A meta-analysis of the Vascular endothelial growth factor polymorphisms associated with the risk of pre-eclampsia

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Abstract

Background: Pre-eclampsia (PE) is a common pregnancy-induced hypertension disease. Some case–control studies reported the association between Vascular endothelial growth factor (VEGF) gene polymorphisms (rs3025039, rs2010963) and PE risk. However, these associations were inconsistent in several studies. Therefore, we conducted this meta-analysis to assess the role of VEGF gene polymorphisms in PE more precisely.

Methods: Eligible studies were searched in PubMed, Embase, Web of Science and Chinese (CNKI and WanFang) databases. Statistical analyses were performed by Stata 12.0 software. Odds ratio (OR) and 95% confidence interval (CI) were used to assess the strength of the association. In addition, subgroup analyses, sensitive analyses and publication bias analyses were performed to further assess this meta-analysis.

Results: Totally, 21 studies were included in the meta-analysis covering 2,018 cases and 2,632 controls. There were significant associations between VEGF polymorphisms (rs3025039,
rs2010963) and PE risk in the overall populations. In the subgroup analyses, we found that rs3025039 polymorphism was associated with the increased risk of PE amongst Chinese. As for rs2010963 polymorphism, a significant association was observed in subgroup of Caucasian.

**Conclusion:** The present study suggested that the two VEGF gene polymorphisms (rs3025039, rs2010963) are associated with increased risk of PE in different ethnic groups, which means that the targets may be useful genetic markers for early prediction of PE.

**Keywords:** VEGF, polymorphisms, pre-eclampsia, meta-analysis, risk.

**Introduction**

Pre-eclampsia (PE), a common pregnancy disease diagnosed by hypertension and proteinuria, occurs in approximately 2%–8% of pregnancies [1, 2]. PE is an important reason for the maternal and fetal morbidity and mortality due to dysfunction of multiple systems and organs, such as liver, kidney and brain [3]. Although its etiology has not been well recognized, PE is now regarded as the result of the combined effect of multiple factors [4, 5]. According to the results of the several epidemiological studies, PE has a substantial heritable component, which is estimated to be a major effect [4, 6].

The VEGF gene, which locates on chromosome 6p21.3, is a key regulator of angiogenesis and vascular function. Therefore, VEGF is vital for the formation of trophoblasts, embryonic vasculature and maternal and foetal blood cells in utero [7]. Abnormal vascular growth and endothelial dysfunction have been proposed to be the part of pathogenesis. Hence, VEGF have drawn the attention of many researchers [8, 9].

The associations between polymorphisms of the VEGF gene and PE have been extensively studied [10-30]. However, the results were somewhat controversial. In 2013, two meta-analyses assessed the associations between four polymorphisms of the VEGF gene and the risk of PE [31, 32]. But the retrieved datasets of these two meta-analyses were not sufficient, and several new studies have been published regarding this relationship between VEGF gene polymorphisms
(rs3025039, rs2010963) and PE [10-15]. In addition, the results published recently remained inconsistent and conflicting, likely owing to heterogeneity of different researches or inadequate sample size. A comprehensive retrieval of the pertinent literature in multiple databases is likely to help assess disease risks more precisely. In view of the mortality of PE, more efficient biomarkers are required for early discovery and prevention in the clinical practice. Therefore, we performed an updated meta-analysis of all eligible studies including English and non-English Journals to investigate the association between VEGF gene polymorphisms and the risk of PE. Moreover, we further divided the cases by ethnic groups, Countries as well as genotyping methods and analyzed sub-group specific associations.

Methods

Identification of literature

This meta-analysis was conducted in accordance with the guidance of the Preferred Reporting Items for Systematic Reviews and Meta- Analyses (PRISMA) statement [33]. The PRISMA Checklist was presented in Supplementary Table S1. The literature search using the electronic databases PubMed, EMBASE, Web of Science, CNKI (Chinese National Knowledge Infrastructure) and WanFang was conducted by two study investigators. The comprehensive search strategies included the Mesh term and Keywords: (‘vascular endothelial growth factor’ or ‘VEGF’), (‘polymorphism’, ‘SNP’ ‘variant’, ‘genotype’ or ‘mutations’), (‘Pre-eclampsia’, ‘Preeclampsia’, ‘Pregnancy Toxemias’, ‘Pregnancy Toxemia’, ‘Edema-Proteinuria-Hypertension Gestosis’, ‘Edema Proteinuria Hypertension Gestosis’, ‘EPH Complex’, ‘EPH Toxemias’, ‘EPH Toxemia’, ‘Proteinuria-Edema-Hypertension Gestosis’ or ‘Proteinuria Edema Hypertension Gestosis’) through January 03, 2019. All eligible studies were retrieved and examined carefully. Review articles and references of other relevant researches were further searched to find additional eligible studies.

Inclusion and Exclusion Criteria
The inclusion criteria were as follows: (a) studies which estimated the associations between VEGF rs2010963 or rs3025039 and the susceptibility to PE; (b) case-control studies or cohort studies of PE; (c) patients must be clinically diagnosed PE (blood pressure ≥140/90 mmHg on two measurements with ≥1+ proteinuria or 300 mg/24 hours after the 20th week of pregnancy); (d) reported the allele frequencies of both cases and controls for different genotypes; (e) genotype distribution in the control group confirmed by Hardy–Weinberg equilibrium (HWE). The exclusion criteria of the meta-analysis were: (a) non-human studies, meta-analysis, comments, letters, reviews, mechanism studies or studies without controls (c) studies with overlapping or incomplete data. When overlapped population between studies was identified, only the newest or most complete article was included in the analysis. According to the corresponding criteria, two independent authors screened the articles.

Data extraction and assessment of methodological quality

Data were extracted by 2 authors independently from each study. The following information was collected: first author, publication year, participants’ Country, ethnicity (categorized as Caucasian, Xanthoderm, Indo-european hybrid), sample size, study design (case-control or cohort), genotyping method, alleles and genotype frequency distribution in cases and controls, and the major conclusion of the study. When incomplete or apparent conflicting data was found in the article, we made an attempt to contact authors. Inconsistencies of data interpretation were resolved with discussion. The Newcastle–Ottawa Quality Assessment Scale (NOS) was employed to evaluate the methodological quality of the identified articles, and scores ranging from 0 (the worst) to 9 (the best) were assigned based on the quality of the studies. The studies with no less than 5 stars were considered to be of high quality.

Statistical Analysis

First, deviation from HWE in the distribution of allele frequencies was estimated again by the chi-square test (determined by p<0.05). Stata 12.0 was used to perform quantitative meta-analysis. The association was estimated with four models: Allele comparison model, Dominant model,
Recessive model, and Homozygote model. The four models of the data analysis were conducted by the random-effects model to prevent exaggerated results. The association between the VEGF rs3025039 or rs2010963 and PE risk was assessed by the raw ORs with 95% CIs. The student’s t-test was used to determine the significance of the crude OR, and p<0.05 was considered statistically significant. In addition, heterogeneity assumption among the included researches was evaluated by the Chi-square and I², which was regarded to be statistically significant if p<0.10. And I² values of 25%, 50%, and 75% were nominally assigned as low, moderate, and high estimates. To insure that any single study did not cause an obvious influence to the whole effects, sensitivity analysis was performed to estimate the validity and stability of the study. In addition, to further analyze the source of the heterogeneity and the specific association between the VEGF polymorphism and PE, studies were also divided into several subgroups on the basis of the Country, the ethnicity of the related population, and the genotyping method. Egger’s test was performed to estimate the potential publication bias.

Results

Study Characteristics

As shown in Fig.1, the PRISMA flowchart demonstrated process of the literature retrieval. 405 studies were identified according to the result of the retrieval strategy and manual searches from PubMed, EMBASE, Web of Science, CNKI and WanFang database. On the basis of our inclusion/exclusion criteria, 142 studies were excluded for the duplication, and 239 studies were excluded as meta-analysis, reviews, mechanism studies or non-relevant research. Then, 24 studied were selected for full-text review. However, three studies were excluded, because 2 studies lacked genotype data and 1 study focused on placental polymorphism. Because most of the studies did not use the rs number to name the SNP, every SNP was manually confirmed by searching in the NCBI according to the sequence in the literature. Finally, 21 studies were included in the meta-analysis. Thereinto, 15 studies assessed the association between VEGF rs3025039 T/C polymorphism and the risk of PE, and 12 studies examined the association between VEGF
rs2010963 C/G polymorphism and the risk of PE. The specific information about the included studies was exhibited in Table 1. The quality evaluation of each study following the NOS is presented in Table 2, which showed all of these studies can be regarded as high-quality studies.

Overall analysis

Overall results of this meta-analysis between the two SNP and pre-eclampsia were displayed in Table 3 and Table 4. In total, we analyzed 1,426 cases and 1,872 controls for rs3025039 with the random effect model, showing a significantly increased risk for the comparison of the T allele to the C allele (OR=1.418, 95% CI=1.060-1.898, p=0.019, Fig.2A). Also, the results of the three genotype models analysis all revealed a significant association between pre-eclampsia and the VEGF rs3025039 (Dominant model: OR=1.637, 95% CI=1.031-2.598, p=0.037, Fig.2B; Recessive model: OR=1.501, 95% CI=1.068-2.109, p=0.019, Fig.2C; Homozygote model: OR=1.819, 95% CI=1.021-3.240, p=0.042, Fig.2D), in which the result of the Recessive model exhibited high heterogeneity (I² = 77.2%) and others were acceptable. An analysis of 1,148 cases and 1,388 controls for rs2010963 showed the C allele in allele comparison model, CC and CG genotype in the recessive model, and CC genotype in the homozygous model increased the risk of PE significantly (Allele comparison model: OR=1.207, 95% CI=1.046-1.394, p=0.010, Fig.3A; Recessive model: OR=1.310, 95% CI=1.044-1.643, p=0.020 Fig.3C; Homozygote model: OR=1.324, 95% CI=1.024-1.713, p=0.032, Fig.3D), however the result of the dominant model did not indicate statistical significance (OR=1.154, 95% CI=0.912-1.460, p=0.232, Fig.3B).

Subgroup analyses

The subgroup analyses were carried out due to the heterogeneity of result and biases of the different subgroups. The results of subgroup analyses were shown in Table 3 and Table 4. First of all, the different Countries of population were divided into three parts including China, Korea and Other Countries in rs3025039 according to the source of the population in studies. For Countries subgroup analyses in rs3025039 polymorphism, a significant correlation was found in the allele model and the three genotype model of the Chinese subgroup, in which the Allele comparison
model showed moderate heterogeneity ($I^2 = 69.5\%$), the Dominant model showed low heterogeneity ($I^2 < 0.001\%$), the Recessive model showed high heterogeneity ($I^2 = 71.6\%$), and the Homozygote model showed moderate heterogeneity ($I^2 = 32.3\%$). However, no significant association was found in the Korea’s subgroup and Other Countries’ subgroup, and relatively high heterogeneity was observed in the almost all models of the two subgroups except in Dominant and Homozygote model of Other Countries’ subgroup, which indicated the differences of population Countries were not the major cause of the heterogeneity for rs3025039 in this meta-analysis. Then, we performed an ethnic restriction including Xanthoderm and Caucasian and Indo-european hybrid for further subgroup analysis in rs3025039. No significant association was observed in the any model, and the heterogeneity of various models in different subgroups showed no significant reduction. Next, subgroup analysis was performed in rs3025039 according to the genotyping methods including MassARRAY system, Sequencing, PCR-RFLP and Snapshot. A significantly increased risk was found in the Sequencing and PCR-RFLP subgroup, but a protective effect was found in the MassARRAY system subgroup with Allele comparison model and Snapshot subgroup with Recessive model. The heterogeneity of all the subgroup in the four models were relatively low, which indicated that the genotyping methods might be the major source of the heterogeneity.

For rs2010963, we performed the subgroup analysis based on ethnicity and genotyping methods, because the overlapping Countries were limited. The subgroups of ethnicity were divided as the same as the subgroups of rs3025039. A significant association was observed in the Caucasian subgroup with both Allele comparison model and Homozygote model (Allele comparison model: OR=1.246, p=0.046; Homozygote model: OR=1.461, p=0.039), and other subgroups showed no obvious difference. Moreover, the heterogeneity of allele comparison model and homozygote model in Caucasian subgroup was low (Allele comparison model: $I^2=38.4\%$, Homozygote model: $I^2=8.9\%$). However, the difference between the overall heterogeneity and subgroup heterogeneity was not apparent, illustrating that the ethnicity was not the important cause of heterogeneity in the meta-analysis. Regarding the genotyping methods subgroups, we divided into three groups: Sequencing, PCR-RFLP and Other methods, owing to the duplicating number of the genotyping methods. The result of the Sequencing subgroup showed a statistically significant in Allele
comparison model and Homozygote model with indistinctive heterogeneity (Allele comparison model: OR=1.620, p=0.032, I²<0.1%; Recessive model: OR=2.328, p=0.005, I²<0.1%). Besides, heterogeneity of the Sequencing and PCR-RFLP subgroups in all models was not significant (I²<0.1%), while heterogeneity of Other methods subgroups was higher. The results suggested that the source of the heterogeneity might be the genotyping methods, consistent with the conclusion above.

**Sensitivity Analysis and Publication Bias**

To confirm the reliability of our results, a sensitivity analysis was performed for the allele model, showing no apparent difference before and after the removal of each study shown in the Fig.4. In addition, publication bias assessed by Egger’s regression test present no any obvious evidence in statistics, which was displayed in the Table 5.

**Discussion**

Although the etiology of preeclampsia is considered to be multi-factorial, genetic factors are thought to be strong determinants of this disease [4, 6]. Early studies reported that VEGF genes were associated with vascular growth and endothelial dysfunction, which may somewhat interpret the development of PE. In recent decades, many researchers have been focusing the role that VEGF gene may play in the cause of PE [34]. However, case-control studies have shown contradictory associations between VEGF gene polymorphisms and PE. The aim of this meta-analysis was to evaluate the association between VEGF rs3025039 and rs2010963 polymorphisms and PE for the use of the biomarkers in the clinical practice and the investigation of the concrete pathomachanism.

We conducted a thorough literature retrieve and review to identify as many relevant studies as possible in our meta-analysis. Compared with previous meta-analyses, we made an effort to gain some improvements in our analysis: first, several studies were not included in previous meta-analyses (Lu Z (2017), Amosco et al. (2016), Salimi et al. (2015), Silva et al. (2014), Zhang
honghui et al. (2014), Procopciuc LM et al. (2014), A. Atis et al. (2012), Chen baoli et al. (2011), Liu shifang. (2010)); second, multiple subgroups were divided to be analyzed; thereby a more adequate statistical power was gained in our study. Similar to the published researches, we found significant associations between the two VEGF gene polymorphisms (rs3025039, rs2010963) and PE, suggesting VEGF gene variants in rs3025039 and rs2010963 loci might be involved in the development of PE. Our results provide evidence of a significantly increased risk about rs3025039 polymorphisms for PE with the four models. Compared to the previous meta-analyses, a significantly increased risk for PE was observed in rs2010963 polymorphisms with less heterogeneity except for the Dominant model. In the stratified analysis by ethnicity and Countries for rs3025039, a significantly increased risk of preeclampsia was observed in studies conducted among Chinese population. As for subgroup analyses of ethnicity in rs2010963, a statistically association was found in the Allele comparison and the Homozygote models of Caucasian.

In addition, the heterogeneity could be accounted for by the subgroup analysis of genotyping methods. For rs3025039 polymorphism, the subgroup analyses of four genotyping methods including MassARRAY system, Sequencing, PCR-RFLP and Snapshot all showed low levels of heterogeneity (I^2 < 40%, p>0.10), where the results of the PCR-RFLP and Sequencing were consistent with the total result (OR > 1, p < 0.05), but different from the result of the other genotyping methods. Similarly, the heterogeneity of the three subgroups covering PCR-RFLP and Sequencing and Other methods was different for rs2010963 polymorphism. The heterogeneity of the PCR-RFLP and Sequencing subgroup was quite low (I^2 < 10%, p > 0.1), whereas the heterogeneity of the other methods subgroup was extensive (I^2 > 40%). The reason could be that studies in each subgroup are relatively few or different genotyping methods may influence the genotyping result. This observation is similar to previous studies, in which differences in genotyping methods might contribute to heterogeneity [35, 36]. The results would be more reliable and accurate if the same appropriate genotyping method was applied in different studies, because different genotyping methods have specialty in different aspects. Genotyping results with new genotyping technologies need be confirmed using direct sequencing. Furthermore, we have made efforts to seek out the potential sources of heterogeneity via sensitivity analysis assess and
publication biases assessment through Egger’s test, demonstrating impact of the individual literature and the publication biases were not obvious. Although the exact pathogenesis of how the SNPs change VEGF and PE susceptibility are not fully understood, a significant correlation between VEGF SNPs (rs3025039 and rs2010963) and PE have been confirmed by our present meta-analysis. At present, several biomarkers have been associated with PE, including soluble endoglin, Flt-1, MAP, PIGF and so on [37-39]. Integration of more reliable biomarkers and figuring out the feasibility in different ethnic groups will increase the accuracy the prediction of the PE, which is quite important for the early prevention of PE. In this study, our results provide the evidence that the status of the VEGF is close to occurrence of PE and the two SNPs of the VEGF could be applied in prediction of PE, particularly different ethnic groups.

Several limitations of our meta-analysis should be acknowledged. Firstly, unpublished reports or studies published in other non-international journals could not be included in the analysis. These problems may have affected the stability of the meta-analysis data. Secondly, the pooled sample sizes for the subgroup analyses among Xanthoderm and Caucasian for both rs2010963, rs3025039 were relatively small (<2,000 for cases), which may limit the statistical power. Thirdly, the recruitment criteria of patients and controls varied in different studies. Finally, gene–gene or gene–environmental interactions were not considered in this study—such as age, smoking, alcohol status, and mental state—which may have influenced the associations between VEGF gene polymorphisms and PE risk. Nevertheless, this meta-analysis improves our understanding of the associations between two polymorphisms of VEGF gene and the risk of PE.

In conclusion, the two VEGF gene polymorphisms are associated with an increased risk of preeclampsia in different ethnic groups respectively. A large number of and high-quality studies are required to establish more precise evidence and minimize the bias in meta-analysis.

Author Contribution

Duan Weicheng: project design, result interpretation, data collection, data validation and manuscript writing. Wang Kang: result interpretation, data collection. Duan Yijie: data collection, project design. Cheng Ping: data validation. Xiong Bo: project design, result interpretation, and
manuscript writing.

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Competing Interests
The authors declare that there are no competing interests associated with the manuscript

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Records identified by searching PubMed, EMBASE, Web of Science, CNKI and wanfang database (update to January, 2019) N=405

Records after duplicates removed, 263 studies retrieved

239 were excluded based on title and abstract

24 articles for full-text review

Excluded for:
- 2 inadequate genotype data
- 1 placental Polymorphism

21 articles included in meta-analysis
**A**

| Study                          | Lower CI Limit | Estimate | Upper CI Limit |
|-------------------------------|----------------|----------|----------------|
| Lu Z (2013)                   |                |          |                |
| Amosco (2016)                 |                |          |                |
| Zhang honghui (2014)          |                |          |                |
| Procopio LM (2014)            |                |          |                |
| Chedraui (2013)               |                |          |                |
| Andrewere (2013)              |                |          |                |
| A. Altis (2012)               |                |          |                |
| Chen bai (2011)               |                |          |                |
| He yun (2011)                 |                |          |                |
| Cunha VM (2010)               |                |          |                |
| Huang yuliang (2009)          |                |          |                |
| Liu shifang (2010)            |                |          |                |
| Shim Jy (2007)                |                |          |                |
| Kim YJ (2007)                 |                |          |                |
| Papazoglou D (2004)           |                |          |                |

1.00  1.42  1.90  2.02

**B**

| Study                          | Lower CI Limit | Estimate | Upper CI Limit |
|-------------------------------|----------------|----------|----------------|
| Amosco (2016)                 |                |          |                |
| Salini (2015)                 |                |          |                |
| Silva (2015)                  |                |          |                |
| Zhang honghui (2014)          |                |          |                |
| Chedraui P (2013)             |                |          |                |
| He yun (2011)                 |                |          |                |
| Garza-Veloz I (2011)          |                |          |                |
| Sandrim VC (2008)             |                |          |                |
| Nagy (2008)                   |                |          |                |
| Kim YJ (2007)                 |                |          |                |
| Bányaisz I (2006)             |                |          |                |
| Papazoglou D (2004)           |                |          |                |

1.00  1.05  1.21  1.39  1.46
Table 1: Study Characteristics of pre-eclampsia cases and controls in the analysis of VEGF polymorphisms

| Author[ref] | Year | Country | Ethnicity | Sources of Controls | Number of CASE | Polymorphisms(s) | Genotyping method | Association findings |
|------------|------|---------|-----------|---------------------|----------------|------------------|-----------------|---------------------|
| Lu Z [10]  | 2017 | China   | Xanthoderm| HB                  | 156            | rs3025039        | Snapshot        | NS                  |
| Amosco [11]| 2016 | Philippine | Xanthoderm| HB                  | 165            | rs3025039, rs2010963 | MassARRAY system | Supportive          |
| Salimi [12]| 2015 | Iran    | Caucasian | HB                  | 192            | rs2010963         | PCR-RFLP        | Supportive          |
| Silva [13] | 2015 | Brazil  | Caucasian | HB                  | 79             | rs2010963         | PCR-RFLP        | NS                  |
| Zhang honghui [14] | 2014 | China | Xanthoderm | HB                  | 58             | rs3025039, rs2010963 | Sequencing      | Supportive          |
| Procopciuc LM [15] | 2014 | Romania | Caucasian | HB                  | 70             | rs3025039         | PCR-RFLP        | Supportive          |
| Chedraui [16] | 2013 | Ecuador | Indo-europea n hybrid | HB | 31 | rs3025039, rs2010963 | Sequencing | NS |
| Andraweere [17] | 2013 | Australia | Caucasian | HB | 174 | rs3025039 | MassARRAY system | NS |
| A. Atis [18] | 2012 | Turkey | Caucasian | HB | 34 | rs3025039 | MassARRAY system | NS |
| Chen baoli [19] | 2011 | China | Xanthoderm | HB | 84 | rs3025039 | PCR-RFLP | Supportive |
| He yun [20] | 2011 | China | Xanthoderm | HB | 61 | rs3025039, rs2010963 | Sequencing | Supportive |
| Garza-Veloz I [21] | 2011 | Mexico | Indo-europea n hybrid | HB | 86 | rs2010963 | PCR-RFLP | NS |
| Cunha VM [22] | 2010 | Brazil | Caucasian | HB | 52 | rs3025039 | PCR-RFLP | NS |
| Liu shifang [23] | 2010 | China | Xanthoderm | HB | 84 | rs3025039 | PCR-RFLP | Supportive |
| Huang yuliang [24] | 2009 | China | Xanthoderm | HB | 128 | rs3025039 | PCR-RFLP | Supportive |
| Sandrim VC [25] | 2008 | Brazil | Caucasian | HB | 94 | rs2010963 | TaqMan-assays | NS |
| Nagy [26] | 2008 | Hungary | Caucasian | HB | 71 | rs2010963 | real-time PCR | NS |
| Shim JY [27] | 2007 | Korea | Xanthoderm | HB | 110 | rs3025039 | PCR-RFLP | Supportive |
| Kim YJ [28] | 2007 | Korea | Xanthoderm | HB | 223 | rs3025039, rs2010963 | Snapshot | NS |
| Banyasz I [29] | 2006 | Hungary | Caucasian | HB | 84 | rs2010963 | PCR-RFLP | Supportive |
| Papazoglou D [30] | 2004 | Sweden | Caucasian | HB | 42 | rs3025039, rs2010963 | PCR-RFLP | Supportive |

HB: hospital-based study, CTR: Control.
TABLE 2 Quality assessment conducted according to the Newcastle-Ottawa Scale for all the included studies.

| Author          | Adequate definition of case | Representativeness of the cases | Selective allocation of controls | Definition of controls | Comparability of cases and controls | Exposure assessment | Same method of ascertainment for cases and controls | Non-response rate | Total score |
|-----------------|-----------------------------|---------------------------------|----------------------------------|------------------------|-------------------------------------|--------------------|-----------------------------------------------------|------------------|-------------|
| Lu Z [10]       | *                           | *                               | *                                | *                      | *                                   | *                  | *                                                   | *                | 6           |
| Amosco [11]     | *                           | *                               | *                                | *                      | *                                   | *                  | *                                                   | *                | 7           |
| Salimi [12]     | *                           | *                               | *                                | *                      | *                                   | *                  | *                                                   | *                | 7           |
| Silva [13]      | *                           | *                               | *                                | *                      | *                                   | *                  | *                                                   | *                | 7           |
| Zhang           | *                           | *                               | *                                | *                      | *                                   | *                  | *                                                   | *                | 7           |
| Honghui         | *                           | *                               | *                                | *                      | *                                   | *                  | *                                                   | *                | 5           |
| Procopciuc LM   | *                           | *                               | *                                | *                      | *                                   | *                  | *                                                   | *                | 7           |
| Chedraui        | *                           | *                               | *                                | *                      | *                                   | *                  | *                                                   | *                | 7           |
| Andraweer e     | *                           | *                               | *                                | *                      | *                                   | *                  | *                                                   | *                | 7           |
| A.Atis          | *                           | *                               | *                                | *                      | *                                   | *                  | *                                                   | *                | 7           |
| Chen Baoli      | *                           | *                               | *                                | *                      | *                                   | *                  | *                                                   | *                | 5           |
| He yun          | *                           | *                               | *                                | *                      | *                                   | *                  | *                                                   | *                | 6           |
| Garza-Veloz I   | *                           | *                               | *                                | *                      | *                                   | *                  | *                                                   | *                | 8           |
| Cunha VM        | *                           | *                               | *                                | *                      | *                                   | *                  | *                                                   | *                | 7           |
| Liu Shifang     | *                           | *                               | *                                | *                      | *                                   | *                  | *                                                   | *                | 6           |
| Huang           | *                           | *                               | *                                | *                      | *                                   | *                  | *                                                   | *                | 5           |
| Yuliang         | *                           | *                               | *                                | *                      | *                                   | *                  | *                                                   | *                | 5           |
| Sandrim VC      | *                           | *                               | *                                | *                      | *                                   | *                  | *                                                   | *                | 8           |
| Nagy            | *                           | *                               | *                                | *                      | *                                   | *                  | *                                                   | *                | 7           |
| Shim Jy         | *                           | *                               | *                                | *                      | *                                   | *                  | *                                                   | *                | 8           |
| Kim YJ          | *                           | *                               | *                                | *                      | *                                   | *                  | *                                                   | *                | 7           |
| Banyasz I       | *                           | *                               | *                                | *                      | *                                   | *                  | *                                                   | *                | 8           |
| Papazoglou D    | *                           | *                               | *                                | *                      | *                                   | *                  | *                                                   | *                | 8           |
Table 3 Main results for the rs3025039 polymorphism with the risk of PE.

| Comparison       | Subgroup         | No | Test of association | Test of heterogeneity |
|------------------|------------------|----|---------------------|----------------------|
|                  |                  |    | OR                  | 95%CI                | P Value   | $^2$ | P Value   |
| T vs C           | Overall          | 15 | 1.418               | 1.060-1.898          | 0.019     | 76.6% | <0.001    |
|                  | China            | 6  | 1.793               | 1.229-2.617          | <0.001    | 69.5% | 0.006     |
|                  | Korea            | 2  | 1.219               | 0.438-3.392          | 0.704     | 93.3% | <0.001    |
|                  | Other Countries  | 7  | 1.189               | 0.738-1.917          | 0.476     | 73.6% | <0.001    |
|                  | Xanthoderm       | 9  | 1.454               | 0.995-2.125          | 0.053     | 83.5% | <0.001    |
|                  | Caucasian        | 5  | 1.361               | 0.758-2.444          | 0.301     | 73.1% | 0.005     |
|                  | Indo-european hybrid | 1 | 1.369               | 0.628-2.984          | 0.429     | /     | /         |
|                  | MassARRAY system | 2  | 0.681               | 0.484-0.958          | 0.028     | <0.1% | 0.483     |
|                  | Sequencing       | 2  | 1.942               | 1.182-3.191          | 0.009     | 20.0% | 0.264     |
|                  | PCR-RFLP         | 9  | 1.961               | 1.594-2.413          | <0.001    | 14.1% | 0.316     |
|                  | Snapshot         | 2  | 0.778               | 0.601-1.007          | 0.056     | <0.1% | 0.566     |
| TT vs CC+CT      | Overall          | 14 | 1.637               | 1.031-2.598          | 0.037     | 22.5% | 0.210     |
|                  | China            | 5  | 2.420               | 1.400-4.180          | 0.002     | <0.1% | 0.436     |
|                  | Korea            | 2  | 1.514               | 0.334-6.873          | 0.591     | 73.6% | 0.052     |
|                  | Other Countries  | 7  | 1.062               | 0.502-2.246          | 0.911     | <0.1% | 0.465     |
|                  | Xanthoderm       | 9  | 1.795               | 0.979-3.291          | 0.059     | 42.1% | 0.098     |
|                  | Caucasian        | 5  | 1.144               | 0.438-2.987          | 0.784     | 1.0%  | 0.400     |
|                  | Indo-european hybrid | 1 | 2.148               | 0.364-12.693         | 0.399     | /     | /         |
|                  | MassARRAY system | 2  | 0.338               | 0.091-1.256          | 0.105     | <0.1% | 0.555     |
|                  | Sequencing       | 2  | 4.378               | 1.525-12.572         | 0.006     | <0.1% | 0.328     |
|                  | PCR-RFLP         | 8  | 2.409               | 1.399-4.150          | 0.002     | <0.1% | 0.980     |
|                  | Snapshot         | 2  | 0.926               | 0.461-1.858          | 0.828     | <0.1% | 0.478     |
| TT +CT vs CC     | Overall          | 15 | 1.501               | 1.068-2.109          | 0.019     | 75.1% | <0.001    |
|                  | China            | 6  | 1.933               | 1.205-3.102          | 0.006     | 71.6% | <0.001    |
|                  | Korea            | 2  | 1.243               | 0.385-4.019          | 0.716     | 92.9% | <0.001    |
|                  | Other Countries  | 7  | 1.260               | 0.724-2.193          | 0.414     | 73.6% | <0.001    |
|                  | Xanthoderm       | 9  | 1.520               | 0.977-2.365          | 0.063     | 82.8% | <0.001    |
|                  | Caucasian        | 5  | 1.513               | 0.769-2.977          | 0.230     | 72.9% | 0.005     |
|                  | Indo-european hybrid | 1 | 1.295               | 0.477-3.515          | 0.612     | /     | /         |
|                  | MassARRAY system | 2  | 0.700               | 0.481-1.019          | 0.063     | <0.1% | 0.317     |
|                  | Sequencing       | 2  | 1.976               | 1.035-3.772          | 0.039     | 12.1% | 0.286     |
|                  | PCR-RFLP         | 9  | 2.186               | 1.678-2.848          | <0.001    | 24.0% | 0.230     |
|                  | Snapshot         | 2  | 0.726               | 0.540-0.978          | 0.035     | <0.1% | 0.697     |
| TT vs CC         | Overall          | 14 | 1.819               | 1.021-3.240          | 0.042     | 45.7% | 0.032     |
|                  | China            | 5  | 3.009               | 1.403-6.453          | 0.005     | 32.3% | 0.206     |
|                  | Korea            | 2  | 1.658               | 0.256-10.750         | 0.596     | 82.3% | 0.017     |
|                  | Other Countries  | 7  | 1.139               | 0.467-2.781          | 0.775     | 24.9% | 0.239     |
|                  | Xanthoderm       | 9  | 2.041               | 0.958-4.346          | 0.064     | 60.6% | 0.013     |
|                  | Caucasian        | 5  | 1.256               | 0.376-4.198          | 0.711     | 32.2% | 0.207     |
|                  | Indo-european hybrid | 1 | 2.267               | 0.362-14.185         | 0.382     | /     | /         |
| Method | n  | Median  | Interquartile range | Mean   | p-value | Median absolute deviation |
|--------|----|---------|----------------------|--------|---------|--------------------------|
| MassARRAY system | 2  | 0.316   | 0.085-1.177          | 0.086  | <0.1%  | 0.605                    |
| Sequencing | 2  | 5.284   | 1.322-21.116         | **0.019** | 33.0%  | 0.222                    |
| PCR-RFLP | 8  | 3.120   | 1.793-5.429          | <0.001 | <0.1%  | 0.921                    |
| Snapshot | 2  | 0.846   | 0.420-1.707          | 0.641  | <0.1%  | 0.462                    |

Presentation with bold indicated a statistical significance.
Table 4: Main results for the rs2010963 polymorphism with the risk of PE

| Comparison    | Subgroup                        | No | Test of association | Test of heterogeneity |
|---------------|---------------------------------|----|---------------------|-----------------------|
|               |                                 |    | OR                  | 95%CI                 | P Value | I²     | P Value |
| C vs G        | Overall                         | 12 | 1.207               | 1.046-1.394           | 0.010   | 26.1%  | 0.188   |
|               | Xanthoderm                      | 4  | 1.178               | 0.879-1.581           | 0.273   | 46.1%  | 0.135   |
|               | Caucasian                       | 6  | 1.246               | 1.004-1.546           | 0.046   | 38.4%  | 0.150   |
|               | Indo-european hybrid             | 2  | 1.199               | 0.817-1.760           | 0.353   | <0.1%  | 0.417   |
|               | Sequencing                      | 2  | 1.620               | 1.044-2.516           | 0.032   | <0.1%  | 0.940   |
|               | PCR-RFLP                        | 6  | 1.149               | 0.971-1.358           | 0.105   | <0.1%  | 0.640   |
|               | Other methods                    | 4  | 1.224               | 0.866-1.731           | 0.253   | 68.1%  | 0.024   |
| CC vs GG+GC   | Overall                         | 12 | 1.154               | 0.912-1.460           | 0.232   | <0.1%  | 0.647   |
|               | Xanthoderm                      | 4  | 0.932               | 0.626-1.387           | 0.729   | <0.1%  | 0.510   |
|               | Caucasian                       | 6  | 1.295               | 0.948-1.768           | 0.104   | <0.1%  | 0.462   |
|               | Indo-european hybrid             | 2  | 1.296               | 0.564-2.975           | 0.541   | <0.1%  | 0.784   |
|               | Sequencing                      | 2  | 0.974               | 0.314-3.020           | 0.964   | <0.1%  | 0.976   |
|               | PCR-RFLP                        | 6  | 1.231               | 0.896-1.690           | 0.200   | <0.1%  | 0.689   |
|               | Other methods                    | 4  | 1.203               | 0.676-2.142           | 0.529   | 43.2%  | 0.152   |
| CC +GC vs GG  | Overall                         | 12 | 1.310               | 1.044-1.643           | 0.020   | 42.6%  | 0.058   |
|               | Xanthoderm                      | 4  | 1.350               | 0.898-2.031           | 0.149   | 52.3%  | 0.098   |
|               | Caucasian                       | 6  | 1.278               | 0.894-1.826           | 0.178   | 56.0%  | 0.045   |
|               | Indo-european hybrid             | 2  | 1.360               | 0.667-2.772           | 0.397   | 33.4%  | 0.221   |
|               | Sequencing                      | 2  | 2.328               | 1.294-4.189           | 0.005   | <0.1%  | 0.901   |
|               | PCR-RFLP                        | 6  | 1.174               | 0.924-1.491           | 0.189   | <0.1%  | 0.663   |
|               | Other methods                    | 4  | 1.322               | 0.804-2.175           | 0.272   | 73.8%  | 0.010   |
| CC vs GG      | Overall                         | 12 | 1.324               | 1.024-1.713           | 0.032   | <0.1%  | 0.622   |
|               | Xanthoderm                      | 4  | 1.134               | 0.734-1.753           | 0.570   | <0.1%  | 0.433   |
|               | Caucasian                       | 6  | 1.461               | 1.019-2.094           | 0.039   | 8.9%   | 0.359   |
|               | Indo-european hybrid             | 2  | 1.377               | 0.557-3.406           | 0.489   | <0.1%  | 0.905   |
|               | Sequencing                      | 2  | 1.555               | 0.479-5.048           | 0.462   | <0.1%  | 0.994   |
|               | PCR-RFLP                        | 6  | 1.287               | 0.907-1.827           | 0.157   | <0.1%  | 0.699   |
|               | Other methods                    | 4  | 1.469               | 0.771-2.799           | 0.242   | 49.0%  | 0.117   |

Presentation with bold indicated a statistical significance
|                  | rs3025039 |               | rs2010963 |               |
|------------------|-----------|---------------|-----------|---------------|
|                  | T vs C    | TT vs CC      | T vs C    | TT vs CC      |
| P value          | 0.319     | 0.678         | 0.126     | 0.847         |
| Egger Test       | [-2.12,   | [-2.05,       | [-0.91,   | [-2.26,       |
| 95% CI           | 6.02]     | 6.60]         | 1.88]     | 4.05]         |
|                  | C vs G    | CC vs CC      | C vs G    | CC vs CC      |
| P value          | 0.182     | 0.153         | 0.518     | 0.227         |
| Egger Test       | [-0.88,   | [-0.43,       | [-2.30,   | [-0.67        |
| 95% CI           | -4.05]    | 4.27]         | 2.50]     |               |
|                  | CC vs GG  | CC+GC vs GG   | CC vs GG  | CC vs GG      |
| P value          | 0.182     | 0.227         | 0.518     | 0.227         |
| Egger Test       | [-2.30,   | [-0.67        | [-2.30,   | [-0.67        |
| 95% CI           | 4.27]     | 2.50]         | 2.50]     |               |
| Section/topic | # | Checklist item                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Reported on or not |
|---------------|---|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------|
| TITLE         |   | Title 1 Identify the report as a systematic review, meta-analysis, or both.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | Y                 |
| ABSTRACT      |   | Structured summary 2 Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.                                                                                                                                                                                                                                                                           | Y                 |
| INTRODUCTION  |   | Rationale 3 Describe the rationale for the review in the context of what is already known.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Y                 |
|               |   | Objectives 4 Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).                                                                                                                                                                                                                                                                                                                                                                                            | Y                 |
| METHODS       |   | Protocol and registration 5 Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.                                                                                                                                                                                                                                                                                                                                                                                  | N                 |
|               |   | Eligibility criteria 6 Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.                                                                                                                                                                                                                                                                                                                                                                          | Y                 |
|               |   | Information sources 7 Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.                                                                                                                                                                                                                                                                                                                                                                         | Y                 |
|               |   | Search 8 Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Y                 |
|               |   | Study selection 9 State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).                                                                                                                                                                                                                                                                                                                                                                               | Y                 |
|               |   | Data collection process 10 Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.                                                                                                                                                                                                                                                                                                                                                                                  | Y                 |
|               |   | Data items 11 List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Y                 |
|               |   | Risk of bias in individual studies 12 Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.                                                                                                                                                                                                                                                                                                                                                               | Y                 |
|               |   | Summary measures 13 State the principal summary measures (e.g., risk ratio, difference in means).                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Y                 |
|               |   | Synthesis of results 14 Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.                                                                                                                                                                                                                                                                                                                                                                                        | Y                 |
|               |   | Risk of bias across studies 15 Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Y                 |
| Additional analyses | 16 | Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified. | Y |
|---------------------|----|-------------------------------------------------------------------------------------------------|----|

**RESULTS**

| Study selection | 17 | Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram. | Y |
|-----------------|----|-----------------------------------------------------------------------------------------------|----|
| Study characteristics | 18 | For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations. | Y |
| Risk of bias within studies | 19 | Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12). | Y |
| Results of individual studies | 20 | For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot. | Y |
| Synthesis of results | 21 | Present results of each meta-analysis done, including confidence intervals and measures of consistency. | Y |
| Risk of bias across studies | 22 | Present results of any assessment of risk of bias across studies (see Item 15). | Y |
| Additional analysis | 23 | Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]). | Y |

**DISCUSSION**

| Summary of evidence | 24 | Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers). | Y |
| Limitations | 25 | Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias). | Y |
| Conclusions | 26 | Provide a general interpretation of the results in the context of other evidence, and implications for future research. | Y |

**FUNDING**

| Funding | 27 | Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review. | Y |

Y, the item was reported in article; N, the item was not reported.
A meta-analysis of the Vascular endothelial growth factor polymorphisms associated with the risk of pre-eclampsia

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Abstract

Background: Pre-eclampsia (PE) is a common pregnancy-induced hypertension disease. Some case-control studies reported the association between Vascular endothelial growth factor (VEGF) gene polymorphisms (rs3025039, rs2010963) and PE risk. However, these associations were inconsistent in several studies. Therefore, we conducted this meta-analysis to assess the role of VEGF gene polymorphisms in PE more precisely.

Methods: Eligible studies were searched in PubMed, Embase, Web of Science and Chinese (CNKI and WanFang) databases. Statistical analyses were performed by Stata 12.0 software. Odds ratio (OR) and 95% confidence interval (CI) were used to assess the strength of the association. In addition, subgroup analyses, sensitive analyses and publication bias analyses were performed to further assess this meta-analysis.

Results: Totally, 21 studies were included in the meta-analysis covering 2,018 cases and 2,632 controls. There were significant associations between VEGF polymorphisms (rs3025039,
rs2010963) and PE risk in the overall populations. In the subgroup analyses, we found that rs3025039 polymorphism was associated with the increased risk of PE amongst Chinese. As for rs2010963 polymorphism, a significant association was observed in subgroup of Caucasian.

**Conclusion:** The present study suggested that the two VEGF gene polymorphisms (rs3025039, rs2010963) are associated with increased risk of PE in different ethnic groups, which means that the targets may be useful genetic markers for early prediction of PE.

**Keywords:** VEGF, polymorphisms, pre-eclampsia, meta-analysis, risk.

**Introduction**

Pre-eclampsia (PE), a common pregnancy disease diagnosed by hypertension and proteinuria, occurs in approximately 2%–8% of pregnancies [1, 2]. PE is an important reason for the maternal and fetal morbidity and mortality due to dysfunction of multiple systems and organs, such as liver, kidney and brain [3]. Although its etiology has not been well recognized, PE is now regarded as the result of the combined effect of multiple factors [4, 5]. According to the results of the several epidemiological studies, PE has a substantial heritable component, which is estimated to be a major effect [4, 6].

The VEGF gene, which locates on chromosome 6p21.3, is a key regulator of angiogenesis and vascular function. Therefore, VEGF is vital for the formation of trophoblasts, embryonic vasculature and maternal and foetal blood cells in utero [7]. Abnormal vascular growth and endothelial dysfunction have been proposed to be the part of pathogenesis. Hence, VEGF have drawn the attention of many researchers [8, 9].

The associations between polymorphisms of the VEGF gene and PE have been extensively studied [10-30]. However, the results were somewhat controversial. In 2013, two meta-analyses assessed the associations between four polymorphisms of the VEGF gene and the risk of PE [31, 32]. But the retrieved datasets of these two meta-analyses were not sufficient, and several new studies have been published regarding this relationship between VEGF gene polymorphisms
(rs3025039, rs2010963) and PE [10-15]. In addition, the results published recently remained inconsistent and conflicting, likely owing to heterogeneity of different researches or inadequate sample size. A comprehensive retrieval of the pertinent literature in multiple databases is likely to help assess disease risks more precisely. In view of the mortality of PE, more efficient biomarkers are required for early discovery and prevention in the clinical practice. Therefore, we performed an updated meta-analysis of all eligible studies including English and non-English Journals to investigate the association between VEGF gene polymorphisms and the risk of PE. Moreover, we further divided the cases by ethnic groups, Countries as well as genotyping methods and analyzed sub-group specific associations.

**Methods**

**Identification of literature**

This meta-analysis was conducted in accordance with the guidance of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [33]. The PRISMA Checklist was presented in Supplementary Table S1. The literature search using the electronic databases PubMed, EMBASE, Web of Science, CNKI (Chinese National Knowledge Infrastructure) and WanFang was conducted by two study investigators. The comprehensive search strategies included the Mesh term and Keywords: (‘vascular endothelial growth factor’ or ‘VEGF’), (‘polymorphism’, ‘SNP’ ‘variant’, ‘genotype’ or ‘mutations’), (‘Pre-eclampsia’, ‘Preeclampsia’, ‘Pregnancy Toxemias’, ‘Pregnancy Toxemia’, ‘Edema-Proteinuria-Hypertension Gestosis’, ‘Edema Proteinuria Hypertension Gestosis’, ‘EPH Complex’, ‘EPH Toxemias’, ‘EPH Toxemia’, ‘Proteinuria-Edema-Hypertension Gestosis’ or ‘Proteinuria Edema Hypertension Gestosis’) through January 03, 2019. All eligible studies were retrieved and examined carefully. Review articles and references of other relevant researches were further searched to find additional eligible studies.

**Inclusion and Exclusion Criteria**
The inclusion criteria were as follows: (a) studies which estimated the associations between VEGF rs2010963 or rs3025039 and the susceptibility to PE; (b) case-control studies or cohort studies of PE; (c) patients must be clinically diagnosed PE (blood pressure $\geq 140/90$ mmHg on two measurements with $\geq 1+$ proteinuria or 300 mg/24 hours after the 20th week of pregnancy); (d) reported the allele frequencies of both cases and controls for different genotypes; (e) genotype distribution in the control group confirmed by Hardy–Weinberg equilibrium (HWE). The exclusion criteria of the meta-analysis were: (a) non-human studies, meta-analysis, comments, letters, reviews, mechanism studies or studies without controls (c) studies with overlapping or incomplete data. When overlapped population between studies was identified, only the newest or most complete article was included in the analysis. According to the corresponding criteria, two independent authors screened the articles.

Data extraction and assessment of methodological quality

Data were extracted by 2 authors independently from each study. The following information was collected: first author, publication year, participants' Country, ethnicity (categorized as Caucasian, Xanthoderm, Indo-european hybrid), sample size, study design (case-control or cohort), genotyping method, alleles and genotype frequency distribution in cases and controls, and the major conclusion of the study. When incomplete or apparent conflicting data was found in the article, we made an attempt to contact authors. Inconsistencies of data interpretation were resolved with discussion. The Newcastle–Ottawa Quality Assessment Scale (NOS) was employed to evaluate the methodological quality of the identified articles, and scores ranging from 0 (the worst) to 9 (the best) were assigned based on the quality of the studies. The studies with no less than 5 stars were considered to be of high quality.

Statistical Analysis

First, deviation from HWE in the distribution of allele frequencies was estimated again by the chi-square test (determined by $p<0.05$). Stata 12.0 was used to perform quantitative meta-analysis. The association was estimated with four models: Allele comparison model, Dominant model,
Recessive model, and Homozygote model. The four models of the data analysis were conducted by the random-effects model to prevent exaggerated results. The association between the VEGF rs3025039 or rs2010963 and PE risk was assessed by the raw ORs with 95% CIs. The student’s t-test was used to determine the significance of the crude OR, and p<0.05 was considered statistically significant. In addition, heterogeneity assumption among the included researches was evaluated by the Chi-square and I², which was regarded to be statistically significant if p< 0.10. And I² values of 25%, 50%, and 75% were nominally assigned as low, moderate, and high estimates. To insure that any single study did not cause an obvious influence to the whole effects, sensitivity analysis was performed to estimate the validity and stability of the study. In addition, to further analyze the source of the heterogeneity and the specific association between the VEGF polymorphism and PE, studies were also divided into several subgroups on the basis of the Country, the ethnicity of the related population, and the genotyping method. Egger’s test was performed to estimate the potential publication bias.

Results

Study Characteristics

As shown in Fig.1, the PRISMA flowchart demonstrated process of the literature retrieval. 405 studies were identified according to the result of the retrieval strategy and manual searches from PubMed, EMBASE, Web of Science, CNKI and WanFang database. On the basis of our inclusion/exclusion criteria, 142 studies were excluded for the duplication, and 239 studies were excluded as meta-analysis, reviews, mechanism studies or non-relevant research. Then, 24 studied were selected for full-text review. However, three studies were excluded, because 2 studies lacked genotype data and 1 study focused on placental polymorphism. Because most of the studies did not use the rs number to name the SNP, every SNP was manually confirmed by searching in the NCBI according to the sequence in the literature. Finally, 21 studies were included in the meta-analysis. Thereinto, 15 studies assessed the association between VEGF rs3025039 T/C polymorphism and the risk of PE, and 12 studies examined the association between VEGF rs2010963...
C/G polymorphism and the risk of PE. The specific information about the included studies was exhibited in Table 1. The quality evaluation of each study following the NOS is presented in Table 2, which showed all of these studies can be regarded as high-quality studies.

**Overall analysis**

Overall results of this meta-analysis between the two SNP and pre-eclampsia were displayed in Table 3 and Table 4. In total, we analyzed 1,426 cases and 1,872 controls for rs3025039 with the random effect model, showing a significantly increased risk for the comparison of the T allele to the C allele (OR=1.418, 95% CI=1.060-1.898, p=0.019, Fig.2A). Also, the results of the three genotype models analysis all revealed a significant association between pre-eclampsia and the VEGF rs3025039 (Dominant model: OR=1.637, 95% CI=1.031-2.598, p=0.037, Fig.2B; Recessive model: OR=1.501, 95% CI=1.068-2.109, p=0.019, Fig.2C; Homozygote model: OR=1.819, 95% CI=1.021-3.240, p=0.042, Fig.2D), in which the result of the Recessive model exhibited high heterogeneity (I² = 77.2%) and others were acceptable. An analysis of 1,148 cases and 1,388 controls for rs2010963 showed the C allele in allele comparison model, CC and CG genotype in the recessive model, and CC genotype in the homozygous model increased the risk of PE significantly (Allele comparison model: OR=1.207, 95% CI=1.046-1.394, p=0.010, Fig.3A; Recessive model: OR=1.310, 95% CI=1.044-1.643, p=0.020 Fig.3C; Homozygote model: OR=1.324, 95% CI=1.024-1.713, p=0.032, Fig.3D), however the result of the dominant model did not indicate statistical significance (OR=1.154, 95% CI=0.912-1.460, p=0.232, Fig.3B).

**Subgroup analyses**

The subgroup analyses were carried out due to the heterogeneity of result and biases of the different subgroups. The results of subgroup analyses were shown in Table 3 and Table 4. First of all, the different Countries of population were divided into three parts including China, Korea and Other Countries in rs3025039 according to the source of the population in studies. For Countries subgroup analyses in rs3025039 polymorphism, a significant correlation was found in the allele model and the three genotype model of the Chinese subgroup, in which the Allele comparison model...
showed moderate heterogeneity ($I^2 = 69.5\%$), the Dominant model showed low heterogeneity ($I^2 < 0.001\%$), the Recessive model showed high heterogeneity ($I^2 = 71.6\%$), and the Homozygote model showed moderate heterogeneity ($I^2 = 32.3\%$). However, no significant association was found in the Korea’s subgroup and Other Countries’ subgroup, and relatively high heterogeneity was observed in the almost all models of the two subgroups except in Dominant and Homozygote model of Other Countries’ subgroup, which indicated the differences of population Countries were not the major cause of the heterogeneity for rs3025039 in this meta-analysis. Then, we performed an ethnic restriction including Xanthoderm and Caucasian and Indo-european hybrid for further subgroup analysis in rs3025039. No significant association was observed in the any model, and the heterogeneity of various models in different subgroups showed no significant reduction. Next, subgroup analysis was performed in rs3025039 according to the genotyping methods including MassARRAY system, Sequencing, PCR-RFLP and Snapshot. A significantly increased risk was found in the Sequencing and PCR-RFLP subgroup, but a protective effect was found in the MassARRAY system subgroup with Allele comparison model and Snapshot subgroup with Recessive model. The heterogeneity of all the subgroup in the four models were relatively low, which indicated that the genotyping methods might be the major source of the heterogeneity.

For rs2010963, we performed the subgroup analysis based on ethnicity and genotyping methods, because the overlapping Countries were limited. The subgroups of ethnicity were divided as the same as the subgroups of rs3025039. A significant association was observed in the Caucasian subgroup with both Allele comparison model and Homozygote model (Allele comparison model: $OR=1.246, p=0.046$; Homozygote model: $OR=1.461, p=0.039$), and other subgroups showed no obvious difference. Moreover, the heterogeneity of allele comparison model and homozygote model in Caucasian subgroup was low (Allele comparison model: $I^2=38.4\%$, Homozygote model: $I^2=8.9\%$). However, the difference between the overall heterogeneity and subgroup heterogeneity was not apparent, illustrating that the ethnicity was not the important cause of heterogeneity in the meta-analysis. Regarding the genotyping methods subgroups, we divided into three groups: Sequencing, PCR-RFLP and Other methods, owing to the duplicating number of the genotyping methods. The result of the Sequencing subgroup showed a statistically significant in Allele
comparison model and Homozygote model with indistinctive heterogeneity (Allele comparison model: OR=1.620, p=0.032, I²<0.1%; Recessive model: OR=2.328, p=0.005, I²<0.1%). Besides, heterogeneity of the Sequencing and PCR-RFLP subgroups in all models was not significant (I²<0.1%), while heterogeneity of Other methods subgroups was higher. The results suggested that the source of the heterogeneity might be the genotyping methods, consistent with the conclusion above.

**Sensitivity Analysis and Publication Bias**

To confirm the reliability of our results, a sensitivity analysis was performed for the allele model, showing no apparent difference before and after the removal of each study shown in the Fig.4. In addition, publication bias assessed by Egger’s regression test present no any obvious evidence in statistics, which was displayed in the Table 5.

**Discussion**

Although the etiology of preeclampsia is considered to be multi-factorial, genetic factors are thought to be strong determinants of this disease [4, 6]. Early studies reported that VEGF genes were associated with vascular growth and endothelial dysfunction, which may somewhat interpret the development of PE. In recent decades, many researchers have been focusing the role that VEGF gene may play in the cause of PE [34]. However, case-control studies have shown contradictory associations between VEGF gene polymorphisms and PE. The aim of this meta-analysis was to evaluate the association between VEGF rs3025039 and rs2010963 polymorphisms and PE for the use of the biomarkers in the clinical practice and the investigation of the concrete pathomachanism.

We conducted a thorough literature retrieve and review to identify as many relevant studies as possible in our meta-analysis. Compared with previous meta-analyses, we made an effort to gain some improvements in our analysis: first, several studies were not included in previous meta-analyses (Lu Z (2017), Amosco et al. (2016), Salimi et al. (2015), Silva et al. (2014), Zhang honghui etal. (2014), Procopciuc LM et al. (2014), A.Atis et al. (2012), Chen baoli et al. (2011), Liu shifang.
second, multiple subgroups were divided to be analyzed; thereby a more adequate statistical power was gained in our study. Similar to the published researches, we found significant associations between the two VEGF gene polymorphisms (rs3025039, rs2010963) and PE, suggesting VEGF gene variants in rs3025039 and rs2010963 loci might be involved in the development of PE. Our results provide evidence of a significantly increased risk about rs3025039 polymorphisms for PE with the four models. Compared to the previous meta-analyses, a significantly increased risk for PE was observed in rs2010963 polymorphisms with less heterogeneity except for the Dominant model. In the stratified analysis by ethnicity and Countries for rs3025039, a significantly increased risk of preeclampsia was observed in studies conducted among Chinese population. As for subgroup analyses of ethnicity in rs2010963, a statistically association was found in the Allele comparison and the Homozygote models of Caucasian.

In addition, the heterogeneity could be accounted for by the subgroup analysis of genotyping methods. For rs3025039 polymorphism, the subgroup analyses of four genotyping methods including MassARRAY system, Sequencing, PCR-RFLP and Snapshot all showed low levels of heterogeneity ($I^2<40\%$, $p>0.10$), where the results of the PCR-RFLP and Sequencing were consistent with the total result (OR $>1$, $p<0.05$), but different from the result of the other genotyping methods. Similarly, the heterogeneity of the three subgroups covering PCR-RFLP and Sequencing and Other methods was different for rs2010963 polymorphism. The heterogeneity of the PCR-RFLP and Sequencing subgroup was quite low ($I^2<10\%$, $p>0.1$), whereas the heterogeneity of the other methods subgroup was extensive ($I^2>40\%$). The reason could be that studies in each subgroup are relatively few or different genotyping methods may influence the genotyping result. This observation is similar to previous studies, in which differences in genotyping methods might contribute to heterogeneity [35, 36]. The results would be more reliable and accurate if the same appropriate genotyping method was applied in different studies, because different genotyping methods have specialty in different aspects. Genotyping results with new genotyping technologies need be confirmed using direct sequencing. Furthermore, we have made efforts to seek out the potential sources of heterogeneity via sensitivity analysis assessment and publication biases assessment through Egger’s test, demonstrating impact of the individual
literature and the publication biases were not obvious. Although the exact pathogenesis of how the SNPs change VEGF and PE susceptibility are not fully understood, a significant correlation between VEGF SNPs (rs3025039 and rs2010963) and PE have been confirmed by our present meta-analysis. At present, several biomarkers have been associated with PE, including soluble endoglin, Flt-1, MAP, PlGF and so on [37-39]. Integration of more reliable biomarkers and figuring out the feasibility in different ethnical groups will increase the accuracy the prediction of the PE, which is quite important for the early prevention of PE. In this study, our results provide the evidence that the status of the VEGF is close to occurrence of PE and the two SNPs of the VEGF could be applied in prediction of PE, particularly different ethnical groups.

Several limitations of our meta-analysis should be acknowledged. Firstly, unpublished reports or studies published in other non-international journals could not be included in the analysis. These problems may have affected the stability of the meta-analysis data. Secondly, the pooled sample sizes for the subgroup analyses among Xanthoderm and Caucasian for both rs2010963, rs3025039 were relatively small (<2,000 for cases), which may limit the statistical power. Thirdly, the recruitment criteria of patients and controls varied in different studies. Finally, gene–gene or gene–environmental interactions were not considered in this study—such as age, smoking, alcohol status, and mental state—which may have influenced the associations between VEGF gene polymorphisms and PE risk. Nevertheless, this meta-analysis improves our understanding of the associations between two polymorphisms of VEGF gene and the risk of PE.

In conclusion, the two VEGF gene polymorphisms are associated with an increased risk of preeclampsia in different ethnic groups respectively. A large number of and high-quality studies are required to establish more precise evidence and minimize the bias in meta-analysis.

**Author Contribution**

Duan Weicheng: project design, result interpretation, data collection, data validation and manuscript writing. Wang Kang: result interpretation, data collection. Duan Yijie: data collection, project design. Cheng Ping: data validation. Xiong Bo: project design, result interpretation, and manuscript writing.
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Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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