RESEARCH ARTICLE

Association Between Three eNOS Polymorphisms and Cancer Risk: a Meta-analysis

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Abstract

Polymorphisms in the endothelial nitric oxide synthase (eNOS) gene may influence the risk of cancer, but the results are still debatable. Therefore, we performed a systematic review to provide a more complete picture and conducted a meta-analysis to derive a precise estimation. We searched PubMed, EMBASE, EBSCO, Google Scholar and China National Knowledge Infrastructure (CNKI) databases until April 2014 to identify eligible studies. Thirty-one studies with cancer patients and controls were included in the meta-analysis. Overall, the polled analysis revealed that the T-786C polymorphism was significantly associated with increased cancer risk under multiple genetic models (C *vs* T: OR=1.135, 95%CI=1.048-1.228; CC *vs* TT: OR=1.278, 95%CI=1.045-1.562; TC *vs*TT: OR=1.136, 95%CI=1.023-1.261; CC+TC *vs* TT: OR=1.159, 95%CI=1.047-1.281; CC *vs* TC+TT: OR=1.204, 95%CI= 1.003-1.447). G894T was associated with significant risk for females (TT *vs* GG: OR=1.414, 95%CI=1.056-1.892; TT *vs* GT+GG: OR=1.356, 95%CI=1.108-1.661) and for breast cancer (T *vs* G: OR=1.097, 95%CI=1.001-1.203; TT *vs* GG: OR=1.346, 95%CI=1.012-1.789; TT *vs* GT+GG: OR=1.269, 95%CI=1.028-1.566). Increased susceptibility was revealed for prostate cancer with 4a/b (ba *vs* bb: OR=1.338, 95%CI=1.013-1.768; aa+ba *vs* bb: OR=1.474, 95%CI=1.002-2.170). This meta-analysis indicated that the eNOS T-786C polymorphism is associated with elevated cancer risk; the G894T polymorphism contributes to susceptibility to breast cancer and cancer generally in females; and the 4a/b polymorphism may be associated with prostate cancer risk.

Keywords: Endothelial nitric oxide synthase - polymorphism - cancer - systematic review - meta-analysis

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Introduction

Cancer is a major public health problem and it is one of the leading causes of death worldwide (Jemal et al., 2011). The global burden of cancer is ever-increasing in both economically developed countries and developing countries (Are et al., 2013). Tremendous efforts have been made to unravel the underlying mechanism of cancer, with the aim to develop optimal prophylactic and therapeutic strategies. The mechanism of developing cancer is still unclear. People generally agree that complex environmental factors and interindividual genetic susceptibility may contribute to cancer development (Perera, 1997). Substantial evidence shows that genetic susceptibility has a significant role in an individual's risk of developing cancer (Dong et al., 2008).

Nitric oxide (NO) is a short-lived, pleiotropic molecule that play complicated roles in tumor biology. NO is generated by three isoforms of NO synthase (NOS): neuronal (nNOS/NOS1), inducible (iNOS/NOS2) and endothelial (eNOS/NOS3) (Burke et al., 2013). Endothelial nitric oxide synthase (eNOS) is constitutively expressed in endothelial or epithelial cells, including a variety of tumours (Ying et al., 2007). The gene encoding for eNOS is located on chromosome 7q36 and contains 26 exons in humans (Marsden et al., 1993), and polymorphisms in the eNOS gene have been widely studied. Till now, a number of polymorphisms and mutations within the eNOS gene have been identified, with the most studied being G894T, T-786C and 4a/b polymorphisms. The G894T (Glu298Asp, rs1799983) polymorphism corresponds to a Glu-Asp change at codon 298 in exon 7. T-786C (rs2070744) polymorphism is a point mutation of thymine to cytosine at nucleotide -786 in the 5'-flanking region of the eNOS gene which could result in a significant reduction in eNOS gene promoter activity and reduce serum NO level significantly (Nakayama et al., 1999). 4a/b polymorphism is a variable number of tandem repeats (VNTR, 27nt) in intron 4 accounts influencing basal plasma NO generation (Wang et al., 1997). Many studies have investigated the influence of eNOS polymorphisms on cancer risk, whereas the results remained conflicting.

Therefore, we conducted comprehensive literature search with the aim to provide an overview of studies

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focusing on the relationship between eNOS polymorphisms and cancer risk. Meanwhile, we performed a meta-analysis combining all available data to estimate the potential associations of eNOS polymorphisms with cancer risk.

Materials and Methods

Literature search

We searched the articles using the search terms "NOS," "eNOS," "NOS3," "polymorphism (s)," "genotype," "variant," "carcinoma," "cancer," "tumor," and "malignancy" in PubMed, EMBASE, EBSCO, Google Scholar and China National Knowledge Infrastructure (CNKI) databases without a language limitation, and the last search updated on 5 April 2014. We evaluated all associated publications to retrieve the most eligible literatures. Their reference lists were hand-searched to find for other relevant publications. Only published studies with the full text articles were included. If the same patient population was included in several publications, the most recent or complete study was used in this meta-analysis.

Inclusion criteria

Studies were included in the meta- analysis if (1) it investigated the association between eNOS polymorphism and cancer risk; (2) the design was case-control study or nested case-control study; (3) data regarding genotype distributions were sufficient to calculate the odds ratio (OR) and its corresponding 95% confidence interval (CI). When studies with overlapping subjects were considered eligible, only the one with a larger number of patients was included. If the data regarding genotype distribution was insufficient, the effort was made to contact its corresponding author.

Data extraction

Two reviewers (Xun Wu and Zifeng Wang) extracted the following information from each included study independently and in duplicate: first author's name, publication year, country, source of the study population, cancer type, polymorphisms studied, source of control, number of cases and controls, match criteria, genotype distribution in cases and controls, and whether or not the genotype distributions among controls were in accordance with Hardy-Weinberg equilibrium (HWE). A third reviewer (Yin Xu) was consulted to reach a consensus if any discrepancy occurred.

Statistical analysis

The summary ORs and their corresponding 95 % CI were calculated to assess the strength of the association between eNOS polymorphism and cancer risk. Z-test was performed to determine the statistical significance of pooled ORs, and p <0.05 was considered significant. Cochran's Q test and the I2 statistic were used to measure heterogeneity across the included studies. A P value of more than 0.05 for the Q test indicated a lack of heterogeneity, and the fixed-effects model was subsequently used to calculate the summary ORs. Otherwise, the random-effects model was applied. Publication bias was estimated by visually assessing the asymmetry of Begg's funnel plot. Furthermore, Egger's test was performed to provide quantitative evidence for the checking of publication bias. Sensitivity analysis was also performed by sequentially omitting individual study to check the stability of the result. P <0.05 was considered statistically significant. All the statistical analysis was performed using STATA12.0 (STATA Corporation, College Station, TX, USA).

Results

Identification and characteristics of included studies

The process of study selection was summarized in the flow diagram (Figure 1). Finally, 31 studies (29 in English and 2 in Chinese) with a total of 9310 cases and 9786 controls were included in this study. It should be noted that Lee et al. studied polymorphisms in Caucasians and African-Americans respectively (Lee et al., 2009). Therefore, we treated them as separate data sets during our analysis. The characteristics of these eligible studies were summarized in Table 1. The most commonly investigated polymorphism was G894T, followed by 4a/b and T-786C, which were reported in 27 (Hefler et al., 2002; Medeiros et al., 2002; Ghilardi et al., 2003; Riener et al., 2004; Conde et al., 2006; Hefler et al., 2006; Lu et al., 2006; Marangoni et al., 2006; Royo et al., 2006; Lee et al., 2007; Chen et al., 2009; Funke et al., 2009; Harman et al., 2009; Lee et al., 2009; Li et al., 2009; Yeh et al., 2009; Chen et al., 2010; Unal et al., 2010; Zintzaras et al., 2010; Ozturk et al., 2011; Ryk et al., 2011; Arikan et al., 2012; Brankovic et al., 2013; Jang et al., 2013; Safarinejad et al., 2013; Verim et al., 2013; Ziaei et al., 2013), 14 (Hefler et al., 2002; Medeiros et al., 2002; Riener et al., 2004; Hefler et al., 2006; Lu et al., 2006; Yeh et al., 2009; Unal et al., 2010; Zintzaras et al., 2010; Ozturk et al., 2011; Sanli et al., 2011; Amasyali et al., 2012; Jang et al., 2013; Safarinejad et al., 2013; Yuan et al., 2013) and 11 (Ghilardi et al., 2003; Conde et al., 2006; Lu et al., 2006; Lee et al., 2007; Marangoni et al., 2008; Yeh et al., 2009; Unal et al., 2010; Ryk et al., 2011; Brankovic et al., 2013; Jang et al., 2013; Safarinejad et al., 2013) studies, respectively. There were 9 studies for prostate cancer, 7 studies for breast cancer, 5 studies for colorectal cancer, 3 studies for bladder cancer, 7 studies for other 5 different cancers and adrenal incidentaloma. Among those 31 studies, there were 22 Caucasian, 6 Asian, 3 fixed and 1 African American studies, respectively. A summary of the meta-analysis findings of the association

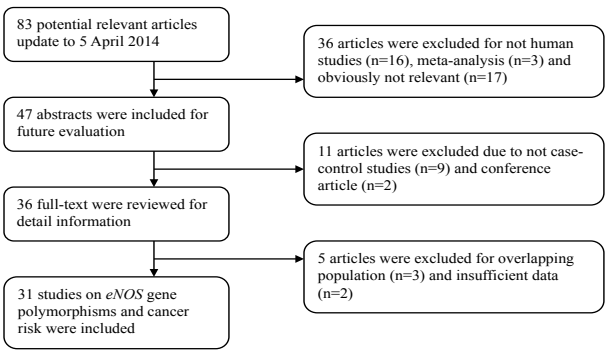

Figure 1. Flow Chart of Literature Search and Selection

between eNOS gene polymorphisms and cancer risks is provided in Table 2.

The association between G894T polymorphism and cancer risk

Data from 25 case-control studies and 2 nested case-control studies comprising 7775 cases and 7817 controls were pooled together for analysis of the G894T polymorphism. Significantly increased cancer risks were found for TT *vs* GG in studies with matched controls enrolled (OR=1.219, 95%CI=1.019-1.457) and femals (OR=1.414, 95%CI=1.056-1.892). Similar situations were found for TT *vs* GT+GG in studies with femals (OR=1.356, 95%CI=1.108-1.661), Asians (OR=2.103, 95%CI=1.133-3.903) and mixed population (OR=1.648, 95%CI=1.056-2.571). In subgroup analysis by cancer type, we found significantly increased breast cancer susceptibility in three models (T *vs* G: OR=1.097, 95%CI=1.001-1.203; TT *vs* GG: OR=1.346, 95%CI=1.012-1.789; TT *vs* GT+GG: OR=1.269, 95%CI=1.028-1.566) (Figure 2).

Sensitivity analysis was performed to assess the influence of each individual study on the pooled ORs by sequential omission of each eligible study. The analysis results showed that the pooled ORs were not significantly affected by any individual study (Figure 3a), thus indicating a robust result of the analysis.

Begg's funnel plot was constructed to evaluate the publication bias of literatures on cancer. The shape of the funnel plot seemed symmetrical, indicating the absence of publication bias (Figure 4a). Furthermore, Egger's test provided statistical evidence for the lack of publication bias (t=0.61, p=0.548).

Table 1. Characteristics of Studies Included in this Meta-Analysis

Author	Country	Ethnicity Gender	Polymorphisms investigated	Cancer type	Source of control	Genotyping method	Case/control	Matching criteria
Harman et al. 2009	Turkey	Caucasian	G894T	Adrenal incidentaloma	Hospital-based	TaqMan	50/30	NA
Ryk et al. 2011	Sweden	Caucasian	G894T;T-786C	Bladder cancer	Population-based	TaqMan, sequencing	262/150	Geographical area, age
Amasyali et al. 2012	Turkey	Caucasian	4a/b	Bladder cancer	Hospital-based	PCR	123/201	Age, gender
Verim et al. 2013	Turkey	Caucasian	G894T	Bladder cancer	Population-based	PCR-RFLP	66/88	Age, gender
Ghilardi et al. 2003	Italy	Caucasian	G894T;T-786C	Breast cancer	Population-based	PCR	71/91	Age
Hefler et al. 2006	German	Caucasian	G894T;4a/b	Breast cancer	Hospital-based	PCR	269/244	Geographical area, age
Lu et al., 2006	America	Caucasian	G894T;T-786C, 4a/b	Breast cancer	Hospital-based	PCR-RFLP, sequencing	421/423	Age
Royo et al. 2006	Spain	Caucasian	G894T	Breast cancer	Population-based	Sequencing	440/321	Age
Lee et al. 2007	Keroa	Asian	G894T;T-786C	Breast cancer	Mixed	SNP-ITTM	1348/944	Age, education
Li et al. 2009	America	Mixed	G894T	Breast cancer	Population-based	TaqMan	428/422	Age, ethnicity
Zintzaras et al. 2010	Greece	Caucasian	G894T;4a/b	Breast cancer	Hospital-based	Sequencing	306/131	NA
Conde et al. 2006	Spain	Caucasian	G894T;T-786C	Colorectal cancer	Population-based	FRET, pyrosequencing	360/550	NA
Funke et al. 2009	Germany	Caucasian	G894T	Colorectal cancer	Population-based	Pyrosequencing	632/604	Age, gender, geographical area
Yeh et al. 2009	Taiwan	Asian	G894T;T-786C, 4a/b	Colorectal cancer	Population-based	PCR	726/736	Age, gender
Arikan et al. 2012	Turkey	Caucasian	G893T	Colorectal cancer	Hospital-based	PCR-RFLP	84/99	NA
Jang et al. 2013	Keroa	Asian	G894T;T-786C, 4a/b	Colorectal cancer	Population-based	PCR	528/509	Age
Ozturk et al. 2011	Turkey	Caucasian	G894T;4a/b	Endometrial cancer	Hospital-based	PCR	89/60	NA
Chen et al. 2010	China	Asian	G894T	Gastric cancer	Population-based	PCR-RFLP	324/200	Age, gender
Unal et al. 2010	Turkey	Caucasian	G894T;T-786C, 4a/b	Gastric cancer	Population-based	PCR	50/98	NA
Yuan et al. 2013	China	Asian	4a/b	Hepatocellular carcinoma	Hospital-based	PCR, sequencing	293/384	Age, gender
Hefler et al. 2002	Austria	Caucasian	G894T;4a/b	Ovarian cancer	Population-based	Pyrosequencing, PCR	130/133	Age
Medeiros et al. 2002	Portugal	Caucasian	G894T;4a/b	Prostate cancer	Population-based	PCR	125/153	Age
Marangoni et al. 2006	Brazil	Mixed	G894T	Prostate cancer	BPH	PCR-RFLP	89/60	NA
Marangoni et al. 2008	Brazil	Mixed	T-786C	Prostate cancer	BPH	PCR-SSCP, sequencing	83/94	NA
Chen et al. 2009	China	Asian	G894T	Prostate cancer	BPH	PCR-RFLP	78/88	Age
Lee et al. 2009	America	Caucasian	G894T	Prostate cancer	Population-based	TaqMan	1213/1433	Age, ethnicity
		African American	G894T	Prostate cancer	Population-based	TaqMan	107/409	Age, ethnicity
Sanli et al. 2011	Turkey	Caucasian	4a/b	Prostate cancer	Population-based	NA	132/158	Age
Brankovic et al. 2013	Serbian	Caucasian	G894T;T-786C	Prostate cancer	Population-based, BPH	PCR-RFLP, CycleSeq Kit	150/250	Age
Safarinejad et al. 2013	Iran	Caucasian	G894T;T-786C, 4a/b	Prostate cancer	Population-based	PCR-RFLP	170/340	Age
Ziaei et al. 2013	Iran	Caucasian	G894T	Prostate cancer	BPH	Sequencing	95/111	Age, BMI
Riener et al. 2004	Austria	Caucasian	G894T;4a/b	Vulvar cancer	Hospital-based	Pyrosequencing, PCR	68/272	NA

Table 2. Stratified Analyses of eNOS Polymorphisms on Cancer Risk

Variables	N	Allele(T *vs* G) OR(95%CI)	Phet	Homozygous(TT *vs* GG) OR(95%CI)	Phet	Heterozygous(GT *vs* GG) OR(95%CI)	Phet	Dominant(TT+GT *vs* GG) OR(95%CI)	Phet	Recessive(TT *vs* GT+GG) OR(95%CI)	Phet
G894T											
Total	27	1.053(0.969-1.143)	0.002	1.178(0.957-1.449)	0.014	1.015(0.946-1.088)	0.06	1.006(0.956-1.060)	0.977	1.105(0.979-1.248)	0.056
HWE	23	1.081(0.991-1.180)	0.003	1.194(0.974-1.464)	0.034	1.047(0.952-1.152)	0.119	1.017(0.960-1.078)	0.974	1.103(0.974-1.250)	0.119
Match	19	1.058(0.991-1.128)	0.296	1.219(1.019-1.457)	0.27	1.034(0.940-1.139)	0.112	1.011(0.958-1.068)	0.949	1.128(0.981-1.297)	0.388
Femal	10	1.112(1.000-1.236)	0.237	1.414(1.056-1.892)	0.2	1.017(0.882-1.172)	0.248	1.007(0.925-1.097)	0.927	1.356(1.108-1.661)	0.14
Ethnicity											
Caucasian	20	1.050(0.956-1.152)	0.018	1.102(0.886-1.370)	0.025	1.045(0.927-1.178)	0.075	1.022(0.950-1.099)	0.844	1.038(0.911-1.182)	0.12
Asian	5	1.020(0.825-1.262)	0.055	2.074(0.881-4.882)	0.251	1.070(0.684-1.672)	0.196	0.992(0.916-1.075)	0.937	2.103(1.133-3.903)	0.278
Mixed	2	1.518(0.684-3.369)	0.011	3.691(0.246-55.335)	0.054	0.950(0.769-1.173)	0.107	0.996(0.801-1.238)	0.537	1.648(1.056-2.571)	0.071
African	1	0.760(0.457-1.265)	0	3.039(0.166-55.566)	0	0.826(0.478-1.428)	0	0.958(0.684-1.343)	0	1.105(0.979-1.248)	0
Cancer type											
Prostate cancer	7	1.063(0.902-1.253)	0.118	1.061(0.779-1.445)	0.368	1.013(0.890-1.153)	0.523	1.006(0.908-1.116)	0.988	1.043(0.831-1.310)	0.346
Breast cancer	7	1.097(1.001-1.203)	0.815	1.346(1.012-1.789)	0.245	1.038(0.918-1.174)	0.575	1.015(0.929-1.108)	0.93	1.269(1.028-1.566)	0.186
Colorectal cancer	5	0.979(0.788-1.216)	0.003	0.916(0.542-1.549)	0.017	1.016(0.889-1.162)	0.147	1.004(0.914-1.103)	0.858	0.944(0.747-1.194)	0.043
Gastric cancer	2	0.749(0.549-1.021)	0.308	0.390(0.077-1.975)	0.242	0.744(0.519-1.065)	0.463	0.912(0.709-1.173)	0.446	0.424(0.127-1.419)	0.324
Bladder cancer	2	1.293(0.852-1.961)	0.126	1.858(0.719-4.799)	0.154	1.391(0.959-2.018)	0.002	1.214(0.880-1.674)	0.014	1.171(0.680-2.018)	0.726
Other cancers	4	1.137(0.663-1.948)	0.016	1.659(0.523-5.256)	0.082	0.835(0.575-1.213)	0.135	0.925(0.689-1.244)	0.681	1.819(0.991-3.341)	0.081
	N	Allele(C *vs* T)		Homozygous(CC *vs* TT)		Heterozygous(TC *vs* TT)		Dominant(CC+TC *vs* TT)		Recessive(CC *vs* TC+TT)	
T-786C											
Total	11	1.135(1.048-1.228)	0.073	1.278(1.045-1.562)	0.302	1.136(1.023-1.261)	0.111	1.159(1.047-1.281)	0.066	1.204(1.003-1.447)	0.54
HWE	9	1.135(1.046-1.231)	0.044	1.265(1.027-1.558)	0.173	1.146(1.030-1.275)	0.104	1.164(1.050-1.291)	0.052	1.182(0.978-1.429)	0.401
Match	8	1.183(1.081-1.296)h	0.118	1.474(1.146-1.895)	0.443	1.174(1.048-1.316)	0.108	1.200(1.075-1.340)	0.071	1.353(1.068-1.715)	0.687
Caucasian	7	1.157(1.045-1.280)	0.073	1.286(1.030-1.604)	0.069	1.192(1.025-1.387)	0.327	1.224(1.060-1.413)	0.158	1.188(0.973-1.450)	0.204
Asian	3	1.112(0.973-1.271)	0.086	1.222(0.715-2.087)	0.989	1.111(0.958-1.290)	0.045	1.118(0.967-1.293)	0.052	1.204(0.706-2.055)	0.998
Mixed	1	0.988(0.644-1.517)	0	1.329(0.468-3.776)	0	0.712(0.373-1.361)	0	0.788(0.421-1.474)	0	1.642(0.627-4.302)	0
Cancer type											
Prostate cancer	3	1.508(1.153-1.972)	0.161	1.677(1.127-2.494)	0.409	1.161(0.886-1.522)	0.04	1.258(0.972-1.628)	0.044	1.563(1.088-2.245)	0.879
Breast cancer	3	1.139(0.998-1.300)	0.126	1.494(1.047-2.130)	0.336	1.091(0.924-1.288)	0.293	1.127(0.960-1.322)	0.15	1.371(0.980-1.918)	0.479
Colorectal cancer	3	1.073(0.940-1.225)	0.038	0.951(0.674-1.342)	0.754	1.144(0.963-1.359)	0.052	1.133(0.959-1.339)	0.044	0.956(0.708-1.291)	0.823
Gastric cancer	1	1.476(0.827-2.634)	0	1.631(0.556-4.785)	0	1.480(0.630-3.476)	0	1.532(0.738-3.180)	0	1.483(0.517-4.257)	0
Bladder cancer	1	1.062(0.777-1.452)	0	0.691(0.263-1.817)	0	1.246(0.836-1.856)	0	1.183(0.803-1.744)	0	0.625(0.242-1.617)	0
	N	Allele(a *vs* b)		Homozygous(aa *vs* bb)		Heterozygous(ba *vs* bb)		Dominant(aa+ba *vs* bb)		Recessive(aa *vs* ba+bb)	
4a/b											
Total	12	1.061(0.862-1.306)	0	1.607(0.901-2.865)	0.014	1.050(0.857-1.287)	0.002	1.081(0.866-1.349)	0	1.508(0.887-2.567)	0.01
Match	9	1.212(0.977-1.502)	0	2.302(1.315-4.028)	0.077	1.129(0.905-1.408)	0.002	1.193(0.941-1.511)	0	2.109(1.264-3.518)	0.147
Femal	4	0.864(0.674-1.109)	0.116	0.817(0.343-1.944)	0.186	1.050(0.857-1.287)	0.417	0.917(0.718-1.171)	0.278	0.816(0.456-1.462)	0.262
Ethnicity											
Caucasian	9	1.087(0.820-1.442)	0	1.489(0.703-3.150)	0.004	1.116(0.848-1.469)	0.002	1.136(0.839-1.537)	0	1.430(0.740-2.767)	0.004
Asian	3	0.968(0.819-1.144)	0.592	1.702(0.761-3.807)	0.579	0.905(0.753-1.088)	0.387	0.933(0.779-1.117)	0.465	1.728(0.773-3.863)	0.55
Cancer type											
Prostate cancer	3	1.515(0.975-2.353)	0.029	3.234(0.724-14.443)	0.018	1.338(1.013-1.768)	0.394	1.474(1.002-2.170)	0.131	3.003(0.721-12.50)	0.025
Breast cancer	3	0.960(0.773-1.194)	0.291	1.181(0.629-2.216)	0.05	0.905(0.700-1.170)	0.289	0.921(0.709-1.197)	0.259	1.206(0.645-2.255)	0.56
Colorectal cancer	2	1.010(0.833-1.225)	0.578	1.686(0.654-4.343)	0.298	0.949(0.760-1.185)	0.295	0.979(0.795-1.204)	0.404	1.717(0.637-4.631)	0.279
Other cancers	4	0.934(0.565-1.545)	0	0.876(0.207-3.714)	0.025	1.031(0.575-1.848)	0.001	0.989(0.535-1.829)	0.404	0.970(0.379-2.480)	0.091

*The random-effects model was applied when the P value was more than 0.05. Otherwise, the fixed-effects model was used, N Number of comparisons, OR odds ratio; CI confidence interval, Phet P value of Q-test for the heterogeneity test

The association between T-786C polymorphism and cancer risk

A total of 11 studies involving 4169 cases and 4185 controls examined the association between T-786C polymorphism and cancer risk, with 7 in Caucasians, 3 in Asians, and 1 in mixed population (Brazilians). Unlike G894T, we observed a significant association between T-786C polymorphism and cancer risk (C *vs* T: OR=1.135, 95%CI=1.048-1.228; CC *vs* TT: OR=1.278, 95%CI=1.045-1.562; TC *vs* TT: OR=1.136, 95%CI=1.023-1.261; CC+TC *vs* TT: OR=1.159, 95%CI=1.047-1.281; CC *vs* TC+TT: OR=1.204, 95%CI=1.003-1.447). When stratified by ethnicity, a significant association between T-786C polymorphism and cancer risk was found in Caucasians. When stratified by cancer type, a significantly increased risk was found in prostate cancer (C *vs* T: OR=1.508, 95%CI=1.153-1.972; CC *vs* TT: OR=1.677, 95%CI=1.127-2.494; CC *vs* TC+TT: OR=1.563, 95%CI=1.088-2.245) and breast cancer (CC *vs* TT: OR=1.494, 95%CI=1.047-2.130) but not in colorectal, gastric, or bladder cancer.

Sensitivity analysis was performed to assess the influence of each individual study on the pooled ORs by sequential omission of each eligible study. The analysis

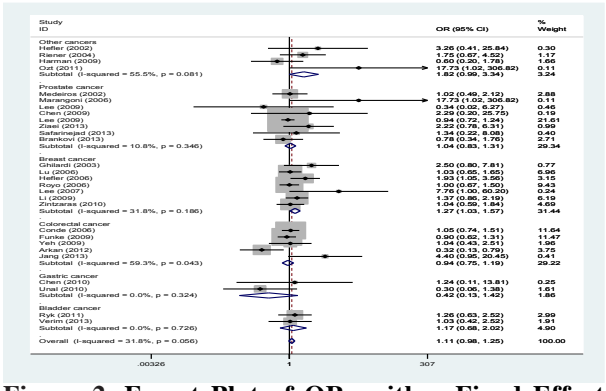

Figure 2. Forest Plot of ORs with a Fixed Effect Model for Association between the eNOS G894T Polymorphism and Overall Cancer Risk under Recessive Model(TT *vs* GT+GG)

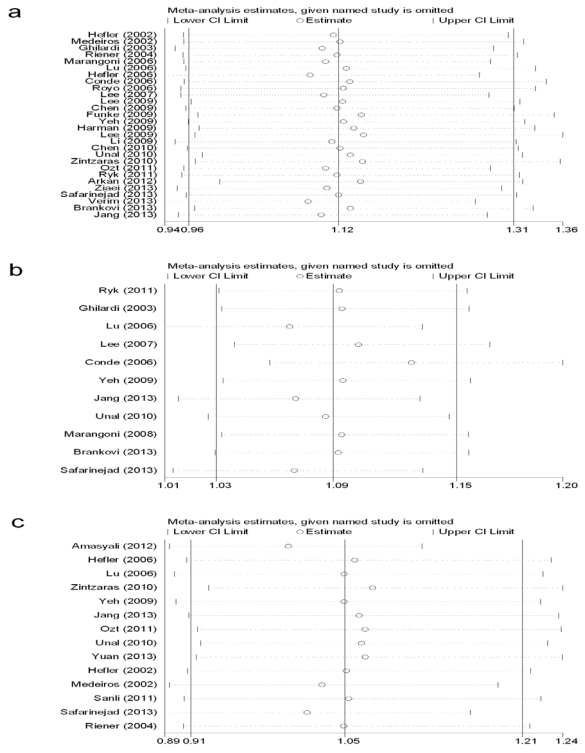

Figure 3. Sensitivity Analysis of Overall OR Ccoefficients for (a G894T TT *vs* GG, b T-786C C *vs* T, c 4a/b aa+ba *vs* bb). Results were calculated by omitting each study in turn. The two ends of the dotted lines represent the 95%CI

results showed that the pooled ORs were not significantly affected by any individual study (Figure 3b), thus indicating a robust result of the analysis.

Begg's funnel plot was constructed to evaluate the publication bias of literatures on cancer. The shape of the funnel plot seemed symmetrical, indicating the absence of publication bias (Figure 4b). Furthermore, Egger's test provided statistical evidence for the lack of publication bias (t=0.48, *p*=0.645).

The association between 4a/b polymorphism and cancer risk

14 studies with 3430 cases and 3842 controls investigated the association between 4a/b polymorphism and cancer risk. All the studies are in HWE. Increased

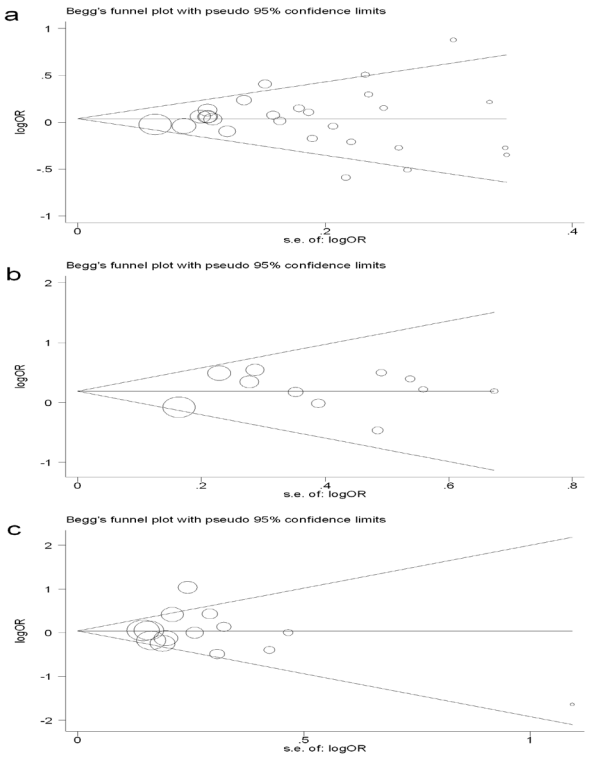

Figure 4. Begg's Funnel Plot for Publication Bias Test. a G894T T *vs* G, b T-786C CC *vs* TC+TT, c 4a/b ba *vs* bb Each point represents a separate study for the indicated association. The circles represent the weight of individual study

cancer risk were detected in studies with matched controls enrolled (aa *vs* bb: OR=2.302, 95%CI=1.315-4.028; aa *vs* ba+bb: OR=2.109, 95%CI=1.264-3.518). Subgroup analysis suggests that increased susceptibility was revealed in prostate cancer (ba *vs* bb: OR=1.338, 95%CI=1.013-1.768; aa+ba *vs* bb: OR=1.474, 95%CI=1.002-2.170) .

Sensitivity analysis was performed to assess the influence of each individual study on the pooled ORs by sequential omission of each eligible study. The analysis results showed that the pooled ORs were not significantly affected by any individual study (Figure 3c), thus indicating a robust result of the analysis.

Begg's funnel plot was constructed to evaluate the publication bias of literatures on cancer. The shape of the funnel plot seemed symmetrical, indicating the absence of publication bias (Figure 4c). Furthermore, Egger's test provided statistical evidence for the lack of publication bias (t=-0.33, *p*=0.745).

Discussion

Recent literature indicates that eNOS can modulate cancer-related events such as angiogenesis, apoptosis, cell cycle, invasion, and metastasis (Ying and Hofseth, 2007). Correlation between eNOS and cancers has been reported (Erdamar et al., 2006; Tu et al., 2006; Yu et al., 2006). Endothelial NOS plays a predominant role in VEGF-induced angiogenesis and vascular permeability (Fukumura et al., 2001). Stress is accepted to constitute a relevant factor in the development of cancer (Reiche et al., 2004). Animal experiments show that eNOS play a pivotal role for eNOS in chronic stress-induced initiation

and promotion of tumour growth (Barbieri et al., 2012). These findings suggest eNOS as a key factor promoting carcinogenesis. Effects of polymorphisms of the eNOS gene on plasma NO concentrations have been reported. The mutant allele of the T-786C and 4a/b polymorphism has been associated with altered eNOS activity and synthesis of NO (Wang et al., 1997; Nakayama et al., 1999). The eNOS polymorphisms might affect the process of carcinogenesis by influencing the expression of eNOS. Till now, many efforts have been made to explore the association between eNOS polymorphisms and cancer risk, whereas the results remain controversial. Here, we conducted a comprehensive meta-analysis to provide a complete picture of the role of eNOS polymorphisms in cancer risk.

By performing meta-analysis with studies involving cases and controls, we didn't find that eNOS G894T polymorphism has an overall association with cancer risk. However, in subgroup analysis, the association was found in females (homozygote comparison and the recessive model) and breast cancer (allele contrast model, homozygote comparison and the recessive model). Three earlier meta-analyses show controversial views for breast cancer (Hao et al., 2010; Yao et al., 2010; Fu et al., 2011). Fu thought Hao's study includes unqualified studies. We agree with Fu's opinion about the overlapping data, but we include Lee's study (Lee et al., 2007) for this meta-analysis. We failed to detect a significant association in other cancer types, which could be partly because the number of included studies for particular cancer type was small. For instance, only two studies discussed the eNOS G894T polymorphism and bladder cancer, and a significant association was found in Verim's research (Verim et al.,2013), but we couldn't find this significance in the pooled OR. We found gender differences in subgroup analysis. Estrogen modulation may explain this phenomenon. It was found that estrogen can active eNOS via MAP kinase-dependent mechanisms (Chen et al., 1999).

As for eNOS T-786C polymorphism, we observed a significantly increased cancer risk in all genetic models by pooling ORs from 11 studies. In subgroup analysis based on ethnicity, elevated cancer risk was detected in Caucasians in four genetic models, while that was not detected in Asians. The different ethnical background and a small number of studies involving Asians may partially explain this difference. When stratified by cancer type, we found a significant association between T-786C polymorphism and increased risk of prostate cancer in three genetic models. That was detected in only homozygote comparison (TT *vs* GG) in breast cancer. Besides, no significant association was found in colorectal cancer.

As for the Intron 4 VNTR (4a/b) polymorphism, significant association was only found with cancer risk in studies with matched controls in homozygote comparison (aa *vs* bb) and recessive comparison (aa+ba *vs* bb). In subgroup analysis based on cancer type, elevated cancer risk was detected in prostate cancer in two genetic models. Interestingly, the minor allele was a in the most studies, while in the study by Ozt et al. (2011), b was the minor allele. We think this diversity may result from a selection bias or different ethnicity background.

We do a comprehensive electronic search for all available eligible studies and provided an overview of the association between eNOS polymorphisms and cancer susceptibility. Still, there were some limitations in our meta-analysis. First, sample size in any given cancer was not sufficiently large, resulting in insufficient power to detect a slight effect on a certain type of cancer. Second, most of included studies are of Caucasian, relative small sample size in Asians might cause the inconspicuousness. Third, selection bias might exist given the fact that the genotype distribution deviated from HWE in some studies. Fourth, due to the original data of the eligible studies was unavailable, it was difficult for us to evaluate the roles of some special environmental factors and lifestyles such as diet, alcohol consumption, and smoking status in developing cancer. Fifth, the influence of bias in the present analysis could not be completely excluded because positive results are supposed to be published much more quickly than articles with "negative" results.

In conclusion, our meta-analysis suggested that the eNOS genetic polymorphisms contribute to the susceptibility of cancers. The eNOS T-786C polymorphism is associated with elevated cancer risk. The G894T polymorphism contributes to susceptibility to breast cancer and femals; and the 4a/b polymorphism may be associated with prostate cancer risk. Large well designed epidemiological studies are needed to validate our findings.

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