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Review Article

Association between β_2-Adrenergic Receptor-16Arg/Gly Gene Polymorphism and Chronic Obstructive Pulmonary Disease Risk: Systematic Review and Meta-Analysis

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Abstract

Background: The association between *β2-adrenergic receptor* (*ADRB2*) -16Arg/Gly polymorphism (rs1042713) and chronic obstructive pulmonary disease (COPD) risk has been investigated in many published studies. However, the results were inconclusive. A meta-analysis was performed to make a more precise estimation of the relationship.

Methods: The PubMed, EMBASE, ISI web of science, the Cochrane Database of Systematic Reviews, and Chinese databases (CNKI, Wanfang Data, CBM, VIP) were searched for published literature. Odds ratios (OR) with 95% confidence interval (CI) were used to assess the strength of association.

Results: Eleven studies, comprising 1,128 COPD patients and 1,182 controls, were included in the meta-analysis. Overall, there was no significant association between the *ADRB2*-16Arg/Gly polymorphism and COPD risk in general population. In the stratification analysis by potential confounding variables, significant associations were observed between the *ADRB2*-16Arg/Gly polymorphism and COPD risk among smoking Asians under the dominant genetic model and allele model (Arg vs. Gly) (dominant model: OR = 1.45, 95% CI = 1.04–2.01, $P = 0.311$ for heterogeneity, $\chi = 2.22$, $P = 0.026$ for OR; allele model: OR = 1.27, 95% CI = 1.03–1.57, $P = 0.209$ for heterogeneity, $\chi = 2.20$, $P = 0.028$ for OR), but not in other subgroups.

Conclusion: This meta-analysis suggested that the *ADRB2*-16Arg/Gly polymorphism might be a potential risk factor for the development of COPD in smoking Asian populations, but not in European descendents, and tobacco smoking probably increased the genetic susceptibility. More studies with larger sample sizes are needed to validate the results.

Keywords: β_2-adrenergic receptor, rs1042713, Tobacco smoking, COPD, Meta-analysis

Introduction

Chronic obstructive pulmonary disease (COPD) is a significant major cause of chronic morbidity and mortality worldwide. It is characterized by incompletely reversible airflow limitation and persistent airway inflammation (1). However, the pathogenesis of COPD has not been fully clarified. The previous studies have revealed that an imbalance of endogenous proteinases and antiproteinases, inflammatory cells, proinflammatory mediators, and oxidative stress were responsible for the pathogenesis of COPD (1, 2). In addition, genetic factors and environmental exposures like tobacco smoke are also involved in the pathogenesis of COPD (1). Of these risk factors, tobacco smoke is the most commonly encountered and important risk factor for COPD, and smokers account for 80~90% of all COPD patients. However, only 10~15% of smokers develop clinically significant COPD (3, 4). Moreover, many COPD patients have a family history and the occurrence of

COPD exhibits the high tendency of familial aggregation (5). These findings indicate that the individual's risk differences to tobacco smoke injury may be related to genetic factors and the genetic factors may also play an important role in the pathogenesis of COPD. Therefore, it is widely believed that COPD arises from an interaction between genetic factors and environmental exposures. A number of candidate gene studies have been carried out to identify genetic susceptibility factors for COPD over the past few years. So far, more than 25 different candidate genes have been tested (6).

β_2-adrenergic agonists are one of the most effective bronchodilators available for the clinical treatment of both COPD and asthma (7). They may induce bronchial relaxation and regulate airway hyperresponsiveness by binding to the *β_2-adrenergic receptor* (*ADRB2*) in airway smooth muscle cells. However, not all the COPD patients have the same response to β_2-agonists, which suggests that there is individual difference in the sensitivity of *ADRB2* among COPD patients. The mechanism leading to this alteration is not still clear. The gene encoding *ADRB2* is located on the chromosome 5 (5q31–q32). To date, at least five polymorphisms of *ADRB2* gene have been described, and they may result in single amino acid substitution at positions of 16, 27, 34, 164, and 19, respectively (8). In particular, the amino acid substitutions at positions 16 (rs1042713, Arg→Gly) and 27 (rs1042714, Gln→Glu) have been shown to affect the receptor function in vitro (9). Previous research also showed that agonist-promoted down-regulation of *ADRB2* was raised in 16Arg/Gly variant (10, 11).

A large number of studies have investigated the association between *ADRB2*-16Arg/Gly polymorphism and COPD risk, but the results were inconsistent. Some studies proposed that Arg homozygote at position 16 was a risk factor for COPD (12-14), whereas other studies found no associations between *ADRB2*-16Arg/Gly polymorphism and COPD (15-19). The discrepancy may be due to the relatively small sample sizes in these studies. Furthermore, some previous published studies may not properly investigate the effects of potential confounding variables, such as other polymorphic loci, ethnicity, environmental exposures (tobacco smoke, occupational dusts and chemicals, outdoor and indoor air pollution, sex, infection, socioeconomic status), and study design (study sample size, source of controls, genotyping methods, quality score).

The purpose of this meta-analysis was to ascertain the association between *ADRB2*-16Arg/Gly polymorphism and COPD risk from all eligible studies by analyzing the effects of potential confounding variables.

Materials and Methods

Search strategy

Since this study was a meta-analysis based on published articles, we did not draft a statement of patient consent or seek the approval of internal review boards. The study was conducted according to the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines (20). We performed a comprehensive search from the electronic literature databases of PubMed, EMBASE, ISI web of science, the Cochrane Database of Systematic Reviews, and Chinese databases (CNKI, Wanfang Data, CBM, and VIP) using the keywords:(*Beta2-adrenoreceptor* OR *β_2-adrenoceptor* OR *ADRB2* OR *Beta2-adrenergic receptor* OR *β_2AR*) AND (SNP OR polymorphism OR single nucleotide polymorphism OR variants) AND (COPD OR chronic obstructive pulmonary disease). No lower cut-off date was applied, and an upper date limit was on June 12, 2013. We retrieved all the eligible studies and checked their bibliographies for other relevant publications. If more than one publication included the same sample, we selected the most recent and complete one in our meta-analysis. Only those with full text articles published in English or Chinese language were included.

Inclusion criteria

The eligible studies had to meet the following criteria: a) case-control, cross-sectional, or cohort studies, b) evaluated *ADRB2*-16Arg/Gly polymorphism and COPD risk, c) provided information

on genotype frequency in COPD cases and controls, d) supplied the definite diagnosis criteria for COPD patients, and e) fulfilled Hardy-Weinberg equilibrium in the genotype distribution of control group.

Data extraction

Two authors (Wang and Li) independently extracted data from the eligible studies according to the same inclusion criteria listed above. Disagreements were resolved by consulting the third author (Chen). The following data were collected from each study: first author, year of publication, country, ethnicity, COPD definition and total number of cases and controls, the smoking status of cases and controls (smokers were defined as the subjects with at least 10 pack-years smoking history, and pack-years were calculated as the average number of cigarettes smoked per day divided by 20 and multiplied by the number of years smoked (13)), genotyping methods, and the distribution of genotype and allele (if there were no direct data in eligible study, we calculated it from the genotype frequencies).

Quality score assessment

The quality of each study was independently assessed by the same two authors (Wang and Li). Quality scoring criteria was modified from the score systems by Thakkinstian (21) and Sun (22). These scores were built on both traditional epidemiologic considerations and genetic issues (23). The total scores ranged from 0 (worst) to 15 (best).

Statistical analysis

All statistical analyses were done using STATA software (version 11.0, Stata Corp, College Station, TX, USA). The strength of the association between *ADRB2*-16Arg/Gly polymorphism and COPD risk was assessed using Odds ratios (OR), with 95% confidence interval (CI). The pooled OR was calculated for dominant model (Arg/Arg vs. Arg/Gly + Gly/Gly), recessive model (Gly/Gly vs. Arg/Gly + Arg/Arg), co-dominant model (Gly/Gly vs. Arg/Arg, Arg/Gly vs. Arg/Arg), and allele model (Arg vs. Gly), respectively. The Cochran's Q statistic for heterogeneity among studies was performed and the I^2 statistic were used to investigate the proportion of variation due to heterogeneity (24). A P value more than 0.10 for the Q statistic or the I^2 less than 50% suggested a lack of heterogeneity among studies, and the pooled OR was calculated by the fixed-effects model (the Mantel-Haenszel method) (25), otherwise, the random-effects model (the DerSimonian and Laird method) was performed (26). Once the pooled OR was calculated, and then Z statistic was used to test the significance of the pooled OR ($P < 0.05$ was considered statistically significant). To explore the sources of heterogeneity, the stratified analyses were carried out by smoking status (Yes/Undefined smoking), ethnicity (European descendent/Asian), study sample size (> 200 subjects/ ≤ 200 subjects), source of controls (healthy smokers/healthy population), genotyping methods, and quality score. Studies with less than 10 scores were excluded in high quality score stratification.

Hardy-Weinberg equilibrium (HWE) was assessed for each study in controls using the chi-square test or a Fisher's exact test ($P < 0.05$ was considered as disequilibrium). If there was deviation from HWE in studies, they would be excluded from this meta-analysis. Sensitivity analysis was conducted to assess the stability of the results by deleting a single study in this meta-analysis at a time. Publication bias was estimated by funnel plots Begg's test and Egger's test ($P < 0.05$ was considered statistically significant publication bias) (27, 28).

Results

Studies characteristics

Twelve published articles evaluating an association between *ADRB2*-16Arg/Gly polymorphism and COPD risk were collected (12-19, 29-32). Of these, one was excluded from our meta-analysis due to lack of its agreement to HWE (29). In addition, two articles were published by the same author using the same data available (17, 30), thus we selected the most recent and complete one (17). Therefore, 10 publications met the inclusion crite-

ria (12-19, 31, 32). Among these publications, one publication (12) included two studies (Hegab AE-1 and Hegab AE-2) according to different ethnicity, so each study in this article was considered separately for pooling analyses. Finally, there were 11 eligible studies in this final analysis. Six studies were involved in Asian subjects and five in European descendent subjects. Detailed characteristics of the studies included in this meta-analysis are given in Table 1 and 2. The literature search procedures are shown in Fig. 1.

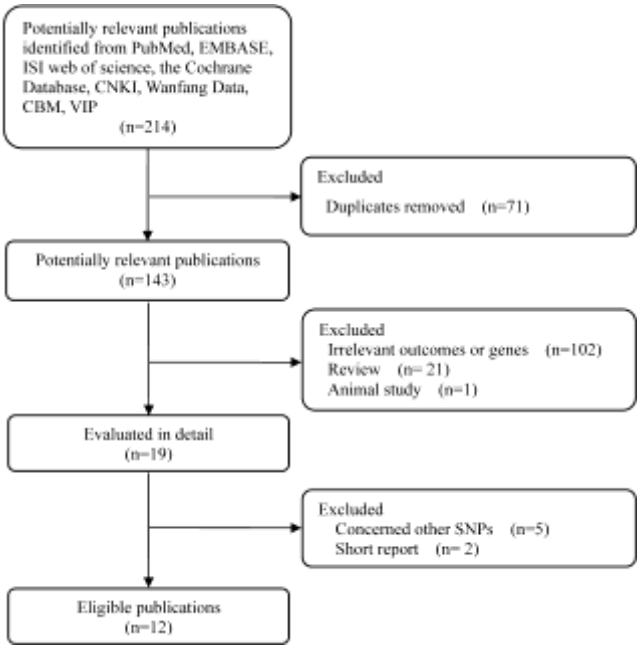

Fig. 1: Flow diagram of the meta-analysis

Overall meta-analysis

Table 3 listed the main results of this meta-analysis. The overall meta-analysis between the *ADRB2*-16Arg/Gly polymorphism and COPD risk included 11 studies involving 1128 COPD patients and 1182 controls. The variant genotypes (Gly/Gly and Arg/Gly) of the 16Arg/Gly were not associated with COPD risk compared with the wild type Arg/Arg homozygote (Gly/Gly vs. Arg/Arg: OR = 0.87, 95% CI = 0.55–1.39, P = 0.001 for heterogeneity; Arg/Gly vs. Arg/Arg: OR = 0.94, 95% CI = 0.68–1.30, P = 0.028 for heterogeneity). Similarly, no significant associations were found under dominant model, recessive model and allele model (dominant model: OR = 1.09, 95% CI = 0.76–1.55, P = 0.002 for heterogeneity; recessive model: OR = 0.91, 95% CI = 0.68–1.24, P = 0.018 for heterogeneity; allele model: OR = 1.07, 95% CI = 0.86–1.34, P = 0.001 for heterogeneity) (Table 3 and Fig. 2).

Stratification analysis

In smoking Asian subgroup, the variant genotypes (Gly/Gly and Arg/Gly) had significant relationship with COPD risk compared with the wild type Arg/Arg homozygote (Gly/Gly vs. Arg/Arg: OR = 0.64, 95% CI = 0.41–0.99, P = 0.153 for heterogeneity, χ = 2.01, P = 0.044 for OR; Arg/Gly vs. Arg/Arg: OR = 0.70, 95% CI = 0.50–0.99, P = 0.607 for heterogeneity, χ = 1.99, P = 0.046 for OR). Similar results were identified among smoking Asians under the dominant model and allele model (dominant model: OR = 1.45, 95% CI = 1.04–2.01, P = 0.311 for heterogeneity, χ = 2.22, P = 0.026 for OR; allele model: OR = 1.27, 95% CI = 1.03–1.57, P = 0.209 for heterogeneity, χ = 2.20, P = 0.028 for OR) (Fig. 3A and B). No statistical association was observed in smoking European descendent subgroup. The stratification analysis by smoking status did not indicate any significant associations, except among undefined smoking populations for recessive model (OR = 0.45, 95% CI = 0.24–0.85, P = 0.389 for heterogeneity, χ = 1.28, P = 0.201 for OR). In other subgroups by high-quality score (≥ 10), ethnicity, study sample size, source of controls and genotyping methods, the OR for each subgroup was not statistically significant. Results have been summarized in Table 3.

Sensitivity analysis and publication bias

A sensitivity analysis was conducted to evaluate the stability of the results by deleting a single study at a time. The results of sensitivity analysis showed that no individual study significantly affected the pooled ORs (Fig. 4). Publication bias was estimated by funnel plots Begg's test and Egger's test. The funnel plot was almost symmetrical and did not reveal any evidence of publication bias (Fig. 5).

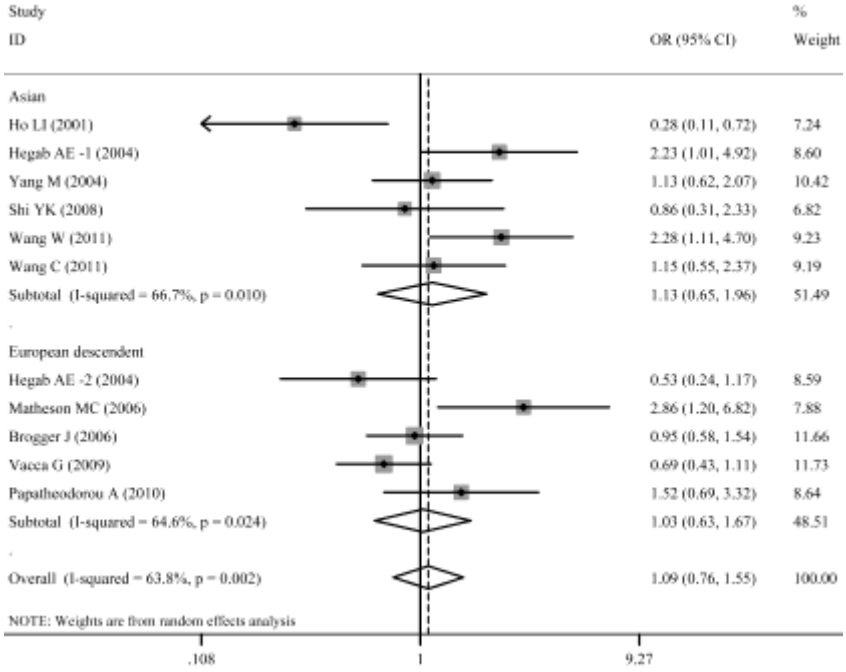

Fig. 2: Forest plot for the association between *ADRB2*-16Arg/Gly and COPD risk under the dominant genetic model. High heterogeneity was existing among studies and the random-effects model was performed

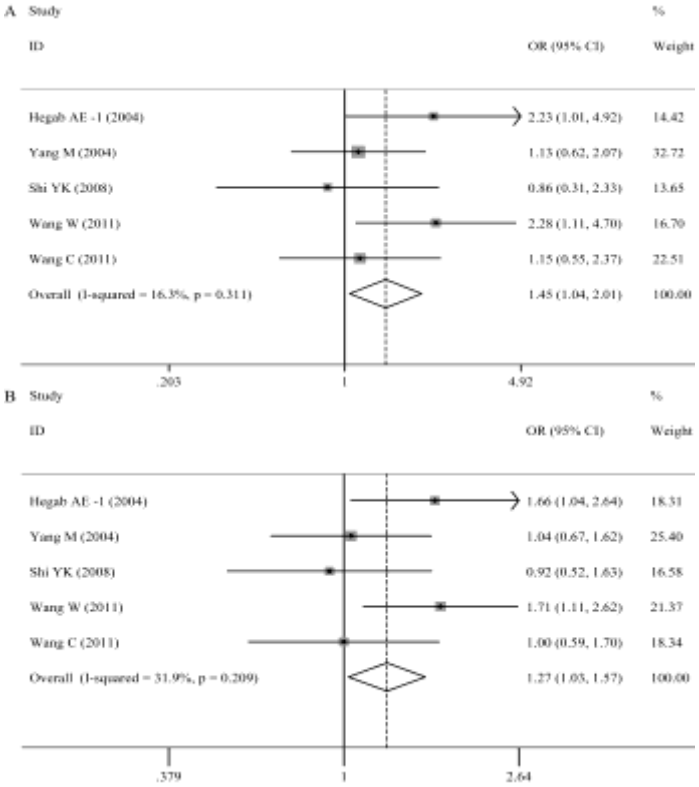

Fig. 3: Forest plot for the association between *ADRB2*-16Arg/Gly and COPD risk among smoking Asians under allele and dominant genetic models. (A) dominant model, (B) allele model, fixed-effects model was used

The Begg's test (dominant model: P = 0.806; Gly/Gly vs. Arg/Arg: P = 0.221; Arg/Gly vs. Arg/Arg: P = 0.806; allele model: P = 0.221) and Egger's test (dominant model: P = 0.931; Gly/Gly vs. Arg/Arg: P = 0.124; Arg/Gly vs. Arg/Arg: P = 0.811; allele model: P = 0.211) also did not show any statistical significance. In addition, the 95% CI (–11.8~11.1) of Egger's test included zero, which suggested that nearly no publication bias was existing among studies.

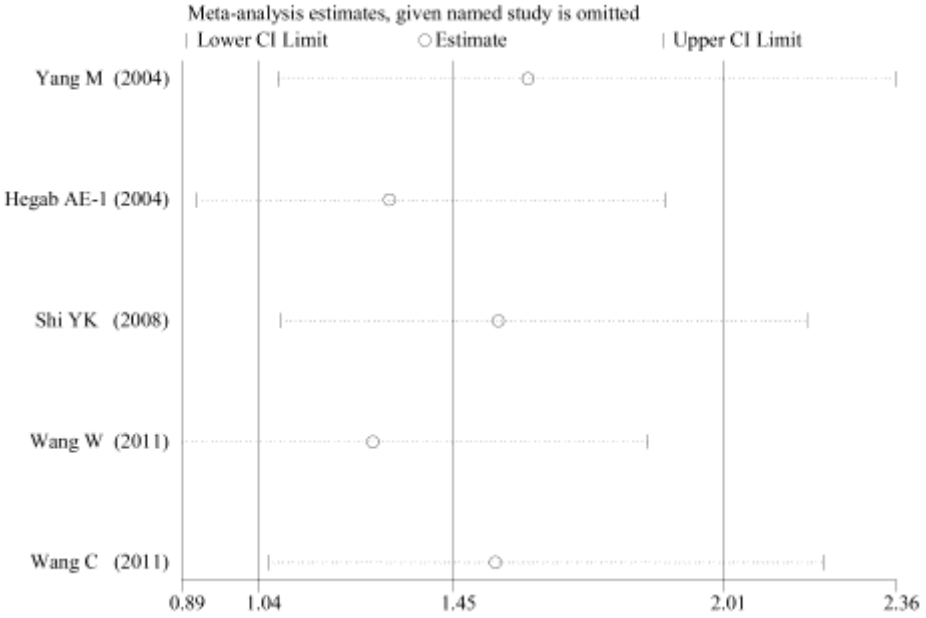

Fig. 4: Sensitivity analysis for the association between the *ADRB2*-16Arg/Gly polymorphism and COPD risk among smoking Asians. Each circle and transverse line represented the pooled OR and 95% CI by deleting the corresponding study

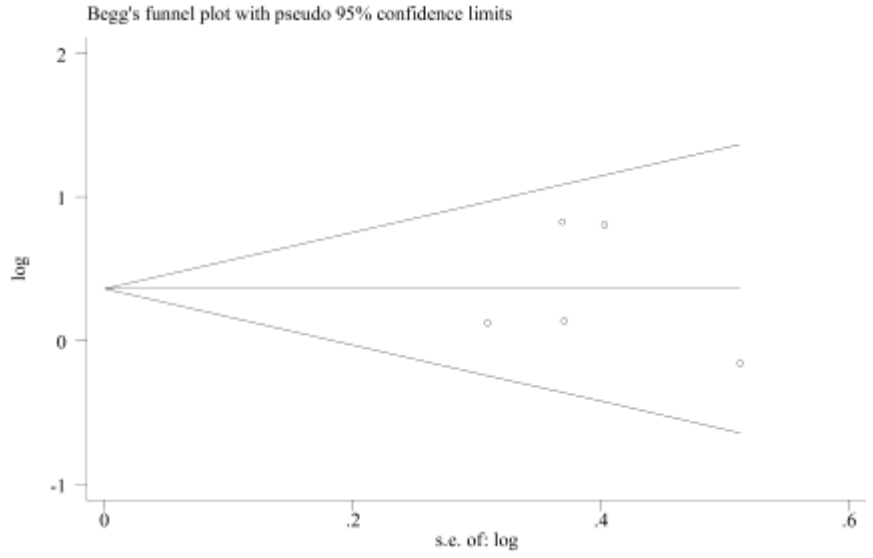

Fig. 5: Begg's funnel plot for publication bias of studies under dominant model. Each circle represented a corresponding study

Table 1: Main characteristics of the studies included in the meta-analysis

First author (Ref.)	Year	Ethnicity/country	Source of controls	Smoking status	HWE	Genotyping methods
Ho LI (32)	2001	Asian/China	Healthy population	Not mentioned	Yes	allele-specific PCR
Hegab AE -1 (12)	2004	Asian/Japan	Healthy smokers	Smokers	Yes	TaqMan allelic discrimination
Hegab AE -2 (12)	2004	European descendent/Egypt	Healthy smokers	Smokers	Yes	TaqMan allelic discrimination
Yang M (15)	2004	Asian/China	Healthy smokers	Smokers	Yes	PCR direct sequencing
Matheson MC (13)	2006	European descendent/Australia	General population	Mixed	Yes	ARMS-PCR
Brogger J (16)	2006	European descendent/Norway	Healthy smokers	Smokers	Yes	TaqMan PCR
Shi YK (17)	2008	Asian/China	Healthy smokers	Smokers	Yes	PCR direct sequencing
Vacca G (31)	2009	European descendent/Germany	Healthy volunteers	Smokers	Yes	allele-specific PCR
Papatheodorou A (19)	2010	European descendent/Greece	Healthy smokers	Smokers	Yes	Nanogen NanoChip® 400
Wang W (14)	2011	Asian/China	Healthy smokers	Smokers	Yes	allele-specific PCR
Wang C (18)	2011	Asian/China	Healthy smokers	Smokers	Yes	allele-specific PCR

COPD, chronic obstructive pulmonary disease; HWE, Hardy-Weinberg equilibrium; ARMS, amplification refractory mutation system; PCR, polymerase chain reaction

Table 2: The distribution of *ADRB2*-16Arg/Gly genotypes, and allelic frequency in COPD patients and controls

First author (Ref.)	Ethnicity/country	Cases/Controls	COPD group					Control group					Quality score
			Genotype			Allele		Genotype			Allele		
			Arg/Arg	Arg/Gly	Gly/Gly	Arg	Gly	Arg/Arg	Arg/Gly	Gly/Gly	Arg	Gly	
Ho LI (32)	Asian/China	65/41	9	48	8	66	64	15	19	7	49	33	8
Hegab AE -1 (12)	Asian/Japan	88/61	29	42	17	100	76	11	32	18	54	68	13
Hegab AE -2 (12)	European descendent/Egypt	106/72	14	46	46	74	138	16	33	23	65	79	13
Yang M (15)	Asian/China	90/82	40	38	12	118	62	34	38	10	106	58	10
Matheson MC (13)	European descendent/Australia	39/221	9	21	9	39	39	21	102	98	144	298	8
Brogger J (16)	European descendent/Norway	238/239	38	121	79	197	279	40	109	90	189	289	13
Shi YK (17)	Asian/China	49/48	9	25	15	43	55	10	24	14	44	52	10
Vacca G (31)	European descendent/Germany	190/172	41	93	56	175	205	49	92	31	190	154	11
Papatheodorou A (19)	European descendent/Greece	111/106	18	49	44	85	137	12	49	45	73	139	12
Wang W (14)	Asian/China	92/80	30	45	17	105	79	14	42	24	70	90	10
Wang C (18)	Asian/China	60/60	26	25	9	77	43	24	29	7	77	43	11

Table 3: Odds ratio and 95% CI for COPD and the *ADRB2*-16Arg/Gly Polymorphism under different genetic models

Variables	N※	Dominant model		Recessive model		Gly/Gly vs. Arg/Arg		Arg/Gly vs. Arg/Arg		Arg vs.Gly	
		OR (95% CI)	*P**	OR (95% CI)	*P**	OR (95% CI)	*P**	OR (95% CI)	*P**	OR (95% CI)	*P**
Total	11	1.09 (0.76,1.55)	0.002	0.91 (0.68,1.24)	0.018	0.87 (0.55,1.39)	0.001	0.94 (0.68,1.30)	0.028	1.07 (0.86,1.34)	0.001
High quality score (≥10)	9	1.10 (0.81,1.50)	0.059	1.01 (0.75,1.37)	0.051	0.95 (0.60,1.49)	0.006	0.92 (0.73,1.16)	0.336	1.04 (0.84,1.30)	0.006
Smoking status											
Yes	9	1.10 (0.81,1.50)	0.059	1.01 (0.75,1.37)	0.051	0.95 (0.60,1.49)	0.006	0.92 (0.73,1.16)	0.336	1.04 (0.84,1.30)	0.006
Undefined smoking	2	0.90 (0.09,8.89)	0.000	0.45 (0.24,0.85)	0.389	0.62 (0.07,5.28)	0.010	1.41 (0.17,11.88)	0.001	1.21 (0.41,3.53)	0.004
Ethnicity											
European descendent	5	1.03 (0.63,1.67)	0.024	1.00 (0.62,1.63)	0.003	0.96 (0.47,1.99)	0.001	1.05 (0.78,1.40)	0.251	1.02 (0.72,1.44)	0.001
Asian	6	1.13 (0.65,1.96)	0.010	0.77 (0.54,1.10)	0.561	0.72 (0.47,1.08)	0.106	0.92 (0.53,1.62)	0.016	1.14 (0.86,1.52)	0.082
Ethnicity (smokers)											
European descendent	4	0.83 (0.62,1.11)	0.229	1.20 (0.78,1.85)	0.029	1.31 (0.73,2.35)	0.037	1.14 (0.84,1.55)	0.520	0.88 (0.66,1.17)	0.030
Asian	5	1.45 (1.04,2.01)	0.311	0.79 (0.54,1.14)	0.424	0.64 (0.41,0.99)	0.153	0.70 (0.50,0.99)	0.607	1.27 (1.03,1.57)	0.209
Study sample size											
>200	4	1.00 (0.75,1.34)	0.029	0.89 (0.51,1.56)	0.004	0.79 (0.34,1.79)	0.001	0.99 (0.73,1.35)	0.232	1.02 (0.87,1.21)	0.001
≤200	7	1.01 (0.60,1.70)	0.005	0.93 (0.69,1.26)	0.232	0.93 (0.51,1.69)	0.024	0.99 (0.60,1.65)	0.015	1.04 (0.78,1.40)	0.016
Source of controls											
Healthy smokers	8	1.19 (0.93,1.51)	0.130	0.90 (0.72,1.12)	0.325	0.83 (0.53,1.28)	0.058	0.86 (0.66,1.11)	0.358	1.11 (0.91,1.37)	0.064
Healthy population	3	0.82 (0.27,2.53)	0.001	0.82 (0.27,2.49)	0.002	0.97 (0.22,4.34)	0.001	1.32(0.47,3.67)	0.006	0.99 (0.49,1.99)	0.001
Genotyping methods											
Allele-specific PCR	4	0.87 (0.41,1.84)	0.003	1.01 (0.50,2.05)	0.024	1.12 (0.43,2.89)	0.007	1.14 (0.55,2.38)	0.007	0.95 (0.61,1.49)	0.005
TaqMan PCR	3	1.03 (0.51,2.09)	0.039	0.93 (0.55,1.57)	0.080	0.93 (0.38,2.27)	0.020	1.03 (0.70,1.51)	0.123	1.05 (0.67,1.64)	0.015
Other methods	4	1.36 (0.92,2.01)	0.256	0.78 (0.54,1.11)	0.204	0.64 (0.31,1.31)	0.097	0.75 (0.50,1.14)	0.618	1.24 (0.98,1.56)	0.114

Other methods: PCR direct sequencing, ARMS–PCR, methodology of the Nanogen NanoChip® 400

Dominant model: (Arg/Arg vs. Arg/Gly + Gly/Gly), Recessive model: (Gly/Gly vs. Arg/Gly + Arg/Arg)

※ Number of comparisons

* *P* value of Q test for heterogeneity test. Random effects model was used when *P* value for heterogeneity test <0.1; otherwise, fixed effects model was used

Discussion

In the present meta-analysis (based on 1128 cases and 1182 control subjects from 11 eligible studies), we demonstrated that there was no significant association between the *ADRB2*-16Arg/Gly polymorphism and COPD risk in the overall populations. We also found that *ADRB2*-Arg homozygotes may be a high risk factor of developing COPD in smoking Asian populations. The results suggest that the presence of Arg allele might be one of the genetic factors susceptible to COPD and that smoking might increase the genetic susceptibility in Asian populations.

There is likely to be a complicated interaction between genetic and environmental factors in the development of COPD (5). In recent years, genomewide association studies (GWAS) have been used as an important tool to identify susceptibility genes and loci associated with COPD (33-35). *ADRB2*, a member of a large super family of cell surface G protein-coupled receptors, has a seven transmembrane domain. It can mediate the actions of catecholamines and β_2-adrenergic agonists (36). Therefore, β_2-adrenergic agonists show significant clinical effects on COPD patients. They may inhibit the proliferation of human airway smooth muscle cells and neutrophil accumulation besides bronchodilation (37, 38). In addition, they can also stimulate mucociliary transport of human bronchial epithelial cells and reduce the mucosal damage (39, 40). Therefore, the responsiveness of *ADRB2* to β_2-adrenergic agonists may play an important role in regulating airway hyperresponsiveness and the development of COPD. However, genetic variation in *ADRB2* can influence desensitization (36). It also has been reported that 16Arg/Gly variant is related to increased agonist-promoted down-regulation (10).

To date, many studies were performed to assess the effects of *ADRB2*-16Arg/Gly single nucleotide polymorphism (SNP) on COPD risk, but the results have been conflicting. These discrepancies could be due to factors as follows: First, a small sample size may lead to under-powering of studies to evaluate statistically significant effects. Second, different ethnicity may be associated with genetic risk, so studies with different populations have shown different results. Third, other potential confounding variables, such as other polymorphic loci, smoking status and study design (source of controls, genotyping methods, quality score), may also help to explain the controversy among these studies. Especially tobacco smoke was an important risk factor for COPD, but many studies have not taken into account it.

Meta-analysis has been recognized as a powerful and effective method to solve a wide variety of clinical problems by summarizing and analyzing the cumulative data of the previous individual studies with small sample size and low statistical power. Pooling the effects of individual studies by meta-analysis, which increases the sample size and statistical power, can help explore accurate associations between genetic variability and disease outcomes. So far, a number of gene polymorphisms have been found to be related to specific disease states by using meta-analysis.

Our results from the present meta-analysis indicated that the *ADRB2*-16Arg/Gly SNP was not associated with COPD risk in the overall populations and high heterogeneity was existing among these studies. In order to explore the sources of heterogeneity, we carried out stratification analysis by the potential confounding variables (i.e. smoking status, ethnicity, study sample size, source of controls, genotyping methods, quality score). Our stratification analysis has tried to clarify the effects of confounding variables on the relationship between 16Arg/Gly polymorphism and COPD risk. However, the results highlighted that there was no significant association between *ADRB2*-16Arg/Gly polymorphism and COPD risk and significant heterogeneity was present among each subgroup, except in smoking Asian populations. In the stratification analysis by smoking Asians, we found there was nearly no heterogeneity among these studies. The results showed that significant associations were observed between the *ADRB2*-16Arg/Gly polymorphism and COPD risk under the dominant genetic model and allele model. The pooled data indicated that 16Gly allele may have a protective effect on COPD and that the Arg homozygotes may be a high risk factor of

developing COPD compared to the carriers of Gly allele in smoking Asian populations. Therefore, these results suggested that the presence of Arg allele might be one of the genetic factors making these patients more susceptible to COPD, and some environmental factors such as smoking might increase the genetic susceptibility in Asian populations. Our findings were in agreement with the results of three studies included to the current meta-analysis (12-14), which showed that the Arg16 homozygous genotype had an increased risk of COPD. However, these results were in contrast to the study by Ho et al. (32), and they found the Arg allele to be less prevalent in COPD patients. Furthermore, some studies of the current meta-analysis indicated that the *ADRB2*-16Arg/Gly polymorphism was not associated with risk of COPD (15-19, 31). The discrepancy may be caused by potential confounding variables. The results of our meta-analysis relied on published data, which may bring about a publication bias. In the current meta-analysis, the shape of funnel plot was symmetrical, and neither Egger's test nor Begg's test showed publication bias.

Interestingly, a recent meta-analysis by Niu et al. also made an estimation of the relationship between *ADRB2* gene polymorphisms and COPD risk (41). But they reported that there was no association between them, and that 16Arg allele was not a risk of morbidity for COPD. Their results were partially inconsistent with the current meta-analysis. A few of factors could explain the difference. First, quality assessment of studies included in the meta-analysis by Niu et al. was not performed. Second, they did not assess the association between *ADRB2*-16Arg/Gly and COPD risk under allele model. Finally, stratification analysis by some potential confounding variables like smoking status was not conducted in order to explore the sources of heterogeneity among studies.

There are several limitations in the present meta-analysis that should be mentioned. For example, we did not have original data of studies included in the current meta-analysis and we were not able to take into account other factors related to COPD like age, gender, air pollution, infection, and socioeconomic status, which may change the risk estimates. Besides, the sample size of the present meta-analysis was relatively small and may not provide sufficient statistical power to assess the association between *ADRB2* gene and susceptibility to COPD. So a more precise analysis allowing for the adjustment by other covariates should be performed if larger sample studies and more individual data are available. Finally, the *ADRB2*-16Arg/Gly is highly linked with other polymorphic loci of the same gene or other genes. Therefore, when investigating the effect of the *ADRB2*-16Arg/Gly SNP on COPD susceptibility in smoking Asian populations, we should consider these factors above.

Sensitivity analysis is used to investigate the influence of a single study on the overall meta-analysis estimate, which may demonstrate whether the results of one meta-analysis are stable. In the current meta-analysis, the results of sensitivity analysis showed that no individual study significantly affected our overall results. Therefore, the results indicated that *ADRB2*-16Arg/Gly polymorphism might be a potential risk factor for the development of COPD and Gly allele was likely to be a protective effect on COPD among smoking Asians, but the consequences should be further validated by the addition of more samples.

Conclusion

In conclusion, our meta-analysis suggests that *ADRB2*-16Arg/Gly polymorphism might be associated with COPD in smoking Asian populations and that tobacco smoking probably can increase the genetic susceptibility of COPD in Asians, but not in European descendents, which gives a new opportunity to investigate the pathogenesis of COPD susceptibility. But further prospective investigations with larger sample studies are necessary.

Ethical considerations

Ethical issues (Including plagiarism, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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References

1. Vestbo J, Hurd SS, Agusti AG et al. (2013). Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *Am J Respir Crit Care Med*, 187(4):347-65.
2. Yamaya A, Osanai K (2011). [Cytokines and proteases involved in pathogenesis of COPD]. *Nihon Rinsho Japanese Journal of Clinical Medicine*, 69(10):1748-53.
3. Sethi JM, Rochester CL (2000). Smoking and chronic obstructive pulmonary disease. *Clin Chest Med*, 21(1):67-86.
4. Mannino DM, Homa DM, Akinbami LJ, Ford ES, Redd SC (2002). Chronic obstructive pulmonary disease surveillance--United States, 1971-2000. *MMWR Surveill Summ*, 51(6):1-16.
5. Sandford AJ, Silverman EK (2002). Chronic obstructive pulmonary disease. 1: Susceptibility factors for COPD the genotype-environment interaction. *Thorax*, 57(8):736-41.
6. Molfino NA (2004). Genetics of COPD. *Chest*, 125(5):1929-40.
7. Chowdhury BA, Dal Pan G (2010). The FDA and safe use of long-acting beta-agonists in the treatment of asthma. *N Engl J Med*, 362(13):1169-71.
8. Reihsaus E, Innis M, MacIntyre N, Liggett SB (1993). Mutations in the gene encoding for the beta 2-adrenergic receptor in normal and asthmatic subjects. *Am J Respir Cell Mol Biol*, 8(3):334-9.
9. Yin K, Zhang X, Qiu Y (2006). Association between beta2-adrenergic receptor genetic polymorphisms and nocturnal asthmatic patients of Chinese Han nationality. *Respiration*, 73(4):464-7.
10. Green SA, Turki J, Innis M, Liggett SB (1994). Amino-terminal polymorphisms of the human beta 2-adrenergic receptor impart distinct agonist-promoted regulatory properties. *Biochemistry*, 33(32):9414-9.
11. Moore PE, Laporte JD, Abraham JH, Schwartzman IN, Yandava CN, Silverman ES, Drazen JM, Wand MP, Panettieri RA, Jr., Shore SA (2000). Polymorphism of the beta(2)-adrenergic receptor gene and desensitization in human airway smooth muscle. *Am J Respir Crit Care Med*, 162(6):2117-24.
12. Hegab AE, Sakamoto T, Saitoh W, Massoud HH, Massoud HM, Hassanein KM, Sekizawa K (2004). Polymorphisms of IL4, IL13, and ADRB2 genes in COPD. *Chest*, 126(6):1832-9.
13. Matheson MC, Ellis JA, Raven J, Johns DP, Walters EH, Abramson MJ (2006). Beta2-adrenergic receptor polymorphisms are associated with asthma and COPD in adults. *Journal of Human Genetics*, 51(11):943-51.
14. Wang W, Yu YJ, Qian R, Mei WX, Huang CP (2011). Association between polymorphisms of IL-13, IL-4, and β2AR genes and the susceptibility to chronic obstructive pulmonary disease (in Chinese). *J Clin Internal Med*, 28(5):332-4.
15. Yang M (2004). Preliminary study on the association between β2AR polymorphisms and COPD in Southwest Han Chinese Subjects (in Chinese). *Master Dissertation, Kunming Medical University*.
16. Brogger J, Steen VM, Eiken HG, Gulsvik A, Bakke P (2006). Genetic association between COPD and polymorphisms in TNF, ADRB2 and EPHX1. *Eur Respir J*, 27(4):682-8.
17. Shi YK, Ma J, Yuan ZJ, Sun T, Li SL (2008). Investigation on the relation between polymorphisms of β2 adrenergic receptor and the chronic obstructive pulmonary disease (in Chinese). *Shandong Med J*, 48(13):9-11.
18. Wang C, Yang AL, Li H, Bi HY, Fu WP, Dai LM, Chen F, Tan F, Fu Q, Tian LY (2011). Association between the β2-adrenoceptor polymorphisms and the older-aged chronic obstructive pulmonary disease with hypertension (in Chinese). *Chinese J Gerontol*, 31(15):2822-4.
19. Papatheodorou A, Makrythanasis P, Kaliakatsos M, Dimakou A, Orfanidou D, Roussos C, Kanavakis E, Tzetis M (2010). Development of novel microarray methodology for the study of mutations in the SERPINA1 and ADRB2 genes-Their association with Obstructive Pulmonary Disease and Disseminated

Bronchiectasis in Greek patients. *Clin Biochem*, 43(1-2):43-50.
20. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thacker SB (2000). Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA*, 283(15):2008-12.
21. Thakkinstian A, McEvoy M, Minelli C et al. (2005). Systematic review and meta-analysis of the association between {beta}2-adrenoceptor polymorphisms and asthma: a HuGE review. *Am J Epidemiol*, 162(3):201-11.
22. Sun K, Li Y, Wei C, Tong Y, Zheng H, Guo Y (2012). Recessive protective effect of ADIPOQ rs1501299 on cardiovascular diseases with type 2 diabetes: A meta-analysis. *Mol Cellular Endocrinol*, 349(2):162-9.
23. Attia J, Thakkinstian A, D'Este C (2003). Meta-analyses of molecular association studies: methodologic lessons for genetic epidemiology. *J Clin Epidemiol*, 56(4):297-303.
24. Higgins JP, Thompson SG (2002). Quantifying heterogeneity in a meta-analysis. *Stat Med*, 21(11):1539-58.
25. Mantel N, Haenszel W (1959). Statistical aspects of the analysis of data from retrospective studies of disease. *J Natl Cancer Inst*, 22(4):719-48.
26. DerSimonian R, Laird N (1986). Meta-analysis in clinical trials. *Control Clin Trials*, 7(3):177-88.
27. Begg CB, Mazumdar M (1994). Operating characteristics of a rank correlation test for publication bias. *Biometrics*, 50(4):1088-101.
28. Egger M, Davey Smith G, Schneider M, Minder C (1997). Bias in meta-analysis detected by a simple, graphical test. *BMJ*, 315(7109):629-34.
29. Ma L, Feng DX, Zhang XY, Shan KR, Diao XY, Li Y, Liu WJ, Wan ZF, Yang J (2006). Association between the genetic polymorphisms of β2-adrenergic receptor and the chronic obstructive pulmonary disease (in Chinese). *Chinese Journal of Practical Internal Medicine*, 26(4):267-9.
30. Chen JX, Shi YK, Li SL, Zhang ZX (2008). The study of β2-AR polymorphisms and chronic obstructive pulmonary disease on relations (in Chinese). *Acta Acad Med Weifang*, 30(1):63-5.
31. Vacca G, Schwabe K, Duck R, Hlawa HP, Westphal A, Pabst S, Grohe C, Gillissen A (2009). Polymorphisms of the beta2 adrenoreceptor gene in chronic obstructive pulmonary disease. *Ther Adv Respir Dis*, 3(1):3-10.
32. Ho LI, Ham HJ, Chen CJ, Tsai NM (2001). Polymorphism of the (beta)2-adrenoceptor in COPD in Chinese subjects. *Chest*, 120(5):1493-9.
33. Boezen HM (2009). Genome-wide association studies: what do they teach us about asthma and chronic obstructive pulmonary disease? *Proc Am Thorac Soc*, 6(8):701-3.
34. Castaldi PJ, Cho MH, Cohn M, Langerman F, Moran S, Tarragona N, Moukhachen H, Venugopal R, Hasimja D, Kao E, Wallace B, Hersh CP, Bagade S, Bertram L, Silverman EK, Trikalinos TA (2010). The COPD genetic association compendium: a comprehensive online database of COPD genetic associations. *Hum Mol Genet*, 19(3):526-34.
35. Silverman EK, Spira A, Pare PD (2009). Genetics and genomics of chronic obstructive pulmonary disease. *Proc Am Thorac Soc*, 6(6):539-42.
36. Shore SA, Moore PE (2003). Regulation of beta-adrenergic responses in airway smooth muscle. *Respir Physiol Neurobiol*, 137(2-3):179-95.
37. Dekkers BG, Pehlic A, Mariani R, Bos IS, Meurs H, Zaagsma J (2012). Glucocorticosteroids and beta(2)-adrenoceptor agonists synergize to inhibit airway smooth muscle remodeling. *J Pharmacol Exp Ther*, 342(3):780-7.
38. Mirza ZN, Kato M, Kimura H, Tachibana A, Fujiu T, Suzuki M, Mochizuki H, Tokuyama K, Morikawa A (2002). Fenoterol inhibits superoxide anion generation by human polymorphonuclear leukocytes via beta-adrenoceptor-dependent and -independent mechanisms. *Ann Allergy Asthma Immunol*, 88(5):494-500.
39. Dowling RB, Johnson M, Cole PJ, Wilson R (1999). Effect of fluticasone propionate and salmeterol on Pseudomonas aeruginosa infection of the respiratory mucosa in vitro. *Eur Respir J*, 14(2):363-9.
40. Wegner CD (2001). Novel mechanistic targets for the treatment of sub-acute and chronic bronchitis. *Current Pharmaceutical Design*, 7(3):199-212.
41. Niu LM, Liang Y, Xu M, Zhang YY, Zhang Y, He B (2012). Effect of polymorphisms in the beta2-adrenergic receptor on the susceptibility and pulmonary function of patients with chronic obstructive pulmonary disease: a meta analysis. *Chin Med J (Engl)*, 125(12):2213-8.