Mahajan *et al.*
Egyptian Journal of Medical Human Genetics (2024) 25:65
https://doi.org/10.1186/s43042-024-00535-0

Egyptian Journal of Medical Human Genetics

META-ANALYSIS

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Association of *VEGF*-2549I/D promoter polymorphism with gastrointestinal tract cancer risk: a meta-analysis

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Abstract

Background Gastrointestinal tract (GIT) cancers are complex disorders affecting millions of people worldwide. The vascular endothelial growth factor (VEGF) helps in the development of different GIT cancers by promoting abnormal angiogenesis in cancer cells. The role of *VEGF*-2549I/D polymorphism in influencing GIT cancer susceptibility has been studied in different populations with inconclusive results. Therefore, the relationship between *VEGF*-2549I/D polymorphism with GIT susceptibility was studied by performing a meta-analysis study.

Methods Various online databases were used for identifying the articles. Based on study selection criteria, five studies on different GIT cancers including 1178 patients and 1520 controls were included in the meta-analysis. The accuracy of the study results was determined by performing a trial sequential analysis.

Results In this study, the *VEGF*-2549I/D polymorphism did not influence the GIT cancer susceptibility in the overall analysis as well as when stratified according to ethnicity ($p > 0.05$). Stratification of all the studies based on the different GIT cancers reported an increased susceptibility to gastric cancer under different genetic models including allele contrast (OR = 1.67, CI = 1.294–2.157, $p = 0.00008$), recessive (OR = 1.68, CI = 1.056–2.660, $p = 0.029$), dominant (OR = 2.49, CI = 1.617–3.823, $p = 0.00003$), over-dominant (OR = 1.52, CI = 1.055–2.177, $p = 0.025$), II vs DD (OR = 2.97, CI = 1.692–5.208, $p = 0.00015$) and ID vs DD model (OR = 2.35, CI = 1.501–3.669, $p = 0.00018$).

Conclusion There was no relationship between *VEGF*-2549I/D promoter polymorphism and GIT cancer susceptibility in the overall population and also in different ethnic groups. Stratification analysis revealed higher susceptibility towards gastric cancer development with *VEGF*-2549I/D polymorphism.

Keywords Vascular endothelial growth factor, Angiogenesis, Gastrointestinal tract cancers, Polymorphism, *VEGF*-2549I/D

Introduction

Cancer is a serious polygenic disease affecting the global population [1]. The development and progression of cancer occur due to the interaction between several environmental stresses, genetic and epigenetic factors [2]. Genetic variation affects the key biological processes involved in oncogenesis [3, 4] and also determines the individual's susceptibility to cancer development [5]. Genome-wide association studies (GWAS) performed on different populations have confirmed the relationship between several genes and cancer susceptibility [6]. It has been documented in the literature that the genetic variation reported in the angiogenesis-related genes might be responsible for differences in the individual's susceptibility towards tumour development [7].

The angiogenesis process, a critical hallmark of cancer, helps in the continuous growth and metastasis of

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the tumour cells [8, 9]. The coordinated functioning of several growth molecules regulates the angiogenesis process [10]. The vascular endothelial growth factor (VEGF*)*, a crucial angiogenic growth mediator, is responsible for the formation of new vasculature, needed for the continuous blood supply to the growing tumour [11, 12]. *VEGF*, a highly polymorphic gene localised on 6p21.3, encodes the pro-angiogenic VEGF protein [13]. The *VEGF* harbours several functional polymorphisms in the promoter and UTRs which alter the gene expression levels of *VEGF* [14–16]. One such functional polymorphism is a 18 bp ins/del polymorphism (−2549 Ins/del), positioned upstream of the *VEGF* promoter. Ins/del polymorphisms are one of the most frequently reported genetic variations, which affect the functions of the regulatory region of the gene [17, 18]. Ins/del polymorphisms have been reported to alter several complex human traits and contribute to the disease development [17, 19]. *VEGF*-2549I/D promoter polymorphism (rs35569394) is an important functional polymorphism, known to regulate VEGF production and activity. The functional relevance of *VEGF*-2549I/D polymorphism has been studied in several studies. It was observed that the *VEGF*-2549 D allele enhanced the transcriptional activity of the *VEGF* promoter [20], whereas the *VEGF*-2549 II genotype increased the VEGF production in the cells of healthy individuals [21]. The *VEGF*-2549I/D polymorphism by regulating the protein levels might influence susceptibilities to cancer development.

VEGF-2549I/D polymorphism has been studied in different gastrointestinal tract (GIT) cancers including oesophageal [22, 23], gastric [24], hepatocellular [25], gall bladder [26] and colorectal cancer [27] with conflicting results. The association of *VEGF*-2549I/D polymorphism with therapy response has been studied in oesophageal [23], colorectal [28, 29] and hepatocellular cancer [30]. Other than the GIT cancers, the association of *VEGF*-2549I/D polymorphism with cancer risk has been reported in renal cell carcinoma [31], bladder [32], urothelial bladder [33], breast [34, 35] and prostate cancer [36].

GIT cancers are among the most prevalent cancers globally [1]. Increased expression of VEGF in tissue and serum has been detected in several GIT cancers including oral [37], gastric [38] and pancreatic [39] cancer. Increased VEGF levels in the patient's serum samples have been associated with tumour aggressiveness and lower overall survival in various GIT cancers like oesophageal [40], pancreatic [41], hepatocellular [42] and gastric cancer [43].

The case–control studies with small sample sizes give limited statistical power to provide a definitive decision. Therefore, to overcome this problem, a detailed structured meta-analysis study investigating the role of *VEGF*-2549I/D polymorphism in modulating GIT cancer susceptibility was performed to identify a more accurate conclusion. The present meta-analysis study is the first study examining the relationship between the *VEGF*-2549I/D polymorphism and GIT cancer risk.

Methods

Study design

This meta-analysis followed the PRISMA guidelines [44] and the study question was prepared according to the PICO criteria [45]. The formulated research study question was whether *VEGF*-2549I/D polymorphism was associated with GIT cancer risk, by comparing the genotypic and allelic distribution between patients suffering from GIT cancers and age, gender and geographical matched healthy controls.

Literature search strategy

We systematically searched all the published research articles evaluating the role of *VEGF* -2549I/D polymorphism in influencing GIT cancer susceptibility by extensively searching several databases such as PubMed, Google Scholar and Embase up to December 2023. The following combination of keywords was used for searching: "VEGF" or "VEGFA", "SNPs", "cancer", "oral", "oesophageal", "gall bladder", "hepatocellular", "gastric", "colon", "colorectal", "pancreatic" and "angiogenesis". The references cited in the identified published studies were independently screened to identify eligible articles, missed during the initial search.

Inclusion criteria

The main selection criteria for employing the five studies in the meta-analysis were:

1) Case–control studies examining the relationship between *VEGF*-2549I/D polymorphism with different GIT cancer risk.
2) Study population: Patients suffering from GIT cancers and healthy controls
3) Genotypic distribution in complete agreement with Hardy–Weinberg equilibrium (HWE) in controls in all of these studies.
4) High or medium quality studies, as per the Newcastle–Ottawa Scale assessment
5) Sufficient genotype data available in the studies to calculate the odds ratio, 95%CIs and p values.

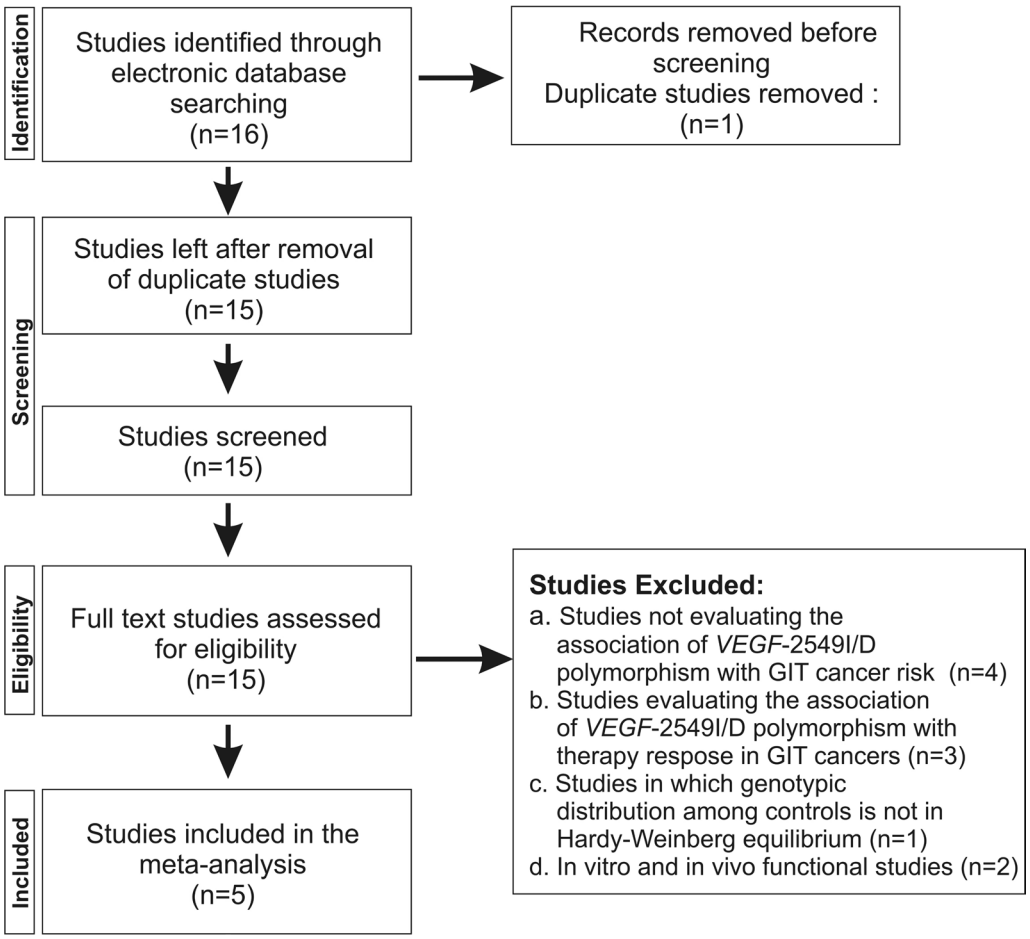

Fig. 1 The PRISMA flowchart of the study selection process

Study selection

All the retrieved research studies were reviewed and analysed carefully by the two authors. The titles and abstract of each research article were screened, and studies following the inclusion criteria were included in the meta-analysis. Studies not following the study selection criteria were removed from the analysis. In case of disagreements between the study selection, the decision to include or exclude the study was only made after author discussions. The PRISMA flowchart depicting the procedure for the selection of the studies is demonstrated in Fig. 1.

Data extraction

The authors carefully extracted the relevant data from each included study in this meta-analysis. The data included: type of gastrointestinal tract cancer, the total number of study subjects, genotypic distribution of *VEGF*-2549I/D polymorphism, HWE p value in controls, population and ethnicity of study subjects, main author's last name along with publishing year and key findings of study.

Quality assessment

The quality of the selected studies was assessed by scoring the studies according to the Newcastle–Ottawa Scale scoring system [46]. Each study was awarded one star under selection (4 items) and exposure (3 items) category, and two stars were given in the comparability category. The studies with an evaluation score of ≥ 7 were considered high-quality studies, 4–6 were considered medium quality, and < 4 were considered low-quality studies.

Statistical analysis

The deviation from the HWE was tested using the chi-squared test among the control subjects ($p > 0.05$). The combined ORs, 95%CIs and p value < 0.05 were calculated to determine the risk of GIT cancers with *VEGF*-2549I/D polymorphism under seven genetic models (allelic, dominant, recessive, over-dominant, II versus DD, II versus ID and ID versus DD comparison model).

Heterogeneity analysis

The Q statistic and I^2 metric tests were performed to calculate the heterogeneity between the selected studies. The fixed effect model was applied in the absence of inter-study heterogeneity. However, in the presence of inter-study heterogeneity, a random effects model was applied for performing the statistical analysis. Higher I^2 values indicated a higher degree of heterogeneity among the studies [47].

Sensitivity analysis

The stability of the results was determined by performing the sensitivity analysis. The analysis was done by sequentially omitting each study and then re-analysing the association with GIT cancer risk to determine the influence of each study on the overall pooled results.

Publication bias

The graphical symmetry of the constructed funnel plots and the probability value for Egger's test were taken as standards to calculate the presence or absence of bias in the study results.

Trial sequential analysis (TSA)

There are more chances of type I and type II errors in the results prepared through a meta-analysis study; therefore, the TSA was performed. The TSA was done for checking the reliability and robustness of the meta-analysis results. The TSA was performed to estimate the required sample size using the online available TSA software 0.9.5.10 Beta. The study was conducted using assumptions of 95%CI, 10% relative risk reduction (RRR), 5% α level and 80% β value. The crossing of both the TSA monitoring boundaries and required information size (RIS) by the cumulative Z values is indicative of a conclusive decision, requiring no further clinical trials. However, if TSA monitoring boundaries are not crossed before reaching the RIS, it indicates that the sample size is inadequate and more studies are needed for a conclusive decision.

Results

Characteristics of included studies

Five case–control studies were selected for analysis after applying the selection criteria. Out of the five studies, four studies included patients from Asian ethnicity, whereas only one study included patients from Caucasian ethnicity. Among the five included studies, two were reported on oesophageal cancer and one study each was reported on gastric, hepatocellular and colorectal cancer. All the included studies followed the Hardy–Weinberg equilibrium assumption in control subjects ($p > 0.05$). All of the studies used the direct-polymerase chain reaction (PCR) method for genotyping *VEGF*-2549I/D polymorphism. The contribution of *VEGF*-2549I/D polymorphism in influencing cancer risk was reported in oesophageal and gastric cancer; however, no relationship was reported with hepatocellular and colorectal cancer. The important salient features of all selected studies are detailed in Table 1.

Table 1 General characteristics of the studies including in the meta-analysis

Cancer	Patients / Controls	Genotypic distribution	HWE p-value in controls	Study included	Population	Ethnicity	Inference	References
		Patients (P): DD/ID/II Controls (C): DD/ID/II						
Oesophageal	200/200	55/104/41 74/101/25	0.29	Yes	North Indian	Asian	↑ risk with II genotype and I allele	[22]
	290/322	59/154/77 44/168/110	0.11	Yes	Han Chinese	Asian	↑ risk with DD genotype and D allele	[23]
Gastric	180/360	34/107/39 132/177/51	0.50	Yes	South Indian	Asian	↑ risk with ID, II genotype and I allele	[24]
Gall Bladder	195/300	32/103/60 54/173/73	0.006	No	North Indian	Asian	No association	[26]
Hepatocellular	206/302	118/73/15 168/114/20	0.91	Yes	Han-Chinese	Asian	No association	[25]
Colorectal	302/336	82/150/70 83/181/72	0.15	Yes	Swedish	Caucasian	No association	[27]

Table 2 Quality assessment of the included studies according to the Newcastle–Ottawa Scale

References	Selection	Comparability	Exposure	Score	Quality
[22]	****	**	***	9	High
[23]	****	-	***	7	High
[24]	***	-	***	6	Medium
[25]	****	**	**	8	High
[27]	****	*	-	5	Medium

Different no of stars were used for rating the studies according to Newcastle-Ottawa qulity assessment scale as explained in material and method section

Assessment of the quality of selected studies

As per the Newcastle–Ottawa scale, out of five studies (Table 2), four studies had high-quality (evaluation score ≥ 7), whereas one study had medium quality (evaluation score = 4–6).

Meta-analysis of VEGF-2549I/D polymorphism in GIT cancer

The current meta-analysis included a total of 1178 patients and 1520 controls to calculate pooled odds ratio (OR). The results indicated that *VEGF*-2549I/D polymorphism was not associated with susceptibility to GIT cancers ($p > 0.05$) in any of the studied genetic model in the overall analysis (Table 3) and on data stratification based on ethnicity (Table 4). Stratification of the data based on the different GIT cancers revealed an increased gastric cancer risk under allele contrast (OR = 1.67, CI = 1.294–2.157, $p = 0.00008$), recessive (OR = 1.68, CI = 1.056–2.660, $p = 0.029$), dominant (OR = 2.49, CI = 1.617–3.823, $p = 0.00003$), over-dominant (OR = 1.52, CI = 1.055–2.177, $p = 0.025$), II vs DD (OR = 2.97, CI = 1.692–5.208, $p = 0.00015$) and ID vs DD (OR = 2.35, CI = 1.501–3.669, $p = 0.00018$) genetic models (Table 5). All the results were graphically displayed as forest plots (Fig. 2).

Assessment of the publication bias and sensitivity analysis

There was no publication bias observed in our study in any of the studied genetic models as evidenced by the graphically constructed funnel plots (Fig. 3). The Egger's test p value ($p > 0.05$) indicated no bias in all studied genetic models. We omitted each study and then re-analysed the results and observed no substantial change in the overall results which further confirms the stability of our results (Fig. 4).

Trial sequential analysis

The allelic model was used as an example for performing the TSA. There was no crossing of the cumulative Z-value with the TSA monitoring boundaries before reaching the required information size in this study (Fig. 5). Therefore, no conclusion can be drawn from the meta-analysis study and more clinical studies are required in future to confirm the reliability of the study.

Assessment of the inter-study heterogeneity

In this meta-analysis study, on account of significant heterogeneity observed among the included studies under the allele contrast, recessive, dominant, II versus DD and ID versus DD genetic model, the random effect model was applied in the statistical analysis. The potential sources of heterogeneity were investigated by performing the subgroup analysis based on ethnicity and different tumour types. After subgroup analysis, significant heterogeneity remained in Asians and in oesophageal cancer subgroup. Other reasons for this heterogeneity could be difference in sample size, gender distributions, lifestyle factors, variations in age of the study participants and different sources of controls.

Discussion

VEGF glycoprotein is considered an important angiogenic activator required for tumour angiogenesis. *VEGF* promoter harbours several single nucleotide polymorphisms (SNPs) which impact the binding of the

Table 3 Association of *VEGF*-2549I/D polymorphism with GIT risk in the overall population

Model	Association test			Heterogeneity			Publication bias
	OR	95% CI	p-value	Model	p-value	I^2	Egger's test p-value
Allele contrast	1.11	0.831- 1.482	0.481	Random	0	0.848	0.387
Recessive	1.18	0.814- 1.713	0.382	Random	0.011	0.693	0.297
Dominant	1.14	0.728- 1.781	0.571	Random	0	0.843	0.464
Over-dominant	1.04	0.887- 1.209	0.659	Fixed	0.171	0.376	0.497
II vs. DD	1.28	0.683- 2.393	0.443	Random	0	0.846	0.448
II vs. ID	1.08	0.876- 1.326	0.477	Fixed	0.206	0.323	0.265
ID vs. DD	1.11	0.734- 1.662	0.633	Random	0.0007	0.793	0.415

OR: Odds Ratio, CI: Confidence Interval

Table 4 Association of *VEGF*-2549I/D polymorphism with GIT cancer risk based on ethnicity

Model	Group	Number of studies	Association test			Heterogeneity			Publication bias
			OR	95% CI	p-value	Model	p-value	I^2	Egger's test p-value
Allele	Overall	5	1.11	0.831–1.482	0.481	Random	0	0.848	0.387
Contrast	Asian	4	1.15	0.783- 1.678	0.484	Random	0	0.882	0.495
	Caucasian	1	0.99	0.791- 1.229	0.901	Fixed	NA	NA	NA
Recessive	Overall	5	1.18	0.814 -1.713	0.382	Random	0.0112	0.693	0.297
	Asian	4	1.22	0.728–2.031	0.455	Random	0.0046	0.770	0.370
	Caucasian	1	1.11	0.762- 1.607	0.596	Fixed	NA	NA	NA
Dominant	Overall	5	1.14	0.728- 1.781	0.571	Random	0	0.843	0.464
	Asian	4	1.22	0.689- 2.155	0.497	Random	0	0.872	0.656
	Caucasian	1	0.88	0.617- 1.255	0.481	Fixed	NA	NA	NA
Over-dominant	Overall	5	1.04	0.887- 1.209	0.659	Fixed	0.1705	0.376	0.497
	Asian	4	1.11	0.926- 1.323	0.264	Fixed	0.2371	0.292	0.931
	Caucasian	1	0.85	0.619- 1.154	0.289	Fixed	NA	NA	NA
II vs. DD	Overall	5	1.28	0.683- 2.393	0.443	Random	0	0.846	0.448
	Asian	4	1.38	0.592- 3.193	0.459	Random	0	0.880	0.564
	Caucasian	1	0.98	0.628- 1.541	0.944	Fixed	NA	NA	NA
II vs. ID	Overall	5	1.08	0.876- 1.326	0.477	Fixed	0.206	0.323	0.265
	Asian	4	1.04	0.818- 1.332	0.730	Fixed	0.1291	0.471	0.259
	Caucasian	1	1.17	0.791- 1.739	0.427	Fixed	NA	NA	NA
ID vs. DD	Overall	5	1.11	0.734- 1.662	0.633	Random	0.0007	0.793	0.415
	Asian	4	1.19	0.714- 1.980	0.506	Random	0.0007	0.825	0.636
	Caucasian	1	0.84	0.577- 1.219	0.357	Fixed	NA	NA	NA

OR: Odds Ratio, CI: Confidence Interval

transcription factors, alter the VEGF expression and contribute to the disease development [15]. So far, six case–control genetic association studies have investigated the relationship of *VEGF*-2549I/D polymorphism with GIT cancer susceptibility. It was reported that the *VEGF*-2549I allele and II genotype conferred increased oesophageal cancer risk in the North-Indian population [22]. In South Indians, individuals carrying *VEGF*-2549ID and II genotype had an increased susceptibility towards gastric cancer [24] The *VEGF*-2549 D allele and DD genotype increased the susceptibility towards oesophageal cancer in Chinese patients [23]. However, studies conducted on North Indian gall bladder cancer patients [26], Chinese hepatocellular cancer patients [25] and Swedish colorectal cancer patients [27] reported no role of *VEGF*-2549I/D polymorphism in determining cancer susceptibility. Apart from GIT cancers, *VEGF*-2549I/D polymorphism has been reported to be associated with risk of several cancers. In North Indians, the *VEGF*-2549ID genotype was associated with a reduced risk of bladder cancer [32] and a higher risk of developing prostate cancer [36]. The *VEGF*-2549II genotype and I allele have been associated with increased risk of breast cancer in North Indians [34]. There was no association of *VEGF*-2549I/D polymorphism with breast cancer risk in the Iranian population [35]. The *VEGF*-2549D allele has been associated with a higher risk of developing renal cell carcinoma in Caucasian subjects [31]. Ben Wafi et al. [33] reported no association of *VEGF*-2549I/D polymorphism with urothelial bladder cancer risk in the Tunisian population. The possible reasons for disagreements in study findings could be differences in the sample size, lifestyle factors and different ethnicities.

The impact of *VEGF*-2549I/D polymorphism on therapy response has been studied in a few GIT cancers. *VEGF*-2549 DD genotype was associated with a complete or partial response to chemotherapy in Han-Chinese oesophageal cancer patients [23] and with better treatment response and longer progression-free survival in Caucasian colorectal cancer (CRC) patients treated with FOLFIRI-cetuximab [29]. On the contrary, in Caucasian, African-American and Hispanic metastatic CRC patients treated with fluorouracil, irinotecan and bevacizumab, no association of *VEGF*-2549I/D polymorphism was reported [28]. *VEGF*-2549I/D polymorphism was not associated with clinicopathological characteristics and overall survival in Korean HCC patients treated with TACE therapy [30].

Table 5 Association of *VEGF*-2549I/D polymorphism with GIT cancer risk based on cancer type

Model	Group	No. of studies	Association test			Heterogeneity			Publication bias
			OR	95% CI	p-value	Model	p-value	I^2	Egger's test p-value
Allele	Overall	5	1.11	0.831- 1.482	0.481	Random	0	0.848	0.387
Contrast	Colorectal	1	0.99	0.791- 1.229	0.901	Fixed	NA	NA	NA
	Oesophageal	2	1.03	0.544- 1.948	0.930	Random	0.0004	0.920	NA
	Gastric	1	1.67	1.294- 2.157	**0.00008**	Fixed	NA	NA	NA
	Hepatocellular	1	0.97	0.730- 1.300	0.858	Fixed	NA	NA	NA
Recessive	Overall	5	1.18	0.814- 1.713	0.382	Random	0.011	0.693	0.297
	Colorectal	1	1.11	0.762- 1.607	0.596	Fixed	NA	NA	NA
	Oesophageal	2	1.10	0.431- 2.781	0.848	Random	0.004	0.881	NA
	Gastric	1	1.68	1.056- 2.660	**0.029**	Fixed	NA	NA	NA
	Hepatocellular	1	1.11	0.553- 2.217	0.774	Fixed	NA	NA	NA
Dominant	Overall	5	1.14	0.728- 1.781	0.571	Random	0	0.843	0.464
	Colorectal	1	0.88	0.617- 1.255	0.481	Fixed	NA	NA	NA
	Oesophageal	2	0.98	0.400- 2.404	0.965	Random	0.003	0.888	NA
	Gastric	1	2.49	1.617- 3.823	**0.00003**	Fixed	NA	NA	NA
	Hepatocellular	1	0.94	0.654- 1.337	0.712	Fixed	NA	NA	NA
Over-dominant	Overall	5	1.04	0.887- 1.209	0.659	Fixed	0.171	0.376	0.497
	Colorectal	1	0.85	0.619- 1.154	0.289	Fixed	NA	NA	NA
	Oesophageal	2	1.05	0.818- 1.341	0.713	Fixed	0.930	0	NA
	Gastric	1	1.52	1.055- 2.177	**0.025**	Fixed	NA	NA	NA
	Hepatocellular	1	0.91	0.626- 1.308	0.596	Fixed	NA	NA	NA
II vs. DD	Overall	5	1.28	0.683- 2.393	0.443	Random	0	0.846	0.448
	Colorectal	1	0.98	0.628- 1.541	0.944	Fixed	NA	NA	NA
	Oesophageal	2	1.061	0.258- 4.354	0.935	Random	0.0003	0.924	NA
	Gastric	1	2.97	1.692- 5.208	**0.00015**	Fixed	NA	NA	NA
	Hepatocellular	1	1.07	0.525- 2.171	0.856	Fixed	NA	NA	NA
II vs. ID	Overall	5	1.08	0.876- 1.326	0.477	Fixed	0.206	0.323	0.265
	Colorectal	1	1.17	0.791- 1.739	0.427	Fixed	NA	NA	NA
	Oesophageal	2	1.07	0.520- 2.185	0.861	Random	0.033	0.781	NA
	Gastric	1	1.27	0.782- 2.047	0.338	Fixed	NA	NA	NA
	Hepatocellular	1	1.17	0.564- 2.433	0.672	Fixed	NA	NA	NA
ID vs. DD	Overall	5	1.11	0.734- 1.662	0.633	Random	0.0007	0.793	0.415
	Colorectal	1	0.84	0.577- 1.219	0.357	Fixed	NA	NA	NA
	Oesophageal	2	0.97	0.487- 1.946	0.940	Random	0.028	0.793	NA
	Gastric	1	2.35	1.501- 3.669	**0.00018**	Fixed	NA	NA	NA
	Hepatocellular	1	0.91	0.626- 1.329	0.630	Fixed	NA	NA	NA

OR: Odds Ratio, CI: Confidence Interval

The role of *VEGF*-2549I/D polymorphism might vary in different GIT cancer types as explained by differences in the pathologies behind different GIT cancer development. In this meta-analysis, we did not observe any relationship between *VEGF*-2549I/D polymorphism and GIT cancer susceptibility in the overall population as well as in the pooled analysis based on ethnicity. However, *VEGF*-2549I/D polymorphism increased the susceptibility to gastric cancer under allele contrast, recessive, dominant, over-dominant, II versus DD and ID versus DD genetic models when data were stratified on the basis of different GIT cancer types. Meta-analysis is an improved statistical study design in genetic association studies. It resolves the disagreements in different study results by combining the findings of case–control studies with small sample size and thereby increases the statistical power of the meta-analysis [48].

The cancerous cells' interaction with the stromal cells creates a tumour environment required for the metastasis, growth and vascularization of tumours in gastric cancer [49–51]. It has been reported that angiogenesis influences tumour progression and prognosis

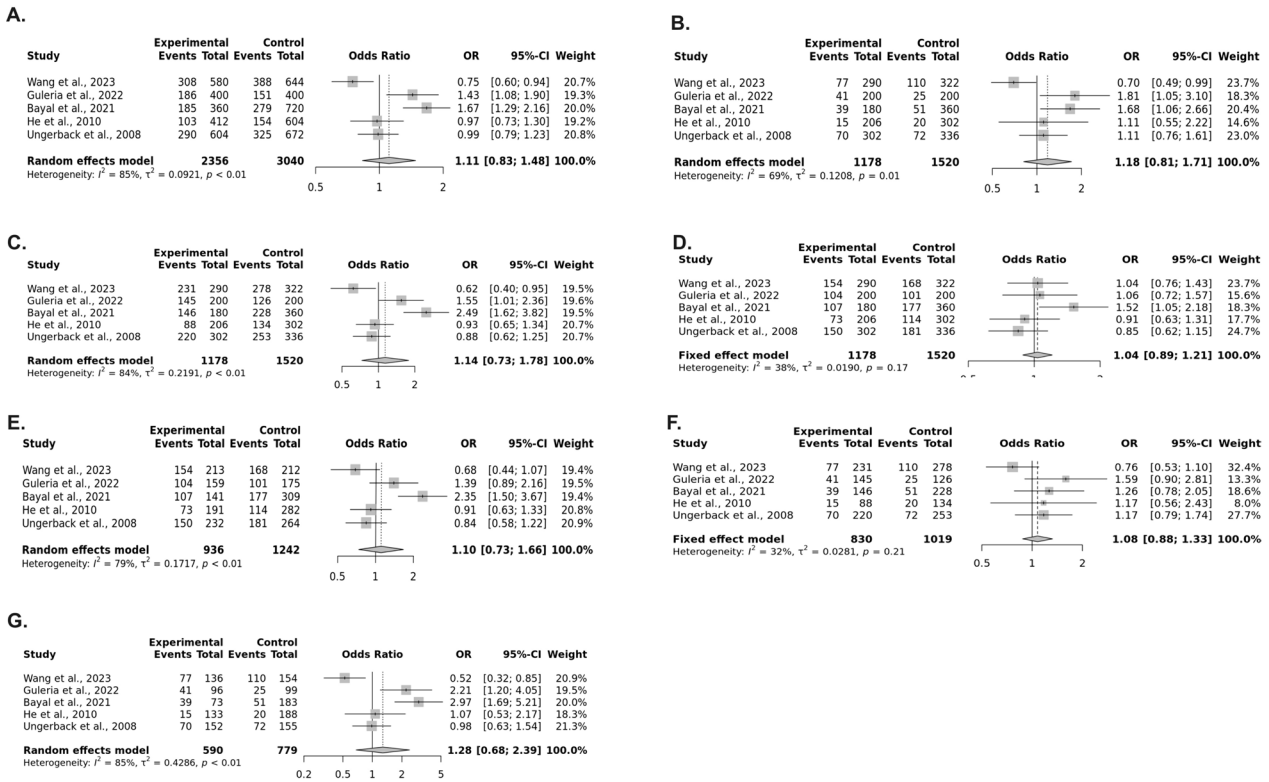

Fig. 2 Forest plots of *VEGF*-2549I/D polymorphism under different genetic models **A** Allele contrast, **B** Recessive, **C** Dominant, **D** Over-dominant, **E** ID vs DD, **F** II vs ID and **G** II vs DD genetic model

in gastric cancer [52]. The correlation of VEGF protein with increased vascularity [53], increased angiogenesis and gastric tumour progression [54–56] has been previously studied. Elevated VEGF expression in tumour has also been correlated with unfavourable prognosis in gastric cancer [53, 57]. Increased VEGF serum levels were associated with distant tissue invasion [58], whereas the higher VEGF plasma levels were associated with increased venous invasion [59] in gastric cancer patients. The *VEGF*-2549I/D polymorphism might regulate the VEGF expression in gastric cancer patients, ultimately affecting cancer susceptibility and prognosis in gastric cancer patients.

The present meta-analysis has several advantages. It is the first meta-analysis report evaluating the role of *VEGF*-2549I/D polymorphism in GIT cancer susceptibility. We confirmed the robustness of the results by performing sensitivity analysis and trial sequential analysis. In this study, publication bias was not observed in any genetic model.

However, some limitations are still present in this meta-analysis. It involved only few case–control studies and the total sample size of the study was not enough as reported by the TSA results. We observed significant heterogeneity in the included studies and could not identify the main source of heterogeneity which might affect results interpretation. Lastly, we did not stratify the studies based on confounding factors like age, body mass index, sex, family history or lifestyle factors involved in GIT development because of unavailability of research data and the small sample size of the studies. Several previous published studies have evaluated the risk of bias of each included study in the randomized clinical trials [60–62]. In the present analysis, we did not calculate the risk of bias for each case–control study included in the meta-analysis.

In conclusion, *VEGF*-2549I/D polymorphism was associated with an increased risk of gastric cancer only. GIT cancers are polygenic multifactorial disorders, influenced by several lifestyle factors including tobacco smoking, diet, physical activity, alcohol consumption, habitat and chemical exposure. Therefore, the study of the synergistic effect of the *VEGF* polymorphisms and the environmental factors is needed in future to explore the potential role of *VEGF* polymorphisms in influencing GIT cancer susceptibility. Larger case–control studies on diverse populations evaluating the combined effect of multiple genetic variants in angiogenesis pathway-associated genes are also needed to understand the exact biological mechanisms behind the GIT cancers development.

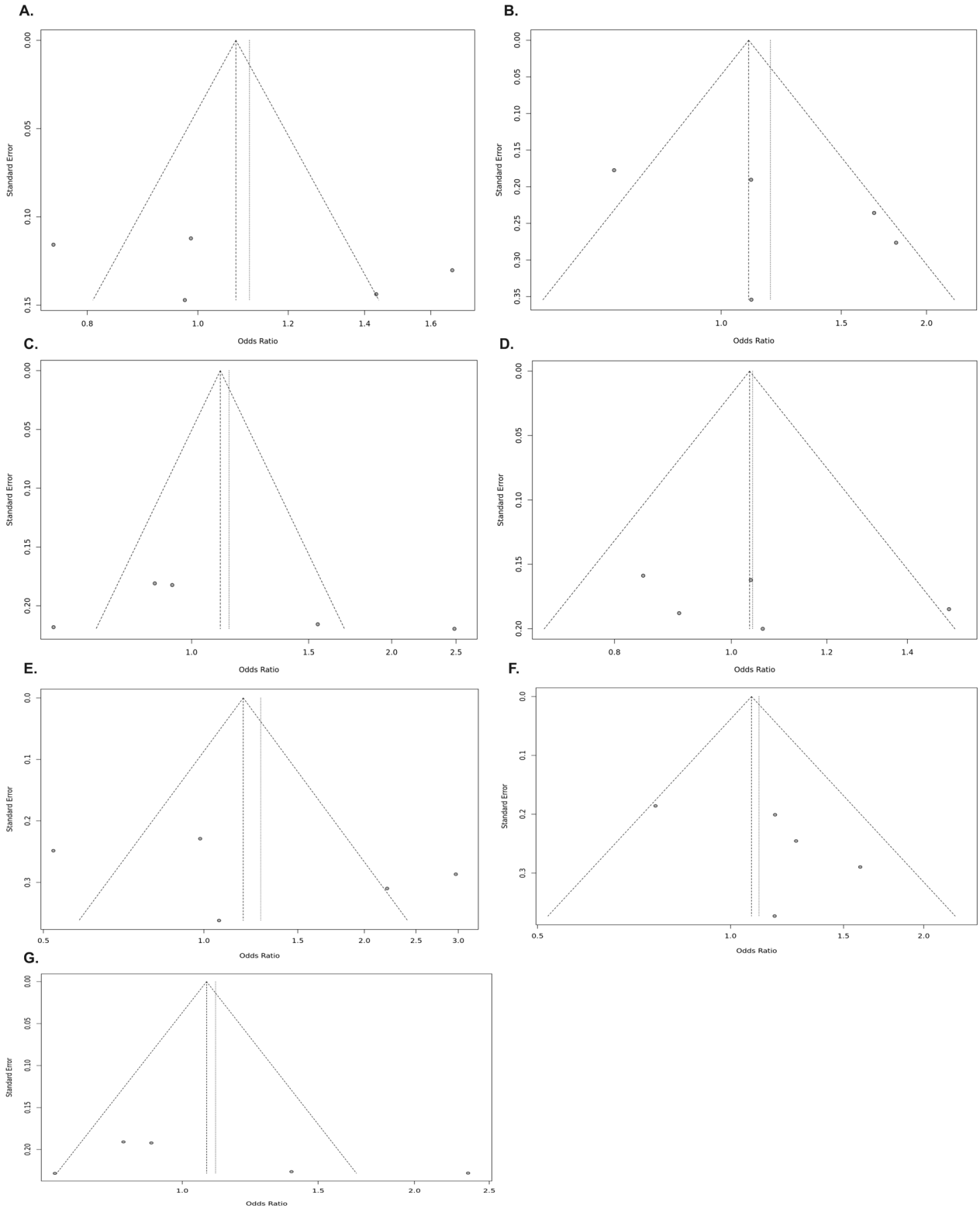

Fig. 3 Funnel plots of *VEGF*-2549I/D polymorphism under different genetic models **A** Allele contrast, **B** Recessive, **C** Dominant, **D** Over-dominant, **E** II vs DD, **F** II vs ID and **G** ID vs DD genetic model

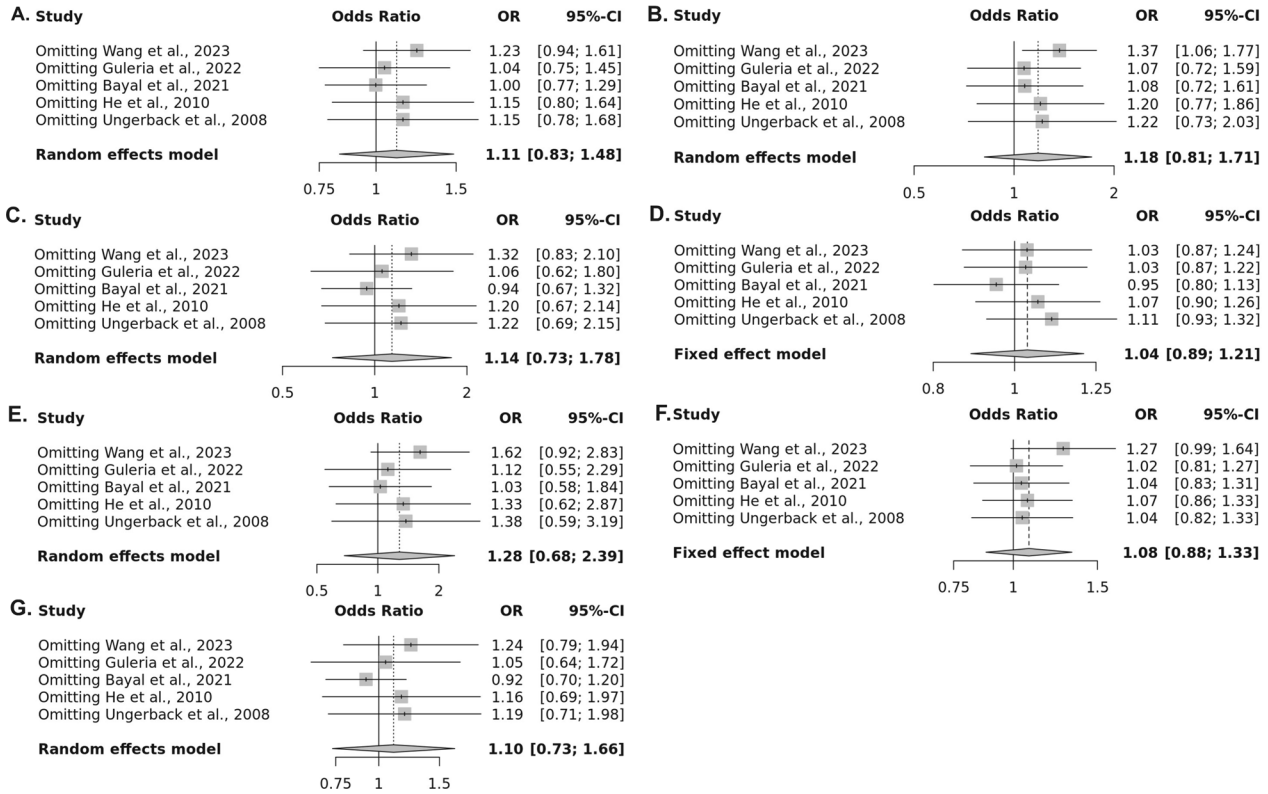

Fig. 4 Sensitivity analysis of *VEGF*-2549I/D polymorphism under different genetic models **A** Allele contrast, **B** Recessive, **C** Dominant, **D** Over-dominant, **E** II vs DD, **F** II vs ID and **G** ID vs DD genetic model

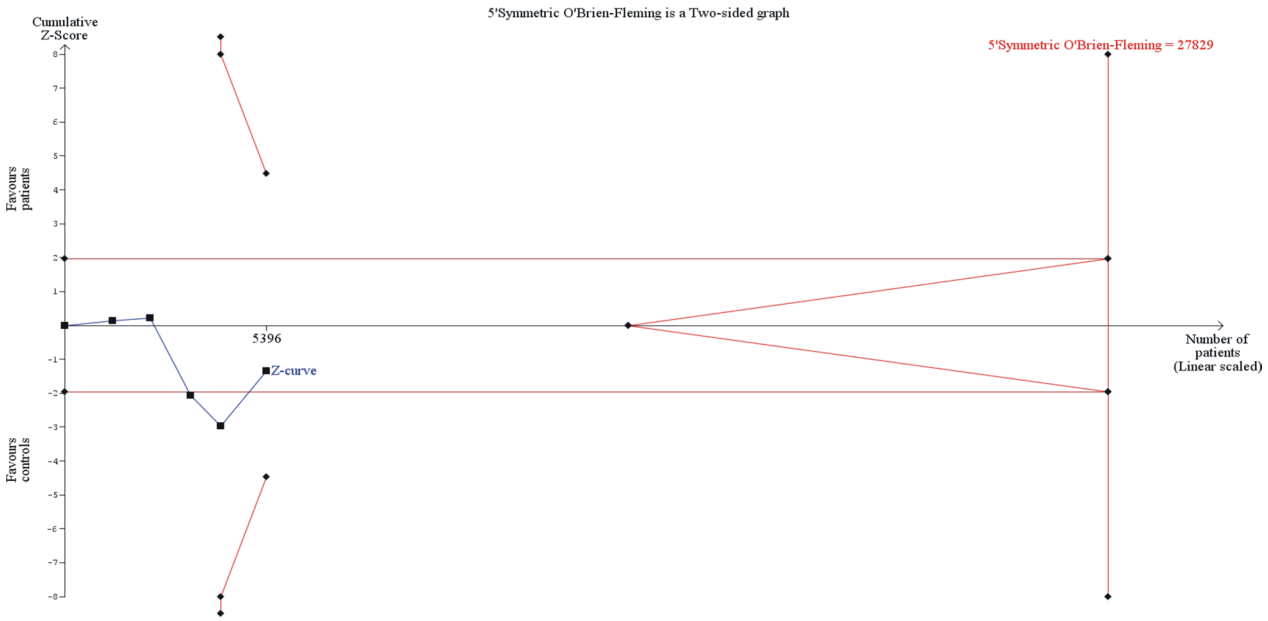

Fig. 5 Trial sequential analysis of *VEGF*-2549I/D polymorphism under allelic model

Abbreviations

GIT	Gastrointestinal tract
VEGF	Vascular endothelial growth factor
GWAS	Genome-wide association studies
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PICO	Participant, intervention, comparison and outcome
HWE	Hardy–Weinberg equilibrium
CIs	Confidence intervals
TSA	Trial sequential analysis
RIS	Required information size
RRR	Relative risk reduction
Direct-PCR	Direct-polymerase chain reaction
SNP	Single nucleotide polymorphism

Author contributions
KG and VS designed the study. DM and KG prepared the manuscript. All authors read and approved the manuscript.

Funding
Not applicable.

Availability of data and materials
All data relevant to this study have been included in the manuscript or uploaded as supplementary information.

Declarations

Ethics approval
Not applicable.

Informed consent
Not applicable

Competing interests
All the authors declare that they have no conflicts of interest.

Received: 3 January 2024 Accepted: 9 June 2024
Published online: 17 June 2024

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