Vascular endothelial growth factor A polymorphisms are associated with increased risk of coronary heart disease: a meta-analysis

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ABSTRACT

Coronary heart disease (CHD) is a common complex disease resulting from the interaction of multiple environmental and genetic factors. To assess the potential relationship of vascular endothelial growth factor (VEGFA) rs699947 C>A, rs3025039 C>T and rs2010963 G>C polymorphisms with CHD risk, a comprehensive meta-analysis was conducted. A systematic search of EMBASE and PubMed online database for publications on VEGFA polymorphisms and risk of CHD was carried out. Crude Odds ratios (ORs) with their 95% confidence intervals (CIs) were calculated to determine the association. A total of ten publications including 22 trials involving 2097 cases and 2867 controls were included in our pooled analysis. Overall, results of the present meta-analysis demonstrated a significant association between VEGFA rs699947 C>A polymorphism and an increased risk of CHD. After stratifying by ethnicity and CHD type, the association was also obtained. A significant association between VEGFA rs3025039 C>T polymorphism and risk of CHD was also found. For VEGFA rs2010963 G>C polymorphism, the polymorphism was associated with MI risk. In conclusion, our findings suggest that VEGFA rs699947 C>A, rs3025039 C>T and rs2010963 G>C polymorphisms are risk factors for CHD. In the future, large sample size and well-designed epidemiologic studies are needed to confirm these conclusions.

INTRODUCTION

Coronary heart disease (CHD) is one of the leading causes of mortality and morbidity worldwide [1, 2]. Besides environmental risk factors (e.g. smoking, drinking, and sedentary lifestyle et al.), genetic factors, such as single-nucleotide polymorphisms (SNPs), may play prominent roles in the development of CHD [3].
Table 1: Characteristics of the eligible studies in the meta-analysis

| Study                      | Year | Country   | Ethnicity | CHD Type                  | No. of cases/controls | Genotype Method                  | Polymorphisms                      |
|----------------------------|------|-----------|-----------|---------------------------|-----------------------|----------------------------------|------------------------------------|
| Han et al.                 | 2015 | China     | Asians    | coronary heart disease    | 144/150               | MALDI-TOF MS                     | rs3025039 C>T and rs2010963 G>C    |
| Moradzadegan et al.        | 2015 | Iran      | Caucasians| coronary heart disease    | 141/369               | PCR-RFLP                         | rs2010963 G>C                       |
| Gu et al.                  | 2013 | China     | Asians    | coronary heart disease    | 435/480               | MALDI-TOF MS                     | rs699947 C>A, rs3025039 C>T and rs2010963 G>C |
| Cui et al.                 | 2013 | China     | Asians    | coronary heart disease    | 242/253               | MALDI-TOF MS                     | rs699947 C>A, rs3025039 C>T and rs2010963 G>C |
| Amoli et al.               | 2012 | Iran      | Caucasians| coronary heart disease    | 50/50                 | ARMS-PCR                         | rs699947 C>A                       |
| Guerzoni et al.            | 2009 | Brazil    | Caucasians| coronary heart disease    | 145/99                | PCR-SSCP                         | rs699947 C>A                       |
| Douvaras et al.            | 2009 | Greece    | Caucasians| myocardial infarction     | 102/98                | PCR-RFLP                         | rs3025039 C>T and rs2010963 G>C    |
| Kangas-Kontio et al.       | 2009 | Finland   | Caucasians| myocardial infarction     | 215/218               | TaqMan                           | rs699947 C>A, rs3025039 C>T and rs2010963 G>C |
| Kangas-Kontio et al.       | 2009 | Finland   | Caucasians| myocardial infarction     | 36/263                | TaqMan                           | rs699947 C>A, rs3025039 C>T and rs2010963 G>C |
| Biselli et al.             | 2008 | Brazil    | Caucasians| coronary heart disease    | 175/108               | PCR-SSCP                         | rs699947 C>A and rs3025039 C>T     |
| Petrovic et al.            | 2006 | Slovenia  | Caucasians| myocardial infarction     | 143/228               | PCR-RFLP                         | rs2010963 G>C                       |

Abbreviations: MALDI-TOF MS, Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry; ARMS-PCR, Amplification Refractory Mutation System-Polymerase Chain Reaction; PCR-SSCP, Polymerase Chain Reaction-Single-Strand Conformational Polymorphism; PCR-RFLP, Polymerase Chain Reaction -Restriction Fragment Length Polymorphism.

[7]. Previous study also found that increased plasma VEGF A levels in CHD patients may indicate the severity of coronary lesion, and it may be adopted as an indicator of the need for revascularization [8, 9]. These results suggested that VEGF A might be involved in the development of CHD.

The VEGF gene, also named as vascular permeability factor, is located on chromosome 6p21.3 and contains eight exons [10]. VEGF family consists of VEGFA, VEGFB, VEGFC, VEGFD, VEGFE, VEGFF and placental growth factor. The human VEGFA gene is very polymorphic (http://www.ncbi.nlm.nih.gov/SNP). And the variants of VEGFA gene may influence the expression between individuals [11]. Functional studies indicated that a number of variants in VEGFA gene were correlated with the level of mRNA and protein expression [12, 13]. Three single nucleotide polymorphisms (SNPs), VEGFA rs699947 (−2578C > A), rs3025039 (+936C > T) and rs2010963 G > C were extensively studied their associations with CHD; however, the results remained inconsistent. Recently, a systematic review and meta-analysis showed that VEGFA rs699947 polymorphism was not associated with CHD [14]. However, in this pooled analysis [14], only three case-control studies focusing on Caucasians were included, the power of this pooled-analyses might be insufficient. Of late, more epidemiologic studies with relatively large sample size focusing on the potential association of VEGFA rs699947 C > A, rs3025039 C > T and rs2010963 G > C polymorphisms with CHD risk were carried out. Considering the potential role of VEGFA rs699947 C > A, rs3025039 C > T and rs2010963 G > C polymorphism for CHD susceptibility, this coverage might increase the statistical power to assess the association of VEGFA rs699947 C > A, rs3025039 C > T and rs2010963 G > C polymorphisms with CHD risk.

RESULTS

Characteristics

There were two independent groups in a paper conducted by Kangas-Kontio et al., we treated them separately [19]. According to the major inclusion and exclusion criteria, ten eligible publications with 22 independent case-control studies [19-28] were included to extract the data. The flow chart of the detailed publication selection is summarized in Figure 1. For VEGFA rs699947 C > A polymorphism, a total of 1,290 CHD cases and 1,456 non-CHD controls from seven independent case-control studies [19-24] were included in this meta-analysis. The year of publication ranged from 2008 to 2013. Two of these studies were conducted in Asians [20, 21] and five studies in Caucasians [19, 22-24]. Using a Goodness-of-fit chi-square calculator, the HWE test was performed; the genotype distributions of controls were all in HWE (P > 0.05). The genotype distributions of controls were all in HWE (P > 0.05). In total, for VEGFA rs3025039 C > T polymorphism, 1,344 CHD cases and 1,563 non-CHD controls from seven independent case-control studies [19-24] were included in this meta-analysis. The year of publication ranged from 2008 to 2013. Two of these studies were conducted in Asians [20, 21] and five studies in Caucasians [19, 22-24]. Using a Goodness-of-fit chi-square calculator, the HWE test was performed; the genotype distributions of controls were all in HWE (P > 0.05). And for VEGFA rs2010963 G > C polymorphism, 1,344 CHD
cases and 2610 non-CHD controls from eight independent case-control studies were included [19-21, 25-28]. The year of publication ranged from 2006 to 2015. Three of these studies were conducted in Asians [20, 21, 26] and five studies in Caucasians [19, 25, 27, 28]. The HWE test was conducted; the genotype distributions of controls were all in HWE (P > 0.05). The characteristics of the included studies are shown in Table 1. The genotype distributions of the VEGF A rs699947 C > A, rs3025039 C > T and rs2010963 polymorphisms in CHD cases and controls are presented in Table 2, Table 3 and Table 4, respectively.

### Quantitative synthesis

Overall, VEGF A rs699947 C > A polymorphism was a risk factor for CHD (A vs. C: OR = 1.19; 95% CI, 1.05 - 1.34; P = 0.005; AA vs. CC: OR = 1.33; 95% CI, 1.03-1.73; P = 0.032 and AA+CA vs. CC: OR = 1.33; 95% CI, 1.12-1.58; P = 0.001; Table 5 and Figure 2). In subgroup analyses by ethnicity, the similar association was found among Asians (AA+CA vs. CC: OR = 1.36; 95% CI, 1.10-1.68; P = 0.005; Table 5). In subgroup analyses by

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**Table 2: Distribution of VEGFA rs699947 C>A polymorphism genotypes and alleles**

| Study          | Year | Case genotype | Control genotype | Case allele | Control allele | HWE |
|---------------|------|---------------|------------------|-------------|----------------|-----|
| Gu et al.     | 2013 | 219 CC        | 178 CA           | 30 AA       | 267 CC         | 174 CA | 31 AA       | 616 C       | 238 A       | 708 C       | 236 A       | YES |
| Cui et al.    | 2013 | 137 CC        | 78 CA            | 27 AA       | 172 CC         | 69 CA  | 12 AA       | 352 C       | 132 A       | 413 C       | 93 A        | YES |
| Amoli et al.  | 2012 | 9 CC          | 27 CA            | 14 AA       | 15 CC          | 26 A   | 9 AA        | 45 C        | 55 A        | 56 C        | 44 A        | YES |
| Guerzoni et al.| 2009 | 34 CC        | 83 CA            | 28 AA       | 29 CC          | 46 CA  | 24 AA       | 151 C       | 139 A       | 104 C       | 94 A        | YES |
| Kangas-Kontio et al. | 2009 | 36 CC    | 104 CA           | 75 AA       | 40 CC          | 101 A  | 70 AA       | 176 C       | 254 A       | 181 C       | 241 A       | YES |
| Kangas-Kontio et al. | 2009 | 4 CC      | 18 CA            | 14 AA       | 53 CC          | 129 A  | 81 AA       | 26 C        | 46 A        | 235 C       | 291 A       | YES |
| Biselli et al.| 2008 | 47 CC        | 96 CA            | 32 AA       | 30 CC          | 51 A   | 27 AA       | 190 C       | 160 A       | 111 C       | 105 A       | YES |

**Abbreviation:** HWE, Hardy–Weinberg equilibrium

**Table 3: Distribution of VEGFA rs3025039 C>T polymorphism genotypes and alleles**

| Study          | Year | Case genotype | Control genotype | Case allele | Control allele | HWE |
|---------------|------|---------------|------------------|-------------|----------------|-----|
| Han et al.    | 2015 | 84 CC         | 55 CT            | 5 TT        | 115 CC         | 31 CT  | 4 TT        | 223 C       | 65 T         | 261 C       | 39 T         | YES |
| Gu et al.     | 2013 | 272 CC        | 142 CT           | 16 TT       | 300 CC         | 159 CT  | 14 TT       | 686 C       | 174 T         | 759 C       | 187 T         | YES |
| Cui et al.    | 2013 | 133 CC        | 95 CT            | 14 TT       | 159 CC         | 86 CT  | 8 TT        | 361 C       | 123 T         | 404 C       | 102 T         | YES |
| Douvas et al. | 2009 | 68 CC         | 30 CT            | 4 TT        | 69 CC          | 27 CT  | 2 TT        | 166 C       | 38 T         | 165 C       | 31 T         | YES |
| Kangas-Kontio et al. | 2009 | 160 CC    | 50 CT            | 5 TT        | 155 CC         | 56 CT  | 7 TT        | 370 C       | 60 T         | 366 C       | 70 T         | YES |
| Kangas-Kontio et al. | 2009 | 23 CC      | 13 CT            | 0 TT        | 184 CC         | 72 CT  | 7 TT        | 59 C        | 13 T         | 440 C       | 86 T         | YES |
| Biselli et al.| 2008 | 133 CC        | 36 CT            | 6 TT        | 83 CC          | 23 CT  | 2 TT        | 302 C       | 48 T         | 189 C       | 27 T         | YES |

**Abbreviation:** HWE, Hardy–Weinberg equilibrium

**Table 4: Distribution of VEGFA rs2010963 G>C polymorphism genotypes and alleles**

| Study          | Year | Case genotype | Control genotype | Case allele | Control allele | HWE |
|---------------|------|---------------|------------------|-------------|----------------|-----|
| Han et al.    | 2015 | 69 CC         | 49 CC            | 26 CG       | 86 CG          | 54 G   | 10 C        | 187 G       | 101 C         | 226 G       | 74 C         | YES |
| Moradzadegan et al. | 2015 | 43 CC    | 65 CG            | 33 CG       | 85 CC          | 197 G  | 87 C        | 151 G       | 131 C         | 367 G       | 371 C         | YES |
| Gu et al.     | 2013 | 144 CC        | 215 CG           | 60 CG       | 154 CC         | 225 G  | 89 C        | 503 G       | 335 C         | 533 G       | 403 C         | YES |
| Cui et al.    | 2013 | 75 CC         | 102 CG           | 65 CG       | 104 CC         | 114 G  | 35 C        | 252 G       | 232 C         | 322 G       | 184 C         | YES |
| Douvas et al. | 2009 | 37 CC         | 49 CG            | 16 CG       | 29 CC          | 55 G   | 14 C        | 123 G       | 81 C         | 113 G       | 83 C         | YES |
| Kangas-Kontio et al. | 2009 | 132 CC    | 72 CG            | 10 CG       | 143 CC         | 67 G   | 8 C         | 336 G       | 92 C         | 353 G       | 83 C         | YES |
| Kangas-Kontio et al. | 2009 | 22 CC      | 10 CC            | 3 CG        | 154 CC         | 90 G   | 19 C        | 54 G        | 16 C         | 398 G       | 128 C         | YES |
| Petrovic et al.| 2006 | 42 CC         | 76 CG            | 25 CG       | 103 CC         | 104 G  | 21 C        | 160 G       | 126 C         | 310 G       | 146 C         | YES |

**Abbreviation:** HWE, Hardy–Weinberg equilibrium
| No. of study | Allelic comparison  | Homozygote comparison | Dominant comparison | Recessive comparison |
|------------|---------------------|-----------------------|--------------------|---------------------|
|            | OR(95%CI) | P  | OR(95%CI) | P  | OR(95%CI) | P  | OR(95%CI) | P  |
| Overall    | 1.19(1.05 - 1.34) | 0.005 | 1.33(1.03 - 1.72) | 0.032 | 1.33(1.12 - 1.58) | 0.001 | 1.14(0.83 - 1.55) | 0.422 |
| Ethnicity  |           |     |           |     |           |     |           |     |
| Asians     | 1.37(0.96 - 1.95) | 0.084 | 1.76(0.75 - 4.14) | 0.192 | 1.36(1.10 - 1.68) | 0.005 | 1.59(0.69 - 3.66) | 0.275 |
| Caucasians | 1.09(0.92 - 1.28) | 0.324 | 1.17(0.84 - 1.64) | 0.361 | 1.28(0.97 - 1.70) | 0.080 | 0.99(0.76 - 1.28) | 0.947 |
| Type of CHD |           |     |           |     |           |     |           |     |
| MI         | 1.15(0.91 - 1.47) | 0.242 | 1.36(0.83 - 2.24) | 0.220 | 1.30(0.83 - 2.03) | 0.245 | 1.15(0.81 - 1.64) | 0.432 |
| Non-MI     | 1.20(0.96 - 1.50) | 0.108 | 1.36(0.84 - 2.21) | 0.213 | 1.34(1.11 - 1.60) | 0.002 | 1.13(0.71 - 1.82) | 0.604 |

Abbreviations: MI: myocardial infarction; CHD: coronary heart disease

| No. of study | Allelic comparison  | Homozygote comparison | Dominant comparison | Recessive comparison |
|------------|---------------------|-----------------------|--------------------|---------------------|
|            | OR(95%CI) | P  | OR(95%CI) | P  | OR(95%CI) | P  | OR(95%CI) | P  |
| Overall    | 1.16(1.01 - 1.33) | 0.035 | 1.40(0.91 - 2.15) | 0.125 | 1.21(0.95 - 1.55) | 0.117 | 1.33(0.87 - 2.04) | 0.189 |
| Ethnicity  |           |     |           |     |           |     |           |     |
| Asians     | 1.34(0.96 - 1.87) | 0.089 | 1.57(0.93 - 2.65) | 0.089 | 1.42(0.91 - 2.22) | 0.119 | 1.46(0.87 - 2.45) | 0.149 |
| Caucasians | 1.01(0.80 - 1.29) | 0.906 | 1.09(0.51 - 2.33) | 0.825 | 1.02(0.77 - 1.33) | 0.914 | 1.09(0.51 - 2.31) | 0.832 |
| Type of CHD |           |     |           |     |           |     |           |     |
| MI         | 0.99(0.75 - 1.30) | 0.926 | 0.91(0.38 - 2.20) | 0.835 | 0.91(0.74 - 1.37) | 0.974 | 0.91(0.38 - 2.17) | 0.823 |
| Non-MI     | 1.29(0.98 - 1.68) | 0.065 | 1.60(0.98 - 2.63) | 0.063 | 1.33(0.93 - 1.90) | 0.113 | 1.50(0.92 - 2.45) | 0.106 |

Abbreviations: MI: myocardial infarction; CHD: coronary heart disease

Table 6: Meta-analysis of the VEGFA rs3025039 C>T polymorphism and CHD

Table 7: Meta-analysis of the VEGFA rs2010963 G>C polymorphism and CHD

the type of CHD, VEGFA rs699947 C > A polymorphism was also associated with risk of non-MI (AA+CA vs. CC: OR = 1.34; 95% CI, 1.11 - 1.60; P = 0.002; Table 5).

For VEGFA rs3025039 C > T polymorphism, this SNP was associated with increased risk of overall CHD in one genetic models (T vs. C: OR = 1.16; 95% CI, 1.01 - 1.33; P = 0.035; Table 6 and Figure 3). However, in a subgroup analysis by ethnicity and the type of CHD, the association was not identified (Table 6).
Table 8: Quality assessment of the included studies

| Study              | Year | Selection Adequate case definition | Selection Representativeness of the cases | Selection Representativeness of the controls | Definition of Controls | Comparability of the cases and controls | Ascertainment of exposure | Same ascertainment method for all cases controls | Non-Response rate | Total stars |
|--------------------|------|------------------------------------|------------------------------------------|-----------------------------------------------|------------------------|-----------------------------------------|--------------------------|-----------------------------------------------|------------------|-------------|
| Han et al.         | 2015 | *                                  | *                                        | —                                             | *                      | **                                     | **                       | *                                             | —                | 8           |
| Moradzadegan et al.| 2015 | *                                  | *                                        | —                                             | *                      | **                                     | *                        | *                                             | —                | 7           |
| Gu et al.          | 2013 | *                                  | *                                        | —                                             | *                      | **                                     | *                        | *                                             | —                | 8           |
| Cui et al.         | 2013 | *                                  | *                                        | —                                             | *                      | **                                     | *                        | *                                             | —                | 7           |
| Amoli et al.       | 2012 | *                                  | *                                        | —                                             | *                      | **                                     | *                        | —                                             | —                | 8           |
| Guerzoni et al.    | 2009 | *                                  | *                                        | —                                             | *                      | **                                     | *                        | *                                             | —                | 8           |
| Douvaras et al.    | 2009 | *                                  | *                                        | —                                             | *                      | **                                     | *                        | —                                             | —                | 7           |
| Kangas-Kontio et al.| 2009| *                                  | *                                        | *                                             | *                      | **                                     | *                        | *                                             | —                | 9           |
| Kangas-Kontio et al.| 2009| *                                  | *                                        | *                                             | *                      | **                                     | *                        | *                                             | —                | 9           |
| Biselli et al.     | 2008 | *                                  | —                                        | —                                             | *                      | **                                     | *                        | —                                             | —                | 7           |
| Petrovic et al.    | 2006 | *                                  | *                                        | —                                             | *                      | **                                     | *                        | —                                             | —                | 8           |

Tests for publication bias

The shape of Begg’s funnel plot test was symmetrical for VEGFA rs699947 C > A, rs3025039 C > T and rs2010963 G > C polymorphisms (rs699947 C > A polymorphism: A vs. C: Begg’s test \( P = 0.764 \); AA vs. CC: Begg’s test \( P = 0.368 \); AA+CA vs. CC: Begg’s test \( P = 0.548 \); rs3025039 C > T polymorphism: T vs. C: Begg’s test \( P = 0.764 \); TT vs. CC: Begg’s test \( P = 0.230 \); TT+CT vs. CC: Begg’s test \( P = 0.548 \); and rs2010963 G > C polymorphism: C vs. G: Begg’s test \( P = 0.100 \).

Figure 1: Flow diagram of studies selection.
test $P = 1.000$; CC vs. GG: Begg’s test $P = 1.000$; CC+GC vs. GG: Begg’s test $P = 1.000$ and CC vs. GG+GC: Begg’s test $P = 0.902$; Figure 5, Figure 6 and Figure 7). The statistical results of Egger’s test still demonstrated there were no evidence of bias for these two SNPs (rs699947 C > A polymorphism: A vs. C: Egger’s test $P = 0.627$; AA vs. CC: Egger’s test $P = 0.257$; AA+CA vs. CC: Egger’s test $P = 0.394$ and AA vs. CC+CA: Egger’s test $P = 0.356$; rs3025039 C > T polymorphism: T vs. C: Egger’s test $P = 0.598$; TT vs. CC: Egger’s test $P = 0.783$; TT+CT vs. CC: Egger’s test $P = 0.475$ and TT vs. CT+CC: Egger’s test $P = 0.660$; rs2010963 G > C polymorphism: C vs. G: Egger’s test $P = 0.608$; CC vs. GG: Egger’s test $P = 0.445$; CC+GC vs. GG: Egger’s test $P = 0.899$ and CC vs. GC+GG: Egger’s test $P = 0.318$).

**Tests for sensitivity analyses**

An independent study involved in the present pooled-analysis was omitted each time to assess the influence of the data-set on the pooled ORs, and the exclusion of anyone did not materially alter the corresponding pooled ORs (Figure 8, Figure 9 and Figure 10, data not shown).

**Tests for heterogeneity**

In some genetic models, we found significant heterogeneity across studies in the present meta-analysis for VEGFA rs699947 C > A, rs3025039 C > T and rs2010963 G > C polymorphisms. Type of CHD and ethnicity were defined as characteristics for evaluation of potential heterogeneity. Results of subgroup analyses demonstrated that studies conducted in Asians and non-MI subgroups may contribute to the major source of heterogeneity for VEGFA rs699947 C > A, rs3025039 C > T and rs2010963 G > C polymorphisms.

**Results of quality assessment**

We used Newcastle-Ottawa Quality Assessment Scale to assess the quality score of the eligible studies. When scores $\geq 7$ stars, the study was considered as high-

![Figure 2: Meta-analysis for the association between VEGFA rs699947 C > A polymorphism and CHD risk (AA+CA vs. CC genetic model, fixed-effects model).](image-url)
quality. The results indicated that all included studies were high-quality, suggesting the reliability of our findings (Table 8).

DISCUSSION

Besides environmental risk factors (e.g. smoking, drinking, and sedentary lifestyle et al.), multiple evidences support a vital role of genetics in determining susceptibility for CHD. The involvement of VEGF in inflammation and neovascularization may underlie the major mechanism responsible for the association between VEGF genotypes and risk of CHD. Recently, several investigations on the molecular epidemiology considering the correlation of VEGF polymorphism with CHD risk were performed; however, the findings remained conflicting. With respect to VEGF polymorphisms, a recent systemic review and meta-analysis with small sample sizes on this issue did not suggest any association between VEGF rs699947 C > A polymorphism and risk of CHD [14]. After that, some case-control studies reported that rs699947 C > A polymorphism in VEGF gene have been implicated in CHD risk, especially in Asians. Thus, we conducted a meta-analysis involving a total of 2097 CHD cases and 2867 controls subjects from ten publications including 22 trails to assess the potential associations between two commonly functional SNPs (rs699947 C > A, rs3025039 C > T and rs2010963 G > C) in VEGF gene and CHD risk.

For VEGF rs699947 C > A polymorphism, seven independent studies focusing on the relationship of this SNP with CHD risk were included. A recent case-control study has reported positive signals of VEGF rs699947 C > A polymorphism with risk of CHD [21]; contrastingly, others showed the variants of VEGF rs699947 C > A polymorphism did not influence risk of CHD [19, 20, 22-24]. As shown in Table 4, VEGF rs699947 C > A polymorphism was identified to be associated with the development of CHD. The A allele carriers indicated higher CHD susceptibility in comparison with the C allele carriers. In subgroup analyses by ethnicity, the similar association was found among Asians, but not Caucasians. Our results were consistent with the findings

![Figure 3: Meta-analysis for the association between VEGFA rs3025039 C > T polymorphism and CHD risk (T vs. C genetic model; fixed-effects model).](image-url)
of a previous meta-analysis [14]. A previous study indicated the expression levels of VEGF mRNA in CHD patients carrying the VEGF rs699947 AA genotype were significantly lower than those who carried the VEGF rs699947 AC or CC genotypes [29]. This study also suggested that CHD patients carrying the VEGF rs699947 A allele might have more chances in developing better coronary collaterals [29]. Gokkusu et al. reported that VEGF might be a cardio-protective factor [30]. In this study, we found that VEGFA rs699947 C > A polymorphism was correlated with increased risk of CHD, suggesting the presence of the A allele, which was associated with lower expression of VEGF mRNA and activity, might lead to the increased risk of CHD.

Rs3025039 C > T polymorphism locates on the 3'-UTR region of VEGFA gene. Thus, it may regulate post-transcription and then influence gene expression. VEGFA rs3025039 C > T polymorphism was well known to influence the secreted levels of VEGFA protein and has been identified to have overt association in most studies [31]. This SNP exhibited a very strong association with epithelial ovarian cancer status and poorer prognosis [31]. A prior study indicated this 3'-UTR polymorphism was associated with the occurrence and severity of diabetic nephropathy [32]. Recently, several case-control studies focused on the association between VEGFA rs3025039 C > T polymorphism and CHD risk. Han et al. reported that VEGFA rs3025039 CT genotype and C allele appeared to be a genetic risk factor for CHD [26]. Cui et al. also found VEGFA rs3025039 C > T polymorphism conferred a borderline increased risk to CHD [21]. As demonstrated in Table 5, the combined evidence suggested that VEGFA rs3025039 C > T polymorphism was a risk factor for overall CHD. In a subgroup analysis by ethnicity and the type of CHD, a borderline increased risk to CHD was also found in Asians and non-MI subgroups (P = 0.089 and P = 0.065, respectively). These findings demonstrated the presence of the T allele may alter mRNA and secreted levels of VEGFA protein and then led to the increased risk of CHD.

Rs2010963 G > C polymorphism is located in the 5'-untranslated region in VEGFA gene. According to previous reports, rs2010963 G > C polymorphism was a genetic marker of microvascular complications in cases...

Figure 4: Meta-analysis for the association between VEGFA 2010963 G > C polymorphism and CHD risk (CC vs. GG genetic model; fixed-effects model).
Figure 5: Begg’s funnel plot of meta-analysis for the association between VEGFA rs699947 C > A polymorphism and CHD risk (AA+CA vs. CC genetic model).

Figure 6: Begg’s funnel plot of meta-analysis for the association between VEGFA rs3025039 C > T polymorphism and CHD risk (T vs. C genetic model).

Figure 7: Begg’s funnel plot of meta-analysis for the association between VEGFA rs2010963 G > C polymorphism and CHD risk (CC vs. GG genetic model).
with type 2 diabetes [33-35]. Compared to those with
*VEGFA* GG and GC genotypes, a remarkably higher
VEGF serum level was found in healthy individuals
with the *VEGFA* rs2010963 CC genotype [33, 36]. The
CC genotype of the rs2010963 G > C polymorphism has
been demonstrated to be related to heart failure induced

Figure 8: Sensitivity analysis of the overall CHD meta-analysis for *VEGFA* rs699947 C > A polymorphism.

Figure 9: Sensitivity analysis of the overall CHD meta-analysis for *VEGFA* rs3025039 C > T polymorphism.

Figure 10: Sensitivity analysis of the overall CHD meta-analysis for *VEGFA* rs2010963 G > C polymorphism.
by acute myocardial infarction [25]. Several studies have investigated the association between \( VEGF \) rs2010963 G > C polymorphism and CHD risk. After meta-analyses in our study, we concluded that the CC genotype of the polymorphism may increase risk of MI.

Similar to other meta-analyses, some potential limitations of our meta-analysis should be acknowledged. First, although bias tests showed there was no significant publication bias in our meta-analysis and a comprehensive literature search was well designed, it is likely that certain unpublished studies might be overlooked. Second, the association of \( VEGF \) rs699947 C > A, rs3025039 C > T and rs2010963 G > C polymorphisms with risk of CHD was assessed based on unadjusted estimates. If the detailed data of individuals were available, a more precise meta-analysis could be carried out. Third, for lack of individual-level data, we did not conduct a further analysis to assess any potential interactions between gene-gene and gene-metabolic traits. Finally, significant heterogeneity between the eligible studies for \( VEGF \) rs699947 C > A, rs3025039 C > T and rs2010963 G > C polymorphisms was found. Our results should be interpreted with very cautions.

In conclusion, our findings indicate that \( VEGF \) rs699947 C > A, rs3025039 C > T and rs2010963 G > C polymorphisms may be risk factors for the development of CHD. As the participants in some subgroup are currently limited, further well-designed studies with larger sample size to investigate the role of these loci are needed. Moreover, interactions of gene-gene and gene-environment should not be ignored.

**MATERIALS AND METHODS**

**Search strategy**

Genetic association publications published before the end of November 15, 2016 on CHD and polymorphisms in \( VEGF \) gene were retrieved through a search of PubMed and EMBASE online databases with keywords: (vascular endothelial growth factor-A or \( VEGF \)) and (polymorphism or variant or SNP) and (coronary artery disease or CAD or coronary heart disease or CHD or myocardial infarction or MI). All bibliographies cited in eligible publications, reviews and meta-analysis were examined to retrieve the potential publications.

**Inclusion and exclusion criteria**

The major criteria of eligible studies were: (a) studies focused on the relationship of \( VEGF \) rs699947 C > A, rs3025039 C > T and rs2010963 G > C polymorphisms with CHD risk; (b) sufficient data were presented to determine the odds ratios (ORs) with their 95% confidence intervals (CIs) and \( P \) value, and (c) the genotyping method, equipment, and protocols used or provided reference were described in publication. Accordingly, publications providing insufficient data, CHD treatment, not case-control design, overlapping data, reviews and meta-analysis were excluded.

**Data extraction**

Two authors (Y. Wang and Q. Huang) reviewed and collected information independently from eligible studies in accordance with the major criteria for inclusion and exclusion mentioned above. The following data: the surname of first author, year of publication, country, ethnicity of the participants, type of CHD [myocardial infarction (MI) or non-MI], genotyping method as well as allele and genotype frequencies, were entered into a database. In case of conflicting evaluations, disagreements over study/data inclusion were resolved by a discussion among all reviewers.

**Quality assessment**

The Newcastle-Ottawa Quality Assessment Scale was harnessed to assess the quality score of the eligible studies. And scores ≥ 7 stars were considered as high-quality study [15].

**Statistical analysis**

A Goodness-of-fit chi-square calculator (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl) was used to examine the deviation from HWE in controls. The strength of correlation between SNPs in \( VEGF \) gene and CHD risk was assessed by ORs with the corresponding 95% CIs. Type of CHD (MI or non-MI) and ethnicity were considered as characteristics for evaluation of potential heterogeneity. Ethnicity group was defined as Asians and Caucasians. We used Chi-square based \( F \)-statistic test and Q statistical test to analyze the potential heterogeneity among the studies. \( P < 0.10 \) or \( F > 50\% \) indicates high heterogeneity, random-effects model (the DerSimonian and Laird method) was used to calculate the pooled ORs and CIs [16]; otherwise, the fixed-effects model (the Mantel-Haenszel method) was used [17]. Funnel plots and Egger’s regression test were harnessed to diagnose the potential publication bias [18], and a \( P < 0.1 \) was defined as statistical significance. Sensitivity analysis, which assessed the influence of each independent study on the pooled ORs with their corresponding 95% CIs, was also carried out to evaluate the stability of our results. All \( P \) values were defined as two-sided at the \( P = 0.05 \) level. All data analysis was performed with Stata 12.0 software for windows (Stata Corporation, College Station, TX).
CONFLICTS OF INTEREST

There is no conflict of interest.

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