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Systematic Review

# VEGF Gene Polymorphisms in Diabetic Peripheral Neuropathy: A Systematic Review and Meta-analysis

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## Abstract

**Background and objectives:** Diabetes mellitus is a global health concern, and one of its most common complications is diabetic peripheral neuropathy (DPN). The vascular endothelial growth factor (VEGF) gene, which influences not only blood vessels but also neurons, has been studied in the occurrence of DPN. Polymorphism of the VEGF gene may affect the VEGF expression. This study aimed to identify, evaluate, and summarize all relevant studies about VEGF gene polymorphisms in DPN.

**Methods:** We performed a systematic review of the association of VEGF gene polymorphisms in patients with diabetic neuropathy based on a comprehensive search of PubMed, ScienceDirect, ProQuest, and EBSCOhost. A meta-analysis was performed on the most studied gene to clarify its association. Newcastle-Ottawa scale (NOS) was used to verify the quality of the evidence. Hardy-Weinberg equilibrium and six other items were used to determine whether the study was eligible for meta-analysis. Odds ratios and standardized mean differences with 95% confidence intervals were used to determine the association.

**Results:** The systematic review included five case-control and three cross-sectional studies with six VEGF gene polymorphisms (VEGF 936C/T, VEGF −7C/T, VEGF −1001G/C, VEGF −1154G/A, VEGF −2678C/A, and VEGF 405G/C) and the final meta-analysis included four studies with a highly studied gene (VEGF 936C/T). Newcastle-Ottawa scale quality appraisal resulted in six good/high quality and two moderate quality studies. Meta-analysis showed that VEGF 936 C/T polymorphism was associated with a decreased risk of diabetic peripheral neuropathy (odd ratio 0.63; 95% confidence interval: 0.49–0.81; $p = 0.0004$).

**Conclusions:** Our meta-analysis suggests that the VEGF 936C/T gene polymorphism is linked to a decreased risk of diabetic peripheral neuropathy. This gene has the potential to be a predictive biomarker for determining who is at a lower risk of developing diabetic peripheral neuropathy. Early preventive efforts should be addressed in patients bearing the 936C allele.

**Keywords:** Vascular endothelial growth factor; Polymorphisms; Diabetic neuropathy; Predictive biomarker.

**Abbreviations:** ARMS-PCR, amplification refractory mutation system-polymerase chain reaction; CB, community-based; CI, confidence interval; DFU, diabetic foot ulcer; DPN, diabetic peripheral neuropathy; FPRP, false-positive report probability; HB, hospital-based; HC, healthy control; HM, homozygous mutation; HtM, heterozygous mutation; HWE, Hardy-Weinberg equilibrium; N/A, not available; NOS, Newcastle-Ottawa scale; OR, odds ratio; PCR, polymerase chain reaction; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-analysis; RevMan, Review Manager; SNP, single nucleotide polymorphism; UTR, untranslated region; VEGF, vascular endothelial growth factor; WT, wild type.

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## Introduction

Diabetes mellitus is a major global health concern that affects 425 million people worldwide and is estimated to reach 628 million by 2045.[1] Diabetic peripheral neuropathy (DPN) is one of the most common complications of diabetes mellitus, affecting 7 to 34.2% of people with type 1 diabetes and 21.3 to 34.5% of people with type 2 diabetes.[2–4] The prevalence ranges from 10% in the first year of diagnosis to 50% 25 years later, DPN is strongly linked to chronicity and long-term complications. Although many DPN patients were asymptomatic, some experienced excruciating pain. When compared to other complications, DPN has the highest rate of years lived with disability at 20,758/100,000 worldwide.[5]

The Toronto Diabetic Neuropathy Expert Group defines DPN as a symmetrical, length-dependent sensorimotor polyneuropathy caused by metabolic and microvessel changes driven by prolonged

**Table 1. Search terms and strategy**

| Source | Search term | Number of Studies |
|---|---|---|
| PubMed | ((VEGF) OR (vascular endothelial growth factor)) AND ((diabetic neuropathy) OR (diabetic polyneuropathy) OR (diabetic neuralgia)) | 348 |
| ScienceDirect | ("VEGF" OR "vascular endothelial growth factor") AND ("diabetic neuropathy" OR "diabetic polyneuropathy" OR "diabetic neuralgia") | 313 |
| ProQuest | (title (VEGF) OR abstract (VEGF) OR title (vascular endothelial growth factor) OR abstract(vascular endothelial growth factor)) AND (title (diabetic neuropathy) OR abstract (diabetic neuropathy) OR title (diabetic polyneuropathy) OR abstract. (diabetic polyneuropathy) OR title (diabetic neuralgia) OR abstract (diabetic neuralgia)) | 69 |
| EBSCOhost | (TI VEGF OR AB VEGF OR TI vascular endothelial growth factor OR AB vascular endothelial growth factor) AND (TI diabetic neuropathy OR AB diabetic neuropathy OR TI diabetic polyneuropathy OR AB diabetic polyneuropathy OR TI diabetic neuralgia OR AB diabetic neuralgia) | 205 |

VEGF, vascular endothelial growth factor.

hyperglycemia exposure and cardiovascular risk factors.[6] Diabetic neuropathic syndromes are caused by various etiologies, ranging from hyperglycemia-induced neuropathy to other microvascular complications of diabetes. The polyol pathway, the hexosamine pathway, the protein kinase C isoforms pathway, and the build-up of advanced glycation end products are all involved in the pathophysiology of DPN. These pathways can lead to mitochondrial redox state imbalance and excess reactive oxygen species formation. Hypoxia and increased protein kinase C ß-isoform may also result in overexpression of the angiogenic proteins VEGF, transforming growth factor-ß1, nuclear factor kappa B and plasminogen activator inhibitor-1, as well as the development of several diabetic complications.[5]

Vascular endothelial growth factors (VEGFs) are a family of secreted polypeptides consisting of VEGF-A, VEGF-B, VEGF-C, VEGF-D, and VEGF-E. The VEGF encoding gene is 14 kb long and is found on chromosome 6p.21.3. It contains eight exons and seven introns. The VEGF-A gene is extremely polymorphic, with variants identified in 148 untranslated regions (UTRs), 209 exons, 779 introns, and 124 near gene.[7] VEGF is produced by macrophages, platelets, keratinocytes, renal mesangial cells, and tumor cells.[8] It binds to its family cognate kinases to stimulate blood vessel and bone formation, develop the lymphatic vessels, stimulate cell migration, wound healing, and neurogenesis.[9] A study of primary cultures of cortical neurons in the central nervous system found that VEGF enhances the width and length of neurites by 30–40%. In an autocrine loop, VEGF drives blood vessel growth, protects motor and sensory neurons, and encourages Schwann cell proliferation and migration in the peripheral nervous system.[10]

Many studies have been conducted to observe the VEGF gene in diabetic populations with or without DPN. Many factors might influence VEGF activity, including hyperglycemia, oxidative stress, and the sorbitol pathway. Some discovered a higher level of systemic VEGF in diabetic patients with DPN or other complications, while some reported lower VEGF-A expression in epidermal VEGF-A biopsy in this population. Genetic polymorphism may affect the functions of genes, including their affinity, gene expression, and level of activity of gene product.[11] Because of the ambiguity, we performed a systematic review of the association of VEGF gene polymorphisms in patients with diabetic neuropathy. Based on the available data, a meta-analysis was performed to determine the relationship between the specific VEGF gene and diabetic neuropathy. The findings could lead to the identification of specific VEGF gene polymorphisms that can be used to predict the risk of diabetic neuropathy.

## Methods

### *Study registration and methodology*

The Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) statement guidelines and a predetermined search strategy were used to identify and collect all potential studies, screen the titles and abstracts, assess full-text articles for eligibility, and include only relevant studies.[12] The study protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO) (CRD42023405483).

### *Literature search*

For this systematic review and meta-analysis, two authors (JB and HS) independently performed a structured literature search from inception until 1 March 2023 to identify all relevant studies related to the VEGF gene polymorphisms in diabetic neuropathy. We did a systematic literature search on PubMed, ScienceDirect, ProQuest, and EBSCOhost. The following search terms were used: ((VEGF) OR (vascular endothelial growth factor)) AND ((diabetic neuropathy) OR (diabetic polyneuropathy) OR (diabetic neuralgia)). Table 1 shows the complete search terms strategy and findings. The search terms included VEGF in general to encompass all possibilities for applicable studies. There were no restrictions on the publication date or language. Following the removal of duplicates, the authors assessed all titles and abstracts and removed articles with full text that could not be accessed. Any disagreements will be settled through consensus. When agreement was not achieved, a neutral third party was asked to help resolve the conflict.

### *Inclusion and exclusion criteria*

The inclusion criteria for the present studies were (1) human case-control and cross-sectional studies, (2) studies with any VEGF gene polymorphism in a population with DPN or diabetic ulcer as a complication of DPN, (3) studies with a clearly stated diagnosis of diabetes (according to Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, WHO expert committee, or American Diabetes Association), (4) and studies with non-neuropathy diabetes or healthy patients as the control group. The following studies were excluded: (1) all types of re-

view studies, case reports, case series, book sections, conference papers, preliminary reports, preclinical studies, and correspondences, (2) studies of VEGF molecules without gene involvement, (3) studies in animal subjects, and (4) studies with no comparison/control group.

### *Data extraction and analysis*

Two authors (JB and HS) independently reviewed the final articles to extract the information about the first author, date published, study design, study population characteristics, number of samples, genotyping method, adjustment factors, and outcome. The specific outcome measures recorded for the systematic review were the detection of VEGF gene polymorphisms of any subtypes in patients with diabetic neuropathy. To ensure the accuracy of the data, we resolved all conflicting data by consensus among all the authors.

### *Study quality assessment*

The Newcastle-Ottawa scale (NOS) is an eight-item validated star-scoring system scale divided into three domains, selection, comparability, and outcome. The NOS was used to assess the quality of nonrandomized studies such as cohort studies, case-control studies, and cross-sectional studies.[13] With the exception of comparability, all items in the study are graded one point each, with a maximum achievable score of nine for a case-control study and eight for a cross-sectional study. In general, scores of 0–3 represent poor quality, 3–5 represent fair quality, and 6 or more represent good/high quality.[14,15]

### *Methodology quality assessment*

Two reviewers (JB and IW) independently evaluated the methodological quality of all clinical studies by scoring them on a scale of 0–10 based on several parameters. Several assessment items were essential to evaluate genomic studies to ensure data accuracy since genetic analyses are prone to laboratory mistakes. In the present study, we assessed ten items on each case-control and cohort study.[16] When the quality score was >5 and the Hardy-Weinberg equilibrium (HWE) $p$-value was >0.05, the study was considered eligible for meta-analysis. The HWE was used to determine whether a population was in equilibrium. A $p$-value < 0.05 suggests that the genotype distribution in the control group was not in equilibrium and that the study must be discarded because of possible genotyping error or selection bias.[16]

### *Meta-analysis*

Pearson's chi-square test was used to assess the relationship between VEGF gene polymorphism distribution in the case/control group. Odds ratios (ORs) with 95% confidence intervals (CIs) for each VEGF gene polymorphism were collected and calculated. The Q statistical test based on the chi-square value was used to investigate interstudy heterogeneity (statistical significance was defined as an $I^2 > 50\%$). When there was no substantial interstudy heterogeneity, the fixed-effect model was employed. Otherwise, the random-effect model was used. In addition, genetic association analysis was done in wild-type dominant and mutant-dominant models. The wild-type dominant model was defined as wild-type homozygote + heterozygote, and the mutant-dominant model was defined as mutant homozygote + heterozygote. A funnel plot was used to measure publication bias if there were at least ten studies. Otherwise, Begg and Mazumdar rank correlation approaches were used for continuous and dichotomous outcomes. To further analyze the possibility of publication bias, a fail-safe N analysis was done. If publication bias was observed, Duval and Tweedie's Trim and Fill Method was used to correct the bias. In addition, sensitivity and meta-regression analyses were carried out to confirm the robustness of this meta-analysis. The leave-one-out method was used to conduct sensitivity analyses in the current meta-analysis. This analysis was carried out by removing one study at a time and observing any changes in the overall effect size of the meta-analysis, which demonstrated how each individual study influenced the overall estimate of the other studies. We performed a meta-regression analysis in which the mean/median age of the population, percentage of males-to-females, and ethnicity are regressed on the occurrence of DPN. All statistical tests were done using comprehensive meta-analysis,[17] Review Manager (RevMan) 5.3 (https://training.cochrane.org/online-learning/core-software/revman), and Jamovi 2.3.21.0 (https://cran.r-project.org; https://cran.r-project.org).

### *Statistical significance assessment*

To determine the magnitude of statistically significant relationships, the false-positive report probability (FPRP) was calculated. Given a statistically significant discovery, there is a probability of no true association between a genetic variant, such as gene polymorphisms employed in this study, and diseases. It is determined not solely by the observed $p$-value, but also by the prior probability that the relationship between the genetic variant and the disease exists and the test's statistical power.[18] NCSS-PASS ver. 11.0.7 (USA) was used for the analysis. Based on the number of observations, ORs, and $p$-values, we first analyzed the statistical power of each association. The FPRP values were then calculated as previously described.[18] A statistically significant relationship with FPRP was defined as $p < 0.5$.

## Results

### *Characteristics of eligible studies*

We identified 936 published articles and imported results into Endnote 20. From the initial search, 348 articles were identified from PubMed, 313 from ScienceDirect, 205 from EBSCOhost, 69 from the ProQuest database, and one additional study from other sources. Of those, 252 were excluded as duplicates, leaving 684 articles to be reviewed. The abstracts were reviewed based on the following criteria: genetic studies (VEGF gene polymorphisms), relevant independent and dependent variables, and human case-control or cross-sectional studies. Thirteen full texts were reviewed, of which five were excluded, a review study, two genetic studies of single nucleotide polymorphisms (SNPs) but not VEGF gene polymorphism, and two genetic studies with no VEGF genomic or allelic data. Figure 1 illustrates the study search strategy and selection techniques in accordance with PRISMA criteria. Baseline study characteristics, including study design, genotyping method, study groups, size, mean/median age, sex (male %), matching criteria, and quality score, are shown in Table 2.[19–26] Healthy control groups were divided into community-based and hospital-based. The subjects in the community-based group were neither inpatients nor outpatients, but the subjects in the hospital-based groups were mostly outpatients (medical checkup patients) with no known disease or abnormal laboratory findings.

### *Characteristics of involved SNP*

Tables 3 and 4 summarize the genotype frequency distribution of

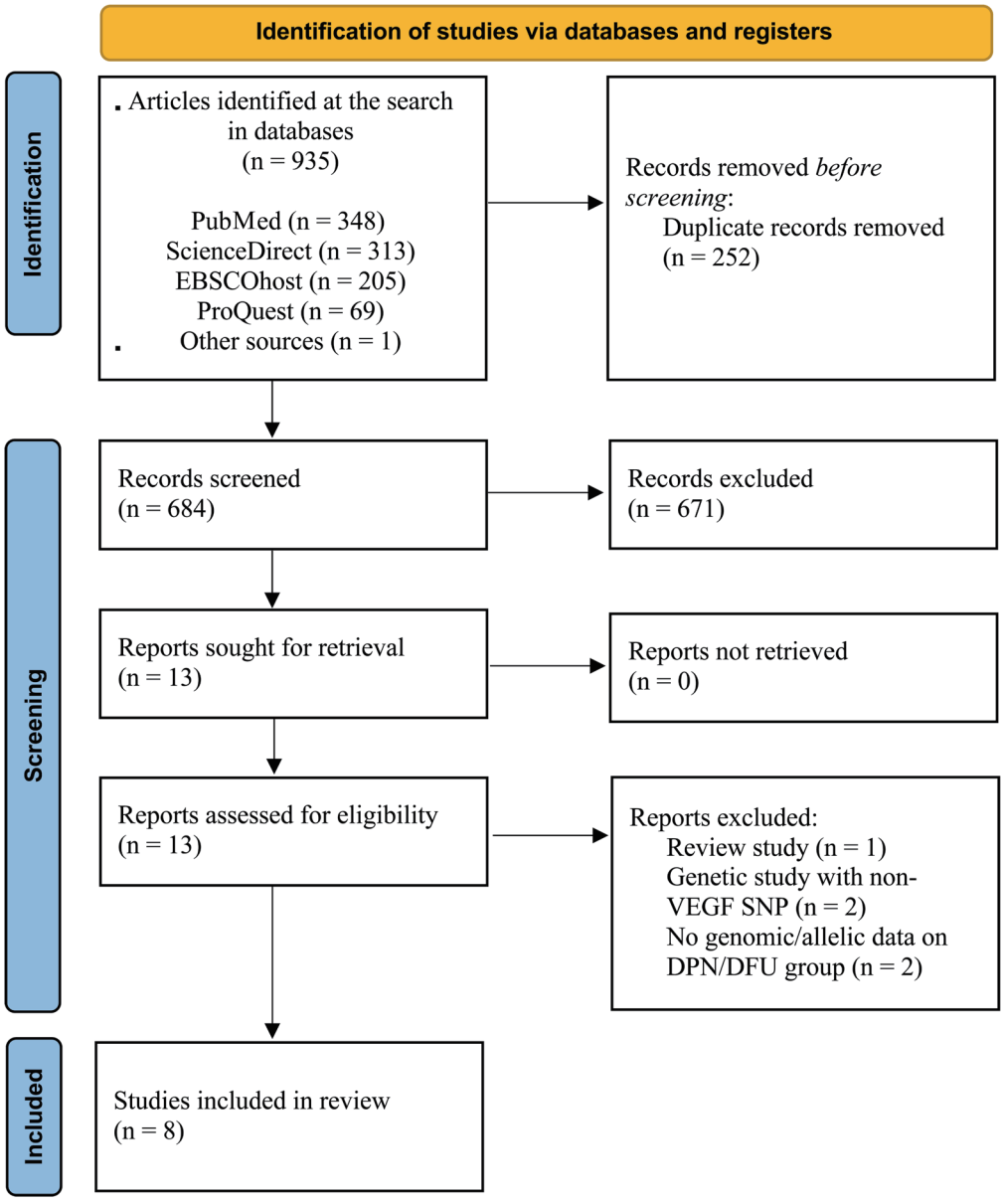


**Fig. 1. Search strategy and study selection following PRISMA guidelines.** DFU, diabetic foot ulcer; DPN, diabetic peripheral neuropathy; SNP, single nucleotide polymorphism; VEGF, vascular endothelial growth factor.

VEGF polymorphism in DPN and diabetic foot ulcer (DFU).[19–26] For subgroup analysis, the study groups were divided into DPN, non-DPN, and healthy control groups.

### *Study quality assessment*

Assessment by the NOS resulted in five case-control studies and one cross-sectional study considered good/high quality with scores of six or more (Tables 5 and 6).[19–26] Two cross-sectional studies were of moderate quality with scores of five. The selection of controls differed across groups as only three case-control studies used healthy populations as controls (two community-based and one hospital-based control groups). Community-based controls are preferred to achieve normal gene distribution among the controls. Two studies matched the duration of diabetes mellitus as necessary for DPN. None of the studies reported any nonrespondents or nonresponse rates.

### *Publication bias*

Funnel plot visualization was observed to be in symmetrical distribution but was not included in the current study owing to having fewer than the recommended number of ten studies. Kendall's Tau was −0.333 ($p = 0.75$) for the Begg and Mazumdar rank correlation test. A high correlation suggests that the funnel plot was asymmetric, which could have been because of publication bias. The current study found no correlation, consistent with the symmetric funnel plot visualization. The Rosenthal approach to fail-safe N analysis yielded 18,000 studies ($p < 0.001$), with a null effect required to reduce the result to nonsignificant. This showed a strong

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**Table 2. Baseline characteristics of the study group**

| Ref no. | Author, year | Country | Ethnicity | Study design | Genotyping method | Study group | Size (n) | Mean/median age, range (years) | Male (%) | Matching criteria | Quality score |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Kim *et al.*, 2009[19] | Korea | Asian | Case-control study | PCR[†] | Diabetic patients with DPN[a] | 106 | 56.1 | 30 | Mean age, and sex | 8/10 |
| | | | | | | Diabetic patients without DPN[a] | 292 | | | | |
| | | | | | | Healthy control (CB) | 526 | 47.3 | 41.8 | | |
| 2. | Tavakkoly-Bazzaz *et al.*, 2010[20] | Iran | Caucasian | Cross-sectional study | ARMS-PCR | Diabetic patients[b] with DPN | 82 | N/A | N/A | Age at the onset of disease, and sex | 6/10 |
| | | | | | | Diabetic patients without DPN | 166 | N/A | N/A | | |
| 3. | Zhang *et al.*, 2014[21] | China | Asian | Case-control study | PCR-RFLP | Diabetic patients[b] with DPN | 204 | 59, 38–85 | 51 | Mean age, sex, and duration of disease | 8/10 |
| | | | | | | Diabetic patients[b] without DPN | 184 | 62, 36–87 | 50 | | |
| | | | | | | Healthy control (HB) | 240 | 60, 41–78 | 51.7 | | |
| 4. | Ghisleni *et al.*, 2015[22] | Brazil | Caucasian | Case-control study | PCR[†] | Diabetic patients[b] with DPN | 98 | 65, 57–71 | 27.6 | N/A | 8/10 |
| | | | | | | Healthy control (CB) | 104 | 55, 50–61 | 81.7 | | |
| 5. | Zitouni *et al.*, 2017[23] | UK | African-Caribbean, Indo-Asian, and Caucasian | Cross-sectional study | PCR[†] | Diabetic patients[*] with DPN | 49 | 67.5 | 65.6 | N/A | 7/10 |
| | | | | | | Diabetic patients[*] without DPN | 264 | 60.6 | 46.6 | | |
| 6. | Barus *et al.*, 2018[24] | Indonesia | Asian | Cross-sectional study | PCR-RFLP | Diabetic patients[c] with DPN | 69 | N/A | N/A | N/A | 7/10 |
| | | | | | | Diabetic patients without DPN | 83 | N/A | N/A | | |
| 7. | Arrendodo-Garcia *et al.*, 2019[25] | Mexico | Mexican | Case-control study | PCR-RFLP | Diabetic patients[c] with DPN | 90 | 60.57, 35–78 | 30 | N/A | 7/10 |
| | | | | | | Diabetic patients without DPN | 128 | 55.37, 30–82 | 25 | | |
| 8. | Dahlan *et al.*, 2019[26] | Indonesia | Asian | Case-control study | PCR[†] | Diabetic patients[c] with DFU | 96 | N/A | 50 | Mean age, sex | 7/10 |
| | | | | | | Diabetic patients without DFU | 101 | N/A | 48.5 | | |

[a]Diagnosis was made according to the report by the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. [b]Diagnosis was made according to the WHO expert committee. [c]Diagnosis was made according to the American Diabetes Association criteria.[*]Absence of ketosis/need of insulin within 1 year of diagnosis. [†]The author did not specify the type of PCR used in the study. ARMS-PCR, amplification refractory mutation system-polymerase chain reaction; CB, community-based; DFU, diabetic foot ulcer; DPN, diabetic peripheral neuropathy; HB, hospital-based; N/A, not available; PCR, polymerase chain reaction; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism.

**Table 3. Genotype frequency distribution of VEGF polymorphism in DPN**

| Ref no. | Author, year | SNP | Genomic/Allelic | Sample size (n) | | | *p* HWE | Included in meta-analysis |
|---|---|---|---|---|---|---|---|---|
| | | | | Case/DPN (%)* | Control/no DPN (%)* | Control/ HC (%)* | | |
| 1 | Kim *et al.*, 2009[19] | VEGF 936C/T | WT (CC) | 69 (64.7) | 190 (65) | 386 (73.3) | 0.35936 | Yes |
| | | | HtM (CT) | 34 (32.4) | 87 (30) | 140 (26.7) | | |
| | | | HM (TT) | 3 (2.9) | 15 (5) | 0 (0) | | |
| | | | Major allele (C) | 172 (80) | 467 (77.4) | 912 (86.6) | | |
| | | | Minor allele (T) | 40 (20) | 117 (22.6) | 140 6(13.4) | | |
| 2 | Tavakkoly-Bazzaz *et al.*, 2010[20] | VEGF –7C/T, | WT (CC) | 61 (74) | 100 (60) | – | 0.86983 | No[b] |
| | | | HtM (CT) | 19 (23) | 57 (34) | – | | |
| | | | HM (TT) | 2 (3) | 9 (6) | – | | |
| | | | Major allele (C) | 141 (86) | 257 (77) | – | | |
| | | | Minor allele (T) | 23 (14) | 75 (23) | – | | |
| | | VEGF –1001G/C | WT (GG) | 79 (97) | 150 (90) | – | 0.70598 | No[b] |
| | | | HtM (GC) | 3 (4) | 16 (10) | – | | |
| | | | HM (CC) | 0 (0) | 0 (0) | – | | |
| | | | Major allele (G) | 161 (98) | 316 (95) | – | | |
| | | | Minor allele (C) | 3 (2) | 16 (5) | – | | |
| | | VEGF –1154G/A | WT (GG) | 42 (51) | 77 (46) | – | 0.51500 | No[b] |
| | | | HtM (GA) | 34 (42) | 77 (46) | – | | |
| | | | HM (AA) | 6 (7) | 12 (8) | – | | |
| | | | Major allele (G) | 118 (72) | 231 (70) | – | | |
| | | | Minor allele (A) | 46 (28) | 101 (30) | – | | |
| | | VEGF –2578C/A | WT (CC) | 22 (27) | 52 (31) | – | 0.95882 | No[b] |
| | | | HtM (CA) | 43 (52) | 82 (50) | – | | |
| | | | HM (AA) | 17 (21) | 32 (19) | – | | |
| | | | Major allele (C) | 87 (53) | 186 (56) | – | | |
| | | | Minor allele (A) | 77 (47) | 146 (44) | – | | |
| 3 | Zhang *et al.*, 2014[21] | VEGF 936C/T | WT (CC) | 159 (77.9) | 115 (62.5) | 141 (58.8) | 0.11987 | Yes |
| | | | HtM (CT) | 39 (19.1) | 59 (32.1) | 83 (34.6) | | |
| | | | HM (TT) | 6 (3) | 10 (5.4) | 16 (6.7) | | |
| | | | Major allele (C) | 357 (87.5) | 289 (78.5) | 365 (76.3) | | |
| | | | Minor allele (T) | 51 (21.5) | 79 (21.5) | 115 (23.8) | | |
| 4 | Ghisleni *et al.*, 2015[22] | VEGF 936C/T | WT (CC) | 25 (83.3) | 55 (80.9) | 73 (71.6) | 0.30974 | Yes |
| | | | HtM (CT) | 4 (13.3) | 12 (17.6) | 25 (24.5) | | |
| | | | HM (TT) | 1 (3.3) | 1 (1.5) | 4 (3.9) | | |
| | | | Major allele (C)[†] | 29 (85.3) | 67 (83.8) | 98 (77.2) | | |
| | | | Minor allele (T)[†] | 5 (14.7) | 13 (16.2) | 29 (22.8) | | |
| | | | Adjusted major allele (C)[†] | 54 (90) | 122 (89.7) | 171 (83.8) | | |

*continued*

**Table 3.** *- continued*

| Ref no. | Author, year | SNP | Genomic/Allelic | Sample size (n) | | | *p* HWE | Included in meta-analysis |
|---|---|---|---|---|---|---|---|---|
| | | | | Case/DPN (%)* | Control/no DPN (%)* | Control/ HC (%)* | | |
| | | | Adjusted minor allele (T)[†] | 6 (10) | 14 (10.3)) | 33 (16.2) | | |
| 5 | Zitouni *et al.*, 2017[23] | VEGF 405G/C | WT (GG) | 16 (45.7) | 119 (45.1) | – | 0.00056 | No[a] |
| | | | HtM (CG) | 18 (51.4) | 134 (50.8) | – | | |
| | | | HM (CC) | 1 (2.9) | 11 (4.2) | – | | |
| | | | Major allele (G) | 50 (70.4) | 372 (70.5) | – | | |
| | | | Minor allele (C) | 21 (29.6) | 156 (29.6) | – | | |
| 6 | Barus *et al.*, 2018[24] | VEGF 936C/T | WT (CC) | 56 (84.8) | 59 (68.6) | – | 0.96638 | Yes |
| | | | HtM (CT)[∂] | 9 (13.7) | 25 (29.1) | – | | |
| | | | HM (TT)[∂] | 1 (1.5) | 2 (2.3) | – | | |
| | | | Major allele (C)[∂] | 121 (91.7) | 143 (83.1) | – | | |
| | | | Minor allele (T)[∂] | 11 (8.3) | 29 (16.9) | – | | |
| 7 | Arrendondo-Garcia *et al.*, 2019[25] | VEGF 936C/T | WT (CC) | 46 (51) | 50 (39) | – | 0.99088 | Yes |
| | | | HtM (CT) | 32 (36) | 66 (52) | – | | |
| | | | HM (TT) | 12 (13) | 12 (9) | – | | |
| | | | Major allele (C) | 124 (69) | 166 (65) | – | | |
| | | | Minor allele (T) | 56 (31) | 90 (35) | – | | |

HWE in these groups was calculated from the total population not from subgroups. *The frequency of various VEGF polymorphisms is represented as a percentage (%) within the same group (case/control, and genotypic/allelic). [†]This study looked at allele frequency, which means that both homozygote and heterozygote genotypes were counted as one allele, as opposed to other studies that counted wild types (like CC) as two major alleles. As a result, we adjusted their allele count to match that of other studies. [∂]We contacted the author to obtain detailed genotypic and allelic data because this study combined heterozygous mutation (HtM) and homozygous mutation (HM) into unified CT+TT in their primary study. [a]Excluded because HWE was <0.05. [b]Excluded because of limited studies on this locus. DPN, diabetic peripheral neuropathy; HC, healthy control; HWE, Hardy-Weinberg Equilibrium; SNP, single nucleotide polymorphism; VEGF, vascular endothelial growth factor; WT, wild type.

**Table 4. Genotype frequency distribution of VEGF polymorphism in DFU**

| Ref no. | Author, year | SNP | Group | Sample size, *n* | | | *p* HWE | Included in the meta-analysis |
|---|---|---|---|---|---|---|---|---|
| | | | | Case/DFU (%)* | Control/no DFU (%)* | Control/ HC (%)* | | |
| 1 | Dahlan *et al.*, 2019[26] | VEGF 405G/C | WT (GG) | 18 (19.9) | 23 (22.8) | – | <0.00001 | No[a] |
| | | | HtM (GC) | 69 (71.9) | 72 (71.3) | – | | |
| | | | HM (CC) | 9 (9.4) | 6 (5.9) | – | | |
| | | | Major allele (G) | 105 (54.7) | 118 (58.4) | – | | |
| | | | Minor allele (C) | 87 (45.3) | 84 (41.6) | – | | |
| | | VEGF –460T/C | WT (TT) | 42 (43.8) | 50 (49.5) | – | 0.20917 | No[b] |
| | | | HtM (TC) | 41 (42.7) | 36 (35.6) | – | | |
| | | | HM (CC) | 13 (13.5) | 15 (14.9) | – | | |
| | | | Major allele (T) | 125 (65.1) | 136 (67.3) | – | | |
| | | | Minor allele (C) | 67 (34.9) | 66 (32.7) | – | | |

*The frequency of various VEGF polymorphisms is represented as a percentage (%) within the same group (case/control, and genotypic/allelic). **HWE in these group were calculated from the non-DPN group as control due to no healthy control. [a]Excluded because HWE was <0.05. [b]Excluded because of limited studies of this locus. DFU, diabetic foot ulcer; DPN, diabetic peripheral neuropathy; HC, healthy control; HM, homozygous mutation; HtM, heterozygous mutation; HWE, Hardy-Weinberg equilibrium; SNP, single nucleotide polymorphism; VEGF, vascular endothelial growth factor; WT, wild-type.

**Table 5. Newcastle-Ottawa scale quality assessment of case-control studies**

| Studies | Selection | | | | Comparability | Outcome | | |
|---|---|---|---|---|---|---|---|---|
| | Adequate case definition | Case representative | Selection of controls | Definition of controls | | Ascertainment of exposure | Same method | Nonresponse rate |
| Kim *et al.*, 2009[19] | ★ | ★ | ★ | ★ | ★☆ | ★ | ★ | ☆ |
| Zhang *et al.*, 2014[21] | ★ | ★ | ☆ | ★ | ★★ | ★ | ★ | ☆ |
| Ghisleni *et al.*,2015[22] | ★ | ★ | ★ | ★ | ☆☆ | ★ | ★ | ☆ |
| Arrendondo-Garcia *et al.*, 2019[25] | ★ | ★ | ☆ | ★ | ★☆ | ★ | ★ | ☆ |
| Dahlan *et al.*, 2019[26] | ★ | ★ | ☆ | ★ | ★★ | ★ | ★ | ☆ |

The number of stars represents the maximum number of points possible in the categories of selection, comparability, and outcome. A black star (★) signifies a given star/point, whereas a white star (☆) represents a failed attempt to obtain a star/point.

**Table 6. Newcastle-Ottawa scale quality assessment of cross-sectional studies**

| Studies | Selection | | | | Comparability | Outcome | |
|---|---|---|---|---|---|---|---|
| | Case representative | Sample size | Nonrespondents | Ascertainment | | Outcome assessment | Statistical test |
| Tavakkoly-bazzaz *et al.*, 2010[20] | ★ | ★ | ☆ | ★ | ★☆ | ★ | ★ |
| Zitouni *et al.*, 2017[23] | ★ | ★ | ☆ | ★ | ☆☆ | ★ | ★ |
| Barus *et al.*, 2018[24] | ★ | ★ | ☆ | ★ | ☆☆ | ★ | ★ |

The number of stars represents the maximum number of points possible in the categories of selection, comparability, and outcome. A black star (★) signifies a given star/point, whereas a white star (☆) represents a failed attempt to obtain a star/point.

significant result in the present study. Duval and Tweedie's Trim and fill method was not performed because no publication bias was observed.

### *Quantitative data synthesis of the association between SNP and DPN*

The pooled ORs from all five studies (four case-control studies and one cross-sectional study) were used to conduct a meta-analysis of the association between VEGF 936C/T polymorphism and DPN. To identify variations in the results, we ran a subgroup analysis solely based on the four case-control studies. The VEGF 936C/T polymorphism was significantly associated with DPN in the mutation-dominant model (Fig. 2), but not in the wild-type dominant model (Fig. 3). The CT + TT genotype was associated with a lower risk of DPN (OR 0.63; 95% CI: 0.49–0.81; $p$ = 0.0004), indicating that it was associated with a lower risk of DPN. The CC + CT genotype showed no association with the occurrence of DPN (OR 1.16; 95% CI: 0.68–2.00; $p$ = 0.58). Interstudy heterogeneity was 43%, which was considered low, so stratified analyses were not performed. The current result was not altered by subgroup analysis, with the VEGF 936C/T polymorphism being significantly associated with DPN only in the mutation-dominant model (OR 0.67; 95% CI: 0.51 – 0.87; $p$ = 0.003) (Fig. 4), not in the wild-type dominant model (Fig. 5)

### *Sensitivity analysis*

The sensitivity analysis of the mutation-dominant model showed the robustness of the results (Table 7).[19,21,22,24,25] The effect size did not vary as observed in the leave-one-out method. The ORs varied from 0.52 to 0.73 with a mean OR of 0.64, comparable to our meta-analysis (OR 0.63; 95% CI: 0.49–0.81; $p$ = 0.0004). Thus, the effect size remained consistent between medium values.

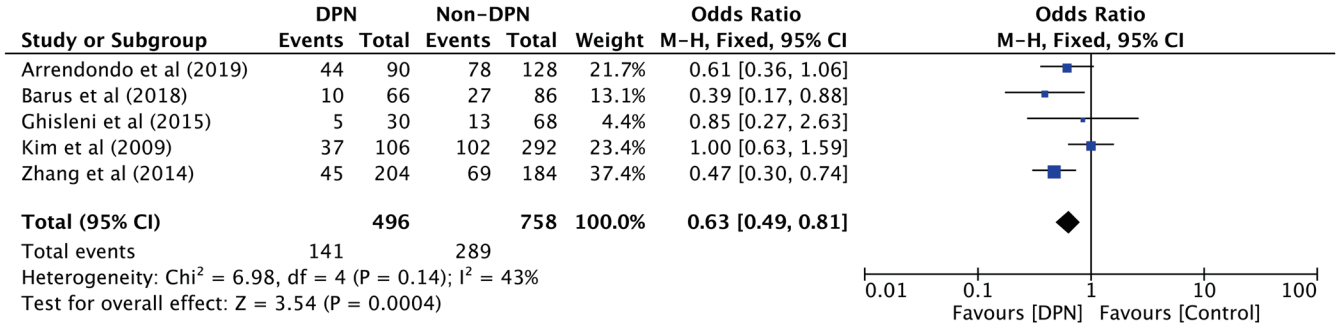



**Fig. 2. Meta-analysis using a mutation-dominant model (CT/TT) of VEGF 936C/T polymorphism.** CI, confidence interval; DPN, diabetic peripheral neuropathy; VEGF, vascular endothelial growth factor.

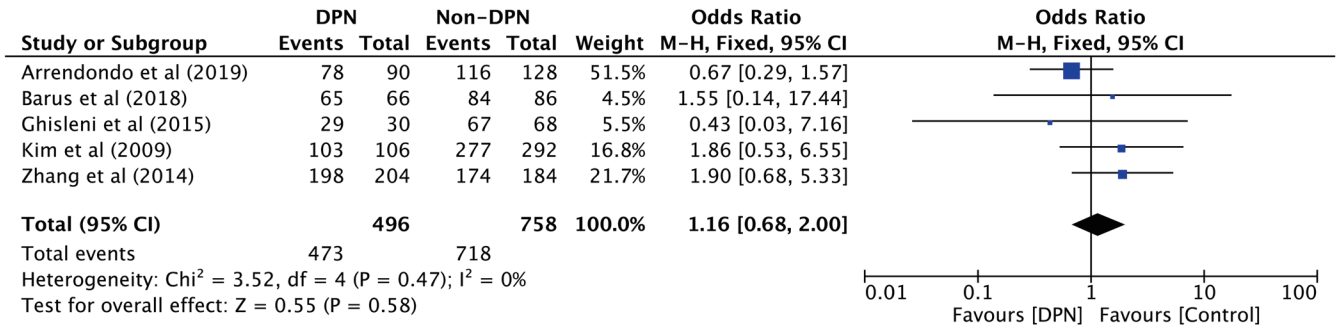



**Fig. 3. Meta-analysis using a wild-type dominant model (CC/CT) of VEGF 936C/T polymorphism.** CI, confidence interval; DPN, diabetic peripheral neuropathy; VEGF, vascular endothelial growth factor.

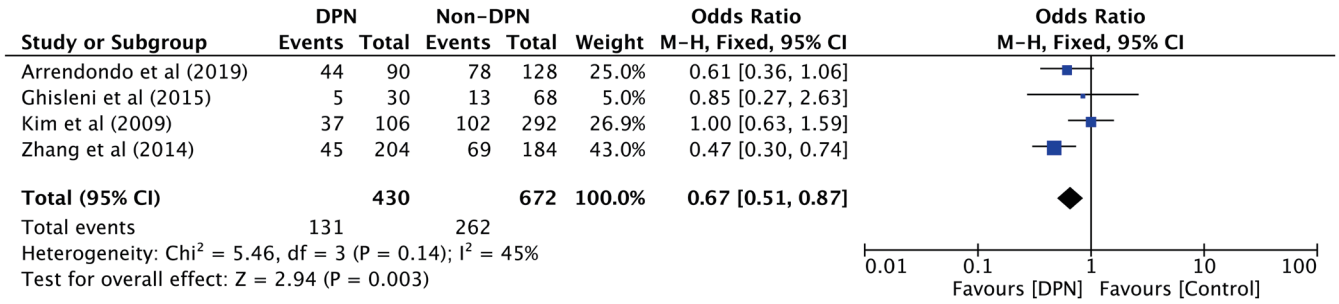



**Fig. 4. Subgroup analysis consisting of all case-control studies of the mutation-dominant model (CT/TT) of VEGF 936C/T polymorphism.** CI, confidence interval; DPN, diabetic peripheral neuropathy; VEGF, vascular endothelial growth factor.

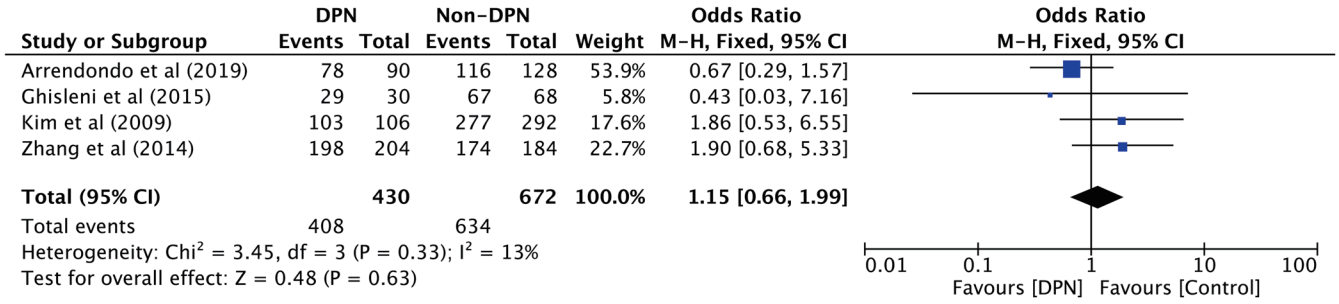



**Fig. 5. Subgroup analysis consisting of all case-control studies of the wild-type dominant model (CC/CT) of VEGF 936C/T polymorphism.** CI, confidence interval; DPN, diabetic peripheral neuropathy; VEGF, vascular endothelial growth factor.

**Table 7. Sensitivity analysis of the mutation-dominant model using the leave-one-out method**

| Author(s) and year | OR | 95% CI | $I^2$ |
|---|---|---|---|
| Arrendondo *et al.*, 2019[25] | 0.64 | 0.48–0.85 | 57% |
| Barus *et al.*, 2018[24] | 0.67 | 0.51–0.87 | 45% |
| Ghisleni *et al.*, 2015[22] | 0.62 | 0.48–0.81 | 55% |
| Kim *et al.*, 2009[19] | 0.52 | 0.38–0.70 | 0% |
| Zhang *et al.*, 2014[21] | 0.73 | 0.53–0.99 | 33% |
| Mean | 0.64 | | 38% |

Each line represents the results obtained by excluding that study. CI, confidence interval; OR, odds ratio.

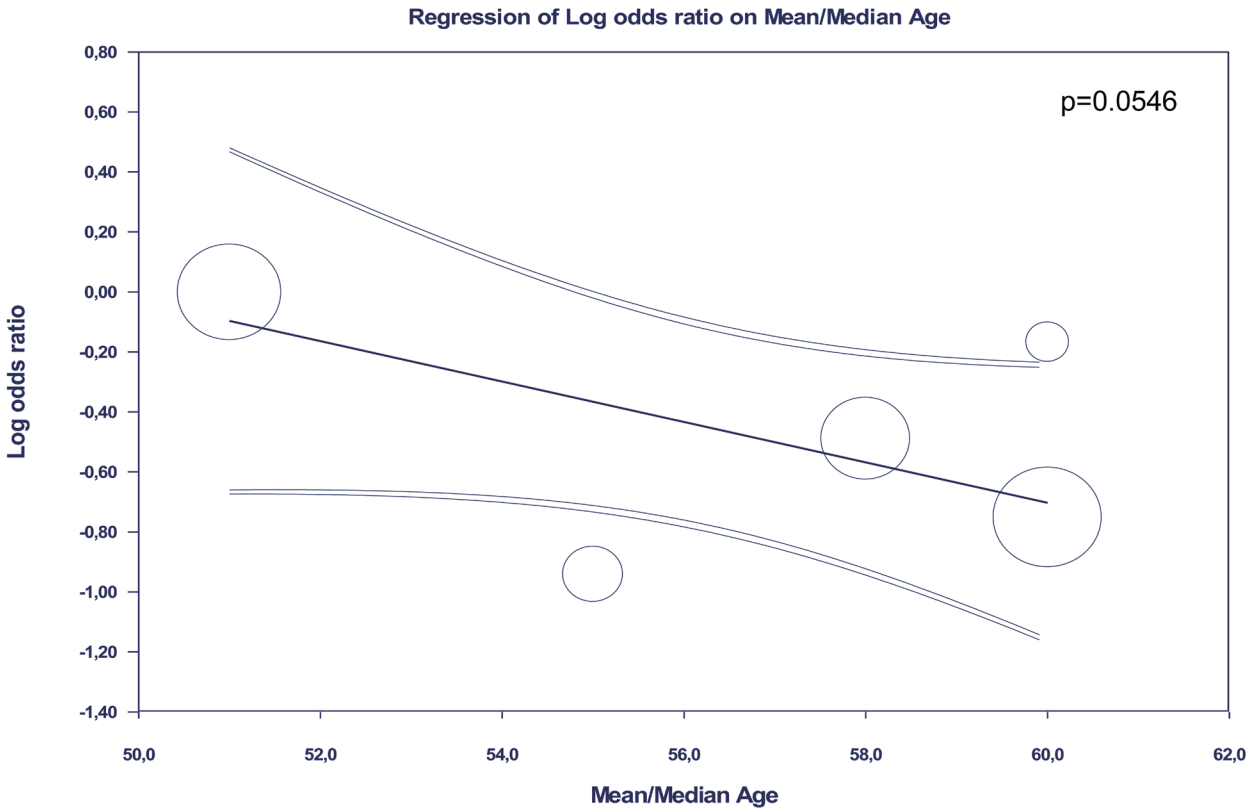
Interstudy heterogeneity was 38% and was considered low.

### *Meta-regression analysis*

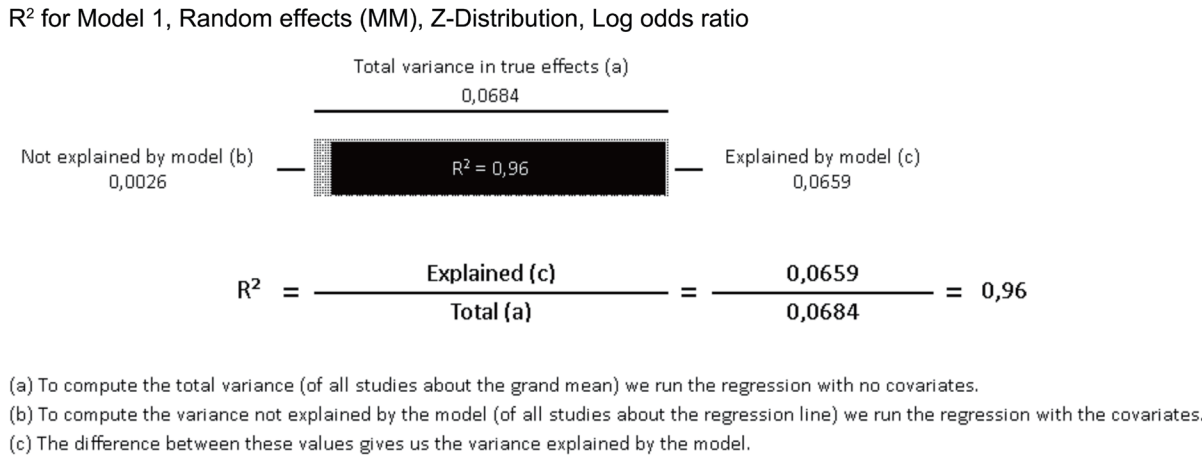
In the current analysis model, no study covariates (mean/median age, male-to-female ratio, and ethnicity) were significantly linked with the relative prevalence of DPN among a variety of study-level factors. We predicted the age of diabetes diagnosis and duration (years) of diabetes to be highly related, although this information was lacking across studies and needed to be explored further. The mean/median age was the closest covariate to a significant result (Fig. 6B). The meta-regression showed that the magnitude of DPN occurrence in the VEGF 936C/T polymorphism was lower with older age (Coefficient −0.0674; 95% CI: −0.1361 to 0.0013; $p = 0.0546$), which explained $R^2 = 95\%$ of the heterogeneity. The goodness of fit test resulted in a good result $\tau^2 = 0.0026$ with very

a

| Model | Study name | Statistics for each study | | | | | Events / Total | | Odds ratio and 95% CI | Weight (Fixed) | Residual (Fixed) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Odds ratio | Lower limit | Upper limit | Z-Value | p-Value | DPN | Non-DPN | 0,10 0,20 0,50 1,00 2,00 5,00 10,00 | Relative weight | Std Residual |
| | Arrendondo | 0,613 | 0,356 | 1,057 | -1,759 | 0,079 | 44 / 90 | 78 / 128 | | 22,02 | -0,13 |
| | Barus et al | 0,390 | 0,173 | 0,879 | -2,270 | 0,023 | 10 / 66 | 27 / 86 | | 9,90 | -1,23 |
| | Ghisleni et | 0,846 | 0,272 | 2,631 | -0,289 | 0,773 | 5 / 30 | 13 / 68 | | 5,08 | 0,51 |
| | Kim et al | 0,999 | 0,627 | 1,592 | -0,005 | 0,996 | 37 / 106 | 102 / 292 | | 30,08 | 2,29 |
| | Zhang et al | 0,472 | 0,302 | 0,737 | -3,305 | 0,001 | 45 / 204 | 69 / 184 | | 32,92 | -1,58 |
| Fixed | | 0,633 | 0,490 | 0,818 | -3,504 | 0,000 | | | | | |




b




(a) To compute the total variance (of all studies about the grand mean) we run the regression with no covariates.
(b) To compute the variance not explained by the model (of all studies about the regression line) we run the regression with the covariates.
(c) The difference between these values gives us the variance explained by the model.

**Fig. 6. Meta-analysis and meta-regression of the occurrence of DPN in the dominant-mutation model of VEGF 936C/T polymorphism.** (a) Forest plot with OR for DPN occurrence following VEGF 936C/T polymorphism in DPN group (case) against control for separate studies and the pooled population. (b) The OR of studies against controls as a function of the mean/median age (years) of participants at enrollment, with $R^2$ = 96%. ORs are represented on a logarithmic scale. A solid line represents the fitted meta-regression function, along with the upper and lower bounds for the 95% mean prediction interval (double line). Individual studies are represented by circles, and the size of each circle is equal to their statistical weight. CI, confidence interval; DPN, diabetic peripheral neuropathy; OR, odds ratio; VEGF, vascular endothelial growth factor.

**Table 8. FPRP analysis of VEGF 936C/T gene polymorphism**

| Genotype | OR (95% CI) | *p*-value | Power | Prior probability* | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 0.25 | 0.1 | 0.01 | 0.001 | 0.0001 | 0.00001 |
| VEGF 936C/T | | | | | | | | | |
| CC+CT vs. TT | 1.02 | 0.94 | 0.9114 | 0.762 | 0.905 | 0.991 | 0.999 | 1 | 1 |
| CT+TT vs. CC | 0.52 | <0.0001 | 0.9114 | *0.001* | *0.003* | *0.031* | *0.242* | 0.762 | 0.970 |

*Significant values are denoted in italic if FPRP was < 0.5. CI, confidence interval; FPRP, false-positive report probability; OR, odds ratio; VEGF, vascular endothelial growth factor.

low heterogeneity ($I^2$ = 2.22%).

### *FPRP analysis*

The positive findings of the meta-analysis were evaluated with an FPRP analysis. VEGF 936C/T was tested in both wild-type dominant and mutation-dominant models. As in a previous study, we used an FPRP of 0.5 because that value demonstrated a significant improvement over current practice. Because of the minimal number of studies and sample size involved, we set the threshold at 0.5.[27] Several significant associations of the VEGF 936C/T gene polymorphism in the mutation-dominant model (prior *p*-values of 0.25/0.1/0.01/0.001) could be noteworthy (Table 8).

## Discussion

A systematic review was carried out to examine the relationship between all published SNPs in the VEGF gene and the risk of developing DPN in the diabetic population. As the most studied polymorphism, VEGF 936 C/T was the subject of a meta-analysis. The T allele in the VEGF 936 C/T polymorphism was linked to fewer occurrences of DPN, showing that it has a protective effect. To the best of our knowledge, this is the first meta-analysis of VEGF SNPs associated with DPN.

The CC (wild-type) genotype was related to increased DPN in diabetes individuals in every research study included in the meta-analysis. A study by Zhang *et al.* found no difference in C or T allele distribution between diabetes patients and healthy controls.[21] A study by Arrendondo *et al.* discovered that the T allele conferred significant protection against DPN.[25] Several studies have not found a link between this polymorphism and DPN susceptibility.[19,22] The inconsistent results may be attributed to the small sample size of individual studies, the wide range of diabetes onset, and other external or environmental factors. Our meta-analysis suggested that it was linked to a decreased risk of DPN, like that reported by Arrendondo *et al.*[25]

VEGF 936 is located in the 3′-UTR of chromosome 6p.21.3, bound to a protein that controls the termination of nucleic acid transcriptions or stop codons. It has been widely investigated for its role in angiogenesis, cell migration, and neurogenesis. The VEGF gene regulates angiogenesis under both physiological and pathologic conditions. This gene controls cell proliferation, differentiation, migration, and survival, as well as nitric oxide production and interaction with other angiogenic factors. The 5′- and 3′-UTRs may not be translated into proteins, but they are a critical regulator of protein synthesis. This regulation occurs by both the cis-regulatory elements and trans-acting factors, both in the UTRs. The changes in this cis-regulatory sequence may alter physiological balance causing several diseases.[28]

The mechanisms by which VEGF gene polymorphism from C to T allele conferred protection against DPN were still unknown. A study by Kim *et al.* found that TT homozygotes and T allele carriers had higher plasma VEGF concentrations in healthy controls and diabetic patients, but the mechanism was not known.[22] We propose several hypotheses that may explain this protective effect of VEGF gene polymorphism in DPN. The first hypothesis is that the increased plasma VEGF concentration may increase the vasa nervorum count, protect peripheral nerves, and promote the restoration of large and small fiber peripheral nerve function. Two findings supported this hypothesis. First, even in the early stages of diabetic neuropathy, abnormalities in the vasa nervorum and nerve fiber loss were observed. The high VEGF concentration in the stage before neuropathy develops is beneficial. Second, in 4 weeks, plasmid DNA encoding the VEGF gene restored severe peripheral neuropathy rats to a state comparable to healthy non-diabetic rats. A similar study in rabbits produced similar results.[29] Our second hypothesis is that the VEGF 936 C/T polymorphism decreases VEGF binding affinity, expression, and inflammation. Increased plasma VEGF levels would compensate for this low affinity, as observed in TT homozygotes and T allele carriers with higher plasma VEGF levels. This hypothesis was supported by the fact that many genetic polymorphism studies have discovered lower affinity following mutation/polymorphism.[30,31] Studies have shown that VEGF and other pro-angiogenic factors can influence the inflammatory process in various ways, including mediating inflammatory cell migration and the production of cytokines by activated endothelial cells. This has been observed in VEGF studies of rheumatoid arthritis and T-cell-mediated immunity diseases.[32]

Possible clinical applications of polymorphic genes like the VEGF gene include use as susceptibility biomarkers in populations with similar genetic profiles. Knowing who is at risk for certain diseases and who is less likely to develop them because of gene polymorphisms allows clinicians to develop more effective personalized preventive strategies or treatments. For example, in the current study, the VEGF-C/T polymorphism conferred protection against DPN, implying that diabetics lacking this polymorphism require more aggressive preventive strategies to avoid developing DPN. Furthermore, advances in genetic testing have enabled us to detect genes using safe and minimally invasive techniques, such as DNA extracted from biological matrices such as urinary sediment, exfoliated buccal cells, and blood matrices.

It should be noted that the current study had some limitations. First, while the sample size for the meta-analysis was adequate, it was considered small, particularly in genetic studies. Second, some studies were prone to selection bias because they only adjusted for ethnicity, age, and sex. Case-control studies that used a diabetic population without DPN as a control only matched the group based on either diabetes onset or diabetes duration, not both. It would be ideal if these studies were conducted in a large community-based populations with matched mean age, sex ratio, onset of diabetes, and duration of diabetes. Other risk factors including systemic diseases that could affect peripheral nerves should also be excluded. Third, stratified analysis using community-based or hospital-based

controls was not performed owing to a lack of data. Only two studies had healthy community-based healthy controls and only one had a hospital-based control. Fourth, because of limited data, many VEGF polymorphisms could not be analyzed, and further research on other key VEGFs is needed for an updated meta-analysis. Finally, the mechanisms underlying how VEGF gene polymorphism affects physiological function remain unknown, necessitating additional molecular studies to elucidate the hypotheses of related mechanisms.

## Conclusion

Our meta-analysis suggests that the VEGF 936C/T gene polymorphism is linked to a decreased risk of DPN. This gene has the potential to be a predictive biomarker for determining who is at a lower risk of developing DPN. Early preventive efforts should be addressed in patients bearing the 936C allele.

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## Conflict of interest

The authors have no conflict of interest to declare.

## Author contributions

Study concept and design (JB, HS, IW, SA); acquisition of data (JB, HS); analysis and interpretation of data (JB, HS, SA); drafting of the manuscript (JB, HS, IW); critical revision of the manuscript for important intellectual content (IW, SA); administrative, technical, or material support (JB, SA); and study supervision (JB, SA). All authors have made a significant contribution to this study and have approved the final manuscript.

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