SYSTEMATIC REVIEW

Association between nitric oxide and cancer and stroke risk: A meta-analysis [version 1; peer review: 2 not approved]

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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

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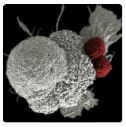
stroke in the T *versus* G model, followed by TT *versus* GG+GT.
Conclusions: The results of the eNOS 894G>T polymorphisms have been correlated with cancer, and in particular, the GT+TT *versus* GG model yielded an odds ratio (OR of 1.96, a 95% CI of 1.22 to 3.15, and a *p*-value of 0.0005. Moreover, 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, meta-analysis, safe work



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

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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* G, 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 heterozygous genotype, where the individual has one copy of the G allele and one copy of the T allele. TT 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 $p \leq 0.05$.

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 I^2 test, which quantifies the degree of heterogeneity. If the I^2 value was less than 25%, it indicated no heterogeneity. If the I^2 value ranged from 25% to 50%, it showed moderate heterogeneity. If the I^2 was greater than 50%, it indicated extreme heterogeneity. The 50% *p*-value indicating the existence of 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.*, 2019) were also excluded because of unavailable genotype frequency. Six studies (Hao, Montiel and Huang, 2010; Yao *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

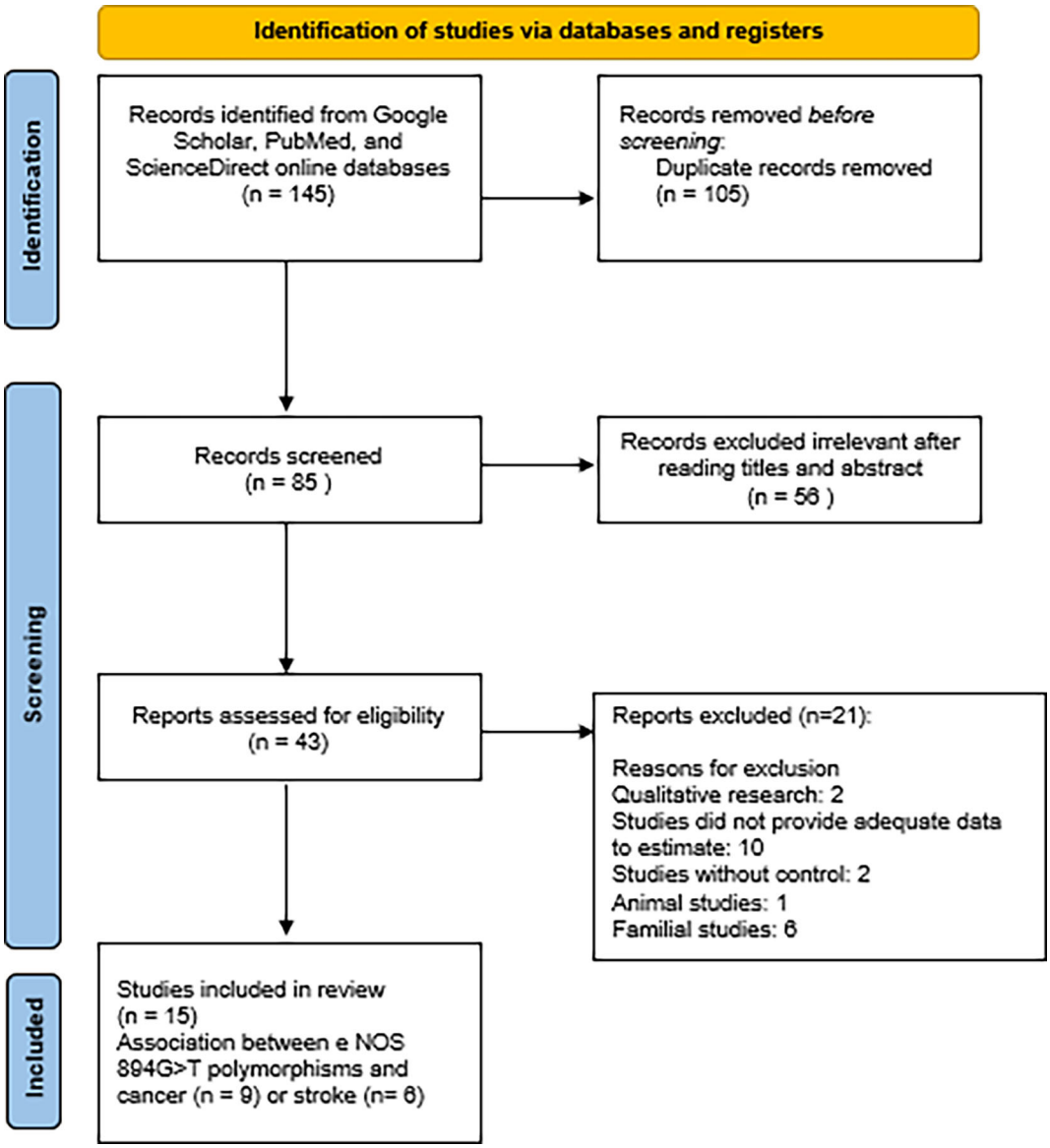

Figure 1. Flow diagram depicting the inclusion process of the studies in the meta-analysis.

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).

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

First Author/Year	Country	Risk	Case/Control	Case					Control				
				Genotype			Allele		Genotype			Allele	
				GG	TT	GT	T	G	GG	TT	GT	T	G
(Adibmanesh *et al.*, 2020)	Iran	Cancer	100/100	28	28	44	100	100	57	6	37	49	151
(Aouf *et al.*, 2019)	Tunisia	Cancer	259/169	149	20	90	130	388	73	18	78	114	224
(Branković *et al.*, 2013)	Serbia	Cancer	150/100	76	9	65	83	217	54	6	40	52	148
(Carkic *et al.*, 2020)	Serbia	Cancer	50/110	21	5	24	34	66	61	7	42	56	164
(Koçer *et al.*, 2020)	Turkey	Cancer	107/100	74	1	32	34	180	65	1	34	36	164
(Su *et al.*, 2018)	Taiwan	Cancer	1044/1200	825	10	209	229	1859	935	15	250	280	2120
(Verim *et al.*, 2013)	Turkey	Cancer	66/88	7	10	49	69	63	31	13	44	70	106
(Yadav *et al.*, 2019)	India	Cancer	179/173	88	20	64	104	240	96	8	59	75	251
(Yanar *et al.*, 2016)	Turkey	Cancer	58/147	18	11	29	51	65	31	35	81	151	143
(Anliaçik *et al.*, 2019)	Turkey	Stroke	112/160	21	14	77	44	40	38	19	103	57	61
(Diakite *et al.*, 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	6	6	45	15	5	3	3	12	8
(Kaur, Uppal and Kaur, 2015)	India	Stroke	120/101	84	6	30	18	83	83	1	17	9	91
(Kumar *et al.*, 2016)	India	Stroke	250/250	164	12	74	20	80	186	5	59	14	86
(Shyu *et al.*, 2017)	Taiwan	Stroke	229/243	151	16	62	21	50	185	7	51	13	87

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

Genetic Models	NS	Pooled ORs (95% CI)	*p* value[a] (Z test)	I^2 (%)	*p*H	*p*E	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

NS: Number of studies, *p*H: *p* heterogenity, *p*E: *p* egger.
[a]The *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)	*p* value[a] (Z test)	I^2 (%)	*p*H	*p*E	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.
[a]The *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 l-arginine to l-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. Koçer *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

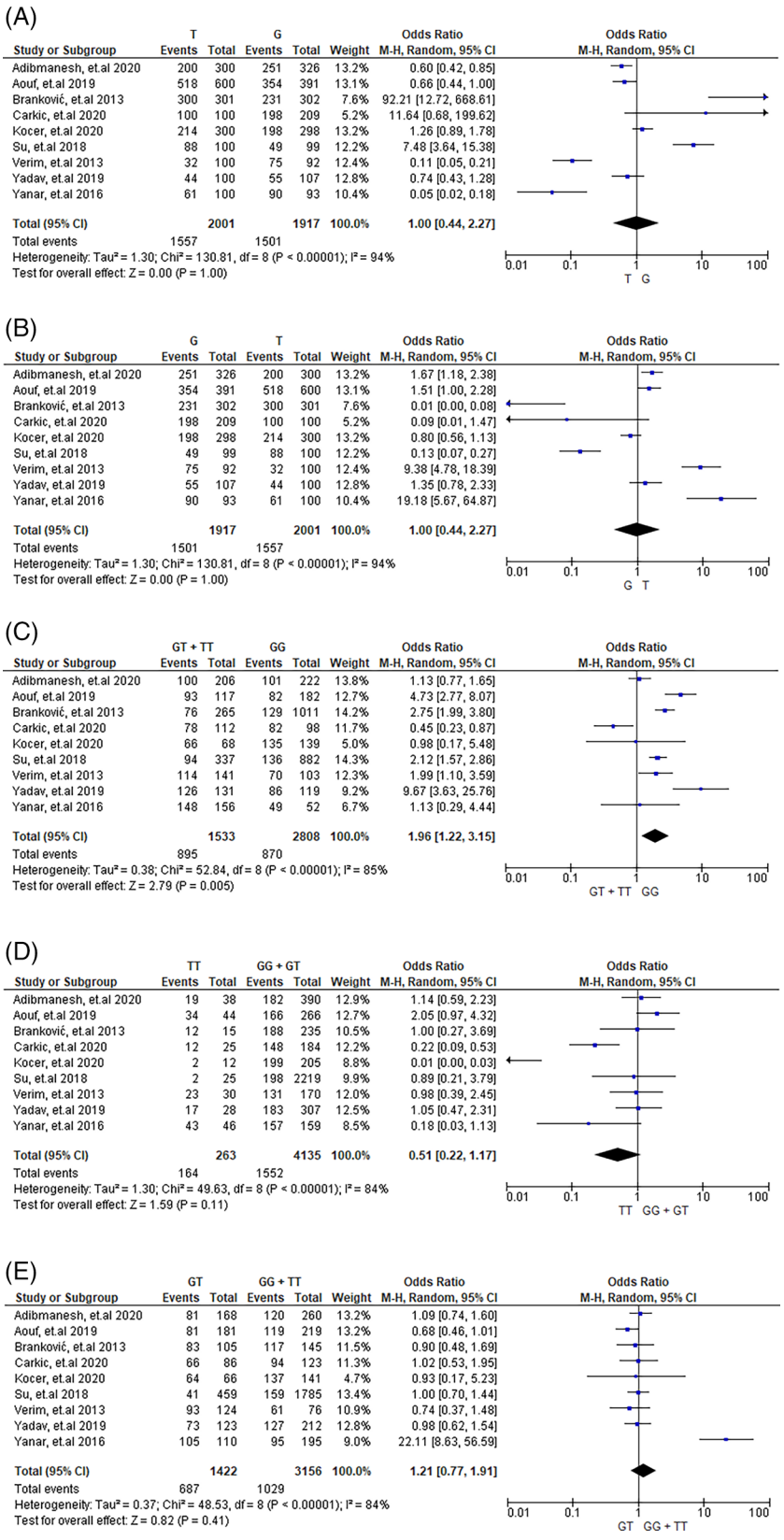

Figure 2. Forest plots depicting the association of eNOS 894G>T polymorphisms with cancer risk in all genetic models, including T vs G, G vs T, GT + TT vs GG, TT vs GG + GT, and GT vs GG + TT, respectively.

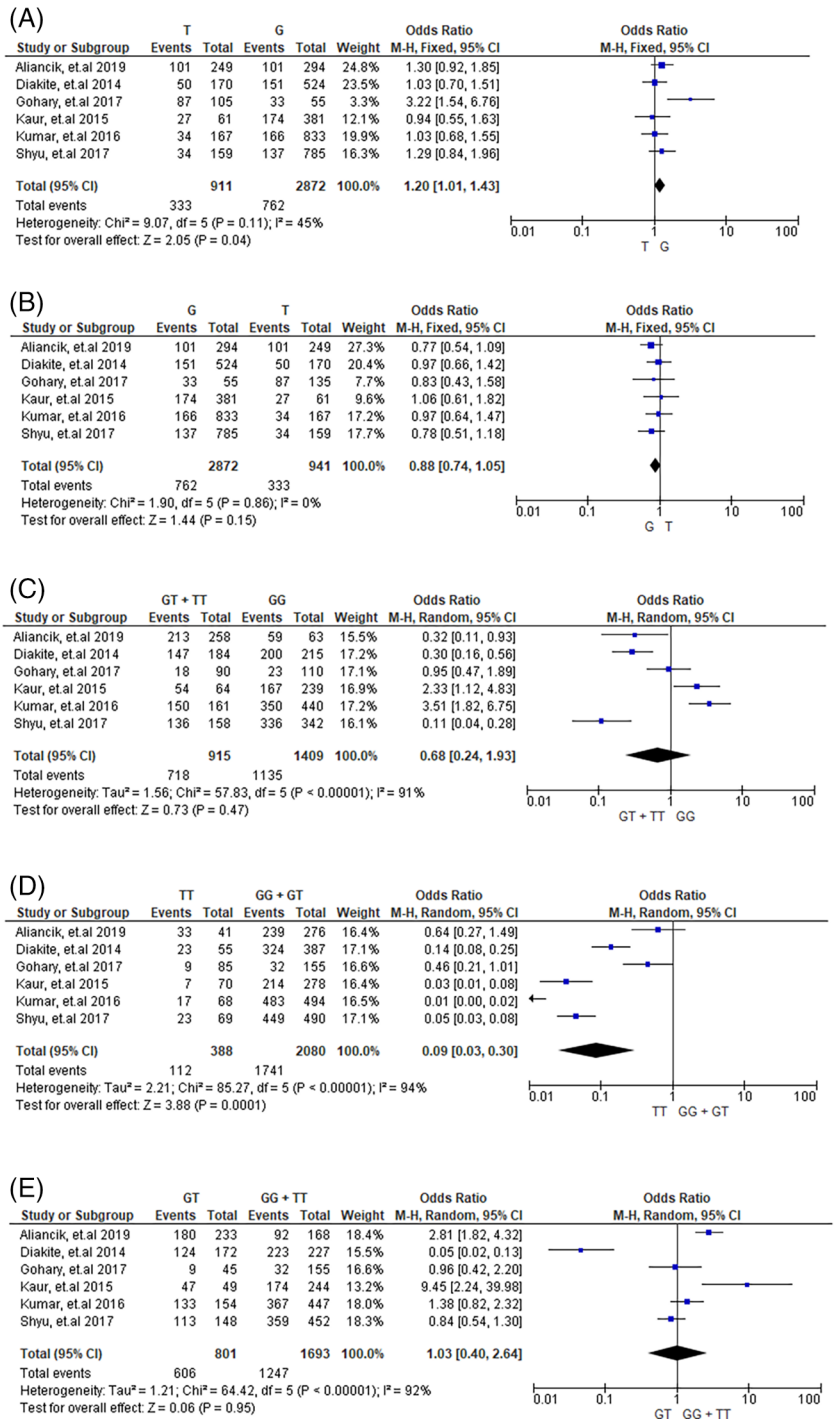

Figure 3. Forest plots illustrating the association of eNOS 894G>T polymorphisms with stroke risk in all genetic models, including T vs G, G vs T, GT + TT vs GG, TT vs GG + GT, and GT vs GG + TT, respectively.

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

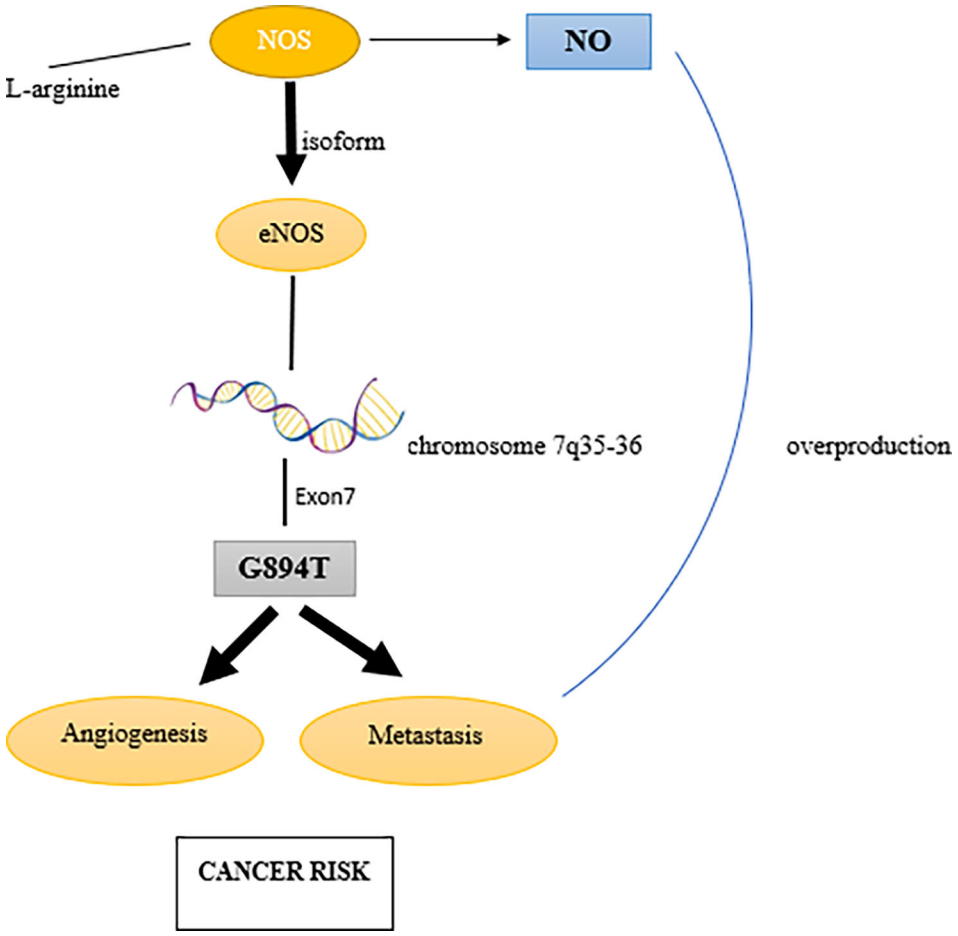

Figure 4. The role of nitric oxide in cancer.

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

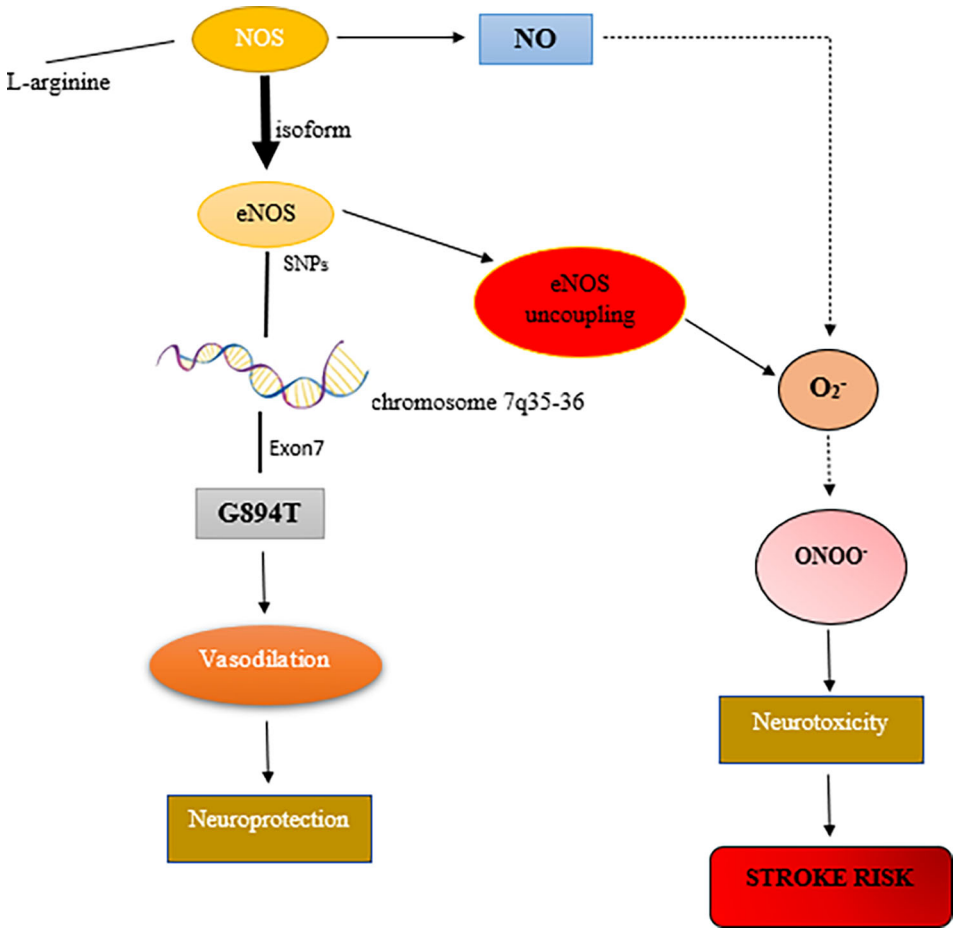

Figure 5. The role of nitric oxide in stroke.

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).

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 meta-analysis.** *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, Gabbouj 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.
PubMed 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.
PubMed Abstract | Publisher Full Text

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 meta-analysis.** *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 Demirdöıen 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

Shyu 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 meta-analysis.** *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, Toptaş 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 meta-analysis 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

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Version 1

Reviewer Report 16 February 2024

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

✗ **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?
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

Eric Tzyy Jiann Chong
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 meta-analysis 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.