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ORIGINAL ARTICLE

Endothelial nitric oxide synthase gene G894T polymorphism and risk assessment for pregnancy-induced hypertension: evidence from 11 700 subjects

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Recent studies have reported the association between endothelial nitric oxide synthase (eNOS) gene G894T polymorphism and pregnancy-induced hypertension (PIH). However, the results have been inconsistent. We conducted a comprehensive meta-analysis to explore this association. A total of 36 case–control studies involving 4028 PIH cases and 7672 controls were ultimately included. In the overall analysis, no association was identified between eNOS gene G894T polymorphism and PIH risk in any of the genetic models. In the subgroup analysis, the results showed that T-allele carriers had a higher risk of PIH than those with the G allele in Asians (G *vs.* T: odds ratio (OR) = 0.76, 95% confidence interval (CI) = 0.63–0.91, *P* = 0.002; GT+TT *vs.* GG: OR = 1.32, 95% CI = 1.09–1.59, *P* = 0.004; TT *vs.* GT+GG: OR = 1.96, 95% CI = 1.26–3.06, *P* = 0.003; TT *vs.* GG: OR = 1.99, 95% CI = 1.27–3.11, *P* = 0.003; GT *vs.* GG: OR = 1.23, 95% CI = 1.05–1.43, *P* = 0.009). For Latin American and African populations, the association between G894T polymorphism and susceptibility to PIH was only observed in the dominant model. However, no association was observed in Europeans and Americans. Therefore, eNOS gene G894T polymorphism was related to PIH risk, especially for Asians.
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Keywords: endothelial nitric oxide synthase; meta-analysis; polymorphism; pregnancy-induced hypertension

INTRODUCTION

Pregnancy-induced hypertension (PIH) is regarded as a multifactorial pregnancy-specific syndrome. It is defined as hypertension with or without proteinuria developing during pregnancy, delivery or the puerperium in previously normotensive women without proteinuria.[1] PIH is classified into four categories: gestational hypertension, preeclampsia (PE), eclampsia and chronic hypertension complicated by PE.[2] It is one of the major causes of maternal morbidity and mortality, leading to 10–15% of maternal deaths, especially in developing countries.[3] According to a WHO (World Health Organization) estimate, one woman dies every 7 min from complications of PIH.[4] However, the exact pathogenesis of PIH remains uncertain. There is some evidence to suggest that PIH may be correlated with immune, genetic and environmental factors and placental abnormalities. Furthermore, it is widely accepted that PIH is a complex genetic susceptibility disease.[2]

Nitric oxide (NO), an important vasodilator in cardiovascular homeostasis, is synthesized from L-arginine by neuronal, inducible and endothelial nitric oxide synthases (NOS1, NOS2 and eNOS/NOS3, respectively).[5] It is mainly involved in vascular smooth muscle relaxation through a cyclic guanosine monophosphate-mediated signal transduction pathway.[6] In addition, eNOS, a chemical product of endothelial NO, has a key role in most NO formation in the cardiovascular system.[7] The hemodynamics of pregnancy mainly depend on eNOS, which contributes to systemic arteriolar vasodilatation; eNOS is responsible for increased blood volume and cardiac output as well as decreased systolic and diastolic blood pressure.[8] Furthermore, single-nucleotide polymorphisms in the NOS3 gene may affect its regulation at the transcriptional, post-transcriptional and post-translational levels, bringing about the abnormal expression of a series of target genes and leading to PIH susceptibility.

The eNOS gene, located on chromosome 7q35-36 in humans, has become a logical candidate gene in the development of PIH. Here, we focus on a G-to-T transversion at nucleotide position 894 of the eNOS, which results in a change to Asp from Glu at amino acid 298 (G894T; Glu298Asp; single-nucleotide polymorphism rs1799983).[9]

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Many recent studies have been performed to explore the eNOS gene polymorphisms related to PIH risk; however, the results have been conflicting. Although several meta-analyses have studied this association, the analyses missed some relevant studies and additional reports have been published since the analyses were conducted. Moreover, these studies focused on specific stages of PIH and therefore did not conduct comprehensive analyses; they were also of limited sample size. Here, we summarize the characteristics of the previous studies and present an objective, accurate and comprehensive review regarding the relationship between eNOS G894T gene polymorphism and PIH.

MATERIALS AND METHODS

Search strategy

To obtain more convincing evidence regarding the association between eNOS G894T gene polymorphism and PIH, we conducted a systematic search of six online databases, with the last search updated on 26 December 2015. The six databases were PubMed, Excerpta Medica Database (Embase), Cochrane Library database, Chinese Biomedical Literature Database (CBM), Chinese National Knowledge Infrastructure (CNKI) and Wanfang Data (WF). All searches were conducted using a combination of subject headings [MEDLINE (MeSH) and Embase (Emtree)] and free-text terms; we determined the final search strategy through several pre-searches. The keywords used in the search strategy were as follows: (pregnancy-induced hypertension OR pregnancy hypertensive disorders OR maternal hypertension OR eclampsia OR pre-eclampsia OR gestational hypertension OR HELLP Syndrome) AND (nitric oxide synthase OR NO Synthase OR eNOS Enzyme OR ecNOS Enzyme OR NOS3 protein). There was no restriction on language or publication years. We also reviewed the reference lists of the identified articles to avoid missing relevant studies.

Inclusion and exclusion criteria

The following inclusion criteria were employed for this meta-analysis: (1) case–control studies; (2) PIH defined as BP $\geqslant$ 140/90 mm Hg on at least two occasions 6 h apart that generally appeared after 20 weeks of gestation and returned to normal 12 weeks postpartum; gestational hypertension defined as BP $\geqslant$ 140/90 mm Hg after 20 weeks of gestation on two occasions at least 6 h apart and no proteinuria in earlier pregnant women; PE defined as gestational hypertension plus significant proteinuria ($\geqslant$0.3 g/24 h); and eclampsia diagnosed as tonic-clonic seizures in pregnant women with BP $\geqslant$ 140/90 mm Hg and proteinuria;[10] (3) involved the polymorphism of eNOS G894T gene (Glu298Asp; rs1799983); (4) could directly or indirectly provide the genotype frequencies related to G894T eNOS in both case and control groups; (5) the distribution of genotype frequencies in control groups was consistent with Hardy–Weinberg Equilibrium (HWE); and (6) published in Chinese or English. The following were the exclusion criteria: (1) research on animals; (2) no suitable control groups; and (3) no detailed data.

The data in this meta-analysis were derived from existing studies; therefore, neither informed consent nor the approval of the ethics committee was required.

Data extraction

Following the inclusion criteria, two authors (Ma and LV) independently selected the literature by reading the titles and abstracts. The full text of each identified article was then read to determine whether it was suitable for inclusion. Disagreements were resolved through consensus or by discussion with a third author (Yang). For each eligible study, the following information was independently extracted by two authors (Ma and LV) and examined by a third author (Yang): name of the first author, date of publication, country of research subjects, sample size of cases and controls, distributions of allele and genotype frequencies in cases and controls, main exclusion criteria of research subjects and HWE in the controls.

Methodological quality assessment

According to the Newcastle-Ottawa-Scale (NOS)[11] on case–control studies, we evaluated the qualities of all included studies based on the object selection, comparability and exposure. A star was described as an appropriate entry, with each star representing one point. The possible quality assessment score ranged from zero to nine points; a high score indicated a good quality study.

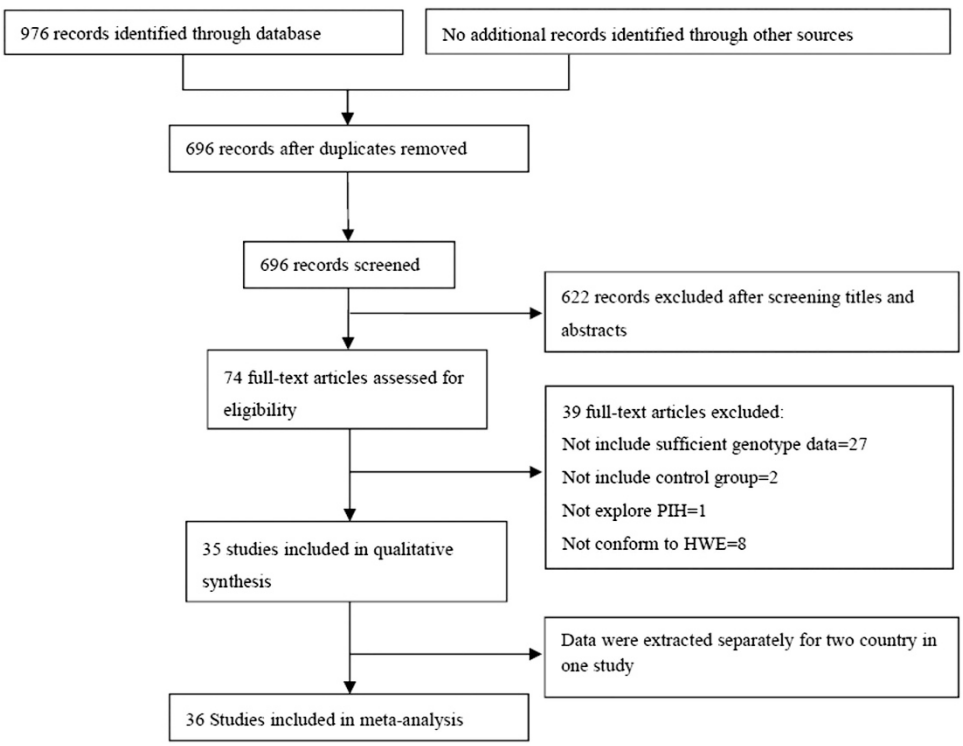

Figure 1 Flow diagram of search strategy and study selection. HWE, Hardy–Weinberg equilibrium; PIH, pregnancy-induced hypertension.

Table 1 Summary of studies included in the meta-analysis

	Sample size			*Test of heterogeneity*				*Test of association*			
Comparisons	*Case*	*Control*	*No. of studies*	X^2	*P-value*	I^2*(%)*	*Model*	*OR*	*95% CI*	Z	*P-value*
Overall											
G *vs.* T	4028	7672	36	74.99	0	53.3	R	0.87	0.77–0.99	2.26	0.024
GT+TT *vs.* GG	4028	7672	36	199.18	0	82.4	R	0.96	0.77–1.20	0.36	0.718
TT *vs.* GT+GG	4028	7672	36	42.31	0.031	36.2	R	1.41	1.05–1.88	2.31	0.021
TT *vs.* GG	4028	7672	36	46.96	0.01	42.5	R	1.39	1.02–1.91	2.06	0.039
GT *vs.* GG	4028	7672	36	55.19	0.016	36.6	R	1.09	0.96–1.24	1.34	0.182
Asians											
G *vs.* T	1943	2582	20	31.78	0.033	40.2	R	0.76	0.63–0.91	3.04	0.002
GT+TT *vs.* GG	1943	2582	20	27.64	0.091	31.3	R	1.32	1.09–1.59	2.87	0.004
TT *vs.* GT+GG	1943	2582	20	13.13	0.285	16.3	F	1.96	1.26–3.06	2.98	0.003
TT *vs.* GG	1943	2582	20	14.73	0.195	25.3	F	1.99	1.27–3.11	3.02	0.003
GT *vs.* GG	1943	2582	20	25.2	0.154	24.6	F	1.23	1.05–1.43	2.61	0.009
Europeans											
G *vs.* T	817	2887	7	15.68	0.016	61.7	R	1.08	0.86–1.36	0.69	0.489
GT+TT *vs.* GG	817	2887	7	16.12	0.013	62.8	R	0.9	0.67–1.21	0.71	0.478
TT *vs.* GT+GG	817	2887	7	7.94	0.242	24.5	F	1.09	0.83–1.43	0.59	0.555
TT *vs.* GG	817	2887	7	10.7	0.098	43.9	R	0.97	0.61–1.52	0.16	0.876
GT *vs.* GG	817	2887	7	14.79	0.022	59.4	R	0.9	0.66–1.21	0.71	0.476
Americans											
G *vs.* T	777	1402	5	14.72	0.005	72.8	R	0.89	0.64–1.23	0.73	0.467
GT+TT *vs.* GG	777	1402	5	9.38	0.052	57.4	R	1.06	0.77–1.45	0.34	0.734
TT *vs.* GT+GG	777	1402	5	14.82	0.005	73	R	1.74	0.77–3.96	1.33	0.185
TT *vs.* GG	777	1402	5	15.86	0.003	74.8	R	1.68	0.71–3.99	1.18	0.24
GT *vs.* GG	777	1402	5	5.89	0.208	32.1	F	1.04	0.84–1.28	0.34	0.732
Latin Americans											
G *vs.* T	357	615	2	2.87	0.09	65.1	R	0.93	0.58–1.49	0.3	0.767
GT+TT *vs.* GG	357	615	2	4.62	0.032	78.3	R	0.28	0.16–0.49	4.47	0
TT *vs.* GT+GG	357	615	2	2.77	0.096	64	R	1.62	0.33–7.99	0.59	0.556
TT *vs.* GG	357	615	2	2.37	0.123	57.9	F	1.39	0.61–3.18	0.77	0.439
GT *vs.* GG	357	615	2	2.36	0.125	57.6	F	1.14	0.85–1.52	0.86	0.389
Africans											
G *vs.* T	134	186	2	2.42	0.12	58.6	F	0.79	0.46–1.39	0.81	0.417
GT+TT *vs.* GG	134	186	2	0.9	0.342	0	F	0.2	0.12–0.33	6.36	0
TT *vs.* GT+GG	134	186	2	0.1	0.749	0	F	1.2	0.17–8.66	0.18	0.856
TT *vs.* GG	134	186	2	0.17	0.685	0	F	1.21	0.17–8.73	0.19	0.852
GT *vs.* GG	134	186	2	2.48	0.115	59.7	F	1.29	0.69–2.42	0.81	0.421

Abbreviations: CI, confidence interval; F, fixed-effect model; OR, odd ratio; R, random-effect model; *vs.*, versus.

Statistical methods

For each selected study, we used the chi-square test to evaluate HWE in control groups.[12] A *P*-value of <0.05 for HWE was considered statistically significant, indicating a violation of HWE. The strength of connection between eNOS G894T gene polymorphism and PIH was evaluated by odds ratios (ORs) with 95% confidence intervals (CIs). Pooled ORs were calculated for allelic contrast (G *vs.* T), recessive model (TT *vs.* GT+GG), dominant model (GT+TT *vs.* GG), homozygote contrast (TT *vs.* GG) and heterozygote contrast (GT *vs.* GG). Therefore, the Bonferroni correction was applied to correct the problem of multiple comparisons by adjusting the alpha significance level.[13] To be more precise, the usual significance level ($a=0.05$) divided by 5 was used in those five comparisons. Therefore, a *P*-value of <0.01 was considered statistically significant in the study.[14] The *Z*-test was performed to determine the significance of the pooled ORs; a 95% CI that included 1 indicated no statistical significance. Subgroup analyses were performed in different ethnicities including Asians, Europeans, Americans, Latin Americans and Africans. A chi-square-based Q test was applied to check the heterogeneity among studies. A *P*-value above 0.10 indicated no significant heterogeneity among studies, and thus, a fixed-effect model (the Mantel–Haenszel method) was applied in the meta-analysis. Otherwise, the random-effects model (the DerSimonian and Laird method) was used.[15,16]

Publication bias among included studies was determined by Begg's funnel plot and Egger's linear regression test. A symmetrical inverted funnel plot strongly suggested no publication bias through visual examination, which took the natural logarithm of OR as the abscissa and the standard error of the natural logarithm of OR as the vertical axis.[17] Egger's test provided statistical evidence of publication bias, and a *P*-value of above 0.05 was considered to represent no significant publication bias.[18] The intercept a represented the

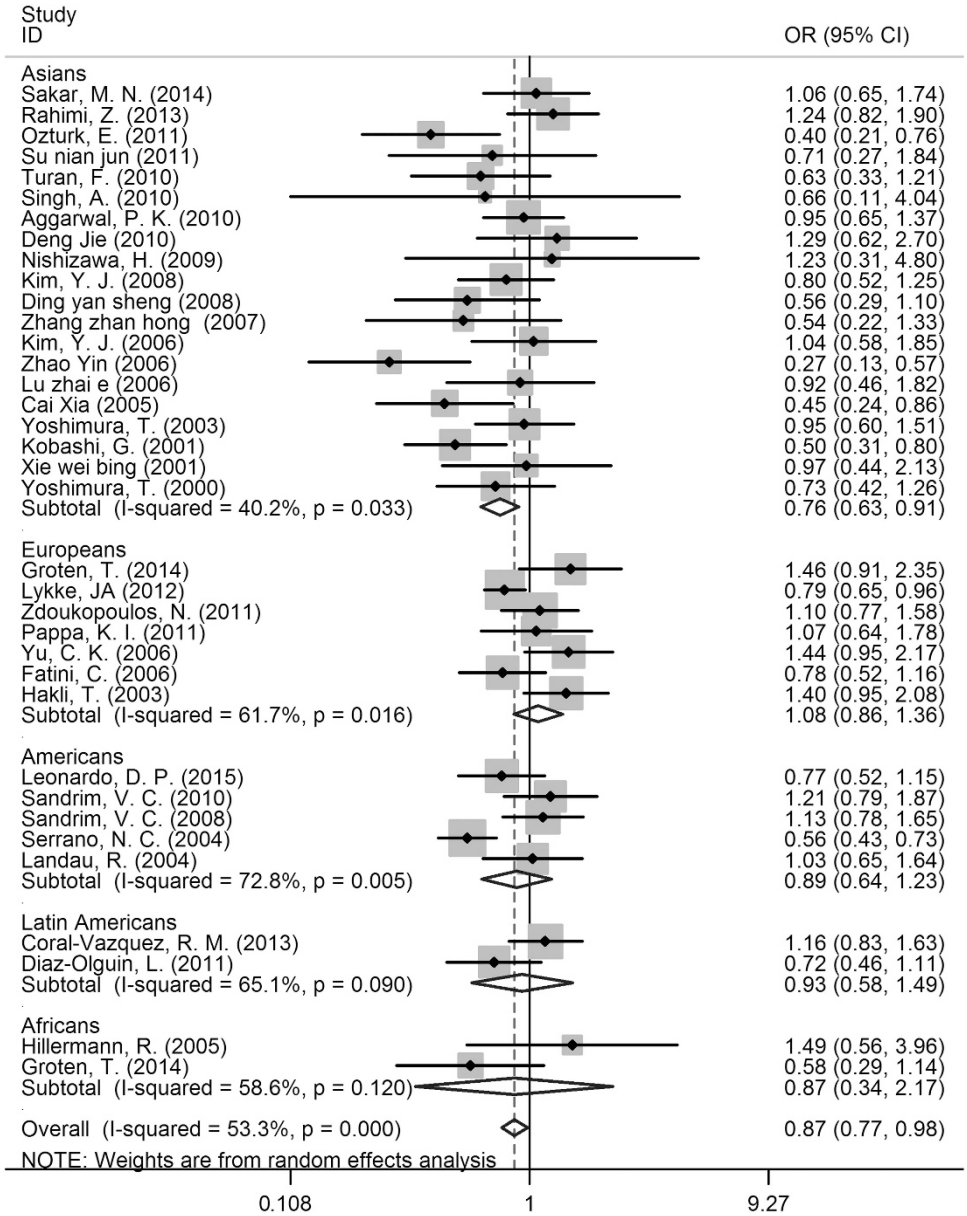

Figure 2 Forest plots for the association between eNOS G894T gene polymorphism and pregnancy-induced hypertension (PIH) of genotype models: for G *vs.* T. CI, confidence interval; *I*-squared, inconsistency; OR, odds ratio. A full color version of this figure is available at *Hypertension Research* journal online.

degree of asymmetry: the more it deviated from zero, the more obvious the asymmetry was. Sensitivity analysis was conducted to explore an individual study's effect on the pooled estimate by removing each study. All statistical analyses were performed using Stata version 12.0 (Stata Corp, College Station, TX, USA).

RESULTS

Characteristics of eligible studies

A total of 976 articles were identified in the keyword search (PubMed: 399; Cochrane Library: 9; Embase: 281; CBM: 90; CNKI: 106; WF: 91). As shown in Figure 1, 35 publications met the inclusion criteria. In the article reported by Groten *et al*,[19] the genotype frequencies in Germany and Ghana were presented separately, so we regarded these data as separate studies in our meta-analysis. Therefore, a total of 36 case–control studies involving 4028 PIH cases and 7672 controls were ultimately included in the meta-analysis. Among the 36 studies, 20 studies were conducted in subjects of Asian descent, 7 in European descent, 5 in American descent, 2 in Latin American descent and 2 in African descent. Several genotyping methods were summarized in the studies, including PCR-restriction, PCR allele-specific oligonucleotide (PCR-ASO), PCR-fragment length polymorphism (PCR-RFLP) and TaqMan (Supplementary Table S1). The results of the HWE test for genotype frequencies in the controls are shown in Supplementary Table S1, and they were all consistent with HWE. The quality scores of the included studies ranged from 5 to 8, and 6 studies scored more than 7 points, whereas 12 studies scored only 5 points (Supplementary Table S2).

Quantitative synthesis

The main results of the meta-analysis for eNOS gene G894T polymorphism and PIH are described in Table 1. We first assessed

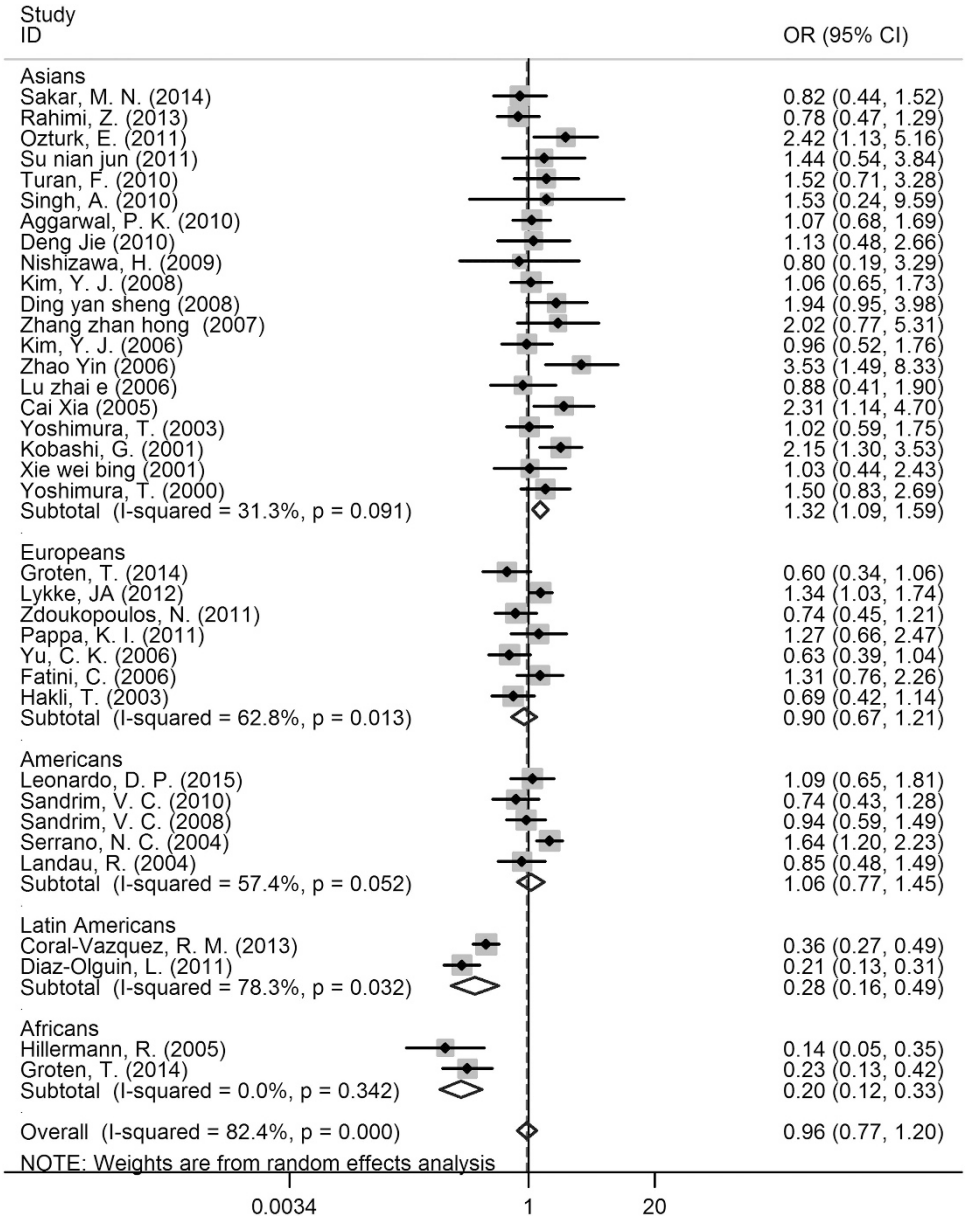

Figure 3 Forest plots for the association between eNOS G894T gene polymorphism and pregnancy-induced hypertension (PIH) of genotype models: for GT+TT *vs.* GG. CI, confidence interval; *I*-squared, inconsistency; OR, odds ratio. A full color version of this figure is available at *Hypertension Research* journal online.

the association among the overall population and then analyzed this association in different ethnicities.

When all included studies were pooled in the meta-analysis, we observed no significant association between eNOS gene G894T polymorphism and PIH in any of the genetic models (G *vs.* T: OR = 0.87, 95% CI = 0.77–0.99, $P = 0.024$; GT+TT *vs.* GG: OR = 0.96, 95% CI = 0.77–1.20, $P = 0.718$; TT *vs.* GT+GG: OR = 1.41, 95% CI = 1.05–1.88, $P = 0.021$; TT *vs.* GG: OR = 1.39, 95% CI = 1.02–1.91, $P = 0.039$; GT *vs.* GG: OR = 1.09, 95% CI = 0.96–1.24, $P = 0.182$) (Figures 2 and 3).

In Asian populations, 20 studies involving 1943 PIH cases and 2582 controls were included. A significant association was shown between eNOS G894T gene polymorphism and PIH risk in all genetic models (G *vs.* T: OR = 0.76, 95% CI = 0.63–0.91, $P = 0.002$; GT+TT *vs.* GG: OR = 1.32, 95% CI = 1.09–1.59, $P = 0.004$; TT *vs.* GT+GG: OR = 1.96, 95% CI = 1.26–3.06, $P = 0.003$; TT *vs.* GG: OR = 1.99, 95% CI = 1.27–3.11, $P = 0.003$; GT *vs.* GG: OR = 1.23, 95% CI = 1.05–1.43, $P = 0.009$).

In Latin American and African populations, the results indicated that G894T polymorphism was significantly associated with the risk of PIH only in the dominant model.

In the American population, no evidence of association between G894T polymorphism and susceptibility to PIH was detected in any of the genetic models (G *vs.* T: OR = 0.89, 95% CI = 0.64–1.23, $P = 0.467$; GT+TT *vs.* GG: OR = 1.06, 95% CI = 0.77–1.45, $P = 0.734$; TT *vs.* GT+GG: OR = 1.74, 95% CI = 0.77–3.96, $P = 0.185$; TT *vs.* GG: OR = 1.68, 95% CI = 0.71–3.99, $P = 0.240$; GT *vs.* GG: OR = 1.04, 95% CI = 0.84–1.28, $P = 0.732$).

Table 2 Egger's linear regression test to evaluate the funnel plot symmetry

	Y axis intercept: a (95% CI)									
	G vs. T		*GT+TT vs. GG*		*TT vs. GT+GG*		*TT vs. GG*		*GT vs. GG*	
Groups	a *(95% CI)*	P-*value*	a *(95% CI)*	P-*value*	a *(95% CI)*	P-*value*	a *(95% CI)*	P-*value*	a *(95% CI)*	P-*value*
Overall	−0.23 (−1.52, 1.06)	0.715	0.80 (−1.48, 3.09)	0.481	0.45 (−0.53, 1.42)	0.357	0.42 (−0.65, 1.49)	0.427	−0.09 (−1.34, 1.16)	0.883
Asian	−1.11 (−3.01, 0.79)	0.236	1.00 (−0.92, 2.92)	0.290	1.79 (0.47, 3.10)	0.013	1.97 (0.59, 3.35)	0.010	0.73 (−1.18, 2.63)	0.432
Europeans	3.22 (−0.14, 6.57)	0.057	−2.86 (−7.37, 1.64)	0.163	−1.78 (−3.44, −0.11)	0.041	−2.15 (−4.11, −0.20)	0.036	−2.47 (−7.04, 2.10)	0.223
Americans	7.32 (−0.76, 15.41)	0.063	−5.51 (−8.68, −2.33)	0.012	−18.87 (−59.10, 21.35)	0.232	−22.33 (−59.11, .44)	0.149	−4.35 (−5.68, −3.03)	0.002

Abbreviations: CI, confidence interval; OR, odds ratio; *P*, *P*-value for Egger's test; *vs.*, versus.

In the subgroup of Europeans, there was also no evidence of association between G894T polymorphism and susceptibility to PIH in any of the genetic models (G *vs.* T: OR = 1.08, 95% CI = 0.86–1.36, *P* = 0.489; GT+TT *vs.* GG: OR = 0.90, 95% CI = 0.67–1.21, *P* = 0.478; TT *vs.* GT+GG: OR = 1.09, 95% CI = 0.83–1.43, *P* = 0.555; TT *vs.* GG: OR = 0.97, 95% CI = 0.61–1.52, *P* = 0.876; GT *vs.* GG: OR = 0.90, 95% CI = 0.66–1.21, *P* = 0.476).

Publication bias diagnostics

Begg's funnel plot and Egger's linear regression test were applied to assess the publication bias of the included studies. The results of Egger's linear regression test are listed in Table 2. The visible symmetrical funnel shape revealed no obvious publication bias in the overall meta-analysis, which was consistent with the results of Egger's test (G *vs.* T: *P* = 0.715; GT+TT *vs.* GG: *P* = 0.481; TT *vs.* GT +GG: *P* = 0.357; TT *vs.* GG: *P* = 0.427; GT *vs.* GG: *P* = 0.883). In the subgroup analysis, evidence of publication bias detected by Egger's test existed in Asians (TT *vs.* GT+GG: *P* = 0.013; TT *vs.* GG: *P* = 0.010), Europeans (TT *vs.* GT+GG: *P* = 0.041; TT *vs.* GG: *P* = 0.036) and Americans (GT+TT *vs.* GG: *P* = 0.012; GT *vs.* GG: *P* = 0.002). However, due to the small number of studies in some subgroups, Egger's linear regression test was not performed.

Sensitivity analysis

Sensitivity analysis was conducted to investigate the effects of each individual study on the overall estimate by removing a single study at a time from the meta-analysis. The results suggested that no single study affected the overall pooled OR.

DISCUSSION

In the present meta-analysis, 36 eligible studies with 4028 PIH cases and 7672 controls were identified and carefully analyzed. In the subgroup analysis of Asian populations, the results in all genetic models indicated that G894T polymorphism was significantly associated with PIH. In African and Latin American populations, we only found evidence of an association in the dominant model. However, in Americans and Europeans, no significant risk of PIH was detected.

In recent years, several studies have focused on clarifying the role of eNOS gene single-nucleotide polymorphisms and their influence on susceptibility to PIH. Meta-analyses of these studies have reported conflicting results. Yu *et al.*[20] performed a meta-analysis of nine studies of the association between G894T and PE and found no significant risk of PE. Medica *et al.*[21] reported a meta-analysis of nine studies of G894T and PE (1055 patients and 1788 controls); their results showed a significantly increased risk in the recessive model (TT *vs.* GT+GG: OR = 1.68, 95% CI = 1.07–2.64, *P* = 0.0239). The meta-analysis of 15 studies conducted by Shaik *et al.*[22] did not find any significant associations between G894T and PE. Chen *et al.*[23] performed a meta-analysis of 13 studies on G894T and PE. Their results suggested that all studies showed significant associations in the dominant model, but the association was not significant for Asians (GT+TT *vs.* GG: OR = 1.09, 95% CI = 0.82–1.45). Qi *et al.*[24] described the relationship of G894T and PE in 26 studies and found that TT was significantly associated with PE risk (TT *vs.* GT+GG: OR = 1.43, 95% CI = 1.13–1.82, *P* = 0.003). In the meta-analysis by Dai *et al.*,[25] no significant association between G894T and PE was found (GT+TT *vs.* GG: OR = 1.07, 95% CI = 0.87–1.30, *P* = 0.52; TT *vs.* GT+GG: OR = 1.25, 95% CI = 0.96–1.63, *P* = 0.10; T *vs.* G: OR = 1.07, 95% CI = 0.89-1.29, *P* = 0.50). Zeng *et al.*[26] performed a meta-analysis of 30 eligible studies with 3503 cases and 6843 controls and showed that the

TT genotype of G894T was associated with an increased risk of PE (TT *vs.* GT+GG: OR = 1.46, 95% CI = 1.21–1.77, $P < 0.001$). While the previous meta-analyses focused on PE, we paid attention to all stages of PIH in this study.

PIH is the most frequent obstetrical complication during pregnancy and includes gestational hypertension, PE and eclampsia. The typical clinical manifestations of PIH include increased blood pressure and proteinuria.[27] The etiology and pathogenic mechanisms of PIH are complex and relate to placental ischemia, oxidative stress, inflammatory activation and genetic involvement.[28] NO, an important endothelium-derived relaxing factor, is mainly produced by the catalytic action of NOS, especially eNOS and NOS1 in the myometrium and placenta.[29] NO is involved in regulating endothelial cell function, blood pressure homeostasis, vasoconstriction and vasodilatation.[5] Furthermore, Metzger *et al.*[30] reported that individual eNOS polymorphisms might have a significant influence on NO formation within a specific haplotype. Bernardi *et al.*[31] indicated that the NO level and oxidative stress decreased in women with PE due to excessive arginase and low superoxide dismutase activity. The variant within exon 7 of the eNOS gene, that is, G-to-T conversion at nucleotide position 894, led to replacement of glutamic acid with aspartic acid at codon 298 (Glu298Asp). Savvidou *et al.*[32] and Leeson *et al.*[33] found that the Glu298Asp polymorphism was associated with endothelial function. In addition, the eNOS Asp298 might reduce NO bioavailability.[34]

Study strengths and limitations

Strengths of the current study include the large sample size, with 4028 PIH cases and 7672 controls, as well as five ethnicities including Asians, Europeans, Americans, Latin Americans and Africans. Meanwhile, the alpha significance level was adjusted to avoid the effect of multiple comparisons. Therefore, our systematic evaluation included more publications and demonstrated a significant association between G894T polymorphism and susceptibility to PIH.

Despite the considerable efforts to determine the association between G894T polymorphism and PIH risk, certain potential limitations should not be ignored. First, PIH is a multifactorial disease resulting from complex interactions between environmental and genetic factors. Most of the included case–control studies lacked environmental information such as living habits and nutritional status. Furthermore, the results were only based on unadjusted analysis. Publication bias existed in some of the subgroup analyses, which might interfere with the reliability of the meta-analysis. PIH has different clinical stages and various degrees of severity, and we could not extract the data on different subclinical forms of PIH in some of the included studies. Moreover, even though the studies focused on a certain clinical type of PIH, there was no severity classification. In addition, publication bias could be attributed to the small sample size and the number of studies in some subgroups, especially Americans. Finally, we only reviewed the studies in English and Chinese and only received detailed data from published studies.

CONCLUSIONS

The results of the current meta-analysis suggest that the eNOS G894T polymorphism was significantly associated with PIH in Asian populations. The results showed that the T-allele carriers had a higher risk of PIH than those with the G allele in Asians. For Latin American and African populations, we only explored the association in the dominant model. However, we did not observe any association in Americans and Europeans. Further well-designed large case–control studies or cohort studies are needed to verify our results.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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