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STATE OF THE ART REVIEW

Association between Endothelial Nitric Oxide Synthase Glu298Asp Gene Polymorphism and Diabetic Nephropathy Susceptibility

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Abstract

The association between endothelial nitric oxide synthase (eNOS) Glu298Asp gene polymorphism and diabetic nephropathy (DN) risk is still controversial. A meta-analysis was performed to evaluate the association between eNOS Glu298Asp gene polymorphism and DN susceptibility. A predefined literature search and selection of eligible relevant studies were performed to collect data from electronic database. Eight articles were identified for the analysis of association between eNOS Glu298Asp gene polymorphism and DN risk. T allele was associated with DN susceptibility in overall populations, in Asians, and for Caucasians (overall populations, $p = 0.005$; Asians, $p = 0.004$; Caucasians, $p = 0.002$). Furthermore, GG genotype might play a protective role against DN onset for overall populations, Asians, Caucasians, and Africans. However, a link between eNOS Glu298Asp gene polymorphism and DN risk was not found in overall populations, Asians, Caucasians, and Brazil population. In conclusion, T allele might become a significant genetic molecular marker for the onset of DN in overall populations, in Asians, and for Caucasians. However, more studies should be performed in the future.

Keywords: diabetic nephropathy, endothelial nitric oxide synthase, Glu298Asp, gene polymorphism, meta-analysis

INTRODUCTION

Diabetic nephropathy (DN) is one of the most common microvascular complications of diabetes and the leading cause of end-stage renal disease.[1] Susceptibility to DN has an inherent genetic basis as evidenced by familial aggregation and ethnic-specific prevalence rates.[2] Some current investigations[3–5] suggested that gene polymorphism might play a key role in the onset of DN.

Nitric oxide (NO) is a ubiquitous vasodilator and an important regulator of renal sodium excretion.[6] Reduced NO generation induces renal injury,[7] and impairment of endothelial NO generation is considered the major deterioration factor for progressive renal disease. NO is produced by inducible nitric oxide synthase.[8] Glu298Asp is an important gene mutation of endothelial nitric oxide synthase (eNOS), and the eNOS Glu298Asp gene polymorphism includes GG (Glu/Glu), GT (Glu/Asp), and TT (Asp/Asp) genotypes and G (Glu) and T (Asp) alleles. We present an epidemiologic study showing that the eNOS Glu298Asp gene polymorphism has been implicated in the etiology of DN. However, the available evidence reported to date is weak, due to sparseness of data or disagreements among studies. There was rare meta-analysis to explore the association of eNOS Glu298Asp gene polymorphism with DN risk. We performed this meta-analysis to investigate the relation between eNOS Glu298Asp gene polymorphism and DN susceptibility, with the intention to provide a much more reliable finding on the significance of the association.

MATERIALS AND METHODS

Search Strategy

The relevant studies were screened from the search engines of PubMed, Embase, Cochrane Library, and CBM-disc (China Biological Medicine Database) on 1 March 2012. The following terms were used to complete the search: "diabetic nephropathy," "DN," "diabetes mellitus nephropathy," "endothelial nitric oxide synthase," "eNOS," and "gene." We also extended search spectrum to the "related articles" and the

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bibliographies of all retrieved studies. If multiple publications of the same data from the same study group occurred, we only recruited the later paper for analysis.

Inclusion and Exclusion Criteria

Inclusion criteria

(1) A case–control study; (2) the outcome had to be DN; and (3) there had to be at least two comparison groups (DN group vs. control group).

Exclusion criteria

(1) Review articles, editorials, and case reports; (2) articles that did not provide the detailed genotype data; (3) investigating the association of other genes with DN; (4) investigating the role of eNOS in diseases; and (5) multiple publications of the same data from the same study group.

Data Extraction and Synthesis

The following information was extracted from each study independently by at least two investigators: first author's surname, year of publication, ethnicity of study population, and the number of cases and controls for eNOS genotype. Frequencies of T allele were calculated for case group and control group from the corresponding genotype distribution. The results were compared and disagreements were resolved by discussion.

Statistical Analysis

Available data were entered into Cochrane Review Manager (RevMan, version 5, Oxford, UK) and analyzed. The pooled statistic was counted using the fixed effects model, but a random effects model was conducted when the p-value of heterogeneity test was less than 0.1.[9] The results were expressed with odds ratios (ORs) for dichotomous data, and 95% confidence intervals (CIs) were also calculated. A p-value of <0.05 was required for the overall OR to be deemed statistically significant.[10,11] I^2 was used to test the heterogeneity between the included studies. We classified the investigations into studies for Caucasians, Asians, Africans, and Brazil population because genotype frequencies and prevalence of DN were different among ethnic groups. In order to avoid excessive comparisons, the OR was calculated by using three methods[12,13]: method 1, allele comparison (T allele vs. G allele); method 2, comparing TT homozygous with the other two combinations (TT vs. TG + GG); method 3, comparing GG genotype with the other two combinations (GG vs. TG + TT). A χ^2-test using a web-based program was applied to determine whether genotype distribution of the control population reported conformed to Hardy–Weinberg equilibrium (HWE) ($p < 0.05$ was considered significant). Sensitivity analysis was performed when studies with controls were not in HWE. All descriptive data were expressed as mean ± SD.

RESULTS

Study Characteristics

The search yielded 165 references from PubMed, Embase, Cochrane Library, and CBM-disc. According to the inclusion and exclusion criteria, eight articles[14–21] were identified for the analysis of the association between eNOS Glu298Asp gene polymorphism and DN susceptibility in our review (Figure 1). Five investigations[15–17,20,21] were conducted in Asians, one study[18] for Caucasians, one[14] in Africans, and one[19] in Brazil population. Six studies[14–19] were reported in English and two reports[20,21] were published in Chinese. The data of our interest were extracted and shown in Table 1. These eight studies contained 850 case series and 1254 controls. The average frequency of T allele was 19.19% in Asian DN patients and 8.68% in controls. For Caucasians, the frequency of T allele was 52.50% in case group and 22.50% for controls. The frequency of T allele in Brazil population was 30.75% in cases and 29.57% for control group. When compared with that in Brazil population, the ratio of cases/controls for average frequency of T allele was markedly elevated in Asians and Caucasians (Asians, cases/controls = 2.21; Caucasians, cases/controls = 2.33; Brazil population, cases/controls = 1.04).

Association of the eNOS Glu298Asp Gene Polymorphisms with DN Risk

In this meta-analysis, a significant association between T allele and DN risk was observed in overall populations (Figure 2 for T allele; Table 2). Furthermore, the GG genotype might be a protective factor against the risk of DN in overall populations (Figure 3 for GG allele;

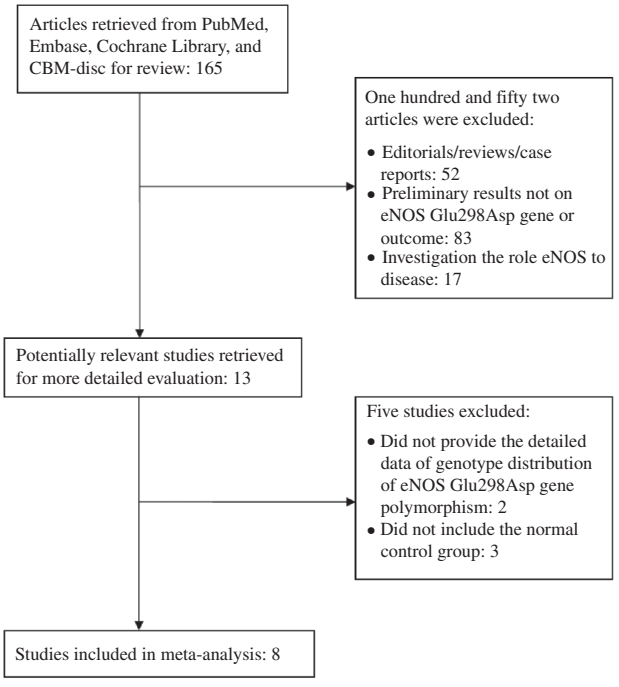

Figure 1. Flowchart of our studies.

Table 1. Characteristics of the studies evaluating the effects of eNOS Glu298Asp gene polymorphism on DN risk.

		DN				Control				Tallele (%)	
First author, year	Ethnicity	TT	TG	GG	Total	TT	TG	GG	Total	DN	Control
Noiri et al. (2002)	African			49	72			251	307		
Nagase et al. (2003)	Asian	2	8	38	48	2	18	250	270	12.5	4.07
Shin et al. (2004)	Asian	0	23	93	116	0	7	52	59	9.91	5.93
Fu et al. (2006)	Asian	0	27	32	59	0	12	49	61	22.88	9.84
Thaha et al. (2008)	Asian	1	31	7	39	2	26	72	100	42.31	15
El-Din and Hamdy (2011)	Caucasian	12	18	10	40	1	7	12	20	52.5	22.5
Santos et al. (2011)	Brazil	32	166	176	374	22	95	118	235	30.75	29.57
Li1 et al. (2011)	Asian	0	17	85	102	1	33	171	205	8.33	8.54

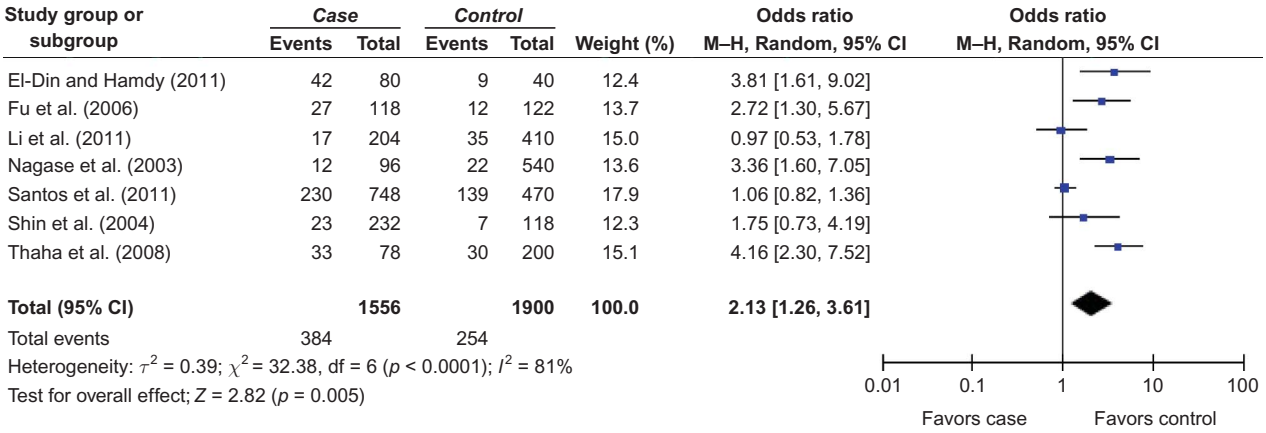

Figure 2. The pooled OR indicated that T allele was associated with DN risk in overall populations.

Table 2. Meta-analysis of the association of eNOS Glu298Asp gene polymorphism with the risk of DN.

Genetic contrasts	Group and studies subgroups		Q-test *p*-value	Model selected	OR (95% CI)	*p*
T versus G	Overall	7	0.0001	Random	2.13 (1.26, 3.61)	0.005
	Asian	5	0.01	Random	2.30 (1.31, 4.07)	0.004
	Caucasian	1	–	Fixed	3.81 (1.61, 9.02)	0.002
	Brazil	1	–	Fixed	1.06 (0.82, 1.36)	0.66
TT versus TG + GG	Overall	7	0.15	Fixed	1.25 (0.76, 2.06)	0.38
	Asian	5	0.43	Fixed	2.04 (0.54, 7.71)	0.29
	Caucasian	1	–	Fixed	8.14 (0.986, 7.94)	0.05
	Brazil	1	–	Fixed	0.91 (0.51, 1.60)	0.73
GG versus TG + TT	Overall	8	0.0001	Random	0.40 (0.23, 0.69)	0.0009
	Asian	5	0.0005	Random	0. 34 (0.15, 0.77)	0.01
	Caucasian	1	–	Fixed	0.22 (0.07,0.70)	0.01
	African	1	–	Fixed	0.45 (0.25, 0.80)	0.007
	Brazil	1	–	Fixed	0.88 (0.64, 1.22)	0.45
Sensitivity analysis						
T versus G	Overall	6	0.0001	Random	1.99 (1.13, 3.49)	0.02
	Asian	4	0.008	Random	2.10 (1.06, 4.19)	0.03
	Caucasian	1	–	Fixed	3.81 (1.61, 9.02)	0.002
	Brazil	1	–	Fixed	1.06 (0.82, 1.36)	0.66
TT versus TG + GG	Overall	6	0.25	Fixed	1.16 (0.69, 1.93)	0.58
	Asian	4	0.75	Fixed	0.99 (0.14, 6.79)	0.99
	Caucasian	1	–	Fixed	8.14 (0.98, 67.94)	0.05
	Brazil	1	–	Fixed	0.91 (0.51, 1.60)	0.73
GG versus TG + TT	Overall	6	0.00001	Random	0.40 (0.19, 0.82)	0.01
	Asian	4	0.0002	Random	0.35 (0.12, 0.99)	0.05
	Caucasian	1	–	Fixed	0.22 (0.07, 0.70)	0.01
	Brazil	1	–	Fixed	0.88 (0.64, 1.22)	0.45

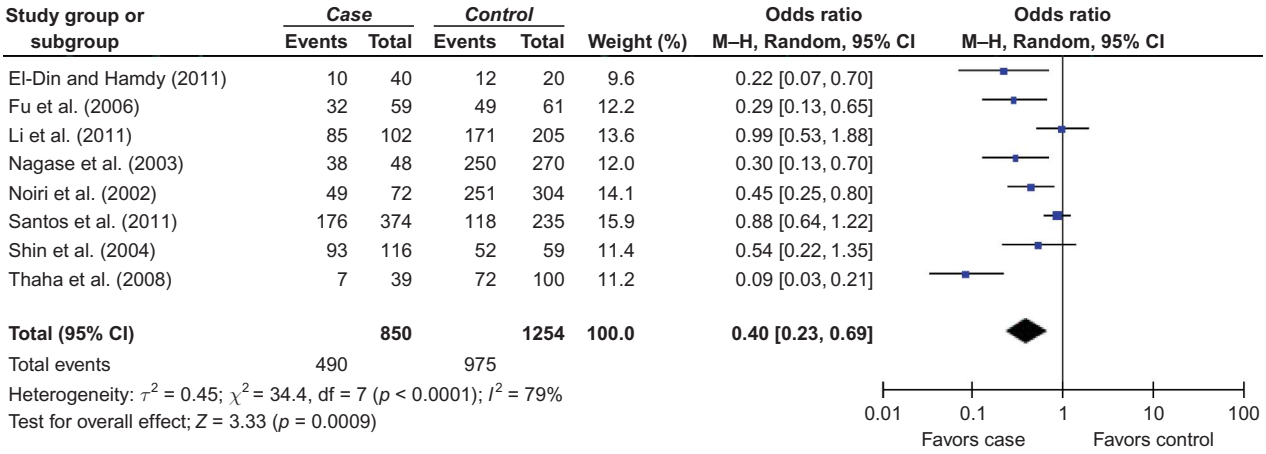

Figure 3. The pooled OR indicated that GG genotype was associated with DN risk in overall populations.

Table 2). However, the association of TT genotype with DN risk was not observed (Table 2).

The ethnical and geopolitical difference might affect the results of our analysis for the association of eNOS Glu298Asp gene polymorphism with DN susceptibility. In order to evaluate this effect, we divided the population by ethnicity. T allele was associated with the risk of DN in Asians and for Caucasians (Table 2). Furthermore, GG genotype might play a protective role against DN risk in Asians, Caucasians, and Africans. However, there was no association between TT genotype and DN susceptibility in Asians and Caucasians (Table 2). Furthermore, there was no association of eNOS Glu298Asp gene polymorphism with DN susceptibility for Brazil population (Table 2).

Sensitivity Analysis

One study[15] from Asians that the genotype distributions in the controls were significantly deviated from HWE was excluded from our sensitive analysis. Furthermore, one study[14] from Africans had not provided the detailed gene distribution data for the control group for HWE test and it was excluded from the sensitive analysis. Finally, four from Asians, one from Caucasians, and one from Brazil population were included for our sensitive analysis.

In the sensitivity analysis for overall populations, we found that the pooled OR for T allele was favorable to DN group (OR = 1.99, 95% CI = 1.13–3.49; Table 2) and the difference was statistically significant ($p = 0.02$). Furthermore, the pooled OR for GG genotype seemed to play a protective role against DN disease (OR = 0.40, 95% CI = 0.19–0.82; Table 2). Interestingly, TT genotype was not associated with the risk of DN in the sensitivity analysis. The results of the sensitivity analysis were consistent with those of the non-sensitivity analysis for overall populations.

Furthermore, those results of the sensitivity analysis in Asians, Caucasians, and Brazil population were consistent with those of the non-sensitivity analysis (Table 2).

DISCUSSION

Damage of endothelial NO generation brought about by gene polymorphism is considered the major deterioration factor for progressive renal disease, such as DN.[14] DN is a major health problem associated with very high morbidity and mortality, and data on the risk factors for the pathogenesis of DN were insufficient. There was rare genetic molecular marker to predict the onset of DN. This meta-analysis was performed to explore whether the eNOS Glu298Asp gene polymorphism could predict the susceptibility of DN.

In this investigation, eight suitable studies were recruited into our meta-analysis: five studies from Asians, one investigation for Caucasians, one from Africans, and one from Brazil population. Our meta-analysis showed that T allele was associated with the onset of DN in overall populations, but TT genotype did not. Furthermore, GG genotype seemed to play a protective role against DN risk. Sensitivity analysis was also performed in our meta-analysis, and we found that the results of the sensitivity analysis were similar to those of the non-sensitivity analysis for overall populations. The results for the overall populations might be robust to some extent.

The geographic and ethnic difference might be an important factor to effect the association of gene polymorphism with the susceptibility of DN. In our study, we found that the average frequency of T allele in controls was 22.50% in Caucasians. Furthermore, the frequency of T allele in Brazil population was 29.57% for control group. However, for Asians, the average frequency of T allele for controls was 8.68%. The disequilibrium of T allele distribution in controls was observed among those different races. The subgroup analysis was conducted to explore the association of eNOS Glu298Asp gene polymorphism with the susceptibility of DN in different races.

In Asians, an association between T allele and the risk of DN was found. The ratio of cases/controls for average frequency of T allele was elevated in Asians (cases/

controls = 2.21). T allele might be a risk factor to predict the risk of DN in Asians. Furthermore, GG genotype seemed to play a protective role against DN risk in Asians. Interestingly, the results of the sensitivity analysis were consistent with those of the non-sensitivity analysis. T allele might be a risk factor for the risk of DN in Asians.

In Caucasians, there was an association between eNOS Glu298Asp gene polymorphism and susceptibility of DN. However, only one study was recruited into our meta-analysis for Caucasians and it was difficult to draw a robust conclusion for Caucasians. However, more studies should be performed in the future.

GG genotype might be a protective factor against the susceptibility of DN for African population. However, there was only one study recruited for African population in this meta-analysis, and the gene distribution of TT and GT was not provided. The conclusion for African population might be less robust. Whether there was an association between eNOS Glu298Asp gene polymorphism and susceptibility of DN, more religious studies should be performed further.

eNOS Glu298Asp gene polymorphism was not associated with the susceptibility of DN in Brazil population. The gene distributions of the included study were in HWE, and the results of the sensitivity analysis were the same as those of the non-sensitivity analysis in Brazil population. However, only one study was included into our meta-analysis and it was difficult to draw a robust conclusion for Brazil population. More studies in Brazil population should be conducted in the future.

There were some meta-analyses to explore the association of eNOS Glu298Asp gene polymorphism with the susceptibility of some diseases in the past years. Su et al.[22] performed a meta-analysis to investigate the association of eNOS Glu298Asp gene polymorphism with recurrent pregnancy loss (RPL) and reported that eNOS Glu298Asp gene polymorphism was significantly associated with RPL. Casas et al.[23] performed a meta-analysis to explore the relationship between eNOS genotype and ischemic heart disease (IHD) and reported that homozygosity for the TT was associated with an increased risk of ischemic heart disease Shaik et al.[24] reported that eNOS Glu298Asp gene polymorphism was not associated with the risk of preeclampsia in women by meta-analysis method. Yu et al.[25] conducted a meta-analysis to study the relationship between eNOS Glu298Asp polymorphism and preeclampsia risk and found that the eNOS Glu298Asp polymorphism was not associated with a significant increased risk of preeclampsia in overall populations, Caucasians, and Asians. In this meta-analysis, the relationship between eNOS Glu298Asp gene polymorphism and DN risk was explored and our results were similar to those of Su et al.[22]

In our investigation, we found that the T allele was associated with DN susceptibility in overall populations, Asians, and Caucasians, and GG genotype might play a protective role against DN susceptibility in overall populations, Asians, Caucasians, and Africans. However, the association was not found in Brazil population. These findings should be regarded cautiously because many other ingredients, such as heterogeneity of enrolled cases, limited statistical power, variable study designs, and different interventions, were closely related to affect the results. Furthermore, the gene polymorphisms of the eNOS 894G>T and −786T>C were also reported to be associated with the developing DN patients.[26] Whether the eNOS Glu298Asp gene polymorphism is just linked with other discrete loci involved in the occurrence of DN is not clear at the moment. Undoubtedly, the limitations mentioned above might affect our final conclusions.

In conclusion, the results of our study support that T allele was associated with DN susceptibility in overall populations, Asians, and Caucasians, and GG genotype might play a protective role against DN susceptibility in overall populations, for Asians, in Caucasians, and in Africans. However, more case–control association investigations on larger, stratified populations are required to further clarify the role of this eNOS Glu298Asp gene polymorphism in DN susceptibility in different ethnicities.

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