

The genetic association between *iNOS* and *eNOS* polymorphisms and gastric cancer risk: a meta-analysis

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Objective: There are a number of susceptible factors for an increased risk of gastric cancer. Nitric oxide (NO) is considered to be associated with the development of a range of cancers. In particular, inducible nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS) are known to play a central role in the production of NO. Published studies relating to the association between *eNOS* rs1799983, rs2070744, and *iNOS* rs2297518 polymorphisms and the risk of gastric cancer risk are conflicting and inconclusive and require further analysis.

Materials and methods: This study involved a meta-analysis of case-control studies relating to *eNOS* rs1799983, rs2070744, and *iNOS* rs2297518 polymorphisms published prior to January 2018. Literature searches were carried out in PubMed, Embase, Web of Science, the Cochrane Library databases, and the Chinese National Knowledge Infrastructure. Pooled odds ratios (ORs) and 95% confidence intervals (CIs) were used to evaluate the strength of association based on genotype data.

Results: A total of 1,356 cases and 1,791 controls were included from nine case-control studies involving *eNOS* rs1799983 (G894T), rs2070744 (T-786C), and *iNOS* rs2297518 (C150T) polymorphisms. Data analysis indicated that *iNOS* rs2297518 was a risk factor for *Helicobacter pylorus*-positive gastric cancer when compared with *H. pylorus*-negative gastric cancer ($p=0.003$, OR [95% CI]=2.19 [1.31–3.66]). In addition, the allelic, dominant, and recessive models of *eNOS* rs2070744 were significantly associated with a risk of gastric cancer (allelic model: $p<0.00001$, OR [95% CI]=0.23 [0.16–0.34]; dominant model: $p<0.00001$, OR [95% CI]=0.25 [0.15–0.42]; recessive model: $p<0.00001$, OR [95% CI]=0.16 [0.08–0.30]). No association was identified between *eNOS* rs1799983 and the risk of gastric cancer ($p>0.05$).

Conclusion: *iNOS* rs2297518 and *eNOS* rs2070744 polymorphisms may represent susceptible factors for gastric cancer.

Keywords: inducible nitric oxide synthase, iNOS, endothelial nitric oxide synthase, eNOS, gastric cancer, meta-analysis

Introduction

Gastric cancer is one of the leading causes of mortality worldwide.^{1,2} Multiple factors contribute to the pathogenesis of gastric cancer including genetic parameters, epigenetic predisposition, and environmental risk factors.^{3–5} Previous studies have reported that *Helicobacter pylorus* is the most common risk factor in the pathology of gastric cancer.^{6,7} However, one study showed that while 50% of the study population was infected with *H. pylorus*, the proportion of patients infected with *H. pylorus* who went on to develop gastric cancer was <5%.⁸ Notably, in South Africa, where the incidence of *H. pylorus* is known to be higher than other areas of the world, the morbidity of

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gastric cancer was shown to be relatively low.⁹ Thus, it is important that we investigate the potential association between genetic predisposition and gastric cancer.

Recent research studies have focused on nitric oxide (NO), a short-lived, small molecule, generated from the physiological transformation of L-arginine to L-citrulline by nitric oxide synthase (NOS).^{10,11} NO plays an important role in vasodilatation,¹² smooth muscle relaxation,¹³ immunity,¹⁴ and tumor angiogenesis.¹⁵ Four isoforms of NOS have been identified and described as inducible nitric oxide synthase (iNOS), neuronal nitric oxide synthase (nNOS), endothelial nitric oxide synthase (eNOS), and mitochondrial nitric oxide synthase (mNOS).¹⁶ eNOS is a calcium (Ca²⁺)-dependent enzyme and was first identified in vascular endothelial cells.¹⁷ It has also been shown that eNOS regulates blood pressure, platelet aggregation, leukocyte adherence, and vascular smooth muscle cell mitogenesis and angiogenesis.^{18–20} Furthermore, iNOS is one of the most important enzymes involved in the metabolic pathway of reactive oxygen and nitrogen species in the presence of *H. pylorus* infection.²¹ It is also important to note that iNOS can produce larger amounts of NO than either eNOS or nNOS.²²

eNOS is encoded by the *NOS3* gene, located in 7q35-q36, and encodes a protein of 1,203 amino acids.²³ To date, a number of polymorphisms have been reported for the *NOS3* gene, including rs1799983, intron 4a/b, and rs2070744; all these have been investigated for their association with the risk of gastric cancer.^{24,25} Significant associations were detected between *eNOS* rs2070744 and the risk of gastric cancer in two different populations.^{25,26} However, no genetic association was observed between *eNOS* rs1799983 and the risk of gastric cancer in Guilan and Chinese populations.^{25,27} In terms of the *iNOS* gene, the rs2297518 polymorphism in exon 16 results in an amino acid substitute, Ser608Leu, and has been reported to be associated with cigarette- and alcohol-induced gastric cancer in the Chinese population²⁸ and *H. pylorus*-related gastric cancer in Iran.²⁹ However, these results were inconsistent and inconclusive.

Considering the limited number of subjects included in previous studies and the inconsistent results arising from different populations, we performed this meta-analysis in order to combine all pertinent published studies and investigate the precise association between *eNOS* rs2070744, rs1799983, and *iNOS* rs2297518 and the pathogenesis of gastric cancer.

Materials and methods

Publication searches and selection criteria

This meta-analysis followed the Cochrane collaboration definition and PRISMA 2009 guidelines for meta-analyses

and systematic reviews.³⁰ Literature searches were carried out in PubMed, Embase, Web of Science, the Cochrane Library databases, and the Chinese National Knowledge Infrastructure. We searched for all relevant publications exploring the relationship between iNOS and eNOS polymorphisms and the risk of gastric cancer (until January 1, 2018). The following were used as search terms: “*iNOS*” or “Inducible nitric oxide synthase” and “*eNOS*” or “endothelial nitric oxide synthase” or “*NOS3*” or “nitric oxide synthase 3” and “polymorphism” or “variation” and “gastric cancer” or “stomach cancer” or “gastric adenocarcinoma.” Our search was not limited by language. In addition, other potentially relevant items of literature were identified by cross-reference within eligible studies.

Inclusion criteria

Studies were included if 1) they had a case–control design; 2) they contained sufficient data to calculate an odds ratio (OR) with a 95% CI; 3) they referred to the association between iNOS and eNOS polymorphisms and the risk of gastric cancer; and 4) the genotypic distributions in control groups were in Hardy–Weinberg equilibrium (HWE).

Exclusion criteria

Studies were excluded if 1) they were duplicated studies, abstracts, letters, reviews, or papers presented in a meeting; 2) if they were unrelated to the genetic association between *iNOS* and *eNOS* polymorphisms and gastric cancer; 3) if there was insufficient genotype data; and 4) if the control group was not in HWE.

Data extraction and quality assessment

Two authors (Jiang and Chen) extracted the data independently. Information arising from each individual study were summarized as follows: first author, year of publication, ethnicity, case and control, mean ages, gender (proportion of males), genotyping methods, sample source, HWE for control groups, state of *H. pylorus*, and the number of patients who smoked or suffered from alcoholism. All included studies were independently evaluated by using the Newcastle–Ottawa Scale³¹ by two authors (Lu and Li). Any discrepancies in the assessment were resolved by another author (Peng).

Statistical analysis

The strength of the association between *iNOS* (rs2297518) and *eNOS* (rs2070744, rs1799983) polymorphisms and the risk of gastric cancer were evaluated by calculating

pooled ORs and their corresponding 95% CIs by using RevMan 5.1 (Copenhagen, Denmark) and Stata 12.0 (StataCorp LP, College Station, TX, USA). Heterogeneity was tested using χ^2 -based Q test and I^2 statistics. When there was no significant heterogeneity across studies ($I^2 < 50\%$), the fixed effects model (Mantel–Haenszel method) was used for meta-analysis. Otherwise, the random effects model (the DerSimonian and Laird method) was used. Sensitivity analysis was then performed to assess the stability of the results. Publication bias was detected using Begg's test and Egger's test. $p < 0.05$ was considered to be statistically significant.

Results

Study selection and characteristics

As shown in Figure 1, a total of 354 relevant publications were retrieved from the selected databases. After screening the titles, 293 of these publications were excluded because they were not related to the genetic association between *iNOS* and *eNOS* polymorphisms and gastric cancer, six were removed due to duplication, 46 were removed because they were reviews, abstracts, letters, or editorials. Finally, nine studies^{21,24–29,32,33} that met the required criteria were

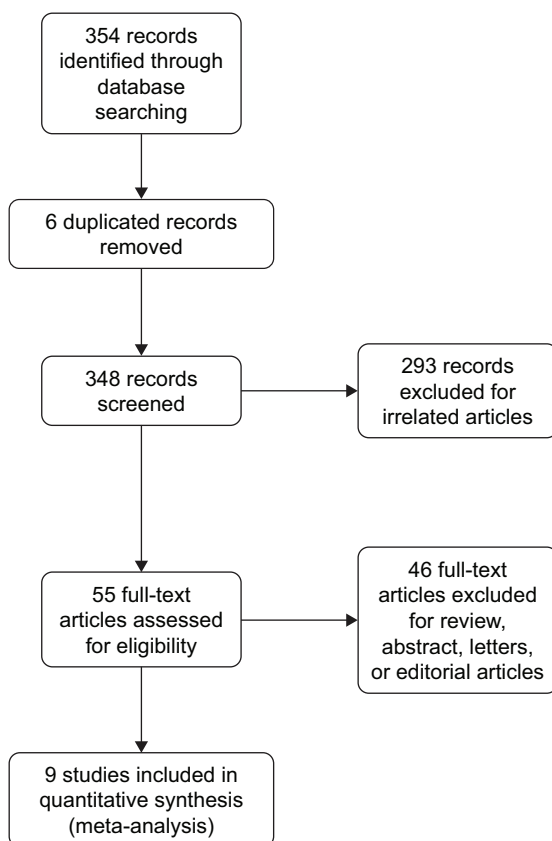


Figure 1 PRISMA flowchart of inclusion and exclusion of studies.

retained for meta-analysis, of which five studies referred to the specific association between *iNOS* rs2297518 and gastric cancer.^{21,28,29,32,33} Two studies referred to the specific associations between *eNOS* rs2070744,^{24,26} rs1799983,^{25,27} and gastric cancer, respectively. The basic characteristics of the enrolled patients are shown in Table 1. The NOS quality assessment of the studies included in this meta-analysis is included in Tables 1 and S1. Only studies with NOS scores > 6 were selected for this meta-analysis.

Results of the meta-analysis

The distributions of *iNOS* rs2297518 in allelic, dominant, and recessive models in the case group were not significantly different from that in the control group (allelic model: $p = 0.73$, OR [95% CI] = 1.09 [0.67–1.75]; dominant model: $p = 0.87$, OR [95% CI] = 1.03 [0.72–1.48]; recessive model: $p = 0.83$, OR [95% CI] = 1.16 [0.31–4.32]) (Figure 2; Table 2). Subgroup analysis, stratified by tests involving an anti-*H. pylorus* immunoglobulin G (IgG) antibody showed that the dominant model of *iNOS* rs2297518 was significantly associated with the risk of gastric cancer in *H. pylorus* CagA-positive cases ($p = 0.003$, OR [95% CI] = 2.19 [1.31–3.66]), but not in gastric cancer patients who were *H. pylorus* CagA-negative ($p = 0.82$, OR [95% CI] = 0.96 [0.65–1.41]) (Table 2).

For *eNOS* rs2070744, significant associations were detected between the allelic, dominant, and recessive models of *eNOS* rs2070744 (allelic model: $p < 0.00001$, OR [95% CI] = 0.23 [0.16–0.34]; dominant model: $p < 0.00001$, OR [95% CI] = 0.25 [0.15–0.42]; recessive model: $p < 0.00001$, OR [95% CI] = 0.16 [0.08–0.30]) (Figure 3; Table 2). However, the frequencies of allelic, dominant, and recessive models for *eNOS* rs1799983 were not significantly different when compared between case and control groups ($p > 0.05$), which showed no significant correlation between *eNOS* rs1799983 and the risk of gastric cancer (Figure 4; Table 2). Because of a lack of data, it was not possible to perform subgroup analyses based on ethnicity, anti-*H. pylorus* IgG antibody status, smoking, and alcoholism.

Sources of heterogeneity

Significant heterogeneity was observed for allelic, dominant, and recessive models of *iNOS* rs2297518 (allelic model: $I^2(\%) = 83$, dominant: $I^2(\%) = 60$, and recessive model: $I^2(\%) = 76$) (Table 2). This level of heterogeneity was contributed predominantly by two studies.^{28,33} Removal of these two studies from our meta-analysis resulted in 0% ($p = 0.90$) heterogeneity, thus showing that this had the highest effect on association between *iNOS* rs2297518 and the risk of gastric cancer.

Table 1 The characteristics of individual studies

Genes	Study	Year	Ethnicity	Case/control	Male, %	Age (mean ± SD)	Genotyping	Sample source	<i>H. pylori</i> CagA (positive/negative)	Smoking	Alcoholism	HWE in control	Quality scores
<i>iNOS</i>	Shen et al ³²	2004	Chinese	143/246	66.67/64.41	59.36±9.29/51.89±10.24	PCR-RFLP	HB	29/115:95/83	54/33	43/38	Yes	8
<i>C105T</i>	Goto et al ²¹	2006	Japanese	202/454	66.3/77.7	NA	PCR-RFLP	HB	NA	NA	NA	Yes	6
	Raffaei et al ²⁹	2012	Iran	159/170	53.0/58.0	61.32±12.5/58.86±14.2	ARFLP	HB	94/65:77/93	33/22	NA	Yes	8
	Shen et al ²⁸	2002	Chinese	143/246	NA	NA	PCR-RFLP	HB	1/2:14/53	NA	NA	Yes	6
	Yu et al ³³	2017	Chinese	80/140	NA	NA	NA	HB	NA	NA	NA	Yes	6
<i>eNOS</i>	Arjmand et al ²⁵	2014	Gulian	87/90	NA	NA	PCR-RFLP	HB	NA	NA	NA	Yes	6
G894T	Chen et al ²⁷	2010	Chinese	324/200	76.0/71.5	58.89±7.87/57.74±8.19	PCR-RFLP	HB	NA	NA	NA	Yes	7
<i>eNOS</i>	Tecder et al ²⁴	2010	Turkey	50/95	56.0/NA	59.27±11.93/NA	PCR-RFLP	PB	NA	NA	NA	NA	7
C786T	Krishnaveni et al ²⁶	2015	Indian	150/150	43.3/42.0	NA	PCR-RFLP	HB	2/148:14/136	113/32	125/64	Yes	7

Abbreviations: *iNOS*, inducible nitric oxide synthase; *eNOS*, endothelial nitric oxide synthase; NA, not available; ARFLP, artificial restriction fragment length polymorphism; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; PB, population-based; HWE, Hardy-Weinberg equilibrium; QA, quality assessment; *H. pylori*, *Helicobacter pylori*; SD, standard deviation.

Sensitivity analysis and publication bias

Sensitivity analysis was performed to assess the influence of an individual study on the overall OR. Analyses showed that ORs were not significantly altered for *iNOS* rs2297518, *eNOS* rs2070744, or rs1799983 (Figure 5). This evidence indicated that the present results were statistically stable and reliable. Funnel plots, Begg's test, and Egger's test were additionally performed to assess publication bias; results revealed that there was no obvious publication bias in our overall analysis for *iNOS* rs2297518 ($p_{\text{egger}}=0.564$; $p_{\text{begg}}=0.327$) and *eNOS* rs1799983 ($p_{\text{egger}}=0.511$; $p_{\text{begg}}=0.602$) (Figure 6; Table 3).

Discussion

In the present meta-analysis, we investigated the genetic associations between *iNOS* rs2297518, *eNOS* rs2070744, and rs1799983 and the risk of gastric cancer. Our combined results showed that the dominant model of *iNOS* rs2297518 was significantly associated with *H. pylori*-positive gastric cancer risk. In addition, *eNOS* rs2070744, but not rs179998, was identified as susceptible factor for the risk of gastric cancer.

Interestingly, the rs2297518 polymorphism in the *iNOS* gene has been reported to be associated with an increased risk for genotype gastric cancer in Asian populations,³³ but not in Iran²⁹ or Japanese populations.²¹ Our meta-analysis failed to find any statistically significant relationship between the allelic, dominant, and recessive models of *iNOS* rs2297518 and the risk of gastric cancer. However, the distribution of the dominant model of *iNOS* rs2297518 among the *H. pylori*-positive gastric cancer group of patients and the *H. pylori*-negative gastric cancer group of patients was statistically significant, which indicated that *H. pylori* infection might play a key role in the development of gastric cancer. Epidemiological studies have also indicated that *H. pylori* infection is a risk factor for gastric cancer.^{34,35} However, most patients with *H. pylori* infection do not appear to develop gastric cancer. The reasons for this inconsistency remain unclear and cannot be explained by bacterial virulence factors alone.

iNOS is shown to be upregulated in response to *H. pylori* infection and leads to excessive release of NO which would then contribute to the development of gastric atrophy.²¹ An earlier hypothesis suggested that a higher level of *iNOS* can cause more oxidative DNA damage, thus increasing the risk of cancer development.³⁶ A number of reports have addressed the high expression of *iNOS* in gastric cancer, which is known to increase as the stage of cancer increases and with lymph node metastasis.^{37,38} Previous research has shown that *iNOS* contributes to *H. pylori*-associated gastric carcinogenesis and that *H. pylori* is associated with

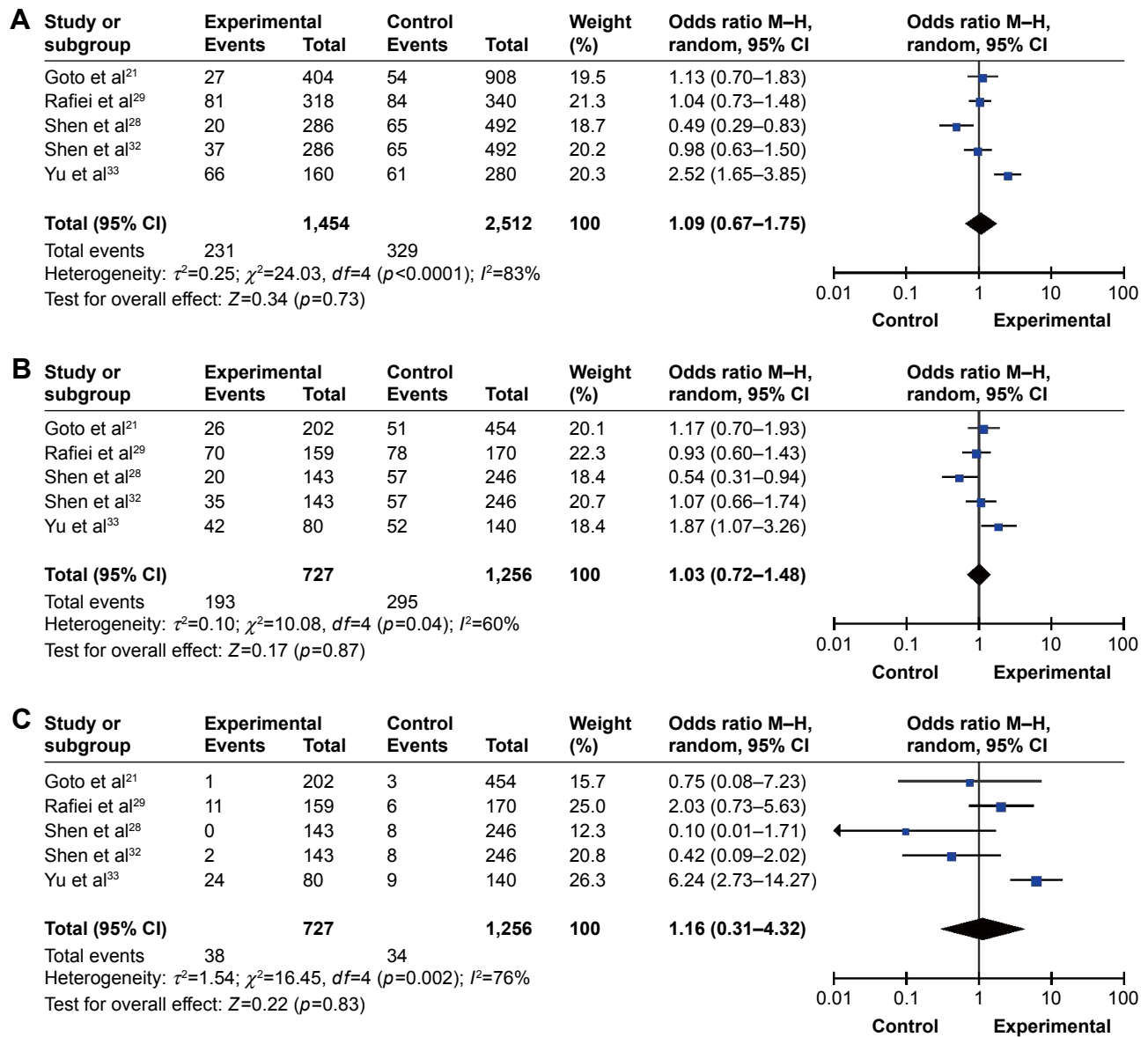


Figure 2 Forest plots of odds ratios for the association between iNOS C150T and gastric cancer. (A) Allelic model; (B) dominant model; (C) recessive model. Abbreviations: iNOS, inducible nitric oxide synthase; CI, confidence interval.

Table 2 The main results of the association between eNOS 894G>T, -786T>C, and iNOS C150T polymorphisms and gastric cancer

SNPs (minor allele)	Genetic model	Number of studies	Numbers		Test of association		Model	Test of heterogeneity	
			Case	Control	OR (95% CI)	p-value		p-value	I ² (%)
iNOS C150T	T	5	1,454	2,512	1.09 (0.67–1.75)	0.73	R	<0.0001	83
	TT/CT+CC	5	727	1,256	1.16 (0.31–4.32)	0.83	R	0.002	76
	TT+CT/CC	5	727	1,256	1.03 (0.72–1.48)	0.87	R	0.04	60
	<i>H. pylorus</i> positive	3	126	181	2.19 (1.31–3.66)	0.003	F	0.22	34
	<i>H. pylorus</i> negative	3	226	402	0.96 (0.65–1.41)	0.82	F	0.20	38
eNOS G894T	T	3	914	776	0.80 (0.62–1.03)	0.09	F	0.57	0
	TT/GT+GG	3	457	388	0.52 (0.22–1.25)	0.14	F	0.56	0
	TT+GT/GG	3	457	388	0.79 (0.59–1.07)	0.13	F	0.60	0
eNOS C786T	T	2	400	482	0.23 (0.16–0.34)	<0.00001	F	0.38	0
	TT/CT+CC	2	200	241	0.16 (0.08–0.30)	<0.00001	F	0.52	0
	TT+CT/CC	2	200	241	0.25 (0.15–0.42)	<0.00001	F	0.41	0

Abbreviations: iNOS, inducible nitric oxide synthase; eNOS, endothelial nitric oxide synthase; OR, odds ratio; CI, confidence interval; R, random model; F, fixed model; NA, not available.

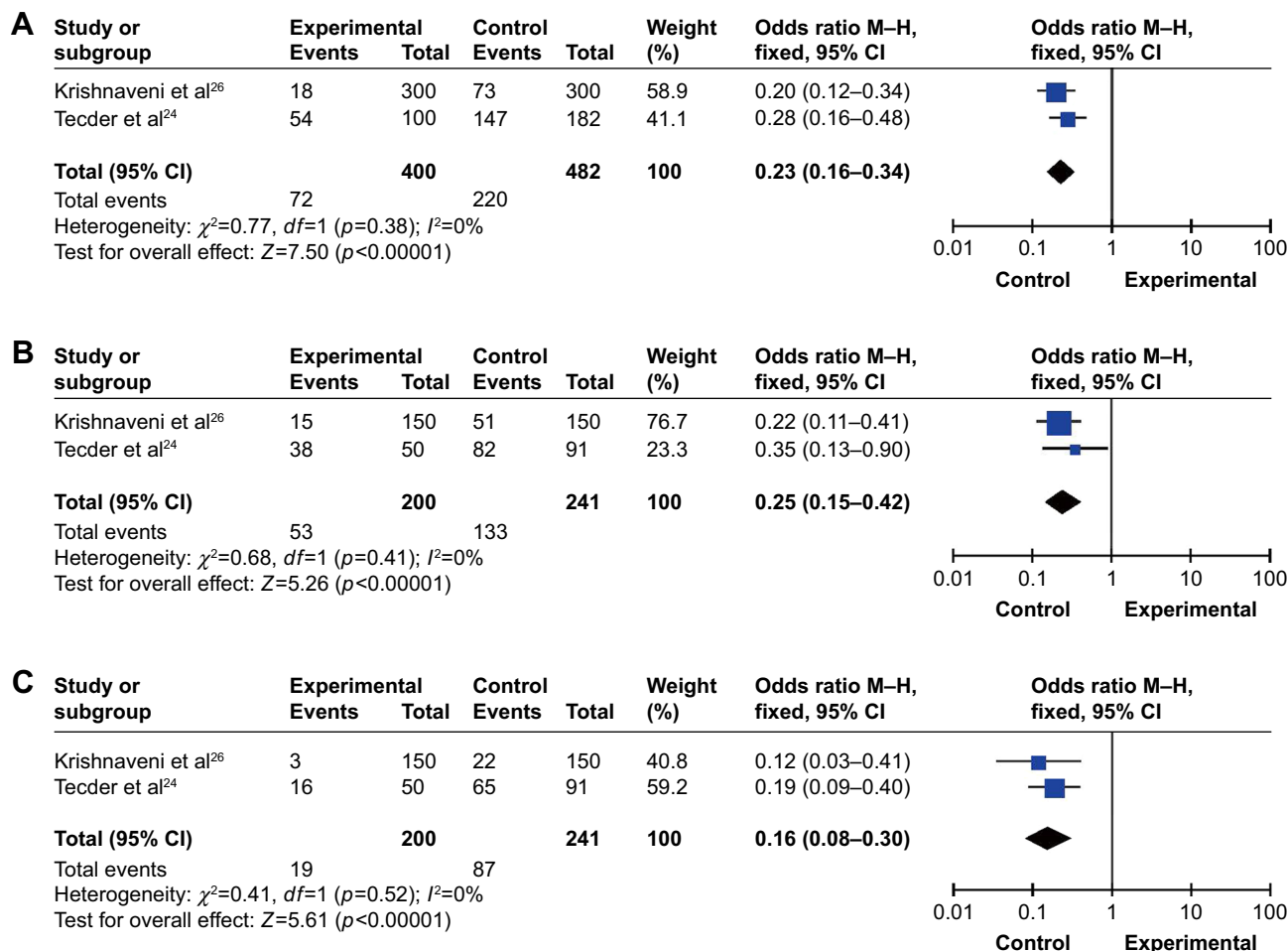


Figure 3 Forest plots of odds ratios for the association between eNOS C786T and gastric cancer. (A) Allelic model; (B) dominant model; (C) recessive model. Abbreviations: eNOS, endothelial nitric oxide synthase; CI, confidence interval.

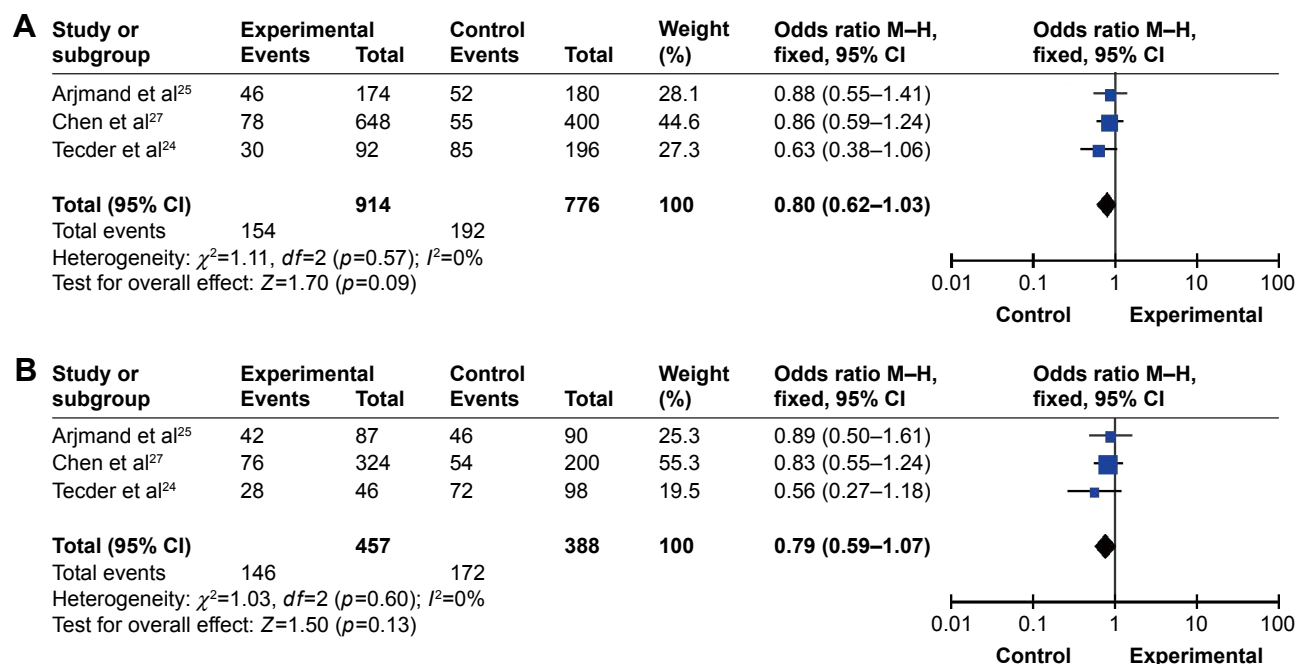


Figure 4 (Continued)

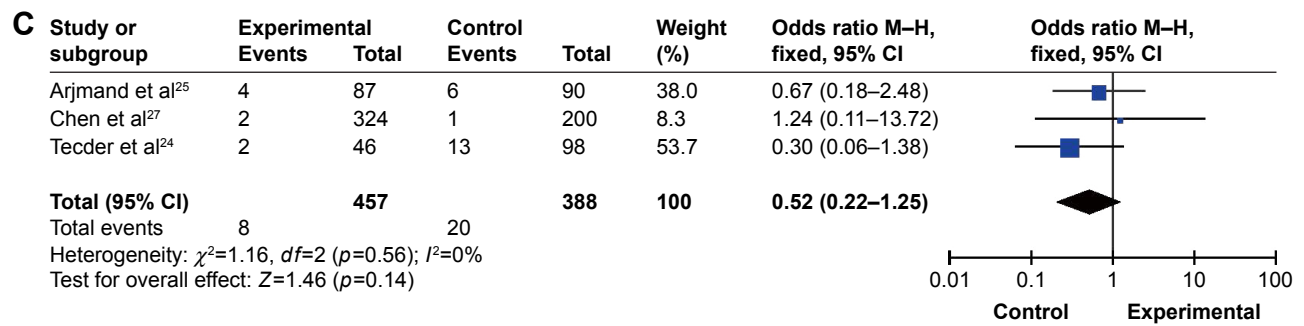


Figure 4 Forest plots of odds ratios for the association between eNOS G894T and gastric cancer. (A) Allelic model; (B) dominant model; (C) recessive model. **Abbreviations:** eNOS, endothelial nitric oxide synthase; CI, confidence interval.

non-cardiac tumors but not with cardiac tumors.³⁹ Several studies have also demonstrated a link between *iNOS* polymorphism and the risk of gastric cancer development. For example, Rafei et al²⁹ reported a significant association between a TT+CT genetic model and *H. pylorus*-positive gastric cancer. However, research conducted by Goto et al²¹ and Shen et al³² could not replicate this result. To our knowledge, the present study is the first to investigate and identify a significant association between *iNOS* rs2297518 and *H. pylorus*-positive gastric cancer risk, which implies that

the *iNOS* rs2297518 polymorphism might affect *H. pylorus* infection and iNOS expression. To confirm this result, it is now necessary to investigate a larger number of subjects in a case-control study.

Both iNOS and eNOS have been reported as an important form of NOS. The *eNOS* rs2070744 polymorphism was found to significantly reduce the rate of mRNA transcription, thus resulting in reduced levels of NO in the serum and stimulating tumor proliferation, angiogenesis, and metastasis.⁴⁰ The *eNOS* rs2070744 polymorphism has been reported to play

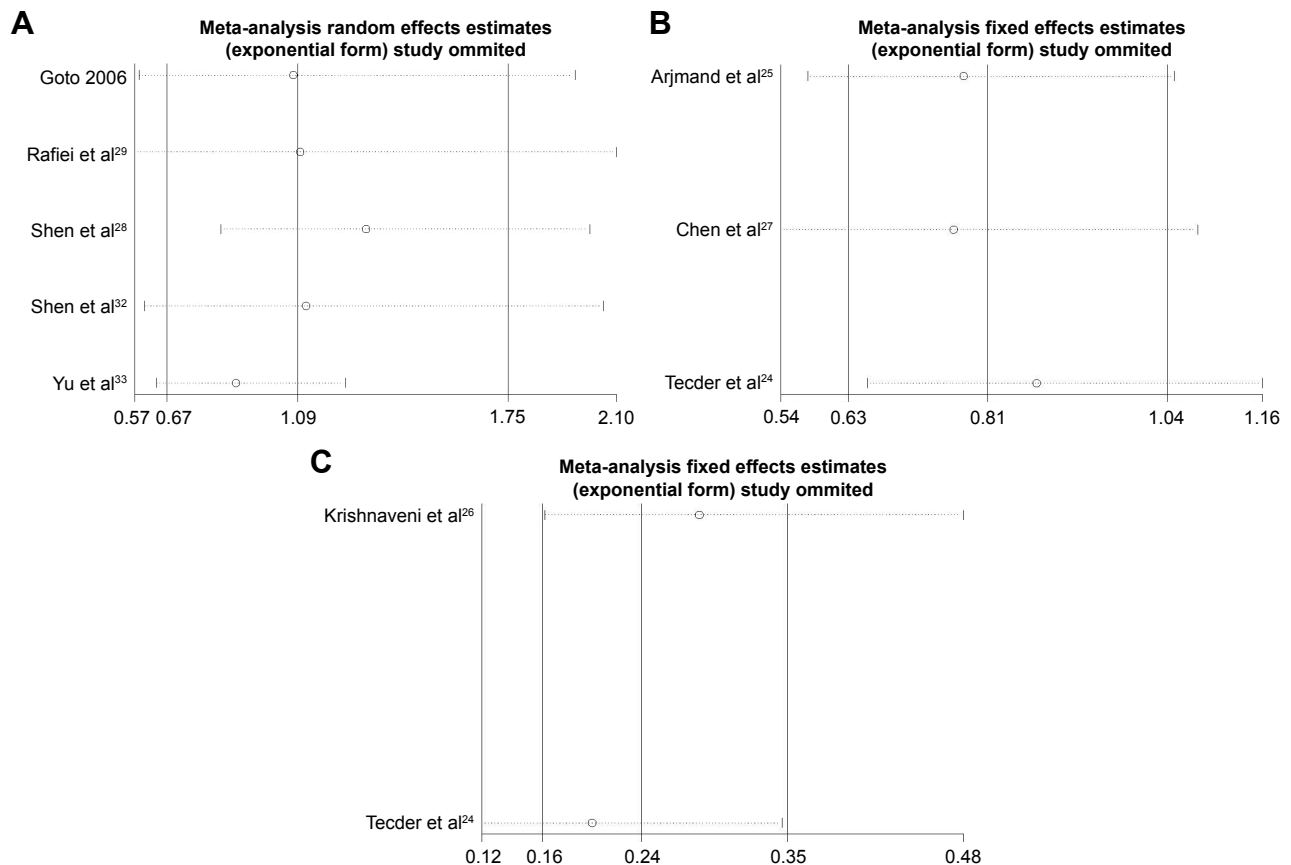


Figure 5 Sensitivity analyses between eNOS C786T, G894T, and iNOS C150T and gastric cancer. (A) iNOS C150T; (B) eNOS C786T; (C) eNOS G894T. **Abbreviations:** eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase.

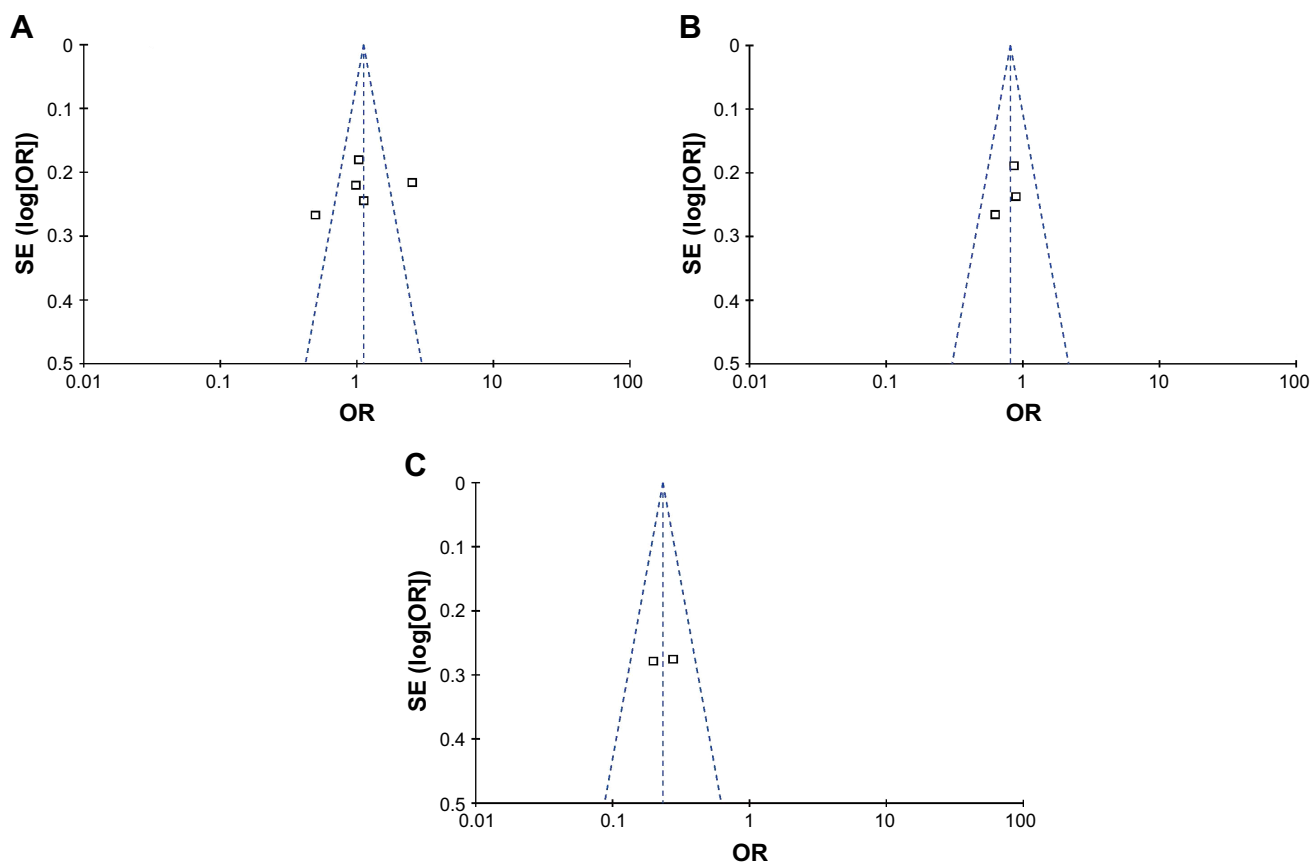


Figure 6 Publication bias of literature for eNOS C786T, G894T, and iNOS C150T was tested by Begg's funnel plot and Egger's test. (A) iNOS C150T; (B) eNOS C786T; (C) eNOS G894T.

Abbreviations: eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase; OR, odds ratio.

a critical role in cardiovascular disease,⁴¹ breast cancer,⁴² colorectal cancer,⁴³ and diabetic neuropathy.⁴⁴ Two previous meta-analyses have investigated the statistical genetic associations between *eNOS* rs2070744 and the risk of cancer; however, results differed between these two studies.^{45,46} This inconsistency may have arisen due to the different number of studies included in two data sets. Two studies^{24,26} included in the present analysis identified a significant association between the rs2070744 polymorphism and gastric cancer, and our combined results were the same as those of the individual studies, which might indicate a potential role for this

variant in the risk of gastric cancer. Notably, the results of the present meta-analysis relating to the correlation between the rs2070744 polymorphism and gastric cancer should be interpreted carefully because the number of studies included was relatively small.

The *eNOS* rs1799983 polymorphism is located in exon 7 of the *NOS3* gene and leads to the amino acid substitution, Glu298Asp, which alters susceptibility to cleavage, while also reducing enzyme activity and basal NO production.⁴⁷ Several previous meta-analyses^{48–50} have investigated the association between the *eNOS* rs1799983 polymorphism and the risk of cancer, although most studies observed negative results. However, no meta-analysis has been conducted on the potential association between the *eNOS* rs1799983 polymorphism and gastric cancer. We included only two studies and found no statistical association of *eNOS* rs1799983 and the risk of gastric cancer, which indicates that the *eNOS* rs1799983 polymorphism might not be a risk factor for gastric cancer.

Although significant associations were detected between *iNOS* rs2297518, *eNOS* rs2070744, and the risk of gastric cancer, several limitations should be taken into account.

Table 3 Egger's test and Begg's test for funnel plot asymmetries of iNOS and eNOS polymorphisms

Models of test	Polymorphisms	iNOS C150T	eNOS G894T	eNOS C786T
Egger's test	p-value	0.564	0.511	NA
	95% CI	-33.78177	-41.67948	NA
		to 22.38564	to 35.79079	
Begg's test	p-value	0.327	0.602	NA

Abbreviations: iNOS, inducible nitric oxide synthase; eNOS, endothelial nitric oxide synthase; OR, odds ratio; CI, confidence interval; NA, not available.

First, the number of studies and subjects included was relatively small. In particular, the association between *eNOS* rs2070744, rs1799983, and gastric cancer was investigated by only two studies enrolled by the present study; this might affect the reliability of our results. Thus, a larger number of studies, with more subjects, are now necessary to confirm our results. Second, the subjects in the present study were mostly of Asian origin. The prevalence of gastric cancer in Europeans and Latin Americans cannot be ignored. However, due to the lack of genetic association studies in Europeans and Latin Americans, we were not able to carry out subgroup analysis stratified by ethnicity. Third, apart from *H. pylorus* infection, smoking, diet, and alcohol abuse were also considered to be risk factors for gastric cancer, although we could not analyze the influence of these factors in regard to the risk of gastric cancer because of insufficient data.

Conclusion

Our meta-analysis showed that the dominant model of *iNOS* rs2297518 may represent a risk factor for *H. pylorus*-positive gastric cancer, while *eNOS* rs2070744, but not rs1799983, represents a protective factor for the risk of gastric cancer. To confirm these results, a larger number of cohorts should be investigated, using a case-control design.

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Disclosure

The authors report no conflicts of interest in this work.

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Supplementary material

Table S1 Newcastle–Ottawa scale for quality assessment of included studies

Study	Year	Selection	Comparability	Outcome
Shen et al ¹	2004	***	**	**
Goto et al ²	2006	***	**	*
Rafiei et al ³	2012	***	**	***
Shen et al ⁴	2002	***	*	**
Yu et al ⁵	2017	***	*	**
Arjmand et al ⁶	2014	***	*	**
Chen et al ⁷	2010	***	**	**
Tecder et al ⁸	2010	***	**	**
Krishnaveni et al ⁹	2015	***	**	**

Note: The number of "*" means the consistency of each study according to the Newcastle–Ottawa Scale.

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