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Meta-Analysis

Endothelial nitric oxide synthase 27VNTR (4b/4a) gene polymorphism and the risk of diabetic microvascular complications in Chinese populations

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Abstract: To access the association of endothelial nitric oxide synthase (eNOS) gene 27VNTR (4b/4a) polymorphism with diabetic microvascular complications (DMI) susceptibility in Chinese populations. Data were retrieved in a systematic manner on PubMed database, ISI Web of Knowledge, the Cochrane Library, Chinese National Knowledge Infrastructure, and Wanfang, as well as manual searching of the references of the identified articles. Odds ratio (OR) and 95% confidence interval were used to evaluate the strength of associations. Potential sources of heterogeneity and publication bias were explored. Twelve published articles with thirteen outcomes including 1484 DMI patients and 1225 diabetic controls were included in the meta-analysis. The results showed no evidence for significant association of 27VNTR (4b/4a) polymorphism with DMI risk in dominant model (OR=1.36, 95% CI=0.90–2.06, *P*=0.15), and similar results were obtained in the allelic, additive and the recessive models (*P*>0.05). Our meta-analysis suggests that eNOS gene 27VNTR (4b/4a) polymorphism might not be a risk factor for DMI in Chinese populations.

Key words: Endothelial nitric oxide synthase; Gene polymorphism; Diabetic microvascular complications.

Introduction

Diabetic microvascular complications (DMI), mainly including diabetic nephropathy (DN) and diabetic retinopathy (DR), have become leading causes of end-stage renal disease and blindness(1). Despite it is well established that duration of diabetes, chronic elevation of blood glucose levels are associated with increased risk of DMI(2, 3), the fact that some individuals develop DMI while others do not under similar environmental exposures suggests that genetic predisposition plays an important role in the pathogenesis of DMI.

Nitric oxide (NO) as a ubiquitous vascular active substance protects vascular endothelium from damage and plays a significant role in renal and retinal vascular function(4, 5). Reduced NO level induces endothelial dysfunction which has been shown to be an important pathophysiologic denominator for DN and DR(6). Endothelial nitric oxide synthase (eNOS) encoded by eNOS gene located on the chromosome 7q35-7q36 is one of the key enzyme in the process of NO synthesis(7). In the 27-bp variable number of tandem repeats (27VNTR) of intron 4, two alleles (mutated, 4a; wild type, 4b) have been identified, and study has shown that this polymorphism may have a certain degree of influence on eNOS expression and activity and further affect the generation of NO(8). Given the suggestion, we hypothesized that this genetic polymorphism may have some relation to DMI.

Studies on the association between 27VNTR (4b/4a) polymorphism in eNOS gene and DMI have been extensively performed in different countries, but the results are still disputable. Considering that potential ethnic difference on the distribution of genotypes and every single study may be inadequate in achieving a comprehensive and reliable conclusion, a meta-analysis therefore been performed to better address the association between 27VNTR polymorphisms and DMI risk in Chinese population.

Materials and Methods

Search strategy

A comprehensive literature research was conducted using research terms ''Endothelial nitric oxide synthase'' or "eNOS, "diabetic retinopathy or DR or diabetic nephropathy or DN or diabetic microvascular complication or DMI" and "gene or polymorphism" in various combinations, with the language limited to English and Chinese. The PubMed database, ISI Web of Knowledge (version 4.5), the Cochrane Library, Chinese National Knowledge Infrastructure, and Wanfang (Chinese) were explored independently by two authors. Reference lists in retrieved articles were also screened. The literature search was updated on April, 2014.

Inclusion and exclusion criteria

The following criteria were used to select the eligible studies: 1) case–control or cohort studies on the association between eNOS 27VNTR (4b/4a) polymorphism and DMI including DR and DN in Chinese population; 2) cases were diabetic patients with DMI, and the controls were diabetic patient without DMI; 3) the numbers of cases and controls reported for each genotype

should be sufficient for calculation of odds ratio (OR) with 95% confidence interval (CI). If multiple studies from the same case series were available, only the study with the latest data was included. Meeting abstracts, case reports, review articles, reports with incomplete data, and studies not written in English or Chinese were excluded.

Data extraction

For each eligible study, the following information was recorded: name of the first author, year of publication, region, sample sizes of cases and controls, genotype distributions of cases and controls, and Hardy-Weinberg equilibrium (HWE) in each control group. Any disagreement between our two authors was resolved by consulting the third.

Statistical analysis

The eNOS 27VNTR (4b/4a) genotypes include 4a/4a, 4a/4b and 4b/4b. The pooled odds ratios (ORs) were calculated for the dominant model [(4a/4a + 4a/4b) *vs*. 4b/4b], the allelic model (4a *vs.* 4b), the additive model (4a/4a *vs.* 4b/4b), and the recessive model [(4a/4a *vs.* (4a/4b + 4b/4b)].

The strength of the relationship between eNOS 27VNTR (4b/4a) polymorphism and DMI risk was assessed by calculating pooled ORs with 95% CIs. The fixed-effects model was used in the absence of between-study heterogeneity; otherwise, a random effects model was adopted. Heterogeneity was assessed by the Q-test and I^2 statistic, statistically significant heterogeneity was considered to be present when $P < 0.05$ and $I^2 > 50\%$. Sensitivity analysis by omitting one study at a time was carried out to assess the stability of the meta-analysis. A funnel plot was performed to look for evidence of publication bias; the funnel plot should be asymmetric when there is publication bias and symmetric in the case of no publication bias. All above statistical analyses were performed using Review Manager (Version 5.1; Cochrane Collaboration, 2011).

Results

Study characteristics

Figure 1 outlines selection process of eligible studies. After the literature searching and the subsequent screening, 13 case–control or cohort studies from 12 qualified papers including 1484 DMI patients and 1225 diabetic controls were included in this meta-analysis(5, 9-19). The distribution of genotypes among controls was consistent with HWE in all but three studies(5, 12, 14). The characteristics of the included studies are listed in Tables 1.

Meta-analysis results

Results of meta-analysis were not showed of any significant relation between eNOS 27VNTR (4b/4a)

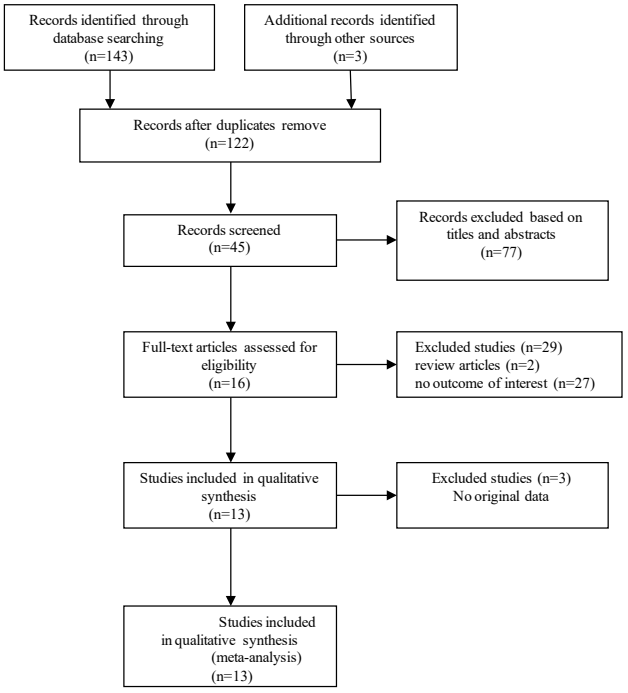

Figure 1. Flowchart of the literature selection process.

Table 1. Characteristics of eNOS 27VNTR(4b/4a) polymorphism genotype distributions for DMI risk in studies included in the meta-analysis.

Study	Geographical location	Case type	Sample size		Case			Control			P_{HWE}
			Case	Control	4b/4b	4a/4b	4a/4a	4b/4b	4a/4b	4a/4a	
Dong et al. 2007①	Shandong	DN	45	42	42	3	0	30	12	0	0.28
Dong et al. 2007②	Singapore Chinese	DN	85	65	66	14	3	56	9	0	0.55
Zhang et al. 2005①	Kunming	DN	180	154	159	21	0	131	21	2	0.29
Xing et al. 2004	Dalian	DN	130	136	110	16	4	128	8	0	0.72
Sun et al. 2004①	Hefei	DN	188	114	136	48	4	101	11	2	0.02
Huang et al. 2003	Fuzhou	DN	141	61	108	33		53	8		>0.05
Luo et al. 2003	Lanzhou	DN	49	35	20	2	27	23	4	8	<0.05
Lin et al. 2002	Tianjin	DN	80	48	71	8	0	41	6	1	0.21
Li et al. 2001	Qingdao	DN	79	64	50	29	0	53	11	0	0.45
Yue et al. 2011	Beijing	DR	154	167	125	27	2	118	45	4	0.91
Li et al. 2010	Beijing	DR	87	79	69	13	5	61	11	7	<0.05
Zhang et al .2005②	Kunming	DR	119	105	108	11	0	98	7	0	0.72
Sun et al. 2004②	Hefei	DR	147	155	108	36	3	133	21	1	0.86

DMI, diabetic microvascular complications; DN, diabetic nephropathy; DR, diabetic retinopathy; NA, not available; PHWE, P value of Hardy-Weinberg equilibrium in control.

polymorphism and the risk of DMI in Chinese populations under any genetic models: dominant model: OR=1.36, 95% CI=0.90–2.06, $P_{heterpgeneity}$<0.0001, Figure 1; allelic model: OR=1.23, 95% CI=0.76–1.99, $P_{heterpgeneity}$<0.00001, Figure 2; additive model:OR=1.58, 95% CI=0.95–2.64, $P_{heterpgeneity}$=0.08, Figure 3; recessive model: OR=1.60, 95% CI=0.96–2.66, $P_{heterpgeneity}$=0.08, Figure 4. After excluding three studies that were not in HWE with the controls, the results remained unchanged (Table2).

Sensitivity analysis

Sensitivity analysis was performed by excluding one study at a time to assess the stability of the meta-analysis in dominant and allelic models which with significant heterpgeneity. After excluding study one by one, the variation ranges of heterogeneity were 64-75% in dominant model and 78-85% in allelic model which suggested that the results of our study are stable and reliable.

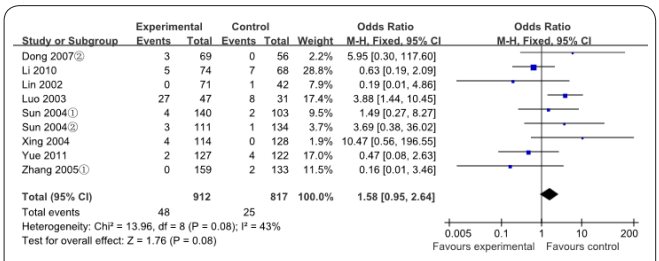

Figure 2. Result of the association between eNOS 27VNTR (4b/4a) polymorphism and DMI susceptibility in dominant model. Our report indicated that no significant association was found.

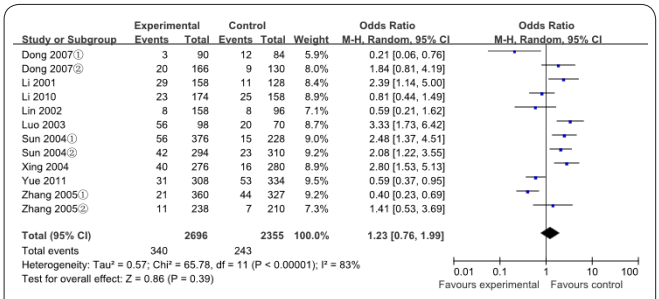

Figure 3. Result of the association between eNOS 27VNTR (4b/4a) polymorphism and DMI susceptibility in allelic model. No positive result was detected in this analysis.

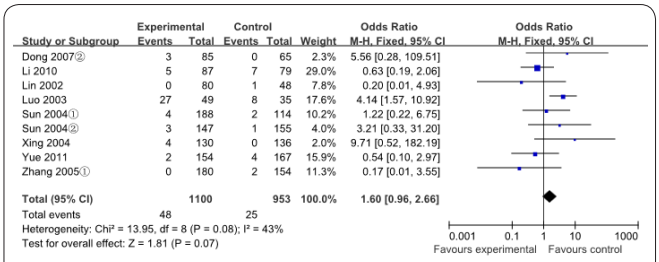

Figure 4. Result of the association between eNOS 27VNTR (4b/4a) polymorphism and DMI susceptibility in additive model. Our result showed that no positive result was detected.

Publication bias evaluation

Publication bias was assayed by visual funnel plot inspection. The shapes of funnel plots were basically symmetric which suggesting no evidence of publication bias among the studies (Figure 5, 6 and 7).

Discussion

The association between eNOS 27VNTR (4b/4a) polymorphism and the risk of DMI has been widely studied in different ethnic groups. However, the conclusion was far from certain in Chinese population because of inconsistent findings and the limited sample size of pre-

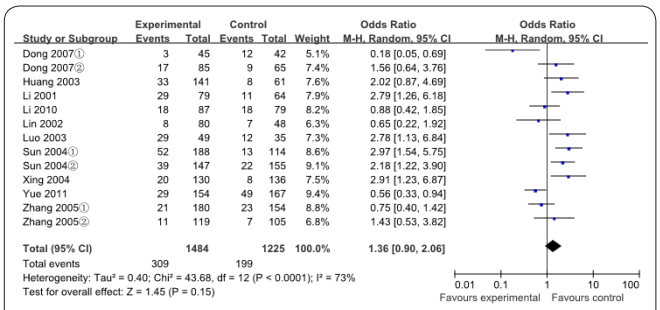

Figure 5. Result of the association between eNOS 27VNTR (4b/4a) polymorphism and DMI susceptibility in recessive model. Our result showed that no positive result was detected.

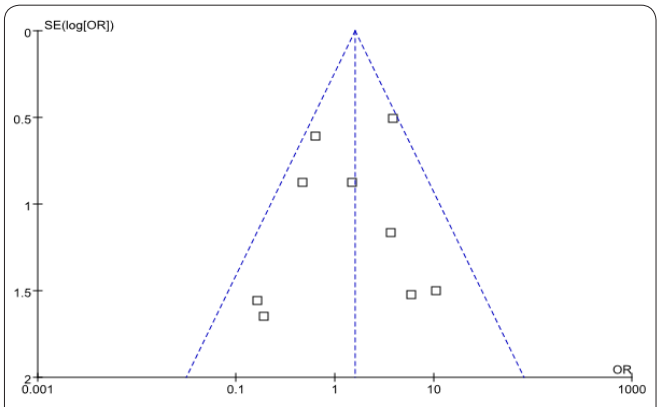

Figure 6. Funnel plot of the evaluation of publication bias in dominant model. The funnel plot should be asymmetric when publication bias exists. No significant publication bias was found in this meta-analysis.

Table 2. Association results of meta-analysis.

Comparisons	Variables	OR (95% CI)	P_{value}	Heterogeneity	
				I^2(%)	P value
4a/4a+4a/4b vs.4b/4b	Totel	1.36 [0.90, 2.06]	0.15	73	<0.0001
	HWE	1.21 [0.75, 1.97]	0.44	73	0.0001
4a vs.4b	Totel	1.23 [0.76, 1.99]	0.39	83	< 0.00001
	HWE	1.06 [0.59, 1.88]	0.85	83	< 0.00001
4a/4a vs.4b/4b	Total	1.58 [0.95, 2.64]	0.08	43	0.08
	HWE	1.32 [0.59, 2.96]	0.5	39	0.14
4a/4a vs. 4a/4b+4b/4b	Total	1.60 [0.96, 2.66]	0.07	43	0.08
	HWE	1.31 [0.58, 2.96]	0.51	33	0.19

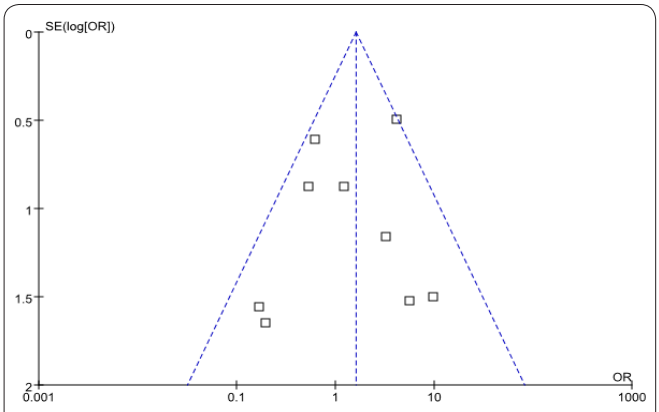

Figure 7. Funnel plot of the evaluation of publication bias in additive model. The funnel plot should be asymmetric when publication bias exists. No significant publication bias was found in this meta-analysis.

vious studies. Here, we employed a meta-analysis to improve statistical power by pooling the related samples.

In this meta-analysis, we summarize the results of 12 case-control studies with 13 eligible outcomes published so far on the association between eNOS gene 27VNTR (4b/4a) polymorphisms and DMI susceptibility in Chinese populations. The results did not detect any relation between eNOS 27VNTR (4b/4a) polymorphism and DMI risk in any genetic models. This negative result may be caused by: 1) the variant of 27VNTR (4b/4a) located in the intron area of eNOS gene which belonging non-coding location; 2) the variant may not a dominant genetic risk, and it has a pathogenic effect on DMI through a interrelated with environmental factors and other discrete loci involved in the occurrence of DMI; 3) the sample sizes included in our meta-analysis are still small, and can't reflect the real relationship between eNOS 27VNTR (4b/4a) polymorphism and DMI risk. However, studies have shown that this variant contributes to the basal levels of plasma NO and produces certain effect to endothelial function(20, 21), moreover, some meta-analysis published previous have provided the evidence of the association between eNOS 27VNTR (4b/4a) polymorphism and DN in Asian populations, DR in African individuals. Thus, we can't exclude the possibility of 4b/a variant in 27VNTR as a functional significant factor to DMI risk.

Significant between-study heterogeneity was found in dominant and allelic models, but no heterogeneity exists in additive and recessive models. The heterogeneity might arise from an indeterminate number of characteristics that vary among studies, such as study quality, characteristics of the subjects, genotyping, clinical heterogeneity (diagnosis for DMI patients) and lifestyle factors etc. could not be ruled out. To clarify the sources of heterogeneity, we conducted a sensitivity analysis and this analysis confirmed the stability of the null association after excluding any one study at a time.

In this meta-analysis, no significant publication bias for 27VNTR (4b/4a) polymorphisms in any of the above-mentioned inherited models, suggesting the associations observed should be stable.

In conclusion, our meta-analysis did not detect any association between eNOS 27VNTR (4b/4a) polymorphism and DMI susceptibility in Chinese populations. The present study is limited by we could not completely preclude the potential biases and confounders in meta-analysis. Further research is warranted to confirm our findings. Further well-designed studies with large sample size are warranted to examine the function of this gene as well as their interactions with other genetic and environmental factors in the development DMI.

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Author's contribution

Involved in design and conduct of study (X.Z., YZ.W., Y.Q.); collection and management of the data (X.Z., YZ.W.); analysis and interpretation of the data (X.Z.); preparation the manuscript (X.Z., Y.Q.); review and approval of the manuscript (X.Z., Y.Q.).

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