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# COX-2 rs5275 and rs689466 polymorphism and risk of lung cancer

## A PRISMA-compliant meta-analysis

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**Abstract**

**Background:** Cyclooxygenase-2 (COX-2) is an inducible enzyme that mediates the synthesis of prostaglandin, which plays an important role in the inflammation response. The overexpression of COX-2 in lung cancer has been found in several studies, suggesting that COX-2 contributes to carcinogenesis. There are many previous case-control studies focused on the association between COX-2 polymorphism and lung cancer risk, however, the conclusion remained controversial.

**Objectives:** We performed this meta-analysis to evaluate the association between COX-2 rs5275 and rs689466 polymorphism and susceptibility to lung cancer.

**Methods:** A systematic literature research was conducted on PubMed, Embase, Cochrane Library, OVID, Web of Science, and Google Scholar up to November 30, 2017. The quality of studies was assessed by Newcastle–Ottawa scale. We combined odds ratios (ORs) and 95% confidence intervals (CIs) in 5 different genetic models for evaluation under a fixed-effect model or random-effect model. Subgroup analysis was performed according to source of control, ethnicity, pathological types, and smoking status. Sensitivity analysis and publication bias were also conducted.

**Results:** Eventually, 14 eligible studies were included in our meta-analysis. We found rs5275 gene polymorphism decreased the risk of lung cancer under heterozygote model (OR: 0.91, 95% CI: 0.84–0.98, $P = .02$). COX-2 rs689466 gene polymorphism was also related to a significantly reduced risk under allele (OR: 0.88, 95% CI: 0.82–0.95, $P = .001$), homozygote (OR: 0.81, 95% CI: 0.68–0.95, $P = .01$), heterozygote (OR: 0.81, 95% CI: 0.72–0.91, $P < .001$), and dominant model (OR: 0.81, 95% CI: 0.72–0.91, $P < .001$), except for recessive model. Subgroup analysis suggested a similar association in Asians, but not in Caucasians. Polymorphism of rs5275 was strongly associated with a reduced risk of lung adenocarcinoma according to stratified analysis by pathological types. Egger test identified no significant publication bias.

**Conclusions:** Our meta-analysis demonstrated that COX-2 rs5275 and rs689466 polymorphism significantly decrease the risk of lung cancer in Asians but not in Caucasians, indicating COX-2 could serve as a potential diagnostic marker for lung cancer.

**Abbreviations:** AD = adenocarcinoma, CI = confidence interval, COX-2 = cyclooxygenase-2, HB = hospital-based, HWE = Hardy–Weinberg equilibrium, NOS = Newcastle–Ottawa Scale, ORs = odds ratios, PB = population-based, PCR-LDR = polymerase chain reaction-ligase detection reactions, PCR-PIRA = polymerase chain reaction-based primer-introduced restriction analysis, PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism, PRISMA = Preferred Reporting Items for Systematic Reviews and Meta-Analyses, PTGS-2 = prostaglandin endoperoxyde synthase-2, RAF = risk allele frequency, RT-qPCR = reverse transcription-quantitative polymerase chain reaction, SCC = squamous cell carcinoma, SM = small cell carcinoma, SNPs = single-nucleotide polymorphisms.

**Keywords:** COX-2, gene polymorphism, lung cancer, rs5275, rs689466

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## 1. Introduction

Lung carcinoma is the leading cause of cancer-associated mortality around the world and the incidence rate has a noteworthy increase during the last century.[1] Although the pathogenesis of lung cancer is fairly complicated and still remains unclear, numerous clinical and experimental investigations have indicated the following risk factors: smoking, environmental pollution, occupation, chronic lung diseases, and genetic susceptibility. With the rapid development of genotyping, the identification of genes that may lead to lung cancer has been successfully conducted in recent years, which could contribute to the investigation of potential mechanisms and formulation of prevention strategies as well as genetic targeted therapy.

Inflammation has been assumed as one of the etiological factors for lung cancer. Cyclooxygenase-2 (COX-2), also named as prostaglandin endoperoxyde synthase-2 (PTGS-2), is an inducible enzyme that transforms arachidonic acid into prostaglandins, which are potent mediators in the inflammation response.[2] COX-2 is not normally expressed but could be activated by growth factors, cytokines, oncogenes, and chemical carcinogens.[3] Accumulated evidence has revealed that COX-2 gene was overexpressed in lung cancer, which suggests that COX-2 contributes to carcinogenesis.[4,5]

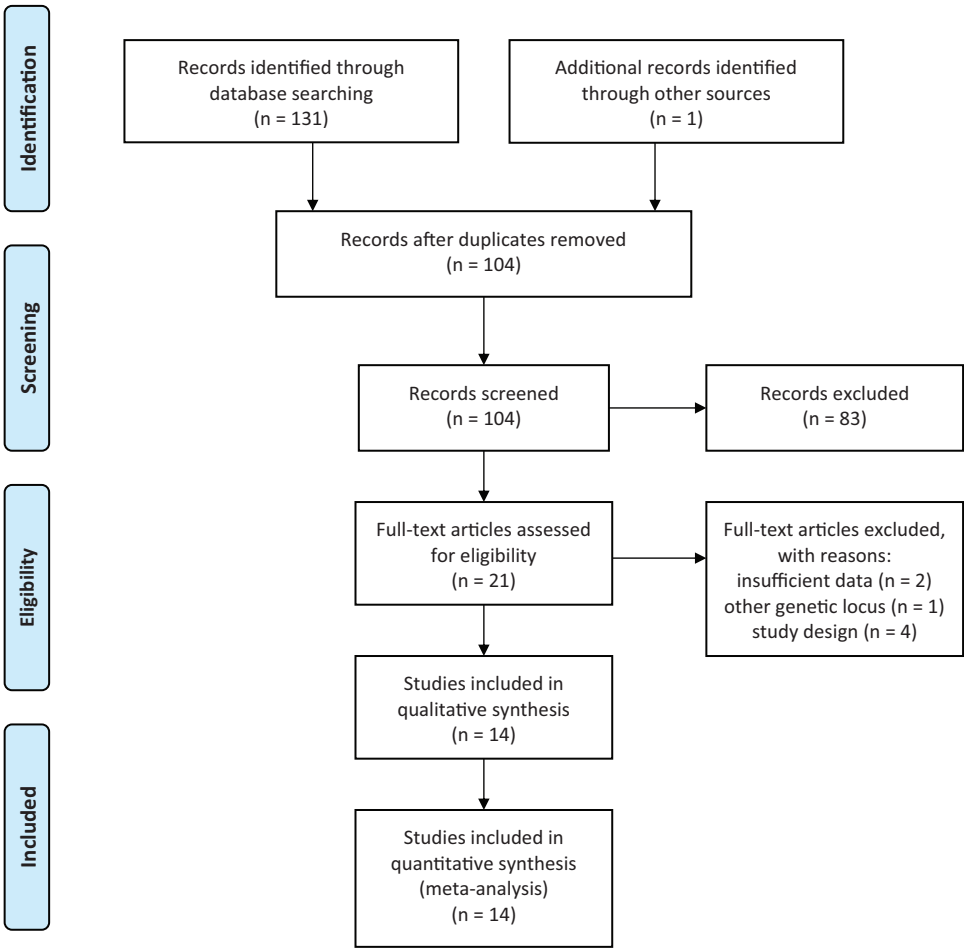
A certain number of single-nucleotide polymorphisms (SNPs) in COX-2 gene may affect the susceptibility to lung cancer through their alteration of enzyme function and expression. The rs689466 polymorphism was revealed to regulate the transcription levels of COX-2,[6] while rs5275 polymorphism may determine the stability of COX-2 mRNA and translation efficiency.[7] Several researches have been published for investigating the connection between rs5275 and rs689466 gene variation and risk of lung cancer, but the outcomes remain controversial. To provide a comprehensive conclusion, we conducted a meta-analysis based on 14 eligible studies[8–21] to evaluate the contribution of these 2 polymorphisms to lung cancer susceptibility.

## 2. Methods

Our meta-analysis was performed based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).[22] The informed consent of the patients and the ethical approval were not required since our article was based on the studies published previously.

### 2.1. Search strategy

A systematic literature search was performed carefully until November 30, 2017 for relevant researches in Pubmed, Embase,




*From:* Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *P*referred *R*eporting *I*tems for *S*ystematic Reviews and *M*eta-*A*nalyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

**Figure 1.** The PRISMA flow diagram of the study inclusion and exclusion.

**Table 1**

**Characteristics of the studies included for meta-analysis.**

| Author | Year | Country | Ethnicity | Source of controls | Sample size | | Genotyping method | Gene polymorphism | HWE test |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Case | Control | | | |
| Campa et al[8] | 2004 | Norway | Caucasian | HB | 250 | 214 | Taqman | rs5275 | .31 |
| Campa et al[9] | 2005 | Europe | Caucasian | HB and PB | 1965 | 1937 | Taqman | rs5275 | .29 |
| Hu et al[10] | 2005 | China | Asian | PB | 322 | 323 | PCR-PIRA | rs5275 | .12 |
| Sorensen et al[11] | 2005 | Denmark | Caucasian | HB | 256 | 268 | Taqman | rs5275 | .38 |
| Park et al[12] | 2006 | Korea | Asian | HB | 582 | 582 | PCR-PIRA | rs5275 | .55 |
| Vogel et al[13] | 2008 | Denmark | Caucasian | PB | 403 | 744 | PCR-probes | rs689466 | .14 |
| | | | | | | | | rs5275 | .96 |
| Liu et al[14] | 2010 | China | Asian | HB | 358 | 716 | PCR-RFLP | rs689466 | .34 |
| | | | | | | | | rs5275 | <.001 |
| Coskunpinar et al[15] | 2011 | Turkey | Caucasian | PB | 231 | 118 | PCR-RFLP | rs689466 | .01 |
| Lim et al[16] | 2011 | China | Asian | HB | 297 | 718 | Taqman | rs5275 | .98 |
| Guo et al[17] | 2012 | China | Asian | HB | 684 | 602 | PCR-LDR | rs689466 | .09 |
| | | | | | | | | rs5275 | .08 |
| Zhang Z et al[18] | 2013 | China | Asian | HB | 956 | 994 | PCR-RFLP | rs689466 | .03 |
| Bhat et al[19] | 2014 | Srinagar | Asian | HB | 190 | 200 | PCR-RFLP | rs5275 | .47 |
| Zhang T et al[20] | 2015 | China | Asian | HB | 60 | 62 | PCR-RFLP | rs689466 | .21 |
| | | | | | | | | rs5275 | .05 |
| Moraes et al[21] | 2017 | Brazil | Caucasian | HB | 104 | 200 | RT-qPCR | rs689466 | .09 |
| | | | | | | | | rs5275 | .11 |

Case-control design was used in all the included studies.

HB = hospital-based, HWE = Hardy–Weinberg equilibrium, PB = population-based, PCR-LDR = polymerase chain reaction-ligase detection reactions, PCR-PIRA = polymerase chain reaction-based primer-introduced restriction analysis, PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism, RT-qPCR = reverse transcription-quantitative polymerase chain reaction, year = publication year.

Cochrane Library, OVID, Web of Science, and Google Scholar. The search strategy included the combination of following items: Polymorphism* or SNP* or mutant or mutation or variant or variation or single-nucleotide polymorphism*; COX2 or COX 2 or COX-2 or cyclooxygenase 2 or cyclooxygenase-2 or PTGS2 or PTGS 2 or PTGS-2 or prostaglandin endoperoxide synthase 2 or prostaglandin endoperoxide synthase-2; (Lung or pulmonary) and (cancer* or neoplasm* or carcinoma*). Furthermore, references of previous articles were manually searched for potential studies.

### 2.2. Inclusion and exclusion criteria

The inclusion criteria were: studies with case-control designs; studies evaluating the potential association between rs5275 and rs689466 polymorphism of COX-2 and lung cancer risk; studies with sufficient data to calculate odds ratios (ORs) and 95% confidence interval (95% CI). When overlapping data existed in more than 1 study, only the study containing larger sample size was included in our meta-analysis.

The exclusion criteria were: review articles, meta-analysis, letters, case reports, articles with abstract only, articles without controls.

### 2.3. Data extraction and quality assessment

All useful data from previous studies was extracted individually by 2 authors (JL and XL). Potential conflicts were resolved by discussion with a third investigator (HM). The extracted information included: last name of the first author, publication year, country and ethnicity of the participants, number of cases and controls, source of controls, genotyping method, genetic locus involved in the studies and genotype distribution. We used 9-point Newcastle

–Ottawa Scale (NOS)[23] to evaluate the quality of studies.

### 2.4. Statistical analysis

For each genetic locus involved in each included study, Hardy–Weinberg equilibrium (HWE) was tested in control group to assess the deviation of genotype distribution. The association between COX-2 polymorphism and lung cancer risk was measured by the pooled ORs and 95% CI with fixed-effect model or random-effect model according to the heterogeneity among studies. We used $\chi^2$ based Q statistic to evaluate the interstudy heterogeneity. A random-effect model (Der Simonian and Laird method)[24] was adopted for pooled ORs when $I^2 >$ 50%, otherwise the fixed-effect model (Mantel–Haenszel method)[25] was taken. Subgroup analysis was further performed to investigate the origin of heterogeneity according to source of control, ethnicity, pathological types, and smoking status. For both rs5275 and rs689466 polymorphism, 5 genetic models were established in overall and subgroup analysis: allele model (C vs T and G vs A), homozygote model (CC vs TT and GG vs AA), heterozygote model (CT vs TT and GA vs AA), dominant model (CC + CT vs TT and GG + GA vs AA), and recessive model (CC vs CT + TT and GG vs GA + AA), respectively. However, the majority of studies only provided the data of CC + CT vs TT or GG + GA vs AA for different pathological types and smoking status, so only dominant model was used in these 2 subgroup analysis. Three articles offered the data of rs5275 gene polymorphism stratified by pathological type, including Squamous cell carcinomas, adenocarcinomas, small cell carcinomas, large cell carcinomas, mixed cell carcinomas, and undifferentiated carcinomas.[10,12,19] We classified the latter 3 as others. The information of other rare pathological types was not found. The stratified analysis by pathological type in rs689466 polymorphism was not mentioned here due to the insufficient data. The data of the majority of eligible studies was based on all the stages of lung cancer. However, the stratified data of different stages was only mentioned in 1 included study,[19] so the related subgroup analysis was not performed. We also conducted sensitivity analysis by observing alternation of combined ORs

**Table 2**

**The results of Newcastle–Ottawa Scale.**

| | Selection | Comparability | Exposure |
|---|---|---|---|
| Campa et al (2004)[8] | ★★★ | ★★ | ★★★ |
| Campa et al (2005)[9] | ★★★★ | ★★ | ★★ |
| Hu et al[10] | ★★★★ | ★★ | ★★★ |
| Sorensen et al[11] | ★★★ | ★★ | ★★★ |
| Park et al[12] | ★★★ | ★★ | ★★★ |
| Vogel et al[13] | ★★★★ | ★★ | ★★★ |
| Liu et al[14] | ★★ | ★★ | ★★★ |
| Coskunpinar et al[15] | ★★★ | ★★ | ★★★ |
| Lim et al[16] | ★★★ | ★★ | ★★★ |
| Guo et al[17] | ★★★ | ★★ | ★★★ |
| Zhang Z et al[18] | ★★ | ★★ | ★★★ |
| Bhat et al[19] | ★★★ | ★★ | ★★★ |
| Zhang T et al[20] | ★★ | ★★ | ★★★ |
| Moraes et al[21] | ★★★ | ★★ | ★★★ |

with absence of each single included study. Publication bias was investigated via Egger test and Begg funnel plot. $P < .05$ indicates that there was statistically significant bias of publication.[26] All the data procession was performed using STATA version12 (Stata Corporation, College Station, TX).

## 3. Results

### 3.1. Study characteristics

The primary search identified 132 records in total, including 1 from the references of the previous articles. There were 104 studies remained for screening after the duplicated articles were filtered. Eighty-three studies were excluded after screening the titles and abstracts. Among the 21 studies left, 2 did not offer sufficient data, 1 discussed other genetic locus of COX-2, and 4 were excluded for different study design. The complete procedures of inclusion and exclusion are shown in Fig. 1. Therefore, there were eventually 14 studies (7 for rs5275 only, 2 for rs689466 only, and 5 for both)[8–21] containing 6658 cases and 7678 controls eligible for our meta-analysis exploring the association between rs5275 and rs689466 polymorphism of COX-2 and lung cancer risk. Table 1 summarizes the main characteristics of the studies included. The ethnicity included Asian (n = 8) and Caucasian (n = 6). There were 3 population-based studies,[10,13,15] 10 hospital-based studies,[8,11,12,14,16–21] and 1 containing both.[9] The sample sizes ranged from 122 to 3902 of all studies involved. For both rs5275 and rs689466 polymorphism, the results of HWE test in control groups are also listed in Table 1. There are 2 studies for rs689466[15,18] and 1 for rs5275[14] not consistent with Hardy–Weinberg equilibrium.

**Table 3**

**COX-2 rs5275 polymorphism genotype distribution and allele frequency in cases and controls.**

| | Genotype (N) | | | | | | | | Allele frequency (N, %) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cases | | | | Controls | | | | Cases | | | Controls | | |
| Author | Total | CC | CT | TT | Total | CC | CT | TT | C | T | RAF | C | T | RAF |
| Campa et al (2004)[8] | 250 | 112 | 107 | 31 | 214 | 50 | 99 | 65 | 331 | 169 | 0.66 | 199 | 229 | 0.46 |
| Campa et al (2005)[9] | 1965 | 224 | 886 | 855 | 1937 | 228 | 904 | 805 | 1334 | 2596 | 0.34 | 1360 | 2514 | 0.35 |
| Hu et al[10] | 322 | 5 | 83 | 234 | 323 | 7 | 107 | 209 | 93 | 551 | 0.14 | 121 | 525 | 0.19 |
| Sorensen et al[11] | 256 | 18 | 111 | 127 | 268 | 27 | 126 | 115 | 147 | 365 | 0.29 | 180 | 356 | 0.34 |
| Park et al[12] | 582 | 25 | 205 | 352 | 582 | 32 | 220 | 330 | 255 | 909 | 0.22 | 284 | 880 | 0.24 |
| Vogel et al[13] | 403 | 38 | 183 | 182 | 744 | 93 | 341 | 310 | 259 | 547 | 0.32 | 527 | 961 | 0.35 |
| Liu et al[14] | 358 | 0 | 119 | 239 | 716 | 0 | 248 | 468 | 119 | 597 | 0.17 | 248 | 1184 | 0.17 |
| Lim et al[16] | 297 | 15 | 100 | 182 | 718 | 28 | 228 | 462 | 130 | 464 | 0.22 | 284 | 1152 | 0.20 |
| Guo et al[17] | 686 | 15 | 185 | 486 | 602 | 32 | 181 | 389 | 215 | 1157 | 0.16 | 245 | 959 | 0.20 |
| Bhat et al[19] | 190 | 4 | 53 | 133 | 200 | 6 | 66 | 128 | 61 | 319 | 0.16 | 78 | 322 | 0.20 |
| Zhang T et al[20] | 60 | 11 | 26 | 23 | 62 | 2 | 32 | 28 | 48 | 72 | 0.40 | 36 | 88 | 0.29 |
| Moraes et al[21] | 104 | 17 | 43 | 44 | 200 | 25 | 106 | 69 | 77 | 131 | 0.37 | 156 | 244 | 0.39 |

Case-control design was used in all the included studies.
RAF = risk allele frequency, risk allele = C allele.

**Table 4**

**COX-2 rs689466 polymorphism genotype distribution and allele frequency in cases and controls.**

| | Genotype (N) | | | | | | | | Allele frequency (N, %) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cases | | | | Controls | | | | Cases | | | Controls | | |
| Author | Total | GG | GA | AA | Total | GG | GA | AA | G | A | RAF | G | A | RAF |
| Vogel et al[13] | 403 | 17 | 124 | 262 | 744 | 24 | 253 | 467 | 158 | 648 | 0.20 | 301 | 1187 | 0.20 |
| Liu et al[14] | 358 | 84 | 172 | 102 | 716 | 178 | 345 | 193 | 340 | 376 | 0.47 | 701 | 731 | 0.49 |
| Coskunpinar et al[15] | 231 | 1 | 57 | 173 | 118 | 0 | 48 | 70 | 59 | 403 | 0.13 | 48 | 188 | 0.20 |
| Guo et al[17] | 684 | 136 | 318 | 230 | 602 | 121 | 320 | 161 | 590 | 778 | 0.43 | 562 | 642 | 0.47 |
| Zhang Z et al[18] | 956 | 183 | 502 | 271 | 994 | 217 | 530 | 247 | 868 | 1044 | 0.45 | 964 | 1024 | 0.48 |
| Zhang T et al[20] | 60 | 20 | 28 | 12 | 62 | 27 | 31 | 4 | 68 | 52 | 0.57 | 85 | 39 | 0.69 |
| Moraes et al[21] | 104 | 3 | 30 | 71 | 200 | 10 | 52 | 138 | 36 | 172 | 0.17 | 72 | 328 | 0.18 |

Case-control design was used in all the included studies.
RAF = risk allele frequency, risk allele = G allele.

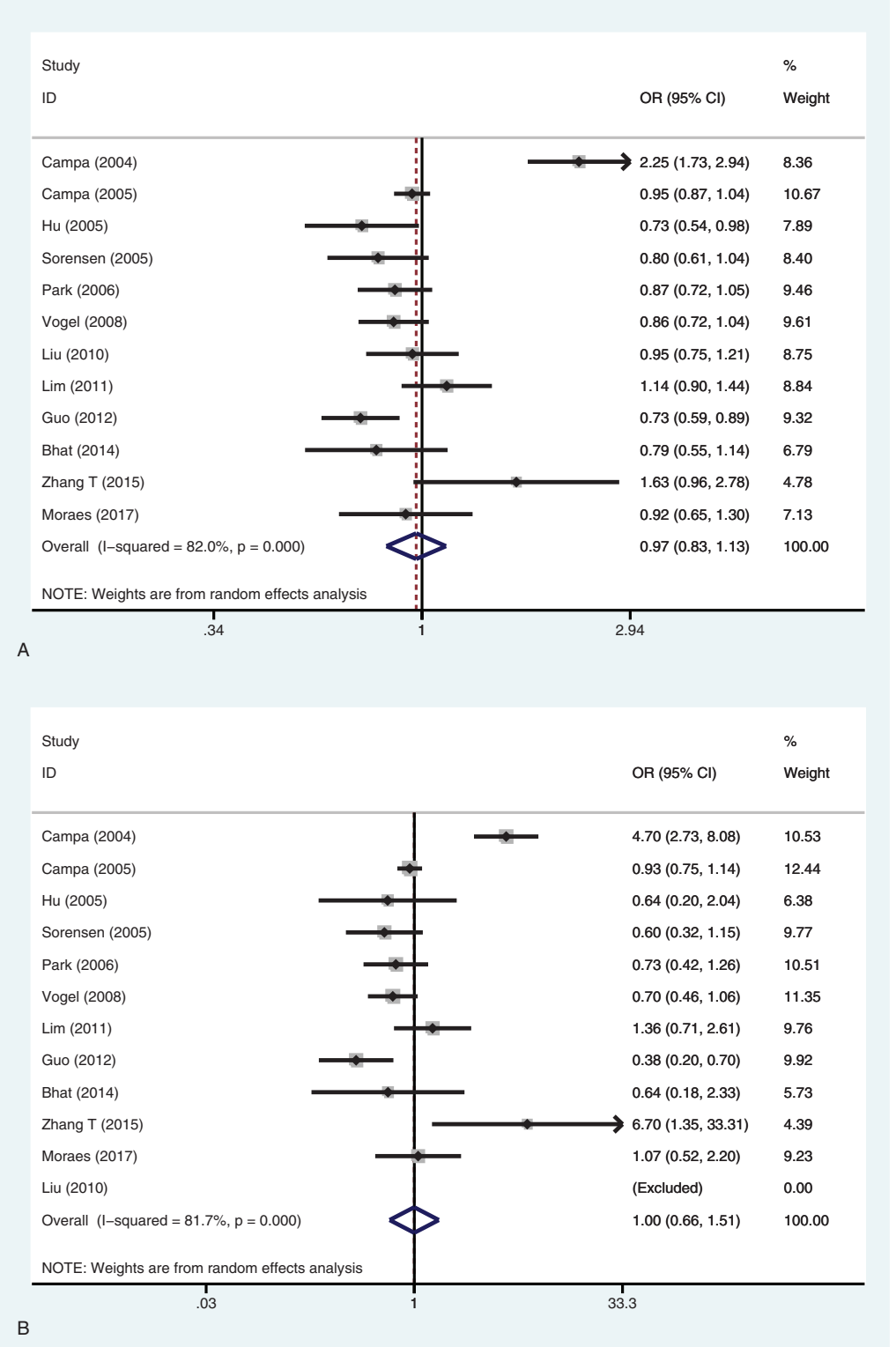



**Figure 2.** Forest plot for association between COX-2 rs5275 gene polymorphism and risk of lung cancer under (A) allele model (C vs T); (B) homozygote model (CC vs TT); (C) heterozygote model (CT vs TT); (D) dominant model (CC + CT vs TT); (E) recessive model (CC vs CT + TT). OR = odds ratio, CI = confidence interval.

Table 2 shows the result of NOS for all the eligible studies. The NOS score of each study involved in our meta-analysis was more than 6 points, which indicated a good quality. The genotype distribution and allele frequency of rs5275 and rs689466 of each study are shown in Tables 3 and 4 respectively.

### *3.2. Quantitative synthesis*

For rs5275 polymorphism, we found a significantly decreased risk of lung carcinoma under heterozygote model (OR: 0.91, 95% CI: 0.84–0.98, $P = .02$, $I^2 = 46.5\%$, $P_{heterogeneity} = .04$) (Fig. 2). However, no significant association was identified under

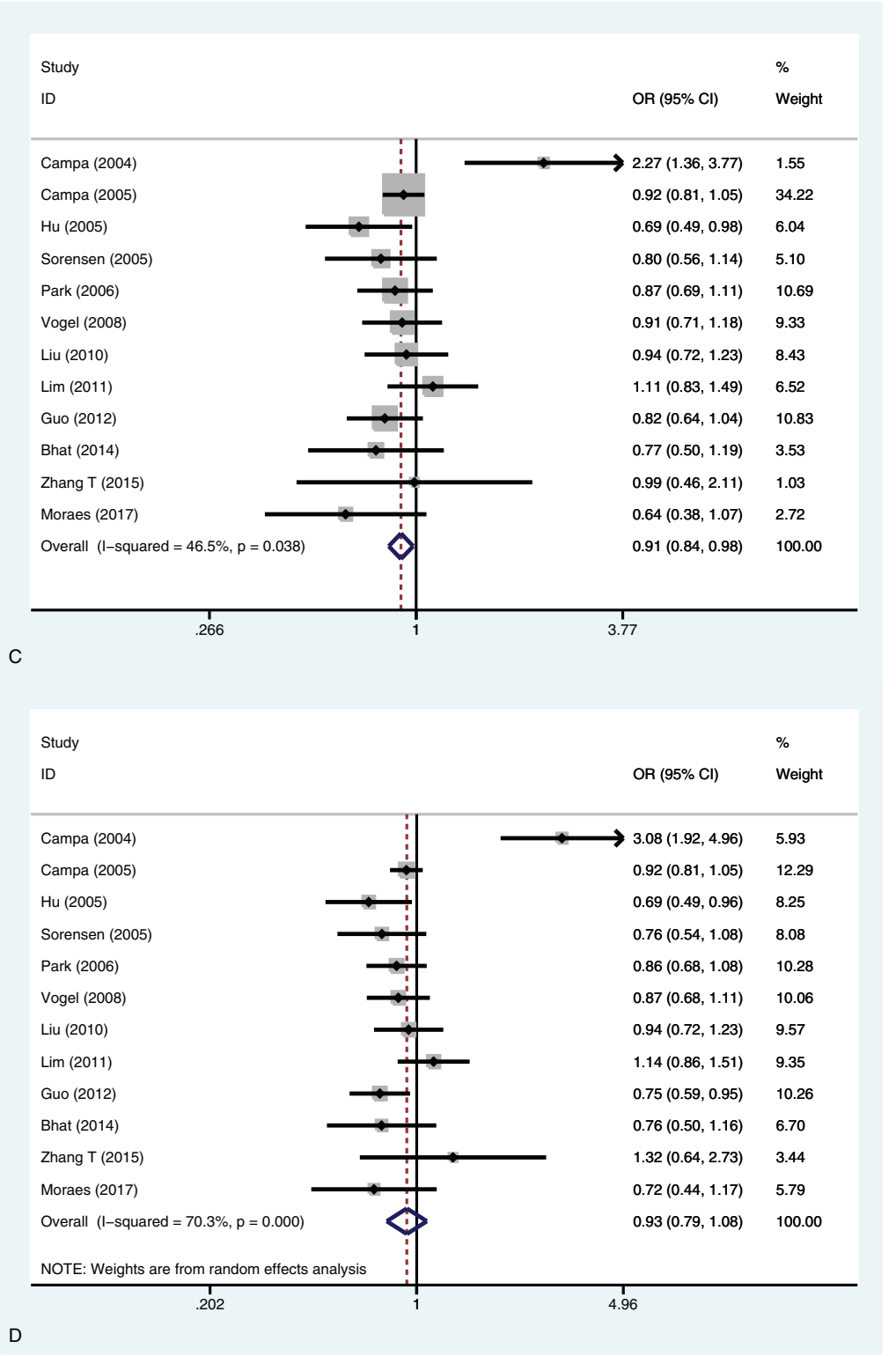



**Figure 2.** (*Continued*)

allele model (OR: 0.97, 95% CI: 0.83–1.13, $P=.70$, $I^2=82.0\%$, $P_{heterogeneity}=.00$), homozygote model (OR: 1.00, 95% CI: 0.66–1.51, $P=.99$, $I^2=81.7\%$, $P_{heterogeneity}=.00$), dominant model (OR: 0.93, 95% CI: 0.79–1.08, $P=.33$, $I^2=70.3\%$, $P_{heterogeneity}=.00$), and recessive model (OR: 1.01, 95% CI: 0.71–1.43, $P=.96$, $I^2=77.3\%$, $P_{heterogeneity}=.00$) (Fig. 2). Since a high heterogeneity was identified among studies, subgroup analysis was performed to investigate the source of heterogeneity. In subgroup analysis categorized by different ethnicity and source of control, there are found significant associations in Asian group

**Table 5**

**Subgroup analysis of association between COX2 rs5275 polymorphism and lung cancer.**

| Subgroup | | Number | ORs | 95% CI | *P* | $I^2$ (%) | Pooling model |
|---|---|---|---|---|---|---|---|
| Source of control | | | | | | | |
| Allele model | HB | 9 | 1.03 | (0.81,1.30) | .83 | 85.9 | Random-effects model |
| | HB/PB | 1 | 0.95 | (0.87,1.04) | .28 | / | Fixed-effects model |
| | PB | 2 | 0.83 | (0.71,0.96) | .02 | 0.0 | Fixed-effects model |
| Homozygote model | HB | 9 | 1.15 | (0.58,2.26) | .70 | 86.1 | Random-effects model |
| | HB/PB | 1 | 0.93 | (0.75,1.14) | .46 | / | Fixed-effects model |
| | PB | 2 | 0.69 | (0.46,1.02) | .06 | 0.0 | Fixed-effects model |
| Heterozygote model | HB | 9 | 0.94 | (0.78,1.12) | .46 | 55.7 | Random-effects model |
| | HB/PB | 1 | 0.92 | (0.81,1.06) | .24 | / | Fixed-effects model |
| | PB | 2 | 0.83 | (0.67,1.02) | .07 | 38.1 | Fixed-effects model |
| Dominant model | HB | 9 | 0.98 | (0.78,1.24) | .89 | 76.6 | Random-effects model |
| | HB/PB | 1 | 0.92 | (0.81,1.05) | .22 | / | Fixed-effects model |
| | PB | 2 | 0.80 | (0.66,0.98) | .03 | 15.1 | Fixed-effects model |
| Recessive model | HB | 9 | 1.12 | (0.64,1.96) | .68 | 81.9 | Random-effects model |
| | HB/PB | 1 | 0.96 | (0.79,1.17) | .72 | / | Fixed-effects model |
| | PB | 2 | 0.73 | (0.50,1.16) | .10 | 0.0 | Fixed-effects model |
| Ethnicity | | | | | | | |
| Allele model | Asian | 7 | 0.90 | (0.77,1.06) | .20 | 61.0 | Random-effects model |
| | Caucasian | 5 | 1.06 | (0.79,1.42) | .71 | 90.6 | Random-effects model |
| Homozygote model | Asian | 7 | 0.86 | (0.47,1.58) | .63 | 67.1 | Random-effects model |
| | Caucasian | 5 | 1.14 | (0.62,2.11) | .68 | 89.2 | Random-effects model |
| Heterozygote model | Asian | 7 | 0.88 | (0.78,0.99) | .03 | 0.0 | Fixed-effects model |
| | Caucasian | 5 | 0.97 | (0.74,1.26) | .80 | 72.6 | Random-effects model |
| Dominant model | Asian | 7 | 0.87 | (0.77,0.97) | .01 | 33.1 | Fixed-effects model |
| | Caucasian | 5 | 1.03 | (0.74,1.44) | .87 | 85.2 | Random-effects model |
| Recessive model | Asian | 7 | 0.90 | (0.50,1.61) | .72 | 65.4 | Random-effects model |
| | Caucasian | 5 | 1.12 | (0.70,1.80) | .63 | 85.1 | Random-effects model |
| Smoking status | | | | | | | |
| Dominant model | Smoker | 5 | 0.93 | (0.61,1.42) | .75 | 88.6 | Random-effects model |
| | Nonsmoker | 5 | 0.96 | (0.70,1.32) | .80 | 55.7 | Random-effects model |
| Pathological types | | | | | | | |
| Dominant model | SCC | 3 | 0.91 | (0.74,1.13) | .40 | 0.0 | Fixed-effects model |
| | AD | 2 | 0.63 | (0.47,0.83) | .001 | 0.0 | Fixed-effects model |
| | SM | 2 | 0.61 | (0.27,1.40) | .25 | 60.6 | Random-effects model |
| | Others | 2 | 0.91 | (0.56,1.50) | .72 | 39.3 | Fixed-effects model |

Others include the large cell, mixed cell carcinomas, or undifferentiated carcinomas.

AD = adenocarcinomas, CI 95% = 95% confidence interval, HB = hospital-based, ORs = odds ratios, PB = population-based, SCC = squamous cell carcinomas, SM = small cell carcinomas.

under heterozygote model (OR: 0.88, 95% CI: 0.78–0.99, $P = .03$, $I^2 = 0.0\%$, $P_{heterogeneity} = .49$) and dominant model (OR: 0.87, 95% CI: 0.77–0.97, $P = .01$, $I^2 = 33.1\%$, $P_{heterogeneity} = .18$) and in population-based group under allele model (OR: 0.83, 95% CI: 0.71–0.96, $P = .02$, $I^2 = 0.0\%$, $P_{heterogeneity} = .35$) and dominant model (OR: 0.80, 95% CI: 0.66–0.98, $P = .03$, $I^2 = 15.1\%$, $P_{heterogeneity} = .28$). However, we found no statistical association in Caucasians and in hospital-based group. We further performed stratified analysis according to pathological type, and only in adenocarcinoma group, there was a stronger relationship with lower OR under dominant model (OR: 0.63, 95% CI: 0.47–0.84, $P = .001$, $I^2 = 0.0\%$, $P_{heterogeneity} = .70$). No association was identified in smokers or nonsmokers subgroup. The complete results of subgroup analysis for rs5275 polymorphism are shown in Table 5.

For rs689466 polymorphism, the pooled analysis indicated a significant association of lower risk with lung cancer under allele model (OR: 0.88, 95% CI: 0.82–0.95, $P = .001$, $I^2 = 19.7\%$, $P_{heterogeneity} = .28$), homozygote model (OR: 0.81, 95% CI: 0.68–0.95, $P = .01$, $I^2 = 0.0\%$, $P_{heterogeneity} = .43$), heterozygote model (OR: 0.81, 95% CI: 0.72–0.91, $P < .001$, $I^2 = 48.1\%$, $P_{heterogeneity} = .07$), and dominant model (OR: 0.81, 95% CI: 0.72–0.91, $P < .001$, $I^2 = 45.6\%$, $P_{heterogeneity} = .09$), but except for recessive model (OR: 0.91, 95% CI: 0.79–1.04, $P = .18$, $I^2 = 0.0\%$, $P_{heterogeneity} = .74$) (Fig. 3). Subgroup analysis was further managed by ethnicity, source of control, and smoking status. Significant associations were identified in hospital-based group and Asian group under all genetic models except for recessive model, but not in population-based group and Caucasian group. There was no significant association in neither smokers nor nonsmokers group. Table 6 shows the intact results of stratified analysis for rs689466 polymorphism.

### 3.3. Sensitivity analysis

We conducted sensitivity analysis to investigate whether the removal of each individual article will subvert the result of our meta-analysis. Figure 4 shows that the result did not alter after each study was omitted, indicating the stability of our consequence (Fig. 4).

### 3.4. Publication bias

Since publication bias is a common problem for each meta-analysis, we performed Egger test and Begg funnel plots to

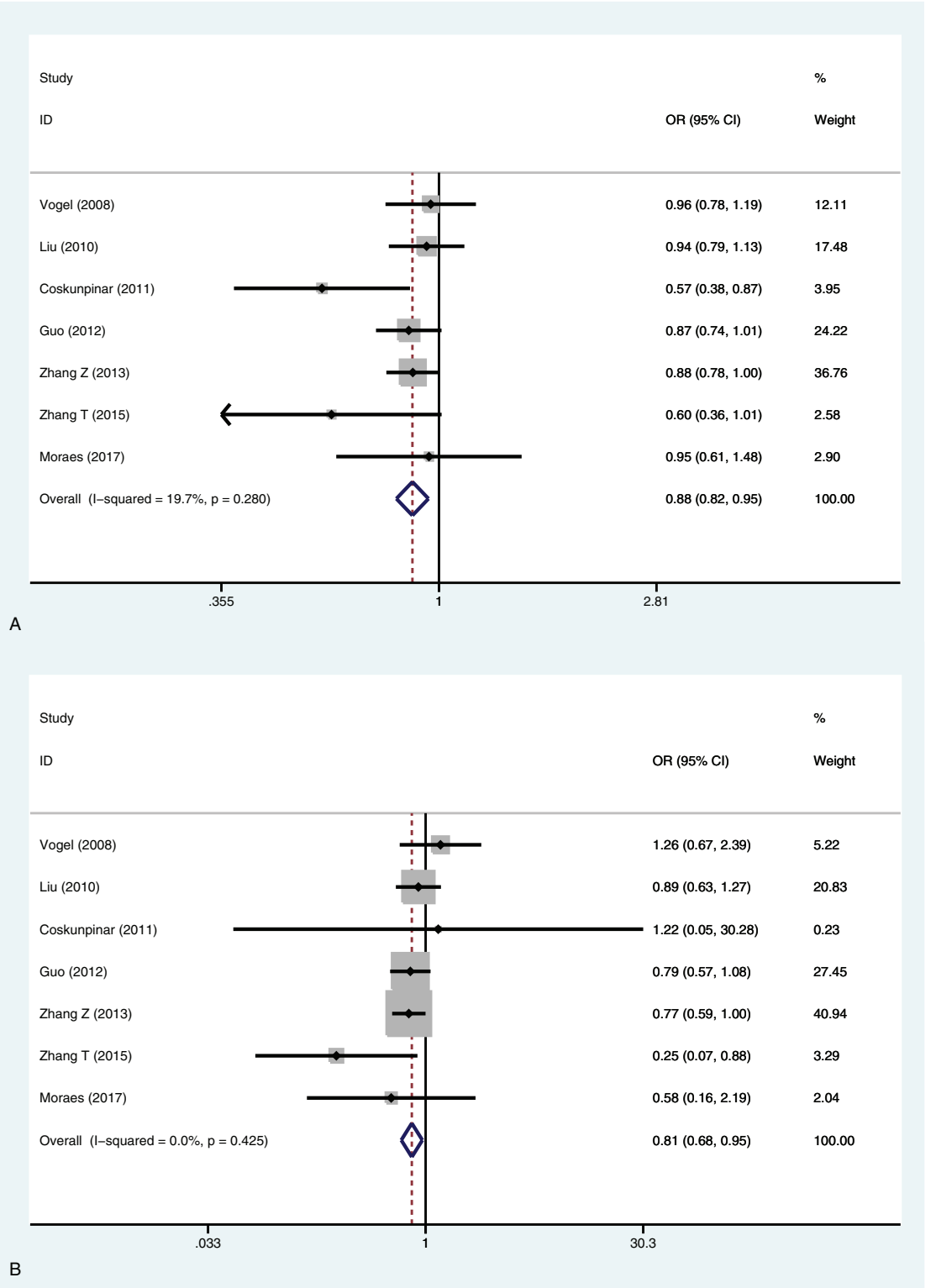



**Figure 3.** Forest plot for association between COX-2 rs689466 gene polymorphism and risk of lung cancer under (A) allele model (G vs A); (B) homozygote model (GG vs AA); (C) heterozygote model (GA vs AA); (D) dominant model (GG + GA vs AA); (E) recessive model (GG vs GA + AA). OR = odds ratio, CI = confidence interval.

evaluate the publication bias. We could see no obvious dissymmetry from the Begg funnel plot visually (Fig. 5), which suggested no evidence of publication bias (Egger test: $P = .88$ for rs5275, $P = .24$ for rs689466).

## 4. Discussion

Many researches focused on the relationship between COX-2 polymorphism and lung cancer risk were reported, however, the conclusion remains controversial. Of the 12 studies[8–14,16,17,19–

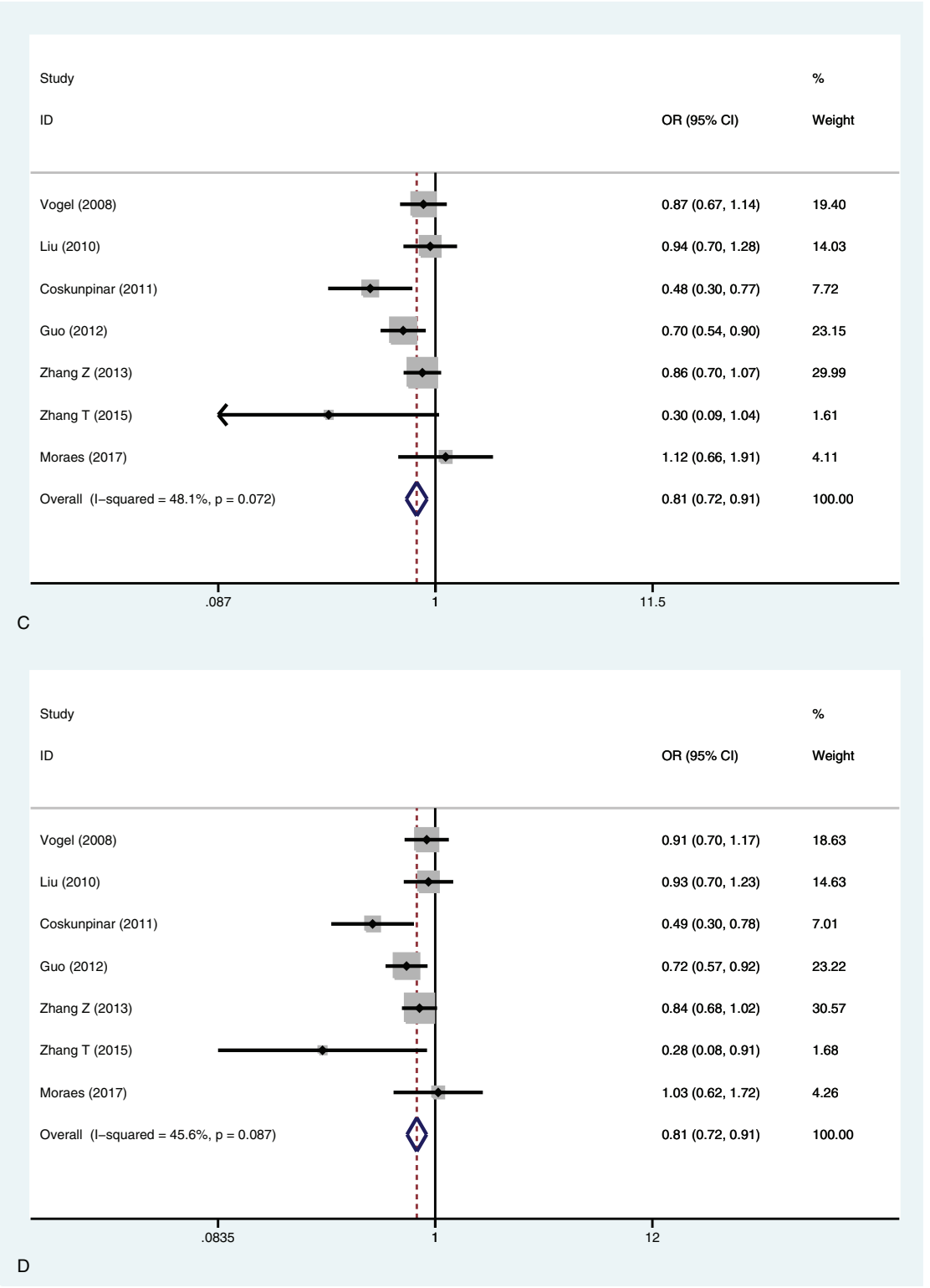



**Figure 3.** (*Continued*)

21] discussing rs5275 polymorphism, 3 studies[8,13,20] revealed an increased risk of lung carcinoma, 3 studies[10,17,19] revealed a decreased risk, while other 6 studies[9,11,12,14,16,21] reported no significant association. Similarly, of the 7 studies[13–15,17,18,20,21] discussing rs689466 polymorphism, 4 studies[15,17,18,20] suggested a reduced risk of lung cancer, but 3 studies[13,14,21] indicated no significant relationship. Due to the conflicting results among these studies which offered a small sample size individually, we performed the present meta-analysis to obtain a comprehensive conclusion.

Fourteen eligible studies[8–21] were consolidated to seek the distribution of rs5275 and rs689466 gene polymorphism to lung

**Table 6**

**Subgroup analysis of association between COX2 rs689466 polymorphism and lung cancer.**

| Subgroup | | Number | ORs | 95% CI | *P* | $I^2$ (%) | Pooling model |
|---|---|---|---|---|---|---|---|
| Source of control | | | | | | | |
| Allele model | HB | 5 | 0.88 | (0.81,0.96) | .004 | 0.0 | Fixed-effects model |
| | PB | 2 | 0.77 | (0.46,1.27) | .30 | 78.4 | Random-effects model |
| Homozygote model | HB | 5 | 0.78 | (0.66,0.93) | .004 | 0.0 | Fixed-effects model |
| | PB | 2 | 1.26 | (0.67,2.36) | .47 | 0.0 | Fixed-effects model |
| Heterozygote model | HB | 5 | 0.83 | (0.72,0.95) | .007 | 38.1 | Fixed-effects model |
| | PB | 2 | 0.67 | (0.37,1.20) | .18 | 78.6 | Random-effects model |
| Dominant model | HB | 5 | 0.82 | (0.72,0.93) | .002 | 31.6 | Fixed-effects model |
| | PB | 2 | 0.69 | (0.38,1.26) | .23 | 80.4 | Random-effects model |
| Recessive model | HB | 5 | 0.89 | (0.77,1.03) | .11 | 0.0 | Fixed-effects model |
| | PB | 2 | 1.33 | (0.72,2.48) | .37 | 0.0 | Fixed-effects model |
| Ethnicity | | | | | | | |
| Allele model | Asian | 4 | 0.88 | (0.81,0.96) | .004 | 0.0 | Fixed-effects model |
| | Caucasian | 3 | 0.83 | (0.61,1.13) | .24 | 58.4 | Random-effects model |
| Homozygote model | Asian | 4 | 0.78 | (0.66,0.93) | .006 | 19.3 | Fixed-effects model |
| | Caucasian | 3 | 1.08 | (0.62,1.88) | .80 | 0.0 | Fixed-effects model |
| Heterozygote model | Asian | 4 | 0.81 | (0.70,0.93) | .004 | 41.7 | Fixed-effects model |
| | Caucasian | 3 | 0.78 | (0.51,1.20) | .26 | 68.8 | Random-effects model |
| Dominant model | Asian | 4 | 0.80 | (0.70,0.92) | .001 | 39.8 | Fixed-effects model |
| | Caucasian | 3 | 0.78 | (0.52,1.17) | .24 | 66.7 | Random-effects model |
| Recessive model | Asian | 4 | 0.89 | (0.77,1.04) | .13 | 0.0 | Fixed-effects model |
| | Caucasian | 3 | 1.11 | (0.64,1.94) | .71 | 0.0 | Fixed-effects model |
| Smoking status | | | | | | | |
| Dominant model | Smoker | 2 | 0.83 | (0.68,1.00) | .06 | 0.0 | Fixed-effects model |
| | Nonsmoker | 2 | 0.84 | (0.65,1.18) | .38 | 0.0 | Fixed-effects model |

95% CI = 95% confidence interval, HB = hospital-based, PB = population-based, ORs = odds ratios.

neoplasm susceptibility. The overall analysis revealed that the variant heterozygote of rs5275 polymorphism reduced the risk of lung cancer significantly compared with wild homozygote. Similar significant association was found in rs689466 gene polymorphism under all genetic models but the recessive. Due to a high heterogeneity, we performed stratified analysis by ethnicity, source of control, pathological type, and smoking status. The subgroup analysis by ethnicity indicated a decreased susceptibility of lung cancer in participants with variant allele in Asian population of rs5275 polymorphism under heterozygote model and dominant model. As for rs689466 polymorphism, the result of subgroup analysis in Asian population was in accordance with the overall analysis. In contrast, no significant association was identified in Caucasian population for both genetic locus. Racial difference in gene polymorphism may be responsible for the outcomes. Previous epidemiology researches showed the incidence rate of lung cancer in Asians was lower than that of Caucasians. Among the histological subtypes, the incidence rate of adenocarcinoma is highest and squamous carcinoma is secondary. The incidence rates of histological subtypes followed the similar patterns to the overall rates.[27,28] Furthermore, we found the histological type may also influence the correlation between gene mutation and lung cancer risk. C allele carriers of rs5275 polymorphism probably have a significantly reduced risk of getting lung adenocarcinoma according to our findings. There were epidemiology researches showing that the proportion of adenocarcinoma among all types of lung cancer is higher in Asians than that in Caucasians,[28] which may possibly cause the difference in the significance of COX-2 polymorphism in lung cancer carcinogenesis between Asians and Caucasians. In the subgroup analysis categorized by source of controls, we also found meaningful differences in population-based group under allele model and dominant model in rs5275 polymorphism, and all models in hospital-based group of rs689466 polymorphism except the recessive model revealed a similar reduced risk. Finally, smoking status may not contribute to the association between COX-2 variation and pulmonary cancer risk, since no difference was found in neither smokers nor nonsmokers group. However, it is noteworthy that the conclusions concerning stratified analysis should be illuminated with caution because of the limited number of relevant studies.

Although 3 studies[14,15,18] in our analysis are not consistent with HWE in controls, the removal of each study (even 2[15,18] in rs689466 polymorphism) did not alter our conclusions during sensitivity analysis. No publication bias was recognized in our study.

Gene targeting therapy has been playing a prominent role in treatment of lung cancer for several years, and the very first step of the therapy is to identify the association between gene mutation and lung cancer susceptibility, as well as the mechanism of it. The human COX-2 gene consists of 9 introns and 10 exons, located in chromosome 1q25.2~25.3.[29] COX-2 is an enzyme involved in the inflammation response and its overexpression in lung cancer has been confirmed according to several previous studies.[4,5] The carcinogenic mechanisms of COX-2 may include: stimulation of cell proliferation, promotion of angiogenesis, inhibition of apoptosis, and mediation of immune suppression.[30–33] The COX-2 rs5275 is located in 3′untranslated region (3′UTR) containing plentiful AU sequences, which can achieve the regulation of posttranscriptional level and mediate the stability of mRNA through regulating its degradation, and thus, control the enzyme level.[34] The COX-2 rs689466 is located in promoter region as a crucial factors of genetic transcription.[35] Therefore, the gene mutations of rs5275 and rs689466 may contribute to the carcinogensis of lung cancer by regulation of COX-2 expression mentioned above.

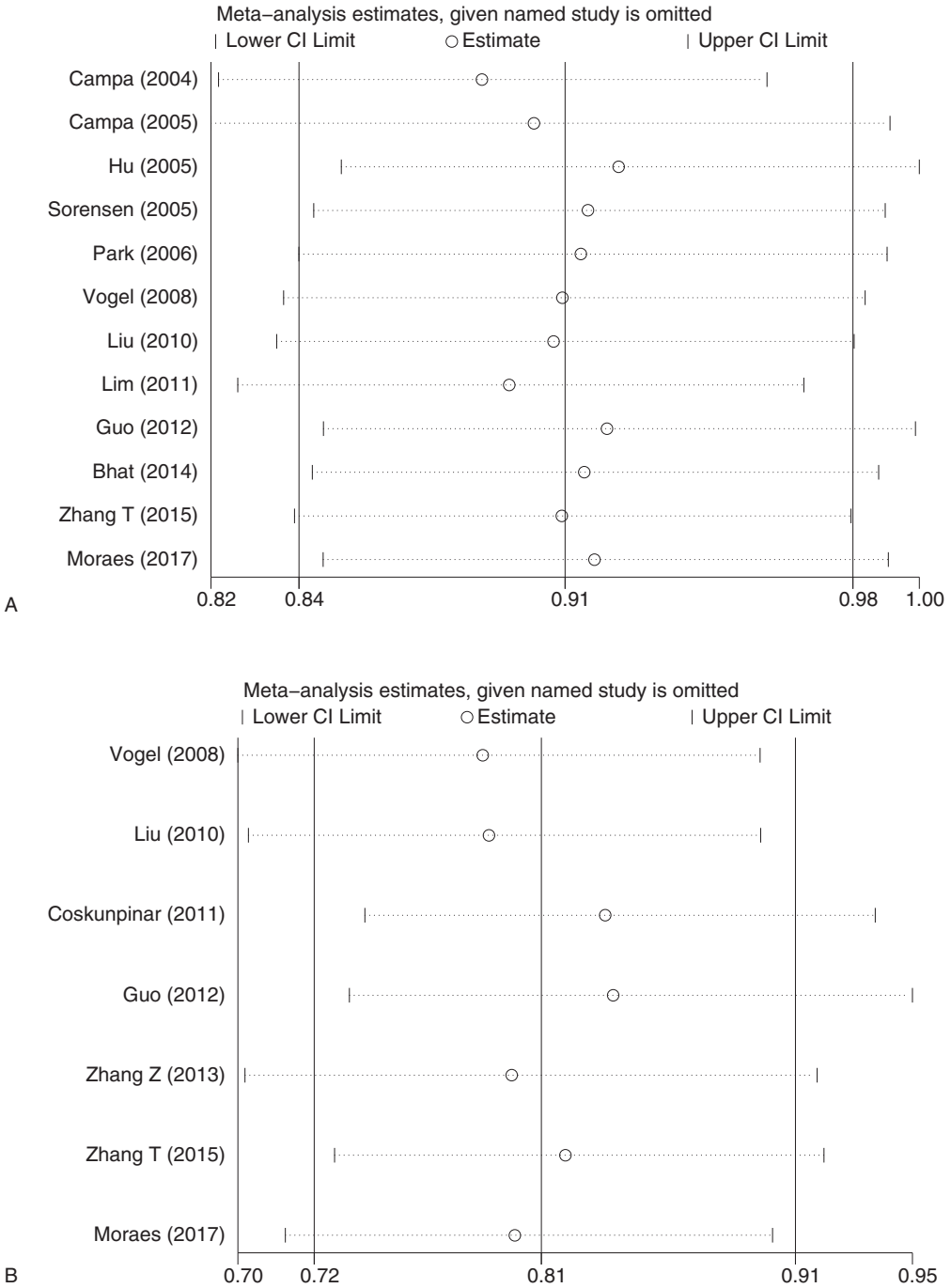



**Figure 4.** Sensitivity analysis of the combined OR for (A) the association between COX-2 rs5275 gene polymorphism and lung cancer risk under heterozygote model; (B) the association between COX-2 rs689466 gene polymorphism and lung cancer risk under dominant model. CI = confidence interval.

Still, several limitations should be admitted when generalizability of our meta-analysis was considered. First, 3 studies deviated from the HWE in controls, despite no alteration of conclusions happened after they were excluded. Second, no studies involving Africans were included, which may lead to selection bias. Third, the combination and interaction of several gene polymorphisms may be a potential influence factor to lung cancer risk, which were not evaluated in our analysis.

Our meta-analysis showed that COX-2 rs689466 polymorphism, especially in Asians, decreases the risk of lung cancer significantly, while COX-2 rs5275 polymorphism may cause a reduced susceptibility to lung cancer to a certain extent with a stronger association in adenocarcinoma cases. Both of them could serve as a potential diagnostic marker for lung cancer, which may further guide prevention strategies as well as genetic targeted therapy. Nevertheless, further evidences from future studies with standardized genotyping methods, multiple pop-

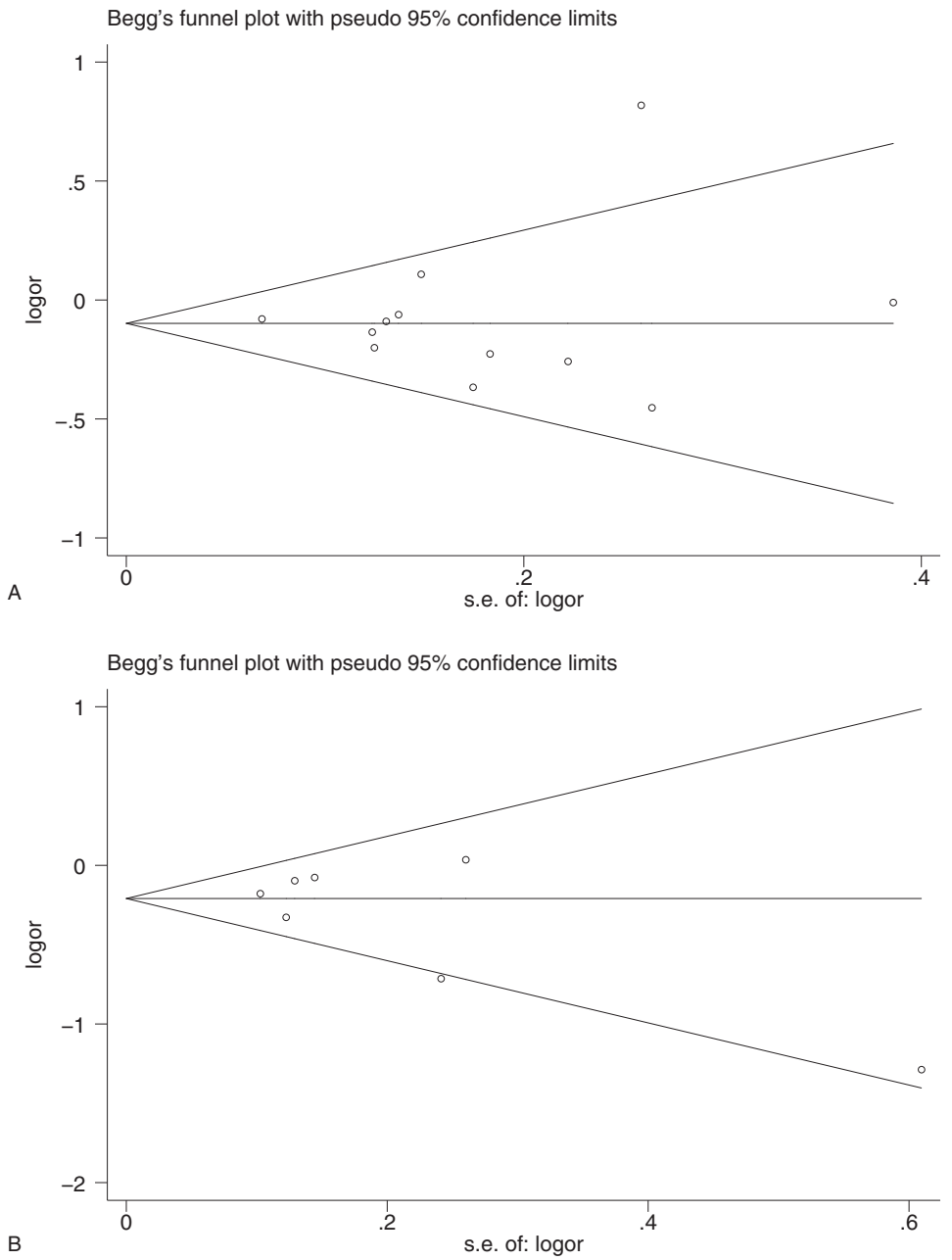



**Figure 5.** Begg funnel plot with pseudo 95% confidence limits for (A) the association between COX-2 rs5275 gene polymorphism and lung cancer risk under heterozygote model ($P = .88$ in Egger test); (B) the association between COX-2 rs689466 gene polymorphism and lung cancer risk under dominant model ($P = .24$ in Egger test).

ulations and pathological types, homogeneous cases, and well-matched controls are needed to corroborate our conclusions.

## Author contributions

JL and XL contributed equally to this work. JL and HM designed the study. JL and XL did the literature search, study quality assessment and data extraction. JiL, XL, XZ and YJ performed the statistical analysis and drafted the tables and figures. JL wrote the first draft of this analysis, and JY, HL and BN helped to finish the final version. All authors approved the conclusions of our study.

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