An association and meta-analysis study of 4 SNPs from beta-2 adrenergic receptor (ADRB2) gene with risk of asthma in children

Xingqing Guo,[1] Hongying Zheng,[2] Chenggang Mao,[1] Enben Guan[1] and Hui Si[1]

Summary

Background: **Although various case-control studies have been conducted to investigate the relationship between ADRB2 gene polymorphisms and asthma risk in different population groups, the results have been conflicting and inconclusive.**

Objective: **We performed a case-control study to investigate the association of 4 SNPs in the ADRB2 gene with risk of asthma in children, and then conducted a meta-analysis by combining the previous studies.**

Methods: **A total of 340 patients and 340 age-matched healthy controls were recruited. All of the subjects were genotyped using the PCR-based invader assay. The case-control study was performed to define the contribution of rs1042713, rs1042714, rs1800888, and rs1042711 to the predisposition of asthma. Additionally, we further conducted a meta-analysis of the study findings together with those of previously reported studies.**

Results: **No significant association was found between the polymorphisms rs1042713, rs1042714, rs1800888, and rs1042711 and asthma in the current case-control study. The meta-analysis confirmed that there was no positive association of these SNPs with asthma in children in Asia, South America, Europe and the overall population.**

Conclusions: **None of the four polymorphisms in ADRB2 gene were associated with a risk of asthma in a current children population.** ***(Asian Pac J Allergy Immunol 2016;34:11-20)***

Keywords: *asthma, single nucleotide polymorphisms, ADRB2, gene, pediatrics*

From 1. Department of Pediatrics, The Affiliated Hospital of Qingdao University, No.1677 Wutaishan Road, Kaifa District, Qingdao, Shan dong Province, 266555, China
2. Clinical Laboratory, The Affiliated Hospital of Qingdao University, No.1677 Wutaishan Road, Kaifa District, Qingdao, Shan dong Province, 266555, China
Corresponding author: Hongying Zheng
E-mail: zhy_pediatrics@126.com
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Introduction

Asthma, which is characterised by variable airway obstruction caused by bronchial hyper-reactivity and airway inflammation, is one of the most common chronic respiratory diseases worldwide. It is a common and combinatorial disorder with genetic, lifestyle and environmental determinants.[1] It is prevalent in developed nations and is becoming an important health issue in many developing countries. Asthma currently affects almost 6.8 million children in the US and 1.1 million in the UK. It accounts for nearly 500,000 hospitalisations, 2 million emergency department visits, about 10,000 paediatric intensive care unit admissions, and 5,000 deaths in the United States each year.[2-4] The high prevalence of asthma in children has been reported in the UK, with an estimated prevalence of 1 in 9.[5] Visits to the doctor because of asthma have more than doubled in the last decade.[4] Despite some advances in treatment, it is a major cause of socioeconomic burden on the healthcare system and results in a poor quality of life for sufferers.

The aetiology of asthma is not yet clear. An increasing number of studies have focused on asthma genetics research.[6-8] Therefore, the identification of asthma susceptibility genes contributing to asthma pathogenesis is important. In a large number of asthma susceptibility genes, the beta-2 adrenergic receptor (ADRB2, also known as beta2-AR) gene has been extensively studied.

ADRB2 is located on chromosome 5q31–32, encodes 413 amino acids, and is an intronless gene.[9] ADRB2 is a non-intronic, relatively small (1.2-kb) gene that encodes a protein of 413 amino acids. 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 activation of beta2-AR can result in the expansion of the small airways, and thus beta2-AR agonists are used in first-line bronchodilator therapy in asthma.[10] It has also been reported that ADRB2 variants are associated with airway hypersensitivity, asthma severity, and the response to medications.[11,12] In the ADRB2 gene, numerous polymorphisms have been identified. The most widely analysed single-nucleotide polymorphisms (SNPs) in the coding gene region include Arg16Gly (A46G, rs1042713), Gln27Glu (C79G, rs1042714), Thr164Ile (C491T, rs1800888) and Arg19Cys (T-47C, rs1042711).[13] 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 leads to down-regulation of B2-AR. B2-AR binds specifically to a class of ligands that can lead to the expansion of the small airways. Thr164Ile is also located in the coding region of theADRB2 gene, which can cause a change in amino acid from threonine (Thr) to isoleucine (Ile). Moreover, studies of the ADRB2 gene have not been confined to coding region polymorphisms alone, as increasingly more studies have begun to pay attention to promoter region polymorphisms. Arg19Cys is located in the 5' leader region that harbours an open reading frame (ORF) in the promoter region of the ADRB2 gene, which can impede the translation of ADRB2 mRNA, and regulate cellular expression of the receptor.[14] Therefore, we chose the above four important SNPs in the current study.

Although various case-control studies have been conducted to investigate the relationship between the ADRB2 gene polymorphisms and asthma risk in different populations, the results have been conflicting and inconclusive[15,16]. To confirm the association between SNPs from the ADRB2 gene and asthma, we performed a case-control study for the association of all 4 SNPs from the ADRB2 gene with risk of asthma in Chinese children, and then conducted a meta-analysis to derive a relatively comprehensive picture of the relationship between the ADRB2 gene polymorphisms and asthma risk in children.

Methods

Study design

This was a hospital-based case–control study conducted in the Department of Paediatrics, The Affiliated Hospital of Qingdao University, between September 2010 and May 2014. The present study was approved by the ethics committee of Qingdao University, and all participants gave written, informed consent.

Subjects

We recruited 680 subjects (340 asthmatic and 340 controls) of the same ethnicity. In screening of asthma, asthma diagnosis was made according to GINA recommendations, based on clinical asthma symptoms and lung function test (bronchodilator responsiveness, exercise-induced hyperresponsiveness). The subjects were excluded because of pneumonia, tuberculosis, disseminated bronchiectasis, bronchiolitis, pneumothorax, pyothorax, immunocompromised status, malignancy and age above 15 years. The control group met the following criteria: (1) 1–15 years of age, no present symptoms or history of asthma or other respiratory disease, (2) no history of atopy and (3) no family history of asthma in mother, father or sibling. Severe asthma was defined as follows: symptoms requiring daily therapy with high-dose inhaled corticosteroids (> 800 budesonide or > 500 fluticasone), despite regular treatment with long-acting 2-agonists and/or leukotriene antagonist and/or theophylline (slow releasing), and 1 or more emergency care visit or oral steroid burst per year.

Genomic DNA extraction and genotyping of ADRB2 variants

We selected the Arg16Gly (rs1042713), Gln27Glu (rs1042714), Thr164Ile (rs1800888) and Arg19Cys (rs1042711) single nucleotide polymorphisms (SNPs) for association analysis. Genomic DNA was extracted from peripheral blood leukocytes using genomic DNA isolation kits (Promega, Madison, WI) according to the manufacturer's instructions. The primers, probes and reaction conditions are available upon request. SNPs were genotyped by the PCR-based invader assay (Third Wave Technologies) using an ABI 7900 (Applied Biosystems, Foster City, CA, WI).[17] Genotyping was done by laboratory personnel blinded to subject status. Of the samples,

10% were tested twice to validate the genotyping results with 100% reproducibility. Two authors independently reviewed the genotyping results, data entry, and statistical analysis.

Meta-analysis

Firstly, studies were included if they were case-control or cohort studies that evaluated the association between the ADRB2 gene polymorphisms and risk of asthma and included data about the genotype distributions or allele frequency of the above 4 SNPs in the ADRB2 gene. Secondly, two of the authors independently extracted th following data from each full-text report using a standard data extraction form. The data extracted from the studies included the title, authors, year of publication, study design, number of cases or controls, ethnicity, genotyping method, genotype distribution, and allele frequency of the 4 SNPs in cases or controls.

Statistical analysis

Standard$^{\chi}$ analysis was used to examine differences in allelic frequencies and genotype distributions of the 4 SNPs between asthmatic patients and controls in case-control study. Hardy–Weinberg equilibrium was tested by a goodness-of-fit$^{\chi}$ test. Meta-analysis was performed with STATA 12.0 (Statacorp, college station, Tex). The association between the 4 SNPs and asthma susceptibility was assessed under the following genetic models, which were treated as a dichotomous variable: (a) A-allele versus a-allele for allele level comparison; (b) AA+Aa versus aa for a dominant model; (c) AA versus Aa+aa for a recessive model, and (d) AA versus aa for the homozygote genotype comparison. Two-sided *P* values less than 0.05 were considered statistically significant. The Q-test and I^2 test were used to assess the effect of heterogeneity. Heterogeneity was considered statistically significant when Q-test (P <0.10) or I^2>50%. If heterogeneity was indicated, data were combined according to the random-effects model; when the Q-test (P >0.10) or I^2<50%, the fixed-effect model was used. Stratified analysis was performed by ethnicity. Publication bias was conducted both visually by using a funnel plot and statistically via Begg funnel plots and Egger's bias test, which measure the degree of funnel plot asymmetry.[18,19]

Results

Demographic details

A total of 680 subjects (340 cases and 340 controls) were successfully genotyped and subjected to statistical analysis. Table 1 represents the demographic characteristics of the studied subjects. Among the case and control group, there were no significant differences in gender, age, weight and height. Severe asthma was reported in 151 (44.4%). The duration of asthma was 14±4.4 months. The medication information in asthma patients was as follows: β2-adrenoceptor in 313 (92.1%); inhaled corticosteroids in 192 (56.5%); leukotriene antagonist in 176 (51.8%); and theophylline in 179 (52.6%).

Table 1. Characteristics of the recruited patients and controls

Characteristics	Asthma group (n = 340)	Control group (n = 340)	*P*-value
Sex, female (n%)	156 (45.9)	171 (50.3)	0.78
Age in month (mean ± SD)	73.5±39.8	76.1±40.0	0.21
Weight in kg (mean ± SD)	18.9±9.0	19.3±9.8	0.79
Height in cm (mean ± SD)	108.9±22.5	110.1±21.4	0.76
BMI (mean ± SD)	17.1±2.5	16.9±2.2	0.41
Severe asthma (n, %)	151 (44.4%)	-	-
Medication			
β2-adrenoceptor (n, %)	313 (92.1%)	-	-
Inhaled corticosteroids (n, %)	192 (56.5%)	-	-
Leukotriene antagonist	176 (51.8%)	-	-
Theophylline	179 (52.6%)	-	-
Duration of disease (months)	14±4.4	-	-

Association of ADRB2 gene variants with asthma compared with controls in the case–control study

The distributions of the alleles and genotypes for the 4 SNPs of the ADRB2 gene (including rs1042713, rs1042714, rs1800888, and rs1042711) were presented in Table 2. Genotype frequencies of the GDM group and the control group conformed to Hardy–Weinberg equilibrium. In accordance with the genome-wide association study (GWAS), no significant association was found between the polymorphisms rs1042713, rs1042714, rs1800888, and rs1042711 and asthma in the current case-control study.

Table 2. Allele and genotype frequencies of the 4 SNPs of ADRB2 gene in a Chinese population

SNP		Genotype (%)			P	Allele (%)		P	OR (95%CI)
Arg16Gly (rs1042713)		AA	AG	GG		A	G		
	Asthma group	84	218	38		386	294		
	Control group	116	184	40	0.27	416	264	0.28	0.83 (0.67-1.03)
Gln27Glu (rs1042714)		CC	CG	GG		C	G		
	Asthma group	176	138	26		490	190		
	Control group	178	120	42	0.25	476	204	0.17	1.11 (0.87, 1.40)
Thr164Ile (rs1800888)		CC	CT	TT		C	T		
	Asthma group	338	2	0		678	2		
	Control group	337	2	1	0.58	676	4	0.56	2.01 (0.37, 10.99)
Arg19Cys (rs1042711)		TT	TC	CC		T	C		
	Asthma group	154	152	34		460	220		
	Control group	162	154	24	0.40	478	202	0.29	0.88 (0.70, 1.11)

Meta-analysis

A total of 96 titles and abstracts were preliminarily reviewed, of which 13 of the published studies eventually satisfied the eligibility criteria.[20-32] All of the included studies investigated the relationship between one of the 4 SNPs and asthma. We therefore performed a meta-analysis by combining previous studies and the present case-control study. Ultimately, 14 studies investigated the relationship between Arg16Gly (rs1042713) and asthma,[20-32] 12 studies studied Gln27Glu (rs1042714),[20,21,23-32] 2 studies focused on Thr164Ile (rs1800888),[21] and 2 studies on Arg19Cys (rs1042711).[24] Characteristics of the studies that were included in the meta-analysis are presented in Table 3.

For Arg16Gly (rs1042713), there was no significant association in any of the genetic model comparisons in the Asia, South American, European and overall populations (Figure 1, Table 4). ForGln27Glu (rs1042714), no evidence of an association with asthma risk was found in the South American and European population in any of the genetic model comparisons. A significant association was found in the Asia population in the dominant model and homozygote model comparison, but not in the other genetic comparisons (Figure 2, Table 4). For Thr164Ile (rs1042713), only two case-control studies in Asia were included. There was no evidence of any association with asthma risk in any of the genetic models in the Asia population (Table 4). For Arg19Cys (rs1042711), only two case-control studies provided genotype distribution data. No significant association was found in the Asia, European and overall population in any of the genetic models (Table 4).

Publication bias

The publication bias test was performed for the Arg16Gly and Gln27Glu polymorphism in all of the model comparisons. No significant publication bias was shown by Begg rank correlation method and Egger weighted regression method.

Discussion

In the present study, the relationship between all 4 related ADRB2 gene polymorphisms and the overall risk of asthma was examined in a case-control study. Furthermore, a meta-analysis was also conducted to evaluate the pooled results of the association between the ADRB2 gene polymorphisms and risk of asthma in children. The pathogenesis of asthma in adults and children may differ, but the exact mechanism remains unknown and needs further detailed research. Therefore, the present case-control study and meta-analysis was performed only in children. The purpose of the present study was to provide more information for asthma candidate gene research in children.

Four ADRB2 polymorphisms were included in the study. The results indicated that Arg16Gly, Gln27Glu, Thr164Ile, and Arg19Cys were not associated with the risk of asthma in the present population. The extensive studies on Arg16Gly and Gln27Glu polymorphisms were based on their localisation within (codon 16) or in the vicinity (codon 27) of 2 tripeptides (N-X-S/T) that form a consensus for N-glycosylation involved in receptor expression on the cell surface and in binding to G_s proteins.[33,34] The functionality of the Arg16Gly

Table 3. Characteristics of included studies in Meta-analysis

Author	Year	Country	Ethnicity	Asthma group		Control group		Diagnostic criteria	Detection method
				Sample size	Age	Sample size	Age		
Zhang XY	2008	China	Asia	217	1-17	50	2-13	The guideline of treatment for bronchial asthma in children	PCR-RFLP
Zhang Z	2012	China	Asia	212	7.7±2.6	52	7.7±2.5	Guidelines of prevention and treatment of bronchial asthma in children	PCR-SSCP
Xie Y	2008	China	Asia	57	5.0±2.8	62	5.3±3.4	The guideline of treatment for bronchial asthma in children	PCR-SSCP
Liao W	2001	China	Asia	50	1.2-11.7	50	2.5-13.2	The Chinese medical association respiratory of bronchial asthma	PCR-RFLP
Zheng BQ	2012	China	Asian	198	0-14	110	0-14	The Chinese medical association respiratory of bronchial asthma	PCR-RFLP
Isaza C	2012	Colombia	South America	109	11.6±5.4	137	11.8±5.2	Standardized questionnaires with detailed questions on the occurrence and severity of symptoms of asthma	PCR
Szczepankiewicz A	2009	Polish	Europe	113	6-18	123	10.0±2.2	GINA recommendations, based on clinical asthma symptoms and lung function test	PCR-RFLP
Chan IH	2008	China	Asia	298	5-18	175	5-18	The American thoracic society guideline	PCR-RFLP
Wang JY	2009	China	Asia	449	7.8±3.8	512	8.4±2.5	2006 global initiative for asthma guideline	Taqman
Lv J	2009	China	Asia	192	3-12	192	3-12	2006 global initiative for asthma guideline	PCR-RFLP
Binaei S	2003	USA	Europe	38	NA	155	NA	The American thoracic society guideline	PCR-RFLP
Leung TF	2002	China	Asia	76	5-15	70	11.3±3.8	The American thoracic society guideline	PCR
Lin YC	2003	China	Asia	80	NA	69	NA	The Chinese medical association respiratory of bronchial asthma	PCR

NA, not available; PCR-RFLP, polymerase chain reaction restriction fragment-length polymorphism; PCR-SSCP, polymerase chain reaction–single strand conformation polymorphism; SD, standard deviation

polymorphism was determined *in vitro* by Green et al., who demonstrated that the Gly allele was associated with more rapid receptor desensitisation in response to β_2-agonists, in comparison to the Arg allele. In the case of the Gln27Glu polymorphism, the Glu variant underlies down-regulation to a lesser degree than Gln in cultures of human primary airway smooth muscle cells after chronic exposure to β_2-agonists. That may suggest a protective role of this variant in regard to receptor desensitisation. In our case-control study and meta-analysis, we did not observe any association between the Arg16Gly and Gln27Glu polymorphisms and asthma in children. The findings of the current study are consistent with previous studies. A meta-analysis showed a non-significant odds ratio for the Arg16Gly and Gln27Glu polymorphisms. Contopoulos-Ioannidis et al. conducted a meta-analysis and found that polymorphisms in the ADRB2 gene are not major risk factors for the development of asthma in the entire population.[11] Although the present study was confined to children, there was no significant association between the Arg16Gly polymorphism or the Gln27Glu polymorphism and the risk of asthma.

No significant association with the risk of asthma was found for the Thr164Ile and Arg19Cys

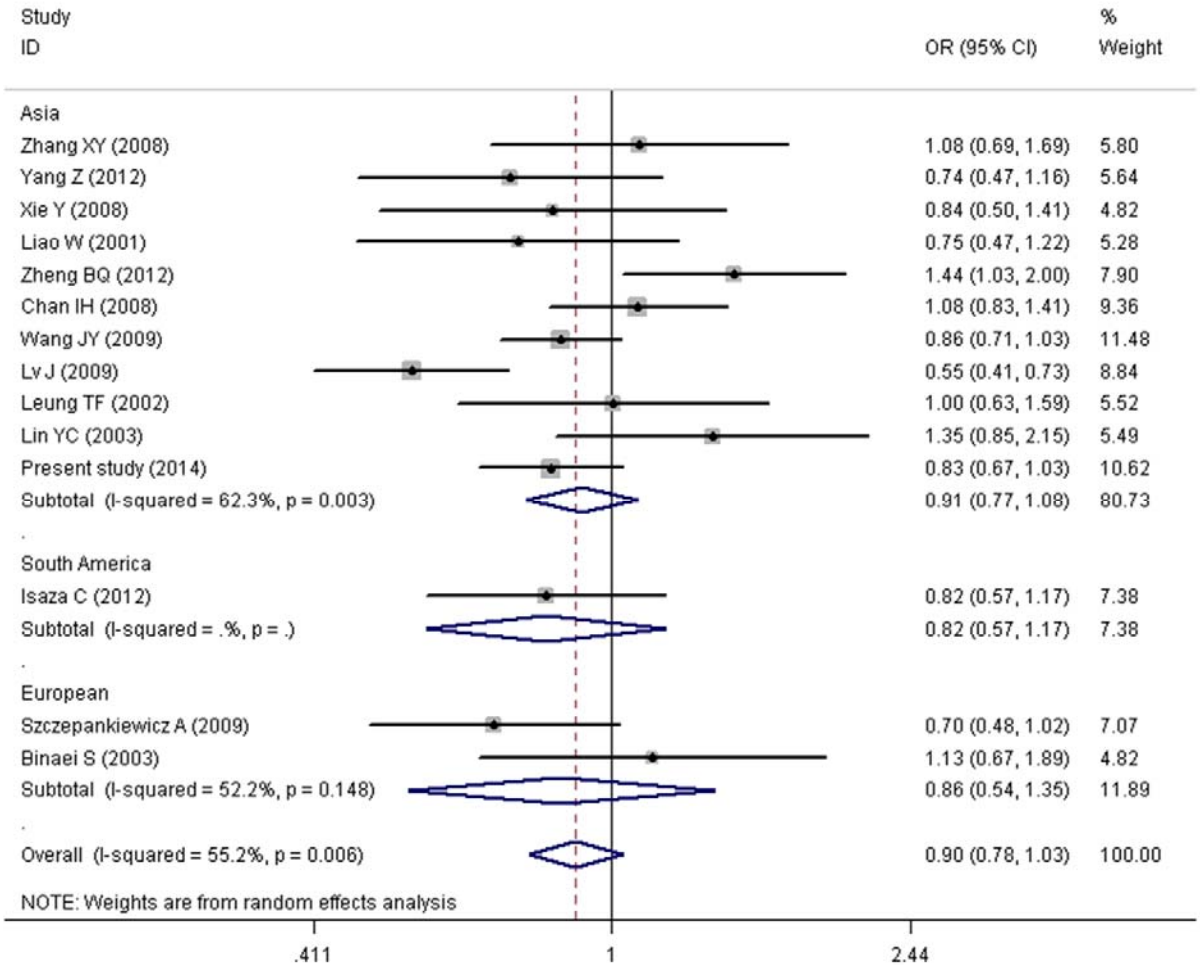

Figure 1. Forest plots of the association between the Arg16Gly (rs1042713) polymorphism and risk of asthma in allele comparison

polymorphisms. Therefore, the Thr164Ile and Arg19Cys polymorphisms may not be involved in the pathogenesis of asthma in children. However, as only two studies were included in the meta-analysis, there may have been insufficient statistical evidence to clarify the association between the Thr164Ile and Arg19Cys polymorphisms and the risk of asthma in children. Thus, further studies are still required.

In the meta-analysis, only the above four SNPs were investigated in the present study. Although several included studies reported an association between other SNPs and the risk of asthma, the limited number of studies which reported certain SNPs will lower the stability of the pooled results. Therefore, we chose the four widely investigated SNPs in the current meta-analysis. Further studies investigating the other SNPs may therefore be required in the future. Further meta-analyses combining the studies which state an association between the other SNPs and the risk of asthma could be conducted when there have been enough relevant studies.

Although Liang et al.[37] conducted a meta-analysis to assess the relationship between ADRB2 gene polymorphisms and asthma risk, all patients including adults and children were included in the study. The different age of patients in individual studies, as an obvious source of clinical heterogeneity, may introduce a risk of bias. Therefore, we performed the current meta-analysis by only including studies of children. However, the results of the present study were similar to those of previous studies.

The limitations of this meta-analysis include the following: (1) The efficacy of the statistics may be further improved by including more studies in the future, especially in the investigation of Thr164Ile and Arg19Cys polymorphisms. (2) In the current meta-analysis, we only included literature published in English and Chinese, thus neglecting studies

Table 4. Results of the pooled ORs in the meta-analysis

SNP	Group	Dominant model comparison			Recessive model comparison			Homozygote model comparison			Allelic comparison		
		OR (95%CI)	P	I^2	OR (95%CI)	P	I^2	OR (95%CI)	P	I^2	OR (95%CI)	P	I^2
Arg16Gly (rs1042713)	Total	0.95 (0.73, 1.23)	0.69	59.3%	0.84 (0.71, 1.00)	0.05	30.3%	0.85 (0.64, 1.12)	0.25	50.1%	0.90 (0.78, 1.03)	0.13	55.2%
	Asia	0.93 (0.69, 1.27)	0.66	61.5%	0.87 (0.71, 1.07)	0.19	41.7%	0.88 (0.63, 1.22)	0.44	56.8%	0.91 (0.77, 1.08)	0.29	62.3%
	South America	0.90 (0.53, 1.52)	0.70	NA	0.70 (0.41, 1.22)	0.21	NA	0.73 (0.39, 1.37)	0.33	NA	0.82 (0.57, 1.17)	0.28	NA
	European	1.20 (0.35, 4.12)	0.78	82.8%	0.67 (0.39, 1.15)	0.15	0.0%	0.83 (0.28, 2.48)	0.74	62.2%	0.86 (0.54, 1.35)	0.51	52.2%
Gln27Glu (rs1042714)	Total	1.74 (1.34, 2.27)	<0.01	0.0%	0.93 (0.80, 1.09)	0.37	0.0%	1.63 (1.23, 2.17)	<0.01	0.0%	1.08 (0.95, 1.22)	0.22	21.6%
	Asia	1.87 (1.38, 2.55)	<0.01	0.0%	0.97 (0.82, 1.15)	0.75	8.9%	1.83 (1.31, 2.54)	<0.01	0.0%	1.12 (0.97, 1.28)	0.12	31.8%
	South America	0.99 (0.26, 3.80)	0.99	NA	0.76 (0.43, 1.34)	0.34	NA	0.92 (0.24, 3.55)	0.91	NA	0.81 (0.50, 1.32)	0.40	NA
	European	1.52 (0.87, 2.66)	0.14	0.0%	0.79 (0.50, 1.23)	0.29	0.0%	1.21 (0.64, 2.29)	0.56	0.0%	1.02 (0.75, 1.40)	0.89	0.0%
Thr164Ile (rs1800888)	Total (Asia)	3.01 (0.12, 74.12)	0.50	NA	3.01 (0.12, 74.12)	0.50	NA	1.47 (0.31, 7.01)	0.63	0.0%	1.86 (0.42, 8.20)	0.42	0.0%
Arg19Cys (rs1042711)	Total	0.87 (0.66, 1.15)	0.34	0.0%	0.92 (0.71, 1.19)	0.53	0.0%	0.69 (0.44, 1.08)	0.11	0.0%	0.88 (0.72, 1.07)	0.21	0.0%
	Asia	0.91 (0.67, 1.23)	0.54	NA	0.91 (0.67, 1.23)	0.54	NA	0.67 (0.38, 1.18)	0.17	NA	0.88 (0.70, 1.11)	0.29	NA
	European	0.70 (0.35, 1.41)	0.32	NA	0.95 (0.57, 1.59)	0.85	NA	0.72 (0.35, 1.52)	0.40	NA	0.88 (0.60, 1.28)	0.50	NA

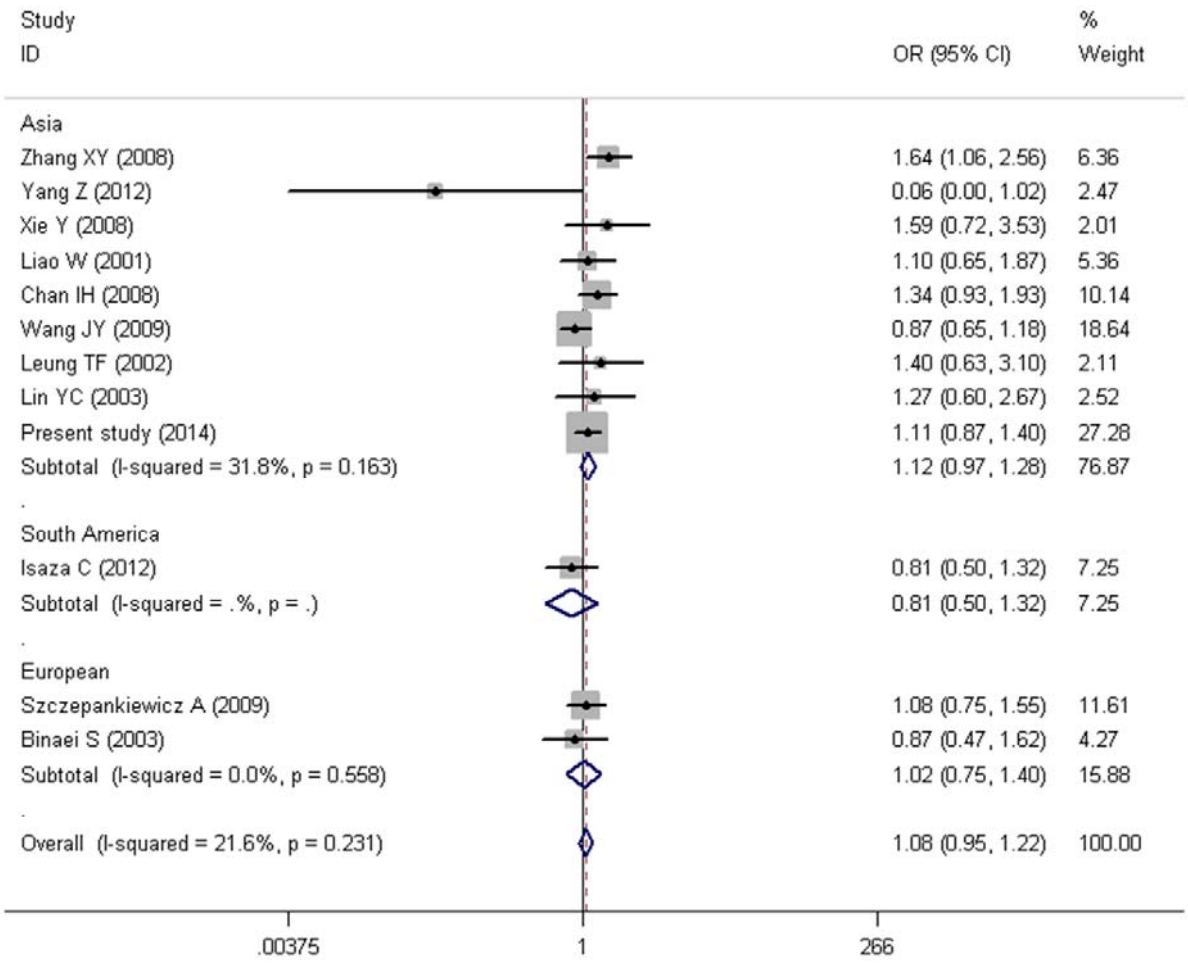

Figure 2. Forest plots of the association between the Gln27Glu (rs1042714) polymorphism and risk of asthma in allele comparison

published in other languages. As a result, language bias may be an issue in the present study. In addition, we may have missed some grey studies, including negative results or unpublished studies. Our reasons for the reluctance to include grey literature included the absence of a peer-review of such unpublished literature. Meta-analysis of unpublished data from interested sources is a controversial issue. (3) Genetic association studies have generated some confusion over the years. Although a meta-analysis may be useful for obtaining a sufficient sample size, controversies may not be resolved without suitable genetic models and standardised genotyping. (4) Although a meta-analysis can extract several similar studies to increase the statistical power, heterogeneity among studies can introduce some bias. Stratification by ethnicity may help to improve homogeneity among studies, but it may also influence statistical power. Most original literature only provides a generic definition of asthma, and does not describe asthma severity and environmental factors in detail, so we cannot supply this information and conduct stratification by other factors. (5) Because of the limited number of included studies, publication bias cannot be assessed for the Thr164Ile and Arg19Cys polymorphisms. Therefore, the results need to be interpreted with caution. (6) In the present case-control study, we did not investigate the phenotype of response to treatment and association between other SNPs and the risk of asthma in children. Further high-quality studies are still required in the future.

Conclusion

The present study suggested that the ADRB2 gene polymorphisms Arg16Gly, Gln27Glu, Thr164Ile and Arg19Cys were not associated with susceptibility to asthma in the current population of children. The further meta-analysis provided additional evidence supporting the above result that these four polymorphisms may not be involved in the risk of asthma in the overall children population. Due to the limited data that are currently available, further well-designed studies with large sample sizes are still required.

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