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VEGF Polymorphisms do Not Contribute to the Risk of Congenital Heart Defect

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Abstract

Objective: To clarify the role of VEGF polymorphisms in CHD, we performed a meta-analysis to determine the association between these three variants and risk of CHD.

Methods: Our meta-analysis included a total of 6, 4, and 6 research articles for each of the C2578A, G1154A, and G634C polymorphisms, respectively. Data extraction and study quality assessment were performed in duplicate. Summary odds ratios (ORs) and 95% confidence intervals (CIs) of allele contrast and genotype contrast were estimated using either a fixed or random effects model. The Q-statistic test was used to identify heterogeneity and a funnel plot was adopted to evaluate publication bias.

Results: Six articles containing 1080 cases and 2289 controls were relevant to C2578A, 4 researches containing 528 cases and 1036 controls were relevant to G1154A, and 6 articles containing 1081 cases and 2281 controls were relevant to G634C. The results of overall meta-analysis showed that none of the VEGF C2578A、G1154A、G634C increased the susceptibility of CHD. In summary, this meta-analysis demonstrates that the three analyzed VEGF polymorphisms do not increase the risk of CHD.

Conclusions: Our meta-analysis suggests that the common VEGF polymorphisms C2578A, G1154A, and G634C do not alter CHD risk.

Keywords: Endothelial growth factor; Polymorphism; Congenital heart defect

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Introduction

Congenital heart defect (CHD) is one of the most common birth deformities, with approximately 0.6-0.8% of live infant births receiving the diagnosis [1]. Despite this prevalence, the pathogenesis of CHD remains unknown. However, a small number of CHD cases are caused by a single gene mutation or other chromosomal aberrations, leaving the remaining 90% CHD diagnoses resulting from a heterogenous etiology including a variety of genetic factors and environmental factors [2].

The heart is the first organ to form and function during development [3]. Numerous signaling pathways contribute to its development, and include vascular endothelial growth factor (VEGF), GATA4, and Nkx2.5 [4-6]. Of these pathways, VEGF signaling has been shown to be linked to CHD, with its spatiotemporal expression pattern indicating a potential role [7]. The VEGF gene is located on chromosome 6p12 and consists of eight exons, which can be alternatively spliced to form a family of proteins [8].

It has been reported that VEGF is required for proper heart morphogenesis at stages [7]. Additionally, VEGF-expressing endothelial cells located in the cushion-forming region may be a unique subpopulation of endothelial cells that are predetermined to transform from endocardium to mesenchyme (EMT) [9]. Importantly, maintaining an appropriate timing and dosage of VEGF during heart development has been shown in animal models to be shared in various cardiovascular developmental defects. This work includes work with transgenic mice heterozygous for the VEGF allele, which showed a two- to three-fold increase in VEGF levels [10]. Past work has also shown that increased VEGF levels during the development of the right ventricular outflow tract can lead to abnormal development of both cushion and myocardial structures [11].

VEGF gene polymorphisms may play a role in susceptibility to congenital valvuloseptal heart defects. It has also been reported that VEGF genetic polymorphisms may also be associated with CHD, including tetralogy of fallot (TOF) [12] and ventricular septal defect (VSD) [13].

Although a variety of VEGF gene polymorphisms have been reported, the most common are C2578A, G1154A, and G634C [14]. Until this point, many studies have focused on the association between VEGF genetic polymorphisms and CHD risk. Among these studies, two meta-analyses have been published [15,16]. Interestingly, Griffin [15] did not find any correlation between VEGF genetic polymorphisms and congenital cardiovascular malformation. However, this work only analyzed allelic polymorphisms, did not include genotypes and genotype haploid, and included no Chinese people in its sample population. Similarly, Li [16] showed that VEGF genetic polymorphisms increased CHD susceptibility, but used a sample population almost entirely of Caucasians. To better address the association between VEGF polymorphisms and CHD, we studied the mutations of VEGF C2578A, G1154A and G634C, which form different allele and genotype genetic unit type and their resulting correlation with CHD.

Materials and Methods

Identification and eligibility of relevant studies

We carried out an online search in PubMed and Web of Science databases for related articles published before March 31, 2016 using the following terms: "congenital heart defects or congenital heart diseases or heart, malformation of heart abnormalities or CHD" and "mutation or polymorphism or variation" and "vascular endothelial growth factor or VEGF". To expand the range of our studies, we also used the same terms in Chinese to search the Chinese National Knowledge Infrastructure (CNKI), Wangfang Database, and Chinese Biology medicine disc (CBM). References of the retrieved articles were also scanned for additional studies.

We included case-controls with human subjects that studied the relationship between VEGF C2578A, G1154A, and G634C mutations and CHD susceptibility in both English and Chinese languages. All phenotypes of CHD, including ventricular septal defect, patent formen ovale, atrial septal defect, patent ductus arteriosus, and coarctation of the aorta were included in this meta-analysis. However, CHD patients who had additional congenital, co-morbid anomalies such as Down syndrome were excluded [17]. Research articles utilizing animal subjects, reviews, commentaries, case reports, and unpublished reports were also excluded [18-20]. Studies that did not provide raw data of allele frequencies in the initial publications were excluded [12], though we attempted to obtain primary data by writing to the authors. Finally, when the research populations overlapped, we avoided repetition by including only the research with the broadest data set for the meta-analysis.

Data extraction

All data were collected independently by two authors (Zhang and Mo) and any discrepancy was resolved by a third co-author (Yu). The following information was collected or counted from each study: first author, year of publication, country of origin, ethnicity, type of CHD, number of cases and controls, counts of alleles in case and control groups in case-control studies, and Hardy-Weinberg equilibrium **(Tables 1-3)**.

Statistical analysis

STATA (version 11.0; StataCorp, College Station, Texas, USA) was used for meta-analysis. All gene models for the VEGF C2578A, G1154A, and G634C mutations were estimated. The existence of heterogeneity between studies was ascertained using a Q-statistic. The pooled odds ratio (OR) was evaluated with models based on either fixed or random effects assumptions. A random effects model was used if a significant Q statistic (P<0.1) indicated heterogeneity in the studies. In all other cases a fixed effects model was used. The 95% confidence interval (CI) of

Table 1 Characteristics of the included studies on VEGF C2578A polymorphism and congenital heart defects (CHD).

Reference	Country of origin	Ethnicity	Type of CHDs	Sample size (Case/Control)	Cases			Controls			HWE
					CC	CA	AA	CC	CA	AA	
Calderon (2009)	Chile	American	All types	61/61	14	32	15	16	33	12	0.50
Smedts et al. [24]	Netherland	European	All types	187/307	53	88	44	71	153	88	0.77
Xie et al. [13]	China	Asian	VSD	222/352	124	83	15	211	124	17	0.82
Stalmans et al. [22]	Germany	European	All types	58/316	12	28	18	97	157	62	0.91
Wang et al. [21]	China	Asian	All types	238/134	135	85	18	66	59	9	0.38
Gu [23]	China	Asian	All types	316/557	272	182	22	305	225	27	0.073

HWE: Hardy-Weinberg equilibrium; VSD: ventricular septal defect

Table 2 Characteristics of the included studies on VEGF G1154A polymorphism and congenital heart defects (CHD).

Reference	Country of origin	Ethnicity	Type of CHDs	Sample size (case/control)	Cases			Controls			HWE
					GG	GA	AA	GG	GA	AA	
Calderon (2009)	Chile	American	All types	61/61	29	26	6	37	19	5	0.27
Smedts et al. [24]	Netherland	European	All types	185/312	90	79	18	134	130	43	0.21
Xie et al. [13]	China	Asian	VSD	222/352	156	60	6	255	89	8	0.94
Stalmans et al. [22]	Germany	European	All types	58/316	16	32	10	152	132	32	0.67

HWE: Hardy-Weinberg equilibrium; VSD: Ventricular septal defect

Table 3 Characteristics of the included studies on VEGF G634C polymorphism and congenital heart defects (CHD).

Reference	Country of origin	Ethnicity	Type of CHDs	Sample size (case/control)	Cases			Controls			HWE
					CC	CA	AA	CC	CA	AA	
Calderon (2009)	Chile	American	All types	61/61	27	28	26	27	26	8	0.66
Smedts et al. [24]	Netherland	European	All types	184/303	77	85	22	131	133	39	0.57
Xie et al. [13]	China	Asian	VSD	222/352	78	118	26	68	181	103	0.47
Stalmans et al. [22]	Germany	European	All types	58/316	30	25	3	147	135	34	0.72
Wang et al. [21]	China	Asian	All types	240/135	90	116	34	50	63	22	0.77
Gu [23]	China	Asian	All types	476/557	153	248	75	183	283	91	0.29

HWE: Hardy-Weinberg equilibrium; VSD: ventricular septal defect

OR was also computed. The distributions of genetypes in the controls were checked for Hardy-Weinberg equilibrium. Begg's and Egger's tests were used to assess the publication bias and P<0.05 was considered as statistically significant. Funnel plots of the VEGF C2578A (for C versus A), G1154A (for G versus A), and G634C (for G versus C) were performed to look for evidence of publication bias. The funnel plot should be asymmetric in the case of publication bias and symmetric in the case of no publication bias.

Results

Characteristics of eligible studies

Figure 1 shows the literature retrieval and research selection processes. We found that 6 articles containing 1080 cases and 2289 controls were relevant to C2578A, 4 articles containing 528 cases and 1036 controls were relevant to G1154A, and 6 articles containing 1081 cases and 2281 controls were relevant to G634C. A total of 4 articles explored a single type of CHD [13,14,21,22], whereas various types of CHD were included in the other two articles [2,23]. Among them, three studies were carried out in China [13,21,24], one in Chile [14], one in Germany [22], and one in the Netherlands [24]. The distributions of the genotypes in the control groups were consistent with Hardy-Weinberg equilibrium (p>0.05) in all six studies in which the control groups were representative **(Tables 1-3)**.

Results of the meta-analysis

We accessed the full genotype distributions and observed that the VEGF C2578A polymorphism was unrelated to CHD in allelic comparisons (A *vs*. C: OR=1.016, 95% CI: 0.851, 1.214; $P_{heterogeneity}$=0.072), homozygote comparison (AA *vs*. CC: OR=1.048, 95% CI: 0.792, 1.386; $P_{heterogeneity}$=0.134, **Figure 2**), dominant model (AA/AC *vs*. CC: OR=0.950, 95% CI: 0.812, 1.112; $P_{heterogeneity}$=0.177), and recessive model (AA *vs*. AC/CC: OR=1.080, 95% CI: 0.842, 1.385; $P_{heterogeneity}$=0.314). Moreover, no significant associations were found in G1154A allelic comparison (A vs. G: OR=1.202, 95% CI: 0.837, 1.725; $P_{heterogeneity}$=0.011), homozygote comparison (AA *vs*. GG: OR=1.302, 95% CI: 0.596, 2.844; $P_{heterogeneity}$=0.036, **Figure 3**), dominant model (AA/AG *vs*. GG: OR=1.310, 95% CI: 0.843, 2.036; $P_{heterogeneity}$=0.020), and recessive model (AA *vs*. AG/GG OR=1.078, 95% CI: 0.630, 1.846; $P_{heterogeneity}$=0.196). We also found no relationship in G634C alleleic comparison with susceptibility to CHD (C vs. G: OR = 0.850, 95% CI: 0.659, 1.097; $P_{heterogeneity}$=0.000), homozygote comparison (CC *vs*. GG: OR=0. 633, 95% CI: 0.357, 1.123; $P_{heterogeneity}$=0. 000, **Figure 4**), dominant model (CC/CG *vs*. GG: OR=0.849, 95% CI: 0.629, 1.145; $P_{heterogeneity}$=0.009), and recessive model (CC *vs*. CG/GG: OR=0.675, 95% CI: 0.435, 1.046; $P_{heterogeneity}$=0.007).

Galbraith plot

We then created a Galbraith plot to graphically assess the sources of heterogeneity of VEGF G634C **(Figure 5)**. Only one study was identified as the main source of heterogeneity [8]. After the outlier study was excluded, we still did not find a connection in G634C allelic comparison and CHD (C *vs*. G: OR=0.979, 95% CI: 0.866, 1.108; $P_{heterogeneity}$=0.974), homozygote comparison (CC *vs*. GG: OR=0. 908, 95% CI: 0.694, 1.188; $P_{heterogeneity}$=0. 787 **Figure 6**), dominant model (CC/CG vs. GG: OR=1.004, 95% CI: 0.842, 1.197; $P_{heterogeneity}$=0.953), and recessive model (CC *vs*. CG/GG: OR=0.890, 95% CI: 0.696, 1.137; $P_{heterogeneity}$=0.817).

Publication bias

Publication bias was assessed using both a Begg's funnel plot and Egger's test. Begg's funnel plots are shown in **Figure 7** (P=0.536 for VEGF C2578A C allele *versus* A allele), **Figure 8** (P=1.000 for VEGF G1154A G allele *versus* A allele), and **Figure 9** (P=1.000 for VEGF G634C G allele versus C allele). Egger's test was then performed for to determine any publication bias (P=0.891 for A allele *versus* C allele, P=0.687 for A allele versus G allele, P=0.910 for C allele versus G allele). No publication biases were found.

Discussion

VEGF is located on chromosome 6q21. 33 and includes eight exons. Alternative splicing can result in several different protein isoforms [8]. Past work has shown that some of VEGF polymorphisms may be associated with differential VEGF expression *in vitro* [25]. Among these, polymorphisms in the VEGF promoter region (0.2578 C>A, rs 69994) and located on VEGFI exon 634 (G>C, rs 2010963) may be associated with the 3' noncoding region [26]. Other studies have also implicated these VEGF mutations disease states, including in CHD. For instance, Vannay et al. [6] found that VEGF polymorphism 634C (+ 405 c) increased the risk of CHD, while Lambrecht et al. [12] showed that VEGF haplotype 2578A/1154A/634G significantly reduced the risk of tetralogy of fallot (TOF), a form of congenital heart disease.

Griffin et al. [15] was the first to perform a study on the relationship between VEGF C2578A, G1154A and G634C polymorphisms and

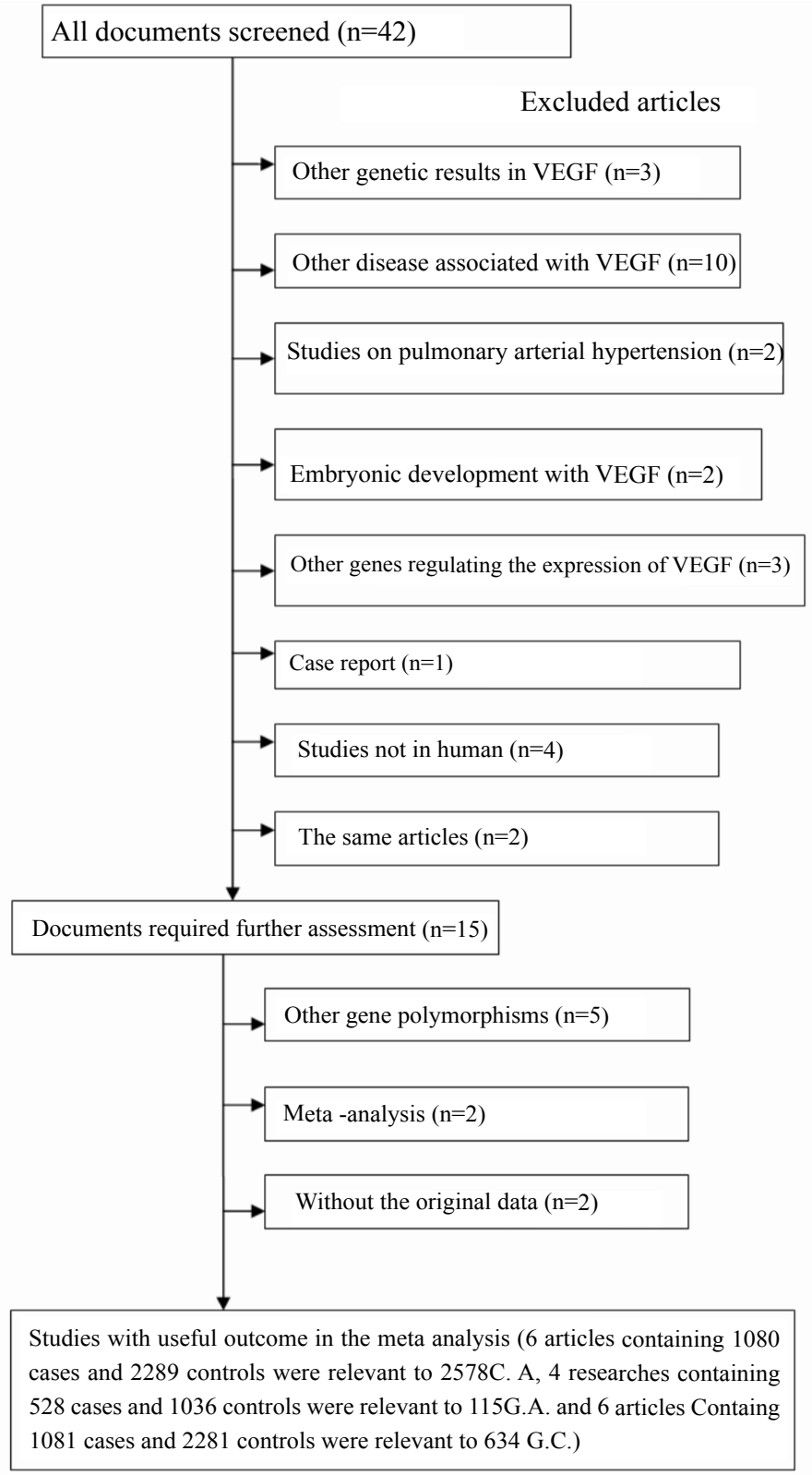

Figure 1 Flow chart of article screening and selection process.

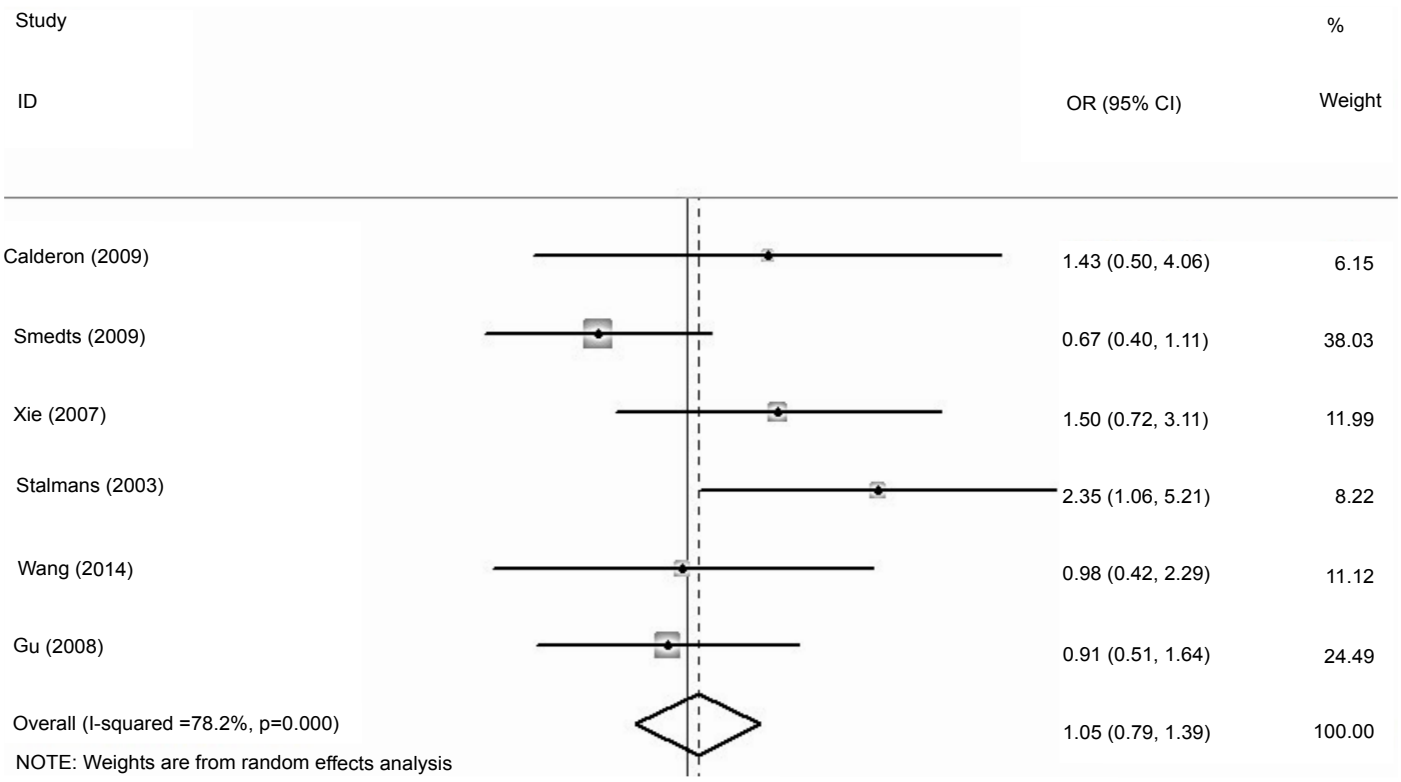

Figure 2 Forest plot of VEGF C2578A polymorphism associated with the CHDs (AA allele *vs.* CC allele)

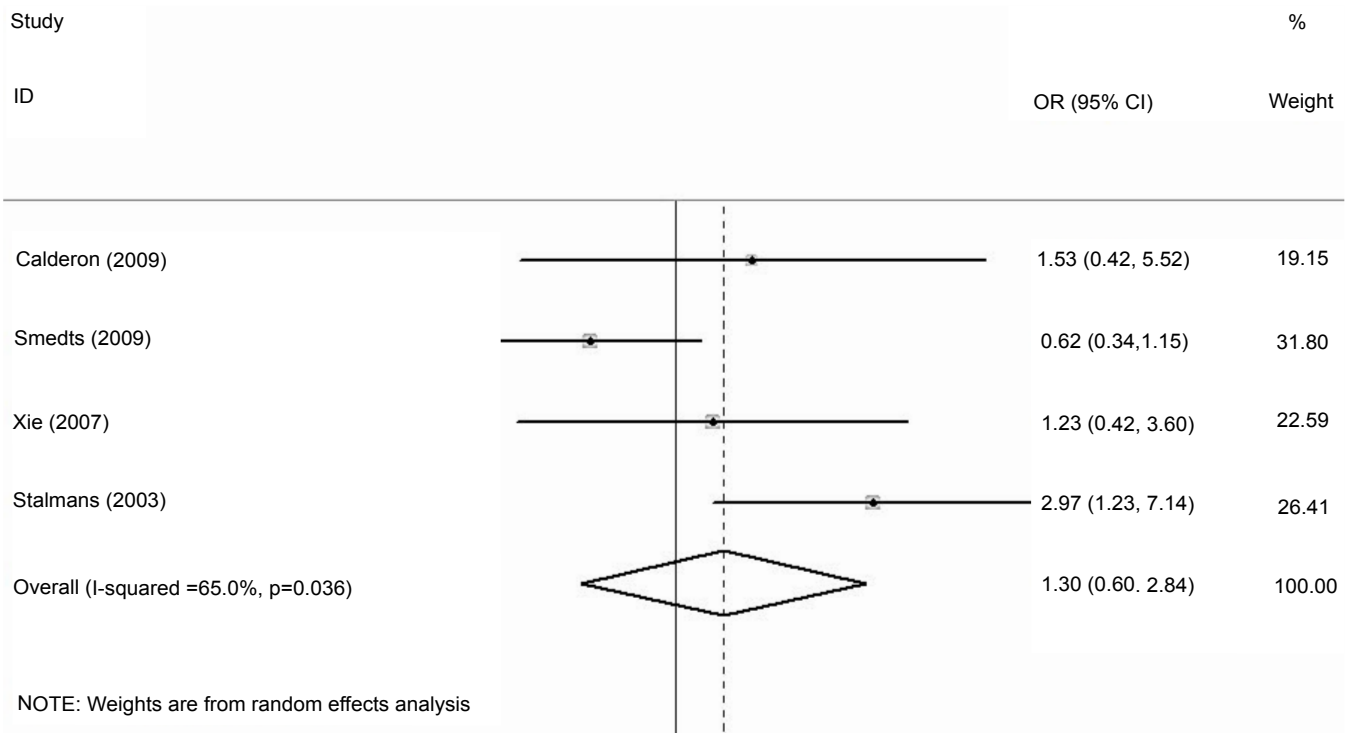

Figure 3 Forest plot of VEGF G1154A polymorphism associated with the CHDs (AA allele *vs*. GG allele).

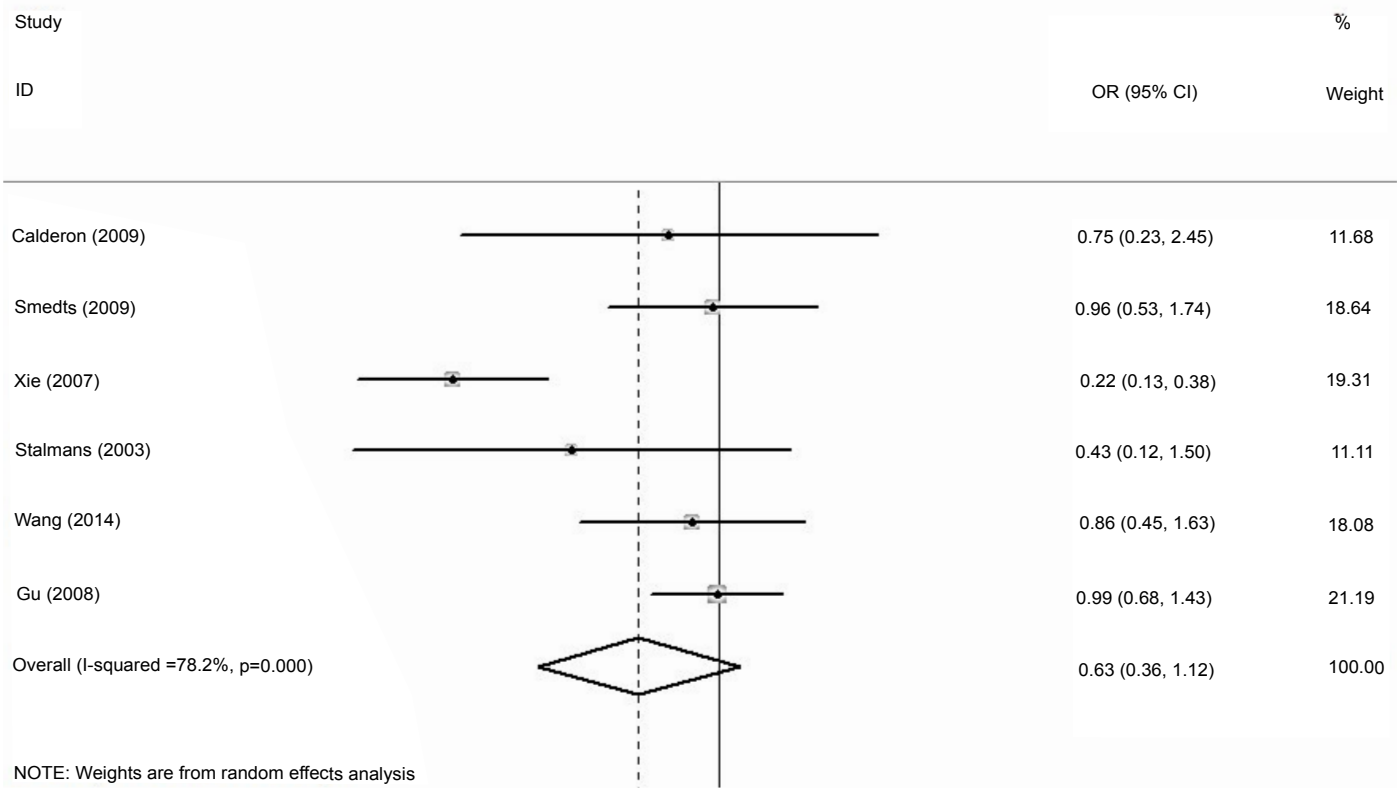

Figure 4 Forest plot of VEGF G634C polymorphism associated with the CHDs (CC allele *vs.* GG allele).

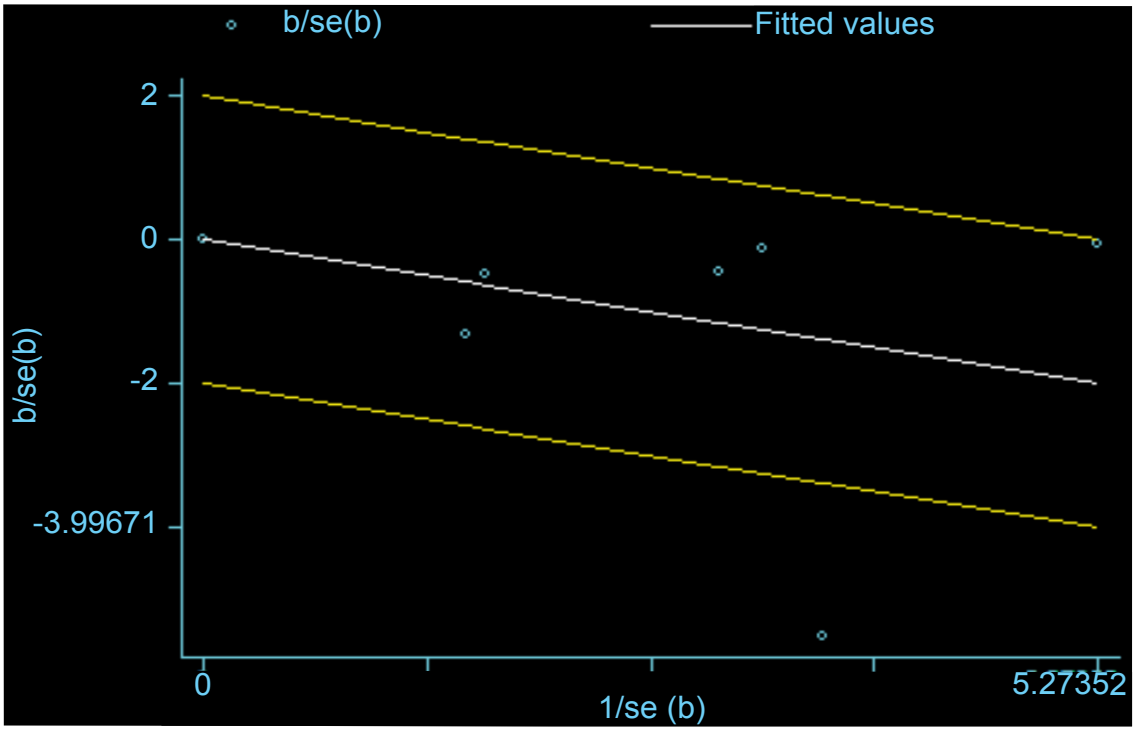

Figure 5 Galbraith plot of VEGF G634C polymorphism associated with the CHDs (CC allele *vs*. GG allele).

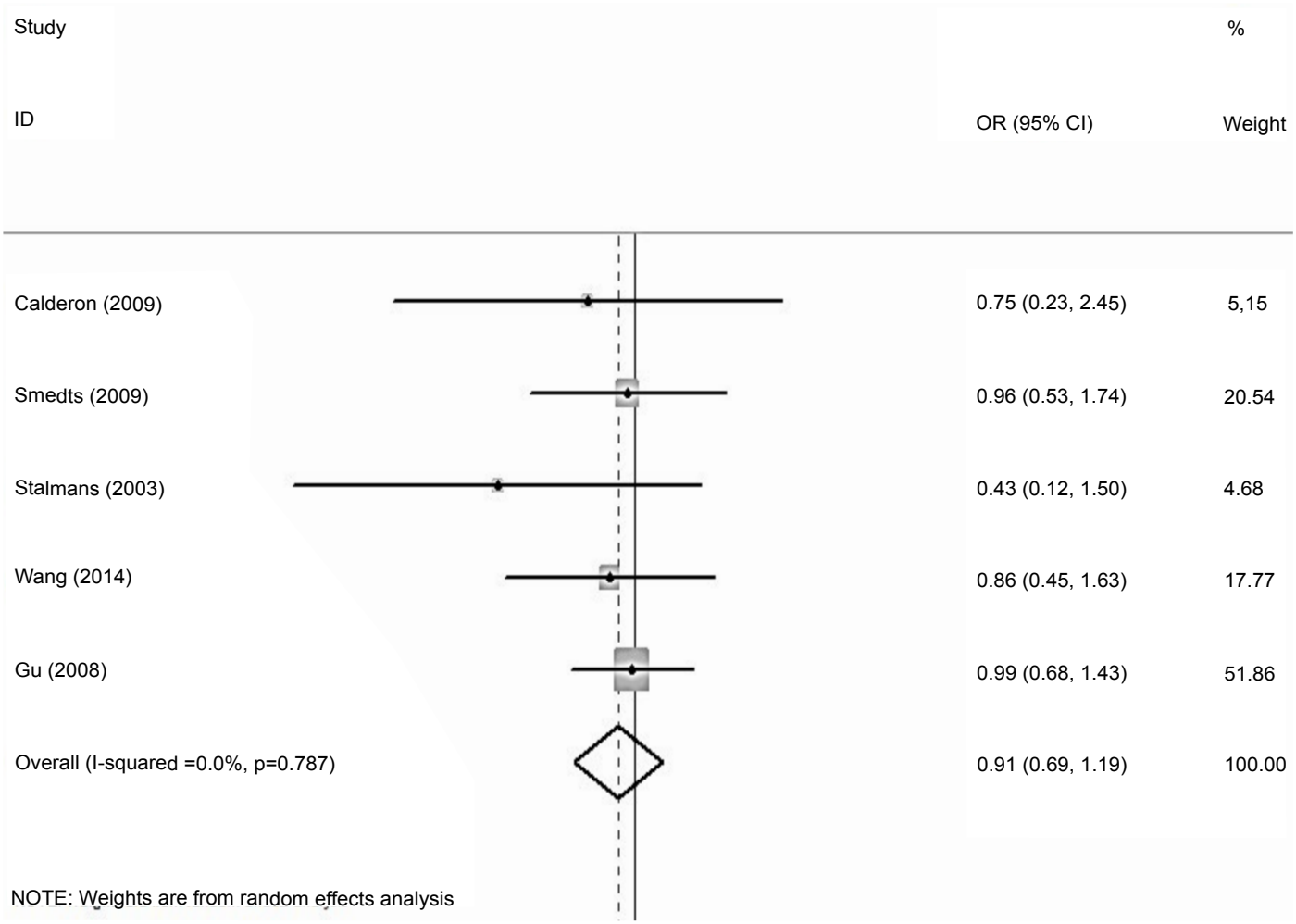

Figure 6 Forest plot of VEGF G634C polymorphism associated with the CHDs (CC allele *vs.* GG allele, after the outlier study were excluded).

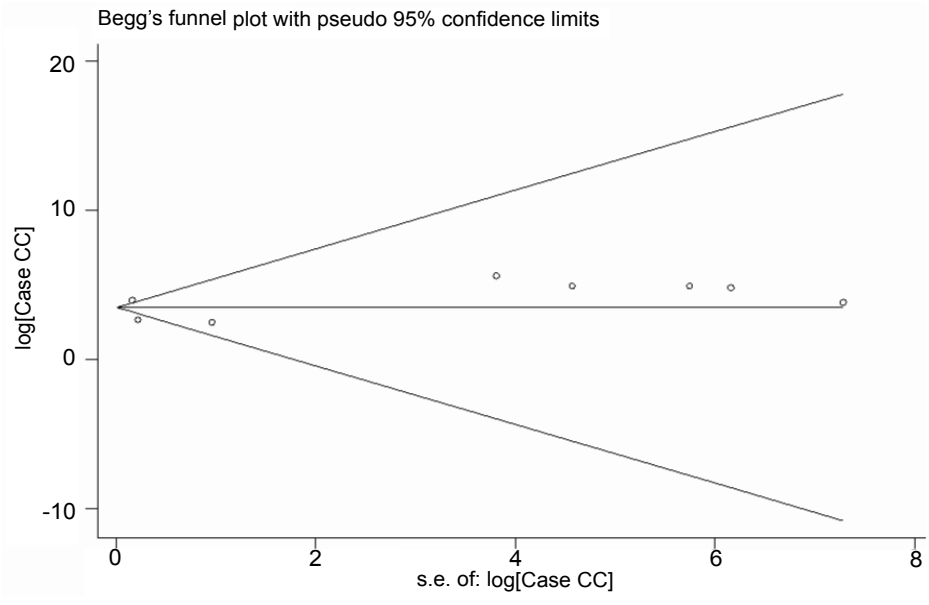

Figure 7 Funnel plot in the meta-analysis of the association between VEGF C2578A polymorphism and the risk of CHDs.

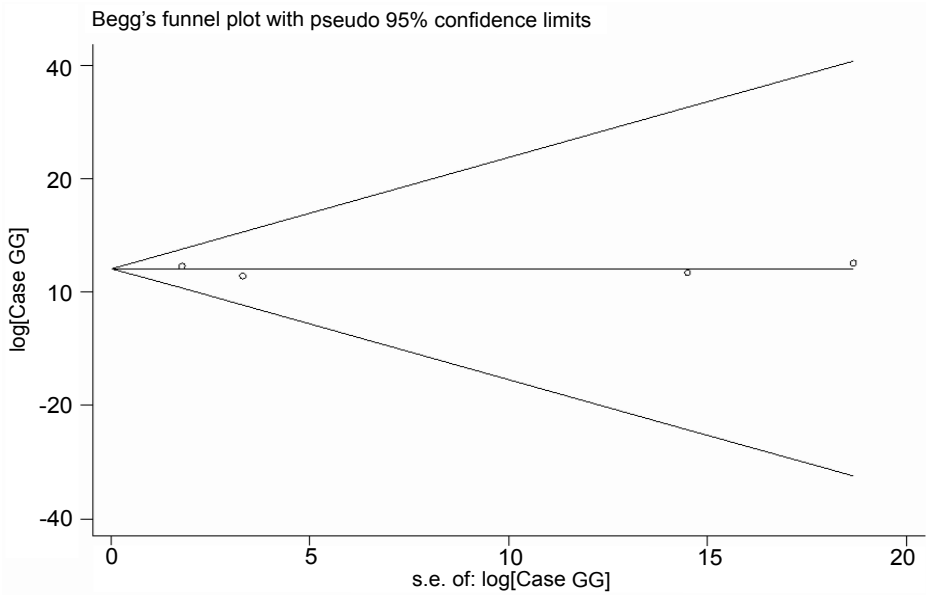

Figure 8 Funnel plot in the meta-analysis of the association between VEGF G1154A polymorphism and the risk of CHDs.

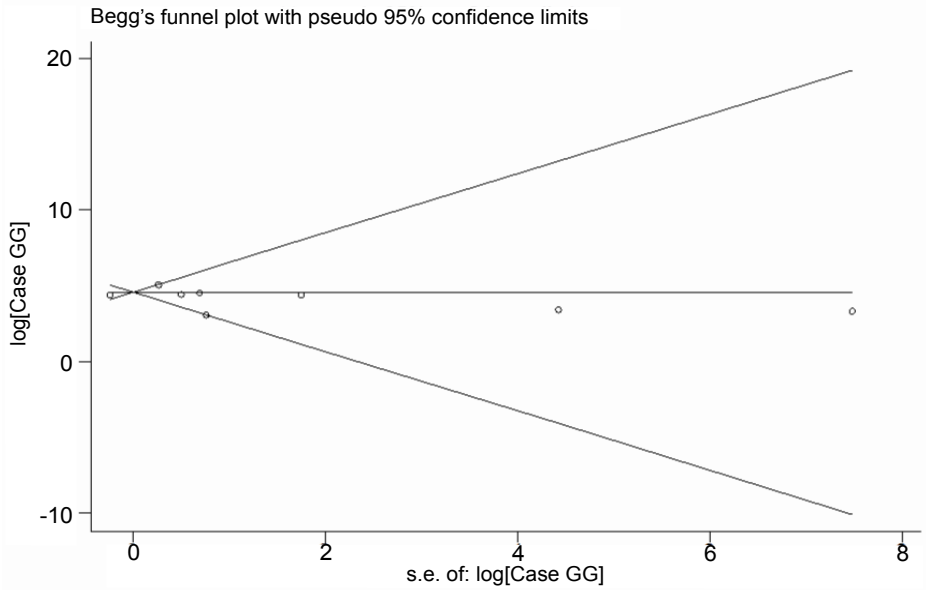

Figure 9 Funnel plot in the meta-analysis of the association between VEGF G634C polymorphism and the risk of CHDs.

the risk of CHD. The results of this work indicated that there was no relationship between these three polymorphisms and CHD. Li et al. [16] found that the allele, genotype, and haplotype of VEGF were identified with an association for susceptibility to CHD. Furthermore, that there were differences between CHD with or without DiGeorge syndrome; namely, that specific haplotypes (CGC) had significant protective effects for reducing the risk for CHD in a non-DiGeorge syndrome population. Given these discrepancies in the literature, the purpose of this meta-analysis was to assess whether or not there was an association between VEGF C2578A, G1154A, and G634C polymorphisms and the risk for CHD.

In this meta-analysis, our results showed that none of the VEGF C2578A, G1154A, and G634C polymorphisms were significantly associated with risk for CHD. However the studies of VEGF C2578A and G1154A polymorphisms were too few to sufficient exam heterogeneity. Therefore, we adopted a Galbraith plot to assess the sources of heterogeneity of VEGF G634C, and eliminated one study based on the result. We conducted our

statistical analysis again, but still failed to find a relationship between VEGF G634C polymorphism and CHD. One reason for this failure could be that only six studies were included in this analysis and that our statistical power was too low to allow for robust statistical conclusions. Moreover, there is significant difference in the genetic background, exposure to environmental factors, and risk factors in life styles between Asian, American, and European populations. Since our meta-analysis included all three of these populations, it is possible that this diversity masked any significant findings.

Future work will need to address some of the limitations present in this meta-analysis. First, all of the studies were carried out using only four different countries, including only Asian, American, and European populations. Second, most of the studies selected grouped all heart defects together. Uniform definitions and categories of CHDs might be needed in later investigations to parse out more specific genetic contributions to each type of CHD. Third, recent work has shown that peri-conceptional use of multivitamins containing folic acid can reduce the incidence of CHD [19,27]. As we did not assess the folate intake of the populations in question, it will be important to include this variable in future work as a potential factor in the prevention of CHDs. Finally, the influence of other environmental factors, such intrauterine infection as well as high doses of radioactive material and/or drugs should also be taken into consideration. Despite these limitations, our meta-analysis offers more evidence for the association (or lack thereof) between VEGF C2578A, G1154A, and G634C gene polymorphisms and the risk of CHD. Collectively, future studies using larger samples and better-matched controls will be needed to further confirm the findings from our meta-analysis.

Conclusion

This meta-analysis did not provide evidence for an association between VEGF C2578A, G1154A, and G634C genetic polymorphisms and CHD risk. These results do not support the hypothesis that VEGF C2578A, G1154A, and G634C polymorphisms may be a susceptibility marker of CHD. However, larger-sized sample studies will be needed in the future to validate our findings. Additionally, other factors such as plasma homocysteine levels, enzymatic activity, parental genotypes, and vitamin complex intake will also need to be included. Finally, more gene-gene and gene-environment interaction studies will be needed in future work, which should lead to a better and more comprehensive understanding of the association between VEGF polymorphisms and CHD risk.

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