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eNOS Genetic Polymorphisms and Cancer Risk

A Meta-Analysis and a Case–Control Study of Breast Cancer

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Abstract: The association between endothelial nitric oxide synthase (eNOS) polymorphisms (intron 4a/b, -786T>C and 894G>T) and cancer risk remains elusive. In addition, no studies focused on their associations with the risk of breast cancer in Chinese Han population. Thus, a meta-analysis was conducted to determine the relationship between eNOS polymorphisms and cancer risk, and then a case–control study in Chinese Han population was performed to assess their associations with breast cancer susceptibility.

Odds ratios (ORs) with 95% confidence intervals (CIs) were used to assess the strength of association. The pooled analysis indicated that *eNOS* intron 4a/b and -786T>C polymorphisms were significantly associated with an increased risk of overall cancer. In subgroup analyses based on cancer type, the significant association was found between *eNOS* intron 4a/b polymorphism and prostate cancer risk, *eNOS* -786T>C polymorphism and risk of prostate, bladder and breast cancers, and *eNOS* 894G>T polymorphism and breast cancer risk. In subgroup analyses based on ethnicity, *eNOS* intron 4a/b and -786T>C polymorphisms were associated with an increased risk of cancer in Caucasians. In consistent with our meta-analysis results, a case–control study in Chinese Han population showed significant associations of *eNOS* -786T>C and 894G>T polymorphisms with the increased risk of breast cancer. In addition, stratified analyses based on pathological type showed that *eNOS* 894G>T polymorphism was only associated with the risk of infiltrative ductal carcinoma. Stratified analyses by tumor stage showed that *eNOS* -786T>C polymorphism was only associated with the risk of tumor stage III and IV.

In conclusion, our meta-analysis and case–control study suggest that *eNOS* -786T>C and 894G>T polymorphisms are associated with the increased risk of breast cancer.

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Abbreviations: CI = confidence interval, eNOS = endothelial nitric oxide synthase, NO = nitric oxide, OR = odds ratio, VEGF = vascular endothelial growth factor.

INTRODUCTION

Nitric oxide (NO) is a short-lived and small molecule, which is closely related to inflammatory status and regarded as a key inflammation mediator. Overproduction of NO can cause DNA damage and inhibit DNA repair.[1] In addition, NO also promotes tumor angiogenesis and metastasis.[2] Therefore, NO plays a significant role in the development of cancer. NO is produced from L-arginine by nitric oxide synthases (NOSs), which have 3 different isoforms and are divided into 2 functional classes. Constitutive class includes endothelial nitric oxide synthase (eNOS) and neuronal-NOS (nNOS) while the other class contains inducible form of NOS (iNOS).[3] eNOS is a Ca^{2+} dependent enzyme and firstly defined in the vascular endothelial cells. Increased expression of eNOS has been noted in the vasculature of various tumor tissues, including bladder, colon, and pancreatic cancers.[4–6] Previous studies have shown that eNOS can modulate cancer-related events, such as angiogenesis, invasion, and metastasis.[7–9] eNOS is a central mediator of several endothelium growth stimulators, such as vascular endothelial growth factor (VEGF) and prostaglandin E2. The former (VEGF) can increase angiogenesis in both $iNOS^{+/+}$ and $iNOS^{-/-}$ mice but not in $eNOS^{-/-}$ mice, suggesting a predominant role of eNOS in VEGF-induced angiogenesis.[7] In addition, an in vivo study has indicated that high eNOS expression is correlated to trophoblast cancer cell vascular invasion.[8] Tumor cells in lung metastatic sites are always strongly eNOS-positive, suggesting that eNOS expression facilitates metastasis.[9]

The gene-encoding eNOS is located on chromosome 7q35 and has more than 168 polymorphisms.[10] Among these polymorphisms, intron 4a/b, -786T>C (rs2070744), and 894G>T (rs1799983) polymorphisms seem to be functional and have been widely investigated for their associations with cancer risk.[10–42] However, results were inconsistent. Therefore, we performed a comprehensive meta-analysis to derive a more precise estimation of the relationship between *eNOS* intron 4a/b, -786T>C, and 894G>T polymorphisms and cancer risk. Additionally, considering that no studies focused on the association of *eNOS* intron 4a/b, -786T>C, and 894G>T polymorphisms with the risk of breast cancer in Chinese Han population, we performed a case–control study to assess the association.

MATERIALS AND METHODS

Meta-Analysis

A comprehensive literature search was performed by PubMed and EMBASE databases with the following key words ''endothelial nitric oxide synthase or eNOS or NOS3,'' ''polymorphism or variation,'' and ''cancer or tumor'' (up to October

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TABLE 1. Distribution of Clinicopathologic Features Among Breast Cancer Cases

Variables	Cases (n = 873)	
	N	%
Pathological type		
Infiltrative ductal carcinoma	713	81.7
Other carcinoma	160	18.3
Stage		
I	226	25.9
II	476	54.5
III	120	13.7
IV	51	5.8

1, 2014). In addition, references of retrieved articles were also screened. The inclusion criteria were as follows: evaluation of the association between *eNOS* polymorphisms and cancer risk; case–control studies; detailed genotype data for estimating of odds ratios (ORs) and 95% confidence intervals (CIs); and no deviation from Hardy-Weinberg equilibrium (HWE) among the controls. As described previously, data were independently extracted from all eligible studies by 2 investigators, and any disagreement was resolved by discussion.[43] The following information was collected from each study: first author, publication year, ethnicity, cancer type, total number of cases and controls, and number of different genotypes in cases and controls.

Case–Control Study

All recruited subjects were ethnically homogenous Han Chinese. A total of 873 patients (age 50.62 ± 10.20) with histopathologically diagnosed breast cancer and 1034 age-matched healthy women (age 51.02 ± 10.79) were consecutively recruited between October 2013 and May 2014 at the Affiliated Hospital of Bengbu Medical College. Clinicopathologic information were collected from medical records and pathology reports (Table 1). Written informed consent was obtained from all participants. The research protocol was approved by the ethics committee of the Affiliated Hospital of Bengbu Medical College. Genotyping method and material of *eNOS* polymorphisms, including primer sequences, PCR program, selected restriction enzymes, and fragment sizes, were presented in Table 2. PCR-RFLP assay was performed for genotyping of -786T>C and 894G>T loci. PCR products were digested by restriction enzyme MspI for -786T>C and BanII for 894G>T overnight according to the manufacture's protocols. The digestion products of -786T>C and 894G>T as well as the amplifying product of intron 4a/b locus were analyzed by electrophoresis on a 3% agarose gel.

Statistical Analysis

Crude ORs with 95% CIs were calculated by the Stata version 12.0 software. Heterogeneity among studies was evaluated using the χ^2-based Cochran Q statistic test. The random effect model was used to estimate a pooled OR when there was heterogeneity between studies ($P_H < 0.05$); otherwise, the fixed effect model was adopted. HWE among the controls was verified using a goodness-of-fit χ^2 test. The association between *eNOS* polymorphisms and cancer risk was examined under

TABLE 2. Genotyping Method and Material of *eNOS* Polymorphisms

Polymorphism	Primer Sequences	PCR Program	Assay	Restriction Enzyme	DNA Fragment Size, bp
intron 4a/b	F-5′-AGGCCCTATGGTAGTGCCTT-3′ R-5′-TCTCTTAGTGCTGTGGTCAC-3′	94°C 5 min, 35 cycles, 94°C 30 seconds, 54°C 30 seconds,72°C 30 seconds, 72°C 5 min	PCR-AGE	–	4b allele: 421 4a allele: 394
-786T>C	F-5′-TGGAGAGTGCTGGTGTACCCCA-3′ R-5′-GCCTCCACCCCCACCCTGTC-3′	94°C 5 minutes, 35 cycles, 94°C 30 seconds, 58°C 30 seconds,72 °C 30 seconds, 72°C 5 minutes	PCR-RFLP	Msp I	T allele: 138 + 42 C allele: 92 + 46 + 42
894G>T	F-5′-AAGGCAGGAGACAGTGGATGGA-3′ R-5′-CCCAGTCAATCCCTTTGGTGCTCA-3′	94°C 5 minutes, 35 cycles, 94°C 30 seconds, 62°C 30 seconds, 72°C 30 seconds, 72 °C 5 minutes	PCR-RFLP	Ban II	G allele: 163 + 85 T allele: 248

F = forward primer, PCR-AGE = polymerase chain reaction and agarose gel electrophoresis, PCR-RFLP = polymerase chain reaction and restriction fragment length polymorphism, R = reverse primer.

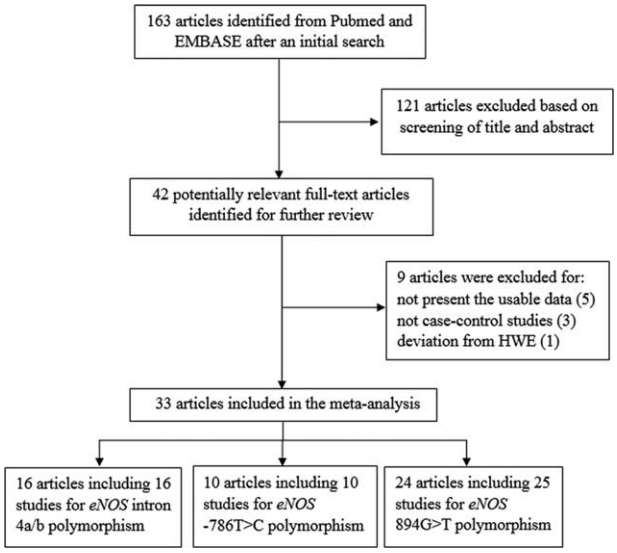

FIGURE 1. A flow chart of the study selection procedure.

allele contrast (4a vs 4b, C vs T, and T vs G), recessive model (4a/4a vs 4a/4b + 4b/4b, CC vs CT + TT, and TT vs TG + GG), dominant model (4a/4a + 4a/4b vs 4b/4b, CC + CT vs TT, and TT + TG vs GG), homozygote contrast (4a/4a vs 4b/4b, CC vs TT, and TT vs GG), and heterozygote contrast (4a/4b vs 4b/4b, CT vs TT, and TG vs GG). Additionally, sensitivity analysis was performed by removing each individual study in turn from the total and reanalyzing the remainder. Finally, the Begg funnel plot and Egger test were employed to investigate the potential publication bias. In case–control study, logistic regression was used to analyze the association between *eNOS* polymorphisms and the risk of breast cancer. These statistical analyses were implemented in Statistic Analysis System software 8.0. All $P < 0.05$ was used as the criterion of statistical significance.

RESULTS

Meta-Analysis of *eNOS* Polymorphisms and Cancer Risk

A total of 33 articles met the inclusion criteria and were included in the meta-analysis (Figure 1 and Table 3). For *eNOS* intron 4a/b polymorphism, 16 studies with 3850 cases and 4180 controls met the inclusion criteria. Among these studies, there were 4 studies of Asians and 12 studies of Caucasians. For *eNOS* -786T>C polymorphism, 10 studies with 4593 cases and 4355 controls were included in the meta-analysis. Four studies were carried out in Asians and 6 studies in Caucasians. For *eNOS* 894G>T polymorphism, there were 25 studies met the inclusion criteria with 9199 cases and 9726 controls. Among these studies, there were 2 studies of Asians, 21 studies of Caucasians, 1 study of African-American population, and 1 study of mixed population.

As shown in Table 4, meta-analysis for *eNOS* intron 4a/b polymorphism showed significant associations in overall cancer. In the subgroup analysis based on cancer type, significant associations were found in prostate cancer. In the subgroup analysis based on ethnicity, significant associations were observed in Caucasians. For *eNOS* -786T>C polymorphism, significant associations were also observed in overall cancer. Subsequently, subgroup analysis by cancer type showed statistically significant associations in breast, prostate, and bladder cancers. Subgroup analysis by ethnicity showed significant associations among Caucasians. For *eNOS* 894G>T polymorphism, no significant associations were found in overall cancer, but stratified analysis by cancer type revealed that *eNOS* 894G>T polymorphism was associated with the risk of breast cancer.

The sensitivity analysis was performed to assess the influence of an individual study on the overall OR. For *eNOS* -786T>C polymorphism, the omission of Lu J study slightly affected the overall OR under recessive model (CC vs CT + TT: OR = 1.22, 95%CI: 0.99–1.50), and so did Jang MJ study under heterozygote contrast (CT vs TT: OR = 1.10, 95%CI: 0.99–1.23).

Both Begg funnel plot and Egger test were conducted to assess the publication bias. The shape of the funnel plot for the overall results seemed symmetrical. Similarly, Egger test showed no evidence of publication bias in the *eNOS* intron 4a/b, -786T>C, and 894G>T polymorphisms (Table 4).

eNOS Polymorphisms and Breast Cancer Risk in Chinese Han Population

As summarized in Table 5, the genotype distributions for *eNOS* intron 4a/b, -786T>C, and 894G>T polymorphisms did not deviate from HWE in the controls ($P_{HWE} = 0.19$, 0.41, and 0.18, respectively). No statistical association was found between the *eNOS* intron 4a/b polymorphism and breast cancer risk. However, *eNOS* -786T>C and 894G>T polymorphisms were associated with breast cancer risk (For *eNOS* -786T>C polymorphism: C vs T, OR = 1.32, 95%CI: 1.02–1.70, $P = 0.04$; CC vs TT, OR = 2.19, 95%CI: 1.01–4.76, $P = 0.05$. For *eNOS* 894G>T polymorphism: TT vs GG, OR = 1.69, 95%CI: 1.19–2.42, $P = 0.00$; TT vs TG + GG, OR = 1.69, 95%CI: 1.18–2.41, $P = 0.00$).

Stratification Analysis of *eNOS* Polymorphisms With Breast Cancer Risk

As summarized in Tables 6 and 7, stratified analysis based on pathological type indicated that *eNOS* -786T>C polymorphism was associated with the risk of infiltrative ductal carcinoma (C vs T: OR = 1.34, 95%CI: 1.02–1.76, $P = 0.03$) and other carcinoma (CC vs TT: OR = 3.40, 95%CI: 1.36–8.46, $P = 0.01$; CC vs CT + TT: OR = 3.37, 95%CI: 1.36–8.37, $P = 0.01$). However, *eNOS* 894G>T polymorphism was only associated with the risk of infiltrative ductal carcinoma (T vs G: OR = 1.25, 95%CI: 1.02–1.52, $P = 0.03$; TT vs GG: OR = 1.73, 95%CI: 1.20–2.50, $P = 0.00$; TT vs TG + GG: OR = 1.71, 95%CI: 1.19–2.47, $P = 0.00$). Furthermore, stratified analysis by tumor stage suggested that *eNOS* -786T>C polymorphism was only associated with the risk of tumor stage III and IV (C vs T: OR = 1.99, 95%CI: 1.35–2.93, $P = 0.00$; CC vs TT: OR = 4.42, 95%CI: 1.97–9.89, $P = 0.00$; CC + CT vs TT: OR = 1.74, 95%CI: 1.12–2.68, $P = 0.01$; CC vs CT + TT: OR = 4.34, 95%CI: 1.94–9.71, $P = 0.00$). However, *eNOS* 894G>T polymorphism was associated not only with the risk of tumor stage I and II (TT vs GG, OR = 1.57, 95%CI: 1.07–2.29, $P = 0.02$; TT vs TG + GG, OR = 2.45, 95%CI: 1.15–5.23, $P = 0.02$), but also with tumor stage III and IV (TT vs GG, OR = 2.14, 95%CI: 1.34–3.40, $P = 0.00$; TT vs TG + GG, OR = 4.56, 95%CI: 1.81–11.52, $P = 0.00$).

TABLE 3. The Main Characteristics of Studies Included in the Meta-Analysis

Study Intron 4a/b	Year	Ethnicity	Cancer Type	Number of Cases/Controls	Case			Control			P_{HWE}
					4a/4a	4a/4b	4b/4b	4a/4a	4a/4b	4b/4b	
Yuan F	2013	Asian	Hepatocellular carcinoma	293/384	3	59	231	2	94	288	0.05
Safarinejad MR	2013	Caucasian	Prostate cancer	170/340	15	54	101	3	88	249	0.11
Ramirez-Patino R	2013	Caucasian	Breast cancer	428/280	3	94	331	2	34	244	0.50
Jang MJ	2013	Asian	Colorectal cancer	528/509	7	87	434	2	98	409	0.13
Amsysli AS	2012	Caucasian	Bladder cancer	123/201	8	63	52	5	59	137	0.65
Oztürk E	2011	Caucasian	Endometrial cancer	89/60	10	31	48	1	16	43	0.72
Sanli O	2011	Caucasian	Prostate cancer	132/158	5	40	87	6	48	104	0.88
Tecder Ünal M	2010	Caucasian	Gastric cancer	46/98	1	10	35	4	28	66	0.64
Zintzaras E	2010	Caucasian	Breast cancer	100/100	3	27	70	4	37	59	0.54
Yeh CC	2009	Asian	Colorectal cancer	713/723	7	115	591	6	112	605	0.75
Lu J	2006	Caucasian	Breast cancer	421/423	15	113	293	13	110	300	0.46
Hefler LA	2006	Caucasian	Breast cancer	269/270	5	68	196	2	75	193	0.07
Riener EK	2004	Caucasian	Vulvar cancer	65/227	0	17	48	3	53	171	0.62
Mediros R	2002	Caucasian	Prostate cancer	125/153	6	32	87	3	29	121	0.43
Hefler LA	2002	Caucasian	Ovarian cancer	130/133	0	40	90	2	34	97	0.61
Cheon KT	2000	Asian	Lung cancer	218/121	2	19	197	1	29	91	0.42
-786T>C					TT	TC	CC	TT	TC	CC	
Safarinejad MR	2013	Caucasian	Prostate cancer	170/340	52	93	25	150	159	31	0.22
Jang MJ	2013	Asian	Colorectal cancer	528/509	395	128	5	418	87	4	0.82
Brankovic A	2013	Caucasian	Prostate cancer	150/100	54	68	28	34	51	15	0.56
Lee SA	2012	Asian	Breast cancer	504/508	409	88	7	415	85	8	0.14
Ryk C	2011	Caucasian	Bladder cancer	334/155	152	142	40	84	63	8	0.38
Yeh CC	2009	Asian	Colorectal cancer	683/726	566	110	7	604	116	6	0.87
Lee KM	2007	Asian	Breast cancer	1364/956	1092	250	22	766	177	13	0.45
Lu J	2006	Caucasian	Breast cancer	421/423	167	200	54	203	185	35	0.43
Conde M	2006	Caucasian	Colorectal cancer	368/547	107	184	77	152	273	122	0.98
Ghilardi G	2003	Caucasian	Breast cancer	71/91	22	35	14	37	42	12	0.99
894G>T					TT	TG	GG	TT	TG	GG	
Ziaei SA	2013	Caucasian	Prostate cancer	78/87	11	23	44	6	33	48	0.92
Safarinejad MR	2013	Caucasian	Prostate cancer	170/340	2	48	120	3	89	248	0.10
Verim L	2013	Caucasian	Bladder cancer	66/88	10	49	7	13	44	31	0.68
Jang MJ	2013	Asian	Colorectal cancer	528/509	9	102	417	2	76	431	0.48
Brankovic A	2013	Caucasian	Prostate cancer	150/100	9	65	76	6	40	54	0.69
Arikan S	2012	Caucasian	Colorectal cancer	84/99	7	42	35	22	50	27	0.90
Oztürk E	2011	Caucasian	Endometrial cancer	89/60	11	31	47	0	18	42	0.17
Ryk C	2011	Caucasian	Bladder cancer	262/150	28	106	128	13	62	75	0.97
Zintzaras E	2010	Caucasian	Breast cancer	100/100	39	46	15	38	50	12	0.47
Funke S	2009	Caucasian	Colorectal cancer	632/604	58	285	289	61	272	271	0.55
Yeh CC	2009	Asian	Colorectal cancer	702/728	10	124	568	10	143	575	0.74
Li Y	2009	Mixed	Breast cancer	489/485	47	200	242	40	209	236	0.51
Lee KM1	2009	Caucasian	Prostate cancer	1088/1293	103	468	517	129	557	607	0.94
Lee KM2	2009	African-American	Prostate cancer	97/373	0	20	77	5	88	280	0.51
Rajaraman P	2008	Caucasian	Acoustic neuroma, Glioma, Meningloma	526/467	54	230	242	61	202	204	0.33
Jacobs EJ	2008	Caucasian	Prostate cancer	1420/1446	129	632	659	164	600	682	0.07
Yang J	2007	Caucasian	Breast cancer	418/409	46	168	204	34	176	199	0.57
Hong CC	2007	Caucasian	Breast cancer	489/485	47	200	242	40	209	236	0.51
Royo JL	2006	Caucasian	Breast cancer	440/321	68	205	167	45	146	130	0.70
Lu J	2006	Caucasian	Breast cancer	421/423	39	193	189	38	186	199	0.56
Hefler LA	2006	Caucasian	Breast cancer	269/244	34	117	118	17	109	118	0.22
Conde M	2006	Caucasian	Colorectal cancer	355/538	60	160	135	87	235	216	0.09
Ghilardi G	2003	Caucasian	Breast cancer	71/91	9	36	26	5	47	39	0.06
Medeiros R	2002	Caucasian	Prostate cancer	125/153	15	61	49	18	65	70	0.63
Hefler LA	2002	Caucasian	Ovarian cancer	130/133	15	57	58	12	61	60	0.53

P_{HWE} >0.05 was considered consistent with HWE. Mixed = White, African-American, Hispanic, Asian, and other/unknown.

TABLE 4. Results of Meta-Analysis Between *eNOS* Polymorphisms and Cancer Risk

				P-Value			Regression Model	
Polymorphism	**Comparison**	**Subgroup**	**N**	P_H	P_Z	P_E	**Random**	**Fixed**
Intron 4a/b	4a vs 4b	Overall	16	0.00	0.24	0.74	1.13 (0.92–1.39)	1.15 (1.05–1.26)
		Breast cancer	4	0.02	0.62		1.09 (0.78–1.52)	1.11 (0.94–1.31)
		Colorectal cancer	2	0.58	0.92		1.01 (0.83–1.22)	1.01 (0.83–1.22)
		Prostate cancer	3	0.03	0.07		1.51 (0.97–2.35)	1.56 (1.25–1.96)
		Other cancer	7	0.00	0.85		1.04 (0.65–1.67)	1.11 (0.94–1.33)
		Caucasian	12	0.00	0.03		1.29 (1.02–1.63)	1.31 (1.17–1.47)
		Asian	4	0.01	0.21		0.82 (0.59–1.12)	0.90 (0.77–1.05)
	4a/4b vs 4b/4b	Overall	16	0.00	0.50	0.97	1.08 (0.87–1.35)	1.08 (0.97–1.20)
		Breast cancer	4	0.01	0.76		1.07 (0.70–1.64)	1.11 (0.91–1.35)
		Colorectal cancer	2	0.30	0.64		0.95 (0.76–1.19)	0.95 (0.77–1.17)
		Prostate cancer	3	0.39	0.04		1.34 (1.01–1.77)	1.34 (1.01–1.76)
		Other cancer	7	0.00	0.92		1.03 (0.60–1.76)	1.06 (0.86–1.29)
		Caucasian	12	0.00	0.06		1.26 (0.99–1.61)	1.27 (1.11–1.45)
		Asian	4	0.01	0.11		0.73 (0.50–1.07)	0.83 (0.70–0.99)
	4a/4a vs 4a/4b + 4b/4b	Overall	16	0.18	0.00	0.50	1.73 (1.12–2.67)	1.81 (1.30–2.53)
		Breast cancer	4	0.75	0.55		1.18 (0.65–2.13)	1.19 (0.67–2.14)
		Colorectal cancer	2	0.28	0.21		1.72 (0.64–4.63)	1.75 (0.73–4.19)
		Prostate cancer	3	0.03	0.13		3.00 (0.72–12.50)	3.27 (1.66–6.45)
		Other cancer	7	0.38	0.08		1.71 (0.80–3.67)	1.77 (0.93–3.35)
		Caucasian	12	0.07	0.00		1.72 (0.97–3.05)	1.84 (1.27–2.67)
		Asian	4	0.73	0.16		1.65 (0.77–3.55)	1.71 (0.81–3.61)
	4a/4a + 4a/4b vs 4b/4b	Overall	16	0.00	0.33	0.93	1.12 (0.89–1.41)	1.12 (1.01–1.25)
		Breast cancer	4	0.01	0.72		1.08 (0.72–1.62)	1.12 (0.93–1.35)
		Colorectal cancer	2	0.40	0.84		0.98 (0.80–1.20)	0.98 (0.80–1.20)
		Prostate cancer	3	0.13	0.00		1.47 (1.00–2.17)	1.50 (1.15–1.96)
		Other cancer	7	0.00	0.84		1.06 (0.61–1.82)	1.09 (0.89–1.32)
		Caucasian	12	0.00	0.04		1.31 (1.01–1.69)	1.32 (1.15–1.50)
		Asian	4	0.01	0.15		0.76 (0.53–1.10)	0.86 (0.72–1.02)
	4a/4a vs 4b/4b	Overall	16	0.08	0.00	0.46	1.81 (1.12–2.93)	1.90 (1.36–2.65)
		Breast cancer	4	0.71	0.57		1.17 (0.65–2.13)	1.19 (0.66–2.13)
		Colorectal cancer	2	0.30	0.22		1.69 (0.65–4.34)	1.74 (0.72–4.17)
		Prostate cancer	3	0.02	0.12		3.23 (0.72–14.44)	3.49 (1.76–6.89)
		Other cancer	7	0.21	0.03		1.72 (0.68–4.35)	2.00 (1.06–3.79)
		Caucasian	12	0.03	0.06		1.83 (0.98–3.43)	1.97 (1.36–2.85)
		Asian	4	0.73	0.19		1.60 (0.75–3.44)	1.66 (0.78–3.51)
-786T>C	C vs T	Overall	10	0.02	0.01	0.36	1.18 (1.05–1.34)	1.16 (1.07–1.26)
		Breast cancer	4	0.16	0.03		1.15 (0.98–1.36)	1.15 (1.02–1.29)
		Colorectal cancer	3	0.04	0.41		1.11 (0.87–1.43)	1.07 (0.94–1.22)
		Prostate cancer	2	0.10	0.01		1.28 (0.88–1.84)	1.32 (1.06–1.64)
		Bladder cancer	1	–	0.02		1.46 (1.08–1.97)	1.46 (1.08–1.97)
		Caucasian	6	0.03	0.02		1.25 (1.04–1.49)	1.21 (1.09–1.35)
		Asian	4	0.16	0.14		1.11 (0.94–1.30)	1.10 (0.97–1.24)
	CT vs TT	Overall	10	0.12	0.01	0.32	1.16 (1.01–1.33)	1.15 (1.03–1.27)
		Breast cancer	4	0.40	0.21		1.10 (0.95–1.27)	1.10 (0.95–1.27)
		Colorectal cancer	3	0.05	0.13		1.15 (0.85–1.54)	1.14 (0.96–1.36)
		Prostate cancer	2	0.05	0.56		1.22 (0.62–2.42)	1.33 (0.96–1.84)
		Bladder cancer	1	–	0.28		1.25 (0.84–1.86)	1.25 (0.84–1.86)
		Caucasian	6	0.23	0.02		1.21 (1.00–1.47)	1.21 (1.03–1.41)
		Asian	4	0.10	0.17		1.12 (0.91–1.37)	1.10 (0.96–1.26)
	CC vs TT	Overall	10	0.13	0.00	0.48	1.45 (1.10–1.92)	1.39 (1.13–1.71)
		Breast cancer	4	0.46	0.01		1.56 (1.11–2.19)	1.55 (1.11–2.18)
		Colorectal cancer	3	0.75	0.78		0.95 (0.67–1.34)	0.95 (0.67–1.34)
		Prostate cancer	2	0.17	0.02		1.72 (0.88–3.34)	1.75 (1.09–2.83)
		Bladder cancer	1	–	0.01		2.76 (1.24–6.18)	2.76 (1.24–6.18)
		Caucasian	6	0.03	0.02		1.63 (1.09–2.43)	1.46 (1.16–1.84)
		Asian	4	0.96	0.58		1.14 (0.71–1.83)	1.14 (0.71–1.83)
	CC vs CT + TT	Overall	10	0.36	0.01	0.36	1.30 (1.06–1.60)	1.28 (1.06–1.55)

Polymorphism	Comparison	Subgroup	N	*P*-Value			Regression Model	
				P_H	P_Z	P_E	Random	Fixed
		Breast cancer	4	0.67	0.03		1.43 (1.03–1.98)	1.43 (1.03–1.97)
		Colorectal cancer	3	0.82	0.77		0.96 (0.71–1.29)	0.96 (0.71–1.29)
		Prostate cancer	2	0.54	0.06		1.54 (0.99–2.37)	1.53 (0.99–2.37)
		Bladder cancer	1	–	0.02		2.50 (1.14–5.48)	2.50 (1.14–5.48)
		Caucasian	6	0.10	0.01		1.43 (1.05–1.95)	1.31 (1.07–1.61)
		Asian	4	0.96	0.62		1.13 (0.70–1.81)	1.13 (0.70–1.81)
	CC + CT vs TT	Overall	10	0.04	0.01	0.32	1.20 (1.04–1.39)	1.18 (1.06–1.30)
		Breast cancer	4	0.19	0.09		1.15 (0.95–1.40)	1.13 (0.98–1.30)
		Colorectal cancer	3	0.04	0.39		1.14 (0.85–1.53)	1.13 (0.96–1.34)
		Prostate cancer	2	0.05	0.42		1.31 (0.68–2.53)	1.42 (1.04–1.94)
		Bladder cancer	1	–	0.07		1.42 (0.97–2.08)	1.42 (0.97–2.08)
		Caucasian	6	0.09	0.00		1.28 (1.03–1.60)	1.27 (1.10–1.48)
		Asian	4	0.11	0.14		1.12 (0.92–1.36)	1.10 (0.97–1.26)
894G>T	T vs G	Overall	25	0.03	0.21	0.06	1.04 (0.98–1.11)	1.02 (0.98–1.07)
		Breast cancer	8	0.84	0.11		1.07 (0.98–1.16)	1.07 (0.98–1.16)
		Prostate cancer	7	0.80	0.72		0.99 (0.92–1.06)	0.99 (0.91–1.06)
		Colorectal cancer	5	0.00	0.85		0.98 (0.79–1.22)	1.00 (0.90–1.10)
		Bladder cancer	2	0.13	0.11		1.29 (0.85–1.96)	1.23 (0.96–1.60)
		Other cancer	3	0.01	0.40		1.22 (0.77–1.93)	1.00 (0.85–1.17)
		Caucasian	21	0.06	0.43		1.04 (0.97–1.11)	1.02 (0.97–1.07)
		Asian	2	0.01	0.56		1.16 (0.71–1.90)	1.11 (0.92–1.33)
		African-American	1	–	0.29		0.76 (0.46–1.26)	0.76 (0.46–1.26)
		Mixed	1	–	0.90		1.01 (0.83–1.23)	1.01 (0.83–1.23)
	TG vs GG	Overall	25	0.37	0.37	0.08	1.03 (0.96–1.10)	1.03 (0.97–1.10)
		Breast cancer	8	0.95	0.93		0.99 (0.89–1.12)	0.99 (0.89–1.12)
		Prostate cancer	7	0.75	0.40		1.04 (0.94–1.16)	1.04 (0.94–1.16)
		Colorectal cancer	5	0.15	0.81		1.02 (0.85–1.22)	1.02 (0.89–1.16)
		Bladder cancer	2	0.00	0.35		2.11 (0.44–10.06)	1.39 (0.96–2.02)
		Other cancer	3	0.47	0.95		1.01 (0.80–1.26)	1.01 (0.81–1.26)
		Caucasian	21	0.46	0.31		1.03 (0.97–1.11)	1.04 (0.97–1.11)
		Asian	2	0.03	0.70		1.09 (0.70–1.71)	1.06 (0.86–1.30)
		African-American	1	–	0.50		0.83 (0.48–1.43)	0.83 (0.48–1.43)
		Mixed	1	–	0.61		0.93 (0.72–1.22)	0.93 (0.72–1.22)
	TT vs GG	Overall	25	0.03	0.28	0.08	1.09 (0.93–1.27)	1.04 (0.93–1.15)
		Breast cancer	8	0.63	0.03		1.23 (1.02–1.49)	1.24 (1.02–1.50)
		Prostate cancer	7	0.67	0.28		0.91 (0.76–1.08)	0.91 (0.76–1.08)
		Colorectal cancer	5	0.02	0.74		0.92 (0.54–1.55)	0.95 (0.74–1.22)
		Bladder cancer	2	0.15	0.11		1.86 (0.72–4.80)	1.65 (0.89–3.03)
		Other cancer	3	0.04	0.62		1.29 (0.48–3.48)	0.98 (0.69–1.39)
		Caucasian	21	0.03	0.38		1.08 (0.91–1.27)	1.02 (0.91–1.14)
		Asian	2	0.09	0.20		1.90 (0.43–8.39)	1.62 (0.78–3.35)
		African-American	1	–	0.45		0.33 (0.02–6.02)	0.33 (0.02–6.02)
		Mixed	1	–	0.56		1.15 (0.72–1.81)	1.15 (0.72–1.81)
	TT + TG vs GG	Overall	25	0.12	0.33	0.08	1.04 (0.96–1.12)	1.03 (0.97–1.10)
		Breast cancer	8	0.91	0.51		1.04 (0.93–1.16)	1.04 (0.93–1.16)
		Prostate cancer	7	0.85	0.71		1.02 (0.92–1.12)	1.02 (0.92–1.12)
		Colorectal cancer	5	0.03	0.99		1.00 (0.80–1.25)	1.01 (0.89–1.15)
		Bladder cancer	2	0.00	0.32		2.07 (0.49–8.81)	1.40 (0.98–2.00)
		Other cancer	3	0.09	0.94		1.13 (0.75–1.70)	1.01 (0.82–1.25)
		Caucasian	21	0.21	0.31		1.04 (0.96–1.13)	1.03 (0.97–1.10)
		Asian	2	0.02	0.63		1.13 (0.69–1.86)	1.09 (0.89–1.33)
		African-American	1	–	0.38		0.78 (0.45–1.35)	0.78 (0.45–1.35)
		Mixed	1	–	0.80		0.97 (0.75–1.24)	0.97 (0.75–1.24)
	TT vs TG + GG	Overall	25	0.09	0.73	0.09	1.05 (0.92–1.21)	1.02 (0.92–1.13)
		Breast cancer	8	0.68	0.02		1.23 (1.03–1.47)	1.23 (1.03–1.47)
		Prostate cancer	7	0.54	0.16		0.88 (0.75–1.05)	0.88 (0.75–1.05)
		Colorectal cancer	5	0.04	0.73		0.92 (0.59–1.45)	0.94 (0.75–1.19)
		Bladder cancer	2	0.73	0.57		1.17 (0.68–2.02)	1.17 (0.68–2.02)
		Other cancer	3	0.05	0.64		1.25 (0.50–3.12)	0.97 (0.70–1.36)
		Caucasian	21	0.08	0.97		1.04 (0.90–1.20)	1.00 (0.90–1.11)

				P-Value			Regression Model	
Polymorphism	**Comparison**	**Subgroup**	**N**	P_H	P_Z	P_E	**Random**	**Fixed**
		Asian	2	0.11	0.20		1.86 (0.46–7.53)	1.61 (0.78–3.34)
		African-American	1	–	0.47		0.34 (0.02–6.27)	0.34 (0.02–6.27)
		Mixed	1	–	0.46		1.18 (0.76–1.84)	1.18 (0.76–1.84)

P_E = *P*-value of Egger test, P_H = *P*-value of heterogeneity test, P_Z = *P*-value of Z test.

TABLE 5. Association of *eNOS* Polymorphisms With Breast Cancer Risk Among Chinese Han Population

Genotype	**Cases (n = 873)**	**Controls (n = 1034)**	**Comparison**	**OR (95% CI)***	***P****
Intron 4a/b			4a vs 4b	1.02 (0.82–1.28)	0.84
4b/4b	722	858	4a/4a vs 4b/4b	1.08 (0.47–2.46)	0.86
4a/4b	140	164	4a/4b vs 4b/4b	1.02 (0.79–1.30)	0.91
4a/4a	11	12	4a/4a + 4a/4b vs 4b/4b	1.02 (0.80–1.29)	0.88
P_{HWE}		0.19	4a/4a vs 4a/4b + 4b/4b	1.08 (0.47–2.45)	0.86
-786T>C			C vs T	1.32 (1.02–1.70)	0.04
TT	751	917	CC vs TT	2.19 (1.01–4.76)	0.05
CT	114	115	CT vs TT	1.21 (0.92–1.60)	0.17
CC	8	2	CC + CT vs TT	1.28 (0.97–1.67)	0.08
P_{HWE}		0.41	CC vs CT + TT	2.17 (1.00–4.72)	0.50
894G>T			T vs G	1.18 (0.98–1.42)	0.09
GG	652	791	TT vs GG	1.69 (1.19–2.42)	0.00
TG	195	232	TG vs GG	1.02 (0.82–1.27)	0.87
TT	26	11	TT + TG vs GG	1.10 (0.89–1.36)	0.36
P_{HWE}		0.18	TT vs TG + GG	1.69 (1.18–2.41)	0.00

* Adjusted for age.

TABLE 6. Genotype Distribution of *eNOS* Polymorphisms in Stratification Analysis

	Cases (n = 873)			**Controls (n = 1034)**		
Intron 4a/b	**4b/4b**	**4a/4b**	**4a/4a**	**4b/4b**	**4a/4b**	**4a/4a**
Pathological type						
Infiltrative ductal carcinoma	586	118	9	858	164	12
Other carcinoma	136	22	2	858	164	12
Stage						
I + II	584	110	8	858	164	12
III + IV	138	30	3	858	164	12
−786T>C	TT	CT	CC	TT	CT	CC
Pathological type						
Infiltrative ductal carcinoma	610	98	5	917	115	2
Other carcinoma	141	16	3	917	115	2
Stage						
I + II	611	89	2	917	115	2
III + IV	140	25	6	917	115	2
894G>T	GG	TG	TT	GG	TG	TT
Pathological type						
Infiltrative ductal carcinoma	523	168	22	791	232	11
Other carcinoma	129	27	4	791	232	11
Stage						
I + II	526	158	18	791	232	11
III + IV	126	37	8	791	232	11

TABLE 7. Stratification Analysis of *eNOS* Polymorphisms With Breast Cancer Risk

	Allele Contrast		Homozygote Contrast		Heterozygote Contrast		Dominant Model		Recessive Model	
	OR (95% CI)*	P*	OR (95% CI)*	P*	OR (95% CI)*	P*	OR (95% CI)*	P*	OR (95% CI)*	P*
Intron 4a/b										
Pathological type										
Infiltrative ductal carcinoma	1.05 (0.84–1.33)	0.65	1.07 (0.45–2.56)	0.87	1.05 (0.81–1.37)	0.69	1.06 (0.82–1.36)	0.68	1.06 (0.45–2.54)	0.89
Other carcinoma	0.88 (0.58–1.36)	0.57	1.11 (0.24–5.02)	0.89	0.84 (0.52–1.36)	0.48	0.86 (0.54–1.37)	0.52	1.14 (0.25–5.17)	0.86
Stage										
I+II	0.99 (0.78–1.25)	0.91	0.97 (0.39–2.39)	0.95	0.99 (0.76–1.28)	0.91	0.98 (0.76–1.27)	0.90	0.97 (0.40–2.39)	0.95
III+IV	1.18 (0.81–1.71)	0.40	1.55 (0.43–5.57)	0.50	1.14 (0.74–1.75)	0.56	1.17 (0.77–1.76)	0.47	1.52 (0.42–5.44)	0.52
-786T>C										
Pathological type										
Infiltrative ductal carcinoma	1.34 (1.02–1.76)	0.03	1.88 (0.83–4.29)	0.13	1.29 (0.97–1.73)	0.08	1.33 (1.00–1.77)	0.05	3.47 (0.67–18.00)	0.14
Other carcinoma	1.21 (0.76–1.94)	0.43	3.40 (1.36–8.46)	0.01	0.92 (0.53–1.60)	0.76	1.08 (0.64–1.81)	0.78	3.37 (1.36–8.37)	0.01
Stage										
I+II	1.16 (0.88–1.54)	0.29	1.22 (0.46–3.25)	0.69	1.16 (0.87–1.56)	0.32	1.17 (0.87–1.57)	0.30	1.21 (0.45–3.23)	0.71
III+IV	1.99 (1.35–2.93)	0.00	4.42 (1.97–9.89)	0.00	1.42 (0.89–2.27)	0.14	1.74 (1.12–2.68)	0.01	4.34 (1.94–9.71)	0.00
894G>T										
Pathological type										
Infiltrative ductal carcinoma	1.25 (1.02–1.52)	0.03	1.73 (1.20–2.50)	0.00	1.10 (0.87–1.38)	0.43	1.18 (0.95–1.47)	0.14	1.71 (1.19–2.47)	0.00
Other carcinoma	0.88 (0.60–1.28)	0.49	1.53 (0.85–2.75)	0.16	0.72 (0.46–1.11)	0.14	0.79 (0.52–1.20)	0.26	1.58 (0.88–2.83)	0.13
Stage										
I+II	1.15 (0.94–1.40)	0.19	1.57 (1.07–2.29)	0.02	1.02 (0.81–1.29)	0.85	1.09 (0.87–1.36)	0.46	2.45 (1.15–5.23)	0.02
III+IV	1.31 (0.95–1.81)	0.10	2.14 (1.34–3.40)	0.00	1.00 (0.68–1.49)	0.99	1.16 (0.80–1.68)	0.42	4.56 (1.81–11.52)	0.00

* Adjusted for age.

DISCUSSION

Multiple lines of evidence supported an important role for genetics in determining cancer risk, and understanding polymorphisms associated with cancer risk may be valuable for providing personalized diagnosis and therapy of certain cancers. Since the identification of *eNOS* intron 4a/b, -786T>C, and 894G>T polymorphisms, an increasing number of studies suggested that *eNOS* intron 4a/b, -786T>C, and 894G>T polymorphisms may play important roles in cancer risk. Epidemiological studies of *eNOS* intron 4a/b, -786T>C, and 894G>T polymorphisms, if large and unbiased, can provide insight into the association between the gene and cancer risk. However, previous results are inconclusive. To derive a more precise estimation of the association, we performed this meta-analysis. The *eNOS* intron 4a/b and -786T>C polymorphisms were significantly associated with overall cancer risk. In contrast, no association was observed between *eNOS* 894G>T polymorphism and overall cancer risk. In subgroup analyses based on cancer type, significant associations were found between *eNOS* intron 4a/b polymorphism and the risk of prostate cancer, *eNOS* -786T>C polymorphism and the risk of prostate, bladder and breast cancers, and *eNOS* 894G>T polymorphisms and the risk of breast cancer. In subgroup analyses based on ethnicity, *eNOS* intron 4a/b and -786T>C polymorphisms were associated with cancer risk in Caucasians.

In current case–control study of 873 patients with breast cancer and 1034 healthy women, we found that *eNOS* -786T>C and 894G>T polymorphisms were associated with breast cancer risk in Chinese Han population, which were consistent with our meta-analysis results. Furthermore, stratified analyses based on pathological type showed that *eNOS* 894G>T polymorphism was only associated with risk of infiltrative ductal carcinoma. In stratified analyses by tumor stage, we found that *eNOS* -786T>C polymorphism was only associated with the risk of tumor stage III and IV.

To a certain extent, our meta-analysis and case–control study still include some limitations, which should be interpreted and taken into consideration. For the present meta-analysis, we did not have original data for all studies to adjust estimates and perform a more precise analysis. For the case–control study, all participants were from hospital, which may result in inherent selection bias.

In conclusion, our meta-analysis and case–control study suggest that *eNOS* -786T>C and 894G>T polymorphisms are associated with the risk of breast cancer. However, our findings need to be further validated in well-designed studies.

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