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--Manuscript Summary--

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Title	Gc-globulin (GC) gene polymorphisms and Chronic Obstructive Pulmonary Disease: a meta-analysis
Running Head	GC gene polymorphisms and COPD: a meta-analysis
Keywords	chronic obstructive pulmonary disease,meta-analysis,risk,Polymorphism,Gc-globulin (vitamin D-binding protein)
Abstract	Background: A number of polymorphisms in Gc-globulin (GC) gene have been implicated in risk of chronic obstructive pulmonary disease (COPD), but the results were controversial. This study aimed to explore the association between GC gene polymorphisms and COPD risk. Methods: Pubmed and Chinese National Knowledge Infrastructure (CNKI) were searched for eligible case-control studies. The pooled odds ratios (ORs) were performed respectively for single allele comparison, dominant/ recessive models analysis and homozygous gene analysis. Subgroup analyses, sensitivity analyses and publication bias were also performed. Results: Eight studies containing 2216 participants were included. Significant results were obtained in single allele comparison: the ORs were 1.47 in GC1F versus GC1S, and 1.77 in GC1F versus GC2. In the dominant/ recessive models analyses, the ORs of recessive and dominant model in GC1F/1S group were 2.18 and 1.46, respectively. In GC1F/2 group, the OR was 2.86 in dominant model, and 2.71 in recessive model. In homozygous genes comparison, the OR was 2.51 in GC1F homozygote versus other genotypes. In subgroup analyses, the same significant results were obtained in Asian population, but not in Caucasian population. The sensitivity analyses showed the stability of the meta-analysis. The results showed no publication bias. Conclusions: There is a close association between COPD and GC gene polymorphisms. The GC1F allele could be a risk factor, the GC1S and GC2 allele may be protective factors in Asian, but not in Caucasians. Future studies are needed to validate our conclusions.
Section Title	Systematic Reviews

Gc-globulin (*GC*) gene polymorphisms and Chronic Obstructive Pulmonary Disease: a meta-analysis

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Running head: GC gene and COPD: a meta-analysis

Abstract

Background: A number of polymorphisms in Gc-globulin (GC) gene have been implicated in risk of chronic obstructive pulmonary disease (COPD), but the results were controversial. This study aimed to explore the association between GC gene polymorphisms and COPD risk. **Methods:** Pubmed and Chinese National Knowledge Infrastructure (CNKI) were searched for eligible case-control studies. The pooled odds ratios (ORs) were performed respectively for single allele comparison, dominant/ recessive models analysis and homozygous gene analysis. Subgroup analyses, sensitivity analyses and publication bias were also performed. **Results:** Eight studies containing 2216 participants were included. Significant results were obtained in single allele comparison: the ORs were 1.47 in GC1F versus GC1S, and 1.77 in GC1F versus GC2. In the dominant/ recessive models analyses, the ORs of recessive and dominant model in GC1F/1S group were 2.18 and 1.46, respectively. In GC1F/2 group, the OR was 2.86 in dominant model, and 2.71 in recessive model. In homozygous genes comparison, the OR was 2.51 in GC1F homozygote versus other genotypes. In subgroup analyses, the same significant results were obtained in Asian population, but not in Caucasian population. The sensitivity analyses showed the stability of the meta-analysis. The results showed no publication bias. **Conclusions:** There is a close association between COPD and GC gene polymorphisms. The GC1F allele

could be a risk factor, the GC1S and GC2 allele may be protective factors in Asian, but not in Caucasians. Future studies are needed to validate our conclusions.

Keywords: Chronic Obstructive Pulmonary Disease; Gc-globulin (vitamin D-binding protein); Polymorphism; risk; Meta-analysis

Introduction

Chronic Obstructive Pulmonary Diseases (COPD) is a disease state characterized by not fully reversible and progressive airflow limitation, which is related to abnormal inflammatory response of the lungs to noxious particles or gases . Cigarette smoking is the most widely recognized risk factor for COPD, but at least one-quarter of COPD patients are non-smokers, and only 15% of smokers develop COPD, suggesting that genetic factors are involved in development of COPD. Besides, COPD is a familial clustering disease, it is reasonable to believe that COPD is the result of interactions of genetic factors and environmental factors. Besides the Alpha 1-Antitrypsin gene, the only confirmed genetic factor, there are also some other genes associated with COPD, by involving in the pathogenesis of COPD . Group-specific component (Gc-globulin, *GC*) gene is one of the candidate genes associated with COPD, by implicating in macrophage activation and augmenting the chemotactic effect of complement-derived molecules on neutrophils, thus influencing the

intensity of the inflammatory reaction.

Gc-globulin proteins is also known as vitamin D-binding protein (DBP) for its function of binding substantial quantities of vitamin D and 25-hydroxyvitamin D. The human *GC* gene is localized on the long arm of chromosome 4 (4q12–q13), and it extends over 35 kb DNA and contains 13 exons and 12 introns. *GC* gene is highly polymorphic, with three common variants (*GC*1F, *GC*1S and *GC*2) and more than 124 rarer variants. Two common point mutation (G→A, C→T) of single nucleotide polymorphisms (SNPs: rs4588 and rs7041) in exon11 result in the three common isoforms and different protein products at positions 416 and 420: GC1F (Asp416, Thr 420), *GC*1S (Glu 416, Thr 420), and *GC*2 (Asp416, Lys420) .

To date, a series of case-control studies have been performed to investigate the relationship between *GC* gene polymorphisms and the risks of COPD, but the results were inconclusive. Some studies showed positive association between COPD risk and *GC* gene , but others offered negative results . Here, we undertook a meta-analysis to evaluate the association between *GC* gene and COPD risk. To the best of our knowledge, there was no meta-analysis evaluating the relationship between *GC* polymorphisms and COPD risk before.

Materials and Methods

Search strategy

We searched for studies evaluating the association between polymorphisms of the *GC* gene and COPD risk. Articles were identified with a search of Pubmed and Chinese National Knowledge Infrastructure (CNKI) (last update February, 2015). The following search terms were used to identify studies: “chronic obstructive pulmonary disease”, “COPD”, “chronic bronchitis”, “emphysema”, “Group-specific component”, “Gc-globulin”, “vitamin D-binding protein” and “DBP”. There was no restriction on languages. References of the retrieved articles were also screened for additional studies.

Study selection

Studies were eligible for inclusion based on the following criteria: (1) studies assessed the association between *GC* gene polymorphism and COPD susceptibility; (2) the design had to be a case–control study; (3) participants in control group were healthy individuals, who were excluded from COPD on the basis of history, symptom and spirometry; (4) studies provided sufficient published data for estimating an odds ratio (OR) with a 95% confidence interval (95%CI). Articles were excluded based on the following condition: (1) family studies and affected compatriots studies, review articles, case reports and case series; (2) genotype frequencies in controls did not conform to Hardy-Weinberg equilibrium (HWE).

Data extraction

Two investigators extracted data from eligible studies independently and resolved controversies by discussion. For each report, we recorded first

author, year of publication, ethnicity, diagnostic criteria of COPD, genotyping method, number and resources of cases and controls, mean age and smoking history (pack-yrs) of cases and controls, gender distribution, forced expiratory volume in one second (FEV_1) and FEV_1/forced vital capacity (FVC) ratio of cases and controls, allele and genotype frequency in cases and controls.

Statistical analysis

GC gene has three distinct alleles in humans, namely *GC*1F, *GC*1S and *GC*2, which could be assembled into six different genotypes (1F-1F, 1F-1S, 1S-1S, 2-1S, 2-1F, 2-2). So the study was conducted using three different groups (1F-1F, 1F-1S, 1S-1S; 1F-1F, 1F-2, 2-2; and 1S-1S, 1S-2, 2-2) following the traditional SNP polymorphism analysis. HWE in the control group was assessed using chi-square test, with $P \leq 0.05$ considered statistically significant. In this analysis, two genetic models (dominant model: 1F-1F+1F-1S versus 1S-1S, 1F-1F+1F-2 versus 2-2, 2-2+1S-2 versus 1S-1S; recessive model: 1F-1F versus 1F-1S+1S-1S, 1F-1F versus 1F-2+2-2, 2-2 versus 1S-2+1S-1S) and allele analysis(1F versus 1S, 1F versus 2, 2 versus 1S) were used. Besides, the comparison between the homozygotes and the other five genotypes (1F-1F versus 1F-1S+1S-1S+2-1S+2-1F+2-2; 1S-1S versus 1F-1F+1F-1S+2-1S +2-1F+2-2; 2-2 versus 1F-1F+1F-1S+1S-1S+2-1S+2-1F) were conducted to assess the function of different homozygotes. The unadjusted OR with 95%CI was used to assess the strength of the association between the GC polymorphisms and COPD risk based on the

genotype frequencies in cases and controls. Pooled ORs were calculated using fixed- or random-effect models. We used a chi squared-based Q-test to assess heterogeneity among studies. P>0.05 was taken to suggest that effect sizes were larger than those expected by chance, indicating the absence of statistical heterogeneity. Therefore, when P>0.05, a pooled OR was calculated for each study using the fixed-effect model. Otherwise, the random effect model was used. The significance of those ORs was assessed using the Z-test. The threshold for significance in the Z-test was defined as P < 0.05.

In order to assess the ethnicity-specific, subgroup analyses were performed by ethnicity. In order to assess the stability of the results, sensitivity analyses were performed through removing one study at a time.

Funnel plots and Egger's linear regression test were used to inspect the potential publication bias, and p<0.05 was considered that significant publication bias existed.

All statistical tests for this meta-analysis were performed using Stata 11.0 (StataCorp, College Station, USA).

Results

Characteristics of the studies

After a computerized search was performed, about 198 studies were identified. This list was reduced to 12 studies after screening the title and

abstract. After we read the full texts of these articles, three of the 12 relevant articles were excluded because they did not present genotype frequency , and a total of nine articles were identified . The studies identified were published between 1990 and 2014, and the sample sizes ranged from 121 to 517. Of the nine studies, seven were published in English, and other two in Chinese . These studies were performed in China, UK, Japan, Korea, Iceland and Canada. The results from chi-square tests showed that genotypic distribution of the controls was in agreement with the HWE except one study (P=0.016) (Table 1). So we removed the data of both cases and controls in this study, and finally a total of eight case-control trials were included in the meta-analysis (Figure 1).

A total of 2216 individuals (809 COPD patients and 1407 control individuals) were included in this meta-analysis. Base on ethnicity, the participants were divided into two groups: 970 in Asian and 1246 in Caucasians. All studies had a case-control design. All the studies used blood samples for DNA extraction. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was the most commonly used genotyping method in these studies. The basic situations of the included studies and the allele and genotype frequency of the case groups and the control groups are shown in Table 1 and Table 2.

Of the eight studies included, most of the studies used the lung function tests as one of the criteria to define COPD, except one study, which appeared

to use a diagnosis of chronic bronchitis or emphysema. In one study , most of the patients were in moderate to severe stage (186/203). Four studies included the moderate to severe COPD patients as the case groups . In one study, the phenotypes of the participants in case group were sub-classified into emphysema (33/102), bronchial hypersecretion with obstruction (BHO) (34/102), and chronic obstruction without hypersecretion (CO) (35/102), according to the radiological signs and sputum production. Other two studies offered no information about the severity of obstruction .

A single allele (GC1F or GC1S or GC2) comparison

When the *GC*1F allele was compared with *GC*2 allele, the result suggested that the *GC*1F was a risk allele for COPD and that GC2 was a potential protective allele (the random effects model: OR=1.77, 95 %CI: 1.20-2.62, I^2=72.2%). For the *GC*1F allele and *GC*1S allele, the summary OR was 1.47 (95 %CI: 1.22-1.77, I^2=0.0 %) in the fixed effects model, which also supported the hypothesis that the *GC*1F allele was a potential risk allele. When the *GC*1S allele was compared with *GC*2 allele, the result suggested no statistical difference between *GC*2 and GC1F allele in the protection of COPD (the random effects model: OR=1.15, 95 %CI: 0.89-1.51, I^2=58.9%). Based on subgroup analysis, significant associations were found in Asian (1F versus 1S: OR=1.53, 95 %CI: 1.22-1.92, I^2=0.0%; 1F versus 2: OR=1.82, 95 %CI: 1.16-2.85, I^2=74.7%). There was no evidence of a significant association in Caucasian (Figure 2, Table 3).

Overall data analysis of two genetic models

In the *GC*1F/1S group (1F-1F, 1F-1S, 1S-1S), significant results were found in the dominant model (OR=1.46, 95 %CI: 1.03-2.07, I^2=15.1%, fixed effects model) and recessive model (OR=2.18, 95 %CI: 1.55-3.08, I^2=11.8%, fixed effects model), which supported that the *GC*1S allele was a potential protective allele and *GC*1F allele was a risk allele(Figure 3, Table 3). In the *GC*1F/2 group (1F-1F, 1F-2, 2-2), significant results were found in the dominant model (OR=2.86, 95% CI=1.24-6.58, I^2=64.3%, random effects model) and recessive model (OR=2.71, 95% CI=1.27-5.79, I^2=75.7%, random effects model), which also supported that the *GC*2 allele was a potential protective allele and *GC*1F allele was a risk allele(Figure 4, Table 3). For *GC*2/1S group (1S-1S, 1S-2, 2-2), we found no significant association in the recessive and dominant models, which was also in line with the results of the single allele comparison(Figure 5, Table 3). Based on subgroup analyses, the results in the Asian were almost in line with those in the total. But in the Caucasian, significant result was only found in 1F-1F versus 1F-2+2-2.

Analysis of homozygous genes (1F-1F or 1S-1S or 2-2) versus other genotypes

While analyzing samples using homozygous genotypes (1F-1F or 1S-1S or 2-2) in comparison with the five remaining genotypes in cases and controls, the OR was 2.51 (95 %CI=1.49-4.26, I^2=60.5%, random effects model) for *GC*1F-*GC*1F homozygotes, which suggested that the *GC*1F allele might confer an

increased risk to COPD. But no significant result was obtained in *GC*1S-*GC*1S and *GC*2-*GC*2 homozygotes. Based on subgroup analysis, significant associations were found in both Asian and Caucasian population for *GC*1F-*GC*1F homozygotes (Asian: OR=2.42, 95 %CI=1.34-4.36, I^2=68.5%; Caucasian: OR=3.24, 95 %CI=1.13-9.28, I^2=36.9%) (Figure 6, Table 3).

Sensitivity analysis

Statistically similar results were obtained after sequentially excluding one study, suggesting the stability of the meta-analyses.

Publication bias

The shape of the funnel plots was symmetrical for all of the results, and the statistic results of Egger's linear regression test also indicated a lack of publication bias (P>0.05) (Table 3).

Discussion

This study provided the first meta-analysis result of a contribution of the *GC* gene to COPD susceptibility. In this meta-analysis, the results of single allele comparison suggested that *GC*1F is a risk factor of COPD, *GC*2 and *GC*1S allele are protective factors for COPD. In the overall data analysis of two genetic models, we found that the carriers of the *GC*1F allele had a higher risk, and the carriers of the *GC*2 and *GC*1S allele played a protect role in COPD. The results of homozygous gene research groups also supported that the *GC*1F-*GC*1F polymorphism contributed to COPD as a risk factor.

Many reports have suggested the potential mechanism of the association

between *GC* gene polymorphisms and COPD. Vitamin D deficiency is common in COPD patients and correlates with severity of COPD . While in contrast to vitamin D, circulating DBP deficiency is inversely related to low FEV_1, therefore, DBP may play an important role in mechanism of COPD. DBP is a 55 kDa protein, which expressed not only in liver, kidney, gonads and fat, but also by neutrophils . Firstly, it was confirmed that DBP plays a pivotal role in modulating monocyte responses to 25OHD3, and that it is associated with its deglycosylation . Therefore, these effects vary according to DBP genotype. The *GC*2 variant is less able to activate macrophages, for the absence of a glycosylated residue at position 420 , so it plays a protectional role in inflammation. However, *GC*1F plays an opposite effect because of its different protein product at position 420. So the different effect may due to the difference of Gc protein oligosaccharide structure. Secondly, DBP can enhance neutrophil chemotactic activity of complement derived C5a and C5a des Arg, which is associated with inflammation. But no significant different neutrophil chemotaxis is found in different allele (*GC*1F, *GC*1S and *GC*2).

Subgroup analysis by ethnicity allowed us to look for potential ethnic differences. In Asain population, the result of the five articles (including 510 cases and 460 controls) suggested that the *GC*1F allele was associated with increased risk of COPD and that *GC*2 and GC1S allele were protective factors of COPD based on allelic contrast and dominant contrast, recessive contrast and homozygote comparison. However, for the Caucasian population, three

articles (299 cases and 947 controls) were included, and significant association was only found in 1F-1F versus 1F-2+2-2, suggesting that 1F homozygote may be a risk factor for COPD in the Caucasian when compared with *GC*2. We found that the association between *GC* polymorphisms and COPD risk varied between ethnicities. The reasons causing the difference may be as followed. Firstly, subgroup analyses identified only a small number of studies, which may have resulted in poor statistical power. Secondly, one of the three Caucasian studies used chronic bronchitis and emphysema as diagnostic criteria without the spirometric criteria, the lack of significant findings in the Caucasian may be attributed to this diagnostic criteria. Thirdly, the sensitivity of individuals to COPD may be affected by different genetic backgrounds and degrees of environmental exposure.

Some limitations of this meta-analysis should be acknowledged. Firstly, all available literature should be included in the meta-analysis, but articles were only identified with a search of Pubmed and CNKI, and we only included literature published in English and Chinese, thus neglecting studies published in other languages. Besides, all the studies included were from the Asian and Caucasian, and most of the significant findings occurred only in the Asian, thus limiting the generalizability of the result. Further studies are required in other ethnic groups, such as Africans and Latinos. Secondly, only three studies are in the Caucasian, and one of them uses a diagnosis of chronic bronchitis or emphysema, without the spirometric criteria, which may lead the

result to be underpowered. Thirdly, significant heterogeneities were still found in a couple of comparisons. After stratified analyses by ethnicity, heterogeneities were partly reduced or removed, suggesting that ethnicity could explain part of the heterogeneities. Lastly, data were only stratified by ethnicity without other factors, such as age and gender, smoking history, and phenotype of the disease, as sufficient information could not be extracted from the studies.

Although there are some limitations, this is the fist meta-analysis concerning the relationship between *GC* gene polymorphisms and COPD risk to date. Our results reveal that the GC1F allele may be a risk factor for COPD, while the presence of the GC2 and GC1S allele may be protective factors against COPD, especially in Asian population. Our results reveal no association between *GC* gene polymorphisms and COPD in the Caucasian, so further case-control studies are needed.

Acknowledgements

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Conflict of interest

We have no conflict of interest**.**

Authors' Contribution

Huan Chen and Lei Zhang were responsible for study selection, Data

extraction and manuscript drafting. Jianquan Zhang, Xiaoning Zhong and Zhiyi He took responsible for design of the study. Huan Chen, Lei Zhang, and Jing Bai were responsible for data extraction and data analysis. Meihua Li, Xiaoning Zhong and Zhiyi He were responsible for data interpretation and manuscript drafting. Zhiyi He is the sponsor for this paper and has full responsibility for this study.

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Table 1. The essential characteristics of the studies we included

Table 2. Allele and genotype frequency of the case groups and the control groups (%)

Table 3. Meta-analysis of the GC gene polymorphisms and risk of COPD

Figure 1. Flow diagram of study selection in this meta-analysis

Figure 2. Forest plots of the association between the GC polymorphism and risk of COPD (single allele comparison)

(a) 1F versus 1S (b) 1S versus 2 (c) 1F versus 2

Figure 3. Forest plots of the association between the GC polymorphism and risk of COPD in GC1F/1S group

(a) dominant model (b) recessive model

Figure 4. Forest plots of the association between the GC polymorphism and risk of COPD in GC1F /2 group

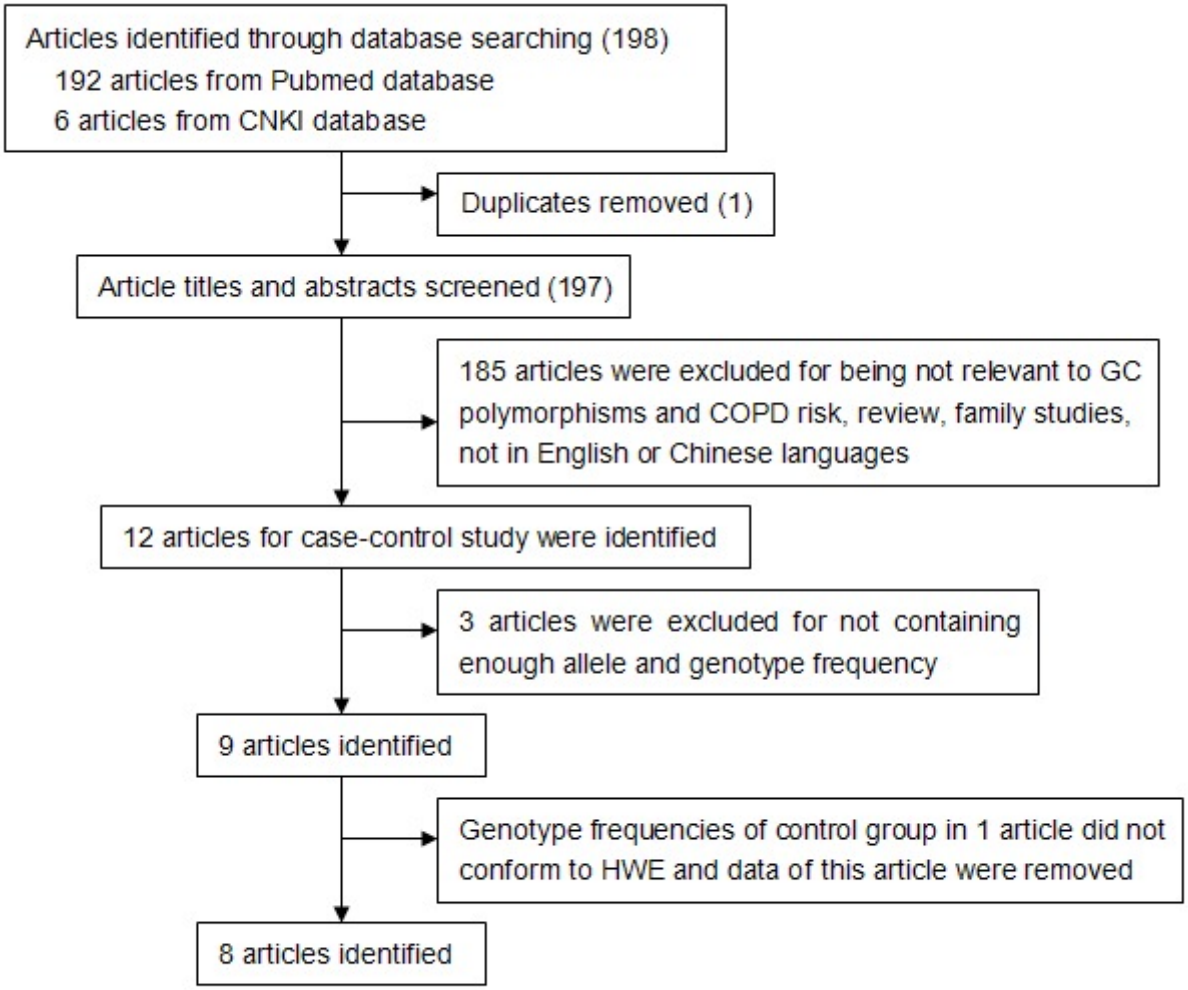
(a) dominant model (b) recessive model

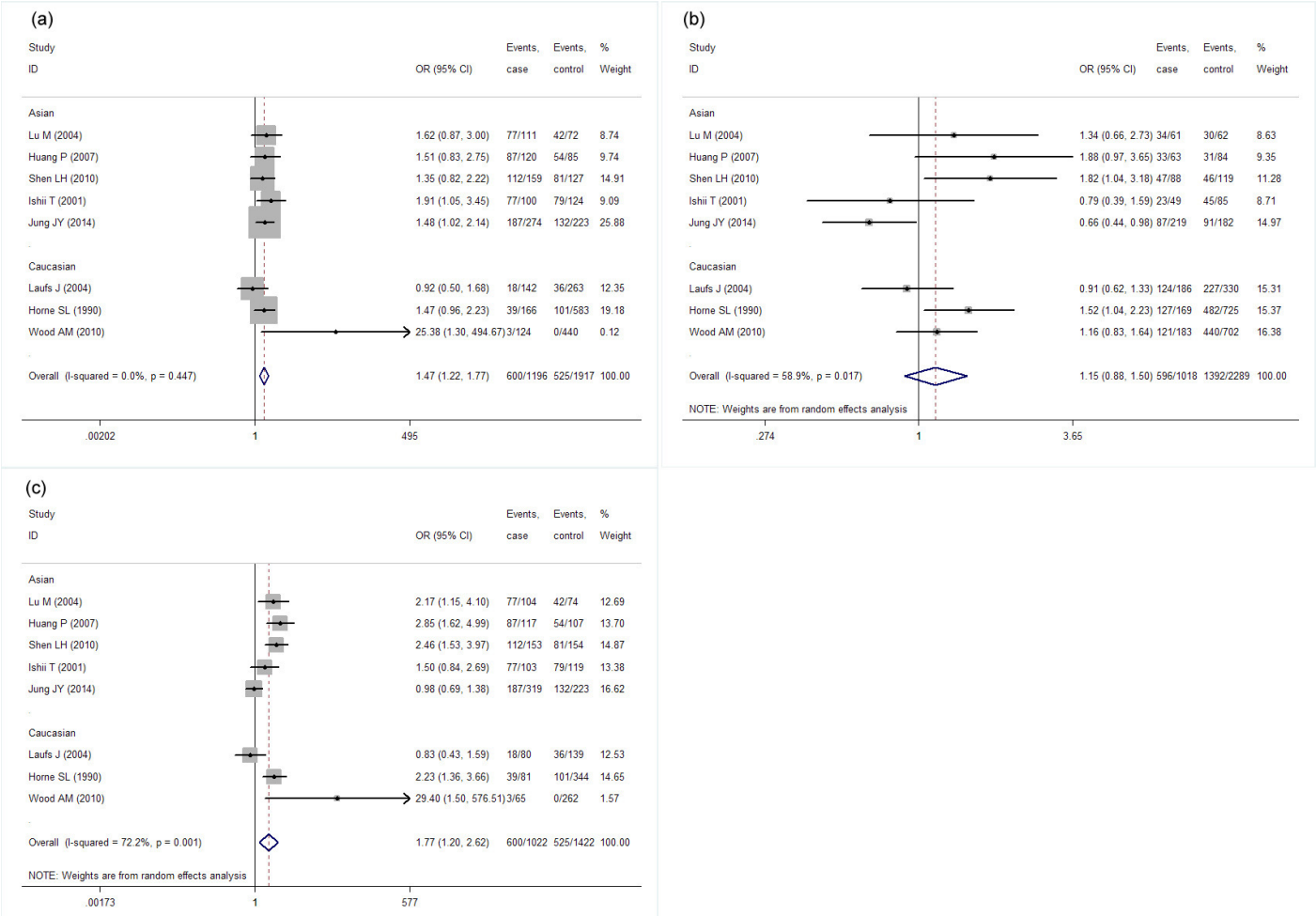
Figure 5. Forest plots of the association between the GC polymorphism and risk of COPD in GC2/1S group

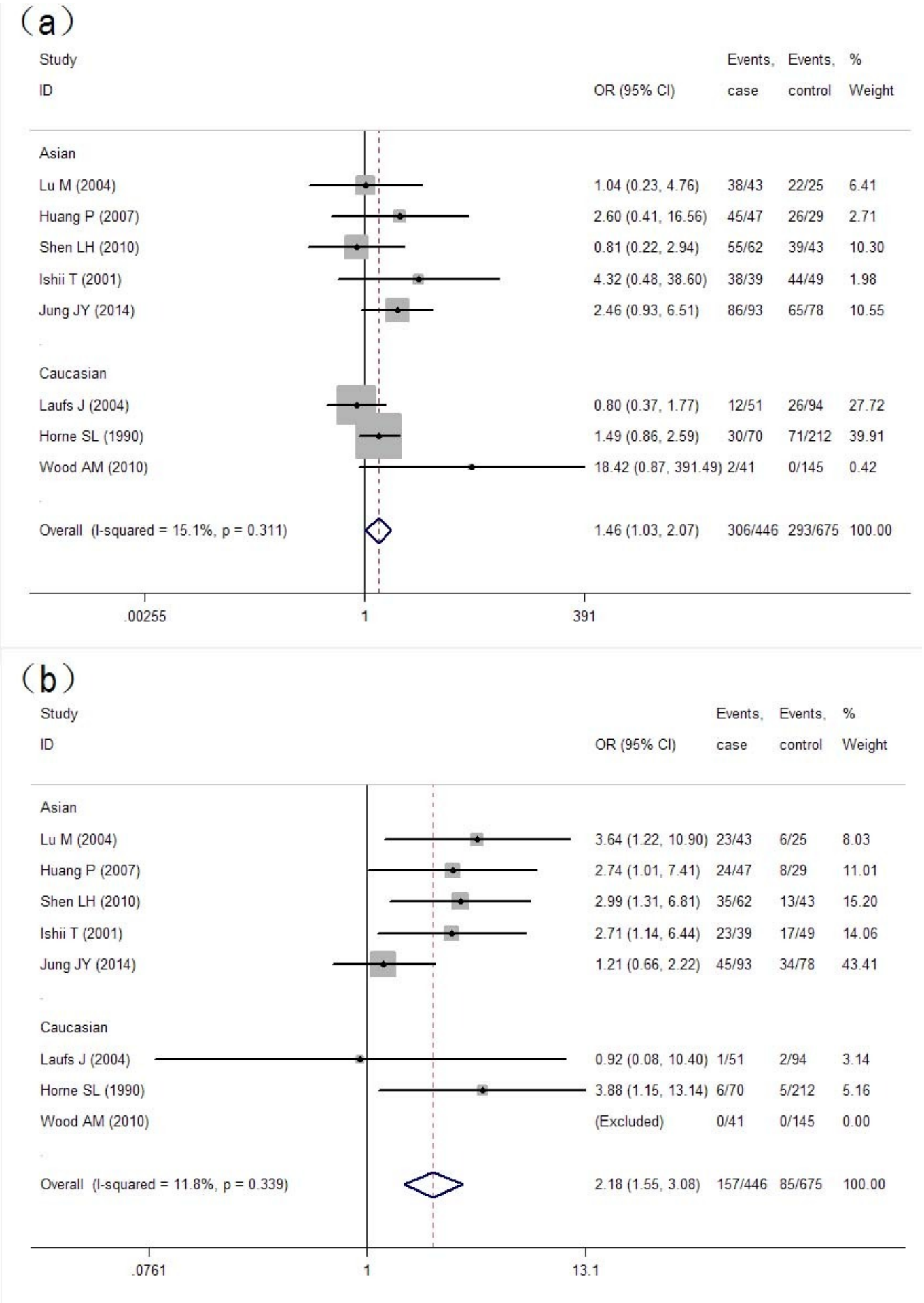
(a) dominant model (b) recessive model

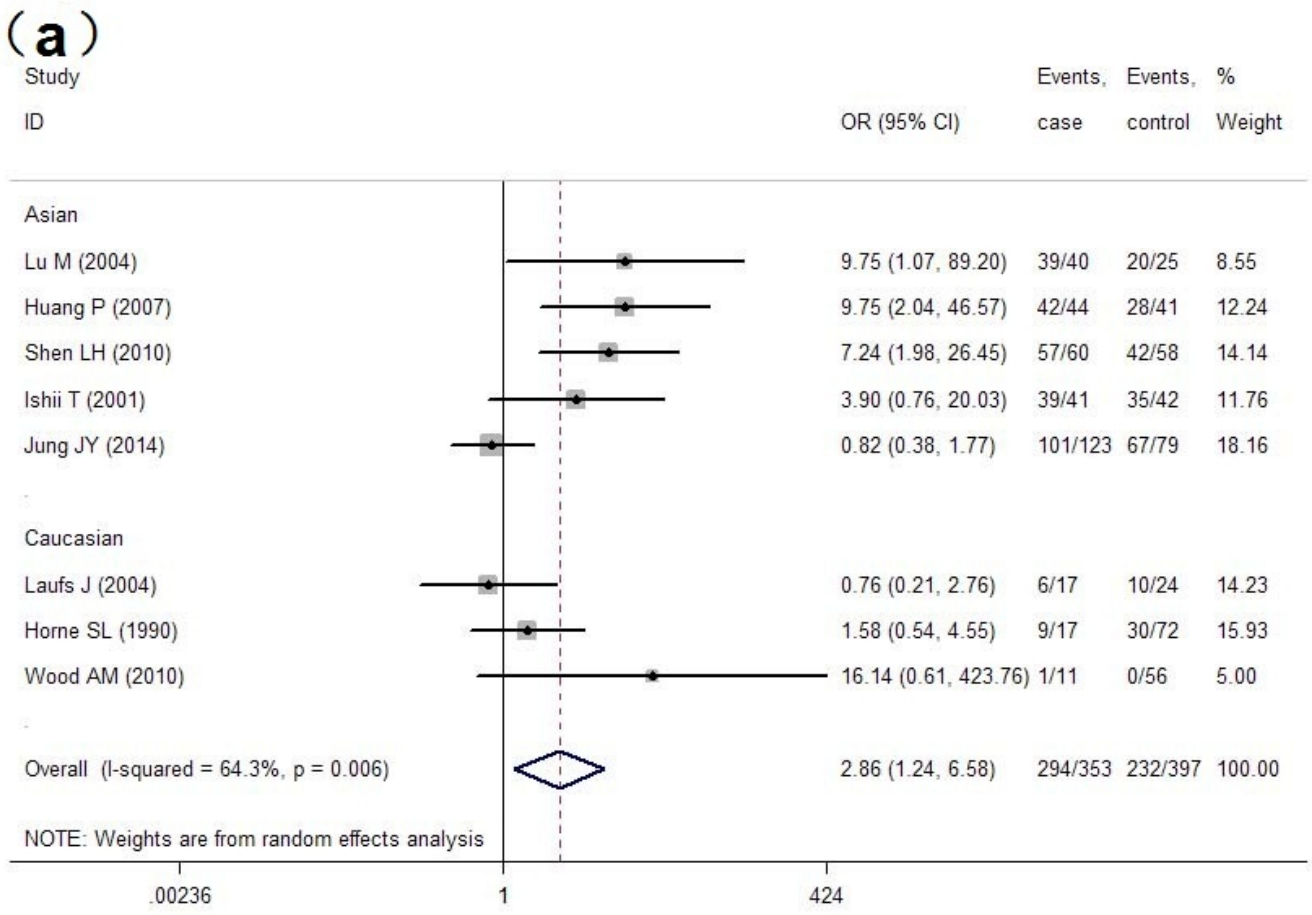
Figure 6. Forest plots of the association between the Q-1 polymorphism and risk of COPD (analysis of homozygous genes versus other genotypes)

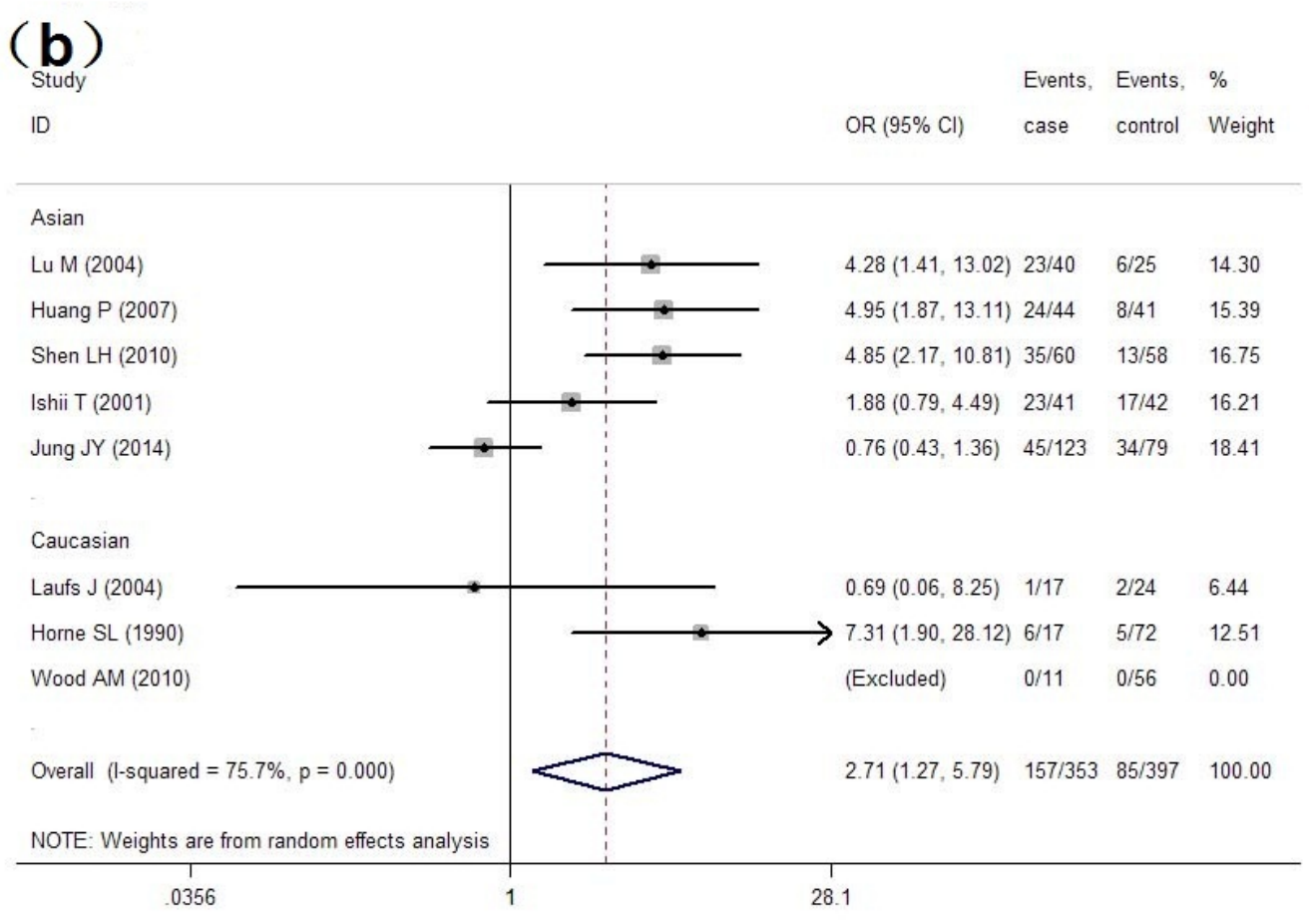
(a) 1F-1F versus 1F-1S+1S-1S+2-1S+2-1F+2-2 (b) 1S-1S versus 1F-1F+1F-1S+2-1S +2-1F+2-2 (c) 2-2 versus 1F-1F+1F-1S+1S-1S+2-1S+2-1F

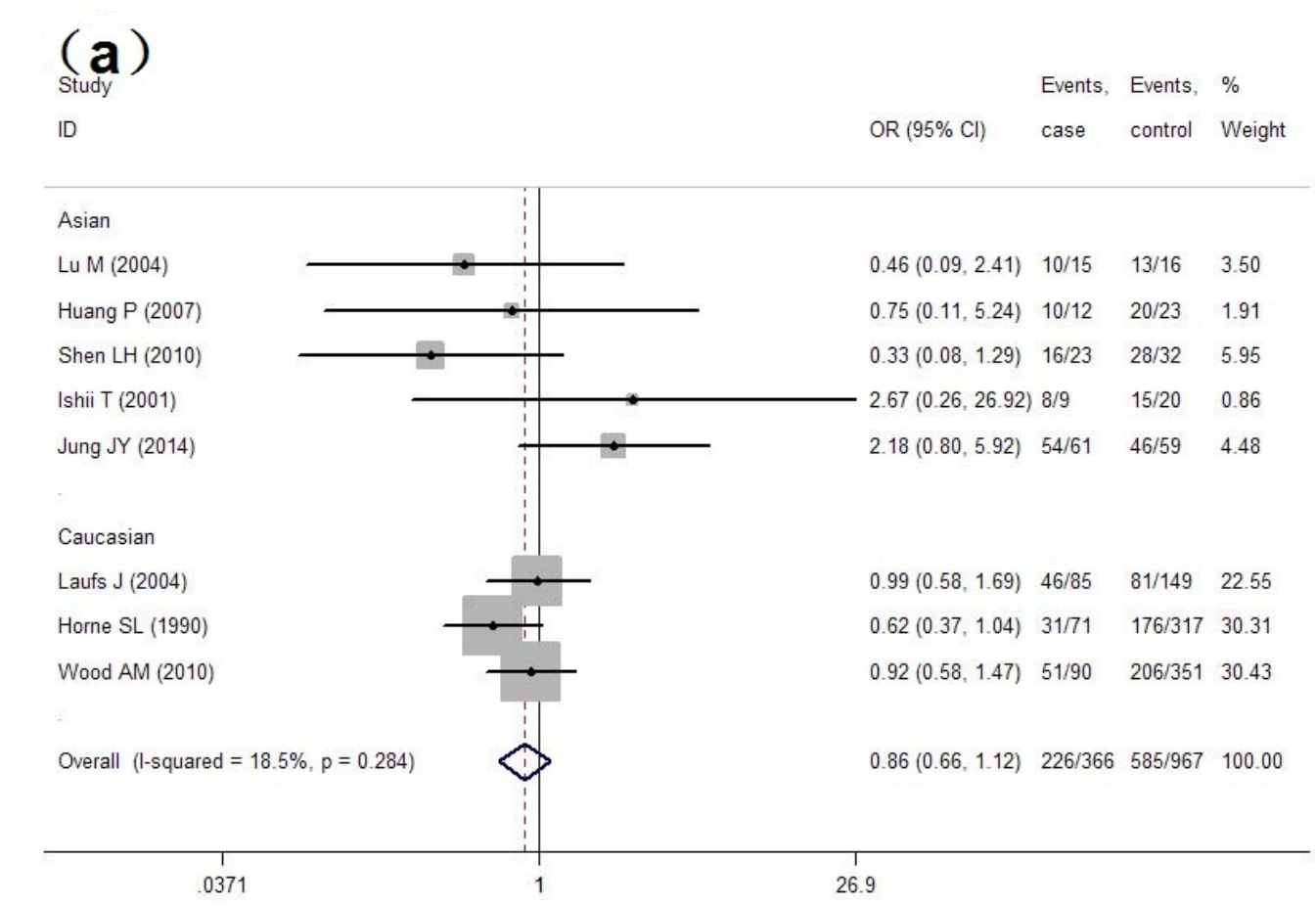

Articles identified through database searching (198)
192 articles from Pubmed database
6 articles from CNKI database
Duplicates removed (1)
Article titles and abstracts screened (197)
185 articles were excluded for being not relevant to GC polymorphisms and COPD risk, review, family studies, not in English or Chinese languages
12 articles for case-control study were identified
3 articles were excluded for not containing enough allele and genotype frequency
9 articles identified
Genotype frequencies of control group in 1 article did not conform to HWE and data of this article were removed
8 articles identified

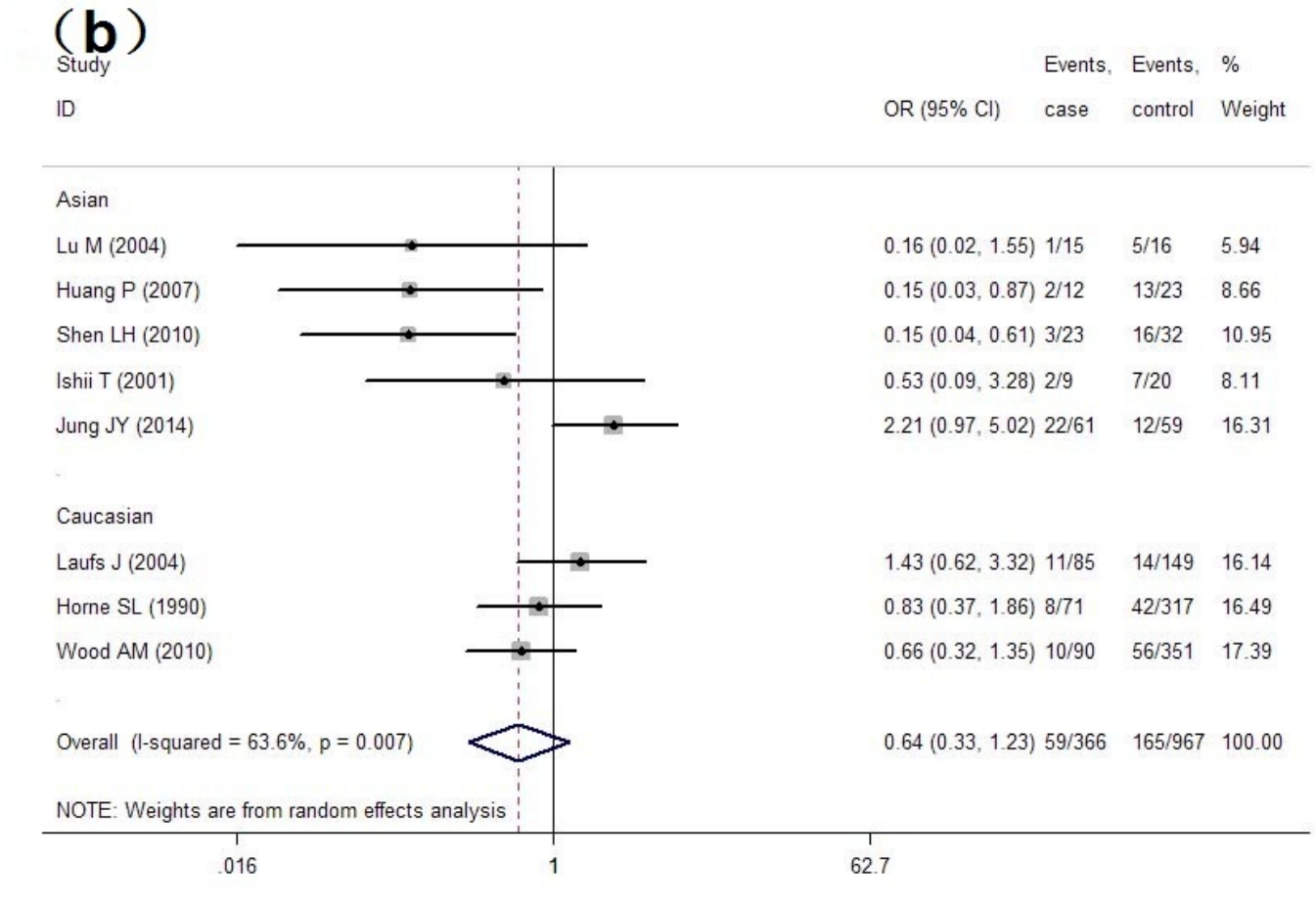

(a)
Study ID
OR (95% CI)
Events, case
Events, control
% Weight
Asian
Lu M (2004) 1.62 (0.87, 3.00) 77/111 42/72 8.74
Huang P (2007) 1.51 (0.83, 2.75) 87/120 54/85 9.74
Shen LH (2010) 1.35 (0.82, 2.22) 112/159 81/127 14.91
Ishii T (2001) 1.91 (1.05, 3.45) 77/100 79/124 9.09
Jung JY (2014) 1.48 (1.02, 2.14) 187/274 132/223 25.88
Caucasian
Laufs J (2004) 0.92 (0.50, 1.68) 18/142 36/263 12.35
Horne SL (1990) 1.47 (0.96, 2.23) 39/166 101/583 19.18
Wood AM (2010) 25.38 (1.30, 494.67) 3/124 0/440 0.12
Overall (I-squared = 0.0%, p = 0.447) 1.47 (1.22, 1.77) 600/1196 525/1917 100.00
.00202
1
495
(b)
Study ID
OR (95% CI)
Events, case
Events, control
% Weight
Asian
Lu M (2004) 1.34 (0.66, 2.73) 34/61 30/62 8.63
Huang P (2007) 1.88 (0.97, 3.65) 33/63 31/84 9.35
Shen LH (2010) 1.82 (1.04, 3.18) 47/88 46/119 11.28
Ishii T (2001) 0.79 (0.39, 1.59) 23/49 45/85 8.71
Jung JY (2014) 0.66 (0.44, 0.98) 87/219 91/182 14.97
Caucasian
Laufs J (2004) 0.91 (0.62, 1.33) 124/186 227/330 15.31
Horne SL (1990) 1.52 (1.04, 2.23) 127/169 482/725 15.37
Wood AM (2010) 1.16 (0.83, 1.64) 121/183 440/702 16.38
Overall (I-squared = 58.9%, p = 0.017) 1.15 (0.88, 1.50) 596/1018 1392/2289 100.00
NOTE: Weights are from random effects analysis
.274
1
3.65
(c)
Study ID
OR (95% CI)
Events, case
Events, control
% Weight
Asian
Lu M (2004) 2.17 (1.15, 4.10) 77/104 42/74 12.69
Huang P (2007) 2.85 (1.62, 4.99) 87/117 54/107 13.70
Shen LH (2010) 2.46 (1.53, 3.97) 112/153 81/154 14.87
Ishii T (2001) 1.50 (0.84, 2.69) 77/103 79/119 13.38
Jung JY (2014) 0.98 (0.69, 1.38) 187/319 132/223 16.62
Caucasian
Laufs J (2004) 0.83 (0.43, 1.59) 18/80 36/139 12.53
Horne SL (1990) 2.23 (1.36, 3.66) 39/81 101/344 14.65
Wood AM (2010) 29.40 (1.50, 576.51) 3/65 0/262 1.57
Overall (I-squared = 72.2%, p = 0.001) 1.77 (1.20, 2.62) 600/1022 525/1422 100.00
NOTE: Weights are from random effects analysis
.00173
1
577


(a)
Study ID
OR (95% CI)
Events, case
Events, control
% Weight
Asian
Lu M (2004) 1.04 (0.23, 4.76) 38/43 22/25 6.41
Huang P (2007) 2.60 (0.41, 16.56) 45/47 26/29 2.71
Shen LH (2010) 0.81 (0.22, 2.94) 55/62 39/43 10.30
Ishii T (2001) 4.32 (0.48, 38.60) 38/39 44/49 1.98
Jung JY (2014) 2.46 (0.93, 6.51) 86/93 65/78 10.55
Caucasian
Laufs J (2004) 0.80 (0.37, 1.77) 12/51 26/94 27.72
Horne SL (1990) 1.49 (0.86, 2.59) 30/70 71/212 39.91
Wood AM (2010) 18.42 (0.87, 391.49) 2/41 0/145 0.42
Overall (I-squared = 15.1%, p = 0.311) 1.46 (1.03, 2.07) 306/446 293/675 100.00
.00255
1
391
(b)
Study ID
OR (95% CI)
Events, case
Events, control
% Weight
Asian
Lu M (2004) 3.64 (1.22, 10.90) 23/43 6/25 8.03
Huang P (2007) 2.74 (1.01, 7.41) 24/47 8/29 11.01
Shen LH (2010) 2.99 (1.31, 6.81) 35/62 13/43 15.20
Ishii T (2001) 2.71 (1.14, 6.44) 23/39 17/49 14.06
Jung JY (2014) 1.21 (0.66, 2.22) 45/93 34/78 43.41
Caucasian
Laufs J (2004) 0.92 (0.08, 10.40) 1/51 2/94 3.14
Horne SL (1990) 3.88 (1.15, 13.14) 6/70 5/212 5.16
Wood AM (2010) (Excluded) 0/41 0/145 0.00
Overall (I-squared = 11.8%, p = 0.339) 2.18 (1.55, 3.08) 157/446 85/675 100.00
.0761
1
13.1


(a)
Study
ID
OR (95% CI)
Events, case
Events, control
% Weight
Asian
Lu M (2004) 9.75 (1.07, 89.20) 39/40 20/25 8.55
Huang P (2007) 9.75 (2.04, 46.57) 42/44 28/41 12.24
Shen LH (2010) 7.24 (1.98, 26.45) 57/60 42/58 14.14
Ishii T (2001) 3.90 (0.76, 20.03) 39/41 35/42 11.76
Jung JY (2014) 0.82 (0.38, 1.77) 101/123 67/79 18.16
Caucasian
Laufs J (2004) 0.76 (0.21, 2.76) 6/17 10/24 14.23
Horne SL (1990) 1.58 (0.54, 4.55) 9/17 30/72 15.93
Wood AM (2010) 16.14 (0.61, 423.76) 1/11 0/56 5.00
Overall (I-squared = 64.3%, p = 0.006) 2.86 (1.24, 6.58) 294/353 232/397 100.00
NOTE: Weights are from random effects analysis
.00236
1
424


(b)
Study
ID
OR (95% CI)
Events, case
Events, control
% Weight
Asian
Lu M (2004) 4.28 (1.41, 13.02) 23/40 6/25 14.30
Huang P (2007) 4.95 (1.87, 13.11) 24/44 8/41 15.39
Shen LH (2010) 4.85 (2.17, 10.81) 35/60 13/58 16.75
Ishii T (2001) 1.88 (0.79, 4.49) 23/41 17/42 16.21
Jung JY (2014) 0.76 (0.43, 1.36) 45/123 34/79 18.41
Caucasian
Laufs J (2004) 0.69 (0.06, 8.25) 1/17 2/24 6.44
Horne SL (1990) 7.31 (1.90, 28.12) 6/17 5/72 12.51
Wood AM (2010) (Excluded) 0/11 0/56 0.00
Overall (I-squared = 75.7%, p = 0.000) 2.71 (1.27, 5.79) 157/353 85/397 100.00
NOTE: Weights are from random effects analysis
.0356
1
28.1


(a)
Study
ID
OR (95% CI)
Events, case
Events, control
% Weight
Asian
Lu M (2004) 0.46 (0.09, 2.41) 10/15 13/16 3.50
Huang P (2007) 0.75 (0.11, 5.24) 10/12 20/23 1.91
Shen LH (2010) 0.33 (0.08, 1.29) 16/23 28/32 5.95
Ishii T (2001) 2.67 (0.26, 26.92) 8/9 15/20 0.86
Jung JY (2014) 2.18 (0.80, 5.92) 54/61 46/59 4.48
Caucasian
Laufs J (2004) 0.99 (0.58, 1.69) 46/85 81/149 22.55
Horne SL (1990) 0.62 (0.37, 1.04) 31/71 176/317 30.31
Wood AM (2010) 0.92 (0.58, 1.47) 51/90 206/351 30.43
Overall (I-squared = 18.5%, p = 0.284) 0.86 (0.66, 1.12) 226/366 585/967 100.00
.0371
1
26.9


(b)
Study
ID
OR (95% CI)
Events, case
Events, control
% Weight
Asian
Lu M (2004) 0.16 (0.02, 1.55) 1/15 5/16 5.94
Huang P (2007) 0.15 (0.03, 0.87) 2/12 13/23 8.66
Shen LH (2010) 0.15 (0.04, 0.61) 3/23 16/32 10.95
Ishii T (2001) 0.53 (0.09, 3.28) 2/9 7/20 8.11
Jung JY (2014) 2.21 (0.97, 5.02) 22/61 12/59 16.31
Caucasian
Laufs J (2004) 1.43 (0.62, 3.32) 11/85 14/149 16.14
Horne SL (1990) 0.83 (0.37, 1.86) 8/71 42/317 16.49
Wood AM (2010) 0.66 (0.32, 1.35) 10/90 56/351 17.39
Overall (I-squared = 63.6%, p = 0.007) 0.64 (0.33, 1.23) 59/366 165/967 100.00
NOTE: Weights are from random effects analysis
.016
1
62.7

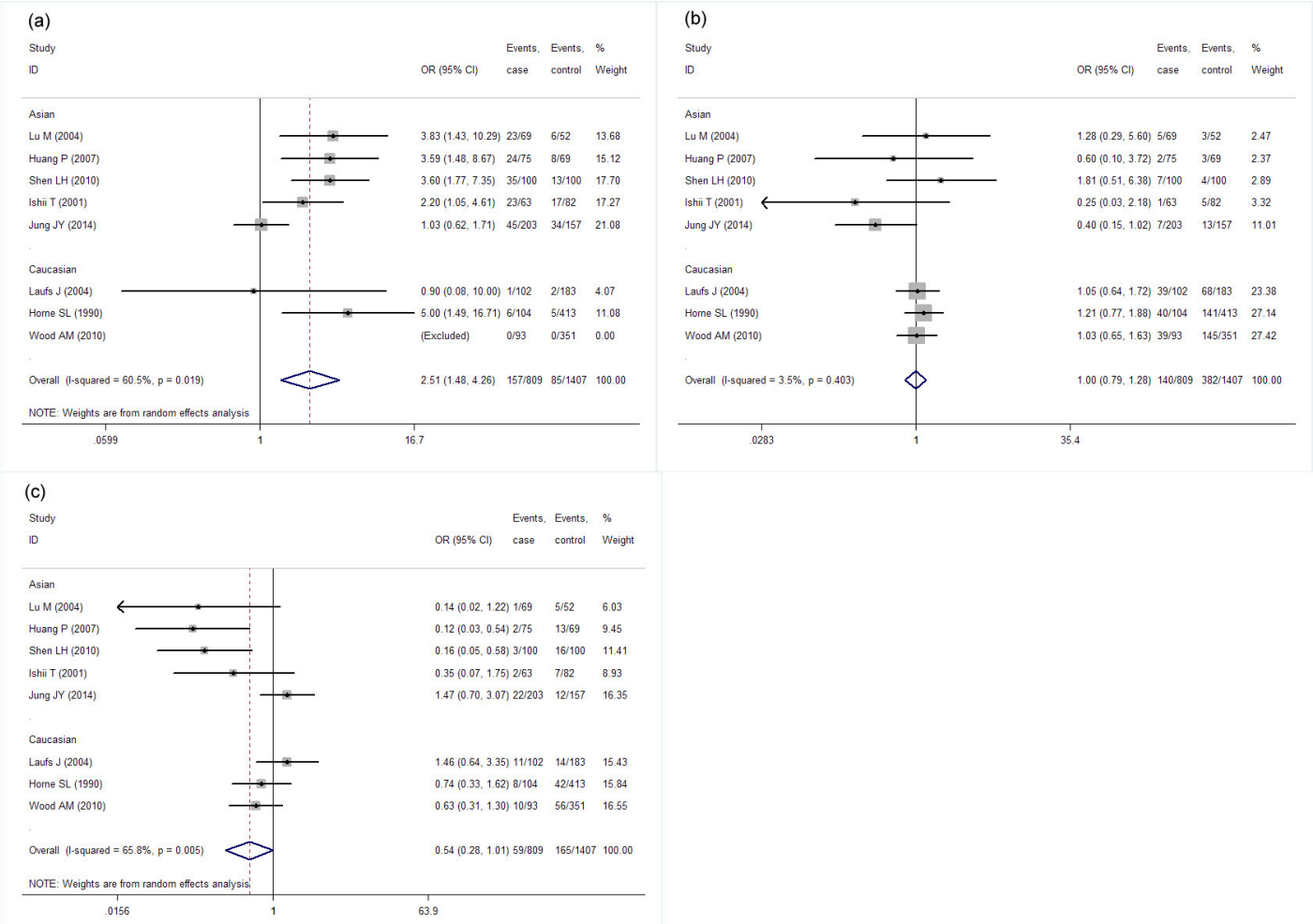

(a)
Study ID
OR (95% CI)
Events, case
Events, control
% Weight
Asian
Lu M (2004) 3.83 (1.43, 10.29) 23/69 6/52 13.68
Huang P (2007) 3.59 (1.48, 8.67) 24/75 8/69 15.12
Shen LH (2010) 3.60 (1.77, 7.35) 35/100 13/100 17.70
Ishii T (2001) 2.20 (1.05, 4.61) 23/63 17/82 17.27
Jung JY (2014) 1.03 (0.62, 1.71) 45/203 34/157 21.08
Caucasian
Laufs J (2004) 0.90 (0.08, 10.00) 1/102 2/183 4.07
Horne SL (1990) 5.00 (1.49, 16.71) 6/104 5/413 11.08
Wood AM (2010) (Excluded) 0/93 0/351 0.00
Overall (I-squared = 60.5%, p = 0.019) 2.51 (1.48, 4.26) 157/809 85/1407 100.00
NOTE: Weights are from random effects analysis
.0599
1
16.7
(b)
Study ID
OR (95% CI)
Events, case
Events, control
% Weight
Asian
Lu M (2004) 1.28 (0.29, 5.60) 5/69 3/52 2.47
Huang P (2007) 0.60 (0.10, 3.72) 2/75 3/69 2.37
Shen LH (2010) 1.81 (0.51, 6.38) 7/100 4/100 2.89
Ishii T (2001) 0.25 (0.03, 2.18) 1/63 5/82 3.32
Jung JY (2014) 0.40 (0.15, 1.02) 7/203 13/157 11.01
Caucasian
Laufs J (2004) 1.05 (0.64, 1.72) 39/102 68/183 23.38
Horne SL (1990) 1.21 (0.77, 1.88) 40/104 141/413 27.14
Wood AM (2010) 1.03 (0.65, 1.63) 39/93 145/351 27.42
Overall (I-squared = 3.5%, p = 0.403) 1.00 (0.79, 1.28) 140/809 382/1407 100.00
.0283
1
35.4
(c)
Study ID
OR (95% CI)
Events, case
Events, control
% Weight
Asian
Lu M (2004) 0.14 (0.02, 1.22) 1/69 5/52 6.03
Huang P (2007) 0.12 (0.03, 0.54) 2/75 13/69 9.45
Shen LH (2010) 0.16 (0.05, 0.58) 3/100 16/100 11.41
Ishii T (2001) 0.35 (0.07, 1.75) 2/63 7/82 8.93
Jung JY (2014) 1.47 (0.70, 3.07) 22/203 12/157 16.35
Caucasian
Laufs J (2004) 1.46 (0.64, 3.35) 11/102 14/183 15.43
Horne SL (1990) 0.74 (0.33, 1.62) 8/104 42/413 15.84
Wood AM (2010) 0.63 (0.31, 1.30) 10/93 56/351 16.55
Overall (I-squared = 65.8%, p = 0.005) 0.54 (0.28, 1.01) 59/809 165/1407 100.00
NOTE: Weights are from random effects analysis
.0156
1
63.9

Table 1. The essential characteristics of the studies we included

First author	*Year*	*Ethnicity*	*Genotyping method*	*Diagnostic criteria of COPD*	*Resources*		*Sample size*		*Mean age*		*Smoking history (pack-yrs)*		*gender distribution (male/female)*		*mean FEV_1 (L)*		*mean FEV_1/FVC (%)*		**HWE**
					Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	
Lu M [17]	2004	Asian	PCR-RFLP	Guidelines of Chronic obstructive pulmonary disease diagnosis and treatment(1997)	local hospital	Healthy smokers (≥400 pack-years) with no history of COPD and a normal spirometry (age ≥50 years)	69	52	67.1±7.4	64.9±8.6	820.4±429.7	690.6±353.5	NG	NG	NG	NG	NG	NG	0.45
Huang P [23]	2007	Asian	PCR-RFLP	Guidelines of Chronic obstructive pulmonary disease diagnosis and treatment(2002)	local hospital	Healthy individuals with a normal spirometry	75	69	60±6	60±6	37±4	37±4	75/0	69/0	**NG**	**NG**	60±4	86±3	**0.095**
Shen LH [20]	2010	Asian	PCR-RFLP	Guidelines of The American Thoracic Society	local hospital	Healthy adults with no history of COPD and spirometry show FEV_1 > 85% pred and FEV_1/FVC > 75%	100	100	62.3±9.7	60.9±8.6	123.5±29.7	25.6±13.3	72/28	66/34	1.28±0.43	3.57±0.65	43.5±12.8	92.8±5.8	0.12
Laufs J [24]	2004	Caucasian	PCR-RFLP	Guidelines of The American Thoracic Society	local hospital	Healthy volunteers from the same area (mostly non-smokers)	102	183	71.7	42.9	38	NG	42/60	105/78	NG	NG	NG	NG	0.84
Ito I [19]	2004	Asian	PCR-RFLP	Guidelines of The American Thoracic Society	local hospital	Healthy smokers (> 20 pack-years) with no history of COPD and a normal spirometry	103	88	67.4±7.8	60.8±12.0	58.3±29.1	41.1±22.2	99/4	72/16	1.18±0.49	2.66±0.86	45.8±10.4	80.1±7.8	0.016
Ishii T [18]	2001	Asian	PCR-RFLP	Guidelines of The American Thoracic Society	local hospital	Healthy, anonymous, ethnically matched volunteers	63	82	68.3±9.9	NG	102.2±40.4	NG	60/3	42/40	**NG**	**NG**	44.3±12.7	NG	0.31
Horne SL [22]	1990	Caucasian	Isoelectric focusing electrophoresis	Chronic bronchitis and emphysema	local hospital	local residents with similar ethnic origins	104	413	NG	NG	NG	NG	NG	NG	**NG**	**NG**	NG	NG	0.34
Wood AM [16]	2010	Caucasian	PCR-RFLP	FEV_1/FVC<0.7and post-bronchodilator FEV_1<80% predicted	the UK national registry	Healthy, anonymous, geographically matched individuals	93	351	NG	NG	NG	NG	NG	NG	**NG**	**NG**	36	NG	0.45
Jung JY [26]	2014	Asian	PCR-RFLP	post-bronchodilator FEV_1/FVC<0.7	KOLD Cohort	Healthy smokers with a normal spirometry	203	157	67	53	46.0±23.4	30.7±17.4	199/4	148/9	1.42±0.49	3.17±0.59	47.5±10.9	78.3±5.1	0.12

Table 2. Allele and genotype frequency of the case groups and the control groups (%)

First author	The frequency of the allele						The frequency of the genotype											
	Cases			Controls			Cases						Controls					
	1F	1S	2	1F	1S	2	1F-1F	1F-1S	1F-2	1S-1S	1S-2	2--2	1F-1F	1F-1S	1F-2	1S-1S	1S-2	2--2
Lu M [17]	77	34	27	42	30	32	23	15	16	5	9	1	6	16	14	3	8	5
Huang P [23]	87	33	30	54	31	53	24	21	18	2	8	2	8	18	20	3	7	13
Shen LH [20]	112	47	41	81	46	73	35	20	22	7	13	3	13	26	29	4	12	16
Laufs J.[24]	18	124	62	36	227	103	1	11	5	39	35	11	2	24	8	68	67	14
Ito I [19]	120	46	40	87	47	42	33	29	25	3	11	2	15	27	30	5	10	1
Ishii T [18]	77	23	26	79	45	40	23	15	16	1	6	2	17	27	18	5	8	7
Horne SL [22]	39	127	42	101	482	243	6	24	3	40	23	8	5	66	25	141	134	42
Wood AM [16]	3	121	62	0	440	262	0	2	1	39	41	10	0	0	0	145	150	56
Jung JY [26]	187	87	132	132	91	91	45	41	56	7	32	22	34	31	33	13	34	12

Table 3. Meta-analysis of the GC gene polymorphisms and risk of COPD

Models	*Ethnicity*	*P*	*OR*	*95%CI*	I^2	*P for heterogeneity*	**P for Egger's test**
1F versus 1S	Asian	0.000	1.53	1.22-1.92	0.0%	0.935	0.133
	Caucasian	0.086	1.34	0.96-1.88	63.4%	0.065	
	Total	0.000	1.47	1.22-1.77	0.0%	0.447	
1S versus 2	Asian	0.533	1.16	0.723-1.87	68.4%	0.011	0.525
	Caucasian	0.129	1.18	0.96-1.32	43.5%	0.171	
	Total	0.290	1.15	0.88-1.50	58.9%	0.017	
1F versus 2	Asian	0.009	1.82	1.16-2.85	74.7%	0.003	0.183
	Caucasian	0.242	1.91	0.65-5.67	78.6%	0.009	
	Total	0.004	1.77	1.20-2.62	72.2%	0.001	
Dominant (1F-1F+1F-1S versus 1S-1S)	Asian	0.062	1.77	0.97-3.21	0.0%	0.537	0.235
	Caucasian	0.222	1.31	0.85-2.04	56%	0.103	
	Total	0.036	1.46	1.03-2.07	15.1%	0.311	
Recessive(1F-1F versus 1F-1S+1S-1S)	Asian	0.000	2.13	1.48-3.07	26.5%	0.245	0.403
	Caucasian	0.061	2.76	0.96-7.99	8.1%	0.297	
	Total	0.000	2.18	1.55-3.08	11.8%	0.339	
Dominant (1F-1F+1F-2 versus 2-2)	Asian	0.023	4.087	1.21-13.81	74.0%	0.004	0.28
	Caucasian	0.414	1.38	0.64-2.98	34.5%	0.217	
	Total	0.014	2.86	1.24-6.58	64.3%	0.006	
Recessive(1F-1F versus 1F-2+2-2)	Asian	0.028	2.59	1.11-6.06	80.5%	0.000	0.305
	Caucasian	0.027	3.614	1.16-11.24	63.8%	0.096	
	Total	0.010	2.71	1.27-5.79	75.7%	0.000	
Dominant (2-2+1S-2 versus 1S-1S)	Asian	0.946	1.02	0.55-1.89	38.5%	0.164	0.932
	Caucasian	0.209	0.83	0.62-1.11	0.0%	0.405	
	Total	0.268	0.86	0.66-1.12	18.5%	0.284	
Recessive(2-2 versus 1S-2+1S-1S)	Asian	0.166	0.38	0.10-1.50	76.4%	0.002	0.068
	Caucasian	0.552	0.87	0.56-1.36	0.0%	0.377	
	Total	0.180	0.64	0.33-1.23	63.6%	0.007	
1F-1F versus 1F-1S+1S-1S+2-1S+2-1F+2-2	Asian	0.003	2.42	1.34-4.36	68.5%	0.013	0.376
	Caucasian	0.028	3.24	1.13-9.28	36.9%	0.208	
	Total	0.001	2.51	1.48-4.26	60.5%	0.019	
1S-1S versus 1F-1F+1F-1S+2-1S +2-1F+2-2	Asian	0.187	0.68	0.38-1.21	21.7%	0.276	0.277
	Caucasian	0.511	1.09	0.84-1.43	0.0%	0.866	
	Total	0.980	1.00	0.79-1.28	3.5%	0.403	
2-2 versus 1F-1F+1F-1S+1S-1S+2-1S+2-1F	Asian	0.054	0.30	0.09-1.02	75.2%	0.003	0.24
	Caucasian	0.410	0.83	0.54-1.29	16.8%	0.301	
	Total	0.053	0.54	0.28-1.01	65.8%	0.005	