Association between VEGF gene polymorphisms (11 sites) and polycystic ovary syndrome risk

Running title: VEGF polymorphisms and polycystic ovary syndrome risk

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Abstract:
Vascular endothelial growth factor (VEGF) plays a critical role in ovarian folliculogenesis and normal reproductive function. So far, several studies focusing on association between VEGF gene polymorphisms and polycystic ovary syndrome (PCOS). However, above association between the VEGF gene polymorphisms and PCOS susceptibility is uncertain. Hence, we performed a timely meta-analysis containing all current publications to make clear this relationship. We searched articles from the PubMed, Embase and Chinese language (WanFang and CNKI) databases that were published up until May 10, 2019. Finally, we obtained 9 studies, containing 29 case-control studies and 11 different polymorphisms. The odds ratios (OR) and 95% confidence intervals (CI) were revealed association strengths. There were significantly decreased associations between rs2010963 (-634), +9812, +405 polymorphisms and PCOS risk. Nevertheless, there existed increased associations between rs699947 (-2578), rs833061, rs1570360 (-1154), rs3025020, rs3025039 polymorphisms and PCOS susceptibility. Our current analysis suggested VEGF gene polymorphisms may be associated with PCOS risk, which is possible to be expected to become biomarkers of early detection for women.

Keywords: VEGF, polycystic ovary syndrome, meta-analysis, polymorphism

Introduction
Polycystic ovarian syndrome (PCOS) is a highly prevalent disorder affecting multiple aspects of a women's overall health, with long-term effects that transcend well beyond the reproductive age [1-3]. Clinically, PCOS is characterized by hyperandrogenism manifested by hirsutism, acne and androgenic alopecia [4]. Patients with PCOS demonstrate reproductive abnormalities [5], marked insulin resistance [6], increased risk for type 2 diabetes mellitus [7], coronary heart disease [8], atherogenic
dyslipidemia [9], cerebrovascular morbidity [10], and anxiety and depression [11]. Although it was first reported in 1935 by Stein et al., the etiology remains unclear. Data from many studies suggest that genetics is very important in the development of PCOS [12].

Vascular endothelial growth factor (VEGF) gene, also known as VEGFA, VPF and MVCD1, locates at 6p21.1 and contains 9 exon counts, and is a member of the PDGF/VEGF growth factor family [13]. It encodes a heparin-binding protein, which exists as a disulfide-linked homodimer. This growth factor induces proliferation and migration of vascular endothelial cells and is essential for both physiological and pathological angiogenesis [14].

VEGF plays a critical role in ovarian folliculogenesis and normal reproductive function [14], highlighted by the findings that women with PCOS had increased serum levels of VEGF, which paralleled increases in Doppler flow velocities within ovarian vessels [15, 16]. High vascularization may result in abnormal growth of the theca interna, the site of androgen steroidogenesis, leading to hyperandrogenism, a hallmark of PCOS [17]. Several single nucleotide polymorphisms (SNPs) were identified within the VEGF gene, of which some were functional, directly affecting VEGF secretion and its serum expression [18-20].

So far, many studies have investigated the association between VEGF polymorphisms and PCOS risk. However, the results were not conclusive or consistent. Considering the vital role of VEGF gene in the development of PCOS, we conducted a timely meta-analysis including 11 SNPs [21-29] to derive a more comprehensive estimation of the association between VEGF gene polymorphisms and PCOS susceptibility to identify some significant biomarkers.

Materials and Methods

Identification and eligibility of relevant studies

We applied the PubMed, Embase, WanFang and CNKI databases using the key words ‘VEGF or VEGFA or VPF’, ‘PCOS or Polycystic ovarian syndrome’ and ‘polymorphism’ or ‘variant’ to identify including studies. The last search was updated on May 10, 2019. Finally, 29 case-control studies about 11 different SNPs were retrieved.

Inclusion criteria and exclusion criteria

Including studies had to meet following criteria: (1) address the correlation between PCOS risk and the VEGF gene SNPs; (2) be a case-control study, and (3) have sufficient genotype (wide type and mutant type) numbers in each case and control group. The following exclusion criteria were used: (1) lack of a control population; (2) lack of available genotype frequency data; and (3) duplicated studies.

Data extraction

The following items were selected: the first author’s last name, the year of publication, the country of origin, the ethnicity of subjects, SNP type, total number of case and control groups, source of control (SOC), the number of each genotype frequency in the case/control groups, the Hardy-Weinberg equilibrium (HWE) in the control group, and the genotyping method. Ethnicity was categorized as Asian, European, Mixed and
African.

**Statistical analysis**

Odds ratio (OR) with 95% confidence intervals (CI) were used to measure the strength of the association between the VEGF gene SNPs and PCOS risk. The statistical significance of the summary OR was determined with the Z-test. A heterogeneity assumption was evaluated among studies using a chi-square-based Q-test. If a P-value of < 0.10 for the Q-test indicated heterogeneity among the studies. If significant heterogeneity was detected, the random-effects model (DerSimonian-Laird method) was used. Otherwise, the fixed-effects model (Mantel-Haenszel method) was applied [30, 31].

We investigated the relationship between genetic variants of the VEGF gene SNPs and PCOS risk by the allelic contrast (1 vs. 2), homozygote comparison (1/1 vs. 2/2), dominant genetic model (1/1+1/2 vs. 2/2), heterozygote comparison (1/2 vs. 2/2) and recessive genetic model (1/1 vs. 1/2+2/2). A sensitivity analysis was performed by omitting studies, one after another, to assess the stability of results. The departure of the VEGF gene SNPs from expected frequencies under HWE was assessed in controls using the Pearson chi-square test (P < 0.05 was considered significant). Funnel plot asymmetry was assessed using Begg’s test and publication bias was assessed using Egger’s test [32], both the P-value < 0.05 is considered as significant. All statistical tests for this meta-analysis were performed with Stata software (version 11.0; StataCorp LP, College Station, TX).

**Network of gene-interaction of VEGF gene**

To more complete understanding of the role of VEGF in PCOS, the network of gene-gene interactions for VEGF gene was utilized through String online server (http://string-db.org/) [33].

**Results**

**Study characteristics**

In total, 73 articles were collected from the PubMed, Embase, CNKI and WanFang databases via a literature search using different combinations of above keywords. As shown in Figure 1, 64 articles were excluded (such as duplications, irrelevant articles, reviews and other gene’s polymorphisms). Finally, nine different articles including 11 SNPs were included in our current meta-analysis (Figure 1). Study characteristics from the published studies on the relationship between the VEGF gene SNPs and PCOS risk are summarized in Table 1. In all the studies, the controls were women under normal pregnancy. The detail of 11 SNPs were rs2010963 or -634 (three case-control studies including 632 cases and 622 controls), +9812 (two case-control studies including 212 cases and 183 controls), +13553 (two case-control studies including 208 cases and 184 controls), -460 (two case-control studies including 263 cases and 285 controls), +405 (two case-control studies including 263 cases and 285 controls), rs699947 or -2578 (four case-control studies including 724 cases and 782 controls), rs833061 (three case-control studies including 618 cases and 673 controls),
rs1570360 or -1154 (four case-control studies including 697 cases and 737 controls), rs833068 (two case-control studies including 500 cases and 540 controls), rs3025020 (two case-control studies including 500 cases and 540 controls), and rs3025039 or +936 (three case-control studies including 586 cases and 628 controls).

**Quantitative synthesis**

Significantly increased association were detected between five VEGF gene SNPs and PCOS susceptibility: rs699947 (Recessive model: OR = 1.74, 95% CI = 1.33-2.27, P = 0.346 for heterogeneity, P < 0.001, Figure 2, Table 2); rs833061 (Recessive model: OR = 1.71, 95% CI = 1.28-2.21, P = 0.794 for heterogeneity, P < 0.001, Figure 2, Table 2); rs1570360 (Recessive model: OR = 1.92, 95% CI = 1.36-2.72, P = 0.231 for heterogeneity, P < 0.001, Figure 2, Table 2); rs3025020 (Homozygote comparison: OR = 1.73, 95% CI = 1.13-2.65, P = 0.920 for heterogeneity, P = 0.011, Figure 3, Table 2); rs3025039 (Dominant model: OR = 1.37, 95% CI = 1.01-1.85, P = 0.235 for heterogeneity, P = 0.042, Figure 4, Table 2).

In opposite, several VEGF gene SNPs acts as a decreased association or protective effect for PCOS risk: rs2010963 (Heterozygote comparison: OR = 0.68, 95% CI = 0.53-0.86, P = 0.339 for heterogeneity, P = 0.002, Figure 5, Table 2); +9812 (Allelic contrast: OR = 0.60, 95% CI = 0.43-0.83, P = 0.892 for heterogeneity, P = 0.002, Figure 3, Table 2); +405 (Homozygote comparison: OR = 0.48, 95% CI = 0.23-1.00, P = 0.327 for heterogeneity, P = 0.050, Figure 6, Table 2).

**Sensitivity analysis and bias diagnosis**

We used a sensitivity analysis to determine whether modifying the meta-analysis inclusion criteria affected the results. No other single study influenced the summary OR qualitatively (data not shown). Egger and Begg’s tests were performed to assess publication bias and the funnel plot symmetry was examined. Finally, no publication bias was observed (data not shown).

**Gene-gene network diagram and interaction of online website**

String online server indicated that VEGF gene interacts with numerous genes. The network of gene-gene interaction has been illustrated in Figure 7.

**Discussion**

A strong association between increased serum VEGF levels and PCOS was previously reported, and a correlation between serum VEGF levels and increased ovarian stromal blood flow in women with polycystic ovaries was suggested [15, 16, 34, 35]. On the other hand, polymorphisms in the VEGF gene may lead to alterations in the production of this protein and may play an important role in the pathophysiology of PCOS, contributing to ovulatory dysfunction, infertility, and ovarian hyperstimulation syndrome, which are commonly observed in women with PCOS [36].

To combine the important of genetic etiology of PCOS, it makes sense to deep study the VEGF gene polymorphisms. There are at least 80 SNPs places in this gene (NCBI Gene association no: NT 007592). Among them, we searched several popular
databases to select more comprehensive case-control studies about SNPs in VEGF gene, which have been reported more than once about PCOS disease. Finally, 11 SNPs (rs2010963 (-634), +9812, +13553, -460, +405, rs699947 (-2578), rs833061, rs1570360 (-1154), rs833068, rs3025020 (-583), rs3025039 (+936)) were identified.

Polymorphisms in the promoter region (loci: -2578, -1154 and -460) or intron 6 (loci: -583) or 5'-untranslated region (loci: +405, +963 and -634) or +534 have been associated with different levels of VEGF expression. It was reported that -2578 C, -460 T and +405 G alleles appear to correlate with altered VEGF expression levels [18, 20, 37-40]. In addition, a strong association between increased serum VEGF levels and PCOS was previously reported, and a correlation between serum VEGF levels and increased ovarian stromal blood flow in women with polycystic ovaries was suggested [15, 16, 34, 35]. Due to above items, these four SNPs have been widely reported in PCOS.

It is the first time to collect such more SNPs at one time, 11 SNPs containing 5203 cases and 5462 controls. The meaningful of our current analysis was that we found five SNPs (rs699947, rs833061, rs1570360, rs3025020, rs3025039) may act as a risk effect for the development of PCOS, moreover, three SNPs (rs2010963, +9812, +405) may have a protective influence for PCOS. Among above results, rs699947, rs3025020 and +405 polymorphisms were consistent with abnormal expression of VEGF gene in serum, may be associated with PCOS risk through the serum VEGF levels. Some factors may be explained: First, different polymorphisms in the same gene may exert different effects on gene expression and function, leading to vary PCOS risks. Secondly, single genes or single environmental factors may not be likely to have direct effects on PCOS susceptibility, but complex interactions between genetic and environmental factors may be involved in the disease development. The last but not the least, if numbers of included studies were small, false-negative results may be detected for each polymorphism [41].

If one woman exists one or more significant following five SNPs (rs699947, rs833061, rs1570360, rs3025020, rs3025039) for VEGF from peripheral blood test, which may indicate that it is possible to increase the occurrence of PCOS for her in present time or at some point in the future. Therefore, it can be offer us some targets to intervene, such as lifestyle modification (reducing the BMI, obesity, high blood pressure, high blood fat and cardiovascular disease) for prevention status, regular gynecological examination (vaginal ultrasound or CT scans or endocrinology) to identify or rule out this disease and carry out treatments as soon as possible (oral contraceptive therapy, ovulation induction, high testosterone therapy, insulin sensitizer, GLP-I receptor agonist therapy, surgical treatment) [42]. To sum up, we wish to use this method to reduce the incidence of PCOS and improve the cure rate of early treatment. In addition, for another three decreased association SNPs (rs2010963, +9812, +405) and these no associated SNPs, it is not necessary to take corresponding monitoring measures at current moment.

In addition, we used the online analysis system-String to predict potential and functional partners (Figure 7). Finally, ten genes were predicted. The average score was very high. Among them, the highest score of association was KDR and FLT1.
(Score = 0.999), however, TGFB1, EGF, IGF1 and NOS3 had the lowest scores (0.993). The action of VEGF is mediated by binding to tyrosine kinase receptors, VEGFR-1 (Fms-like tyrosine kinase: FLT1) and VEGFR-2 (kinase domain-containing receptor: KDR) [43]. Pan et al. demonstrated that HIF1A-mediated VEGF expression might be an important mechanism regulating ovarian luteal development in mammals in vivo, which may provide new strategies for fertility control and for treating PCOS [44]. Additional, IGF-1, TGFB1, STAT3 and NOS3 were just suggested to participate in the development of PCOS [45-48], rather than combined with VEGF. Above information predicted FLT1, KDR, and HIF1A may influence VEGF and regulate the PCOS development, which maybe become intervention and treatment target genes in the future.

Several limitations in our current analysis should be considered. First, unadjusted OR was used. Secondly, control sources were not all health women. Thirdly, only four databases were searched for study retrieval, few relevant studies may be omitted. Fourthly, gene-gene interaction was missing. Fifth, other confounding factors such as age, BMI, lifestyle, total cholesterol, free androgen index, triglycerides and environment were not included and analyzed.

In summary, in the present meta-analysis, VEGF gene polymorphisms may be associated with PCOS susceptibility.

Conflicts of interest
The authors report no conflicts of interest.

Author Contribution
LW conceived the study. LH searched the databases and extracted the data. LH analyzed the data. LH wrote the draft of the paper. LW reviewed the manuscript.

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Figure legends

**Figure 1.** Flowchart illustrating the search strategy used to identify association studies for VEGF gene polymorphisms and PCOS risk.

**Figure 2.** Forest plot of PCOS risk associated with VEGF gene polymorphisms (rs699947, rs833061, rs1570360) (Recessive model) in the whole. The squares and horizontal lines correspond to the study-specific OR and 95% CI. The area of the
squares reflects the weight (inverse of the variance). The diamond represents the summary OR and 95% CI.

Figure 3. Forest plot of PCOS risk associated with VEGF gene polymorphisms (rs3025020 and +9812) (Homozygote comparison) in the whole. The squares and horizontal lines correspond to the study-specific OR and 95% CI. The area of the squares reflects the weight (inverse of the variance). The diamond represents the summary OR and 95% CI.

Figure 4. Forest plot of PCOS risk associated with VEGF gene polymorphism (rs3025039) (Dominant model) in the whole. The squares and horizontal lines correspond to the study-specific OR and 95% CI. The area of the squares reflects the weight (inverse of the variance). The diamond represents the summary OR and 95% CI.

Figure 5. Forest plot of PCOS risk associated with VEGF gene polymorphism (rs2010963) (Heterozygote comparison) in the whole. The squares and horizontal lines correspond to the study-specific OR and 95% CI. The area of the squares reflects the weight (inverse of the variance). The diamond represents the summary OR and 95% CI.

Figure 6. Forest plot of PCOS risk associated with VEGF gene polymorphism (+405) (Allelic contrast) in the whole. The squares and horizontal lines correspond to the study-specific OR and 95% CI. The area of the squares reflects the weight (inverse of the variance). The diamond represents the summary OR and 95% CI.

Figure 7. Human VEGF interactions network with other genes obtained from String server. At least 10 genes have been indicated to correlate with VEGF gene. KDR: vascular endothelial growth factor receptor 2; FLT1: vascular endothelial growth factor receptor 1; NRP2: neuropilin-2; HIF1A: hypoxia-inducible factor 1-alpha; NRP1: neuropilin-1; STAT3: signal transducer and activator of transcription 3; TGFB1: transforming growth factor beta-1; EGF: pro-epidermal growth factor; IGF1: insulin-like growth factor 1; NOS3: nitric oxide synthase, endothelial.
| Author  | Year | Country | Ethnicity | SNPs          | Case Control | SOC | Cases   | Controls | HWE | Genotype |
|---------|------|---------|-----------|---------------|--------------|-----|---------|----------|-----|----------|
| Lee     | 2008 | Korea   | Asian     | rs2010963 (-634) | 132 99       | HB  | 26 60 46 | 20 45 34 | 0.47 | TaqMan   |
| Almawi  | 2016 | Bahrain | Asian     | rs2010963 (-634) | 382 393      | PB  | 57 142 183 | 42 190 161 | 0.01 | TaqMan   |
| Huang   | 2018 | China   | Asian     | rs2010963 (-634) | 118 130      | HB  | 13 45 60 | 19 64 47 | 0.71 | PCR-LDR |
| Lee     | 2008 | Korea   | Asian     | +9812         | 132 99       | HB  | 6 36 90 | 12 29 58 | 0.01 | TaqMan   |
| Ding    | 2009 | China   | Asian     | +9812         | 80 84        | HB  | 6 24 50 | 16 24 44 | 0.001| sequencing |
| Lee     | 2008 | Korea   | Asian     | +13553        | 128 100      | HB  | 4 35 89 | 10 31 59 | 0.06 | TaqMan   |
| Ding    | 2009 | China   | Asian     | +13553        | 80 84        | HB  | 0 35 45 | 0 30 54 | 0.046| sequencing |
| Vural   | 2009 | Turkey  | European  | -460          | 137 155      | HB  | 18 64 55 | 29 74 52 | 0.76 | F-LHPLC |
| Guruvaiah| 2014 | India   | Asian     | -460          | 126 130      | HB  | 27 59 40 | 25 72 33 | 0.2  | sequencing |
| Vural   | 2009 | Turkey  | European  | +405          | 137 155      | HB  | 3 44 90 | 4 39 112 | 0.78 | F-LHPLC |
| Guruvaiah| 2014 | India   | Asian     | +405          | 126 130      | HB  | 10 46 70 | 19 59 52 | 0.73 | sequencing |
| Salem   | 2016 | Tunisia | African   | rs699947(-2578) | 118 150      | HB  | 20 63 35 | 29 76 45 | 0.76 | TaqMan   |
| Almawi  | 2016 | Bahrain | Asian     | rs699947(-2578) | 382 393      | PB  | 64 183 135 | 50 178 165 | 0.85 | TaqMan   |
| Gomes   | 2019 | Brazil  | Mixed     | rs699947(-2578) | 87 84        | HB  | 27 38 22 | 18 41 25 | <0.001| PCR-RFLP |
| Vural   | 2009 | Turkey  | European  | (-2578)       | 137 155      | HB  | 22 63 52 | 25 78 52 | <0.001| F-LHPLC |
| Salem   | 2016 | Tunisia | African   | rs833061       | 118 150      | HB  | 30 55 33 | 32 76 42 | 0.82 | TaqMan   |
| Almawi  | 2016 | Bahrain | Asian     | rs833061       | 382 393      | PB  | 78 174 130 | 71 190 132 | 0.85 | TaqMan   |
| Huang   | 2018 | China   | Asian     | rs833061       | 118 130      | HB  | 10 45 63 | 8 42 80 | 0.44 | PCR-LDR |
| Salem   | 2016 | Tunisia | African   | rs1570360 (-1154) | 118 150      | HB  | 19 42 57 | 18 57 75 | 0.17 | TaqMan   |
| Author   | Year | Region | Ethnicity | SNP          | Genotype | Cases | Controls | p-value | Method       |
|----------|------|--------|-----------|--------------|----------|-------|----------|---------|--------------|
| Almawi   | 2016 | Bahrain | Asian     | rs1570360(-1154) | 382      | 393   | PB 45    | 140     | 197          | 44 | 131          | 218          | <0.001 | TaqMan       |
| Li       | 2014 | China  | Asian     | rs1570360(-1154) | 110      | 110   | HB 3     | 29      | 78           | 5  | 30           | 65           | 0.53   | PCR-RFLP     |
| Gomes    | 2019 | Brazil | Mixed     | rs833068      | 87       | 84    | HB 7     | 24      | 56           | 1  | 31           | 52           | <0.001 | TaqMan       |
| Salem    | 2016 | Tunisia | African  | rs833068      | 382      | 390   | PB 51    | 175     | 156          | 34 | 200          | 156          | 0.006  | TaqMan       |
| Almawi   | 2016 | Bahrain | Asian     | rs3025020(-583) | 118      | 150   | HB 10    | 40      | 68           | 8  | 52           | 90           | 0.89   | TaqMan       |
| Salem    | 2016 | Tunisia | African  | rs3025020(-583) | 382      | 393   | PB 54    | 149     | 179          | 35 | 155          | 203          | 0.49   | TaqMan       |
| Almawi   | 2016 | Bahrain | Asian     | rs3025039(+936) | 118      | 150   | HB 3     | 27      | 88           | 4  | 19           | 127          | 0.005  | TaqMan       |
| Salem    | 2016 | Tunisia | African  | rs3025039(+936) | 382      | 393   | PB 5     | 81      | 296          | 7  | 68           | 318          | 0.141  | TaqMan       |
| Gomes    | 2019 | Brazil  | Mixed     | rs3025039(+936) | 86       | 85    | HB 70    | 16      | 25           | 60 | PCR-RFLP     |

**Table 1 Basic information for included studies of the association between polymorphisms in VEGF gene and PCOS susceptibility.**

HWE: Hardy–Weinberg equilibrium; HB: hospital-based; SOC: source of control; SNPs: single nucleotide polymorphism; PCR-RFLP: polymerase chain reaction and restrictive fragment length polymorphism; PCR-LDR: polymerase chain reaction-ligase detection reaction; F-LHPLC: fluorescence-labelled hybridization probes in a Light-Cycler; * 1/1: mutant genotype, 1/2: heterozygous, 2/2: wide type.
| Variables   | N   | Case/Control | Allelic contrast OR(95% CI) $P_h$ | Heterozygote comparison OR(95% CI) $P_h$ | Dominant model OR(95% CI) $P_h$ | Recessive model OR(95% CI) $P_h$ |
|------------|-----|--------------|----------------------------------|----------------------------------------|----------------------------------|----------------------------------|
| rs2010963  | 3   | 632/622      | 0.89(0.75-1.05) $0.242$          | 0.98(0.69-1.38) $0.233$                | 0.68(0.53-0.86) $0.339$          | 1.68(1.22-2.30) $0.197$          |
| (-634)     | 2   | 212/183      | 0.60(0.43-0.83) $0.892$          | 0.33(0.16-0.68) $0.974$                | 0.002                            | 0.009                            |
| +9812      | 2   | 208/184      | 0.002                            | 0.003                                  | 0.899                            | 0.152                            |
| +13553     | 2   | 208/184      | 0.86(0.40-1.85) $0.031$          | 0.991                                  | 0.425                            | 0.047                            |
| +405       | 2   | 263/285      | 0.84(0.66-1.07) $0.487$          | 0.72(0.44-1.18) $0.413$                | 0.75(0.51-1.10) $0.626$          | 0.74(0.52-1.06) $0.938$          |
| -460       | 2   | 263/285      | 0.86(0.41-1.77) $0.012$          | 0.90(0.38-2.15) $0.018$                | 0.86(0.34-2.13) $0.009$          | 0.85(0.42-1.37) $0.430$          |
| +405       | 2   | 263/285      | 0.673                            | 0.050                                  | 0.819                            | 0.731                            |
| rs699947   | 4   | 724/782      | 1.13(0.98-1.31) $0.258$          | 1.28(0.94-1.73) $0.333$                | 1.17(0.88-1.56) $0.596$          | 1.15(0.93-1.42) $0.389$          |
| (-2578)    | 4   | 618/673      | 1.09(0.93-1.28) $0.582$          | 1.18(0.85-1.63) $0.809$                | 1.01(0.79-1.29) $0.460$          | 1.06(0.84-1.34) $0.484$          |
| rs833061   | 3   | 618/673      | 1.08(0.91-1.27) $0.541$          | 1.23(0.85-1.76) $0.259$                | 1.15(0.79-1.68) $0.170$          | 1.05(0.85-1.30) $0.578$          |
| rs1570360  | 4   | 697/737      | 0.294                            | 0.325                                  | 0.958                            | 0.620                            |
| (-1154)    | 4   | 500/540      | 1.09(0.90-1.28) $0.509$          | 1.28(0.85-1.94) $0.239$                | 1.11(0.65-1.89) $0.071$          | 1.05(0.82-1.35) $0.256$          |
| rs833068   | 2   | 500/540      | 1.24(1.03-1.50) $0.719$          | 1.73(1.13-2.65) $0.920$                | 1.07(0.83-1.39) $0.823$          | 1.18(0.93-1.51) $0.744$          |
| (583)      | 2   | 500/540      | 1.27(0.97-1.27) $0.276$          | 0.87(0.35-2.18) $0.724$                | 1.43(1.05-1.96) $0.212$          | 1.37(1.01-1.85) $0.235$          |
| rs3025039  | 3   | 586/628      | 0.087                            | 0.766                                  | 0.025                            | 0.042                            |

Table 2 Total and stratified subgroup analysis for VEGF gene polymorphisms and PCOS susceptibility.
$P_{h}$: value of $Q$-test for heterogeneity test; $P$: $Z$-test for the statistical significance of the OR.
Finally, there were nine papers including 11 polymorphisms of VEGF gene in our meta-analysis.
| Study ID     | OR (95% CI)       | Weight |
|-------------|-------------------|--------|
| rs699947    |                   |        |
| Salem (2016)| 1.17 (0.63, 2.18) | 9.28   |
| Almawi (2016)| 2.04 (1.38, 3.04) | 17.29  |
| Gomes (2019)| 2.28 (1.15, 4.49) | 5.63   |
| Vural (2009)| 1.39 (0.75, 2.59) | 8.54   |
| Subtotal    | 1.74 (1.33, 2.27) | 40.76  |
| rs333061    |                   |        |
| Salem (2016)| 1.70 (0.97, 2.99) | 9.29   |
| Almawi (2016)| 1.64 (1.15, 2.33) | 24.33  |
| Huang (2019)| 2.34 (0.90, 6.10) | 2.69   |
| Subtotal    | 1.71 (1.28, 2.27) | 36.31  |
| rs1570360   |                   |        |
| Salem (2016)| 2.21 (1.11, 4.39) | 5.31   |
| Almawi (2016)| 1.72 (1.11, 2.66) | 15.27  |
| Li (2014)   | 0.90 (0.21, 3.83) | 1.99   |
| Gomes (2019)| 11.81 (1.43, 97.77)| 0.37   |
| Subtotal    | 1.92 (1.36, 2.72) | 22.93  |
| Overall     | 1.77 (1.49, 2.10) | 100.00 |
### Study

| ID       | OR (95% CI)     | Weight |
|----------|----------------|--------|
| rs3025039 (+936) | 1.88 (1.03, 3.46) | 20.86  |
| Salem (2016)    | 1.23 (0.87, 1.74) | 79.14  |
| Almawi (2016)   |                |        |
| Subtotal (I-squared = 29.1%, p = 0.235) | 1.37 (1.01, 1.85) | 100.00 |
| Overall (I-squared = 29.1%, p = 0.235)  | 1.37 (1.01, 1.85) | 100.00 |

- 289
- 1
- 3.46
Study

ID

OR (95% CI)     Weight

rs2010963 (-634)

Lee (2008)      0.99 (0.55, 1.77)     13.92

Almawi (2016)   0.66 (0.49, 0.89)     63.97

Huang (2019)    0.55 (0.32, 0.94)     22.11

Subtotal (I-squared = 7.5%, p = 0.339)

0.68 (0.53, 0.86)     100.00

Overall (I-squared = 7.5%, p = 0.339)

0.68 (0.53, 0.86)     100.00
