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ORIGINAL PAPER

An Updated Meta-Analysis of the Effects of the Endothelial Nitric Oxide synthase Gene G894T Polymorphism and Erectile Dysfunction Risk

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Abstract Erectile dysfunction (ED) is a common disorder leading to a serious and negative impact on the patient's quality of life. The gene encoding endothelial nitric oxide synthase (eNOS) is an interesting candidate gene for understanding the physiopathology of ED. However, an association between *eNOS G894T* polymorphism and ED risk is uncertain and should be updated. Therefore, a meta-analysis of the current literature was necessary to clarify this relationship. We searched Pubmed and China National Knowledge Infrastructure (CNKI) (last search updated on Dec 12, 2013) using 'nitric oxide synthase,' 'polymorphism or variant,' 'genotype,' and 'ED' as keywords. We also searched reference lists of studies corresponding to the inclusion criteria for the meta-analysis. These studies involved the total number of 1,445 ED men and 1,459 healthy control men subjects. Odds ratio (OR) and 95 % confidence intervals (CIs) were used to evaluate this relationship. Statistical analysis was performed with STATA10.0. In the overall analysis, significantly decreased associations between ED risk and *eNOS G894T* polymorphism were found. Moreover, in the subgroup analysis based on ethnicity, similar significant associations were detected in both Caucasians (such as GG+GT vs. TT: OR 0.92, 95 %CI 0.86–0.97) and Asians (such as GG+GT vs. TT: OR 0.24, 95 % CI 0.07–0.85). The Egger's test did not reveal the presence of a publication bias. Our investigations demonstrate that *eNOS G894T* polymorphism might protect men against ED risk. Further studies based on larger sample size and gene−environment interactions should be conducted.

Keywords Erectile dysfunction · eNOSG894T · Polymorphism · Meta-analysis · Ethnicity

Introduction

Erectile dysfunction (ED) is a common disorder characterized by the inability to achieve or maintain an erection sufficiently rigid for satisfying sexual intercourse. According to epidemiological data, the prevalence of ED is approximately 5–35 % [1]. The risk for the development of ED increases due to factors, such as diabetes mellitus, hormonal dysregulation, hypertension, hyper-cholesterolaemia, vascular insufficiency, psychogenic factors, neurological dysfunctions due to surgery and side effects of drug and radiation therapy [2, 3]. A great deal of work has been performed in an effort to identify candidate genes and single nucleotide polymorphisms (SNPs) in genes that are associated with ED [4–7].

To the best of our knowledge, the function of the penis is regulated by corporeal smooth muscle relaxation and contraction. Relaxation of the penis has been shown to be mediated by nitric oxide (NO). Nitric oxide is produced by a group of enzymes termed nitric oxide synthases (NOSs) that use L-arginine as their substrate [8, 9]. To date, three forms of NOS, labeled after their location have been recognized, including neuronal NOS, eNOS, and inducible NOS [10]. Decreased expression and/or activity of these

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enzymes have been demonstrated in some pathologic disease processes to be associated with the manifestation of ED [11–13]. This implies that the NOS gene and its polymorphisms play a significant role within penile tissues and ED.

The human endothelial NOS (eNOS) gene contains 26 exons spanning about 21 kb of genomic DNA and is located on chromosome 7q36 [14]. In position 894 of exon seven of the eNOS gene, a G to T polymorphism has been described (rs1799983, genotype TT: mutant, GT and GG: wild type), which leads to a change of aspartate (Asp) to glutamate (Glu) in position 298 of the protein [15]. Several studies have reported that the *Asp298* variant is associated with diminished eNOS activity or decreased basal NO production [16], and can be considered as an increased risk of some chronic diseases, such as diabetes, cardiovascular diseases, sleep apnea and ED [16–18]. In contrast, *Glu298* or *G894* allele may be a protective biomarker in above diseases.

Several epidemiologic studies have examined the associations between *eNOS* gene *G894T* polymorphism with a potential functional significance and risk of ED. However, results have been inconsistent across these studies. Some studies reported this gene polymorphism was a risk factor for ED; however, different opinions were published that this polymorphism was a protect factor or had no relationship for ED risk. The objective of our study was to examine associations between *eNOS G894T* polymorphism and risk of ED in larger samples [4, 7, 16, 19–23] by an updated meta-analysis.

Materials and Methods

We searched the PubMed and CNKI databases for all articles on the association between *eNOS G894T* polymorphism and ED risk up to Dec 12, 2013. The medical subject headings and keywords used for the search were 'erectile dysfunction,' 'polymorphism or variant,' 'genotype,' and 'penile function.' The electronic searching was supplemented by checking reference lists from the identified articles and reviews for additional studies.

Eligible studies had to meet the following criteria: (1) study was designed using the methodology of a case–control study; (2) the association between the *eNOS G894T* polymorphism and ED risk was explored; (3) Sexual functions were determined by the 5-item version of the International Index of Erectile Function (IIEF-5). All subjects completed the IIEF-5 to confirm the diagnosis of ED and evaluate the severity according to the total IIEF-5 score and (4) patients with ED were diagnosed as those having an IIEF-5 score of <22 [24, 25]. The major exclusion criteria were (1) duplicate data, the study with the largest number and the latest information of participants was included; (2) abstract, comment, review and editorial and (3) no sufficient data were reported.

The following items were collected: first author's last name, year of publication, country of origin, race, source of control (hospital-based, HB, and population-based, PB), Hardy–Weinberg equilibrium (HWE) of control group, total number for cases/controls, genotype methods, and mean ± SD of participants both in case and control groups.

Statistical Analysis

The strength of the association between the *eNOS G894T* polymorphism and ED risk was measured by ORs with 95 % CIs. Pooled ORs were obtained from a combination of single studies by allelic contrast (G-allele vs. T-allele), homozygote comparison (GG vs. TT), heterozygote comparison (GT vs. TT), the recessive model (GG vs. GT+TT), and the dominant model (GG+GT vs. TT), respectively. The heterogeneity among the studies was checked using the χ^2 based Q statistic and considered statistically significant at $P < 0.10$. When $P > 0.10$, the pooled OR of each study was calculated using the fixed-effects model (the Mantel–Haenszel method), which weighs the studies by the inverse of the variance of the estimates; otherwise, the random-effects model (the DerSimonian and Laird method) was used [26, 27]. The significance of the pooled OR was determined by the Z-test, and $P < 0.05$ was considered statistically significant. The departure of frequencies of *eNOS G894T* polymorphism from expectation under HWE was assessed by the χ^2 test in controls and a $P < 0.05$ was considered as significant disequilibrium. Publication bias was diagnosed with Egger's linear regression method and Begg's funnel plot. The P value less than 0.05 in Egger's linear regression indicated the presence of potential publication bias [28]. All statistical tests for this meta-analysis were performed with Stata software, version 10.0 (STATA Corp., College Station, TX, USA), and all tests were two-sided.

Results

Twenty three articles were collected from the PubMed and CNKI databases via a literature search using different combinations of key terms. Fifteen relevant studies were excluded (review/meta-analysis, duplication, different site polymorphisms). As shown in Fig. 1, eight articles (1,445 cases and 1,459 controls) were identified [4, 7, 16, 19–23]. Study characteristics of published studies on the relationship between *eNOS G894T* and ED risk are summarized in Table 1. The distribution of genotypes among all controls was consistent with HWE, except Deng et al. [23].

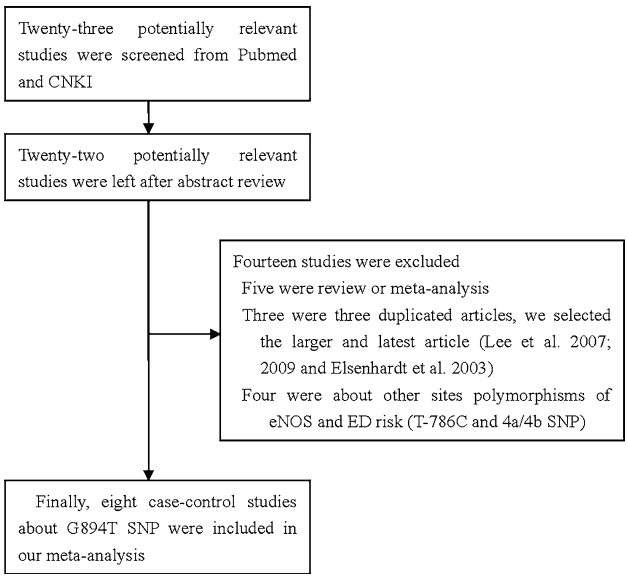

Fig. 1 Flowchart illustrating the search strategy used to identify association studies of the *eNOS G894T* polymorphism and ED risk

In the overall analysis, significantly decreased associations were observed between ED risk and the *eNOS G894T* polymorphism in different genetic models: allelic contrast (OR 0.54, 95 % CI 0.39–0.76, $P_{\text{heterogeneity}} = 0.000$, $P = 0.000$), homozygote comparison(OR 0.33, 95 % CI 0.16–0.68, $P_{\text{heterogeneity}} = 0.007$, $P = 0.003$), heterozygote comparison (OR 0.90, 95 % CI 0.85–0.95, $P_{\text{heterogeneity}} = 0.198$, $P = 0.000$), the recessive model (OR 0.56, 95 % CI 0.37–0.86, $P_{\text{heterogeneity}} = 0.000$, $P = 0.000$), and dominant genetic model (OR 0.42, 95 % CI 0.23–0.76, $P_{\text{heterogeneity}} = 0.043$, $P = 0.004$) (Table 2).

In the stratified analysis by ethnicity subgroup, significantly decreased associations were also found among ED risk and *eNOS G894T* polymorphism in both Asians (allelic contrast: OR 0.41, 95 % CI 0.29–0.58, $P_{\text{heterogeneity}} = 0.088$, $P = 0.000$, Fig. 2, homozygote comparison: OR 0.19, 95 % CI 0.05–0.77, $P_{\text{heterogeneity}} = 0.025$, $P = 0.020$, the recessive model: OR 0.43, 95 % CI 0.27–0.68, $P_{\text{heterogeneity}} = 0.041$, $P = 0.000$, and dominant genetic model: OR 0.24, 95 % CI 0.07–0.85, $P_{\text{heterogeneity}} = 0.046$, $P = 0.027$, Fig. 3), and Caucasians (heterozygote comparison: OR 0.88, 95 % CI 0.79–0.97, $P_{\text{heterogeneity}} = 0.710$, $P = 0.010$, and dominant genetic model: OR 0.92, 95 % CI 0.86–0.97, $P_{\text{heterogeneity}} = 0.192$, $P = 0.003$, Fig. 3) (Table 2). Similarly, obvious relationships were detected between ED risk and eNOS G894T gene polymorphism in both 'source of control' groups (Table 2).

The sensitivity analysis was performed to assess the influence of each individual study on the pooled OR by sequential removal of individual studies. Although the genotype distributions in one study [23] did not follow the HWE, the corresponding overall summary OR was not altered with/without including the study (data not shown). The results suggested that no individual study affected the overall OR. The Begg's funnel plot and Egger's test were performed to assess the publication bias. As shown in Table 3, the shapes of the funnel plots did not reveal any obvious asymmetry in any of the comparison models. Then, the Egger's test was used to provide statistical evidence of funnel plot symmetry. The results still did not suggest any evidence of publication bias (allelic contrast: $t = -0.14$, $P = 0.893$, homozygote comparison: $t = -1.32$, $P = 0.235$; heterozygote comparison: $t = 1.08$, $P = 0.320$, the recessive model: $t = 0.19$, $P = 0.854$, and dominant genetic model: $t = -1.32$, $P = 0.234$, Table 3; Figs. 4, 5).

Discussion

Erectile dysfunction is an important entity of daily urology practice, effecting a high percentage of males in various degrees and increasing with aging. Several factors have

Table 1 Study characteristics from published studies on the relationship between *G894T* polymorphisms in *eNOS* gene and ED

First author	Year	Country	Ethnicity	Source of control	Case	Control	Method	HWE Of control	Mean ± SD	
									Case	Control
Deng	2009	China	Asian	HB	94	100	PCR-RFLP	0.000	56.4 ± 6.7	54.2 ± 7.1
Eisenhardt	2010	Germany	Caucasian	HB	455	108	PCR-CRFA	0.386	56.9 ± 11.7	57.1 ± 2.2
Erol	2009	Turkey	Caucasian	HB	64	82	PCR-RFLP	0.184	51.3 ± 7.2	50.2 ± 7.6
Safarinejad	2011	Iran	Asian	PB	322	318	RT fluorescence PCR	0.111	55.7 ± 12.2	53.2 ± 13.6
Lee	2012	Taiwan	Asian	PB	297	293	PCR-RFLP	0.133	56.1 ± 4.6	54.5 ± 3.3
Peskircioglu	2007	Turkey	Caucasian	PB	96	167	PCR-RFLP	0.576	52.0 ± 15.2	NA
Andersen	2010	Brazil	Mixed	PB	64	329	Allele specific PCR	0.923	52.7 ± 16.0	38.1 ± 12.3
Rosas-Vargas	2004	Mexico	Mixed	PB	53	62	PCR-RFLP	0.243	46.0 ± 8.7	43.2 ± 7.5

PCR-CRFA PCR and consecutive restriction fragment analysis, *RT fluorescence PCR* real-time fluorescence PCR using the Light, *HB* hospital-based, *PB* population-based

Table 2 Total and stratified analysis of *G894T* polymorphisms in *eNOS* gene on ED

Variables	N^a	Case/Control	G-allele vs. T-allele			GT vs. TT		
			OR (95 % CI)	P_h^b	P^c	OR (95 % CI)	P_h^b	P^c
Total	8	1,445/1,459	**0.54 (0.39–0.76)**	**0.000**	**0.000**	**0.90 (0.85–0.95)**	**0.198**	**0.000**
Ethnicity								
Caucasian	3	615/357	0.61 (0.36–1.05)	0.005	0.073	**0.88 (0.79–0.97)**	**0.710**	**0.010**
Asian	3	713/711	**0.41 (0.29–0.58)**	**0.088**	**0.000**	0.44 (0.12–1.60)	0.064	0.214
Mixed	2	117/391	0.72 (0.37–1.39)	0.096	0.326	1.01 (0.91–1.13)	0.448	0.792
Source of control								
HB	3	613/290	0.54 (0.27–1.09)	0.001	0.087	0.91 (0.82–1.01)	0.682	0.082
PB	5	832/1,169	**0.54 (0.36–0.80)**	**0.001**	**0.002**	**0.90 (0.84–0.96)**	**0.056**	**0.001**

Variables	N	GG+GT vs. TT			GG vs. TT			GG vs. GT+TT		
		OR (95 % CI)	P_h^b	P^c	OR (95 % CI)	P_h^b	P^c	OR (95 % CI)	P_h^b	P^c
Total	8	**0.42 (0.23–0.76)**	**0.043**	**0.004**	**0.33 (0.16–0.68)**	**0.007**	**0.003**	**0.56 (0.37–0.86)**	**0.000**	**0.000**
Ethnicity										
Caucasian	3	**0.92 (0.86–0.97)**	**0.192**	**0.003**	0.39 (0.14–1.06)	0.069	0.065	0.65 (0.29–1.46)	0.002	0.302
Asian	3	**0.24 (0.07–0.85)**	**0.046**	**0.027**	**0.19 (0.05–0.77)**	**0.025**	**0.020**	**0.43 (0.27–0.68)**	**0.041**	**0.000**
Mixed	2	1.00 (0.95–1.05)	0.399	0.993	0.99 (0.91–1.08)	0.445	0.835	0.69 (0.29–1.65)	0.064	0.405
Source of control										
HB	3	**0.94 (0.90–0.99)**	**0.673**	**0.013**	**0.91 (0.85–0.98)**	**0.575**	**0.012**	0.62 (0.26–1.44)	0.001	0.267
PB	5	**0.37 (0.14–0.96)**	**0.022**	**0.041**	**0.28 (0.09–0.83)**	**0.009**	**0.022**	**0.53 (0.33–0.84)**	**0.002**	**0.007**

Bold values indicate significant association

[a] Number of comparisons

[b] *P* value of *Q*-test for heterogeneity test

[c] *P* value of *Z*-test for significant test

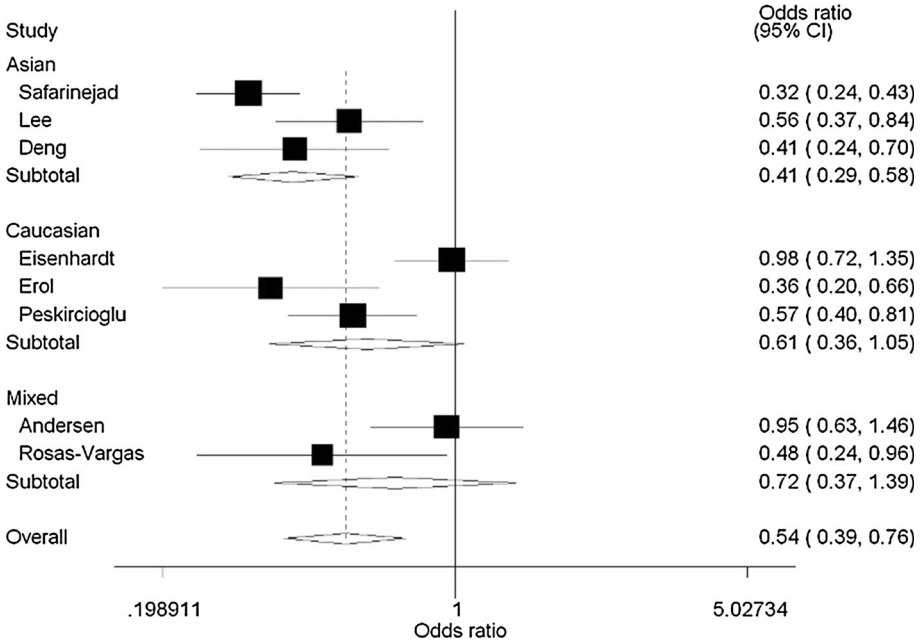

Fig. 2 Forest plot of ED risk associated with the *eNOS G894T* polymorphism (G-allele vs. T-allele) in ethnicity subgroup. The *squares* and *horizontal lines* correspond to the study-specific OR and 95 % CI. The area of the *squares* reflects the weight (inverse of the variance). The *diamond* represents the summary OR and 95 % CI

been shown to be related to ED prevalence, including age, hypertension, diabetes, cardiac disease, vascular insufficiency, cigarette smoking, and lipid disorders [29–31]. In addition to established risk factors, genetic risk factors may have important roles in the pathogenesis of ED. In recent years, there has been an increasing awareness among

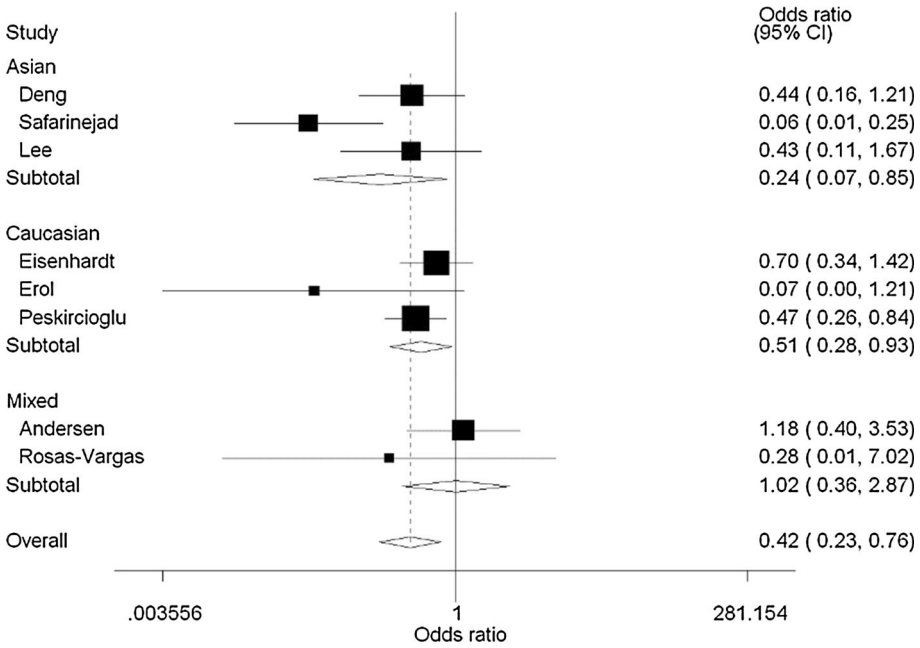

Fig. 3 Forest plot of ED risk associated with the *eNOS G894T* polymorphism (GG+GT vs. TT) in ethnicity subgroup. The *squares* and *horizontal lines* correspond to the study-specific OR and 95 % CI. The area of the *squares* reflects the weight (inverse of the variance). The *diamond* represents the summary OR and 95 % CI

Table 3 Publication bias tests (Begg's funnel plot for publication bias test)

Genetic type	Coefficient	Standard error	*t*	*P* value	95 % CI of intercept
G-allele vs. T-allele	−0.455	3.253	−0.14	0.893	(−8.413, 7.504)
GT vs. TT	1.278	1.178	1.08	0.320	(−1.606, 1.161)
GG vs. TT	−1.778	1.347	−1.32	0.235	(−5.074, 1.518)
GG+GT vs. TT	−1.378	1.419	−1.32	0.234	(−3.927, 1.172)
GG vs. GT+TT	0.626	3.269	0.19	0.854	(−3.374, 8.627)

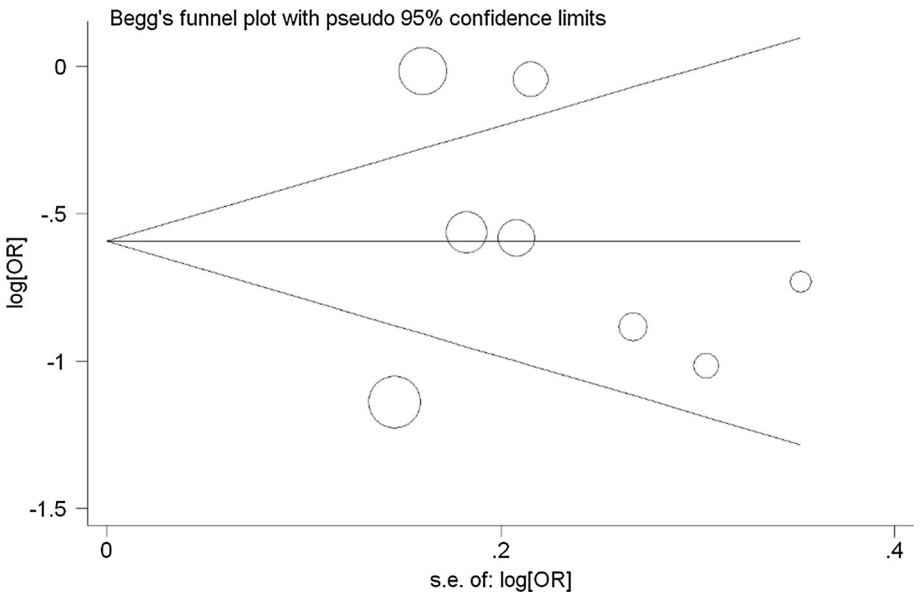

Fig. 4 Begg's funnel plot for publication bias test for (G-allele vs. T-allele). Each *point* represents a separate study for the indicated association. Log [OR], natural logarithm of OR. *Horizontal line*, mean effect size

scientists and clinicians of the incorporation of genetic tests into the diagnostic arsenal of several pathological conditions. This advance in the genetic determinants underlying inter-individual variability has been momentous for revolutionizing multiple scientific and clinical approaches in the sense that genetic determinants and personalized

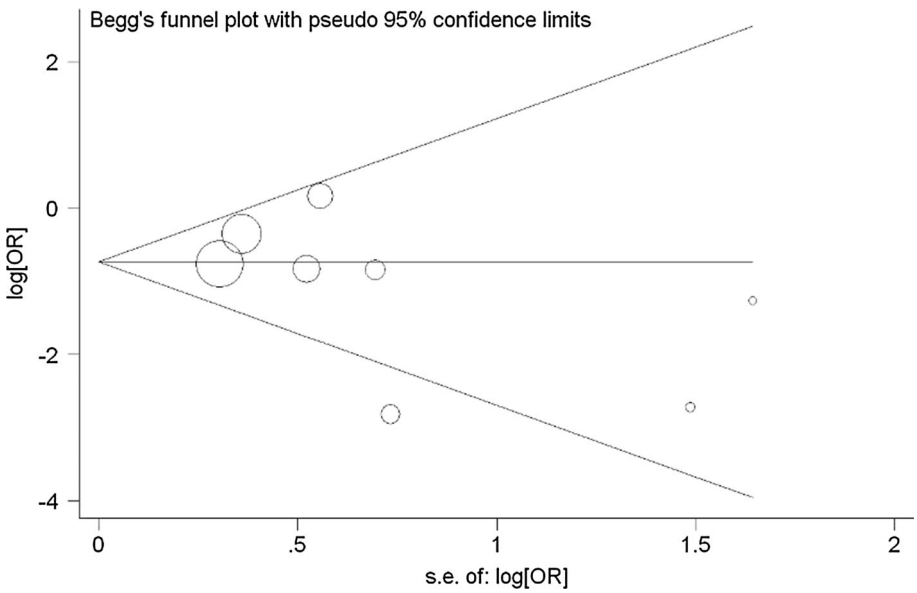

Fig. 5 Begg's funnel plot for publication bias test for (GG+GT vs. TT). Each *point* represents a separate study for the indicated association. Log [OR], natural logarithm of OR. *Horizontal line*, mean effect size

medicine have the potential for providing a beneficial end point for abnormal cases and diseases and for improving drug safety and efficacy. [7, 32] Identification of these genetic risk factors is expected to enhance our understanding of the molecular basis for ED. There is limited information about gene polymorphisms in patients with ED, and available data in this regard are conflicting.

The overall goal of meta-analysis is to combine the results of earlier studies to arrive at summary conclusions about a body of research. It is most useful in summarizing prior research when individual studies are small, and when they are too small to yield a valid conclusion. To the best of our knowledge, this is an updated meta-analysis to explore the association between *eNOS G894T* gene polymorphism and ED risk, involving about 1, 445 ED men and 1,459 healthy men. The main finding of this study is that GG genotype and/or G-allele in *eNOS* gene *894* site polymorphism could protect individuals against ED risk in Asians and Caucasians, but not mixed. *eNOS G894T* polymorphism occurs at different frequencies among various ethnic groups. This difference in distribution could contribute to the lack of well-replicated results across patient populations of different ethnicities. [33, 34] In the future, it could be improved by comparing the distribution of this polymorphism in larger cohorts of patients with ED versus controls across various ethnic backgrounds.

Several epidemiological studies have investigated the association between *eNOS* gene *G894T* polymorphism and ED, but the results were inconclusive. Eisenhardt et al. [20] reported that the risk to develop ED is not influenced by the genotypes in the *eNOS G894T* polymorphism, suggesting no relationship between this polymorphism and ED risk. Similarly, Andersen et al.[16] also reported that no statistically significant differences were found in the frequency of *eNOS G894T* polymorphism in ED patients vs controls. In contrast, the data of Lee et al. [21] showed a higher prevalence of the *G894T T*-allele in patients with ED than in the controls (OR 1.76, 95 % CI 1.11–2.80, $P < 0.05$), suggesting this polymorphism is a strong predictor of the predisposition to ED in addition to traditional risk factors. Moreover, according to the logistic regression analysis, patients with *eNOS 894 T*-allele have a 3.074-fold risk for ED (OR 3.07, 95 % CI 1.44–6.54) [7]. Our study showed similar conclusions with the above two studies.

We first analyzed the overall association between *eNOS* gene *G894T* polymorphism and ED: GG genotype or G-allele was a protect factor for ED. Because of the coupled and complementary association between G-allele and T-allele, in contrast, TT genotype and/or T-allele were risk factors of ED. Then, we analyzed the association stratified analysis by ethnicity, the similar conclusion was found in Asians and Caucasians. Finally, in case–control studies whose controls were from both HB and PB, significant association was also found. Our study may help scientists detect this SNP in *G894T* gene in healthy men and to find high-risk group of ED in the future.

We have put considerable efforts and resources into testing possible association between *G894T* gene polymorphism and ED risk, however, there are still some limitations. First, although we collected all the eligible studies, the sample size of the included studies was not large enough, which could increase the likelihood of type I and type II errors. Second, gene–gene and gene−environment interactions were not analyzed. It is possible that specific environmental and lifestyle factors may alter those associations between gene polymorphism and ED. Third,

the distribution of genotypes in the controls of one study [23] was not consistent with the HWE, which would affect the power of our conclusions.

Conclusion

In summary, our meta-analysis showed the evidence that *eNOS G894T* polymorphism was associated with significantly decreased risk for ED risk. To fully characterize the influence of the *G894T* polymorphism on the susceptibility of ED and to provide progress toward the understanding of the role of genetic factors in the physiopathology of this condition, further studies are needed in large, standardized, and ethnically diverse populations.

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Conflict of interest The authors have no conflicts of interest and received no additional funding for this study.

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