Lack of association between endothelial nitric oxide synthase gene polymorphisms with vasculitis: a meta-analysis

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ABSTRACT

Objective. *We carried out this meta-analysis to evaluate the relationship between eNOS polymorphisms (G894T, T-786C, and intron-4ba) and vasculitis.*

Methods. *We systematically searched PubMed, EMBASE and the Cochrane Library for related genetic association studies. The associations between the G894T, T-786C and intron 4ba polymorphisms of eNOS and vasculitis were conducted using the recessive model and the dominant model. Odds ratio (OR) with 95% confidence interval (CI) of each study were calculated. Cochran's Q test was used to evaluate the between-study heterogeneity.*

Results. *A total of 17 studies were included in our study. Twelve studies with 1213 cases and 1499 controls were included in the G894T association study. The pooled OR of T allele compared to C allele in recessive model was 1.19 (95%CI: 0.76–1.87, p=0.44) in dominant model and was 1.25 (95%CI: 0.70–2.23, p=0.56) in recessive model, respectively. Nine studies with 910 cases and 1062 controls were included in the intron -4ba association study. The pooled OR of b allele compared with intron-4a allele was 1.02 (95%CI: 0.60–1.72, p=0.95) in dominant model and was 0.84 (95%CI: 0.58–1.21, p=0.35) in recessive model. No association was found between T-786C and vasculitis in both the dominant 0.81(95% CI: 0.59–1.11, p=0.19) and recessive model 0.87 (95%CI: 0.55–1.36, p=0.53).*

Conclusion. *The eNOS G894T, T-786C and intron4ba polymorphisms are not associated with vasculitis.*

Introduction

Vasculitis is not referred to a particular disease but rather a group of disease that trigger inflammatory response in large, medium, and small vessels. Vasculitis is usually characterised by inflammation and destruction of blood vessels, leading to endothelial cell activation and ischaemia of dependent tissue (1). Though the pathogenesis of vasculitis is poorly understood, genetic factors have been implicated in the pathogenesis of systemic vasculitis (2) and have even been considered to be a determinant of these diseases (3-5).

NO is produced during the conversion of L-arginine to L-citrulline by different isoforms of NO synthases (NOS). The decrease of NO concentrations could reflect reduced eNOS expression and bioavailability as a consequence of decreased endothelial cell survival and endothelial dysfunction (6). NO is closely related to inflammatory status and regarded as a key inflammation mediator (7). In vasculitis patients, vascular endothelial function is impaired (8), moreover, diminished NO levels were also observed (9). However, conflicting associations between NO concentration levels and vasculitis have also been described. In the study by Iscan *et al*., NO levels in BD patients were demonstrated to be significantly higher than those of healthy subjects (10). One reason for increased NO production is speculated to be that of inflammatory processes which function as a stimuli of NO production. Endothelial nitric oxide synthase (eNOS) is a constitutive enzyme expressed in endothelial cells. Combined with genetic mechanisms of vasculitis pathogenesis, many researches have attempted to discover the association between eNOS polymorphisms and vasculitis. However, the results have been inconsistent. The aim of our present study is to explore whether the eNOS polymorphisms have association with the development of vasculitis using meta-analysis.

Methods

Literature search strategy

We searched PUBMED, EMBASE and the Cochrane Library from Sep-

tember 3, 2014 for relevant available articles, using key words: ("Nitric Oxide Synthase Type III"[Majr]) AND "Vasculitis"[Majr], "endothelial nitric oxide", "vasculitis", combined with "polymorphism". No language restrictions were applied. References lists of relevant papers were also screened.

Study selection criteria

Two investigators independently applied the selection criteria to each reference identified by the search strategy. A third reviewer resolved any discrepancies regarding study eligibility or quality. The inclusion criteria are as follows: (1) Case-control or cohort study of unrelated individuals, (2) Both case and control groups had to come from the same area, (3) The genotypes and allele frequencies of cases and controls were available and genotype frequencies in control groups were within Hardy-Weinberg equilibrium and (4) Two investigators independently extracted data. The following information was collected: year of publication, ethnic of the studied population, genotype frequencies, male percentage, mean ages of case and control groups.

Statistical analysis

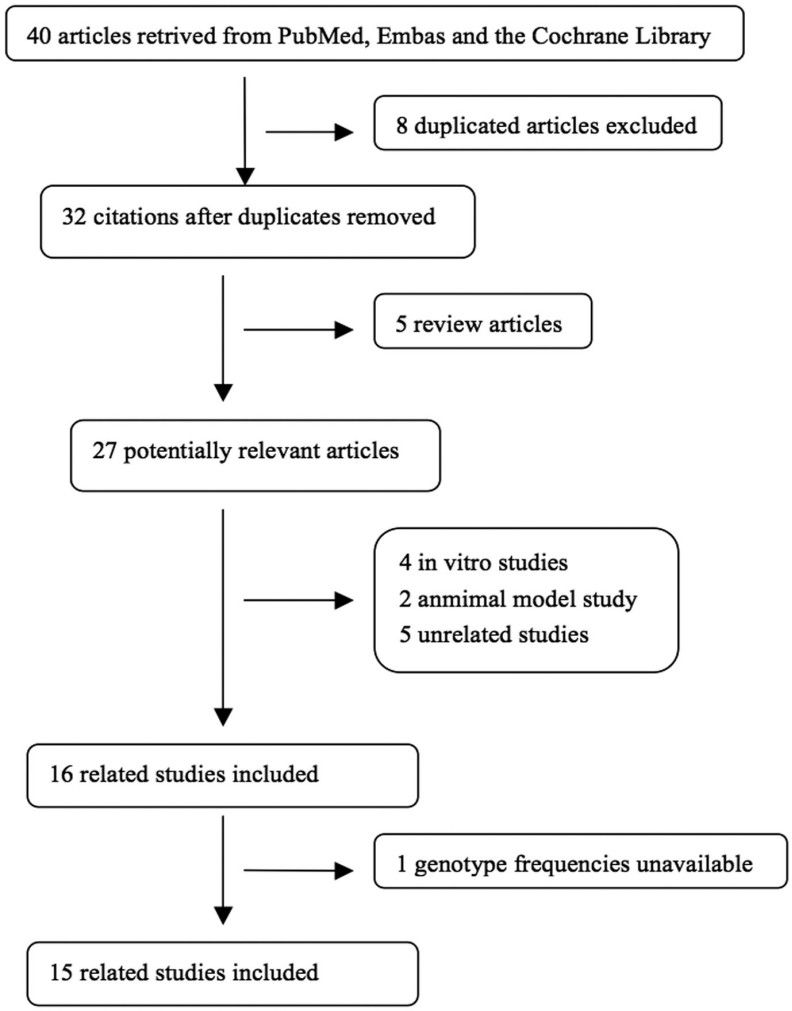
Odds ratio (OR) with 95% confidence interval (CI) of each study were calculated. We used Cochran's Q test to evaluate the between-study heterogeneity. I^2 <50% indicates that studies were homogeneous and fixed effects model (FEM) was used. Otherwise, random effects model (REM) was used. Funnel plots and Egger regression test were used to assess publication bias. Analysis was done by using REVMAN software (version 5.0; Cochrane Collaboration, Oxford, UK) and Stata software (version 9.0; Stata Corporation, College Station, TX). Two tailed p<0.05 was considered statistically significant.

Results

Studies included in the meta-analysis

A systematic search was concluded in PUBMED, EMBASE and the Cochrane Library using the search strategy. A total of 40 articles were retrieved, and 16 potential related articles obtained, however, one study was excluded for the unavailable of genotype frequencies (11). Finally, 15 studies were included. Table I shows the flow chart of candidate and eligible papers selecting. Table II describes the characteristics of studies.

Table I. Detailed procedure of study selection in the meta-analysis.



Meta-analysis of relationships between vasculitis and the eNOS G894T, T-786C and intron-4ba polymorphisms and vasculitis

There are 13 studies (12-24) with 1213 cases and 1499 controls included in the analysis of the G894T polymorphism and vasculitis. The pooled OR of C allele compared with G allele in G894T was 1.19 (95%CI: 0.76–1.87, p=0.44) in dominant model (Fig. 1) and 1.25 (95%CI: 0.7–2.23, p=0.46) in recessive model (Fig. 2). No association was found between vasculitis and the eNOS G894T polymorphism using the recessive or dominant models.

Nine studies with 910 cases and 1062 controls were included in the analysis of intron-4ba polymorphism and vasculitis (13, 14, 17, 19, 20, 23-26). The pooled OR of 4a compared with 4b allele was 1.02 (95%CI: 0.60–1.72, p=0.95) in the dominant model (Fig. 3) and 0.84 (95%CI: 0.58–1.2, p=0.35) in the recessive model (Fig. 4). Meta-analysis showed no association between vasculitis and the eNOS intron-4ba polymorphism using the recessive or dominant models.

Four studies with 373 cases and 443 controls, were enrolled in the analysis of T-786C polymorphism and vasculitis (13, 14, 19, 21). The pooled OR of T compared with C allele was 0.81(95% CI: 0.59-1.11, p=0.19) in the dominant model (Fig. 5) and 0.87 (95%CI: 0.55-1.36, p=0.53) in the recessive model (Fig. 6). No association was found between vasculitis and eNOS T-786C

Table II. Characteristics of studies included in the meta-analysis.

First author	Year of publication	Ethnicity	Disease	Age	Gender case (M/F)/ con (M/F)	Sample size case *vs*. con	G894T case *vs*. con (ww/ht/vv)	T-786C case *vs*. con (ww/ht/vv)	intron 4ba case *vs*. con (ww/ht/vv)
Di B	2012	Chinese	HSPN	NM*	NM*	NE	0/0/160 *vs*. 3/55/79	NE	43/52/65 *vs*. 16/61/60
Adigüzel Y	2010	Trukese	TAO	30.3/34.5	NM	58/102	25/28/5 *vs*. 24/39/39	NE	NE
Dursun A	2009	Trukese	BD	NM*	(36/37) *vs*. (47/43)*	73/90	NE	NE	48/23/2 *vs*. 75/15/0
Ben Dhifallah	2008	Tunisian	BD	39.5/41.3*	(93/42) *vs*. NM*	135/157	45/58/32 *vs*. 37/71/49	NE	NE
Oksel F	2006	Trukese	BD	38.3/NM	(73/59) *vs*. NM	132/91	44/36/20 *vs*. 65/29/6	NE	NE
Kara N	2006	Trukese	BD	34.8/48.4	(49/43) *vs*. (53/47)	92/100	55/35/3 *vs*. 59/34/7	NE	NE
Karasneh JA	2005	Trukese	BD	NM	(112/81) *vs*. (61/45)	193/106	112/63/14 *vs*. 54/45/6	98/76/17 *vs*. 43/44/15	148/39/4 *vs*. 63/39/2
Amoli MM	2004	Spanish	HSP	NM	NM	49/98	22/16/7 *vs*. 35/45/17	19/20/10 *vs*. 37/58/22	39/9/1 *vs*. 71/25/2
Salvarani C	2003	Italians	GCA	73.3/NM*	(20/71) *vs*. NM*	91/133	15/63/13 *vs*. 51/63/19	NE	57/28/6 *vs*. 92/37/4
Kimu JU	2003	Koreans	BD+ vasculitis	38.6 / 35.0/40.6	(31/61) *vs*. (30/50	92/80	60/29/3 *vs*. 71/9/0	NE	75/17/0 *vs*. 62/17/1
Amoli MM	2003	English	GCA	NM	NM	55/98	15/31/11 *vs*. 35/45/17	17/27/11 *vs*. 37/58/22	43/12/0 *vs*. 71/25/2
Salvarani C	2002	Italians	BD	NM	NM	73/135	1/51/21 *vs*. 35/78/22	NE	51/21/1 *vs*. 89/33/13
Kara N	2006	Trukese	BD	34.8/48.4	49/53 *vs*. 43/47	92/100	54/35/3 *vs*. 59/34/7	NE	NE
Nakao K	2007	Japanese	BD	42.7/52.8	58/20 *vs*. 46/61	78/107	68/10/0 *vs*. 93/14/0	64/13/1 *vs*. 88/18/1	63/14/1 *vs*. 85/20/2
Brodmann M	2002	Austrilian	TAO	NM	NM	42/149	19/18/5 *vs*. 76/61/12	NE	NE

*matched; con: control; NM: not mentioned; NE: not evaluated; M/F: male/female; ww: wild type; ht: heterozygotes; vv: homozygous variants. BD: Behcet's disease; GCA: giant cell arteritis; HSP: Henoch-Schönlein purpura; HSPN: Henoch-Schönlein purpura nephritis; TAO: thromboangiitis obliterans.

polymorphism using the recessive or dominant models.

Heterogeneity and publication bias

Significant between-study heterogeneity was found in analysis of G894T (I^2= 78% in dominant model; I^2= 76% in recessive model) and the dominant model of intron-4ba (I^2= 81%), whereas no significant heterogeneity was found in the recessive model of intron-4ba (I^2= 2%) and T-786C (I^2= 0% in dominant recessive models).

No publication bias was detected in any of the dominant and recessive models. (Egger's test: for G894T: p=0.055 in dominant model, p=0.208 in recessive model; for intron-4ba: p=0.055 in dominant model; p=0.771 in recessive model; for T-786C: p=0.209 in dominant model, p=0.671 in recessive model).

Discussion

A negative association between gene polymorphisms implicated in well-established inflammatory pathways and vasculitis has already been reported (27). In addition, our meta-analysis found that the eNOS polymorphisms did not increase the susceptibility to vasculitis.

Low levels of NO constitutively generated from eNOS, is essential for a good endothelial function and integrity (28). Polymorphisms in eNOS might alter enzyme activity and basal NO production, thus modify the susceptibility to vasculitis. G894T, located at exon 7, leading to the conversion of glutamic acid to aspartic acid (29), alters the primary structure of the protein, which could lead to functional changes of the enzyme. T-786C polymorphism is a point mutation of thymine to cytosine in the promoter region, reducing the transcription rate of promoter by approximately 50% (30). The 27-bp variable number of tandem repeat (VNTR) polymorphism (4a/4b) within intron 4 is associated with alterations in promoter activity (31). Compared with the

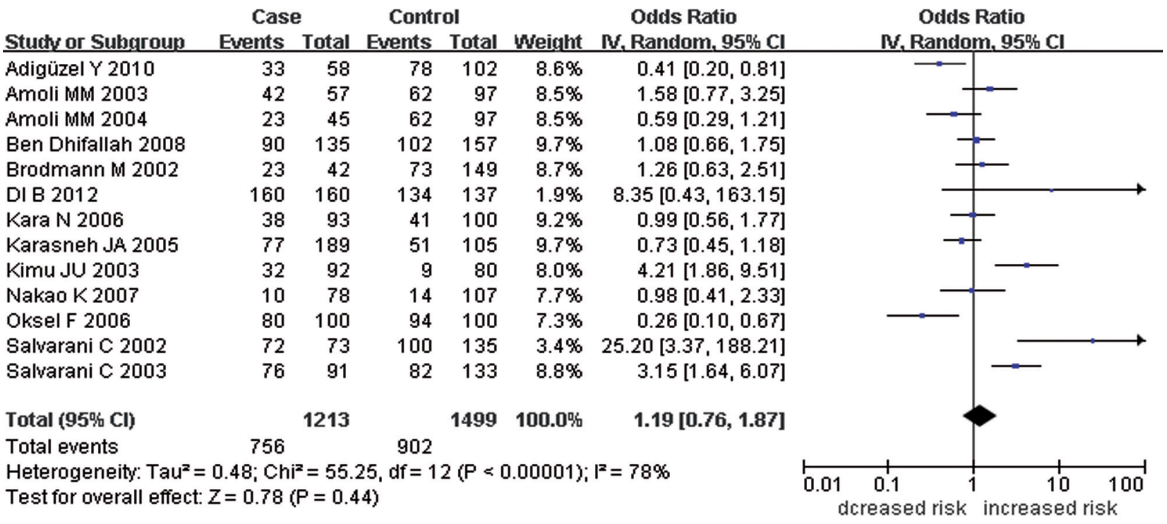


Fig. 1. The relationship between G894T and vasculitis in meta-analysis, dominant model. Events: Carriers of 894T; Total: Total number of cases and controls.

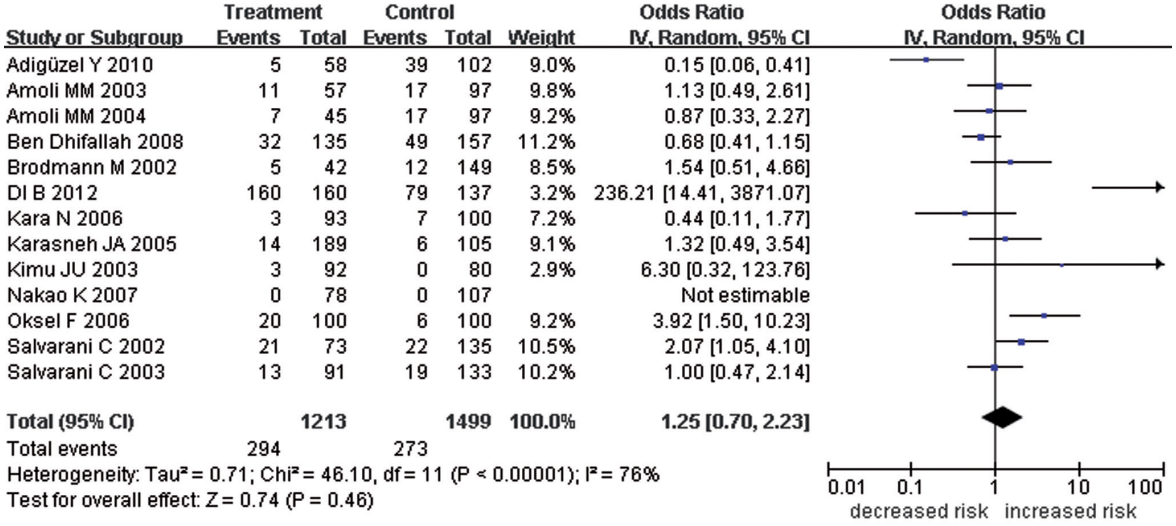


Fig. 2. The relationship between G894T and vasculitis in meta-analysis, recessive model. Events: Carriers of 894T; Total: Total number of cases and controls.

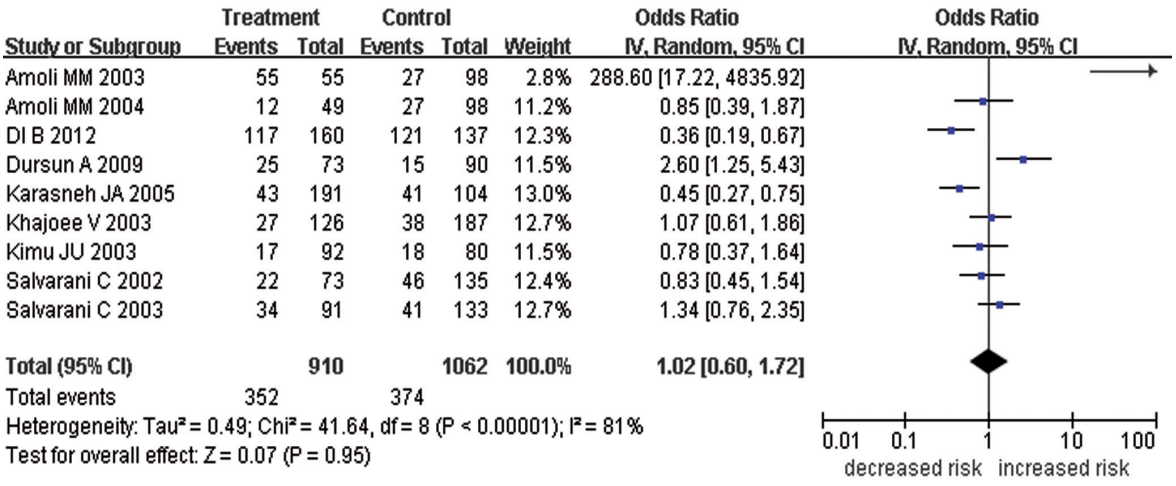


Fig. 3. The relationship between intron-4ba and vasculitis in meta-analysis, dominant model. Events: Carriers of intron-4a; Total: Total number of cases and controls.

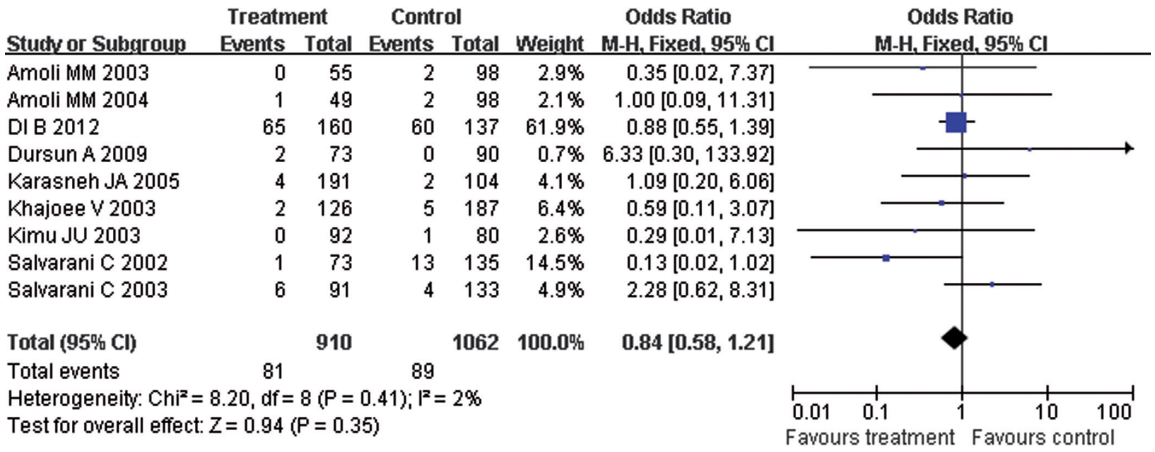


Fig. 4. The relationship between intron-4ba and vasculitis in meta-analysis, recessive model. Events: Carriers of intron-4a; Total: Total number of cases and controls.

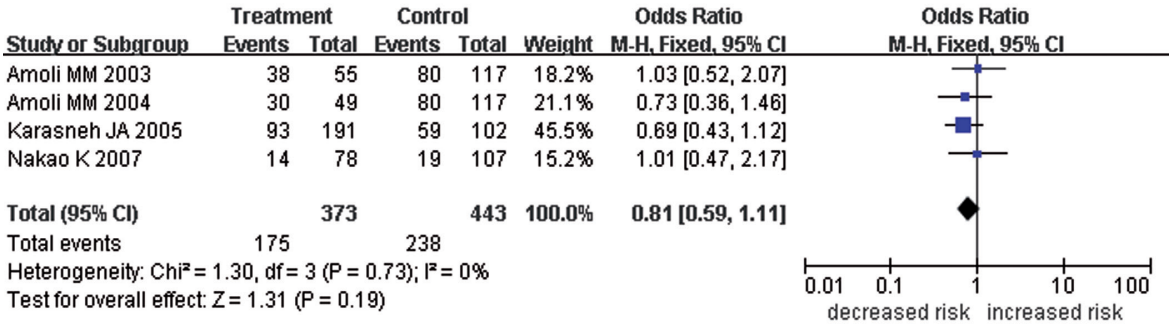


Fig. 5. The relationship between T-786C and vasculitis in meta-analysis, dominant model. Events: Carriers of -786C; Total: Total number of cases and controls.

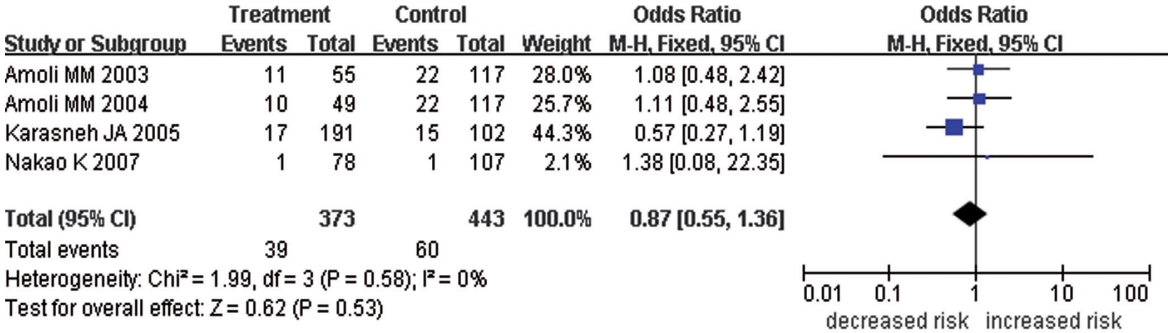


Fig. 6. The relationship between T-786C and vasculitis in meta-analysis, recessive model. Events: Carriers of -786C; Total: Total number of cases and controls.

common intron-4b allele carriers, levels of eNOS mRNA and protein concentrations are lower in the rare intron-4a allele carriers (31). The findings of Schoeb *et al*. strongly indicate that eNOS serves as a negative regulator of vasculitis because eNOS depletion accelerates the onset of disease and increases the number and distribution of affected vessels in the kidney (32). However, in the meta-analysis by Lee *et al*., eNOS G894T and intron -4ba polymorphisms are not associated with BD (33). Moreover, our study did not support such a hypothesis that eNOS polymorphism associated with risk of vasculitis.

However, we cannot completely exclude a potential implication of eNOS gene polymorphisms in the susceptibility to vasculitis. In this regard, most studies of systemic vasculitis were based on small number of patients. In addition, it is possible that eNOS gene polymorphisms may not imply a direct risk for vasculitis but the interaction between eNOS polymorphisms and other genes may have some kind of influence in the risk of vasculitis. It was the case for cardiovascular disease in rheumatoid arthritis where some interactions between NOS gene polymorphisms and HLA-DRB1 alleles conferred an increased risk of developing cardiovascular events in patients with this chronic disease associated with accelerated atherosclerosis. There are limitations in our study. All the studies included explore the association between single polymorphisms and vasculitis. However, the association can be modified by the presence of another polymorphism

and interactions between polymorphisms may provide more information than single polymorphism analysis. For example, homozygous intron-4a genotype was identified as a predisposed factor for acute coronary syndrome, and this relationship can be intensified by the presence of the -786CC genotype (34). Therefore, studies on gene-gene and gene-environment interactions are also needed to further elucidate the role of eNOS polymorphisms and the eNOS gene in the susceptibility of vasculitis. The studies included are all case-control studies, which may increase the false-positive result, and large prospective studies are required.

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