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The Endothelial Nitric Oxide Synthase Gene T-786C Polymorphism Increases Myocardial Infarction Risk: A Meta-Analysis

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Background: Polymorphisms of the endothelial nitric oxide synthase (eNOS) gene are reportedly associated with myocardial infarction (MI) risk. However, definitive evidence of this association is lacking. In this study, we investigated the potential association of eNOS gene polymorphisms with MI risk by conducting a meta-analysis of studies evaluating this association.


Material/Methods: PubMed, Web of Knowledge, ScienceDirect, China National Knowledge Infrastructure (CNKI), WanFang, and Database of Chinese Scientific and Technical Periodicals (VIP) were searched for relevant studies. Pooled odds ratios (OR) with 95% confidence interval (CI) were calculated to evaluate the association of eNOS gene T-786C and 4b4a polymorphisms with MI risk.

Results: Fifteen studies with 8,067 controls and 4,923 MI cases were included in the final meta-analysis. In the overall analysis, T-786C (rs2070744) polymorphism was associated with MI risk ($p < 0.05$, OR=1.69, 95% CI: 1.53–1.86 for T vs. C; $p < 0.05$, OR=2.76, 95% CI: 2.03–3.75 for TT vs. CC; $p < 0.05$, OR=1.74, 95% CI 1.56–1.95 for TT vs. (CT + CC); $p < 0.05$, OR=2.43, 95% CI: 1.79–3.30 for (CT + TT) vs. CC). In addition, a significant association between 4b4a VNTR polymorphism and MI risk was observed. On sub-group analyses by ethnicity, a significant increase in MI risk was observed separately for Asian and Caucasian populations for T-786C polymorphism, but not for the 4b4a polymorphism.

Conclusions: In this meta-analysis, T-786C polymorphism of the eNOS gene was associated with the risk of MI, especially in the Asian populations.

MeSH Keywords: **Myocardial Infarction • Nitric Oxide Synthase Type III • Polymorphism, Single Nucleotide**

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Background

Cardiovascular disease (CVD) is a major cause of death globally, with myocardial infarction (MI) accounting for a large proportion of CVD-related morbidity. MI refers to necrosis of cardiac myocytes due to blockage of coronary blood flow [1,2]. Although up to 90% of all MI patients are known to survive the first MI, mortality associated with re-infarction is very high. MI results from thrombus formation in coronary blood vessels. Such thrombus may cause a stable coronary atherosclerotic lesion to transform into a ruptured plaque [3]. The process of thrombus formation depends on a number of factors that include inflammatory cells, endothelium, endothelin, cytokines, metalloproteinases, cholesterol and platelets, T-lymphocytes, and phospholipase A2 [4]. A large number of activated inflammatory cells can be detected in the ruptured plaque lesions [5]. A previous study has documented that platelet-to-lymphocyte ratio might be a prognostic marker in patients with ST-elevated acute myocardial infarction (AMI) [6].

Cardiac troponin T (cTnT) or I (cTnI) are sensitive and cardio-specific diagnostic markers for MI [7]. Despite biotechnological advances, the currently known molecular markers are poor predictors of MI risk. Nitric oxide (NO) is a critical mediator of the cardiovascular system. NO plays an important role as an antiproliferative, antiplatelet, and antiatherogenic, and as an anti-inflammatory agent [8]. L-arginine is oxidized by the eNOS enzyme to produce L-citrulline and NO. The production of NO decreases after changing in the activity of eNOS. The eNOS gene is located on human chromosome 7q36 and consists of 26 exons with a size of approximately 21 kb. The polymorphisms in 4b4a VNTR of intron 4 and T-786C in the promoter region of the eNOS gene lead to common variants, which have an important effect on the expression of the eNOS gene [9]. The 4b4a variants of the eNOS gene are of two types: "a" allele (five tandem 27-bp repeats) and "b" allele (four tandem 27-bp repeats) [10]. The T-786C variant of the eNOS gene is located in the upstream position -786 [11]. Since disruption of eNOS function in mice can lead to the occurrence of MI, eNOS has been regarded as a risk factor for MI. In addition, several studies have found a significant association of eNOS T-786C and 4b4a polymorphisms with MI risk in humans. However, inconsistent results among different ethnic populations and the wide variability in study designs and sample sizes has, to a large extent, prevented any definitive evidence from being drawn. Hence, to determine a clear association between eNOS T-786C and 4b4a polymorphisms and MI risk, we collected relevant data from previous genetic studies for this meta-analysis.

Material and Methods

Literature search

A literature search for studies that had evaluated the association of eNOS T-786C and 4b4a polymorphisms with MI risk was conducted in PubMed, Web of Knowledge, ScienceDirect, CNKI, WanFang, and Database of Chinese Scientific and Technical Periodicals (VIP). The reference period for the literature search ended with June, 2015. The keywords used were "endothelial nitric oxide synthase gene", "Nitric Oxide Synthase Type III", "eNOS", "NOS3", "T-786C", "rs2070744", "4b4a", "4a/b", "Polymorphism, Genetic", "polymorphism", "polymorphisms", "Myocardial Infarction", "MI", "mutation" and "variant". All included studies had been approved by the Medical Ethics Committee.

Selection criteria

The inclusion criteria were: 1) case-control or cohort studies that evaluated the association of eNOS T-786C and 4b4a polymorphisms with MI risk; 2) available genotype data for eNOS T-786C and 4b4a variants.

Studies were excluded if they had any of the following attributes: 1) literature review and meta-analysis; 2) studies without raw genotype data or allele data; 3) genotype data in the control group deviated from the Hardy-Weinberg equilibrium (HWE); 4) lack of English and Chinese versions of the publication; 5) animal studies.

Quality score assessment

Two investigators independently assessed the quality of included studies using the Newcastle-Ottawa scale (NOS), which ranged between zero and nine stars. Studies with a score of ≥ 7 stars were considered to be of high quality.

Data extraction

Two investigators independently retrieved the articles and extracted relevant information on the basis of the PRISMA guide. This included the surname of the first author, year of publication, country of origin, ethnicity, number of cases/control subjects, frequencies of genotype in the case and control groups, genotyping methods used, study design (HCC, hospital-based case-control study; PCC, population-based case-control study), and disease subtype. Any discrepancy was discussed and resolved with a third investigator.

Statistical analysis

The software Stata 12.0 (Stata Corp LP, USA) was used to evaluate the association between eNOS gene T-786C and 4b4a

polymorphisms with MI risk. The odds ratios (ORs) and the 95% confidence interval (95% CI) were calculated under four genetic models: homozygous model, allele model, recessive model, and dominant model. Inter-group differences in the distribution of genotype frequency in cases and controls were assessed by the chi square (χ^2) test; $p < 0.05$ was considered to be statistically significant. χ^2 test based on Q statistics and I^2 test were calculated to assess heterogeneity. The value of $p < 0.05$ or $I^2 > 50\%$ was considered as indicative of significant heterogeneity. The random effect model (DerSimonian Laird method) was performed in case of significant heterogeneity, or the fixed effect model (Mantel-Haenszel method) was employed. Publication bias was evaluated with Begg's funnel plots; the symmetry of funnel plots was assessed using Egger's normal regression test. The stability of the results was assessed by sensitivity analysis by sequentially excluding one study at a time to observe the effect on the ORs. Any study in which the genotype frequencies in the control group did not conform to the HWE was excluded.

Results

Characteristics of included studies

Out of a total of 59 (five reviews, three letters, one case report and 50 clinical studies) publications retrieved on literature search, only 14 studies met the inclusion criteria and were included in the meta-analysis [12–25]. In the preliminary screening, 31 studies were excluded after review of full text, as these were unrelated to eNOS T-786C, 4b4a polymorphisms, or MI risk. Three publications were excluded because their language was not English or Chinese. Further, seven publications with no genotypic information about eNOS T-786C 4b4a polymorphisms were removed in the process of information extracting. Genotype data pertaining to the control groups was statistically analyzed with χ^2 test for HWE and 2 studies were excluded as they did not conform to HWE. Due to presence of two datasets in a clinical study, a total of 15 datasets with 8,067 controls and 4,923 cases were included in the meta-analysis. Among seven case-control studies for eNOS 4b4a polymorphism, two studies investigated Asians [12,13] and another two studies investigated Caucasian populations [14,15]. Of the seven publications about polymorphism of eNOS T-786C, five reports were on Asians [19–21,23,25] and two were on Caucasian populations [22,24]. In addition, the studies that investigated the association of AMI with eNOS T-786C and 4b4a polymorphisms were also included (Figure 1, Tables 1, 2).

Meta-analysis results

Eight studies were included to assess the association of eNOS T-786C polymorphism with risk of MI. Significant statistical

association was found between T-786C polymorphism and MI risk (T vs. C: OR=1.69, 95% CI: 1.53–1.86, $p < 0.05$; TT vs. CC: OR=2.76, 95% CI: 2.03–3.75, $p < 0.05$; TT vs. (CT+CC): OR=1.74, 95% CI: 1.56–1.95, $p < 0.05$; (CT+TT) vs. CC: OR=2.43, 95% CI: 1.79–3.30, $p < 0.05$). For the seven publications that investigated the association of eNOS 4b4a polymorphism with MI risk, significant association was found for the four genetic models in the overall meta-analysis (b vs. a: OR=1.23, 95% CI: 1.10–1.37, $p < 0.05$; bb vs. aa: OR=1.73, 95% CI: 1.17–2.56, $p < 0.05$; bb vs. (ab+aa): OR=1.66, 95% CI: 1.13–2.45, $p < 0.05$; (ab+bb) vs. aa: OR=1.22, 95% CI: 1.08–1.39, $p < 0.05$).

The subgroup analysis stratified the datasets by ethnicity, disease type, and study design type. A significant association of eNOS T-786C polymorphism with MI risk was observed for Asian and Caucasian populations. The same results were observed for the subgroup analysis when stratified by type of disease and study design. However, association of eNOS 4b4a polymorphism with MI risk was not statistically significant in both these populations. In subgroup analyses for 4b4a polymorphism, stratified by disease type and study design, a significant association was found only between MI and HCC (Tables 3, 4).

Heterogeneity, publication bias and sensitivity analysis

To check for heterogeneity among studies, the forest plot was used to show the values of I^2 and p . In the allele model and dominant genetic model for eNOS T-786C and 4b4a polymorphisms, significant heterogeneity was found and the random effect model was used. Further, stratified analysis was performed to understand the factors contributing to the heterogeneity. The results suggested that heterogeneity disappeared on stratified analysis based on ethnicity and study design type (Figures 2, 3).

The Begg's funnel plot and Egger's test were performed to assess any publication bias affecting the results of the meta-analysis. No publication bias was observed for analysis of eNOS 4b4a polymorphism, while significant publication bias existed for eNOS T-786C polymorphism. We had to perform subgroup analysis to eliminate the effect of publication bias. In the sensitivity analysis, the overall ORs did not change significantly on elimination of one study at a time from the analysis.

Discussion

Genetic polymorphisms of the eNOS gene are good candidates for predicting the risk associated with cardiovascular diseases such as coronary artery disease, atrial fibrillation, and essential hypertension [26–28]. eNOS G894T is one of the most common variants of eNOS, the others being T-786C and 4b4a mutations. A significant association of eNOS G894T polymorphism

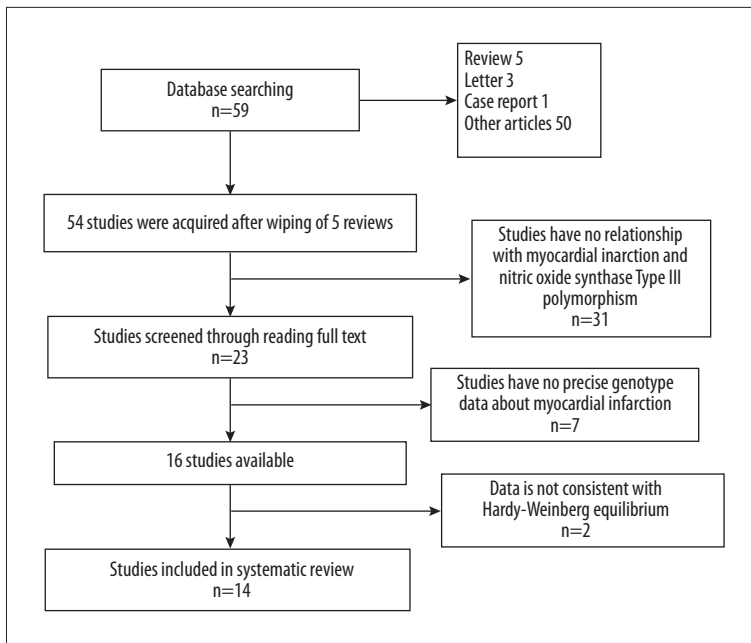


Figure 1. PRISMA flow chart.

Table 1. Characteristics of studies included in the meta-analysis for eNOS 4b4a polymorphism.

Studies	Nationality	Ethnicity	Disease	Control			Case			Control type	P for HWE	NOS score
				4b4b	4b4a	4a4a	4b4b	4b4a	4a4a			
Sahoko, 1998 [12]	Japan	Asians	MI	446	97	7	339	107	9	HB	0.51	8
Kiyoshi, 1998 [13]	Japan	Asians	AMI	284	68	5	174	48	4	HB	0.69	8
Andreas, 2002 [14]	Germany	Caucasians	MI	371	144	13	934	306	25	PB	0.83	7
Cine, 2002 [15]	Turkish	Caucasians	MI	149	55	2	143	55	9	PB	0.21	7
Sampaio, 2007 [16]	Brazil	Mixed	AMI	67	32	4	82	29	4	PB	0.94	8
Riadh, 2012 [17]	Tunisian	Mixed	MI	185	61	4	190	105	15	HB	0.69	7
Amani, 2013 [18]	Tunisian	Mixed	MI	164	58	3	187	101	15	HB	0.40	8

MI – myocardial infarction; AMI – acute myocardial infarction; HB – hospital-based controls; PB – population-based controls.

Table 2. Characteristics of studies included in the meta-analysis for eNOS T-786C polymorphism.

Studies	Nationality	Ethnicity	Disease	Control			Case			Control type	P for HWE	NOS score
				TT	TC	CC	TT	TC	CC			
Masafumi, 2000 [19]	Japan	Asians	MI	179	16	0	276	79	4	PB	0.55	8
Takagi, 2001 [20]	Japan	Asians	MI	3127	748	43	353	95	6	PB	0.82	7
Inho, 2006 [21]	Korea	Asians	MI	643	150	10	91	36	2	HB	0.71	7
Sampaio, 2007 [16]	Brazil	Americans	AMI	68	37	10	42	47	15	HB	0.14	7
Anna, 2009 [22]	Polish	Caucasians	MI	83	44	7	177	86	15	HB	0.71	7
Annan, 2012 [23]	India	Asians	AMI	202	105	14	164	112	11	PB	0.94	7
Aggeliki, 2013 [24]	Greece	Caucasians	MI	70	27	6	41	49	17	HB	0.14	7
Xu, 2013 [25]	Chinese	Asians	MI	209	46	4	234	82	8	HB	0.43	8

MI – myocardial infarction; AMI – acute myocardial infarction; HB – hospital-based controls; PB – population-based controls.

Table 3. Results of meta-analysis showing the association between eNOS T-786C polymorphism and MI risk.

	T vs. C		TT vs. CC		TT vs. (CT+CC)		(TT+CT) vs. CC	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Total	1.69 (1.53, 1.86)	<0.05	2.76 (2.03, 3.75)	<0.05	1.74 (1.56, 1.95)	<0.05	2.43 (1.79, 3.30)	<0.05
Caucasians	1.36 (1.03, 1.78)	<0.05	1.73 (0.88, 3.40)	0.11	1.40 (1.00, 1.95)	0.05	1.56 (0.80, 3.04)	0.19
Asians	1.48 (1.32, 1.66)	<0.05	1.70 (1.11, 2.61)	<0.05	1.56 (1.38, 1.77)	<0.05	1.54 (1.00, 2.35)	<0.05
AMI	1.33 (1.06, 1.66)	<0.05	1.42 (0.79, 2.55)	0.24	1.46 (1.11, 1.93)	<0.05	1.22 (0.69, 2.17)	0.49
MI	1.58 (1.41, 1.77)	<0.05	2.73 (1.90, 3.94)	<0.05	1.60 (1.41, 1.81)	<0.05	2.48 (1.73, 3.57)	<0.05
PCC	1.41 (1.23, 1.61)	<0.05	1.63 (0.98, 2.70)	0.06	1.47 (1.26, 1.71)	<0.05	1.50 (0.90, 2.48)	0.12
HCC	1.83 (1.57, 2.13)	<0.05	2.83 (1.85, 4.33)	<0.05	1.92 (1.61, 2.30)	<0.05	2.40 (1.57, 3.66)	<0.05

MI – myocardial infarction; AMI – acute myocardial infarction; HCC – hospital-based case-control study; PCC – population-based case-control study; OR – odds ratio; CI – confidence interval.

Table 4. Results of meta-analysis showing the association between eNOS 4b4a polymorphism and MI risk.

	b vs. a		bb vs. aa		bb vs. (ab+aa)		(bb+ab) vs. aa	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Total	1.23 (1.10, 1.37)	<0.05	1.73 (1.17, 2.56)	<0.05	1.66 (1.13, 2.45)	<0.05	1.22 (1.08, 1.39)	<0.05
Caucasians	1.32 (1.06, 1.64)	<0.05	1.54 (0.70, 3.41)	0.28	1.45 (0.66, 3.20)	0.35	1.35 (1.06, 1.72)	0.05
Asians	0.92 (0.78, 1.10)	0.37	1.09 (0.59, 2.03)	0.77	1.13 (0.61, 2.09)	0.69	0.89 (0.73, 1.09)	0.25
AMI	1.07 (0.80, 1.44)	0.64	1.22 (0.46, 3.20)	0.69	1.20 (0.46, 3.15)	0.71	1.07 (0.77, 1.48)	0.69
MI	1.24 (1.10, 1.40)	<0.05	1.85 (1.19, 2.86)	<0.05	1.77 (1.14, 2.73)	<0.05	1.23 (1.08, 1.42)	<0.05
PCC	0.90 (0.76, 1.06)	0.20	1.01 (0.58, 1.77)	0.96	1.06 (0.61, 1.84)	0.85	0.87 (0.72, 1.04)	0.13
HCC	1.58 (1.36, 1.85)	<0.05	2.74 (1.59, 4.74)	<0.05	2.47 (1.43, 4.25)	<0.05	1.62 (1.36, 1.92)	<0.05

MI – myocardial infarction; AMI – acute myocardial infarction; HCC – hospital-based case-control study; PCC – population-based case-control study; OR – odds ratio; CI – confidence interval.

with MI risk in Asian populations has been reported earlier [29], which is consistent with our findings.

To the best of our knowledge, this is the first meta-analysis to evaluate the association of eNOS T-786C and 4b4a polymorphisms with MI risk. We eliminated a study due to publication bias in order to decrease the heterogeneity and to ensure the accuracy of results [18]. In the overall analysis, both eNOS gene variants T-786C and 4b4a were significantly associated with MI risk. In order to decrease the heterogeneity present in different studies, we conducted subgroup analysis in Asian and Caucasian populations. The success of this approach suggested that the genotype data of different ethnicities cannot be combined for statistical analysis. A significant association was observed for T-786C polymorphism in every genetic model of Asian populations. The same results were not detected in Caucasian populations. Thus, differences in

ethnicity might be a reason for differences in the MI risk association. The stratified analysis of disease type and study design type showed a significant association between eNOS T-786C polymorphism and MI risk. According to the results of the funnel plots and subgroup analysis, the presence of ethnicity might lead to the discrepancy in results of association for T-786C as compared to the study design type in this meta-analysis. As a consequence, we separately analyzed the data of T-786C polymorphism in different ethnicities.

Although a significant association was observed between eNOS 4b4a polymorphism and MI risk, significant heterogeneity and publication bias existed with respect to the allele model and dominant genetic model. On subgroup analysis, a significant association was found only in the subgroup of HCC and MI. Further, the small sample size in the meta-analysis also limited the statistical power.

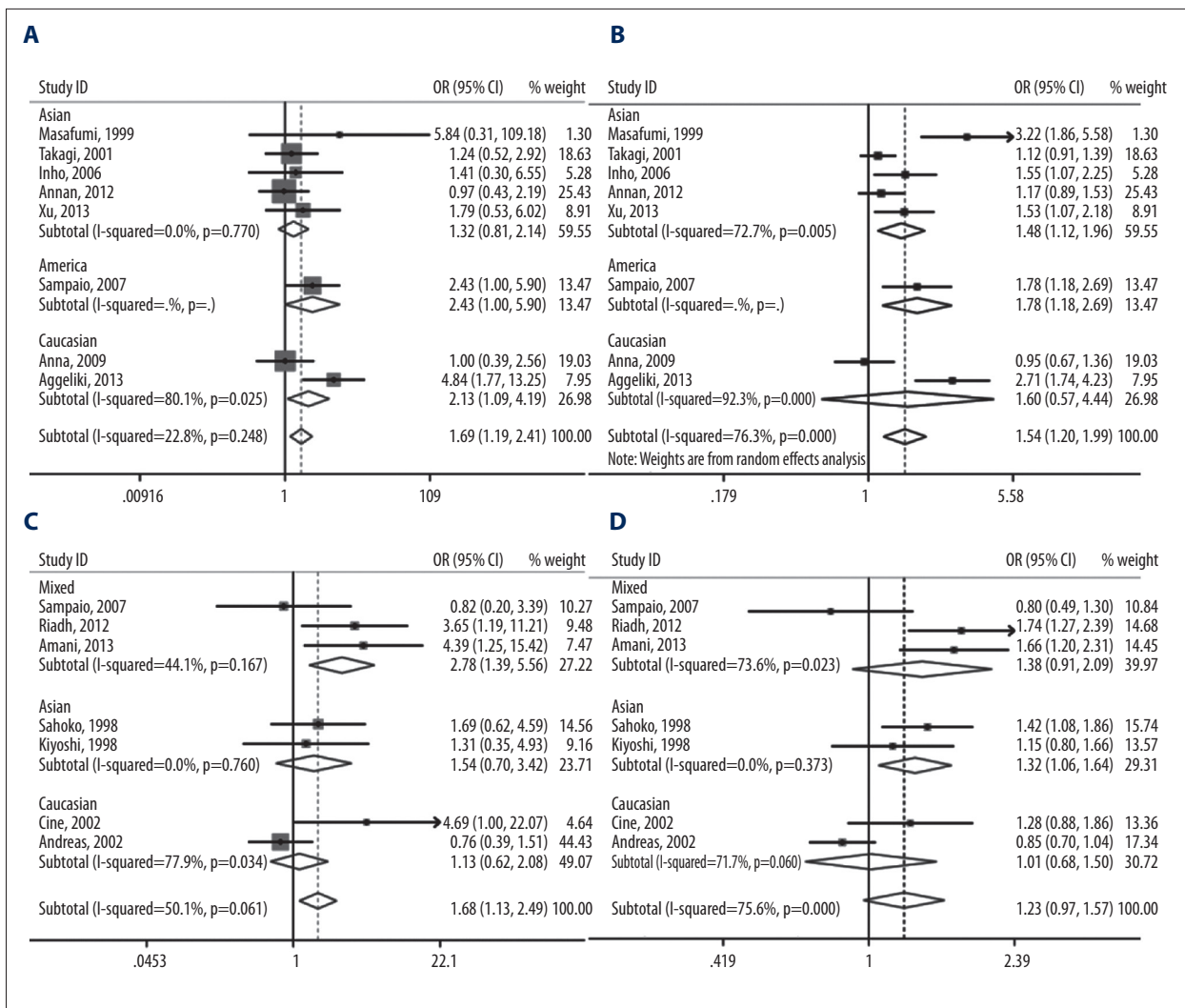


Figure 2. Meta-analysis for the association between susceptibility to myocardial infarction and eNOS T-786C 4b4a polymorphisms. (A) TT vs. CC; (B) T vs. C; (C) 4bb vs. 4aa; (D) 4b vs. 4a.

The results indicate that subgroup analysis based on ethnicity and disease type was necessary for the meta-analysis, especially when the datasets contained heterogeneity and publication bias. From the previous studies, a significant association between both eNOS T-786C and 4b4a polymorphisms with MI risk was not determined. For instance, five studies included in this meta-analysis indicated a significant association of eNOS T-786C polymorphism with MI risk [16,19,21,24,25], and four studies revealed significant association of eNOS 4b4a polymorphism with MI risk [12,15,17,18]. In these studies concerning eNOS T-786C polymorphism, the study populations included Americans, Asians, and Caucasians, and a significant correlation of eNOS T-786C polymorphism with MI risk was found. But the age distribution in the case and control groups had significant differences in some studies [16,21]. In addition, the difference in gender ratio, BMI, hypertension, and smoking were other factors which affected the association between gene polymorphism and MI risk.

On the other hand, the studies of Takagi et al., Inho et al., and Annan et al. did not show any significant association between eNOS T-786C polymorphism and MI risk [20,22,23]. The populations of these three studies were from Japan, Poland, and India. Masafumi et al. conducted their study on the Japanese population, but reached a different conclusion in which eNOS T-786C polymorphism was significantly associated with MI risk [19]. Although the results have been controversial, several clinical studies have reported a significant association of T-786C polymorphism with coronary spasm, coronary artery disease, and stenosis [30–32]. We also found an inconsistency in our results, where the T allele of T-786C appeared to protect against MI, and the carrier of CC was more likely to suffer from MI. Furthermore, the eNOS 4b4a polymorphism has been reported as being associated with MI risk due to the presence of 4a mutation. However, heterogeneity among studies of eNOS 4b4a polymorphism might limit the accuracy of the results.

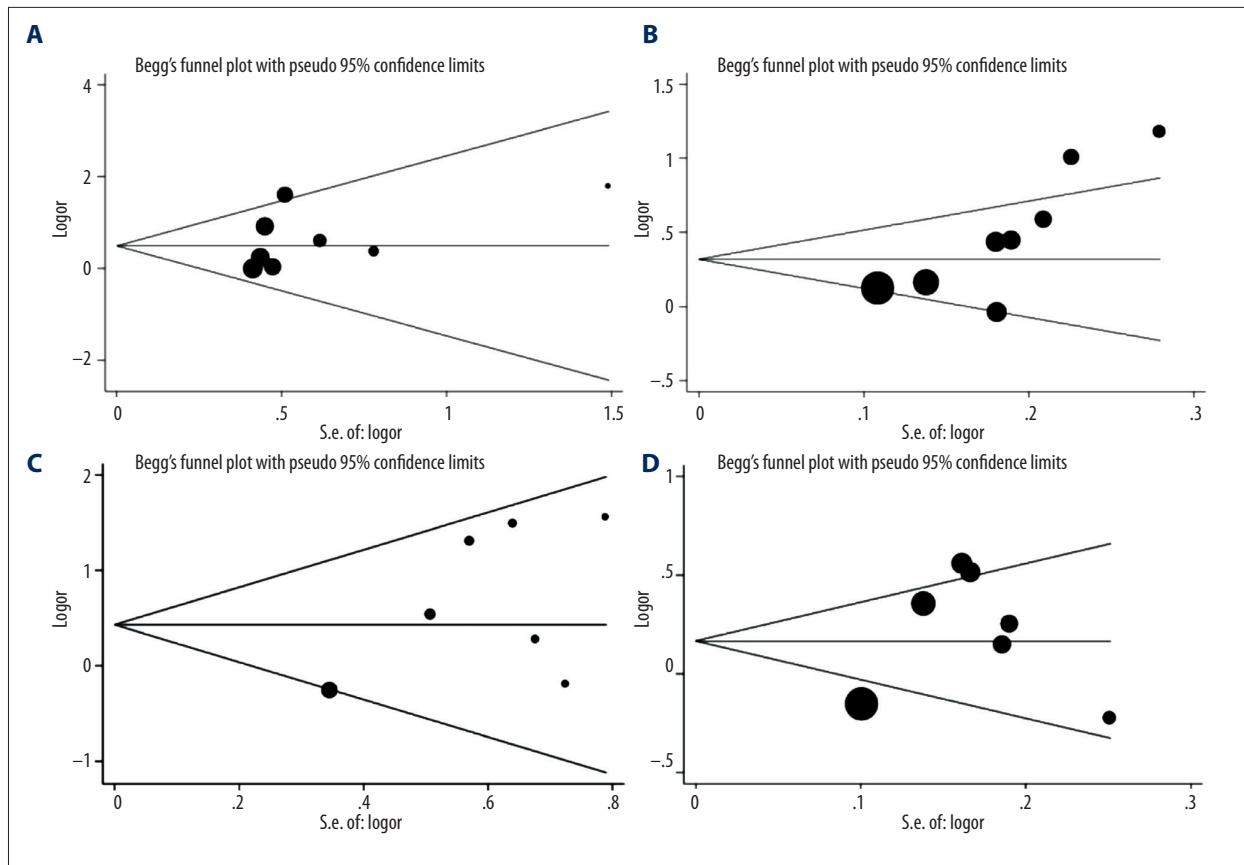


Figure 3. Begg's funnel plot for publication bias of eNOS T-786C 4b4a polymorphisms and myocardial infarction risk. (A) TT vs. CC; (B) T vs. C; (C) 4bb vs. 4aa; (D) 4b vs. 4a.

In addition, MI risk is known to be affected by genetic diversity and factors such as age, smoking, hypertension, diabetes, etc. The gene-gene and gene-environment interactions could have a great influence on MI risk in particular. The reasons for differences in results among ethnicity might be due to differences in exposure to various environmental factors [33]. The clinical information in this meta-analysis has been collected but could not be statistically analyzed because of the prevalence of large heterogeneity. Furthermore, the studies included in the meta-analysis involved confounding factors such as smoking and AMI, but we believe the large sample sizes could offset the impacts for the association of eNOS T-786C polymorphism with MI risk [20,21,23]. Therefore, large population studies with sufficient clinical information are needed to verify these results.

The key limitations of the present meta-analysis include presence of significant heterogeneity, publication bias, and small sample sizes. The sources of heterogeneity might be age,

smoking, ethnicity, sample size, and country. Analysis of clinical data and environmental influences appear to be the key considerations for polymorphism studies. Moreover, the meta-analysis indicated a significant association between T-786C polymorphism and MI risk but could not explain the existence of this causal relationship. Hence, gene functional studies are required to be conducted.

Conclusions

This meta-analysis suggests that eNOS T-786C polymorphism might be a high risk factor for MI, especially in Asian populations. Further research needs to be conducted on the potential association of eNOS 4b4a polymorphism with risk of MI.

Declaration of interest

None.

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