism with asthma; (2) a clear definition of asthma; (3) a case—control study; (4) published in either English or Chinese; and (5) sufficient published data for calculating odds ratios (ORs) with their 95% confidence intervals (CIs).

Exclusion criteria

The exclusion criteria were as follows: (1) duplicate publications (only the latest publication with the most complete or updated data was selected); (2) incomplete information; (3) insufficient data; (4) review articles or conference literatures.

Data extraction

Data were extracted by two reviewers (GF and LF) independently according to the pre-specified data extraction form. The following information was extracted from each study: first author, population (country, ethnicity), source of controls, case/control sample size, genotype counts for cases and controls, and evidence of Hardy-Weinberg equilibrium (HWE). If the essential data were not reported, we attempted to contact the author of the relevant studies to obtain these. The authors did not respond, so incomplete articles were excluded. Differences, if any, were resolved by consensus after discussion.

Quality assessment for individual studies

The Newcastle-Ottawa Scale (NOS) was used to assess the methodological quality of the individual studies by the two reviewers (GF and YC)(21). Each study was evaluated and scored based on three criteria: selection (4 stars), comparability (2 stars), and exposure (3 stars). The NOS point ranged from 0 to 9 stars. Disagreement, if any, was resolved by discussion with a third reviewer (HZ).

Data analysis

We first assessed the HWE in the control group using the chi-square test(22). The P value, OR and corresponding 95% CI were calculated using Mantel-Haenszel statistics under the allele, homozygote, heterozygote, dominant, or recessive models. This differs from the published protocol as there is no explicit additive model; therefore, this was replaced with homozygote and heterozygote models. *P* values of less than 0.05 were considered to represent statistically significant associations between glucocorticoid receptor gene polymorphisms and asthma. Heterogeneity across individual studies was analyzed by the Cochran's-Q statistic and the I^2 statistic ($P \leq 0.10$ and $I^2 \geq 50\%$ indicated the significance of heterogeneity) (23, 24). A fixed-effect model was selected with no significant heterogeneity among studies. Otherwise, a random-effect model was used(23-26). Subsequently, subgroup analyses were performed based on ethnicity to explore the sources of heterogeneity. The potential publication bias was evaluated by Begg's funnel plot and Egger's test(27). All statistical analyses were conducted using the "meta" package (version 4.9-0) of R version 3.4.3 and RStudio version 1.0.44.

Table 1. Search result.

Data base	Search strategy	Numbers
PubMed	(Glucocorticoid Receptors) AND asthma	535
EMBASE	'asthma':ab,ti AND 'glucocorticoid receptor':ab,ti	533
Web of Science	TI=asthma AND TS=((Glucocorticoid Receptors)	452
CNKI	((Key word= glucocorticoid receptor) OR (Key word= glucocorticoid receptor gene)) AND (Title= asthma)	137
Cochrane Library	asthma:ti,ab,kw and Glucocorticoid Receptor:ti,ab,kw (Word variations have been searched)	102

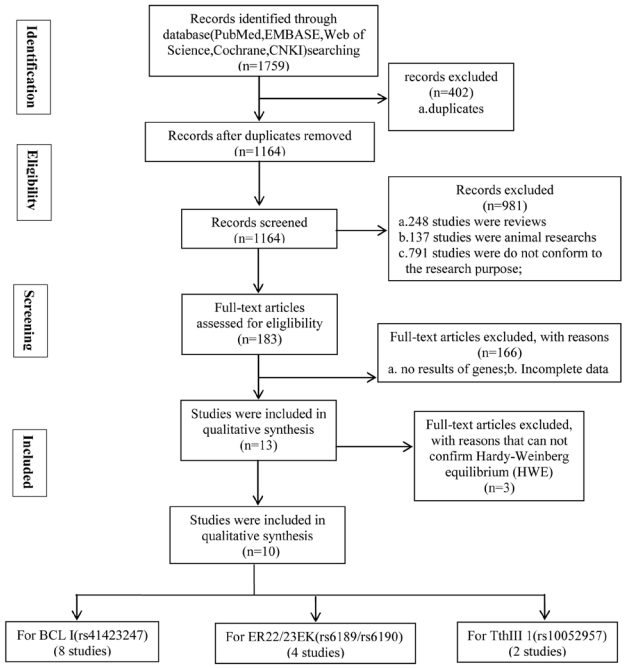

Figure 1. Flow chart depicting the selection of eligible studies during the meta-analysis.

Results

Selection and characteristics of studies

A total of 1759 potential articles were retrieved through electronic databases, including EMBASE (n = 533), PubMed (n = 535), Web of Science (n = 452), Cochrane Library (n = 102), and CNKI (n = 137), during initial searching. The study selection process is detailed in Fig 1. After 402 duplicated articles were removed and 981 articles were excluded by screening the title and abstract, 791 articles were found to be unrelated and 248 articles were reviews. The remaining 183 articles were full-text-reviewed, and 166 were excluded,for no result of genes and no completed data,7 articles were excluded for can not HWE. Finally, 14 studies reported in 10 articles (28-37)fulfilled the inclusion criteria and were included in the present meta-analysis.

The selected study characteristics and data are listed in Table 2. In addition, among the fourteen studies, three glucocorticoid receptor gene loci polymorphisms associated with ASTHMA susceptibility were reported, including *Bcl*I (1121 cases and 691 controls, from eight studies)(28-35). ER22/23EK (897 cases and 606 controls, from four studies)(28, 29, 36, 37), and TthIII1 (398 cases and 346 controls, from two studies)(28, 34). Various genotyping methods were utilized, including polymerase chain (PCR)- restriction fragment length polymorphism (RFLP), TaqMan, and SNPscan. All studies showed that the distribution of gene followed the HWE in Table 2. Furthermore, the NOS scores of all studies ranged from 7 to 9 stars.

Association between polymorphism *Bcl*I and ASTHMA susceptibility

The genetic association between polymorphism *Bcl*I and susceptibility to ASTHMA was measured. The *P* value of Cochran's-Q statistic were lower than 0.1, and those of I^2 were more than 50% for all genetic models; therefore, we applied the random-effect model to all genetic models. Overall, no significant association was found for heterozygote model(GC versus GG: OR (95% CI) = 0.71 (0.43–1.17))and dominant model(CC + GC versus GG: OR (95% CI) = 0.57(0.30–1.06)),but not in the allele model(C versus G: OR (95% CI) = 0.63 (0.40–0.97)), homozygote model(CC versus GG: OR (95% CI) = 0.41 (0.17–0.97)), and recessive model(CC versus CG + GG: OR (95% CI) = 0.54 (0.34–0.88)).The main meta-analysis results are shown in detail in Fig 2 and Table 3.

In addition, subgroup analyses by ethnicity showed no significant association in the Chinese population; this outcome was consistent with that observed in Caucasians. The main meta-analysis results are shown in detail in Fig 3 and Table 3. After subgroup analyses by ethnicity, the *P* value of Cochran's-Q statistic of all genetic models were lower than 0.1, and that of I^2 was more than 50% for all genetic models; therefore, we applied the random-effect model to all genetic models. Although, subgroup analyses by ethnicity in the Chinese showed the *P* value of Cochran's-Q statistic of the recessive model was greater than 0.1, and that of I^2 was less than 50%, we also chose the random-effect model for conservative estimates.

Subgroup analyses by age showed significant association in the adults (>=18 years); this outcome was different with that observed in the minors. In the adults, significant association was found for allele model(C versus G: OR (95% CI) = 0.48 (0.26–0.89)), homozygote model(CC versus GG: OR (95% CI) = 0.23 (0.07–0.74)), dominant model(CC + GC versus GG: OR (95% CI) = 0.35(0.12–0.99)) and recessive model(CC versus CG + GG: OR (95% CI) = 0.40 (0.22–0.75)),but not in the heterozygote model(GC versus GG: OR (95% CI) = 0.46 (0.20–1.06)).The main meta-analysis results are shown in detail in Fig 4 and Table 3. After subgroup analyses by age, the *P* value of Cochran's-Q statistic of all genetic models were lower than 0.1, and that of I^2 was more than 50% for all genetic models; therefore, we applied the random-effect model to all genetic models. Although, subgroup analyses by age of the adults in recessive model and of the minors in heterozygote

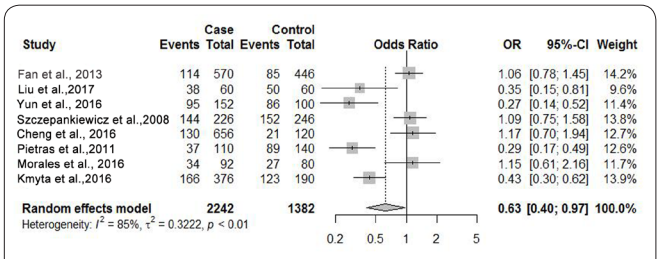

Figure 2. Association between polymorphism BclI and ASTHMA susceptibility (Allel model).

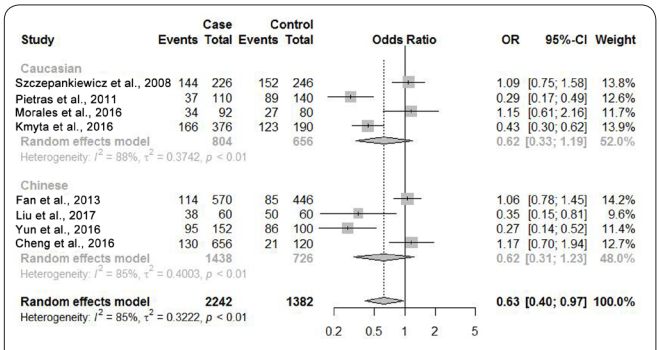

Figure 3. Subgroup analyses by ethnicity.

Table 2. Characteristics of studies included in the meta-analysis

Author Public year	Country	Ethnicity studied	Case						Control						P-HWE	Quality
BclI			**Age (mean±s)**	**Sex (Female /male)**	**n**	**CC**	**CG**	**GG**	**Age (mean±s)**	**Sex (Female /male)**	**n**	**CC**	**CG**	**GG**		
Fan et al., 2013 (50)	China	Chinese	2~12	100/185	285	12	90	183	18~35	110/113	223	10	65	148	0.41	4/1/3
Liu et al., 2017 (51)	China	Chinese	29.4±11.5	12/18	30	14	10	6	28.5±12.7	13/17	30	21	8	1	0.82	4/2/3
Yun et al., 2016 (52)	China	Chinese	9.0±2.9	22/54	76	37	21	18	9.1±3.0	22/54	50	38	10	2	0.23	3/2/3
Szczepankiewicz et al., 2008 (53)	Poland	Caucasian	12.0±3.3	45/68	113	43	58	12	9.8±2.0	64/59	123	47	58	18	0.99	4/1/3
Cheng et al., 2016 (54)	China	Chinese	43.4±20.7	116/212	328	20	90	218	43.9±19.6	29/31	60	3	15	42	0.29	3/2/3
Pietras et al., 2011 (55)	Poland	Caucasian	50.8±13.7	42/17	55	6	25	24	63.1±5.0	34/36	70	28	33	9	0.88	3/2/3
Morales et al., 2016 (56)	Chile	Caucasian	3.9±0.9	19/27	46	4	26	16	4.2±0.8	24/16	40	3	21	16	0.27	3/2/3
Kmyta et al., 2016 (57)	Ukraine	Caucasian	46.2±0.8	124/64	188	43	80	65	44.1±1.5	50/45	95	40	43	12	0.93	2/2/3
ER22/23EK			**Age (mean±s)**	**Sex (Female /male)**	**n**	**GG**	**AG**	**AA**	**Age (mean±s)**	**Sex (Female /male)**	**n**	**GG**	**AG**	**AA**	**P-HWE**	**Quality**
Szczepankiewicz et al., 2008 (53)	Poland	Caucasian	12.0±3.3	45/68	113	107	6	0	9.8±2.0	64/59	123	111	12	0	0.57	4/1/3
Cheng et al., 2016 (54)	China	Chinese	43.4±20.7	116/212	328	0	15	313	43.9±19.6	29/31	60	0	3	57	0.84	3/2/3
Panek et al., 2013 (36)	Poland	Caucasian	48.8±16.0	147/88	235	222	13	0	45.7±16.3	142/74	216	202	14	0	0.62	4/2/3
Panek et al., 2012 (58)	Poland	Caucasian	46.1±16.1	138/69	221	208	13	0	48.9±15.8	140/81	207	195	12	0	0.66	3/2/3
TthIII1			**Age (mean±s)**	**Sex (Female /male)**	**n**	**AA**	**AG**	**GG**	**Age (mean±s)**	**Sex (Female /male)**	**n**	**AA**	**AG**	**GG**	**P-HWE**	**Quality**
Fan et al., 2013 (50)	China	Chinese	2~12	100/185	285	2	42	241	18~35	110/113	223	0	36	187	0.18	4/1/3
Szczepankiewicz et al., 2008 (53)	Poland	Caucasian	12.0±3.3	45/68	113	16	57	40	9.8±2.0	64/59	123	11	58	54	0.41	4/1/3

Table 3. Summary of pooled ORs in the meta-analysis.

SNP	N	Allele model			Heterozygote model			Homozygote model			Dominant model			Recessive model		
		OR(95%CI)	I2(%)	P-H	OR(95%CI)	I2(%)	P-H	OR(95%CI)	I2(%)	P-H	OR(95%CI)	I2(%)	P-H	OR(95%CI)	I2(%)	P-H
BclI		C/G			GC/GG			CC/GG			CC+GC/GG			CC/GC+GG		
Overall	8	0.63(0.40-0.97)	85.1	<0.0001	0.71(0.43-1.17)	66.2	0.0042	0.41(0.17-0.97)	78.1	<0.0001	0.57(0.30-1.06)	80.4	<0.0001	0.54(0.34-0.88)	60.5	0.0132
Ethnicity																
Caucasian	4	0.62(0.33-1.19)	87.7	<0.01	0.65(0.28-1.50)	75.2	<0.01	0.40(0.10-1.54)	85.6	<0.01	0.55(0.20-1.50)	84.1	<0.01	0.52(0.24-1.13)	74.7	<0.01
Chinese	4	0.62(0.31-1.23)	84.5	<0.01	0.88(0.50-1.56)	43.4	0.15	0.43(0.12-1.49)	69.7	0.02	0.64(0.29-1.42)	72.0	0.01	0.57(0.29-1.11)	48.8	0.12
Age																
Adults	4	0.48(0.26-0.89)	81.4	<0.01	0.46(0.20-1.06)	68.4	0.02	0.23(0.07-0.74)	72.4	0.01	0.35(0.12-0.99)	82.2	<0.01	0.40(0.22-0.75)	46.6	0.13
Minors	3	0.71(0.31-1.66)	86.1	<0.01	0.97(0.41-2.30)	50.9	0.13	0.62(0.12-3.07)	77.5	0.01	0.74(0.23-2.40)	75.7	0.02	0.66(0.27-1.64)	69.5	0.04
No comparability	1	1.06(0.78-1.45)	--	--	1.12(0.76-1.65)	--	--	0.97(0.41-2.31)	--	--	1.10(0.76-1.59)	--	--	0.94(0.40-2.21)	--	--
ER22/23EK		A/G			AG/GG			AA/GG			AA+AG/GG			AA/AG+GG		
Overall	4	0.84(0.54-1.31)	0	0.75	0.80(0.49-1.30)	0	0.59	--	--	--	0.80(0.49-1.30)	0	0.59	--	--	--
Ethnicity																
Caucasian	3	0.81(0.50-1.30)	0	0.6	0.80(0.49-1.30)	0	0.59	--	--	--	0.80(0.49-1.30)	0	0.59	--	--	--
Chinese	1	1.10(0.31-3.84)	--	--	--	--	--	--	--	--	--	--	--	--	--	--
TthIII1		A/G			AG/GG			AA/GG			AA+AG/GG			AA/AG+GG		
Overall	2	1.19(0.89-1.60)	0	0.32	1.07(0.75-1.54)	4.6	0.31	2.10(0.91-4.82)	0	0.67	1.14(0.80-1.63)	21.4	0.26	1.81(0.83-3.95)	0	0.59

P-H: *P* value of heterogeneity.

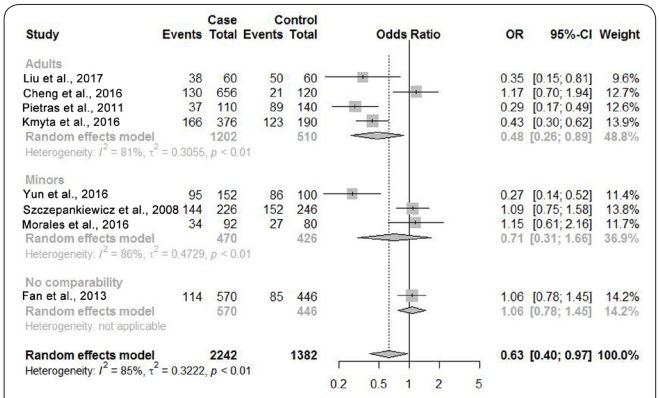

Figure 4. Subgroup analyses by age.

model showed the *P* value of Cochran's-Q statistic of the recessive model was greater than 0.1, and that of I^2 was less than 50%, we also chose the random-effect model for conservative estimates.

The weight of F.FAN 2013(34) was the largest in the allele model in Fig 2, heterozygote model and dominant model. After omitting F.FAN 2013, sensitivity analysis showed significant association in the allele model(C versus G: OR (95% CI) = 0.57 (0.35–0.94)), homozygote model(CC versus GG: OR (95% CI) = 0.35 (0.13–0.94)) and recessive model(CC versus CG + GG: OR (95% CI) = 0.50 (0.30–0.85)),but not in the heterozygote model and dominant model. After omitting Z.L.YUN 2016(33) and V.V.Kmyta 2016(32), none of models were found significant association. The main sensitivity analysis results are shown in detail in Fig 5 and Table 4.

Association between polymorphism ER22/23EK and ASTHMA susceptibility

We additionally pooled analyses of polymorphism ER22/23EK and ASTHMA; these are shown in Fig 6 and Table 3. The *P* value of Cochran's-Q statistic of all genetic models were greater than 0.1 and I^2 was less than 50%, indicating that there was no significant heterogeneity between the studies; therefore, the fixed-effect model was implemented. Overall, no significant associations were detected in the allele model, heterozygote model, and dominant model (A versus G: OR (95% CI) = 0.84 (0.54-1.31), AG versus GG: OR (95% CI) = 0.80 (0.49-1.30), AA + AG versus GG: OR (95% CI) = 0.80 (0.49-1.30)).

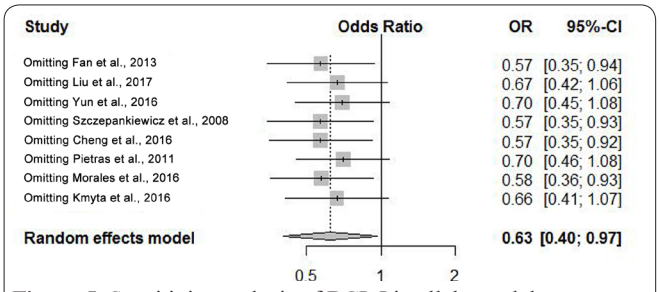

Figure 5. Sensitivity analysis of BCL I in allele model.

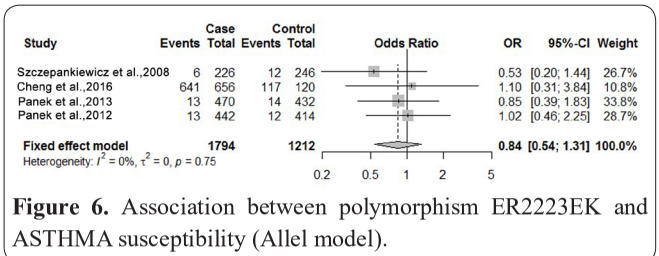

Figure 6. Association between polymorphism ER2223EK and ASTHMA susceptibility (Allel model).

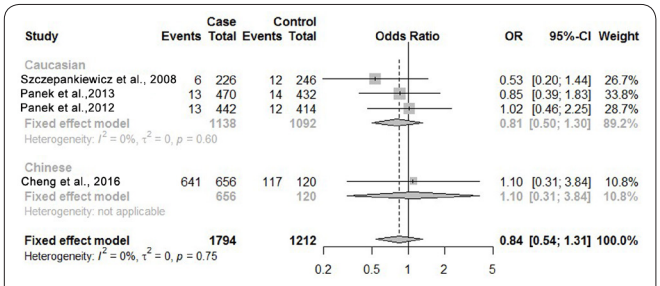

Figure 7. Subgroup analyses by ethnicity.

Furthermore, subgroup analyses by ethnicity showed no significant association of the allele model in the Chinese population and no significant associations were observed for the allele model, heterozygote model, and dominant model in Caucasians. The main sensitivity analysis results are shown in detail in Fig 7 and Table 3.

Szczepankiewicz 2008 (28) was the only study in the minors and Z.Cheng 2016(29) was the only study in the Chinese. After omitting Szczepankiewicz 2008(28), sensitivity analysis showed no significant association in the allele model, heterozygote model and dominant model. After omitting Z.Cheng 2016(29), sensitivity analysis also showed no significant association in the allele model, heterozygote model and dominant model. Regardless of age and ethnicity, the outcome is stable. The main sensitivity analysis results are shown in detail in Fig 8 and Table 4.

Association between polymorphism TthIII1 and ASTHMA susceptibility

The association between polymorphism TthIII1 and the risk of ASTHMA was analyzed in two studies. The *P* value of Cochran's-Q statistic for all genetic models was greater than 0.1, and that of I^2 less than 50%, indicating that there was no significant heterogeneity between the studies; therefore, the fixed-effect model was applied.There were no notable associations for any of the genetic models (A versus G: OR (95% CI) = 1.19 (0.89–1.60), AG versus GG: OR (95% CI) = 1.07 (0.75–1.54), AA versus GG: OR (95% CI) = 2.10 (0.91–4.82), AA + AG versus GG: OR (95% CI) = 1.14 (0.80–1.63), AA versus AG + GG: OR (95%CI) = 1.81 (0.83–3.95)) (Fig 9 and Table 3).

Publication bias

There was no publication bias with regards to the association between *Bcl*I, ER22/23EK, and TthIII1 po-

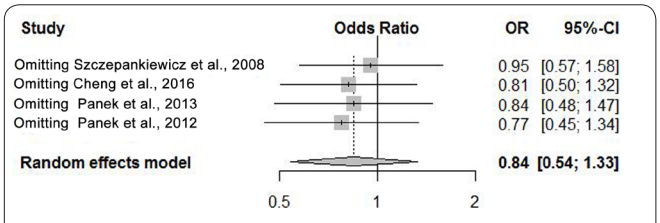

Figure 8. Sensitivity analysis of ER2223EK in allele model.

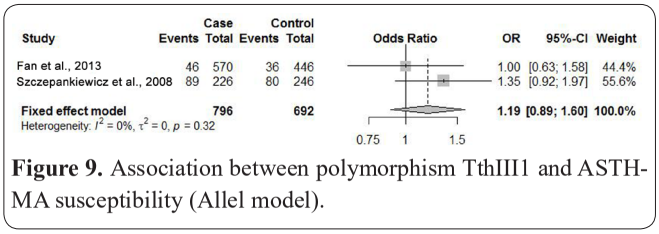

Figure 9. Association between polymorphism TthIII1 and ASTHMA susceptibility (Allel model).

Table 4. Sensitivity analysis.

SNP	Allele model			Heterozygote model			Homozygote model			Dominant model			Recessive model		
	OR(95%CI)	I^2(%)	*P*-E	OR(95%CI)	I^2(%)	*P*-E	OR(95%CI)	I^2(%)	*P*-E	OR(95%CI)	I^2(%)	*P*-E	OR(95%CI)	I^2(%)	*P*-E
BclI	**C/G**			**GC/GG**			**CC/GG**			**CC+GC/GG**			**CC/GC+GG**		
Omitting Fan et al., 2013 (50)	**0.57(0.35- 0.94)**	**84.3**	**0.03**	0.62(0.33- 1.15)	65.7	0.13	**0.35(0.13- 0.94)**	**78.8**	**0.04**	0.48(0.23- 1.04)	79.9	0.06	**0.50(0.30- 0.85)**	**62.9**	**0.01**
Omitting Liu et al., 2017 (51)	0.67(0.42- 1.06)	86.4	0.08	0.74(0.45- 1.24)	68.8	0.26	0.46(0.18- 1.14)	80.3	0.09	0.62(0.33- 1.16)	81.9	0.14	**0.57(0.34- 0.97)**	**65.0**	**0.04**
Omitting Yun et al., 2016 (52)	0.70(0.45- 1.08)	84.2	0.11	0.77(0.46- 1.28)	67.1	0.31	0.48(0.19- 1.20)	78.8	0.12	0.66(0.35- 1.22)	80.1	0.18	0.60(0.36- 1.00)	60.1	0.05
Omitting Szczepankiewicz et al., 2008 (53)	**0.57(0.35- 0.93)**	**85.3**	**0.03**	0.62(0.36- 1.09)	68.0	0.10	**0.33(0.13- 0.83)**	**74.5**	**0.02**	**0.48(0.24- 0.97)**	**81.9**	**0.04**	**0.47(0.29- 0.76)**	**46.9**	**0.00**
Omitting Cheng et al., 2016 (54)	**0.57(0.35- 0.92)**	**86.0**	**0.02**	0.63(0.35- 1.15)	69.6	0.13	**0.35(0.13- 0.89)**	**79.4**	**0.03**	0.49(0.23- 1.02)	82.1	0.06	**0.50(0.30- 0.83)**	**62.7**	**0.01**
Omitting Pietras et al., 2011 (55)	0.70(0.46- 1.08)	82.6	0.11	0.82(0.51- 1.34)	59.5	0.43	0.53(0.23- 1.23)	73.5	0.14	0.69(0.38- 1.25)	76.1	0.22	**0.62(0.40- 0.98)**	**51.2**	**0.04**
Omitting Morales et al., 2016 (56)	**0.58(0.36- 0.93)**	**86.5**	**0.02**	0.64(0.36- 1.13)	70.0	0.13	**0.35(0.14- 0.90)**	**80.2**	**0.03**	0.50(0.25- 1.00)	82.6	0.05	**0.52(0.31- 0.85)**	**64.3**	**0.01**
Omitting Kmyta et al., 2016 (57)	0.66(0.41- 1.07)	84.1	0.09	0.83(0.51- 1.35)	55.8	0.46	0.46(0.17- 1.22)	76.9	0.12	0.66(0.35- 1.23)	76.0	0.19	0.58(0.33- 1.02)	62.4	0.06
ER22/23EK	**A/G**			**AG/GG**			**AA/GG**			**AA+AG/GG**			**AA/AG+GG**		
Omitting Szczepankiewicz et al., 2008 (53)	0.95(0.57- 1.58)	0	0.85	0.92(0.53- 1.62)	0	0.78	--	--	--	0.92(0.53- 1.62)	0	0.78	--	--	--
Omitting Cheng et al., 2016 (54)	0.81(0.50- 1.32)	0	0.40	0.81(0.49- 1.32)	0	0.39	--	--	--	0.81(0.49- 1.32)	0	0.39	--	--	--
Omitting Panek et al., 2013 (36)	0.84(0.48- 1.47)	0	0.55	0.78(0.41- 1.49)	2.9	0.45	--	--	--	0.78(0.41- 1.49)	2.9	0.45	--	--	--
Omitting Panek et al., 2012 (58)	0.77(0.45- 1.34)	0	0.36	0.71(0.38- 1.31)	0	0.27	--	--	--	0.71(0.38- 1.31)	0	0.27	--	--	--

lymorphisms and ASTHMA susceptibility, as identified using the Begg's funnel plot or Egger's regression test (Table 5). Funnel plots of the above three genetic loci in all genetic models were symmetrical (Fig 10). Owing to the limited number of studies of TthIII1 in all genetic models and ER22/23EK in recessive model included, funnel plot analysis and the Egger's test were not carried out.

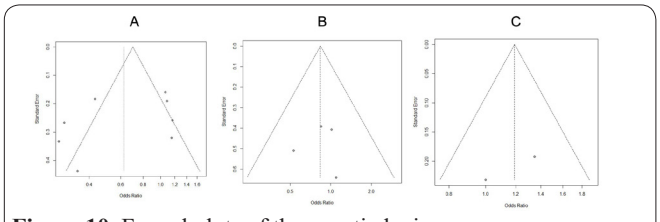

Figure 10. Funnel plots of the genetic loci.

Discussion

In the present meta-analysis, we evaluated the association between single nucleotide polymorphisms (SNPs) in the following glucocorticoid receptor genes: *Bcl*I (1121 cases and 691 controls), ER22/23EK (897 cases and 606 controls), TthIII1 (398 cases and 346 controls) and ASTHMA. Overall analysis indicated that there was no association between ER22/23EK, TthIII1 and ASTHMA susceptibility for any of the genetic models. However, we identified a negative relationship between *Bcl*I and ASTHMA in the allele model, homozygote model, and recessive model. Subgroup analyses by age indicated that there was a negative relationship between *Bcl*I and ASTHMA for allele model, homozygote model, dominant model and recessive model in the adults.

The biological effects of GC need to be mediated by GR. GC can inhibit the activation of many inflammatory cells and factors through binding to receptors, thereby reducing vascular permeability and reducing airway hyperresponsiveness. Hurley et al.(16)found that mutations in the NR3C1 gene can cause familial glucocorticoid resistance. The NR3C1 gene polymorphism can also cause overexpression of related cytokines (such as IL-4) or various key molecules that induce hormone resistance(38), leading to decreased GC sensitivity.

The BclI polymorphism refers to a mutation located 646 nucleotides downstream of exon 2 of the NR3C1 gene: the C mutation to G (TGATCA is mutated to TGATGA) and it may produce two fragments of 2.2 kb and 3.9 kb in length(39). In 2006, Anja Rogausch et al. (40) studied the Bcl 1 polymorphism and smoking behavior. The study subjects were COPD and bronchial asthma. The results showed that the existence of the polymorphism was related to the degree of nicotine dependence. Patients with this polymorphism had a greater degree of dependence on nicotine than the control group. Harriet Corvol et al. (41)studied the patients with pulmonary fibrosis and found that the Bcl I polymorphism may be related to the inflammatory response in the lung tissues of patients with pulmonary fibrosis regulation, so that the GC exerts normal anti-inflammatory effects and is disturbed, so that the lung fibers Patients with accelerated pulmonary function were accelerated. At present, the results of the BclI polymorphism are quite different. In this study, 10(28-36, 42)studies that were qualitatively included after screening were selected, and two studies(36, 42) that did not meet HWE were excluded. The results showed that BCL I polymorphisms negatively related to asthma.

The ER22/23EK polymorphism is located in exon 2 of the GR gene and is a two-linked single-nucleotide mutation in codons 22 and 23, where mutation at codon 22 (GAG mutation to GAA) does not cause Amino acid changes all encode glutamate, but the latter mutation (AGG mutation to AAG) changes arginine to lysine(43). Current research shows that almost all the studies on the association between ER22/23EK polymorphism and asthma are negative. The results of meta-analysis were excluded from the two papers(34, 42)which did not meet the HWE study. The results were analyzed for ER22/23EK polymorphism showed not related to asthma.

The N363S polymorphism is located on the exon 2 of the GR gene. The base A at position 1220 is replaced by G. The encoded amino acid 363 changes from asparagine to serine, and the serine residue provides phosphorylation sites. Amines do not provide a phosphorylation site and hyperphosphorylation increases GR gene expression and may therefore increase the sensitivity of GR to GC(37). Huizenga NA(44) found that mutation of aspartic acid to serine on codon 363 of GR gene No. 2 resulted in increased sensitivity of GR to glucocorticoids and sensitivity of glucocorticoid receptors to glucocorticoids. Increased sexuality**(45)**. There is very little research on N363S polymorphisms and bronchial asthma. A total of 5(28, 29, 34, 37, 42) studies in this study found that the N363S polymorphism was associated with asthma. However, unfortunately, when extracting data, it was found that only one(28) accorded with HWE balance, and the others were not in compliance, so they were excluded. Look forward to higher quality research later.

The TthIII1 polymorphism is a mutation located at position 3807 in the GR gene upstream of the 5' region of the GR gene (C mutation is T), which may produce two fragments of 3.4 kb and 3.8 kb in length(43). Studies have found no interaction between TthIII1 and N363S and BclI, but all ER22/23EK polymorphism carriers also carry the TthIII1 T mutant, suggesting that the TthIII1 polymorphism may not function by itself (46) . In this study, a total of 5 articles (28, 29, 34, 36, 42) were included in the study on the relationship between TthIII1 polymorphism and asthma. Only two

Table 5. Begg's funnel plot and Egger's test of the meta-analysis.

SNP	Allele model		Heterozygote model		Homozygote model		Dominant model		Recessive model	
	Begg's	Egger	Begg's	Egger	Begg's	Egger	Begg's	Egger	Begg's	Egger
BclI	0.22	0.31	0.22	0.15	0.62	0.66	0.14	0.13	1	0.95
ER22/23EK	1	0.91	0.60	0.29	--	--	0.60	0.29	--	--
TthIII1	--	--	--	--	--	--	--	--	--	--

articles(28, 34) were included, and the remaining three articles were included.

To explore the heterogeneity among the studies, we conducted a subgroup analysis for *Bcl*I and ER22/23EK. For *Bcl*I and ER22/23EK, subgroup analysis by ethnicity did not reveal an association in either population. However, subgroup analysis by age for *Bcl*I revealed an association between adults and minors. A gender disparity exists in asthma and changes with age. As children, boys have an increased prevalence of asthma compared to girls (47). In contrast, as adults, women have an increased prevalence of asthma compared to men.This switch in asthma prevalence coincides with puberty, suggesting that sex hormones are important in asthma pathogenesis (48, 49). This article failed to analyze the reasons for this factor, there is no report of genetic polymorphism related literature.

We conducted a sensitivity analysis for *Bcl*I and ER22/23EK. Cases and controls were not matched by age in FAN et al. (34). After omitting FAN et al. (34), sensitivity analysis showed significant association between *Bcl*I and ASTHMA in the allele model, homozygote model and recessive model. For ER22/23EK, Szczepankiewicz et al. (21) was the only study in the minors and Cheng et al. (29) was the only study in the Chinese. After omitting Szczepankiewicz et al. (28), sensitivity analysis showed no significant association in the allele model, heterozygote model and dominant model. After omitting Cheng et al. (29), sensitivity analysis also showed no significant association in the allele model**,** heterozygote model and dominant model. Regardless of age and ethnicity, the outcome is stable.

To the best of our knowledge, the present work is the first to evaluate the association between glucocorticoid receptor gene polymorphisms and ASTHMA susceptibility via a meta-analysis. Our meta-analysis has several strengths. We did not limit the specific loci; all previously reported SNPs were included for analysis. According to the results of NOS, the methodological quality of the study was high. Moreover, we identified the main source of heterogeneity via subgroup analysis. Finally, no publication bias was identified by either Begg's funnel plot or Egger's regression test, except in the case of TthIII1.

The present study also has several limitations. For instance, the number of included studies was very low for TthIII1, which limited further analysis. Secondly, we were unable to extract sufficient adjustment data for certain factors, such as the types of asthma.

In summary, our meta-analysis comprehensively and systematically evaluated the association between glucocorticoid receptor gene polymorphisms and ASTHMA susceptibility. The results suggested that ER22/23EK, and TthIII1 polymorphisms are not associated with susceptibility to ASTHMA, while *Bcl*I polymorphisms are significantly associated with ASTHMA in the allele model, homozygote model and recessive model. In adults, *Bcl*I polymorphisms are also significantly associated with ASTHMA in the allele model, homozygote model, dominant model and recessive model. Given the limited sample size, the conclusions of this study should be treated with caution, and large sample studies are necessary in the future. These results will be useful for genetic testing of ASTHMA susceptibility in *Bcl*I. In addition, ASTHMA susceptible populations can be identified by genetic testing and the corresponding measures can be taken to protect them.

Conflict of interest

The authors declare that they have no conflict of interest.

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