Background and Objective: Vascular endothelial growth factor (VEGF) is one of the key initiators and regulators of angiogenesis and it plays a vital role in the onset and development of malignancy. The association between VEGF gene polymorphisms and lung cancer risk has been extensively studied in recent years, but currently available results remain controversial or ambiguous. The aim of this meta-analysis is to investigate the associations between four common VEGF polymorphisms (i.e., $-2578C>A$, $-460C>T$, $+936C>T$, and $+405G>C$) and lung cancer risk.

Methods: A comprehensive search was conducted to identify all eligible studies to estimate the association between VEGF polymorphisms and lung cancer risk. Crude odds ratios (ORs) with 95% confidence intervals (CIs) were used to evaluate the strength of this association.

Results: A total of 14 published case-control studies with 4,664 cases and 4,571 control subjects were identified. Our meta-analysis provides strong evidence that VEGF $-2578C>A$ polymorphism is capable of increasing lung cancer susceptibility, especially among smokers and lung squamous cell carcinoma (SCC) patients. Additionally, for $+936C>T$ polymorphism, increased lung cancer susceptibility was only observed among lung adenocarcinoma patients. In contrast, VEGF $-460C>T$ polymorphism may be a protective factor among nonsmokers and SCC patients. Nevertheless, we did not find any association between $+405C>G$ polymorphism and lung cancer risk, even when the groups were stratified by ethnicity, smoking status or histological type.

Conclusion: This meta-analysis recommends more investigations into the relationship between $-2578C>A$ and $-460C>T$ lung cancer risks. More detailed and well-designed studies should be conducted to identify the causal variants and the underlying mechanisms of the possible associations.
At least 30 single nucleotide polymorphisms (SNPs) in \( VEGF \) gene have been identified and described, and some have even been shown to affect the expression of \( VEGF \) gene [17,18]. Several previously published meta-analyses showed that \( VEGF+936C>T \) (rs3025039), one of the most common polymorphisms, was not associated with gastric cancer [19–21], colorectal cancer [22], or breast cancer [23–25]. Additionally, these published meta-analyses also showed that other three common \( VEGF \) polymorphisms, \(-1154G>A\) (rs1570360), \(-634G>C\) (rs2010963) and \(-460C>T\) (rs833061), were not associated with colorectal cancer [26] or breast cancer [24], whereas the \( VEGF-634G/C \) polymorphism was found to be associated with gastric cancer [20]. In recent years, four common polymorphisms in \( VEGF \) gene, \(-2578C>A\), \(-460C>T\), \(+936C>T\), and \(+405C>G\), have been described in several literatures to appear to be involved in the development of lung cancer [27–31]. However, the results remain controversial or inconclusive. To the best of our knowledge, there were no published meta-analyses investigating the association between \( VEGF \) gene polymorphisms and lung cancer susceptibility. Therefore, we performed a meta-analysis of all eligible case-control or cohort studies to investigate whether these functional \( VEGF \) polymorphisms are associated with any increased risk of lung cancer and whether the associations are modulated by smoking status, histological type or other risk factors. We hope our meta-analysis can potentially be important in early lung cancer.
identification and become part of the therapeutic strategies in combating lung cancer.

**Materials and Methods**

**Literature Search**

Relevant papers for this meta-analysis were systematically identified through literature searches on PubMed, Embase, Web of science and Chinese National Knowledge Infrastructure (CNKI), and Chinese Biomedical Literature Database (CBM) of publications published up to March 9, 2013 relating to VEGF gene polymorphisms and lung cancer risk. As the main search criteria, we used combinations of the following terms: "VEGF", "vascular endothelial growth factor A", "vascular permeability factor", "vascular endothelial growth factor", "lung neoplasms", "pulmonary neoplasms", "bronchial neoplasms", "lung cancer", "bronchial neoplasm", "genetic polymorphism", "single nucleotide polymorphism", "SNP", "mutant", "gene variation". We also reviewed the reference lists of articles retrieved to identify relevant publications.

**Inclusion and Exclusion Criteria**

Our meta-analysis included genetic association studies fulfilling the following inclusion criteria: (a) a case-control, cohort or cross-sectional study must evaluated at least one of four polymorphisms of VEGF gene and lung cancer risk; (b) the diagnosis of lung cancer patients was confirmed pathologically and controls were confirmed as cancer-free patients; (c) inclusion of sufficient data on the size of the sample, odds ratio (OR), and 95% confidence interval (CI) and (d) articles were published in the English or Chinese language.

Studies were excluded when they represented duplicates of previous publications, or were meta-analyses, case report, letters, reviews or editorial articles. Studies investigating the progression, severity, phenotype modification, response to treatment, or survival were also excluded. Additionally, when data was included in multiple studies using the same case series, either the study with the largest sample size or most recent publication was selected. Finally, family-based studies were excluded because of different design settings. Any disagreements on study inclusion were resolved through discussions between the authors. To ensure the rigour of the current meta-analysis, it was designed and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement. The relevant checklist is shown in Supplement S1.

**Data Extraction**

All data from the included studies were extracted independently by two investigators, using a piloted data standardized form (when it came to conflicting evaluations, an agreement was settled after a discussion): the first author’s surname, year of publication, country of origin, published language, gender of study individuals and ethnic subgroups, study design, number of subjects, smoking status, histological types of lung cancer, SNP genotyping methods, genotyping method and detected sample, allele and genotype frequencies, and evidence of Hardy-Weinberg equilibrium (HWE) in controls. In addition, we also compared key study characteristics such as location, study time and authorship to determine the existence of multiple publications from the same study.

**Quality Assessment of Included Studies**

Two authors independently assessed the quality of the published articles according to the modified STROBE quality score systems [32]. Forty assessment items matching with the quality appraisals were used in this meta-analysis, with scores ranging from 0 to 40. Scores of 0–20, 20–30 and 30–40 were defined as low, moderate and high quality, respectively. The two authors resolved their differences through discussions; if no agreement could be reached, a third author decided on a decision. The modified STROBE quality score system is available in Supplement S2.

**Statistical Analysis**

Crude ORs together with their corresponding 95% CIs were used to calculate and assess the strength of association between VEGF gene polymorphisms and lung cancer risk under five genetic models: allele, dominant, recessive, homozygous, and heterozygous models. The deviation of frequency from those expected under Hardy-Weinberg equilibrium (HWE) was assessed by Chi-squared goodness of fit tests in controls. We explored inter-study variation through prespecified subgrouping of studies according to ethnicity (ie, Caucasian or Asian), gender (ie, female or male), smoking status (ie, smoker or non-smoker), and histological type of lung cancer (ie, adenocarcinoma, squamous cell carcinoma (SCC), and small cell carcinoma (SCLC), where applicable. The statistical significance of the pooled OR was assessed with a Z test. Between-study variation and heterogeneity were estimated using Cochran’s Q-statistic, with P<0.05 as a cutoff for statistically significant heterogeneity [33].

We also quantified the effect of heterogeneity with the I² test (ranges from 0 to 100%), which represents the proportion of inter-study variability that can be attributed to heterogeneity rather than to chance [34]. The fixed effects model (Mantel-Haenszel method) was used, except when a significant Q-test (P<0.05) or I²>50% indicated the existence of heterogeneity among studies; otherwise, the random effects model (DerSimonian-Laird method) was applied for meta-analysis. In order to ensure the reliability of our results, sensitivity analysis was performed by omitting individual studies. Begger’s funnel plots were used to detect publication bias. In addition, Egger’s linear regression test, which measures funnel plot asymmetry via a natural logarithm scale of OR, was also used to evaluate publication bias [35]. All P-values were two-sided. Analyses were conducted with STATA Version 12.0 software (Stata Corp, College Station, TX).

**Results**

**The Characteristics of Included Studies**

Our initial literature search yielded 546 reports, which included 13 population-based [28–31,36–44] and one hospital-based [27] case-control studies meeting the inclusion criteria based on the search criteria for lung cancer susceptibility linking to at least one of four common SNPs of VEGF gene, −2578C>A, −460C>T, +936C>T, and +405C>G. The flow diagram of the selection of studies and specific reasons for exclusion from the meta-analysis are shown in Figure 1. We studied four VEGF SNPs in 4,664 unrelated lung cancer cases and 4,571 unrelated controls from 14 case-control studies. In the eligible studies, there were 12 studies of subjects of Asian descent and only two studies of subjects of Caucasian descent. All included studies extracted DNA from peripheral blood and the VEGF polymorphisms were determined by classic PCR-RFLP in 12 studies, by TaqMan in 1 study, and by PIRA-PCR in another study. SNP genotypes were tested for departures from HWE for controls and all SNPs were in HWE. The qualities of the included studies were moderately high, with a STROBE score of greater than 20. The selected study characteristics were summarized in Table 1. The evaluation of the associations between VEGF −2578C>A, −460C>T, +936C>T, and +405C>G polymorphisms and lung cancer risk are presented in Tables 2, 3, 4 and 5.
A total of 7 studies with 22 data sets involving 1,596 cases and 1,857 controls were included in the pooled analysis. All subjects were of Asian ethnicity. Meta-analysis results showed that a statistically significant correlation was found between \(2578C>A\) polymorphism and susceptibility to lung cancer in Asians under allele and homozygous models (for OR = 1.31, 95%CI = 1.10–1.57, \(P = 0.003\); OR = 1.79, 95%CI = 1.30–2.46, \(P<0.001\)).

### Table 1. Main characteristics and methodological quality of all eligible studies.

| First author | Year | Country | Ethnicity | Simple size (Case/Control) | Enrolled patients | Source of control | Characteristics of controls (matched for) | Sample | Genotype method | SNP ID | Alias name | STROBE scores |
|--------------|------|---------|-----------|-----------------------------|------------------|------------------|------------------------------------------|--------|----------------|--------|------------|--------------|
| Lee et al    | 2005 | Korea   | Caucasian | 432/432 Lung cancer         | Blood PCR-RFLP   | rs3025039 +936 C/T | Patients (Age and gender)                | Blood  | PCR-RFLP       | rs3025039 | +936 C/T  | 27/40         |
|             |      |         |           |                             |                   |                  |                                          |        |                |         |            |              |
| Zhai et al   | 2008 | USA     | Caucasian | 1900/1458 NSCLC             | Blood Taqman     | rs3025039 +936 C/T | Patients (residence and ethnicity)      | Blood  | Taqman         | rs3025039 | +936 C/T  | 25/40         |
|             |      |         |           |                             |                   |                  |                                          |        |                |         |            |              |
| Tan et al    | 2008 | China   | Asian     | 276/279 NSCLC              | Blood PCR-RFLP   | rs3025039 +936 C/T | Patients (age and gender)                | Blood  | PCR-RFLP       | rs3025039 | +936 C/T  | 21/40         |
|             |      |         |           |                             |                   |                  |                                          |        |                |         |            |              |
| Wang et al   | 2008 | China   | Asian     | 377/416 Lung cancer         | Blood PCR-RFLP   | rs699947 -2578 C/A | Patients (age and gender)                | Blood  | PCR-RFLP       | rs699947 | -2578 C/A | 20/40         |
|             |      |         |           |                             |                   |                  |                                          |        |                |         |            |              |
| Liang et al  | 2009 | China   | Asian     | 171/172 Lung cancer         | Blood PCR-RFLP   | rs3025039 +936 C/T | Patients (age and gender, and ethnicity) | Blood  | PCR-RFLP       | rs3025039 | +936 C/T  | 23/40         |
|             |      |         |           |                             |                   |                  |                                          |        |                |         |            |              |
| Liu et al    | 2010 | China   | Asian     | 172/184 Lung cancer         | Blood PCR-RFLP   | rs699947 -2578 C/A | Patients (age and gender)                | Blood  | PCR-RFLP       | rs699947 | -2578 C/A | 22/40         |
|             |      |         |           |                             |                   |                  |                                          |        |                |         |            |              |
| Yuan et al   | 2011 | China   | Asian     | 251/255 Lung cancer         | Blood PIRA-PCR   | rs699947 -2578 C/A | Patients (age and gender)                | Blood  | PIRA-PCR       | rs699947 | -2578 C/A | 21/40         |
|             |      |         |           |                             |                   |                  |                                          |        |                |         |            |              |
| Li et al     | 2012a| China   | Asian     | 150/150 Lung cancer         | Blood PCR-RFLP   | rs3025039 +936 C/T | Patients (age, gender, and ethnicity)    | Blood  | PCR-RFLP       | rs3025039 | +936 C/T  | 25/40         |
|             |      |         |           |                             |                   |                  |                                          |        |                |         |            |              |
| Naik et al   | 2012 | India   | Asian     | 100/150 Lung cancer         | Blood PCR-RFLP   | rs3025039 +936 C/T | Patients (age, gender, and ethnicity)    | Blood  | PCR-RFLP       | rs3025039 | +936 C/T  | 26/40         |
|             |      |         |           |                             |                   |                  |                                          |        |                |         |            |              |
| Gao et al    | 2012 | China   | Asian     | 200/204 Lung cancer         | Blood PCR-RFLP   | rs3025039 +936 C/T | Patients (age, gender, and ethnicity)    | Blood  | PCR-RFLP       | rs3025039 | +936 C/T  | 23/40         |
|             |      |         |           |                             |                   |                  |                                          |        |                |         |            |              |
| Li et al     | 2012b| China   | Asian     | 50/50 Lung cancer           | Blood PCR-RFLP   | rs3025039 +936 C/T | Patients (age, gender, and ethnicity)    | Blood  | PCR-RFLP       | rs3025039 | +936 C/T  | 21/40         |
|             |      |         |           |                             |                   |                  |                                          |        |                |         |            |              |
| Liu et al    | 2012 | China   | Asian     | 260/260 Lung cancer         | Blood PCR-RFLP   | rs3025039 +936 C/T | Patients (age, gender, and ethnicity)    | Blood  | PCR-RFLP       | rs3025039 | +936 C/T  | 23/40         |
|             |      |         |           |                             |                   |                  |                                          |        |                |         |            |              |
| Naykoo et al | 2013 | India   | Asian     | 199/401 Lung cancer         | Blood RT-PCR     | rs3025039 +936 C/T | Patients (age, gender, and ethnicity)    | Blood  | RT-PCR         | rs3025039 | +936 C/T  | 27/40         |
|             |      |         |           |                             |                   |                  |                                          |        |                |         |            |              |
| Sun et al    | 2013 | China   | Asian     | 126/160 Lung cancer         | Blood PCR-RFLP   | rs3025039 +936 C/T | Patients (age, gender, and ethnicity)    | Blood  | PCR-RFLP       | rs3025039 | +936 C/T  | 26/40         |

Abbreviations: NSCLC, non-small cell lung cancer; PCC, population-based case-control study; HCC, hospital-based case-control study; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; PIRA, primer-introduced restriction analysis; SNPs, single nucleotide polymorphisms.

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### VEGF –2578C>A Polymorphism and Risk of Lung Cancer

A total of 7 studies with 22 data sets involving 1,596 cases and 1,857 controls were included in the pooled analysis. All subjects were of Asian ethnicity. Meta-analysis results showed that a statistically significant correlation was found between \(2578C>A\) polymorphism and susceptibility to lung cancer in Asians under allele and homozygous models (for OR = 1.31, 95%CI = 1.10–1.57, \(P = 0.003\); OR = 1.79, 95%CI = 1.30–2.46, \(P<0.001\)). We
### Table 2. Subgroup analyses for the associations of VEGF –2578C/A with susceptibility to lung cancer in Asians.

| Subgroups                  | No. of case/ control | Allele model (A allele vs. C allele) | Dominant model (AAAC vs. CC) | Recessive model (AA vs. CCAC) | Homozygous model (AA vs. CC) | Heterozygous model (AA vs. AC) |
|----------------------------|----------------------|-------------------------------------|------------------------------|-------------------------------|-----------------------------|-------------------------------|
|                           | OR (95%CI)           | P value                             | OR (95%CI)                   | P value                       | OR (95%CI)                  | P value                       |
| Smoking status             |                      |                                     |                              |                               |                             |                               |
| Smoker                     | 583/221              | 2.18 (1.55–3.09)                    | <0.001                       | 0.005                         | 7.31 (2.14–25.0)            | 0.002                         | 3.71 (1.06–13.0)              |
| Non-smoker                 | 434/390              | 1.20 (0.89–1.63)                    | 0.236                        | 0.525                         | 1.19 (0.48–2.92)            | 0.703                         | 0.85 (0.34–2.10)              |
| Histological type          |                      |                                     |                              |                               |                             |                               |                               |
| Adenocarcinoma             | 305/611              | 1.46 (1.04–2.04)                    | 0.028                        | 0.221                         | 1.40 (0.44–4.40)            | 0.568                         | 0.78 (0.25–2.46)              |
| SCC                        | 374/611              | 1.90 (1.43–2.52)                    | <0.001                       | 0.041                         | 5.64 (2.79–11.4)            | <0.001                        | 3.97 (1.94–8.10)              |
| SCLC                       | 152/611              | 1.10 (0.72–1.70)                    | 0.654                        | 0.859                         | 0.93 (0.21–4247)            | 0.928                         | 0.80 (0.17–3.69)              |
| Country of origin          |                      |                                     |                              |                               |                             |                               |                               |
| China                      | 1397/1456            | 1.25 (1.05–1.49)                    | 0.014                        | 0.378                         | 1.00 (1.42–2.81)            | <0.001                        | 2.06 (1.07–3.97)              |
| India                      | 199/401              | 1.75 (1.37–2.24)                    | <0.001                       | <0.001                        | 1.07 (0.24–4.87)            | 0.128                         | 1.08 (1.03–1.19)              |
| Overall                    | 1596/1857            | 1.31 (1.01–1.57)                    | 0.003                        | 0.141                         | 1.79 (1.30–2.46)            | <0.001                        | 1.37 (0.50–3.76)              |

Abbreviations: OR, odds ratio; CI, confidence intervals. doi:10.1371/journal.pone.0075123.t002

### Table 3. Subgroup analyses for the associations of VEGF –460C/T with susceptibility to lung cancer.

| Subgroups                  | No. of case/ control | Allele model (T allele vs. C allele) | Dominant model (TT+CT vs. CC) | Recessive model (TT vs. CC+CT) | Homozygous model (TT vs. CC) | Heterozygous model (TT vs. CT) |
|----------------------------|----------------------|-------------------------------------|------------------------------|-------------------------------|-----------------------------|-------------------------------|
|                           | OR (95%CI)           | P value                             | OR (95%CI)                   | P value                       | OR (95%CI)                  | P value                       |
| Ethnicity                  |                      |                                     |                              |                               |                             |                               |                               |
| Caucasian (USA)            | 2332/1890            | 0.99 (0.91–1.09)                    | 0.951                        | 0.516                         | 0.599                       | 0.679                         | 0.94 (0.82–1.08)              |
| Asian (China)              | 837/879              | 0.90 (0.64–1.28)                    | 0.572                        | 0.367                         | 0.72 (0.35–1.49)            | 0.373                         | 1.09 (0.68–1.75)              |
| Smoking status             |                      |                                     |                              |                               |                             |                               |                               |
| Smoker                     | 842/303              | 0.86 (0.68–1.09)                    | 0.224                        | 0.519                         | 0.84 (0.62–1.13)            | 0.474                         | 0.85 (0.61–1.17)              |
| Non-smoker                 | 580/416              | 0.65 (0.42–1.01)                    | 0.056                        | <0.001                        | 0.423                       | <0.001                        | 0.88 (0.37–2.07)              |
| Histological type          |                      |                                     |                              |                               |                             |                               |                               |
| Adenocarcinoma             | 676/1151             | 0.86 (0.72–1.04)                    | 0.116                        | 0.021                         | 0.91 (0.72–1.16)            | 0.458                         | 0.70 (0.45–1.09)              |
| SCC                        | 843/1151             | 0.81 (0.68–0.96)                    | 0.013                        | 0.077                         | 0.78 (0.63–0.97)            | 0.026                         | 0.69 (0.46–0.94)              |
| SCLC                       | 356/1151             | 0.96 (0.75–1.23)                    | 0.759                        | 0.454                         | 1.00 (0.74–1.36)            | 0.999                         | 0.89 (0.50–1.60)              |
| Overall                    | 3167/2769            | 0.94 (0.79–1.12)                    | 0.495                        | 0.415                         | 0.98 (0.73–1.32)            | 0.788                         | 0.89 (0.60–1.33)              |

Abbreviations: OR, odds ratio; CI, confidence intervals. doi:10.1371/journal.pone.0075123.t003
Table 4. Subgroup analyses for the associations of VEGF +936C/T with susceptibility to lung cancer under dominant model.

| Subgroups        | No. of case/control | Dominant model (TT+CT vs. CC) | OR (95%CI) | P value |
|------------------|---------------------|-------------------------------|------------|---------|
| **Ethnicity**    |                     |                               |            |         |
| Caucasian        | 2332/1890           | OR = 1.25, 95%CI: 1.05–1.49   | 0.937      | 0.038   |
| Asian            | 856/1202            |                               | 0.338      |         |
| **Gender**       |                     |                               |            |         |
| Male             | 1203/784            | OR = 0.66, 95%CI: 0.43–1.00   | 0.927      | 0.360   |
| Female           | 1018/966            |                               | 0.266      |         |
| **Smoking status**|                     |                               |            |         |
| Smoker           | 155/64              | OR = 0.86, 95%CI: 0.53–1.41   | 0.526      | 0.052   |
| Non-smoker       | 166/258             |                               | 0.084      |         |
| **Histological type** |               |                               |            |         |
| Adenocarcinoma   | 310/754             | OR = 0.98, 95%CI: 0.73–1.29   | 0.858      |         |
| SCC              | 316/754             |                               | 0.526      |         |
| SCLC             | 113/754             |                               | 0.19       |         |
| Overall          | 3288/3092           | OR = 1.19, 95%CI: 0.86–1.63   | 0.301      |         |

Abbreviations: OR, odds ratio; CI, confidence intervals.
doi:10.1371/journal.pone.0075123.t004

also performed a stratified analysis based on the geographic region of the studies; the results were persistent in both Chinese (for allele model: OR = 1.25, 95%CI: 1.05–1.49, P = 0.014; recessive model: OR = 1.88, 95%CI: 1.27–2.79, P = 0.002; homozygous model: OR = 2.00, 95%CI: 1.42–2.81, P < 0.001; heterozygous model: OR = 2.06, 95%CI: 1.07–3.97, P = 0.032; and India populations (for allele model: OR = 1.75, 95%CI: 1.37–2.24, P < 0.001; dominant model: OR = 1.26, 95%CI: 0.54–11.6, P < 0.001; heterozygous model: OR = 1.08, 95%CI: 1.03–1.19, P < 0.001) (Figure 2A). Additionally, a stratified analysis according to smoking status was performed using the information on packs of cigarettes smoked multiply by years of smoking; the variant allele is significantly correlated with increased risk of lung cancer among smoker subgroup(for allele model: OR = 2.18, 95%CI: 1.55–3.09, P < 0.001; dominant model: OR = 1.67, 95%CI: 1.17–2.39, P = 0.005; homozygous model: OR = 2.31, 95%CI: 2.14–25.0, P = 0.002; heterozygous model: OR = 3.71, 95%CI: 1.06–13.0, P = 0.041), while not in nonsmokers (P > 0.05 for all comparisons) (Figure 2B). Furthermore, we also stratified the case group by histological types and the data indicated the presence of the variant allele was the most strongly associated with SCC (for allele model: OR = 1.90, 95%CI: 1.43–2.52, P < 0.001; dominant model: OR = 1.39, 95%CI: 1.01–1.89, P = 0.041; recessive model: OR = 4.87, 95%CI: 2.47–9.61, P < 0.001; homozygous model: OR = 5.64, 95%CI: 2.79–11.4, P < 0.001; heterozygous model: OR = 3.97, 95%CI: 1.94–8.0, P < 0.001) (Figure 2C).

VEGF –460C>T Polymorphism and Risk of Lung Cancer

VEGF –460C>T polymorphism was investigated in six studies with a total of 3,167 cases and 2,769 controls. There was no evidence of association between –460C>T polymorphism and lung cancer risk (for allele model: OR = 0.94, 95%CI: 0.79–1.12, P = 0.495; dominant model: OR = 0.86, 95%CI: 0.59–1.24, P = 0.415; recessive model: OR = 0.98, 95%CI: 0.73–1.32, P = 0.788; homozygous model: OR = 0.89, 95%CI: 0.60–1.33,

Table 5. Subgroup analyses for the associations of VEGF +405C/G with susceptibility to lung cancer.

| Subgroups        | No. of case/control | Allele model (G allele vs. C allele) | OR (95%CI) | P value |
|------------------|---------------------|-------------------------------------|------------|---------|
| **Ethnicity**    |                     |                                     |            |         |
| Caucasian        | 2332/1890           | OR = 1.25, 95%CI: 1.05–1.49         | 0.937      | 0.038   |
| Asian            | 856/1202            |                                     | 0.338      |         |
| **Smoking status**|                     |                                     |            |         |
| Smoker           | 1203/784            | OR = 0.66, 95%CI: 0.43–1.00         | 0.927      | 0.360   |
| Non-smoker       | 1018/966            |                                     | 0.266      |         |
| **Histological type** |               |                                     |            |         |
| Adenocarcinoma   | 310/754             | OR = 0.98, 95%CI: 0.73–1.29         | 0.858      |         |
| SCC              | 316/754             |                                     | 0.526      |         |
| SCLC             | 113/754             |                                     | 0.19       |         |
| Overall          | 3288/3092           | OR = 1.19, 95%CI: 0.86–1.63         | 0.301      |         |

Abbreviations: OR, odds ratio; CI, confidence intervals.
doi:10.1371/journal.pone.0075123.t005
Figure 2. Forest plot of ORs for the association between VEGF−2578C>A polymorphism and susceptibility to lung cancer in subgroup analysis based on country of origin (A), smoking status (B), and histological type (C) under the dominant model.

doi:10.1371/journal.pone.0075123.g002
Figure 3. Forest plot of ORs for the association between VEGF -460C>T polymorphism and susceptibility to lung cancer in subgroup analysis based on ethnicity (A), smoking status (B), and histological type (C) under the dominant model.

doi:10.1371/journal.pone.0075123.g003
Figure 4. Forest plot of ORs for the association between VEGF +936C>T polymorphism and susceptibility to lung cancer in subgroup analysis based on ethnicity (A), gender (B), smoking status (C), and histological type (D) under the dominant model. doi:10.1371/journal.pone.0075123.g004
heterozygous model: OR = 1.01, 95%CI: 0.79–1.28, P = 0.969). Again, lung cancer cases and controls did not significantly differ in the subgroup analyses based on ethnicity (data not shown) (Figure 3A). However, when stratified analysis by smoking status was performed, a lower prevalence of the variant allele was observed among nonsmokers (for dominant model: OR = 0.32, 95%CI: 0.18–0.55, P<0.001; recessive model: OR = 0.35, 95%CI: 0.20–0.61, P<0.001) (Figure 3B). Additionally, further analysis on histological type was performed, and we found that −460C>T polymorphism was significantly associated with decreased risk of SCC (for allele model: OR = 0.81, 95%CI: 0.68–0.96, P = 0.013; dominant model: OR = 0.71, 95%CI: 0.48–0.97, P = 0.026; homozygous model: OR = 0.69, 95%CI: 0.46–0.94, P = 0.015; heterozygous model: OR = 0.81, 95%CI: 0.65–0.99, P = 0.020), but not in adenocarcinoma and SCLC patients (P>0.05 for all comparisons) (Figure 3C).

VEGF +936C>T Polymorphism and Risk of Lung Cancer

Eight studies investigated the association between +936C>T polymorphism and lung cancer susceptibility with a total of 3,288 cases and 3,092 controls. We did not find any association between +936C>T genotype and lung cancer risk under the dominant model (OR = 1.19, 95%CI: 0.86–1.63, P = 0.301), even when the groups were stratified by ethnicity (Caucasian: OR = 0.99, 95%CI: 0.80–1.22, P = 0.9372; Asian: OR = 1.28, 95%CI: 0.77–2.12, P = 0.338), gender (male: OR = 1.00, 95%CI: 0.81–1.23, P = 0.972; female: OR = 1.12, 95%CI: 0.92–1.38, P = 0.266), or smoking status (smokers: OR = 0.74, 95%CI: 0.59–1.14, P = 0.360; nonsmokers: OR = 1.56, 95%CI: 1.01–2.43, P = 0.052). Nevertheless, in the subgroup analysis on histological subtype, increased lung cancer susceptibility was shown among the adenocarcinoma subgroup (OR = 1.24, 95%CI: 1.03–1.44, P = 0.012) (Figure 4).

VEGF +405C>G Polymorphism and Risk of Lung Cancer

A total of six studies involving 3,035 cases and 2,880 controls were included in the pooled analysis. The overall data did not show a marked association between +405C>G polymorphism and lung cancer risk in any genetic model, indicating that individuals with G allele might not have an increased lung cancer risk compared with those who carry wild-type C allele (for allele model: OR = 1.03, 95%CI: 0.80–1.31, P = 0.840; dominant model: OR = 1.23, 95%CI: 0.60–2.50, P = 0.573; recessive model: OR = 0.815, 95%CI: 0.62–1.05, P = 0.108; homozygous model: OR = 0.92, 95%CI: 0.62–1.37, P = 0.689; heterozygous model: OR = 0.70, 95%CI: 0.46–1.07, P = 0.099). Likewise, stratified analyses were also conducted according to ethnicity, smoking status and histological type of cancer. Unfortunately, there was no statistical difference in genotype distributions between cases and controls, and overall and different subgroups (P>0.05 for all comparisons; data not shown).

Sensitivity Analysis and Publication Bias

Sensitivity analysis was performed to assess the influence of each study on the pooled ORs by omitting individual studies. The analysis results suggested that no individual study significantly altered the pooled ORs in VEGF −2578C>A (A), −460C>T (B), +936C>T (C) and +405C>G (D) polymorphisms. The dots represent specific studies for the indicated association.
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Figure 5. Funnel plots showed symmetric distribution. Log OR is plotted against the standard error of log OR for studies on VEGF −2578C>A (A), −460C>T (B), +936C>T (C) and +405C>G (D) polymorphisms. The dots represent specific studies for the indicated association.

Begger’s funnel plot and Egger’s linear regression test were performed on the metadata to assess publication bias of the individual studies. The shapes of the funnel plots did not reveal any evidence of obvious asymmetry in VEGF −2578C>A (A),
−460C>T (B), +936C>T (C), and +405C>G (D) polymorphisms (Figure 5). Egger’s test also displayed no significant statistical evidence of publication bias (−2578C>A: t = 0.99, P = 0.369; −460C>T: t = 0.53, P = 0.623; +936C>T: t = 0.28, P = 0.786; +405C>G: t = −1.08, P = 0.339).

Discussion

Evidence from preclinical and clinical studies shows that VEGF, as a predominant angiogenic factor in human cancers, plays a vital role in the carcinogenesis pathway, which has been proved to be a key step in tumor occurrence, progression and prognosis [12,45]. Several functional polymorphisms of VEGF gene have been confirmed to be correlated with high levels of VEGF protein in cancer cells and high tumor angiogenic activity, and they also contribute to the susceptibility and severity of cancer, including lung cancer [36]. Although cigarette smoking is the major cause of lung cancer, only a small fraction of smokers develop this disease during their lifetime, which suggests that both genetic factors and lifestyle risk factors are modulating individual susceptibility to lung cancer risk. A study by Koukourakis et al. reported that non-small cell lung cancer patients with specific VEGF gene polymorphisms develop tumors with low VEGF expression and poor vascularization [46]. In recent years, the associations between VEGF and risk of lung cancer have been extensively investigated, obtaining conflicting results. Therefore, we employed a meta-analysis to explore a more precise evaluation for the associations. To our knowledge, this is the first meta-analysis on this topic.

The present meta-analysis, including 4,664 cases and 4,571 controls from 14 published case-control studies, explored the association between VEGF −2578C>A, −460C>T, +936C>T, and +405C>G polymorphisms and lung cancer risk. According to our pooled analysis, −2578C>A polymorphism may have a correlation with increased lung cancer risk. This finding may be biologically plausible since Koukourakis et al. observed that −2578CC was associated with lower VEGF expression and lower vascular density in lung cancer tissues compared to the −2578C C/A [46]. When lung cancer cases were stratified by histological subtype, the data indicated that the presence of −2578A was strongly associated with SCC, while similar finding was not observed in SCLC and adenocarcinoma. Although a research reported by Jin et al. demonstrated that −2578AA genotype was significantly associated with low histologic grade tumors [47], the reason for such a divergence of VEGF expression and angiogenic status in tumors of similar histologic type and differentiation remains obscure. Thus, more studies should be conducted to further examine the underlying mechanism. Furthermore, the stratified analysis according to smoking status revealed that −2578A is significantly correlated with increased risk of lung cancer among smokers, suggesting that this polymorphism may not be an independent risk factor, but perhaps an effect modifier that acts synergistically with smoking in lung cancer risk.

As for VEGF −460C>T polymorphism, the overall data did not show a marked association of this polymorphism with lung cancer risk in any genetic model, even in the subgroup analyses according to ethnicity. However, when stratified analysis by smoking status and histological type were performed, a lower prevalence of −460T allele was observed among non-smokers, lung adenocarcinoma cases, and SCC cases. Some clinical evidence suggests that cigarette smoking may stimulate both angiogenesis and VEGF expression, which exacerbates the rapid cancer progression effect of angiogenesis [48,49]. Thus, it is possible that cigarette smoke and VEGF activate multiple effects in lung cancer. For VEGF +936C>T, +405C>G polymorphisms, we found no overall association between these two polymorphisms or its interaction with smoking on lung cancer risk in any genetic model. When stratified analyses were conducted according to ethnicity and histological types of cancer, increased lung cancer susceptibility was only observed among the adenocarcinoma subgroup for +936C>T polymorphism, while there was no statistical difference in genotype distributions between cases and controls for any different subgroups for +405C>G polymorphism. Actually, there exist conflicting reports in some literatures regarding the exact function of the +405G/C polymorphism. Some clinical studies suggested that +405C allele has been associated with lower VEGF production, while some groups reported higher VEGF levels or even no association with +405C/C genotype [17,50,51]. Thus, whether these polymorphisms are truly functional requires further investigation through confirmatory studies and in vitro functional assays.

The current meta-analysis has several limitations that should be noted. First, the sample size in the present study was relatively small, so small, but potential, genetic effects may not be detectable. A small sample size may not have enough statistical power to explore the real association, especially in subgroup analysis. Additionally, as with other complex traits, lung cancer risk may also be modulated by several other genetic markers beyond VEGF, and our meta-analysis emphasized that elucidating the pathogenesis of lung cancer would demand an investigation into the association for many gene variants that may constitute distinct pathophysiological pathways. Third, we identified two studies from Caucasian populations and obtained no data from African populations, thus the two racial groups need to be further studied in the future. Therefore, the results should ideally be confirmed in further studies to strengthen the conclusions. Aside from the limitations listed above, our meta-analysis still has some strength. To the best of our knowledge, this is the first meta-analysis on the relationship between VEGF gene polymorphisms and lung cancer. We also explored inter-study variations by prespecified subgrouping of studies according to ethnicity, smoking status, gender, and histological type among cases. Furthermore, although this meta-analysis does not accommodate all previously published data, they are limited compared to the evidence that we generated.

In conclusion, this meta-analysis provides strong evidence that VEGF −2578C>A polymorphism is capable of increasing lung cancer susceptibility, especially among smokers and lung SCC patients. Additionally, for +936C>T polymorphism, increased lung cancer susceptibility was only observed among lung adenocarcinoma patients. In contrast, VEGF −460C>T polymorphism may be a protective factor among nonsmokers, lung adenocarcinoma and SCC patients. However, we did not find any association between +405C>G polymorphism and lung cancer risk, even when the groups were stratified by ethnicity, smoking status or histological type. More detailed and well-designed studies with larger population and different ethnicities are needed to further evaluate these associations.

Supporting Information

Supplement S1 PRISMA Checklist. (DOC)

Supplement S2 Modified STROBE quality score systems. (DOC)

Author Contributions

Conceived and designed the experiments: LL, KJC, HZ. Performed the experiments: LL, WHC. Analyzed the data: XFP. Contributed reagents/materials/analysis tools: LL, KJC. Wrote the paper: LL, KJC, WHC, XFP, HZ.
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