

Original article

Association between VEGF polymorphisms (–460 T/C and +936 C/T) and retinopathy of prematurity risk: A meta-analysis

Swati Shukla^{a,1}; Manzoor Ahmad Malik^{a,1}; Parijat Chandra^b; Jasbir Kaur^{a,*}**Abstract**

Purpose: Vascular endothelial growth factor (VEGF) contributes to the development of retinopathy of prematurity (ROP). A number of studies investigated the association of ROP with VEGF –460 T/C and +936 C/T polymorphisms but the results were conflicting. In order to derive a more precise estimation of the associations, we performed a meta-analysis of the relationship between VEGF –460 T/C and +936 C/T polymorphisms with ROP in all published studies.

Methods: A literature search was performed systematically using electronic databases. Published literature from PubMed and other databases was retrieved. The odds ratio (OR) with 95% confidence interval (CI) was used to estimate the pooled effect. Each –460 T/C and +936 C/T polymorphism included four case-control studies including case/control 249/308 and 179/250 respectively.

Results: Through literature search, we found that both VEGF –460 T/C and +936 C/T polymorphisms were not associated with ROP risk at allelic, co-dominant, dominant and recessive models.

Conclusions: This meta-analysis suggests that the VEGF –460 T/C and +936 C/T polymorphism might contribute to genetic susceptibility of ROP. The association between VEGF –460 T/C and +936 C/T polymorphism and ROP risk awaits further investigation.

Keywords: VEGF, ROP, Polymorphism, Meta-analysis

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Introduction

Retinopathy of prematurity (ROP) is a disorder in which retinal blood vessels fail to develop normally, sometimes resulting in visual impairment and blindness in premature infants. In the early stages of retinal vascular development, premature neonates are exposed to higher oxygen levels eliminating physiological hypoxia, thus down-regulating angiogenic factors that are required for the growth of the

vasculature. ROP occurs in premature infants with disruption of this angiogenic phase. Vascular endothelial growth factor (VEGF) plays an important role in both physiological and pathologic angiogenesis. Discovery of growth factors acting on the vascular endothelium has coincided with application of powerful new genetic approaches to the problem of vascular development. A number of studies have investigated and observed that VEGF messenger ribonucleic acid (mRNA) and protein were significantly higher in ROP, which supported

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a key role for VEGF in the pathological angiogenesis in ROP.^{1,2}

The human VEGF gene (OMIM 192240) is located on chromosome 6p12. Many polymorphisms of the VEGF gene have been reported, although most are relatively rare. Single nucleotide polymorphisms (SNPs) in the VEGF -460 T/C (rs833061) and +936 C/T (rs3025039) in the respective 5' and 3' untranslated region (UTR) have been reported in different populations.³ Some studies have found association of VEGF -460 T/C or +936 C/T polymorphism with ROP risk,⁴⁻⁶ but some other studies show no association between VEGF -460 T/C or +936 C/T polymorphism and risk of ROP.⁷⁻¹¹ These studies revealed a conflicting conclusion, probably due to the relatively small size of subjects, since individual studies are usually underpowered in detecting the effect of low penetrance genes. Therefore, in this study we conducted a meta-analysis to investigate the association between VEGF -460 T/C and +936 C/T polymorphism and the risk for ROP.

Materials and methods

Identification and eligibility of relevant studies

To identify all articles that examined the association of VEGF -460 T/C and +936 C/T polymorphism with ROP, we conducted a literature search in the PubMed, EMBASE, Cochrane Library, Google, dogpile and CBM database, up to July 2015 using the following terms and keywords: "VEGF", "vascular endothelial growth factor", "-460 T/C", "rs833061", "+936 C/T", "rs3025039" polymorphism" and "retinopathy of prematurity". Additional studies were identified by a manual search from other sources (e.g., Web of Knowledge), references of original studies or review articles on this topic.

Inclusion and exclusion criteria

The inclusion criteria were as follows: (1) studies are limited to VEGF -460 T/C and +936 C/T polymorphism and ROP; (2) Independent case-control studies using either a hospital-based or a population based design; (3) complete data with genotype and allele frequencies; (4) the literature having a comprehensive statistical index, sufficient data for estimating an odds ratio (OR) with 95% confidence interval (CI); (5) the genotype frequency of cases and controls was within Hardy-Weinberg equilibrium (HWE); (6) papers published in English and (7) all the studies included were according to tenets of the Declaration of Helsinki.

The major reasons for exclusion of studies were (1) studies which were not possible to extract data from the published results; (2) studies that did not report appropriate outcomes; (3) Duplicated studies were also excluded; (4) case-only studies; (5) all three genotype frequency missing and (6) family based studies.

Data extraction

Authors independently reviewed all the potentially relevant papers through assessing the eligibility of each article and abstracting data with standardized data-abstraction forms. For each study, the following information was

extracted: name of the first author; publication year; ethnicity; sample size; types of disease; sources of samples; genotyping methods; and the minor allele frequency in the controls with the Hardy-Weinberg Equilibrium (HWE) *p*-value, respectively. Disagreements were resolved through discussion. The characteristics of these studies included in this meta-analysis on the association of ROP with VEGF -460 T/C and +936 C/T polymorphism are shown in [Tables 1 and 2](#). The VEGF -460 T/C and +936 C/T polymorphism genotype distributions from each study is presented in [Tables 3 and 4](#) respectively.

Statistical analysis

ORs and relevant 95% CIs were computed using review manager (comprehensive meta-analysis version 3).¹² ORs were used to measure association across the studies. The risk of VEGF -460 T/C CC genotype on retinopathy of prematurity was evaluated by comparing with their reference wild type homozygote and then evaluated the risks of TC + CC vs. TT and TT + TC vs. CC on retinopathy of prematurity, assuming dominant and recessive effects of the variant C allele, respectively. And the risk of VEGF + 936 C/T and TT genotype on retinopathy of prematurity was evaluated by comparing with their reference wild type homozygote and then evaluated the risks of CT + TT vs. CC and CC + CT vs. TT on retinopathy of prematurity, assuming dominant and recessive effects of the variant T allele, respectively. If moderate or high level heterogeneity exists, a random-effects meta-analysis was performed, unless using fixed-effects models. Publication bias was assessed by visually inspecting a funnel plot. A *p* value less than 0.05 was considered statistically significant.¹³

Results

Association of the VEGF -460 T/C polymorphism with ROP

This meta-analysis included four eligible studies of the association of the VEGF -460 T/C polymorphism with ROP. The genetic models all used fixed-effects models. In the overall analysis, no significant associations were found in any of the comparison models including the allele model: T vs. C (OR = 0.931, 95% CI = 0.726–1.195, *P* = 0.576) ([Fig. 1](#)), dominant model: TC + CC vs. TT (OR = 0.895, 95% CI = 0.629–1.273, *P* = 0.536) ([Fig. 2](#)), recessive model: TT + TC vs. CC (OR = 1.048, 95% CI = 0.646–1.699, *P* = 0.849) ([Fig. 3](#)) and co-dominant model: TT vs. CC (OR = 0.911, 95% CI = 0.537–1.544, *P* = 0.729) ([Fig. 4](#)).

Association of the VEGF +936 C/T polymorphism with ROP

A total of four studies were included in this meta-analysis. No significant associations between the VEGF +936 C/T polymorphism with ROP were identified in any comparison models, including the allele model: C vs. T (OR = 0.882, 95% CI = 0.610–1.274, *P* = 0.502) ([Fig. 5](#)), dominant model: CT + TT vs. CC (OR = 0.866, 95% CI = 0.558–1.344, *P* = 0.521) ([Fig. 6](#)), recessive model: CC + CT vs. TT (OR = 1.139, 95% CI = 0.487–2.665, *P* = 0.764) ([Fig. 7](#)) and

Table 1. Characteristics of studies included in the VEGF –460 T/C (rs 833061) meta-analysis.

Study	Year	Country	Ethnicity	Genotyping method	Sample	Case	Control	p-Value	References
Vannay et al.	2005	Hungary	Caucasian	Real-time PCR	Blood	86	115	–	PMID: 15635051
Shastry et al.	2007	USA	Caucasian	PCR-RFLP, Sequencing	Blood	61	61	0.892	PMID: 17119993
Kwinta et al.	2008	America	Caucasian	PCR-RFLP	Blood	60	101	0.024	PMID: 18546007
Kaya et al.	2013	Turkey	Asian	PCR-RFLP	Blood	42	31	0.87	PMID: 23094709

Table 2. Characteristics of studies included in the VEGF +936 C/T (rs 3025039) meta-analysis.

Study	Year	Country	Ethnicity	Genotyping method	Sample	Case	Control	p-Value	References
Cooke et al.	2004	United Kingdom	Caucasian	PCR, SSCP, RFLP	Buccal swab	91	97	0.15	PMID: 15161830
Yagi et al.	2011	Japan	Asian	Taq Man	Buccal mucosa	30	34	0.012	–
Kalmeh et al.	2013	Iran	Asian	PCR-RFLP	Blood	15	66	0.65	PMID: 23644986
Gismondi et al.	2013	USA	Caucasian	PCR	Blood	43	53	0.11	PMID: 22227643

Table 3. VEGF –460 T/C (rs833061) polymorphism genotype distribution of each study included in the meta-analysis.

Author/Year	Genotype frequency						Allele frequency				Dominant model				Recessive model			
	Cases			Controls			Cases		Controls		Cases		Controls		Cases		Controls	
	TT	TC	CC	TT	TC	CC	T	C	T	C	TT	TC + CC	TT	TC + CC	CC	TT + TC	CC	TT + TC
Vannay et al. (2005)	27	47	12	28	61	26	101	71	117	113	27	59	28	87	12	74	26	89
Shastry et al. (2007)	27	27	7	28	26	7	81	41	82	40	27	34	28	33	7	54	7	54
Kwinta et al. (2008)	32	18	10	55	36	10	82	38	146	56	32	28	55	46	10	50	10	91
Kaya et al. (2013)	17	18	7	11	16	4	52	32	38	24	17	25	11	20	7	35	4	27

Table 4. VEGF +936 C/T (rs3025039) polymorphism genotype distribution of each study included in the meta-analysis.

Author/Year	Genotype frequency						Allele frequency				Dominant model				Recessive model			
	Cases			Controls			Cases		Controls		Cases		Controls		Cases		Controls	
	CC	CT	TT	CC	CT	TT	C	T	C	T	CC	CT + TT	CC	CT + TT	TT	CC + CT	TT	CC + CT
Cooke et al. (2004)	67	19	5	68	21	8	153	29	157	37	67	24	68	29	5	86	8	89
Yagi et al. (2007)	14	14	2	27	7	0	42	18	61	7	14	16	27	7	2	28	0	34
Kalmeh et al. (2013)	12	1	2	43	15	8	25	5	101	31	12	3	43	23	2	13	8	58
Gismondi et al. (2013)	31	11	1	28	23	2	73	13	79	27	31	12	28	25	1	42	2	51

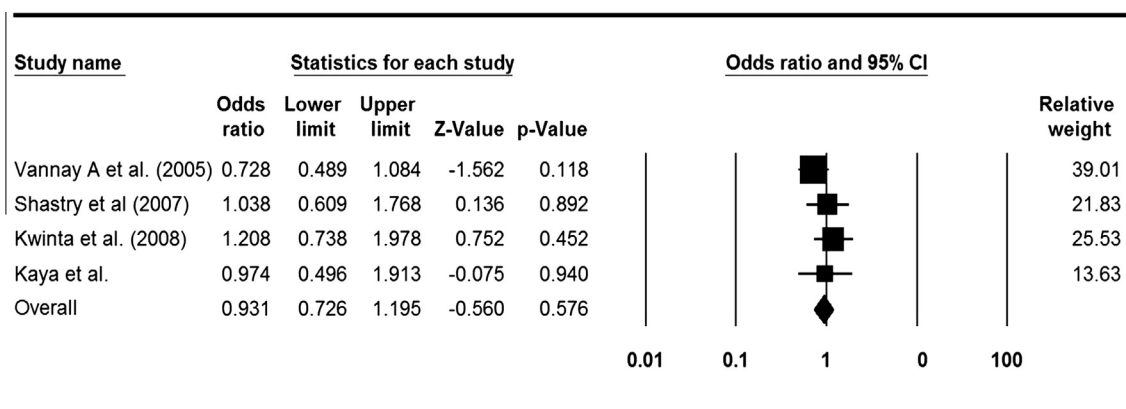


Figure 1. Meta-analysis under allelic model (T vs. C) for the association between ROP risk and the VEGF –460 T/C polymorphism. The squares and horizontal lines correspond to OR and 95% CI of specific study, and the area of squares reflects study weight (inverse of the variance). The diamond represents the pooled OR and its 95% CI.

co-dominant model: CC vs. TT (OR = 0.819, 95% CI = 0.347–1.931, P = 0.648) (Fig. 8).

Sensitivity analysis and publication bias

Individual studies were consecutively excluded in the sensitivity analysis to investigate whether the obtained results

were robust. The analysis showed that the results obtained in the meta-analysis were statistically robust, because the corresponding combined ORs in all of the separate subgroup analyses were relatively stable when deleting any individual study. The influence of CC genotype at –460 T/C locus on ROP was taken as the analysis index and inverted funnel plot was drawn (Fig. 9); TT genotype at +936 C/T locus on ROP

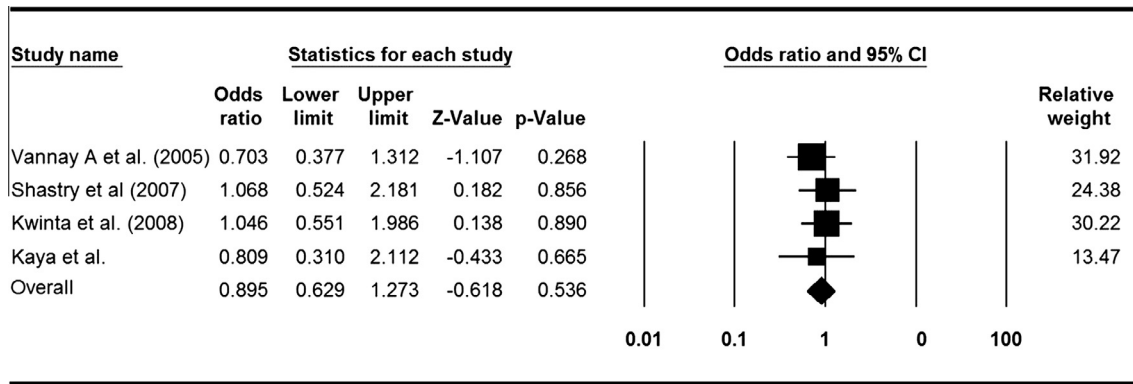


Figure 2. Meta-analysis under dominant model (TC + CC vs TT) for the association between DR risk and the VEGF -460 T/C polymorphism.

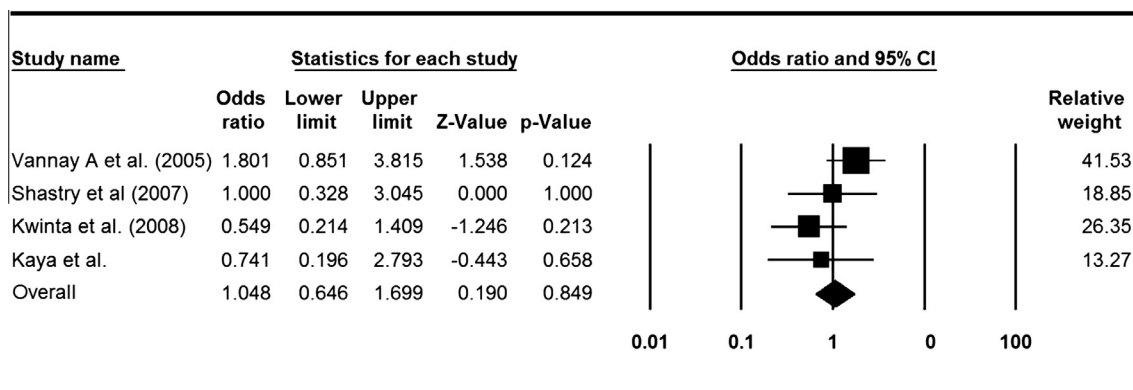


Figure 3. Meta-analysis under recessive model (TT + TC vs. CC) for the association between ROP risk and the VEGF -460 T/C polymorphism.

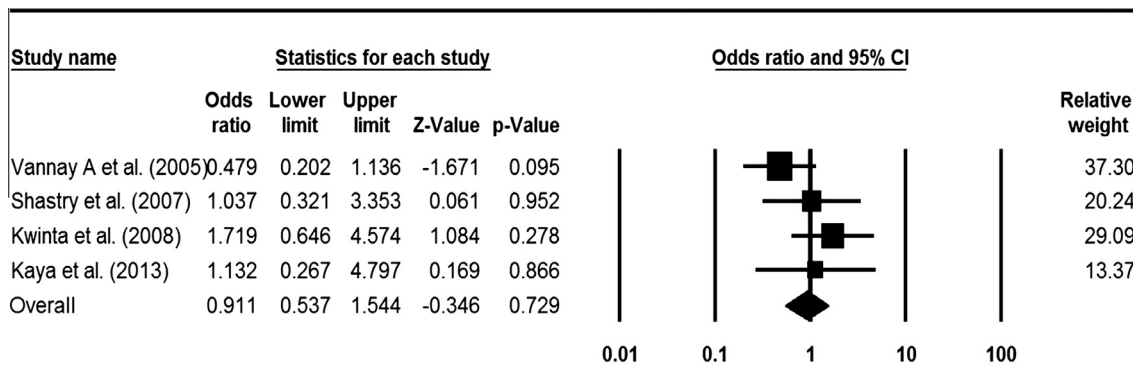


Figure 4. Forest plots of the association between VEGF -460 T/C (Co-dominant model; TT vs. CC) polymorphism and ROP risk.

was taken as the analysis index and inverted funnel plot was drawn (Fig. 10). Due to the small amount of the included research and imperceptible distribution trends, the inverted funnel plot showed trend symmetry, indicating that the publication bias was not big.

Discussion

As a major factor in angiogenesis, VEGF has attracted attention because of its involvement in abnormalities of vascular development, retinal detachment, and ROP. In the present study, we systemically reviewed all available published studies and performed a meta-analysis to explore

the association between the VEGF -460 T/C and +936 C/T polymorphisms and susceptibility to ROP. Our meta-analysis showed that VEGF -460 T/C and +936 C/T polymorphisms were not associated with ROP risk. The deviation most likely indicates a genotyping assay problem with an erroneous gain/loss of homozygous genotypes. The commonly used polymerase chain reaction-restriction fragment length polymorphism analysis for genotyping is reported to have poor accuracy and reproducibility¹⁴ and may underlie this finding. Conversely, effects of sample selection and differences in biological and environmental complexity between samples could also hinder efforts to replicate association in most of the studies which are statistically

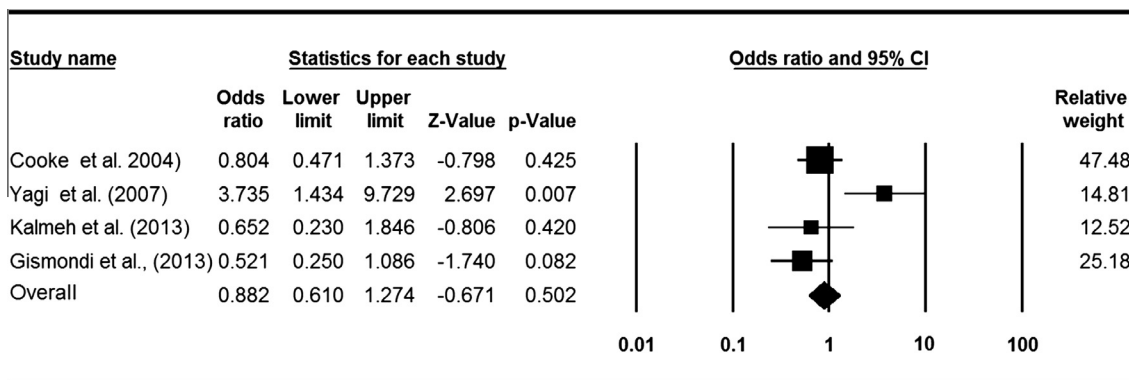


Figure 5. Meta-analysis under allelic model (C vs. T) for the association between ROP risk and the VEGF +936 C/T polymorphism.

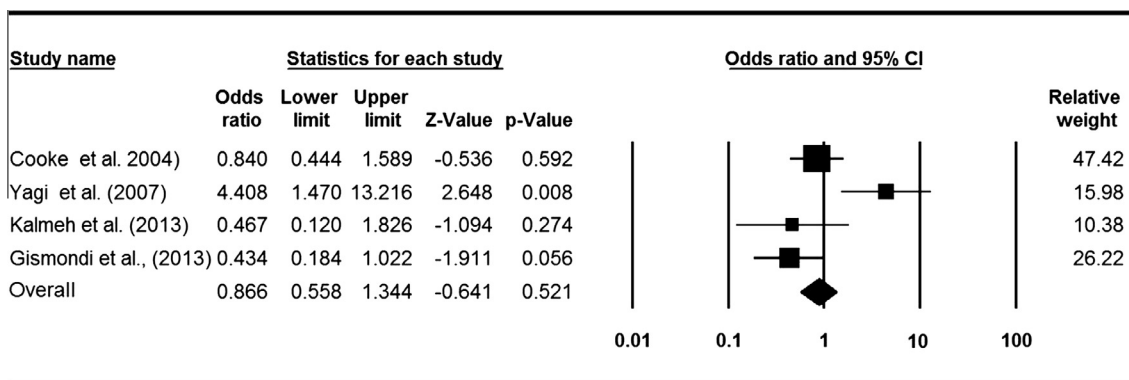


Figure 6. Meta-analysis under dominant model (CT + TT vs CC) for the association between ROP risk and the VEGF +936 C/T polymorphism.

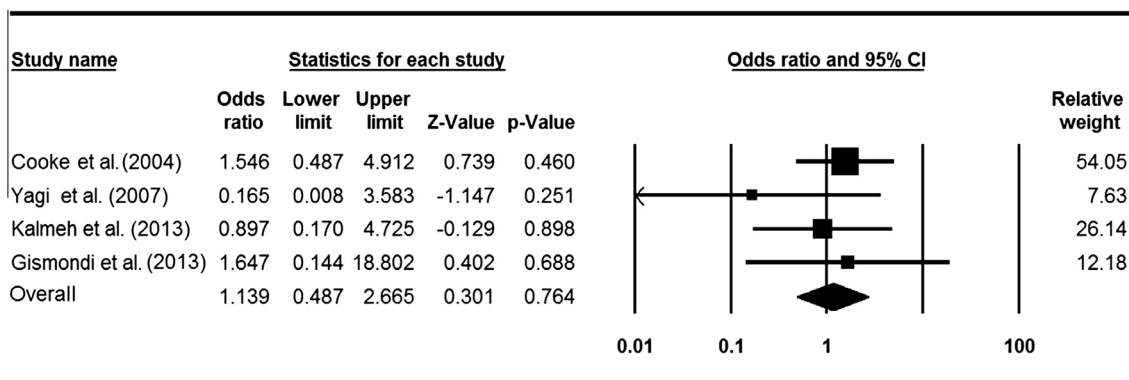


Figure 7. Meta-analysis under recessive model (CC + CT vs. TT) for the association between ROP risk and the VEGF +936 C/T polymorphism.

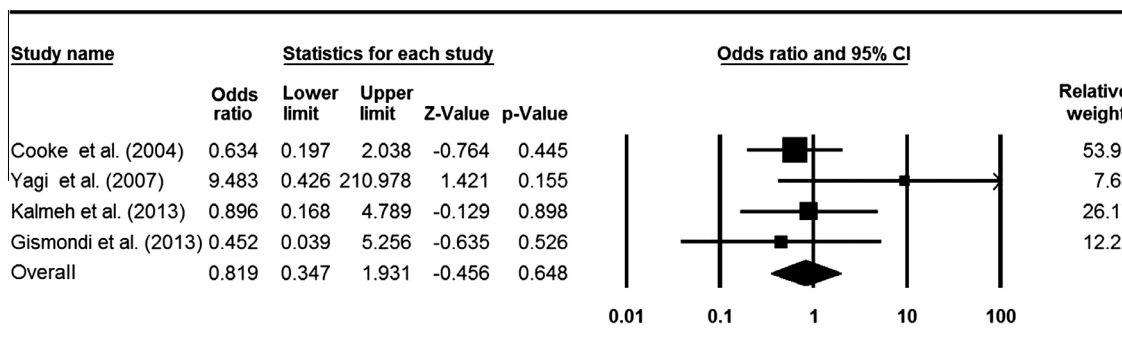


Figure 8. Forest plots of the association between VEGF +936 C/T (Co-dominant model; CC vs. TT) polymorphism and ROP risk.

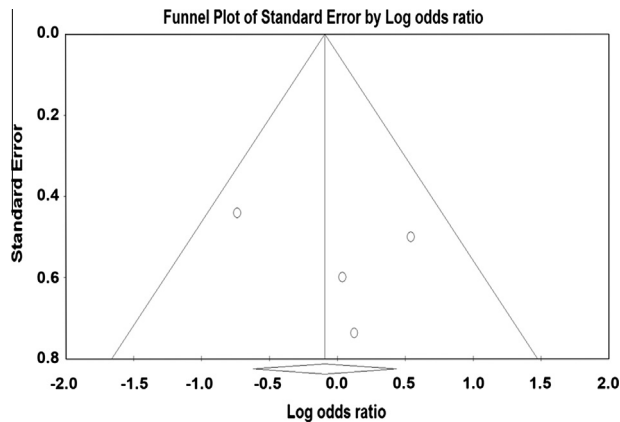


Figure 9. Funnel plots showed symmetric distribution. Log OR is plotted against the standard error of log OR for studies on VEGF -460 T/C.

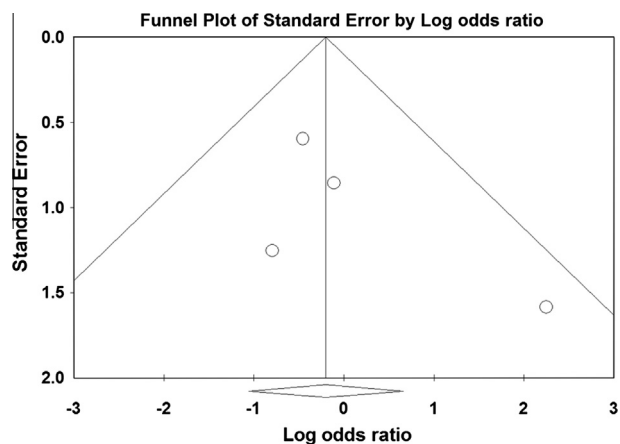


Figure 10. Funnel plots showed symmetric distribution. Log OR is plotted against the standard error of log OR for studies on VEGF $+936$ C/T.

VEGF -460 T/C and $+936$ C/T polymorphism and genetic predisposition to ROP.

Conflict of interest

The authors declared that there is no conflict of interest.

Acknowledgment

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underpowered. The meta-analysis helps researchers to deal with the diversity of the published data but in general cannot do justice to complex human diseases, which involve multiple genetic and environmental determinants.¹⁵ However, in the total combined data, no evidence for association between the VEGF -634 G/C polymorphism genotyped and risk of ROP was observed. Therefore, the different results across studies may result from small sample size and/or genotyping technique rather than ethnic differences. Since the studies included were very limited, it is necessary to validate the association between VEGF -634 G/C CC polymorphism and ROP risk in future studies. A well-designed meta-analysis can provide valuable information for researchers, policymakers, and clinicians.

In conclusion, the present meta-analysis suggested that VEGF -460 T/C and $+936$ C/T polymorphism may not be associated with risk for ROP. More epidemiologic studies are suggested to further ascertain the relationship between