SYSTEMATIC REVIEW



Association between nitric oxide and cancer and stroke risk: A

meta-analysis [version 1; peer review: 2 not approved]

Abdul Rohim Tualeka^{1,3}, Juliana Jalaludin^{2,}, Janvier Gasana^{1,3}, Nor Ashikin Sopian^{1,4}, How Ran Chao^{1,5}, Mohd Yusmaidie^{1,6}, Velu Perumal^{1,7}, Suardi Zurimi⁸, Pudji Rahmawati⁹, Ahsan Ahsan¹⁰, Salsabila Novianti¹¹

¹Department of Occupational Health and Safety, Faculty of Public Health, Universitas Airlangga, Surabaya, East Java, 60115, Indonesia

²Department of Environmental and Occupational Health, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia

³Department of Environmental & Occupational Health, College of Public Health, Health Sciences Center Kuwait University, Kuwait University, Kuwait City, Shadadiya, Kuwait

⁴Occupational Safety and Health Program, Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang, Kuantan, Pahang, 26300, Malaysia

⁵Department of Environmental Science and Engineering, National Pingtung University of Science and Technology, Pingtung, Neipu, Taiwan

⁶Department of Toxicology, Advanced Medical & Dental Institute, Universiti Sains Malaysia, Bertam Kepala Bartas, Penang, 13200, Malaysia

⁷Department of Industrial Design, Faculty of Design and Architecture, Universiti Putra Malaysia, Serdang, Selangor, 43400, Malaysia ⁸Politeknik Kesehatan Kemenkes Maluku, Maluku, Indonesia

⁹Department of Development of Islamic Society, Universitas Islam Negeri Sunan Ampel Surabaya, Surabaya, East Java, Indonesia ¹⁰Faculty of Nurse, Universitas Brawijaya, Malang, East Java, Indonesia

¹¹Department of Environmental Health, Faculty of Public Health, Universitas Airlangga, Surabaya, East Java, 60115, Indonesia

V1First published: 13 Nov 2023, 12:1467
https://doi.org/10.12688/f1000research.134992.1Open Peer ReviewSecond version: 28 Mar 2024, 12:1467
https://doi.org/10.12688/f1000research.134992.2Approval Status ××Latest published: 10 Jun 2024, 12:1467
https://doi.org/10.12688/f1000research.134992.31

Abstract

Background: Numerous case-control studies have been carried out to test the mechanism by which nitric oxide, specifically the polymorphism 894G>T in the eNOS gene, or endothelial nitric oxide synthase, raises the possibility of stroke and cancer. **Methods:** The aim of this meta-analysis was to describe the correlation between cancer and stroke risk with nitric oxide, by implementing a comprehensive search in various digital databases, including Science Direct, PubMed, and Google Scholar, in the period 2012-2023 to observe the published results of all related studies. **Results:** The meta-analysis included a total of fifteen case-control studies. These studies involved 3,019 cases and 3,333 controls in total. This study found that the GG *versus* GT+TT genotype of eNOS 894G>T polymorphism was significantly positively correlated with cancer risk. Additionally, the significance of this association was further attributed to the specific type of polymorphism involved, as well as the risk of

	1	2
version 3		
(revision)		
10 Jun 2024		
version 2		
(revision)		view
28 Mar 2024		view
version 1	×	×
13 Nov 2023	view	view

1. Eric Tzyy Jiann Chong ២, Universiti

Malaysia Sabah, Sabah, Malaysia

stroke in the T *versus* G model, followed by TT *versus* GG+GT. **Conclusions**: The results of the eNOS 894G>T polymorphisms have been correlated with cancer, and in particular, the GT+TT *versus* GG model yielded an odds ratio (OR of 1.96, a 95% CI of 1.22 to 3.15, and a *p*-value of 0.0005. Moreover, the mentioned polymorphisms were found to be associated with stroke risk in the T *versus* G model, which had an OR of 1.20; 95% CI of 1.01 to 1.43 with a *p*-value of 0.04; and TT *versus* GG+GT with an OR of 0.09; 95% CI of 0.03 to 0.30 with a *p*-value of 0.0001.

Keywords

Nitric oxide, eNOS 894G>T, polymorphism, cancer, stroke, metaanalysis, safe work



This article is included in the Oncology

gateway.

2. Katrina Miranda, University of Arizona, Arizona, USA

Any reports and responses or comments on the article can be found at the end of the article.

Corresponding author: Abdul Rohim Tualeka (abdul-r-t@fkm.unair.ac.id)

Author roles: Tualeka AR: Conceptualization, Funding Acquisition, Methodology, Project Administration, Resources, Validation, Writing – Original Draft Preparation, Writing – Review & Editing; Jalaludin J: Writing – Review & Editing; Gasana J: Writing – Review & Editing; Sopian NA: Writing – Review & Editing; Chao HR: Writing – Review & Editing; Yusmaidie M: Writing – Review & Editing; Perumal V: Supervision, Validation, Writing – Original Draft Preparation; Zurimi S: Writing – Review & Editing; Rahmawati P: Writing – Review & Editing; Ahsan A: Writing – Review & Editing; Novianti S: Project Administration, Writing – Original Draft Preparation

Competing interests: No competing interests were disclosed.

Grant information: This research was funded by Lembaga Penelitian dan Pengabdian Kepada Masyarakat Universitas Airlangga, grant number 333/UN3.15/PT/2023.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2023 Tualeka AR *et al*. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Tualeka AR, Jalaludin J, Gasana J *et al.* Association between nitric oxide and cancer and stroke risk: A metaanalysis [version 1; peer review: 2 not approved] F1000Research 2023, **12**:1467 https://doi.org/10.12688/f1000research.134992.1

First published: 13 Nov 2023, 12:1467 https://doi.org/10.12688/f1000research.134992.1

Introduction

Nitric oxide, or NO, is a chemical compound found in organisms such as mammals. For example, in humans, NO acts as a signaling molecule in various physiological and pathological processes in the body and simultaneously, becomes a diatomic free radical, produced by the enzymatic activity of NOS itself on the L-Arginine compound, yielding the production of L-citrulline along with NO (Korde Choudhari *et al.*, 2013). The NOS family consists of three members, namely eNOS, nNOS, and finally iNOS (Sachdev, 1999). NO influences the coagulation process, neuronal activity, and cerebral blood flow (Korde Choudhari *et al.*, 2013). NO has the potential to induce cellular inflammation, which can delay the onset of stroke. Additionally, NO can act as a carcinogen, increasing cancer risk.

NO plays a substantial role in cancer's progression and development. NO promotes cancer progression and metastasis via polyamine synthesis or inhibition of NO-mediated tumor cytotoxicity (Gào and Schöttker, 2017; Gào *et al.*, 2019). The roles and functions of NO have been extensively investigated in numerous types of cancer. The response to hypoxia involves NO, which plays a crucial role in inducing angiogenesis and promoting cancer cell defense, and is attributed to the mutagenic behavior exhibited by NO. When cells are exposed to NO for a considerable duration, it is commonly a result of iNOS being produced during chronic inflammation, which gives it a role in carcinogenesis (Utispan and Koontongkaew, 2020). iNOS is considered to have an impact on the mechanism of carcinogenesis.

iNOS plays a multifaceted role in tumor building through its involvement in genetic changes, angiogenesis, proliferation, metastasis, and immunosuppression (Erlandsson *et al.*, 2018). Several studies have proven NO's influence on both illnesses. NO has been demonstrated to contribute to lung cancer's progression (Chen *et al.*, 2008). NO can potentially promote the progression of pulmonary carcinoma through a process called protein nitration. NO can also cause head-neck cancer in smokers and people with alcohol use disorders (Patel *et al.*, 2009). The subtype NO, which is iNOS/NOS2, is considered to be correlated with a raised risk of development of prostate cancer (Aaltoma, Lipponen and Kosma, 2001). NO can also result in the onset of breast cancer (Loibl *et al.*, 2002).

NO has a critical part in regulating cerebral circulation and modulating neuronal activity. Microvascular endothelial cells in the brain, also known as the endothelium, are capable of producing and releasing various vasoactive substances, among which NO. The continuous production of NO by the endothelium in basal situations and its reactions to vasoactive stimuli provide knowledge of the complex regulation of cerebral circulation and the maintenance of vascular health in the brain. This dysfunction in NO production and release could be a factor in the progression of stroke. Stroke refers to a clinical condition characterized by unexpected loss of cerebral responsibility as a result of vascular pathology in the brain (Demaerschalk *et al.*, 2016). NO is essential to stroke as an important signaling molecule. The harmful effects of NO derived from iNOS and nNOS primarily stem from the generation of nitrates and free radicals (Zhao *et al.*, 2000). nNOS and iNOS are involved in causing nerve injury during both the beginning and final stages of brain ischemia. Conversely, when eNOS is activated, it has a neuroprotective effect (Chen *et al.*, 2017).

The NOS isoform responsible for producing NO in the vascular endothelium is known as endothelial NOS (eNOS), which in its isoform is expressed through cells and actively contributes to normal vascular tone in physiological conditions. eNOS has also been extensively investigated in the context of carcinogenesis, particularly its involvement in mediating tumor maintenance (Lim *et al.*, 2008). Furthermore, limited levels of NO produced by eNOS can have a neuroprotective effect on stroke through increased vasodilation and cerebral circulation (Yang *et al.*, 2019). Recently, several single nucleotide polymorphisms have been found in eNOS, among which 894G>T in exon 7.

Several case-control studies aimed to check whether the NO correlation with cancer and stroke risk exists, especially its polymorphism eNOS, as well as how these factors may impact the development of cancer and stroke risk. Hence, we conducted a meta-analysis to provide a clearer understanding of the association between NO levels and both cancer risks.

Methods

Search strategy

The research method used was a meta-analysis. Our meta-analysis adhered to the criteria recommended in the Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines, or PRISMA. To discover relevant primary articles, we performed a comprehensive search of digital databases, including PubMed, Science Direct, and Google Scholar, to identify all relevant studies on the correlation of NO especially eNOS 894>GT, and risk to cancer and stroke. The search period was limited from December 2022 to January 2023. We applied the following keywords: NO or NOS or eNOS or "eNOS 894G>T" AND "polymorphism" AND "cancer risk" AND "stroke risk". To ensure a comprehensive review of the literature, we conducted a thorough examination of the reference lists included in the recognized literature.

Inclusion and exclusion standard

Determination of inclusion and exclusion criteria followed PICOS (Problem, Intervention, Comparison, Outcome, and Study design). The included studies were carefully examined to ensure their relevance and quality for our study. No national restrictions were imposed, meaning studies from all countries were considered eligible for inclusion. The research that met the eligibility criteria was carefully selected for our analysis: 1) Articles published in the English language that investigated the correlation between NO, especially eNOS 894G>T, and the risk of cancer and stroke; 2) Designed as a case-control study; 3) Articles that provided detailed data on genotype and allele frequencies of eNOS gene polymorphisms, which has sufficient data for the calculation of the odds ratio (OR) and the confidence interval of 95%. Therefore, studies were excluded according to as the following criteria: 1) Qualitative research; 2) No available genotype frequency; 3) Studies without control; 4) Meta-analysis studies; and 5) Animal studies.

Data extraction

The data in all studies were extracted when sufficient criteria were met. We then used Microsoft Excel to record the year of publication, the last name of the authors, control, and case sample sizes, and the country of the study. The results were then compared after being extracted, and an assessment was carried out along with the resolution of matters that were not appropriate through consensus. We extracted data from the nine articles meeting eligibility criteria for cancer risk and the seven articles for stroke risk association with NO.

Statistical analysis

A statistical review was implemented using RevMan, Cochrane with version 5.4 to investigate the association between NO and the risk of stroke and cancer. Crude ORs and a CI of 95% were utilized. Pooled ORs were computed for various genetic models of the eNOS G894T gene polymorphism, including GT+TT *versus* GG, GT *versus* GG, TT *versus* GG, T *versus* GG, and G *versus* T. The eNOS gene encodes for endothelial nitric oxide synthase and has a polymorphism at position 894G>T that can result in GG, GT, or TT variants (Buldreghini *et al.*, 2010). G represents the homozygous wild-type genotype, where the individual has two copies of the G allele. GT represents the homozygous variant genotype, where the individual has two copies of the T allele (Hinz *et al.*, 2013). The calculation of pooled ORs allowed to perform a Z test with a significance level of $p \le 0.05$.

The presence of heterogeneity among the studies included was assessed with a Q test score. If there was no significant hterogeneity, *i.e.*, p>0.10, the effect model was applied consistently. Otherwise, when (p<0.10), the random-effects model was utilized. The diversity of the included research was assessed using the I² test, which quantifies the degree of heterogeneity. If the I² value was less than 25%, it indicated no heterogeneity. If the I² value ranged from 25% to 50%, it showed moderate heterogeneity between the studies and a random effects model (Mantel-Haenszel technique) was implemented; conversely, if no significant heterogeneity was found, the fixed effect model is applied. Publication bias assessment was not conducted in this study based on the limited number of articles included in meta-analysis (less than 10).

Results

Study processing

The flow diagram in Figure 1 summarises the study workflow. A total of 145 articles were identified in the databases. After removing duplicates, a total of 105 studies remained. These studies were further screened by reviewing the titles and abstracts, leading to the exclusion of 56 articles that did not meet the predetermined exclusion and inclusion conditions. After carefully examining the full text of the remaining 43 records, an additional 21 articles were excluded based on both exclusion and inclusion conditions. Ten other studies (da Costa Escobar Piccoli *et al.*, 2012; Jang *et al.*, 2013; Rah *et al.*, 2013; Akhter *et al.*, 2014; Kang *et al.*, 2014; Özçelik *et al.*, 2014; Ben Chaaben *et al.*, 2015; Hung *et al.*, 2019; Lee, 2019; Tsay *et al.*, 2013; Guo, 2014; Zhao *et al.*, 2014; Abedinzadeh *et al.*, 2020; Akbar *et al.*, 2022) were not included in the analysis because they were meta-analysis studies.

Finally, 15 qualified articles met the eligibility criteria. Nine case-control studies examined the association between NO and cancer risk, and six case-control studies analyzed the association between NO and stroke risk. Table 1 shows the features of the 15 studies incorporated in our analysis.

Meta-analysis

A total of 15 studies investigating an association between NO, cancer, and stroke risk, especially, the endothelial nitric oxide G894T polymorphism, were included. In our analysis, we identified a total of 2,013 cases and 2,187 control

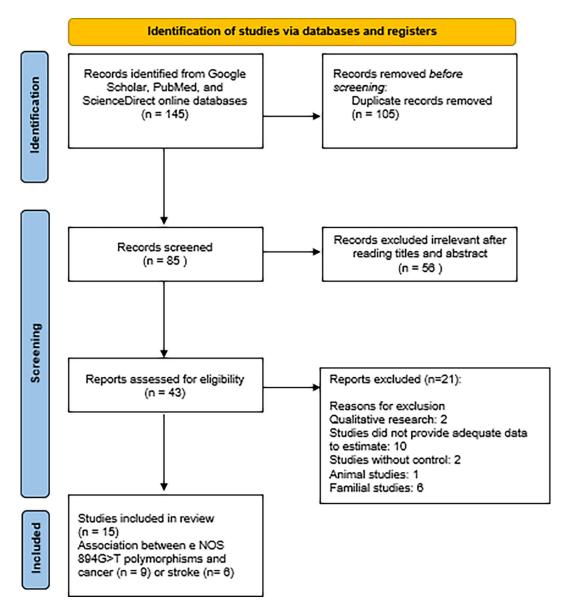


Figure 1. Flow diagram depicting the inclusion process of the studies in the meta-analysis.

subjects for assessing cancer risk, as well as 1006 cases and 1146 control subjects for evaluating stroke risk. Table 2 presents the aggregated outcome polymorphism through meta-analysis and its association with cancer risk. The results showed a substantial correlation of cancer risk and the eNOS polymorphism G894T, with comparisons of T *versus* G yielding an OR of 1.00 (95% CI 0.44 to 2.27, where p=1.00), G *versus* T, where the OR was 1.00 (95% CI 0.44 to 2.27) with p=1.00, and TT *versus* GG+GT, where the OR was 0.51 (95% CI is 0.22 to 1.17 and p=0.11), comparison of GT *versus* GG+TT with an OR of 1.21; 95% CI is 0.77 to 1.91, and p=0.41).

Table 3 presents the consolidated findings of the meta-analysis, demonstrating the significant association between the eNOS G894T gene and the risk of stroke where the comparison is T *versus* G, namely the OR of 1.20 (95% CI=1.01 to 1.43) with a *p* of 0.04 and the TT *versus* GG+ GT comparison with an OR of 0.08 (95% CI, namely 0.03 to 0.30), where *p* is 0.0001. However, a significant correlation was not found in G *versus* T, where the OR is 0.88 (95% CI, namely 0.74 to 1.05) with *p*=0.15, and in TT *versus* GG+GT, where the OR was 0.68 (95% CI was 0.24 to 1.93 and *p*=0.47) compared with GT *versus* GG+TT, where the OR was 1.03 (95% CI was 0.40 to 2.64, and *p*=0.95).

First Author/Year	Country	Risk	Case/Control	Case					Control	_			
				Genotype	/pe		Allele		Genotype	/pe		Allele	
				99	F	G	F	U	90	F	GT	F	U
(Adibmanesh <i>et al.</i> , 2020)	Iran	Cancer	100/100	28	28	44	100	100	57	9	37	49	151
(Aouf <i>et al.</i> , 2019)	Tunisia	Cancer	259/169	149	20	06	130	388	73	18	78	114	224
(Branković <i>et al.</i> , 2013)	Serbia	Cancer	150/100	76	6	65	83	217	54	9	40	52	148
(Carkic <i>et al.</i> , 2020)	Serbia	Cancer	50/110	21	ß	24	34	66	61	7	42	56	164
(Koçer <i>et al.</i> , 2020)	Turkey	Cancer	107/100	74	-	32	34	180	65	-	34	36	164
(Su <i>et al.</i> , 2018)	Taiwan	Cancer	1044/1200	825	10	209	229	1859	935	15	250	280	2120
(Verim <i>et al.</i> , 2013)	Turkey	Cancer	66/88	7	10	49	69	63	31	13	44	70	106
(Yadav <i>et al.</i> , 2019)	India	Cancer	179/173	88	20	64	104	240	96	∞	59	75	251
(Yanar <i>et al.</i> , 2016)	Turkey	Cancer	58/147	18	11	29	51	65	31	35	81	151	143
(Anliaçik <i>et al.</i> , 2019)	Turkey	Stroke	112/160	21	14	77	4	40	38	19	103	57	61
(Diakite <i>et al.</i> , 2014)	Morocco	Stroke	165/182	83	16	66	30	70	117	7	58	20	81
(El Gohary, El Azab and Kamal El-Din, 2017)	Egypt	Stroke	30/10	18	9	9	45	15	ъ	m	m	12	∞
(Kaur, Uppal and Kaur, 2015)	India	Stroke	120/101	84	9	30	18	83	83	-	17	6	91
(Kumar <i>et al.</i> , 2016)	India	Stroke	250/250	164	12	74	20	80	186	ß	59	14	86
(Shyu <i>et al.</i> , 2017)	Taiwan	Stroke	229/243	151	16	62	21	50	185	7	51	13	87

Table 1. Characteristics and genetic frequencies derived the studies included in the meta-analysis.

Genetic Models	NS	Pooled ORs (95% CI)	<i>p</i> value ^a (Z test)	<i>I</i> ² (%)	рН	ρE	Method
T vs G	9	1.00 (0.44,2.27)	1.00	94	<0.00001	0.17497	Ramdom model
G vs T	9	1.00 (0.44,2.27)	1.00	94	<0.00001	0.34809	Ramdom model
GT+TT vs GG	9	1.96 (1.22,3.15)	0.005	85	<0.00001	0.42245	Ramdom model
TT vs GG+GT	9	0.51 (0.22-1.17)	0.11	84	<0.00001	0.03886	Ramdom model
GT vs GG+TT	9	1.21 (0.77-1.91)	0.41	84	<0.00001	0.13326	Ramdom model

Table 2. The relationship of cancer risk with eNOS G894T polymorphism in summary.

NS: Number of studies, *p*H: *p* heterogenity, *p*E: *p* egger.

^aThe *p* value of the Z test was found to be less than 0.05.

Table 3. The relationship of stroke risk with eNOS G894T polymorphism in summary.

Genetic models	NS	Pooled ORs (95% CI)	<i>p</i> value ^a (Z test)	I ² (%)	рН	ρE	Method
T versus G	6	1.20 (1.01,1.43)	0.04	45	0.11	0.11583	Fixed model
G versus T	6	0.88 (0.74,1.05)	0.15	0	0.86	0.17541	Fixed model
GT+TT versus GG	6	0.68 (0.24-1.93)	0.47	91	<0.00001	0.17523	Ramdom model
TT versus GG+GT	6	0.09 (0.03-0.30)	0.0001	94	<0.00001	0.44879	Ramdom model
GT versus GG+TT	6	1.03 (0.40-2.64)	0.95	92	<0.00001	0.29785	Ramdom model

NS: Number of studies, pH: p heterogenity, pE: p egger.

^aThe p value of the Z test was found to be less than 0.05, indicating statistical significance.

Heterogeneity among studies

Heterogeneity was observed among the studies in every allele and gene, as depicted in Figures 2 and 3 (T *versus* G, G *versus* T, GT+TT *versus* GG, TT *versus* GG + GT, and GT *versus* GG + TT). Tables 2 and 3 provide information on the selected model (random or fixed effect) utilized in order to review universal genetic model correlations.

Discussion

NO acts as a crucial part of numerous pathological and psychological processes. NO is extensively implicated in various events related to cancer, including angiogenesis, metastasis, invasion, and apoptosis, which various studies have investigated; results have provided evidence that increased concentrations of NO within cancer cells can effectively suppress tumor angiogenesis and metastasis (Zhao *et al.*, 2014). Conversely, low levels of NO in tumor cells may facilitate tumor growth by reducing NO-induced apoptosis (Heller, 2008). These observations suggest that the effects of NO on cancer development may be strongly dependent on the local NO concentration. NO is the result of three types of NOS isoforms, namely nNOS, eNOS, and iNOS. These enzymes facilitate the conversion of 1-arginine to 1-citrulline through oxidation (Vanini, Kashfi and Nath, 2015). eNOS is one of the three isoforms of NOS responsible for synthesizing NO in humans. Moreover, this particular isoform is closely linked to angiogenesis, which is associated with NO synthesis in both normal and cancerous cells (Song *et al.*, 2013). A polymorphism was found in the gene encoding eNOS that could alter the production of NO. The G894T variation, also known as the guanine polymorphism to thymine with position 894 and exon 7 (rs1799983), is of particular interest (Akbar *et al.*, 2022).

From several studies that were selected for our meta-analysis, the polymorphism was correlated with increased cancer risk in both African, European, and Asian countries. Adibmanesh *et al.* (2020) stated that the eNOS polymorphism 894G>T showed a significant correlation with colorectal cancer existing in the T allele genotype. Aouf *et al.* (2019) stated that NOS3 G894T substantially increases the risk of nasopharyngeal carcinoma (NPC) in a population from Tunisia. Furthermore, research conducted by Branković *et al.* (2013) suggested that NOS3 894G>T genetic polymorphisms were not associated with the risk of prostate tumor in a community in Serbia but may be relevant as prognostic factors for the progression of prostate cancer and patients' outcome. In the study by Carkic *et al.* (2020), the results proved that eNOS had a significant impact on oral squamous cell cancer (OSCC) in the Serbian population. Kocer *et al.* (2020) stated that there was no significant association between the eNOS G894T gene and the risk of lung cancer, where *p* was greater than 0.05. Research by Su *et al.* (2018) found no relationship between OSCC and eNOS holotypes in Taiwan. Research by Verim *et al.* (2013) proved the existence of NOS3 is crucial in increasing the susceptibility to bladder cancer within the

	т		G			Odds Ratio	Odds Ratio
Study or Subgroup	Events		Events			M-H, Random, 95% CI	M-H, Random, 95% Cl
dibmanesh, et.al 2020	200	300	251	326	13.2%	0.60 [0.42, 0.85]	
Aouf, et.al 2019	518	600	354	391	13.1%	0.66 [0.44, 1.00]	
Branković, et.al 2013	300	301	231	302	7.6%	92.21 [12.72, 668.61]	
Carkic, et.al 2020	100	100	198	209	5.2%	11.64 [0.68, 199.62]	
<ocer, 2020<="" et.al="" td=""><td>214</td><td>300</td><td>198</td><td>298</td><td>13.2%</td><td>1.26 [0.89, 1.78]</td><td>+--</td></ocer,>	214	300	198	298	13.2%	1.26 [0.89, 1.78]	+ - -
Su, et.al 2018	88	100	49	99	12.2%	7.48 [3.64, 15.38]	
/erim, et.al 2013	32	100	75	92	12.4%	0.11 [0.05, 0.21]	
/adav, et.al 2019	44	100	55	107	12.8%	0.74 [0.43, 1.28]	
′anar, et.al 2016	61	100	90	93	10.4%	0.05 [0.02, 0.18]	
Fotal (95% CI)		2001		1917	100.0%	1.00 [0.44, 2.27]	+
Fotal events	1557		1501				
Heterogeneity: Tau ² = 1.30	0; Chi ² = 1	30.81,	df = 8 (P	< 0.000	001); l ² = 9	34%	0.01 0.1 1 10 1
Fest for overall effect: Z = 1	0.00 (P = 1	1.00)					'0.01 0.1 i 10 1 TG
B)							
B)	G		т			Odds Ratio	Odds Ratio
B) Study or Subgroup	G Events	Total	T Events	Total	Weight	Odds Ratio M-H, Random, 95% CI	Odds Ratio M-H, Random, 95% Cl
Study or Subgroup Adibmanesh, et.al 2020	Events 251	326	Events 200	300	13.2%	M-H, Random, 95% Cl 1.67 [1.18, 2.38]	
Adibmanesh, et.al 2020 Adibmanesh, et.al 2020	Events 251 354	326 391	Events 200 518	300 600	13.2% 13.1%	M-H, Random, 95% Cl 1.67 [1.18, 2.38] 1.51 [1.00, 2.28]	M-H, Random, 95% Cl
Adibmanesh, et.al 2020 Adibmanesh, et.al 2020 Aouf, et.al 2019 Branković, et.al 2013	Events 251 354 231	326 391 302	Events 200 518 300	300 600 301	13.2% 13.1% 7.6%	M-H, Random, 95% Cl 1.67 [1.18, 2.38] 1.51 [1.00, 2.28] 0.01 [0.00, 0.08]	M-H, Random, 95% Cl
Adibmanesh, et.al 2020 Adibmanesh, et.al 2020	Events 251 354	326 391 302 209	Events 200 518	300 600	13.2% 13.1% 7.6% 5.2%	M-H, Random, 95% Cl 1.67 [1.18, 2.38] 1.51 [1.00, 2.28]	M-H, Random, 95% Cl
Study or Subgroup Adibmanesh, et.al 2020 Aouf, et.al 2019 Branković, et.al 2013 Carkic, et.al 2020 Kocer, et.al 2020	Events 251 354 231	326 391 302	Events 200 518 300	300 600 301	13.2% 13.1% 7.6% 5.2% 13.2%	M-H, Random, 95% Cl 1.67 [1.18, 2.38] 1.51 [1.00, 2.28] 0.01 [0.00, 0.08]	M-H, Random, 95% Cl
Adibmanesh, et.al 2020 Adibmanesh, et.al 2020 Aouf, et.al 2019 Branković, et.al 2013 Carkic, et.al 2020	Events 251 354 231 198 198 49	326 391 302 209 298 99	Events 200 518 300 100	300 600 301 100 300 100	13.2% 13.1% 7.6% 5.2% 13.2% 12.2%	M-H, Random, 95% Cl 1.67 [1.18, 2.38] 1.51 [1.00, 2.28] 0.01 [0.00, 0.08] 0.09 [0.01, 1.47]	M-H, Random, 95% Cl
Study or Subgroup Adibmanesh, et.al 2020 Aouf, et.al 2019 Branković, et.al 2013 Carkic, et.al 2020 Kocer, et.al 2020	Events 251 354 231 198 198	326 391 302 209 298 99 92	Events 200 518 300 100 214 88 32	300 600 301 100 300 100 100	13.2% 13.1% 7.6% 5.2% 13.2% 12.2% 12.4%	M-H, Random, 95% CI 1.67 [1.18, 2.38] 1.51 [1.00, 2.28] 0.01 [0.00, 0.08] 0.09 [0.01, 1.47] 0.80 [0.56, 1.13]	M-H, Random, 95% Cl
Study or Subgroup Adibmanesh, et.al 2020 Aouf, et.al 2019 Branković, et.al 2013 Carkic, et.al 2020 Kocer, et.al 2020 Su, et.al 2018 Verim, et.al 2013 Yadav, et.al 2019	Events 251 354 231 198 198 49 75 55	326 391 302 209 298 99 92 107	Events 200 518 300 100 214 88 32 44	300 600 301 100 300 100 100 100	13.2% 13.1% 7.6% 5.2% 13.2% 12.2% 12.4% 12.8%	M-H, Random, 95% CI 1.67 [1.18, 2.38] 1.51 [1.00, 2.28] 0.01 [0.00, 0.08] 0.09 [0.01, 1.47] 0.80 [0.56, 1.13] 0.13 [0.07, 0.27] 9.38 [4.78, 18.39] 1.35 [0.78, 2.33]	M-H, Random, 95% Cl
Study or Subgroup Adibmanesh, et.al 2020 Aouf, et.al 2019 Branković, et.al 2013 Carkic, et.al 2020 Kocer, et.al 2020 Su, et.al 2018 /erim, et.al 2013	Events 251 354 231 198 198 49 75	326 391 302 209 298 99 92	Events 200 518 300 100 214 88 32	300 600 301 100 300 100 100	13.2% 13.1% 7.6% 5.2% 13.2% 12.2% 12.4%	M-H, Random, 95% Cl 1.67 [1.18, 2.38] 1.51 [1.00, 2.28] 0.01 [0.00, 0.08] 0.09 [0.01, 1.47] 0.80 [0.56, 1.13] 0.13 [0.07, 0.27] 9.38 [4.78, 18.39]	M-H, Random, 95% Cl
Study or Subgroup Adibmanesh, et.al 2020 Aouf, et.al 2019 Branković, et.al 2013 Carkic, et.al 2020 Kocer, et.al 2020 Su, et.al 2018 Verim, et.al 2013 Yadav, et.al 2019	Events 251 354 231 198 198 49 75 55	326 391 302 209 298 99 92 107	Events 200 518 300 100 214 88 32 44	300 600 301 100 300 100 100 100	13.2% 13.1% 7.6% 5.2% 13.2% 12.2% 12.4% 12.8%	M-H, Random, 95% CI 1.67 [1.18, 2.38] 1.51 [1.00, 2.28] 0.01 [0.00, 0.08] 0.09 [0.01, 1.47] 0.80 [0.56, 1.13] 0.13 [0.07, 0.27] 9.38 [4.78, 18.39] 1.35 [0.78, 2.33]	M-H, Random, 95% Cl
Study or Subgroup dilbmanesh, et.al 2020 Aquí, et.al 2019 Branković, et.al 2013 Carkic, et.al 2020 Gocer, et.al 2020 Su, et.al 2018 Verim, et.al 2013 fadav, et.al 2019 fanar, et.al 2016	Events 251 354 231 198 198 49 75 55	326 391 302 209 298 99 92 107 93	Events 200 518 300 100 214 88 32 44	300 600 301 100 300 100 100 100	13.2% 13.1% 7.6% 5.2% 13.2% 12.2% 12.4% 12.8% 10.4%	M-H, Random, 95% CI 1.67 [1.18, 2.38] 1.51 [1.00, 2.28] 0.01 [0.00, 0.08] 0.09 [0.01, 1.47] 0.80 [0.56, 1.13] 0.13 [0.07, 0.27] 9.38 [4.78, 18.39] 1.35 [0.78, 2.33] 19.18 [5.67, 64.87]	M-H, Random, 95% Cl
Study or Subgroup Adibmanesh, et.al 2020 Aour, et.al 2019 Sanković, et.al 2013 Sarkic, et.al 2020 Socer, et.al 2020 Su, et.al 2018 Zerim, et.al 2013 Yanar, et.al 2016 Total (95% CI)	Events 251 354 231 198 198 49 75 55 90 1501	326 391 302 209 298 99 92 107 93 1917	Events 200 518 300 100 214 88 32 44 61	300 600 301 100 300 100 100 100 2001	13.2% 13.1% 7.6% 5.2% 13.2% 12.2% 12.4% 12.8% 10.4% 100.0%	M-H, Random, 95% CI 1.67 [1.18, 2.38] 1.51 [1.00, 2.28] 0.01 [0.00, 0.08] 0.09 [0.01, 1.47] 0.80 [0.56, 1.13] 0.13 [0.07, 0.27] 9.38 [4.78, 18.39] 1.35 [0.78, 2.33] 19.18 [5.67, 64.87] 1.00 [0.44, 2.27]	M-H, Random, 95% Cl

(C)

(A)

(0)	GT +	П	GG			Odds Ratio	Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% CI	M-H, Random, 95% CI
Adibmanesh, et.al 2020	100	206	101	222	13.8%	1.13 [0.77, 1.65]	
Aouf, et.al 2019	93	117	82	182	12.7%	4.73 [2.77, 8.07]	
Branković, et.al 2013	76	265	129	1011	14.2%	2.75 [1.99, 3.80]	-
Carkic, et.al 2020	78	112	82	98	11.7%	0.45 [0.23, 0.87]	
Kocer, et.al 2020	66	68	135	139	5.0%	0.98 [0.17, 5.48]	
Su, et.al 2018	94	337	136	882	14.3%	2.12 [1.57, 2.86]	-
Verim, et.al 2013	114	141	70	103	12.3%	1.99 [1.10, 3.59]	
Yadav, et.al 2019	126	131	86	119	9.2%	9.67 [3.63, 25.76]	
Yanar, et.al 2016	148	156	49	52	6.7%	1.13 [0.29, 4.44]	
Total (95% CI)		1533		2808	100.0%	1.96 [1.22, 3.15]	◆
Total events	895		870				
Heterogeneity: Tau ² = 0.38	; Chi² = 5	2.84, d	f=8(P <	0.0000	01); I ² = 85	i%	
Test for overall effect: Z = 2	.79 (P = 1	0.005)					0.01 0.1 1 10 100 GT+TT GG

(D)

(D)	Π		GG +	ст		Odds Ratio	Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% Cl	M-H, Random, 95% Cl
Adibmanesh, et.al 2020	19	38	182	390	12.9%	1.14 [0.59, 2.23]	
Aouf, et.al 2019	34	44	166	266	12.7%	2.05 [0.97, 4.32]	
Branković, et.al 2013	12	15	188	235	10.5%	1.00 [0.27, 3.69]	
Carkic, et.al 2020	12	25	148	184	12.2%	0.22 [0.09, 0.53]	
Kocer, et.al 2020	2	12	199	205	8.8%	0.01 [0.00, 0.03]	<u>←</u>
Su, et.al 2018	2	25	198	2219	9.9%	0.89 [0.21, 3.79]	
Verim, et.al 2013	23	30	131	170	12.0%	0.98 [0.39, 2.45]	
Yadav, et.al 2019	17	28	183	307	12.5%	1.05 [0.47, 2.31]	_ _
Yanar, et.al 2016	43	46	157	159	8.5%	0.18 [0.03, 1.13]	
Total (95% CI)		263		4135	100.0%	0.51 [0.22, 1.17]	-
Total events	164		1552				
Heterogeneity: Tau ² = 1.30): $Chi^2 = 4$	9.63. d	f=8(P<	0.0000	01); I ² = 84	%	
Test for overall effect: Z = 1	.59 (P =	D.11)					0.01 0.1 1 10 100 TT GG + GT

(E)

(E)	CT		CC .			Odda Datia	Odda Patia	
< / /	GT		GG +			Odds Ratio	Odds Ratio	
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% Cl	M-H, Random, 95%	CI
Adibmanesh, et.al 2020	81	168	120	260	13.2%	1.09 [0.74, 1.60]		
Aouf, et.al 2019	81	181	119	219	13.2%	0.68 [0.46, 1.01]		
Branković, et.al 2013	83	105	117	145	11.5%	0.90 (0.48, 1.69)		
Carkic, et.al 2020	66	86	94	123	11.3%	1.02 [0.53, 1.95]		
Kocer, et.al 2020	64	66	137	141	4.7%	0.93 [0.17, 5.23]		-
Su, et.al 2018	41	459	159	1785	13.4%	1.00 [0.70, 1.44]	+	
Verim, et.al 2013	93	124	61	76	10.9%	0.74 [0.37, 1.48]		
Yadav, et.al 2019	73	123	127	212	12.8%	0.98 [0.62, 1.54]		
Yanar, et.al 2016	105	110	95	195	9.0%	22.11 [8.63, 56.59]		
Total (95% CI)		1422		3156	100.0%	1.21 [0.77, 1.91]	•	
Total events	687		1029					
Heterogeneity: Tau ² = 0.37	; Chi² = 4	8.53, d	f= 8 (P <	0.0000	01); l² = 84	1%	0.01 0.1 1	10 100
Test for overall effect: Z = 0).82 (P = I	0.41)					GT GG+T	

Figure 2. Forest plots depicting the association of eNOS 894G>T polymorphisms with cancer risk in all genetic models, including T vs G, G vs T, GT + TT vs GG, TT vs GG + GT, and GT vs GG + TT, respectively.

(A)	т		G			Odds Ratio			Odds Ratio		
Study or Subgroup		Total	Events	Total	Weight	M-H, Fixed, 95% Cl			H, Fixed, 95%	CI	
Aliancik, et.al 2019	101	249	101	294	24.8%	1.30 [0.92, 1.85]			+		
Diakite, et.al 2014	50	170	151	524	23.5%	1.03 [0.70, 1.51]			_ _		
Gohary, et.al 2017	87	105	33	55	3.3%	3.22 [1.54, 6.76]			—•		
Kaur, et.al 2015	27	61	174	381	12.1%	0.94 [0.55, 1.63]			_		
Kumar, et.al 2016	34	167	166	833	19.9%						
	34 34					1.03 [0.68, 1.55]			I.		
Shyu, et.al 2017	34	159	137	785	16.3%	1.29 [0.84, 1.96]					
Total (95% CI)		911		2872	100.0%	1.20 [1.01, 1.43]			•		
Total events Heterogeneity: Chi ² =	333 9.07, df =	5 (P = 1	762 = *1); 1	45%		1	0.01				
Test for overall effect:							0.01	0.1	T G	1'0	100
(B)			-								
· /	G	Total	T	Total	Mainht	Odds Ratio			Odds Ratio		
Study or Subgroup	Events		Events			M-H, Fixed, 95% CI		M	-H, Fixed, 95%		
Aliancik, et.al 2019	101	294	101	249	27.3%	0.77 [0.54, 1.09]					
Diakite, et.al 2014	151	524	50	170	20.4%	0.97 [0.66, 1.42]			-		
Gohary, et.al 2017	33	55	87	135	7.7%	0.83 [0.43, 1.58]					
Kaur, et.al 2015	174	381	27	61	9.6%	1.06 [0.61, 1.82]			-		
Kumar, et.al 2016	166	833	34	167	17.2%	0.97 [0.64, 1.47]			-		
Shyu, et.al 2017	137	785	34	159	17.7%	0.78 [0.51, 1.18]					
Total (95% CI)		2872		941	100.0%	0.88 [0.74, 1.05]			•		
Total events	762	£ /D -	333				L				
Heterogeneity: Chi ² = Test for overall effect:				= 0%			0.01	0.1	і ст	10	100
									61		
(C)	GT + T	т	GG			Odds Ratio			Odds Rat	io	
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% C	1	M	-H, Random,	95% CI	
Aliancik, et.al 2019	213	258	59	63	15.5%	0.32 [0.11, 0.93	1	_			
Diakite, et.al 2014	147	184	200	215	17.2%	0.30 [0.16, 0.56		-			
Gohary, et.al 2017											
		90	23	110	171%	0.95 (0.47 1.89	n		_		
	18	90 64	23	110 230	17.1%	0.95 [0.47, 1.89					
Kaur, et.al 2015	54	64	167	239	16.9%	2.33 [1.12, 4.83					
Kaur, et.al 2015 Kumar, et.al 2016]			► ◆	
Kaur, et.al 2015 Kumar, et.al 2016 Shyu, et.al 2017	54 150	64 161 158	167 350	239 440 342	16.9% 17.2% 16.1%	2.33 [1.12, 4.83 3.51 [1.82, 6.75 0.11 [0.04, 0.28	- 	_		•	
Kaur, et.al 2015 Kumar, et.al 2016 Shyu, et.al 2017 Total (95% CI) Total events	54 150 136 718	64 161 158 9 1 5	167 350 336 1135	239 440 342 1409	16.9% 17.2% 16.1% 100.0%	2.33 (1.12, 4.83 3.51 (1.82, 6.75 0.11 (0.04, 0.28 0.68 (0.24, 1.93	- 		-	•	
Kaur, et.al 2015 Kumar, et.al 2016 Shyu, et.al 2017 Total (95% CI) Total events Heterogeneity: Tau ² =	54 150 136 718 1.56; Chi ²	64 161 158 915 = 57.8	167 350 336 1135 3, df = 5	239 440 342 1409	16.9% 17.2% 16.1% 100.0%	2.33 (1.12, 4.83 3.51 (1.82, 6.75 0.11 (0.04, 0.28 0.68 (0.24, 1.93	- 	-			10
Kaur, et.al 2015 Kumar, et.al 2016 Shyu, et.al 2017 Total (95% CI) Total events	54 150 136 718 1.56; Chi ²	64 161 158 915 = 57.8	167 350 336 1135 3, df = 5	239 440 342 1409	16.9% 17.2% 16.1% 100.0%	2.33 (1.12, 4.83 3.51 (1.82, 6.75 0.11 (0.04, 0.28 0.68 (0.24, 1.93)))]		GT+TT GG		10
Kaur, et.al 2015 Kurnar, et.al 2016 Shyu, et.al 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect: 2	54 150 136 718 1.56; Chi [≇] Z = 0.73 (F	64 161 158 915 = 57.8	167 350 336 1135 3, df = 5	239 440 342 1409 (P < 0.0	16.9% 17.2% 16.1% 100.0%	2.33 (1.12, 4.83 3.51 (1.82, 6.75 0.11 (0.04, 0.28 0.68 (0.24, 1.93)))]		Odds Rat	io	10
Kaur, et.al 2015 Kumar, et.al 2016 Shyu, et.al 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect: 2	54 150 136 718 1.56; Chi [≇] Z = 0.73 (F	64 161 158 915 = 57.8 = 0.47	167 350 336 1135 3, df = 5 7)	239 440 342 1409 (P < 0.0	16.9% 17.2% 16.1% 100.0%	2.33 j1.12, 4.83 3.61 j1.82, 6.75 0.11 [0.04, 0.28 0.68 [0.24, 1.93	0 0 0 0.01			io	10
Kaur, et al 2015 Kumar, et al 2016 Shyu, et al 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect: J (D) Study or Subgroup	54 150 136 718 1.56; Chi [≇] Z = 0.73 (F	64 161 158 915 = 57.8 = 0.47	167 350 336 1135 3, df = 5 7) GG + (239 440 342 1409 (P < 0.0	16.9% 17.2% 16.1% 100.0% 00001); I ² Weight	2.33 j1.12, 4.83 3.51 j1.82, 6.75 0.11 [0.04, 0.28 0.68 [0.24, 1.93 = 91%	0 0 0 0 0.01		Odds Rat	io	10
Kaur, et al 2015 Kurnar, et al 2016 Shyu, et al 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect: J (D) Study or Subgroup Aliancik, et al 2019	54 150 136 718 1.56; Chi ^a Z = 0.73 (F TT <u>Events</u>	64 161 158 915 = 57.8 P = 0.47	167 350 336 1135 3, df = 5 7) GG + (<u>Events</u>	239 440 342 1409 (P < 0.0	16.9% 17.2% 16.1% 100.0%	2.33 j1.12, 4.83 3.51 [1.82, 6.75 0.11 [0.04, 0.28 0.68 [0.24, 1.93 = 91% Odds Ratio <u>M-H, Random, 95% (</u> 0.04 [0.27, 1.45	9 9 9 0.01		Odds Rat	io	10
Kaur, et al 2015 Kurnar, et al 2016 Shyu, et al 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect 2 (D) Study or Subgroup Aliancik, et al 2019 Diakite, et al 2014	54 150 136 718 1.56; Chi [≠] Z = 0.73 (F TT <u>Events</u> 33 23	64 161 158 915 = 57.8 P = 0.47 Total 41 55	167 350 336 1135 3, df = 5 7) GG + (<u>Events</u> 239 324	239 440 342 1409 (P < 0.0 6T <u>Total</u> 276 387	16.9% 17.2% 16.1% 100.0% 00001); I ² <u>Weight</u> 16.4% 17.1%	2.33 [1.12, 4.83 3.51 [1.82, 6.75 0.11 [0.04, 0.28 0.68 [0.24, 1.93 = 91% Odds Ratio <u>M.H. Random, 95% (</u> 0.64 [0.27, 1.44 0.14 [0.08, 0.22	0 0 0 0 0 0 0 0 0 0		Odds Rat	io	10
Kaur, et al 2015 Kumar, et al 2016 Shyu, et al 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect : (D) Study or Subgroup Aliancik, et al 2019 Diakite, et al 2014 Gohary, et al 2017	54 150 136 718 1.56; Chi² Z = 0.73 (F TT <u>Events</u> 33 23 9	64 161 158 915 = 57.8 P = 0.47 Total 41 55 85	167 350 336 1135 3, df = 5 7) GG + (<u>Events</u> 239 324 32	239 440 342 1409 (P < 0.0 (P < 0.0 5T <u>Total</u> 276 387 155	16.9% 17.2% 16.1% 100.0% 00001); I ² <u>Weight</u> 16.4% 17.1% 16.6%	2.33 [1.12, 4.83 3.61 [1.82, 6.75 0.11 [0.04, 0.28 0.68 [0.24, 1.93 = 91% Odds Ratio <u>M.H. Random, 95% (</u> 0.64 [0.27, 1.44 0.14 [0.08, 0.22 0.66 [0.21, 1.01	0 0 0 0 0 0 0 0 0		Odds Rat	io	10
Kaur, et al 2015 Kurnar, et al 2016 Shyu, et al 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect : D) Study or Subgroup Aliancik, et al 2019 Diakite, et al 2017 Gonary, et al 2015	54 150 136 718 1.56; Chi [≇] Z = 0.73 (F TT <u>Events</u> 33 23 9 9 7	64 161 158 915 = 57.8 = 0.47 Total 41 55 85 70	167 350 336 1135 3, df = 5 7) GG + (<u>Events</u> 239 324 32 214	239 440 342 1409 (P < 0.0 5T <u>Total</u> 276 387 155 278	16.9% 17.2% 16.1% 100.0% 00001); I ² <u>Weight</u> 16.4% 17.1% 16.6% 16.4%	2.33 j1.12, 4.83 3.51 j1.82, 6.75 0.11 [0.04, 0.28 0.68 [0.24, 1.93 = 91% Odds Ratio <u>M-H, Random, 95% C</u> 0.64 [0.27, 1.45 0.14 [0.08, 0.22 0.46 [0.21, 1.01 0.33 [0.01, 0.06	0 0 0 0 0 0 0 0 0 0 0 0 0 0		Odds Rat	io	10
Kaur, et al 2015 Kumar, et al 2016 Shyu, et al 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect ; D) Study or Subgroup Allancik, et al 2019 Diakite, et al 2019 Gohary, et al 2015 Kumar, et al 2016	54 150 136 718 1.56; Chi² Z = 0.73 (F TT <u>Events</u> 33 23 9	64 161 158 915 = 57.8 P = 0.47 Total 41 55 85	167 350 336 1135 3, df = 5 7) GG + (<u>Events</u> 239 324 32	239 440 342 1409 (P < 0.0 (P < 0.0 5T <u>Total</u> 276 387 155	16.9% 17.2% 16.1% 100.0% 00001); I ² <u>Weight</u> 16.4% 17.1% 16.6%	2.33 [1.12, 4.83 3.61 [1.82, 6.75 0.11 [0.04, 0.28 0.68 [0.24, 1.93 = 91% Odds Ratio <u>M.H. Random, 95% (</u> 0.64 [0.27, 1.44 0.14 [0.08, 0.22 0.66 [0.21, 1.01	0 0 0 0 0 0 0 0 0 0 0 0 0 0		Odds Rat	io	10
Kaur, et al 2015 Kurnar, et al 2016 Shyu, et al 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect : CD) Study or Subgroup Aliancik, et al 2019 Diakite, et al 2019 Diakite, et al 2017 Kurnar, et al 2015 Kurnar, et al 2017	54 150 136 718 1.56; Chi [≆] Z = 0.73 (F TT <u>Events</u> 33 23 9 7 7 17	64 161 158 915 5 = 57.8 P = 0.47 Total 41 55 85 70 68 69	167 350 336 1135 3, df = 5 7) GG + (<u>Events</u> 239 324 322 214 483	239 440 342 1409 (P < 0.0 T Total 276 387 155 278 494 490	16.9% 17.2% 16.1% 100.0% 00001); I ² <u>Weight</u> 16.4% 16.4% 16.5%	2.33 j1.12, 4.83 3.51 [1.82, 6.75 0.11 [0.04, 0.28 0.68 [0.24, 1.93 = 91% Odds Ratio M.H, Random, 95% O 0.64 [0.27, 1.45 0.46 [0.21, 1.01 0.33 [0.01, 0.08 0.01 [0.00, 0.02 0.05 [0.03, 0.08	0.01 0.01 0.01		Odds Rat	io	10
Kaur, et al 2015 Kurnar, et al 2016 Shyu, et al 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect : (D) Study or Subgroup Atlancik, et al 2019 Diaktie, et al 2019 Diaktie, et al 2017 Kurnar, et al 2016 Shyu, et al 2017 Total (95% CI) Total events	54 150 136 718 1.56; Chi [≇] Z = 0.73 (F TT <u>Events</u> 33 9 7 7 7 7 7 7 17 23	64 161 158 915 = 57.8 P = 0.47 Total 41 55 70 68 69 388	167 350 336 1135 3, df = 5 7) GG + (<u>Events</u> 239 324 32 214 483 449 1741	239 440 342 1409 (P < 0.0 (P < 0.0 0 5T Total 276 387 155 278 494 490 2080	16.9% 17.2% 16.1% 100.0% 00001); I ² 16.4% 17.1% 16.6% 16.5% 17.1% 100.0%	2.33 [1.12, 4.83 3.51 [1.82, 6.75 0.11 [0.04, 0.28 0.68 [0.24, 1.93 = 91% Odds Ratio <u>M-H, Random, 95% (</u> 0.64 [0.27, 1.46 0.14 [0.08, 0.22 0.46 [0.21, 1.01 0.33 [0.01, 0.06 0.01 [0.00, 0.07 0.05 [0.03, 0.06	0.01 0.01 0.01		Odds Rat	io	10
Kaur, et al 2015 Kumar, et al 2016 Shyu, et al 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect ; (D) Study or Subgroup Aliancik, et al 2019 Diakite, et al 2019 Diakite, et al 2019 Shyu, et al 2015 Shyu, et al 2017 Total (95% CI) Total (95% CI) Total events Heterogeneity: Tau ² =	54 150 136 718 1.56; Chi [≠] Z = 0.73 (F TT Events 33 23 9 7 17 23 9 7 17 23 112 2.21; Chi [≠]	64 161 158 915 = 57.8 = 0.47 Total 41 55 85 70 68 69 388 *= 85.2	167 350 336 11135 3, df = 5 7) GG + (<u>Events</u> 239 324 32 214 483 449 1741 7, df = 5	239 440 342 1409 (P < 0.0 (P < 0.0 0 5T Total 276 387 155 278 494 490 2080	16.9% 17.2% 16.1% 100.0% 00001); I ² 16.4% 17.1% 16.6% 16.5% 17.1% 100.0%	2.33 [1.12, 4.83 3.51 [1.82, 6.75 0.11 [0.04, 0.28 0.68 [0.24, 1.93 = 91% Odds Ratio <u>M-H, Random, 95% (</u> 0.64 [0.27, 1.46 0.14 [0.08, 0.22 0.46 [0.21, 1.01 0.33 [0.01, 0.06 0.01 [0.00, 0.07 0.05 [0.03, 0.06	0.01 0.01 0.01		Odds Rat	io	
Kaur, et.al 2015 Kurnar, et.al 2016 Shyu, et.al 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect 2 (D)	54 150 136 718 1.56; Chi [≠] Z = 0.73 (F TT Events 33 23 9 7 17 23 9 7 17 23 112 2.21; Chi [≠]	64 161 158 915 = 57.8 = 0.47 Total 41 55 85 70 68 69 388 *= 85.2	167 350 336 11135 3, df = 5 7) GG + (<u>Events</u> 239 324 32 214 483 449 1741 7, df = 5	239 440 342 1409 (P < 0.0 (P < 0.0 0 5T Total 276 387 155 278 494 490 2080	16.9% 17.2% 16.1% 100.0% 00001); I ² 16.4% 17.1% 16.6% 16.5% 17.1% 100.0%	2.33 [1.12, 4.83 3.51 [1.82, 6.75 0.11 [0.04, 0.28 0.68 [0.24, 1.93 = 91% Odds Ratio <u>M-H, Random, 95% (</u> 0.64 [0.27, 1.46 0.14 [0.08, 0.22 0.46 [0.21, 1.01 0.33 [0.01, 0.06 0.01 [0.00, 0.07 0.05 [0.03, 0.06			Odds Rat	io 95% CI 10	10
Kaur, et al 2015 Kumar, et al 2016 Shyu, et al 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect ; D) Study or Subgroup Diakite, et al 2019 Diakite, et al 2019 Diakite, et al 2019 Diakite, et al 2016 Shyu, et al 2017 Total (95% CI) Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect.	54 150 136 718 1.56; Chi [≠] Z = 0.73 (F TT Events 33 23 9 7 17 23 9 7 17 23 112 2.21; Chi [≠]	64 161 158 915 = 57.8 = 0.47 Total 41 55 85 70 68 69 388 *= 85.2	167 350 336 11135 3, df = 5 7) GG + (<u>Events</u> 239 324 32 214 483 449 1741 7, df = 5	239 440 342 1409 (P < 0.0 (P < 0.0 0 5T Total 276 387 155 278 494 490 2080	16.9% 17.2% 16.1% 100.0% 00001); I ² 16.4% 17.1% 16.6% 16.5% 17.1% 100.0%	2.33 [1.12, 4.83 3.51 [1.82, 6.75 0.11 [0.04, 0.28 0.68 [0.24, 1.93 = 91% Odds Ratio <u>M-H, Random, 95% (</u> 0.64 [0.27, 1.46 0.14 [0.08, 0.22 0.46 [0.21, 1.01 0.33 [0.01, 0.06 0.01 [0.00, 0.07 0.05 [0.03, 0.06			Odds Rat	io 95% CI 10	
Kaur, et al 2015 Kumar, et al 2016 Shyu, et al 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect ; (D) Study or Subgroup Aliancik, et al 2019 Diakite, et al 2019 Diakite, et al 2019 Diakite, et al 2016 Shyu, et al 2017 Total (95% CI) Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect. (E)	54 150 136 1.56; Chi [#] Z = 0.73 (f TT <u>Events</u> 33 23 9 7 7 7 7 7 7 23 112 2.21; Chi [#] Z = 3.88 (f	64 161 158 915 = 57.8 P = 0.47 Total 41 55 85 69 388 P = 0.00 68 69 388 P = 0.00	167 350 1135 3, df=5 ') GG + (<u>Events</u> 239 324 483 449 1741 1741 7, df=5 001) GG + (239 440 342 1409 (P < 0.0 6T Total 276 387 155 278 494 490 2080 (P < 0.1	16.9% 17.2% 16.1% 100.0% 00001); P <u>Weight</u> 16.4% 16.4% 16.5% 17.1% 100.0%	2.33 [1.12, 4.83 3.51 [1.82, 6.75 0.11 [0.04, 0.28 0.68 [0.24, 1.93 = 91% Odds Ratio M-H, Random, 95% C 0.64 [0.27, 1.44 0.44 [0.27, 1.44 0.44 [0.27, 1.44 0.44 [0.27, 1.44 0.44 [0.27, 1.08, 0.27 0.46 [0.21, 1.01 0.03 [0.01, 0.06 0.01 [0.00, 0.07 0.05 [0.03, 0.08 0.09 [0.03, 0.30] = 94%			Odds Rat H, Random,	io 95% CI 	
Kaur, et al 2015 Kumar, et al 2016 Shyu, et al 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect <i>:</i> (D) Study or Subgroup Aliancik, et al 2019 Diakite, et al 2017 Kaur, et al 2015 Shyu, et al 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect : (E) Study or Subgroup	54 150 136 718 1.56; Chi ² Z = 0.73 (F TT Events 33 9 7 7 7 7 7 7 7 23 112 2.21; Chi ² Z = 3.88 (I GT Events	64 161 158 915 = 57.8 = 0.47 41 55 85 70 68 89 388 = 85.2 P = 0.0 Total	167 350 1135 3, df = 5 239 324 214 483 449 17411 7, df = 5 001) 66 + Events	239 440 342 1409 (P < 0.0 (P < 0.0 (P < 0.0 2080 (P < 0.1 2080 (P < 0.1) 2080	16.9% 17.2% 18.1% 00001); P Weight 16.4% 16.4% 16.4% 16.4% 16.4% 17.1% 100.0% Weight	2.33 [1.12, 4.83 3.51 [1.82, 6.75 0.11 [0.04, 0.28 0.68 [0.24, 1.93 = 91% Odds Ratio <u>M.H. Random, 95% (</u> 0.64 [0.27, 1.44 0.14 [0.08, 0.22 0.46 [0.27, 1.44 0.14 [0.08, 0.22 0.46 [0.27, 1.01 0.03 [0.01, 0.02 0.05 [0.03, 0.03 0.09 [0.03, 0.30 = 94% Odds Ratio <u>M.H. Random, 95%</u>	0.01 0.01 0.01 0.01 0.01 0.01		Odds Rat LH, Random,	io 95% CI 	
Kaur, et al 2015 Kumar, et al 2016 Shyu, et al 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect ; (D) Study or Subgroup Aliancik, et al 2019 Diakite, et al 2019 Diakite, et al 2019 Diakite, et al 2016 Shyu, et al 2017 Total (95% CI) Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect. (E)	54 150 136 1.56; Chi [#] Z = 0.73 (f TT <u>Events</u> 33 23 9 7 7 7 7 7 7 23 112 2.21; Chi [#] Z = 3.88 (f	64 161 158 915 = 57.8 P = 0.47 Total 41 55 85 69 388 P = 0.00 68 69 388 P = 0.00	167 350 1135 3, df=5 ') GG + (<u>Events</u> 239 324 483 449 1741 1741 7, df=5 001) GG + (239 440 342 1409 (P < 0.0 6T Total 276 387 155 278 494 490 2080 (P < 0.1	16.9% 17.2% 16.1% 100.0% 00001); P <u>Weight</u> 16.4% 16.4% 16.5% 17.1% 100.0%	2.33 [1.12, 4.83 3.51 [1.82, 6.75 0.11 [0.04, 0.28 0.68 [0.24, 1.93 = 91% Odds Ratio M-H, Random, 95% C 0.64 [0.27, 1.44 0.14 [0.08, 0.22 0.46 [0.21, 1.01 0.03 [0.01, 0.06 0.01 [0.00, 0.03 0.05 [0.03, 0.08 0.09 [0.03, 0.30] = 94%	0.01 0.01 0.01 0.01 0.01 0.01		Odds Rat H, Random,	io 95% CI 	
Kaur, et al 2015 Kumar, et al 2016 Shyu, et al 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect <i>:</i> (D) Study or Subgroup Aliancik, et al 2019 Diakite, et al 2017 Kaur, et al 2015 Shyu, et al 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect : (E) Study or Subgroup	54 150 136 718 1.56; Chi ² Z = 0.73 (F TT Events 33 9 7 7 7 7 7 7 7 23 112 2.21; Chi ² Z = 3.88 (I GT Events	64 161 158 915 = 57.8 = 0.47 41 55 85 70 68 89 388 = 85.2 P = 0.0 Total	167 350 1135 3, df = 5 239 324 214 483 449 17411 7, df = 5 001) 66 + Events	239 440 342 1409 (P < 0.0 (P < 0.0 (P < 0.0 2080 (P < 0.1 2080 (P < 0.1) 2080	16.9% 17.2% 18.1% 00001); P Weight 16.4% 16.4% 16.4% 16.4% 16.4% 17.1% 100.0% Weight	2.33 [1.12, 4.83 3.51 [1.82, 6.75 0.11 [0.04, 0.28 0.68 [0.24, 1.93 = 91% Odds Ratio <u>M.H. Random, 95% (</u> 0.64 [0.27, 1.44 0.14 [0.08, 0.22 0.46 [0.27, 1.44 0.14 [0.08, 0.22 0.46 [0.27, 1.01 0.03 [0.01, 0.02 0.05 [0.03, 0.03 0.09 [0.03, 0.30 = 94% Odds Ratio <u>M.H. Random, 95%</u>	1 1 1 1 1 1 1 1 1 1 1 1 1 1		Odds Rat H, Random,	io 95% CI 	
Kaur, et al 2015 Kumar, et al 2016 Shyu, et al 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect : (D) Study or Subgroup Atlancik, et al 2019 Diakite, et al 2017 Kaur, et al 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect : (E) Study or Subgroup Atlancik, et al 2019	54 150 136 718 1.56; Chi ² Z = 0.73 (F TT Events 33 23 9 7 17 23 112 2.21; Chi ² Z = 3.88 (f GT Events 180	64 161 158 915 = 57.8 = 0.47 Total 41 55 70 68 69 388 = 85.2 P = 0.00 Total 233	167 350 1135 3, df = 5 7 9 GG + (<u>Events</u> 239 244 32 214 483 449 1741 1,7,df = 5 001) GG + (<u>Events</u> 264 2001)	239 440 342 1409 (P < 0.0 (P < 0.0 7 7 155 278 494 490 2080 (P < 0.0 (P < 0.0 7 7 155 278 494 490 2080 (P < 0.0 7 7 155 278 2080 2080 2080 2080 2080 2080 2080	16.9% 17.2% 18.1% 100.0% 00001); P 16.4% 16.4% 16.6% 16.4% 16.5% 17.1% 100.0% 100.0% 100001); P Weight 18.4%	2.33 [1.12, 4.83 3.51 [1.82, 6.75 0.11 [0.04, 0.28 0.68 [0.24, 1.93 = 91% Odds Ratio M-H, Random, 95% O 0.64 [0.27, 1.44 0.44 [0.27, 1.44 0.44 [0.27, 1.44 0.44 [0.21, 1.01 0.03 [0.01, 0.03 0.01 [0.00, 0.07 0.05 [0.03, 0.06 0.09 [0.03, 0.30 = 94% Odds Ratio M-H, Random, 95% 2.81 [1.82, 4.3 0.05 [0.02, 0.1			Odds Rat H, Random,	io 95% CI 	
Kaur, et al 2015 Kurmar, et al 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect : (D) Study or Subgroup Aliancik, et al 2019 Diakite, et al 2017 Total events Heterogeneity: Tau ² = Test for overall effect : (E) Study or Subgroup Aliancik, et al 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect : (E) Study or Subgroup Aliancik, et al 2014 Gonary, et al 2014 Gonary, et al 2014 Gonary, et al 2014	54 150 136 718 1.56; Chi ² Z = 0.73 (F TT Events 33 23 9 7 17 23 112 2.21; Chi ² Z = 3.88 (f GT Events 180 124 9 9	64 161 158 915 = 57.8 P = 0.47 41 55 85 70 68 89 388 = 85.2 P = 0.07 Total 233 172 45 24 55 55 57 57 57 57 57 57 57 57	167 350 1135 3, df = 5 7) GG + (t <u>Events</u> 239 324 433 2214 483 449 1741 7, df = 5 001) GG + (t <u>Events</u> 239 324 449 1741 7, df = 5 001)	239 440 342 1409 (P < 0.0 (P < 0.0 (P < 0.0 2080 (P < 0.1 2080 (P < 0.1 155 278 494 490 2080 (P < 0.1 TT <u>Total</u> 168 207 155	16.9% 17.2% 18.1% 00001); P Weight 16.4% 17.1% 16.6% 16.4% 16.6% 17.1% 100.0% 17.1% 100.0% 18.4% 15.5%	2.33 [1.12, 4.83 3.51 [1.82, 6.75 0.11 [0.04, 0.28 0.68 [0.24, 1.93 = 91% Odds Ratio <u>M-H, Random, 95% C</u> 0.64 [0.27, 1.44 0.14 [0.08, 0.22 0.46 [0.27, 1.44 0.14 [0.08, 0.22 0.46 [0.27, 1.01 0.03 [0.01, 0.02 0.04 [0.03, 0.03 0.01 [0.00, 0.02 0.05 [0.03, 0.03 0.09 [0.03, 0.30 = 94% Odds Ratio <u>M-H, Random, 95%</u> 2.81 [1.82, 4.3 0.05 [0.02, 0.1 0.96 [0.42, 2.2	(1) (1) (1) (1) (1) (1) (1) (1)		Odds Rat H, Random,	io 95% CI 	
Kaur, et al 2015 Kumar, et al 2016 Shyu, et al 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect : D) Study or Subgroup Aliancik, et al 2019 Diakite, et al 2019 Diakite, et al 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect : E) Study or Subgroup Aliancik, et al 2019 Diakite, et al 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect : Aliancik, et al 2019 Diakite, et al 2019 Diakite, et al 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect :	54 150 136 718 1.56; Chi ² Z = 0.73 (F TT Events 33 23 9 9 7 7 17 23 112 2.21; Chi ² Z = 3.88 (f GT Events 180 124 9 47	64 161 158 915 = 57.8 > = 0.47 Total 41 55 85 69 388 ≈ = 85.2 P = 0.0 Total 233 172 45 49	167 350 1135 3, df=5 ") GG + (C <u>Events</u> 239 324 483 422 214 483 449 1741 7, df=5 0001) GG + (C <u>Events</u> 239 324 483 449 1741 7, df=5 233 32 213 174	239 440 342 1409 (P < 0.0 T Total 276 387 155 278 494 490 2080 (P < 0.1 TT Total 168 227 155 278 494 490 2080 (P < 2.1)	16.9% 17.2% 16.1% 100.0% 00001); P 16.4% 17.1% 16.4% 17.1% 100.0% 00001); P 100.0% 00001); P 10.0% 16.5% 16.5% 16.5%	2.33 [1.12, 4.83 3.51 [1.82, 6.75 0.11 [0.04, 0.28 0.68 [0.24, 1.93 = 91% Odds Ratio <u>M-H, Random, 95% (</u> 0.64 [0.27, 1.46 0.14 [0.08, 0.22 0.46 [0.21, 1.01 0.33 [0.01, 0.06 0.01 [0.00, 0.07 0.05 [0.03, 0.08 0.09 [0.03, 0.30 = 94% Odds Ratio <u>M-H, Random, 95%</u> 2.81 [1.82, 4.3 0.05 [0.02, 0.1 0.96 [0.42, 2.2 9.45 [2.24, 33.9	21 21 21 21 21 21 21 21 21 21		Odds Rat H, Random,	io 95% CI 	
Kaur, et al 2015 Kurmar, et al 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect : (D) Study or Subgroup Aliancik, et al 2019 Diakite, et al 2017 Total events Heterogeneity: Tau ² = Test for overall effect : (E) Study or Subgroup Aliancik, et al 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect : (E) Study or Subgroup Aliancik, et al 2014 Gonary, et al 2014 Gonary, et al 2014 Gonary, et al 2014	54 150 136 718 1.56; Chi ² Z = 0.73 (F TT Events 33 23 9 7 17 23 112 2.21; Chi ² Z = 3.88 (f GT Events 180 124 9 9	64 161 158 915 = 57.8 P = 0.47 41 55 85 70 68 89 388 = 85.2 P = 0.07 Total 233 172 45 24 55 55 57 57 57 57 57 57 57 57	167 350 1135 3, df = 5 7) GG + (t <u>Events</u> 239 324 433 2214 483 449 1741 7, df = 5 001) GG + (t <u>Events</u> 239 324 449 1741 7, df = 5 001)	239 440 342 1409 (P < 0.0 (P < 0.0 (P < 0.0 2080 (P < 0.1 2080 (P < 0.1 155 278 494 490 2080 (P < 0.1 TT <u>Total</u> 168 207 155	16.9% 17.2% 18.1% 00001); P Weight 16.4% 17.1% 16.6% 16.4% 16.6% 17.1% 100.0% 17.1% 100.0% 18.4% 15.5%	2.33 [1.12, 4.83 3.51 [1.82, 6.75 0.11 [0.04, 0.28 0.68 [0.24, 1.93 = 91% Odds Ratio <u>M-H, Random, 95% (</u> 0.64 [0.27, 1.44 0.14 [0.08, 0.22 0.46 [0.21, 1.01 0.03 [0.01, 0.03 0.01 [0.00, 0.03 0.05 [0.03, 0.03 0.09 [0.03, 0.30 = 94% Odds Ratio <u>M-H, Random, 95%</u> 2.81 [1.82, 4.3 0.05 [0.02, 0.1 0.96 [0.42, 2.2 9.45 [2.24, 39.9 1.38 [0.82, 2.3	() () () () () () () () () ()		Odds Rat H, Random,	io 95% CI 	
Kaur, et al 2015 Kumar, et al 2016 Shyu, et al 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect : (D) Study or Subgroup Aliancik, et al 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect : (E) Study or Subgroup Aliancik, et al 2019 Aliancik, et al 2019 Aliancik, et al 2019 Aliancik, et al 2017 Cotal 2019 Aliancik, et al 2019 Aliancik, et al 2014 Gohany, et al 2015 Kumar, et al 2015 Kumar, et al 2015 Kumar, et al 2016 Shyu, et al 2017	54 150 136 1.56; Chi ² Z = 0.73 (f TT Events 33 9 7 7 7 23 112 2.21; Chi ² Z = 3.88 (f GT Events 180 124 47 133	64 181 158 915 = 57.8 = 0.47 41 55 85 85 70 68 85 83 88 82 85.2 P = 0.01 233 172 45 233 172 45 172 45 172 45 172 45 172 45 172 45 172 45 172 172 172 172 172 172 172 172 172 172	167 350 336 1135 3, df = 5 7 9 324 32 214 483 449 17411 17411 17411 17741 17741 17741 17741 230 322 233 322 2134 367	239 440 342 1409 (P < 0.0 6T Total 276 387 278 494 490 2080 (P < 0.1 2080 (P < 0.1 10 10 10 10 10 10 10 10 10 10 10 10 10	16.9% 17.2% 18.1% 100.0% 00001); P 16.4% 16.4% 16.4% 16.4% 16.5% 17.1% 100.0% 10.0% 10.0% 10.0% 10.0% 10.0% 10.5%	2.33 [1.12, 4.83 3.51 [1.82, 6.75 0.11 [0.04, 0.28 0.68 [0.24, 1.93 = 91% Odds Ratio M-H, Random, 95% (0 0.64 [0.27, 1.44 0.44 [0.27, 1.44 0.44 [0.27, 1.44 0.44 [0.27, 1.44 0.44 [0.27, 1.44 0.45 [0.21, 1.00 0.03 [0.01, 0.00 0.05 [0.03, 0.00 0.05 [0.03, 0.00 0.09 [0.03, 0.30 = 94% Odds Ratio M-H, Random, 95% 2.81 [1.82, 4.3 0.05 [0.02, 0.1 0.96 [0.42, 2.2 9.45 [2.24, 3.99 1.38 [0.82, 2.3 0.84 [0.54, 1.3	1 1 1 1 1 1 1 1 1 1 1 1 1 1		Odds Rat H, Random,	io 95% CI 	
Kaur, et al 2015 Kumar, et al 2016 Shyu, et al 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect : D) Study or Subgroup Aliancik, et al 2019 Charle, et al 2017 Kaur, et al 2016 Shyu, et al 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect : E) Study or Subgroup Aliancik, et al 2019 Diakite, et al 2017 Total (95% CI) Total 2014 Sohary, et al 2017 Kaur, et al 2017 Kaur, et al 2014 Sohary, et al 2017 Kaur, et al 2017 Kaur, et al 2017 Kaur, et al 2015 Shyu, et al 2017 Kaur, et al 2015 Shyu, et al 2017 Total (95% CI)	54 150 136 718 1.56; Chi ² Z = 0.73 (F TT Events 33 9 7 7 7 7 7 7 7 23 112 2.21; Chi ² Z = 3.88 (I GT Events 180 124 4 9 47 133 3113	64 181 158 915 = 57.8 = 0.47 Total 41 55 85 85 85 85 85 85 85 915 - 0.47 - 0	167 350 1135 3, df = 5 7) GG + t Events 239 324 483 449 17411 17411 17411 17411 17411 17415 2001)	239 440 342 1409 (P < 0.0 6T Total 276 387 278 494 490 2080 (P < 0.1 2080 (P < 0.1 10 10 10 10 10 10 10 10 10 10 10 10 10	16.9% 17.2% 18.1% 00001); P 00001); P 16.4% 16.4% 16.4% 17.1% 100.0% 00001); P 00001); P 00001); P 18.4% 13.2% 18.0%	2.33 [1.12, 4.83 3.51 [1.82, 6.75 0.11 [0.04, 0.28 0.68 [0.24, 1.93 = 91% Odds Ratio <u>M-H, Random, 95% (</u> 0.64 [0.27, 1.44 0.14 [0.08, 0.22 0.46 [0.21, 1.01 0.03 [0.01, 0.03 0.01 [0.00, 0.03 0.05 [0.03, 0.03 0.09 [0.03, 0.30 = 94% Odds Ratio <u>M-H, Random, 95%</u> 2.81 [1.82, 4.3 0.05 [0.02, 0.1 0.96 [0.42, 2.2 9.45 [2.24, 39.9 1.38 [0.82, 2.3	1 1 1 1 1 1 1 1 1 1 1 1 1 1		Odds Rat H, Random,	io 95% CI 	
Kaur, et al 2015 Kumar, et al 2016 Shyu, et al 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect : D) Study or Subgroup Aliancik, et al 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect : E) Study or Subgroup Aliancik, et al 2017 Total (95% CI) Total events Heterogeneity: Tau ² = Test for overall effect : E) Study or Subgroup Aliancik, et al 2017 Diakite, et al 2017 Aliancik, et al 2017 Mainacik, et al 2017 Aliancik, et al 2017 Kaur, et al 2015 Kumar, et al 2015 Kumar, et al 2016 Shyu, et al 2017	54 150 136 718 1.56; Chi ² Z = 0.73 (F TT Events 33 23 9 7 7 7 7 7 7 7 7 7 7 7 7 7	64 161 158 915 = 57.8 = 0.47 Total 41 55 85 69 388 69 388 69 388 41 233 172 233 172 49 154 49 154 85 85 85 85 85 85 85 85 85 85	167 336 1135 3, df = 5 7) GG + (<u>Events</u> 239 324 483 32 214 483 32 214 483 32 214 489 1741 1741 1741 92 223 32 32 217 4367 92 2233 33 359 92 2233 323 92 2233 323 92 2233 324 5 92 2233 324 5 92 2233 324 5 92 2234 325 92 224 7 92 2233 324 5 92 224 7 92 224 7 92 223 93 224 439 92 22 22 23 92 22 22 14 439 92 22 14 439 92 22 14 439 92 22 14 439 92 22 14 439 92 22 14 439 92 22 14 439 92 22 14 439 92 22 14 439 92 22 14 439 92 22 17 4 32 23 23 23 23 23 23 23 23 23 23 23 23	239 440 342 1409 (P < 0.0 6T Total 276 387 155 278 494 490 2080 (P < 0.1 (P < 0.1 155 278 494 490 2080 (P < 0.1 155 278 494 490 2080 (P < 0.0 155 278 490 2080 2080 2080 2080 2080 2080 2080 20	16.9% 17.2% 16.1% 100.0% 00001); P Weight 16.4% 16.4% 16.6% 16.4% 16.4% 16.5% 100.0% Weight 18.4% 15.5% 18.3% 18.3%	2.33 [1.12, 4.83 3.51 [1.82, 6.75 0.11 [0.04, 0.28 0.68 [0.24, 1.93 = 91% Odds Ratio <u>M.H. Random, 95% (</u> 0.64 [0.27, 1.44 0.14 [0.08, 0.22 0.46 [0.21, 1.01 0.03 [0.01, 0.00 0.01 [0.00, 0.02 0.05 [0.03, 0.30 0.09 [0.03, 0.30 = 94% Odds Ratio <u>M.H. Random, 95%</u> 2.81 [1.82, 4.3 0.05 [0.02, 0.1] 0.95 [0.22, 2.2 9.45 [2.24, 39.9 1.38 [0.82, 2.3 0.84 [0.54, 1.3 1.03 [0.40, 2.6]	1 1 1 1 1 1 1 1 1 1 1 1 1 1		Odds Rat H, Random,	io 95% CI 	

Figure 3. Forest plots illustrating the association of eNOS 894G>T polymorphisms with stroke risk in all genetic models, including T vs G, G vs T, GT + TT vs GG, TT vs GG + GT, and GT vs GG + TT, respectively.

Turkish population. Based on the research by Yadav *et al.* (2019), it is suggested that eNOS 894G > T polymorphisms play a role in influencing the risk of epidermoid cell cancer of the head and neck in the population of North India. Additionally, a study conducted by Yanar *et al.* (2016) suggests a potential association between the G894T variation of NOS3 and the possibility of laryngeal cancer (LC), possibly due to the involvement of impaired redox homeostasis.

The role of nitric oxide in cancer can be seen in Figure 4. Based on Figure 4, overproduction of NO can facilitate tumor angiogenesis and metastasis. The NOS isoforms that produce NO in the vascular endothelium are defined as

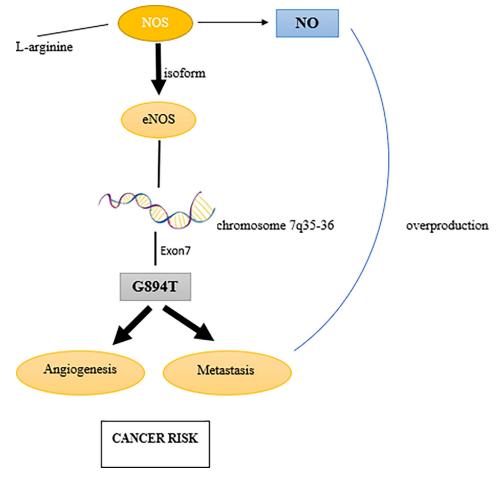


Figure 4. The role of nitric oxide in cancer.

endothelial NOS (eNOS), which is found in the endothelium and carries out a crucial role in regulating vascular tone under normal conditions, which is involved in carcinogenesis and contributes to tumor protection (Lim *et al.*, 2008). One possible explanation for the role of this enzyme in cancer progression is that reduced eNOS enzyme activity may lead to a functional decrease in NO levels within the tumor microenvironment, thereby promoting tumor growth. Recently, single nucleotide polymorphisms (SNPs) have been discovered in the eNOS gene. One of these SNPs, located at exon 7 (894G>T). Regarding to the functional role of NO in regulating angiogenesis in cancer, it is possible that this SNP might be positively correlated with the cancer progress by affecting NO synthesis.

Stroke ranks as the second-leading major contributor to mortality and disability in adults, after coronary heart disease (WHO, 2020). Stroke is a multifactorial disease; Epidemiological studies and animal experiments have provided indications of a genetic impact on the development of ischemic stroke (IS) (Hassan and Markus, 2000). Family history also serves as a crucial factor in assessing the potential for stroke. Endothelial NO, synthesized by eNOS, acts as a significant part of regulating blood flow and exhibits anti-proliferation and anti-inflammatory substances. eNOS polymorphism has a significant impact on endothelial dysfunction. The G894T variant of eNOS has been implicated in the development of diverse conditions, consisting of cardiovascular diseases and erectile dysfunction. A compromised NO-dependent vasomotor response is believed to be involved in the pathophysiology of stroke (Kaur, Uppal and Kaur, 2015). Because of its significant role in vascular physiology, genetic mutations may contribute to stroke pathogenesis by altering the expression and enzymatic activity of eNOS.

External influences that affect eNOS cause cancer and stroke through chronic stress. eNOS activity is regulated by adrenaline (Seya *et al.*, 2006; Kou and Michel, 2007; Figueroa *et al.*, 2009; Barbieri *et al.*, 2012). Prolonged stress can act as a contributing factor in the onset and advancement of cancer. Stress is also considered a relevant factor in cancer development (Antoni *et al.*, 1978; Chida *et al.*, 2008; Desaive and Ronson, 2008). eNOS plays an essential role in

ensuring vascular homeostasis, which includes regulating vascular integrity, blood flow, cell adhesion, angiogenesis, vascular permeability, immune response, and metabolism. Additionally, chronic stress can elevate the production of specific growth factors that enhance blood supply (Heid, 2014). This can accelerate the progression of cancerous tumors. Furthermore, stress can lead to increased cardiac burden, elevated blood pressure, and raised levels of sugar and fat in the bloodstream (Heart and Stroke, 2023). These factors can elevate the risk of cerebral blood clot formation, resulting in increased susceptibility to stroke.

From for the studies examined for the present meta-analysis, the presence of the eNOS polymorphism 894G>T has been correlated with a raised susceptibility to stroke in individuals of African, Asian, and European ancestry. Research conducted by Anliaçik *et al.* (2019) indicates there is no significant relationship between eNOS G894T and ischemia stroke among the Anatolia population. Diakite *et al.* (2014) stated that a significant relationship has been observed between the eNOS polymorphism 894G>T and ischemia stroke found in dominant, recessive, and additive models in the Moroccan population. Furthermore, research conducted by El Gohary, El Azab, and Kamal El-Din (2017) stated that no significant association was found between the eNOS polymorphism G894T and immediate stroke in Egyptian patients.

Research conducted by Kaur, Uppal, and Kaur (2015) stated that the G894T variant has been found to be associated with ischemic stroke and may contribute to ischemic stroke susceptibility in the Northern Indian population Kumar *et al.* (2016) suggest that the G894T eNOS can be a determinant of ischemic stroke, mainly for the large vessel disease (LVD) subtype, in the Northern Indian population. Additionally, a study by Shyu *et al.* (2017) reported that genotypic polymorphisms of the eNOS G894T polymorphism were given or used as an optimization of the risk of atherosclerotic stroke in Taiwan.

The role of nitric oxide in stroke can be seen in Figure 5. Based on Figure 5, low levels of NO derived from eNOS may exert neuroprotection in stroke by promoting vasodilatation and increasing cerebral blood flow (Yang *et al.*, 2019). However, at the same time, there is an enhancement in superoxide production due to eNOS uncoupling. This leads to a

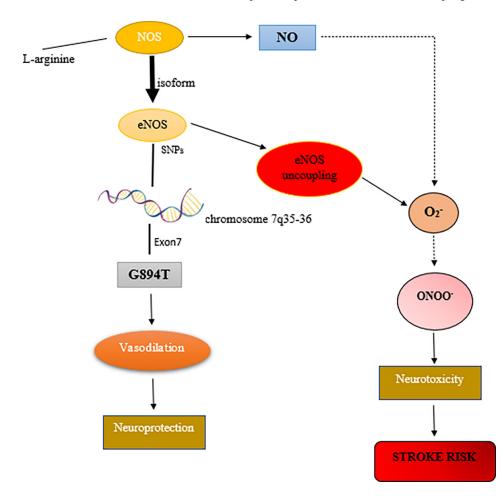


Figure 5. The role of nitric oxide in stroke.

significant increase in peroxynitrite formation, which damages lipids, proteins, and DNA and can trigger activation of poly adenosine diphosphate rribose (ADP-ribose) polymerase (PARP), which all contribute to neurotoxicity in stroke.

We conducted a meta-analysis considering the association or correlation of nitric oxide, especially NOS 894G>T polymorphism with stroke, and cancer risk. A total of 15 studies included 3,333 controls and 3,019 cases were selected for this meta-analysis. Overall, eNOS polymorphism 894G>T was found to be significantly correlated with increased cancer risk in the GG versus GT+TT genetic model. However, there were no substantial associations in the genetic model as examined in G versus T, T versus G, GT versus GG+TT, and TT versus GG+GT. The polymorphism has a relationship with a substantially higher risk of stroke in the genetic models of both TT versus GG+GT and T versus G.

Conclusions

In conclusion, the recent meta-analysis found that nitric oxide-related polymorphisms with the eNOS 894G>T gene are associated with a substantial risk of cancer in the total population based on the GG vs. GT+TT genetic model and significantly correlated with the manifestation of stroke in the genetic models T vs. G, and TT vs. GG + GT. G vs. T, and GG + GT vs. TT. Considering the conclusion, these results should be reassessed in the coming days through studies with a larger sample population.

Data availability

Underlying data

All underlying data are available as part of the article and no additional source data are required.

Reporting guidelines

Zenodo: PRISMA Checklist for "Association between nitric oxide and cancer and stroke risk: A meta-analysis", https:// doi.org/10.5281/zenodo.8031323 (Tualeka et al., 2023).

Data are available under the terms of the Creative Commons Zero "No rights reserved" data waiver (CC0 1.0 Public domain dedication).

References

Aaltoma SH, Lipponen PK, Kosma VM: Inducible nitric oxide synthase (iNOS) expression and its prognostic value in prostate cancer. Anticancer Res. 2001; 21(4B): 3101-3106. **PubMed Abstract**

Abedinzadeh M, Dastgheib SA, Maleki H, et al.: Association of endothelial nitric oxide synthase gene polymorphisms with susceptibility to prostate cancer: A comprehensive systematic review and metaanalysis. Urol. J. 2020; 17(4): 329-337. PubMed Abstract | Publisher Full Text

Adibmanesh A. Bijanzadeh M. Mohammadzadeh G. et al.: The correlation of endothelial nitric oxide synthase gene rs1799983 polymorphisms with colorectal cancer. Int. J. Cancer Manag. 2020; 13(5) **Publisher Full Text**

Akbar KR, Permatasari AI, Suwarno B, et al.: Lack of association between endothelial nitric oxide synthase (eNOS) G894T gene polymorphism and the risk of bladder cancer A meta-analysis. Arch. Hell. Med. 2022; 39(5): 647-653.

Akhter MS, Biswas A, Rashid H, et al.: Screening of the NOS3 gene identifies the variants 894G/T, 1998C/G and 2479G/A to be associated with acute onset ischemic stroke in young Asian Indians. J. Neurol. Sci. 2014; 344(1-2): 69-75

PubMed Abstract | Publisher Full Text

Anliaçik SÖ, Tokgöz S, Zamani AG, et al.: Investigation of the relationship between ischemic stroke and endothelial nitric oxide synthase gene polymorphisms [G894T, intron 4 VNTR and T786C]. Turk. J. Med. Sci. 2019; 49(2): 589-594.

PubMed Abstract | Publisher Full Text | Free Full Text

Antoni MH, Lutgendorf SK, Cole SW, et al.: Pain pathways and mechanisms. Anaesthesia. 1978; 33(10): 935-944. **Publisher Full Text**

Aouf S. Laribi A. Gabboui S. et al.: Contribution of Nitric oxide synthase 3 genetic variants to nasopharyngeal carcinoma risk and progression in a Tunisian population. Eur. Arch. Otorhinolaryngol. 2019; 276(4): 1231-1239.

Publisher Full Text

Barbieri A, Palma G, Rosati A, et al.: Role of endothelial nitric oxide synthase (eNOS) in chronic stress-promoted tumour growth. J. Cell. Mol. Med. 2012; 16(4): 920-926.

PubMed Abstract | Publisher Full Text | Free Full Text

Branković A, Brajušković G, Nikolić Z, et al.: Endothelial nitric oxide synthase gene polymorphisms and prostate cancer risk in serbian population. Int. J. Exp. Pathol. 2013; 94(6): 355-361. Med Abstract | Publisher Full Text | Free Full Text

Buldreghini E, Mahfouz RZ, Vignini A, et al.: Single Nucleotide Polymorphism (SNP) of the Endothelial Nitric Oxide Synthase (eNOS) Gene (Glu298Asp Variant) in Infertile Men With Asthenozoospermia. J. Androl. 2010; 31(5): 482-488.

PubMed Abstract | Publisher Full Text

Carkic J, Nikolic N, Nisevic J, et al.: Endothelial nitric oxide synthase polymorphisms/haplotypes are strong modulators of oral cancer risk in serbian population. J. Oral Sci. 2020; 62(3): 322-326. PubMed Abstract | Publisher Full Text

Ben Chaaben A, Mariaselvam C, Salah S, et al.: Polymorphisms in oxidative stress-related genes are associated with nasopharyngeal carcinoma susceptibility. Immunobiology. 2015; 220(1): 20-25. **Publisher Full Text**

Chen GG, Tak WL, Xu H, et al.: Increased inducible nitric oxide synthase in lung carcinoma of smokers. Cancer. 2008; 112(2): 372-381

PubMed Abstract | Publisher Full Text

Chen ZQ, Mou RT, Feng DX, et al.: The role of nitric oxide in stroke. Med. Gas Res. 2017; 7(3): 194-203.

PubMed Abstract | Publisher Full Text | Free Full Text

Chida Y, Hamer M, Wardle J, et al.: Do stress-related psychosocial factors contribute to cancer incidence and survival? Nat. Clin. Pract. Oncol. 2008: 5(8): 466-475.

PubMed Abstract | Publisher Full Text

da Costa Escobar Piccoli J, Manfredini V, Hamester FI, et al.: Interaction Between Endothelial Nitric Oxide Synthase Gene Polymorphisms (-786T>C. 894G>T and Intron 4 a/b) and Cardiovascular Risk Factors in Acute Coronary Syndromes. Arch. Med. Res. 2012; 43(3): 205-211. Publisher Full Text PubMed Abstract

Demaerschalk BM, Kleindorfer DO, Adeoye OM, et al.: Scientific Rationale for the Inclusion and Exclusion Criteria for Intravenous Alteplase in Acute Ischemic Stroke A Statement for Healthcare Professionals from the American Heart Association/American Stroke Association. Stroke 2016: 47: 581-641.

PubMed Abstract | Publisher Full Text

Desaive P, Ronson A: Stress spectrum disorders in oncology. Curr. Opin. Oncol. 2008: 20(4): 378-385.

Publisher Full Text

Diakite B, Hamzi K, Slassi I, et al.: G894T endothelial nitric oxide synthase polymorphism and ischemic stroke in Morocco. Meta Gene. 2014; 2(1): 349-357

PubMed Abstract | Publisher Full Text | Free Full Text

Erlandsson A, Carlsson J, Andersson SO, et al.: High inducible nitric oxide synthase in prostate tumor epithelium is associated with lethal prostate cancer. *Scand. J. Urol.* 2018; **52**(2): 129–133. PubMed Abstract | Publisher Full Text

Figueroa XF, Poblete I, Fernández R, *et al.*: **NO production and eNOS phosphorylation induced by epinephrine through the activation of** β-adrenoceptors. *Am. J. Physiol. Heart Circ. Physiol.* 2009; **297**(1): . H134-H143

PubMed Abstract | Publisher Full Text

Gào X, Schöttker B: Reduction-oxidation pathways involved in cancer development: A systematic review of literature reviews. Oncotarget. 2017; 8(31): 51888-51906.

PubMed Abstract | Publisher Full Text | Free Full Text

Gào X, Xuan Y, Benner A, et al.: Nitric Oxide Metabolites and Lung Cancer Incidence: A Matched Case-Control Study Nested in the ESTHER Cohort. Oxidative Med. Cell. Longev. 2019; 2019: 1–9. PubMed Abstract | Publisher Full Text | Free Full Text

El Gohary EA, El Azab AA, Kamal El-Din MMM: Study of Relationship between G894T Variant of Endothelial Nitric Oxide Synthase Gene and Acute Ischemic Stroke. Egyptian J. Hosp. Med. 2017; 69(1): 1607–1613. Publisher Full Text

Guo X: Endothelial nitric oxide (eNOS) gene G894T and VNTR polymorphisms are closely associated with the risk of ischemic stroke development for Asians: Meta-analysis of epidemiological studies. Mol. Biol. Rep. 2014; 41(4): 2571-2583.

PubMed Abstract | Publisher Full Text

Hao Y, Montiel R, Huang Y: Endothelial nitric oxide synthase (eNOS) 894 G>T polymorphism is associated with breast cancer risk: A metaanalysis. Breast Cancer Res. Treat. 2010; 124(3): 809-813. PubMed Abstract | Publisher Full Text

Hassan A, Markus HS: Genetics of ischaemic stroke. J. Neurol. Neurosurg. Psychiatry. 2000; 84(12): 1302-1308. **Publisher Full Text**

Heart&Stroke: Stress basics. 2023.

Heid M: How stress affects cancer risk. 2014.

Heller A: Apoptosis-inducing high •NO concentrations are not sustained either in nascent or in developed cancers. ChemMedChem. 2008; 3(10): 1493-1499. Publisher Full Text

Hinz J, Schöndorf D, Bireta C, et al.: The eNOS 894G/T gene polymorphism and its influence on early and long-term mortality after on-pump cardiac surgery. J. Cardiothorac. Surg. 2013; 8(1): 199. PubMed Abstract | Publisher Full Text | Free Full Text

Hung WC, Wu TF, Ng SC, *et al*.: **Involvement of endothelial nitric oxide** synthase gene variants in the aggressiveness of uterine cervical cancer. *J. Cancer.* 2019; **10**(12): 2594–2600.

PubMed Abstract | Publisher Full Text | Free Full Text

Jang MJ, Jeon YJ, Kim JW, *et al*.: Association of eNOS polymorphisms (-786T>C, 4a4b, 894G>T) with colorectal cancer susceptibility in the Korean population. Gene. 2013; 512(2): 275-281. PubMed Abstract | Publisher Full Text

Kang MK, Kim OJ, Jeon YJ, et al.: Interplay between polymorphisms in the endothelial nitric oxide synthase (eNOS) gene and metabolic syndrome in determining the risk of ischemic stroke in Koreans. J. Neurol. Sci. 2014; 344(1-2): 55-59. PubMed Abstract | Publisher Full Text

Kaur K, Uppal A, Kaur A: An exonic G894T variant of endothelial nitric oxide synthase gene as a risk factor for ischemic stroke in North Indians. Acta Neurobiol. Exp. 2015; 75(4): 339-350. PubMed Abstract

Koçer C, Benlier N, Balci SO, et al.: The role of endothelial nitric oxide synthase gene polymorphisms in patients with lung cancer. *Clin. Respir. J.* 2020; **14**(10): 948–955. PubMed Abstract | Publisher Full Text

Korde Choudhari S, Chaudhary M, Bagde S, et al.: Nitric oxide and cancer: A review. World J. Surg. Oncol. 2013; 11: 1–11. PubMed Abstract | Publisher Full Text | Free Full Text

Kou R, Michel T: Epinephrine regulation of the endothelial nitric-oxide synthase: Roles of RAC1 and β3-adrenergic receptors in endothelial no signaling. J. Biol. Chem. 2007; 282(45): 32719–32729. PubMed Abstract | Publisher Full Text

Kumar A, Misra S, Kumar P, *et al.*: **Association between Endothelial nitric** oxide synthase G894T gene polymorphism and risk of ischemic stroke in North Indian population: A case-control study. Neurol. Res. 2016; 38(7): 575-579.

PubMed Abstract | Publisher Full Text

Lee P-C: A Case-Control Study and Meta-Analysis of the Association of eNOS rs1799983 SNP with Stroke Risk. Med. Health. 2019; 14(1): 118-134.

Publisher Full Text

Lim KH, Ancrile BB, Kashatus DF, et al.: Tumour maintenance is mediated by eNOS. Nature. 2008; 452(7187): 646-649.

PubMed Abstract | Publisher Full Text | Free Full Text

Loibl S, Von Minckwitz G, Weber S, *et al.*: **Expression of endothelial and inducible nitric oxide synthase in benign and malignant lesions of the** breast and measurement of nitric oxide using electron paramagnetic resonance spectroscopy. Cancer. 2002; 95(6): 1191-1198. PubMed Abstract | Publisher Full Text

Özçelik AT, Can Demirdöaen B, Demirkaya Ş, et al.: Importance of NOS3 genetic polymorphisms in the risk of development of ischemic stroke in the Turkish population. Genet. Test. Mol. Biomarkers. 2014; 18(12): 797-803

PubMed Abstract | Publisher Full Text

Patel JB, Shah FD, Shukla SN, et al.: Role of nitric oxide and antioxidant enzymes in the pathogenesis of oral cancer. J. Cancer Res. Ther. 2009; 5(4): 247-253.

PubMed Abstract | Publisher Full Text

Rah H, Jeon YJ, Lee WS, *et al.*: Association of nitric oxide synthase gene polymorphisms (-786T>C, 4a4b, 894G>T) with primary ovarian insufficiency in Korean women. *Maturitas.* 2013; **74**(2): 160–165. PubMed Abstract | Publisher Full Text

Sachdev K: Clinical Pathology and Clinical Bacteriology. J. Clin. Pathol. Bacteriol. 1999; 22(1).

Publisher Full Text

Seya Y, Fukuda T, Isobe K, et al.: Effect of norepinephrine on RhoA, MAP kinase, proliferation and VEGF expression in human umbilical vein endothelial cells. Eur. J. Pharmacol. 2006; 553(1-3): 54-60. PubMed Abstract | Publisher Full Text

Shvu HY. Chen MH. Hsieh YH, et al.: Association of eNOS and Cav-1 gene polymorphisms with susceptibility risk of large artery atherosclerotic stroke. PLoS One. 2017; 12(3): e0174110-e0174112. PubMed Abstract | Publisher Full Text | Free Full Text

Song Y, Zhao XP, Song K, et al.: Ephrin-A1 Is Up-Regulated by Hypoxia in Cancer Cells and Promotes Angiogenesis of HUVECs through a Coordinated Cross-Talk with eNOS. PLoS One. 2013; 8(9): e74464-e74468

PubMed Abstract | Publisher Full Text | Free Full Text

Su CW, Chien MH, Lin CW, et al.: Associations of genetic variations of the endothelial nitric oxide synthase gene and environmental carcinogens with oral cancer susceptibility and development. *Nitric* Oxide. 2018; **79**(May): 1–7.

PubMed Abstract | Publisher Full Text

Tsay MD, Hsieh MJ, Wang SS, et al.: Impact of endothelial nitric oxide synthase polymorphisms on urothelial cell carcinoma development. Urol. Oncol. 2019; **37**(4): 293.e1–293.e9. PubMed Abstract | Publisher Full Text

Tualeka AR, Jalaludin J, Gasana J, et al.: PRISMA Checklist for Article Association between nitric oxide and cancer and stroke risk: A metaanalysis. Zenodo. 2023. **Publisher Full Text**

Utispan K, Koontongkaew S: High nitric oxide adaptation in isogenic primary and metastatic head and neck cancer cells. *Anticancer Res.* 2020; **40**(5): 2657-2665.

Publisher Full Text

Vanini F, Kashfi K, Nath N: The dual role of iNOS in cancer. Redox Biol. 2015; 6(August): 334-343.

PubMed Abstract | Publisher Full Text | Free Full Text

Verim L. Toptas B. Özkan NE. et al.: Possible relation between the NOS3 gene GLU298ASP polymorphism and bladder cancer in Turkey. Asian Pac. J. Cancer Prev. 2013; 14(2): 665-668. PubMed Abstract | Publisher Full Text

WHO: The top 10 causes of death. World Health Organization; 2020.

Yadav SK, Gupta S, Yadav A, et al.: Endothelial nitric oxide synthase gene polymorphisms modulate the risk of squamous cell carcinoma of head and neck in north Indian population. Meta Gene. 2019; 21(October 2018): 100575.

Publisher Full Text

Yanar K, Çakatay U, Aydin S, et al.: Relation between Endothelial Nitric **Oxide Synthase Genotypes and Oxidative Stress Markers in Larynx**

Cancer. Oxidative Med. Cell. Longev. 2016; 2016: 1–8. PubMed Abstract | Publisher Full Text | Free Full Text

Yang C, Hawkins KE, Doré S, *et al*.: **Neuroinflammatory mechanisms of blood-brain barrier damage in ischemic stroke.** 2019.

Yao YS, Chang WW, Jin YL, *et al*.: An updated meta-analysis of endothelial nitric oxide synthase gene: Three well-characterized polymorphisms with ischemic stroke. *Gene*. 2013; **528**(2): 84–92. PubMed Abstract | Publisher Full Text Zhao C, Yan W, Zu X, et al.: Association between endothelial nitric oxide synthase 894G-T polymorphism and prostate cancer risk: a metaanalysis of literature studies. *Tumor Biol.* 2014; **35**(12): 11727-11733. PubMed Abstract | Publisher Full Text

Zhao X, Haensel C, Araki E, *et al*.: Gene-dosing effect and persistence of reduction in ischemic brain injury in mice lacking inducible nitric oxide synthase. *Brain Res.* 2000; 872(1–2): 215–218. PubMed Abstract | Publisher Full Text

Open Peer Review

Current Peer Review Status: 🗙 🗙

Version 1

Reviewer Report 16 February 2024

https://doi.org/10.5256/f1000research.148090.r233438

© **2024 Miranda K.** This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Katrina Miranda

University of Arizona, Arizona, USA

The manuscript by Tualeka and colleagues provides a meta-analysis on the correlation of a polymorphism in the eNOS gene on cancer and stroke. The analysis itself is fine. However, presentation of the complex relationship of NO and cancer or stroke is cursory. References include randomly chosen reviews rather than the original literature. It would be beneficial to include an expert on NO in preparation of a revision. For example, the levels of NO produced by the isoforms is not well presented. The polymorphism is also not described sufficiently. Lately, in Figure 5, if eNOs is uncoupled, where does the NO come from to produce peroxynitrite? In sum, more details are needed in this manuscript.

Are the rationale for, and objectives of, the Systematic Review clearly stated?

Yes

Are sufficient details of the methods and analysis provided to allow replication by others? $\ensuremath{\mathsf{Yes}}$

Is the statistical analysis and its interpretation appropriate?

Yes

Are the conclusions drawn adequately supported by the results presented in the review? Partly

If this is a Living Systematic Review, is the 'living' method appropriate and is the search schedule clearly defined and justified? ('Living Systematic Review' or a variation of this term should be included in the title.)

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: NO and cancer

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 12 February 2024

https://doi.org/10.5256/f1000research.148090.r233428

© **2024 Chong E.** This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Eric Tzyy Jiann Chong 回

Biotechnology Research Institute, Universiti Malaysia Sabah, Sabah, Malaysia

The manuscript by Tualeka et al. aims to associate the eNOS rs1799983 SNP with the risk of cancer and stroke using a meta-analysis approach. The authors concluded that this SNP is associated with the risk of cancer and stroke in some genetic model comparisons. Overall, the manuscript is poorly written, with many unclear statements and grammatical errors, although it has been reviewed and edited by many authors. There are many concerns that should be addressed by the authors.

Title

- The current title is not specific enough to reflect the contents of the manuscript. Please include the specific gene and polymorphism in the title.

Abstract

-Why is the objective of the study is written in the Methods instead of the Background?

- The Results section is not clear. Among the 3019 cases and 3333 controls, how many are derived from cancer and stroke cases, respectively?

- What is meant by "significantly positively correlated with cancer risk"? Does it mean an increase or decrease in risk?

-The conclusion is more like describing the results of this study than a conclusion. Please rephrase.

Introduction

- The authors mentioned that several SNPs have been identified in the eNOS gene but failed to justify why they focused on the rs179983 SNP.

- What is meant by "....especially its polymorphism eNOS, as well as....."?

-What is meant by "...and both cancer risks" in the last sentence of this section?

Methods

- What is meant by "The search period was limited from December 2022 to January 2023"? Were only studies published within this period included in the meta-analysis?

- The authors did not include some essential keywords in the literature search, such as

"rs1799983", "carcinoma", and "cerebrovascular *disease*", which are frequently used in scientific publications.

- Please remove the G vs. T allelic model in the statistical analysis, as it did not yield any meaningful results. The T. vs. G allelic model is sufficient to determine if the recessive T allele is a risk factor for cancer or stroke susceptibility.

- The authors mentioned that a publication bias test cannot be performed due to fewer than 10 studies. This is incorrect, as more than or equal to 3 studies are sufficient for a publication bias test.

- The quality scoring of the studies is not included in the meta-analysis.

- A sensitivity analysis is not included in the meta-analysis.

Results

- It is very confusing to look at the Figure 1 alone. For example, the reports assessed for eligibility are 43, after excluding 21 reports, it should remain at 22. Why are studies included in the metaanalysis only 15?

-The caption for Figures 2 and 3 is not clear. What does A-E mean?

- Many studies published between the years 2012-2023 are not included in the meta-analysis. A few examples are listed below. This shows that the literature search is not comprehensive, probably due to the keywords used.

i) Fadi et al. 2018. World Journal of Neuroscience, 8(1): 98-107.

ii) Phneh et al. 2019. Medicine & Health, 14(1): 118-134.

iii) Jelel et al. 2020. Biological Research for Nursing, 23(3): 408-17.

Discussion

- The second, sixth, and seventh paragraphs are restating the findings of previous studies; they are not comparing to the data of this meta-analysis.

- The last paragraph should be removed and replaced with a paragraph that states the limitations of this study.

Are the rationale for, and objectives of, the Systematic Review clearly stated?

Partly

Are sufficient details of the methods and analysis provided to allow replication by others? Partly

Is the statistical analysis and its interpretation appropriate?

Partly

Are the conclusions drawn adequately supported by the results presented in the review? Partly

If this is a Living Systematic Review, is the 'living' method appropriate and is the search schedule clearly defined and justified? ('Living Systematic Review' or a variation of this term should be included in the title.)

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Gene polymorphisms, meta-analysis, risk association, molecular epidemiology, medical biotechnology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

