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Association Between the VEGF Gene Polymorphism and Diabetic Retinopathy Risk: a Meta-Analysis of 4216 Individuals

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Abstract. Aim: This study aimed to evaluate the association between gene vascular endothelial growth factor (VEGF)-634C/G polymorphism and diabetic retinopathy. **Methods:** Case–control and cohort studies about the association between gene VEGF polymorphism and diabetic retinopathy (DR) were searched on the Internet from the English and Chinese databases. Heterogeneity analysis was performed on the included studies using the *Q* test. RevMan5.3 was used to perform the meta-analysis. The outcomes across included studies were performed using fixed-effects and/or random-effects models.. Publication bias was assessed using a funnel plot. **Results:** A total of 14 studies were screened, and 2418 cases were found to have DR and 1798 case–controls had diabetes without retinopathy. The total odds ratio (OR) of gene VEGF-634C/G risk allele (C vs G) was 1.09 [95% confidence interval (CI) (0.90–1.31)], $P = 0.40$, $P_{(\text{heterogeneity})}<0.00001$; the total OR of genotype CC vs CG + GG was 1.28 [95% CI (0.97–1.68)], $P = 0.08$, $P_{(\text{heterogeneity})} = 0.01$; the total OR of CG vs CC + GG was 1.04 [95% CI (0.92–1.18)], $P = 0.55$, $P_{(\text{heterogeneity})} = 0.22$. **Conclusion:** No statistical significance existed between gene VEGT 634C/G and diabetic retinopathy.

Introduction

The basic symptoms of diabetic retinopathy (DR), the severest capillary complication due to type 2diabetes mellitus, are impaired blood–retinal barrier (BRB), leakage of retinal vessel, nonperfusion of retinal vasculature, and neovascularization. Its incidence is 30%–60%. DR is harmful to human health and the main ophthalmopathy leading to blindness. The single-nucleotide polymorphism (SNP) of gene vascular endothelial growth factor (VEGF) can change gene transcription and translation. Moreover, it affects protein expression, leading to an individual difference in susceptibility to some diseases. DR has been studied at the genetic and molecular levels in the last decade with the application of sequencing technique of human genome project and the study of SNP and sequence-specific oligonucleotide probe. Following the research on human genome project, studying DR pathogenesis at the genetic level has become a breakthrough point to prevent and treat DR. At present, a number of study reports exist about gene VEGF-634C/G polymorphism and DR. However, patients with DR show heterogeneity because of different ethnicities and different areas. Every study result shows diversification; even some studies yielded contrast conclusion.

Therefore, a meta-analysis was performed to comprehensively evaluate quantitatively the former literature about the relationship between this risk locus and DR, to gain more reliable evidence of the connection between gene VEGF-634C/G polymorphism and DR and provide evidence-based medicine-proof for the future research of the association between gene VEGF and DR.

Methods

Literature retrieval Case–control and cohort studies about the relationship between gene VEGF-634C/G polymorphism and DR were retrieved by searching the following English and Chinese databases on the Internet: PubMed, Springer Link, WANG journal, and CNKI. The literature was comprehensively and systematically retrieved using the key words "vascular endothelial growth factor," "diabetic retinopathy," and "gene polymorphism" in Chinese and "VEGF," "DR," and "SNP" in English, and tracing relative references. Retrieving deadline was August 26, 2017.

Inclusion and exclusion criteria (1) Studies were included if research types were case–control study and cohort study; (2), participants were randomly selected without limiting to age, gender, and family history; (3) each study calculated odds ratio (OR) and 95% confidence interval (CI); (4) for one author, only one study of higher quality in a different language was included.

Literature was selected based on the inclusion and exclusion criteria, and the quality was evaluated. DR was considered as a case group and diabetic without retinopathy (DWR) as a control group. Genotype frequency of the two groups was considered as statistical data, and the difference in the genotype distribution and allele frequency of VEGF-634C/G polymorphism of the DR and DWR groups was systematically assessed.

Data extraction and statistical analysis Extraction and reorganization of data: After including the relevant studies, the standard information was extracted. Then, a database was established by collating data and filling the meta-analysis information table (Table 1), according to the primary literature date (OR and 95% CI).

Statistical software and procedures RevMan5.3 was used to perform meta- analysis after filling the data in the Excel sheet. The heterogeneity of the included literature was performed using the O test and I^2 statistics. In the case of no significant heterogeneity, Mantel–Haenszel fixed-effects model (peto) was used to combine data and calculate OR and 95% CI. In the case of significant heterogeneity among the included studies, derSimonian–Laird random-effects model (D-L) was used; a funnel plot was used to evaluate the publication bias of the included literature.

Results

Basic information of literature A total of 31 studies were searched at primary retrieval: 8 in Chinese and 23 in English. After screening all the literature, 14 case–control studies about the relationship between geneVEGF-634C/G polymorphism and DR, 2418 DR cases, and 1798 DWR controls were selected.

Table 1. Basic information of the relationship between gene VEGF-34C/G polymorphism and DR from the studies included in meta-analysis.

Author	Time	Group	Sample	Allele frequency	OR [95% CI]	Genotype frequency CC/ CG/ GG
Awata[2]	2002	DR+	176	0.477	1.68 [1.18–2.38]	0.27/0.54/0.253
		DR–	118	0.352		0.103/0.5/0.398
Awata[3]	2005	DR+	175	0.477	1.45 [1.08–1.93]	0.217/0.52/0.263
		DR–	203	0.387		0.148/0.468/0.369
Suganthalakshmi[4]	2006	DR+	120	0.5833	0.89 [0.60–1.32]	0.175/0.808/0.017
		DR–	90	0.6111		0.3/0.644/0.056
Buraczynska[5]	2007	DR+	195	0.32	1.06 [0.73–1.55]	0.1/0.45/0.45
		DR–	91	0.31		0.09/0.46/0.45
Szaflik[6]	2008	DR+	148	0.58	1.39 [0.90–2.13]	0.24/0.68/0.09
		DR–	58	0.5		0.1/0.8/0.1
Globočnik[7]	2008	DR+	206	0.367	1.13 [0.82–1.55]	0.116/0.5/0.384
		DR–	143	0.339		0.105/0.469/0.426
Uthra[8]	2009	DR+	131	0.288	1.07 [0.69–1.68]	0.075/0.425/:0.5
		DR–	82	0.259		0.076/0.367/0.557
Kontio[9]	2009	DR+	126	0.22	0.95 [0.62–1.47]	0.6/0.32/0.61
		DR–	96	0.26		0.5/0.41/0.54/0.26
Nakamura[10]	2009	DR+	176	0.418	0.85 [0.65–1.11]	0.19/0.45/0.36
		DR–	289	0.457		0.20/0.51/0.29
Chun[11]	2010	DR+	253	0.417	1.00 [0.70–1.43]	0.164/0.515/0.321
		DR–	134	0.422		0.181/0.485/0.335
Yangying[12]	2010	DR+	176	0.38	0.95 [0.68–1.34]	0.15/0.47/0.38
		DR–	109	0.30		0.05/0.5/0.45
Yang[13]	2011	DR+	129	0.434	1.46 [1.02–2.10]	0.147/0.574/0.279
		DR–	139	0.453		0.19/0.285/0.453
Yazen F[14]	2012	DR+	232	0.466	0.43 [0.30–0.61]	0.233/0.466/0.302
		DR–	144	0.438		0.215/0.444/0.34
Choudhuri1[15]	2014	DR+	175	0.311	1.74 [1.16–2.62]	0.12/0.383/0.497
		DR–	102	0.206		0.039/0.333/0.626

Heterogeneity analysis Heterogeneity test results of gene VEGF-634C/G allele C/G, genotype CC vs CG + GG, and CG vs CC + GG are presented in Table 2. Allele C vs G ($P < 0.00001$) and genotype CC vs CG + GG ($P = 0.01$) had heterogeneity, but genotype CG vs CC + GG had no heterogeneity ($P = 0.22$)

Table 2. Heterogeneity test results of geneVEGF-634C/G.

	Q	I^2	*P*
C vs G	50.46	74%	<0.00001
CC vs CG + GG	26.58	51%	0.01
CG vs CC + GG	16.62	22%	0.22

Association between gene VEGF-634C/G polymorphism and DR According to the heterogeneity analysis, the random-effects model was used to calculate the OR (95% CI) of C allele and genotype CC vs CG + GG. The OR (95% CI) of allele C was 1.09 (0.90–1.31), illustrating no statistically significant association between allele C and DR ($Z = 0.85$, $P = 0.40$) (Figure 1). The OR (95% CI) of genotype CC vs CG + GG was 1.28 (0.97–1.68), illustrating no statistically significant association between CC vs CG + GG and DR ($Z = 1.75$, $P = 0.08$) (Figure 2). The fixed-effects model was used for studies showing no heterogeneity in every research of genotype CG vs CC + GG, t The OR (95% CI) was 1.04 (0.92–1.18), and no statistically significant association existed between genotype CG vs CC + GG ($Z = 0.60$, $P = 0.55$) (Figure 3).

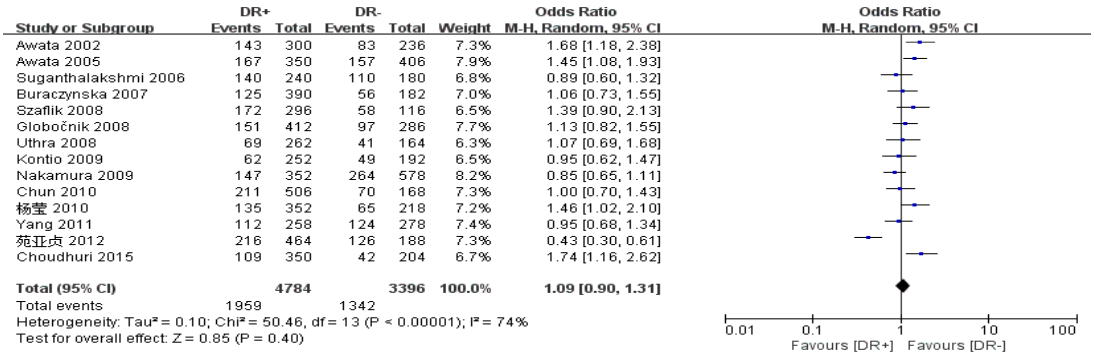


Figure 1. Forest plot of meta-analysis of the association between VEGF-634C/G risk allele (C vs G) and DR.

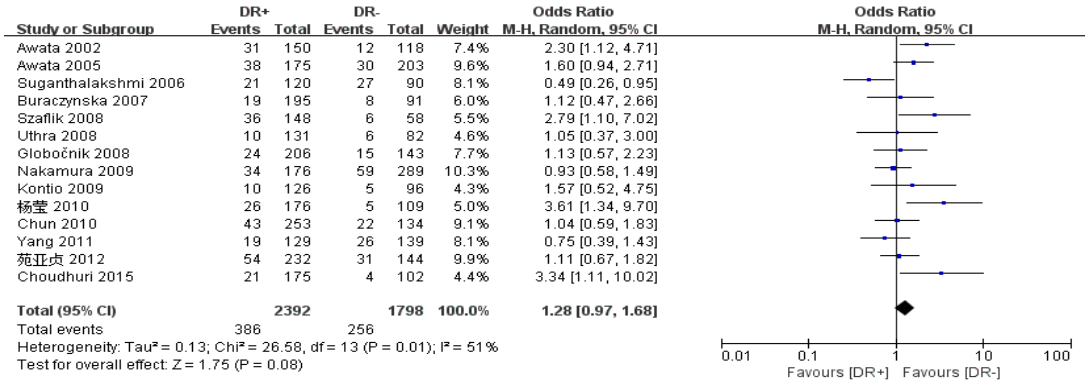


Figure 2. Forest plot of meta-analysis of the association between VEGF-634C/G genotype (CC vs CG + GG) and DR.

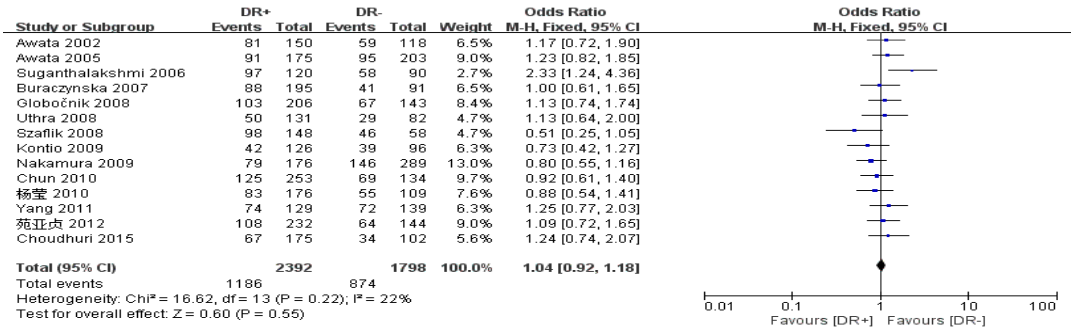
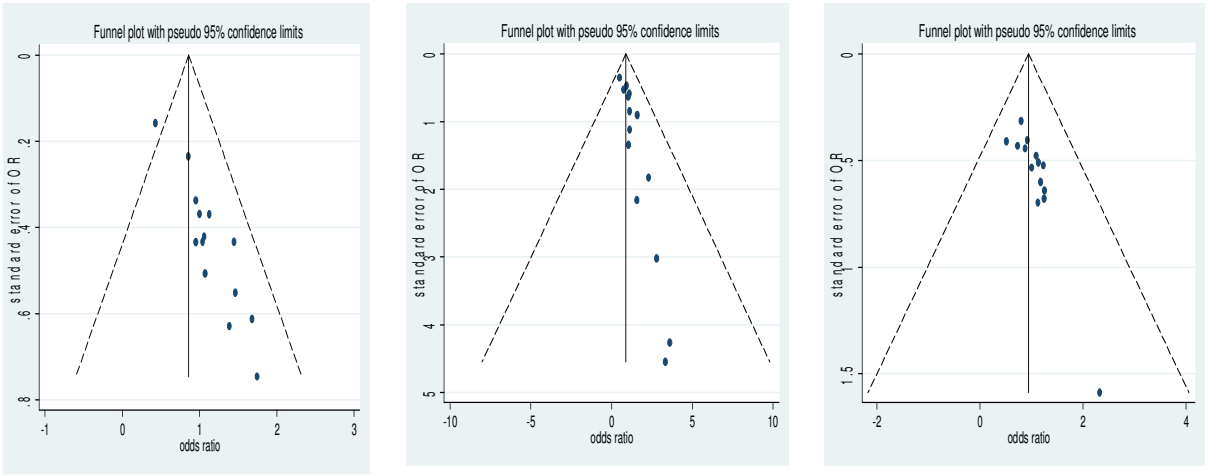


Figure 3. Forest plot of meta-analysis of the association between VEGF-634C/G genotype (CG vs CC + GG) and DR.

Publication bias With OR of every study as horizontal axis and $1/SE_{OR}$ as vertical axis, every circle of the funnel plot represented one independent research. The funnel plot for ORs was displayed from the research funnel plot of the association between gene VEGF-634C/G allele (C/G) (Fig. 4A), genotype (CC/CG + GG) (Fig. 4B), genotype (CG/CC + GG) (Fig. 4C), and DR, and no evidence of obvious asymmetry was observed.



A B C

Figure 4. Funnel plot analyses for odds ratios (ORs). Panel A, funnel plot analysis for ORs of allele C compared with those of allele G in the studies analyzed. Panel B, funnel plot analysis for ORs of genotype CC compared with those of genotype CG + GG in the studies analyzed. Panel C, funnel plot analysis for ORs of genotype CG compared with those of genotype CC + GG in the studies analyzed.

Discussion

DR is the severest chronic microangiopathy of diabetic ophthalmopathy[1, 2], the main reason for blindness in humans[3]**.** VEGF, the strongest and most direct and distinct endotheliocyte selective mitosis-promoting factor and pro-angiogenesis factor, is considered as the main factor of demagogue BRB [4]. It plays an important role in the occurrence and development of DR. Gene VEGF is located on chromosome 6p21.3; its coding area contains eight exons and seven introns[5]**.** The gene that codes VEGF is 14 kb long and belongs to the platelet-derived growth factor family [6].

VEGF has been found to be the closest gene to SNP in DR-specific gene research[7]. SNP-634C is the most researchful loci. In 2002, Awata[8] team selected 268 diabetic cases [118 cases without DR, 80 non-proliferative diabetic retinopathy (NPDR), and 70 proliferative diabetic retinopathy (PDR)] from the Japanese population. After studying the polymorphism of seven loci of VEGF, it was found that only genotype CC of -634C/G loci had a significant association with DR. Thus, VEGF5'-untranslated region-634C/G polymorphism is an independent risk factor in DR. However, further studies on the association yielded similar[9-12] and opposite results[13-21]. Even in one country without ethnic diversity, different results were obtained. Therefore, the degree of gene VEGF-634C/G polymorphism and its association with DR were systematically analyzed using evidence-based medicine at allele and genotype levels in the present study. A total of 14 case–control studies were retrieved from various populations: three cases each from China[10, 11, 20], India[12, 13, 17], and Japan[8, 9, 18]; two cases from Portland[14, 16]; and one case each from Slovenia[15], Finland[19], and Korea[20]. The meta-analysis showed that -634C/G polymorphism C allele and genotypes CC vs CG + GG and CG vs CC + GG had no statistically significant association with DR. The result further verified most former results [13-21].

Meta-analysis is a summary analysis method that can combine various similar research results. In statistics, this analysis can increase sample size and improve effectiveness, especially when various researches yield different or not statistically significant results. Meta-analysis can yield closest comprehensive analysis results. Case–control study literature about the relationship between gene VEGF-634C/G polymorphism and DR was screened, literature quality was analyzed, heterogeneity test was performed, a suitable model was chosen, and then the relationship was comprehensively analyzed in the present study. However, this meta-analysis had some limitations. (1) Only 14 cases were selected; limited number of samples would have affected the analysis result. (2) As this meta-analysis screened only Chinese and English literature, the exclusion of literature in other languages would have limited the results. (3) The longer the course of diabetes, the more the chances of DR. However, the relationship between the patient course of diabetes and DR was not analyzed, and hence this meta-analysis result might have errors. (4), Primary data came from different laboratories. Hence, different test techniques, test levels, and other factors would have affected the results of this meta-analysis, leading to research bias.

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