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# Endothelial NO Synthase Gene Polymorphisms and Hypertension

## A Meta-Analysis

Elias Zintzaras, Georgios Kitsios, Ioannis Stefanidis

**Abstract**—Studies investigated the association between endothelial NO synthase gene polymorphisms and hypertension-reported contradicted or nonconclusive results. A meta-analysis of 35 genetic association studies that examined the relation between hypertension and the G894T, 4a/b, T786C, and G23T polymorphisms of the endothelial nitric oxide synthase gene was carried out. Subgroup analysis by ethnicity and potential sources of heterogeneity and bias were explored. The meta-analysis included genotype data on 7779/10 498, 2216/3222, 2491/3913, and 833/587 cases/controls for G894T, 4b/a, T786C, and G23T, respectively. For the 4b/a polymorphism, overall, the heterogeneity between studies was not significant ( $P=0.82$ ), and the allele b was associated with a 15% decreased risk of hypertension relative to allele a (odds ratio: 0.85; 95% CI: 0.74 to 0.98). Overall and in whites, the recessive model for allele b produced significant results (odds ratios: 0.78; 95% CI: 0.68 to 0.90 and OR: 0.76 95% CI: 0.62 to 0.92, respectively), whereas the dominant model produced nonsignificant results. In studies involved East Asians and blacks, an association was not demonstrated. Regarding the G894T, T786C, and G23T polymorphisms, in no case (ie, overall, in whites, or in East Asians) was a statistically significant association and heterogeneity found. There was no substantial source of bias in the selected studies. In conclusion, there is evidence of association only between 4b/a polymorphism and hypertension; however, studies exploring combinations of the polymorphisms may help us better understand the genetics of hypertension. (*Hypertension*. 2006;48:700-710.)

**Key Words:** nitric oxide ■ polymorphism ■ meta-analysis

Hypertension is a multifactorial disease involving both environmental and genetic components.<sup>1</sup> Clinical and animal studies have suggested that an alteration in NO metabolism may be a contributing factor in the pathogenesis of hypertension.<sup>2-4</sup> Thus, abnormalities in the activity of the enzyme endothelial NO synthase (eNOS) that synthesize NO in endothelial cells may lead to NO deficiency. The gene encoding eNOS is located on chromosome 7 (7q35-q36) and contains 26 exons with an entire length of 21kb. G894T (Glu298Asp in exon 7), intron 4 (4b/a), (T-786)C (T786C), and G23T are the most clinically relevant polymorphisms in the *eNOS* gene that have been described.<sup>5</sup>

The genetic association studies that examined whether the polymorphisms in the *eNOS* gene are associated with hypertension have provided controversial or inconclusive results, partly because each study involved few cases and few controls and, therefore, there was not enough information to demonstrate association. Furthermore, the interpretation is complicated by the fact that different populations, sampling strategies, genotyping procedures, and number of loci included in the analyses have been used. To shed some light on these contradictory results,

as well as to decrease the uncertainty of the effect size of estimated risk, a meta-analysis<sup>6</sup> of all of the available studies related the G894T (Glu298Asp), 4b/a, (T-786)C (T786C), and G23T polymorphisms and their associations with hypertension for various genetic contrasts was carried out. In addition, the heterogeneity between studies and the existence of potential bias were explored. Cumulative and recursive cumulative meta-analyses were also performed.

## Methods

### Selection of Studies

All of the studies published before May 2006 were identified by extended computer based searches of PubMed and EMBASE databases. The following search criterion was used: (“eNOS” or “endothelial nitric oxide synthase” or “NOS3”) and “polymorphism” and (“hypertension” or “blood pressure”). The retrieved studies were then read in their entirety to assess their appropriateness for inclusion in the meta-analysis. All of the references cited in the studies were also reviewed to identify additional published work not indexed by the PubMed and EMBASE databases. Case reports, editorials, and review articles were excluded. The search was restricted to articles in English.

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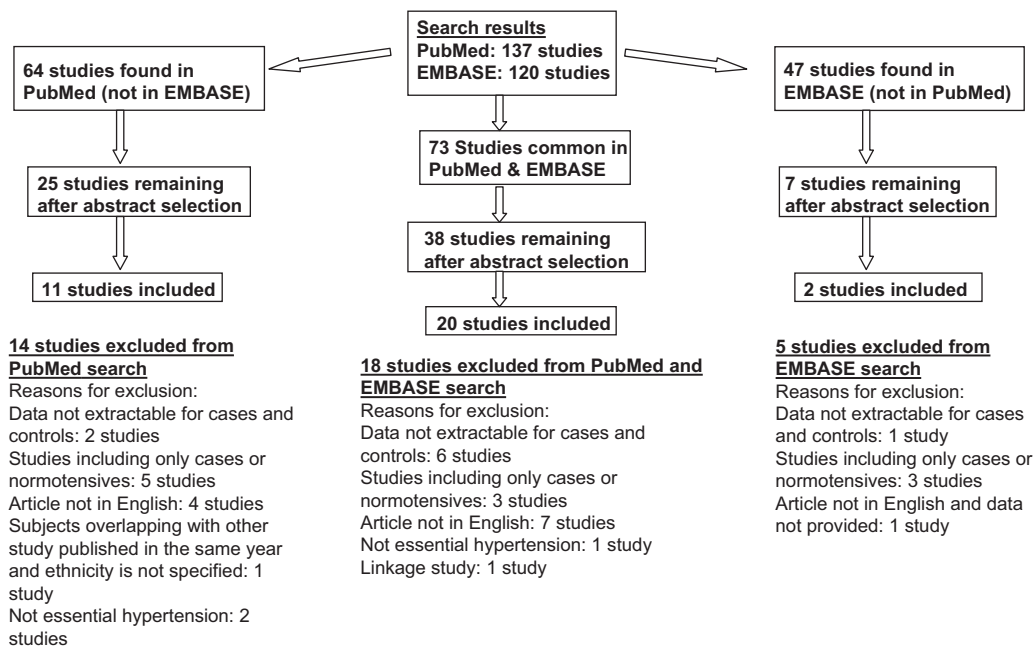
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**Figure 1.** Flow chart of retrieved studies and studies excluded, with specification of reasons.

Case-control studies that determined the distribution of G894T, 4b/a, T786C, and G23T genotypes in cases with essential hypertension and in a healthy/normotensive control group were eligible for inclusion in the meta-analysis. Case subjects were considered to be hypertensive if their systolic or diastolic blood pressure was  $\geq 140$  mm Hg or  $\geq 90$  mm Hg, respectively; were reported to have hypertension; or were on antihypertensive therapy. In studies with overlapping cases/controls, the most recent study, the largest in size study, or the study with more information on origin of cases/controls was included in the meta-analysis. Only studies in human subjects that have used validated genotyping methods were considered. Finally, genome scans were excluded because they investigate linkage.<sup>7,8</sup>

## Data Extraction

From each study, the following information was abstracted: first author, journal, year of publication, ethnicity of the study population, demographics, clinical characteristics, matching, validity of the genotyping method, and the number of cases and controls for each G894T, 4b/a, T786C, and G23T genotype. The frequencies of the alleles and the genotypic distributions were extracted or calculated for both the cases and the controls. In addition, it was recorded whether the genotyping in each study was blinded to clinical status. When studies investigated  $>1$  polymorphism, information on linkage disequilibrium and haplotype estimation was recorded.

## Meta-Analysis

Before the main analysis, the significance of association for: (1) the allele contrast (G894T G versus T, 4b/a b versus a, T786C T versus C, and G23T G versus T), (2) the recessive (G894T GG versus GT+TT, 4b/a bb versus ba+aa, T786C TT versus TC+CC, and G23T GG versus GT+TT), and (3) dominant models (G894T GG+TG versus TT, 4b/a bb+ba versus aa, T786C TT+TC versus CC, and G23T GG+GT versus TT) were evaluated for each study separately. All of the associations were indicated as odds ratios (ORs) with the corresponding 95% CI. Based on the individual ORs, a pooled OR was estimated.

The heterogeneity between studies in terms of degree of association was tested using the Q-statistic, which is the weighted sum of squares deviations of the OR study estimates from the pooled estimate.<sup>8-10</sup> When the ORs are homogeneous, Q follows a  $\chi^2$

distribution with  $r-1$  ( $r$  is the number of studies) degrees of freedom. If  $P < 0.10$ , then the heterogeneity was considered significant. Heterogeneity was quantified with the  $I^2$  metric [ $I^2 = (Q - d.f.) / Q$ ], which is independent of the number of studies in the meta-analysis.<sup>11</sup>  $I^2$  takes values between 0% and 100% with higher values denoting greater degree of heterogeneity ( $I^2 = 0\%$  to 25%: no heterogeneity;  $I^2 = 25\%$  to 50%: moderate heterogeneity;  $I^2 = 50\%$  to 75%: large heterogeneity;  $I^2 = 75\%$  to 100%: extreme heterogeneity). The pooled OR was estimated using fixed effects (FEs; Mantel-Haenszel) and random effects (REs; DerSimonian and Laird) models.<sup>12</sup> The calculation of pooled OR and Q used as weighting factor ( $w_i$ ) the inverse variance of  $\theta_i = \ln OR$  (ie,  $w_i = 1/\text{var}[\theta_i]$ ) of each study  $i$ . RE modeling assumes a genuine diversity in the results of various studies and incorporates to the calculations a between-study variance. Therefore, when there is heterogeneity between studies, the pooled OR was estimated using the RE model.

A cumulative and recursive cumulative meta-analysis was carried out for each polymorphism to evaluate the trend of RE OR for the genetic contrast under investigation in time.<sup>13,14</sup> In cumulative meta-analysis, studies were chronologically ordered by publication year, then the pooled OR was obtained at the end of each year, that is, at each information step. In recursive cumulative meta-analysis, the relative change in pooled OR in each information step (OR in next year/OR in current year) was calculated. Thus, cumulative and recursive cumulative meta-analysis provide a framework for updating a genetic effect from all of the studies and a measure of how much the genetic effect changes as evidence accumulates.

A differential magnitude of effect in large versus small studies for the genetic contrast under investigation was checked using the Egger regression test for funnel plot asymmetry and the Begg-Mazumdar test, which is based on Kendall's  $\tau$ .<sup>15-18</sup> Given that these tests are underpowered, they were always considered statistically significant for  $P < 0.10$  rather than for  $P < 0.05$ .<sup>15,16</sup>

In addition to the main (or overall) analysis, which included all of the available data, subgroup analysis for each ethnicity was also performed. Ethnicity was categorized into 2 main groups: (1) white descents, and (2) East Asian descents.<sup>19</sup> However, the consistency of genetic effects across these traditionally defined ethnicities does not necessarily mean that ethnicity-specific genetic effects are exactly the same. The distribution of the genotypes in the control group was tested for Hardy-Weinberg equilibrium (HWE;  $P \geq 0.05$ ).<sup>7</sup> Studies with controls not in HWE were subjected to a sensitivity analysis.<sup>20</sup> In sensitivity analysis, the effect of excluding specific studies was

TABLE 1. Characteristics of Eligible Studies Considered in the Meta-Analysis

Reference	Ethnicity	Polymorphisms	Cases				
			No. (M/F)	Age, y, mean±SD	BMI, mean±SD	SBP, mm Hg, mean±SD	DBP, mm Hg, mean±SD
52	White	G23T	198 (97/101)	53.0±10.4	25.4±3.4	161±22	100±14
24	White	G894T, G23T	309 (212/97)	49.4±1.2	25.7±0.4	157.0±1.5	95.8±1.2
25	East Asian	G894T 4b/a, G23T	623 (346/277) 218	56.3±11.6	23.8±5	162±18	93.5±13.8
41	East Asian	4b/a	123 (75/48)	53.53±10.1	24.26±3.85	178.4±22.8	106.7±14.4
26	East Asian	G894T	549 (305/244)	63.1±9.8	23.7±2.8	164.0±18.2	100.8±9.2
27	White	G894T	91 (nr)	nr	nr	nr	nr
		4b/a	112 (nr)				
28	East Asian	G894T, 4b/a	183 (83/100)	56.9±9.6	25.4±3.5	160±20	95±11
50	East Asian	T786C	401 (230/171)	56.5±11.5	23.9±5.1	161.8±18.5	93.6±13.7
44	East Asian	4b/a	107 (82/25)	53.1±0.91	nr	nr	nr
29	East Asian	G894T, T786C	1539 (nr)	60.1±12.3*	22.7±2.9*	nr	nr
38	White	G894T	119 (82/37)	59.4±15.6	26.8±3.6	144.3±14.1	90.7±7.8
39	White	G894T	116	nr	nr	nr	nr
23	White	G894T	43 (nr)	29.3±5.0*	26.4±5.8*	nr	nr
	Blacks	G894T	49 (nr)	28.6±5.2*	29.4±8.0*	nr	nr
45	East Asian	4b/a	80 (nr)	59.7±10.1*	23.4±2.2*	nr	nr
30	White	G894T	505 (248/257)	51.4±6.1	28.7±4.8	154.8±20.2	93.2±10.8
4	White	T786C	106 (106/0)	53±11.4	nr	151±13	94±10
53	White	G23T	108 (74/34)	nr	nr	nr	nr
42	White	4b/a	217	59±9	28.22±3.49	148±26	86±14
51	White	T786C	137 (115/22)	49±9	26.4±2.7	151±11	99±5
46	White	4b/a	82 (0/82)	35.5±8.9	nr	nr	nr
47	East Asian	4b/a	110 (nr)	54.4±8.6*	23.1±3.1*	nr	nr
32	White	G894T, T786C	105 (71/34)	52.08±8.31	34.27±6.21	nr	nr
31	East Asian	G894T	135 (nr)	37.1±8.5*	22.2±2.7*	nr	nr
40	Arabs	G894T	20 (nr)	nr	nr	nr	nr
33	White	G894T	172 (115/57)	49.2±12.1	26.9±3.4	nr	nr
34	White	G894T	2229 (1333/896)	57.4±14.3	28.9±4.6	151.7±17.6	90.0±10.8
35	White	G894T	79 (nr)	54.3±12.3	nr	nr	nr
		4b/a	109 (66/43)				
36	White	G894T, 4b/a	93 (46/47)	44.2±4.7	27.3±3.9	nr	nr
43	White	4b/a	255 (144/111)	64.6±9.9	29.3±5.2	148±22	84±11
48	Turks	4b/a	129 (53/76)	45.0±6.4	nr	nr	nr
49	East Asian	4b/a	195 (90/105)	nr	nr	nr	nr
1	White, black	G894T, T786C 4b/a	112 (44/68)	nr	nr	nr	nr
		G894T 4b/a-T786C	91 (37/54)				
37	Mixed	G894T	617	45.2±11.0*	26.0±4.8*	nr	nr

(Continued)

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; nr, not reported.

\*Data concerned both cases and controls.

examined. Analyses were performed using Meta-Analyst (Lau), StatsDirect (Microsoft Corp), and CVF90 with the Institute of Museum and Library Sciences library.<sup>21,22</sup>

## Results

### Eligible Studies

The literature review identified 137 titles in PubMed and 120 in EMBASE that met the search criteria. The full

articles of the retrieved studies were read to assess their appropriateness for meta-analysis according to the inclusion criteria. Data from 33 articles that investigated the association between any of the G894T, 4a/b, T786C, and G23T polymorphisms and hypertension met the inclusion criteria, and they were included in the meta-analysis. Figure 1 presents a flow chart of retrieved studies and studies excluded, with specification of reasons. Two arti-

TABLE 1. (Continued)

Controls				
No. (M/F)	Age, y, mean±SD	BMI, mean±SD	SBP, mm Hg, mean±SD	DBP, mm Hg, mean±SD
106 (47/59)	44.3±9.5	22.4±2.5	123±10	76±8
123 (77/55)	44.3±2.1	24.4±0.7	123.7±2.0	76.5±1.4
703 (403/700) 240	54.2±13.2	23.1±2.7	125.8±11.9	76.9±10.6
120 (89/31)	51.5±7.2	22.96±2.89	115.9±12.4	70.9±10.6
513 (310/203)	54.8±11.3	22.4±2.8	117.2±12.7	75.0±9.2
149 (nr) 184 (nr)	nr	nr	nr	nr
193 (67/126)	54.4±11.9	23.2±3.0	125±10	76±8
456 (267/189)	54.0±13.2	23.0±2.6	125.5±11.3	73.7±9.6
362 (248/114)	50.5±0.44	nr	nr	nr
2516 (nr)	60.1±12.3*	22.7±2.9*	nr	nr
85 (48/37)	55.6±14.3	26.5±3.3	125.7±12.0	81.2±7.1
222	nr	nr	nr	nr
678 (nr)	29.3±5.0*	26.4±5.8*	nr	nr
251 (nr)	28.6±5.2*	29.4±8.0*	nr	nr
75 (nr)	59.7±10.1*	23.4±2.2*	nr	nr
519 (257/262)	51.9±6.2	26.5±4.2	142.8±20.7	85.8±10.7
599 (599/0)	44±8.3	nr	125±15	81±10
118 (nr)	nr	nr	nr	nr
202	57±8	26.61±3.55	127±18	75±10
48 (30/18)	42±16	23.8±3.2	123±10	78±7
188 (188F)	33.6±7.8	nr	nr	nr
722 (nr)	54.4±8.6*	23.1±3.1*	nr	nr
94 (nr)	nr	nr	nr	nr
789 (nr)	37.1±8.5*	22.2±2.7*	nr	nr
10 (nr)	37±7	nr	nr	nr
200 (117/83)	45.3±11.3	25.3±3.1	nr	nr
1990 (758/1232)	42.4±14.9	25.4±4.2	120.9±10.9	76.8±6.9
102 (60/42) 84 (nr)	38±1.5	nr	nr	nr
201 (89/112)	31.5±4.7	24.5±3.9	nr	nr
140 (81/59)	60.3±11.8	27.6±4.7	131±12	79±8
129 (70/59)	35.6±6.8	nr	nr	nr
182 (74/108)	48±10	nr	nr	nr
113 (78/35)	nr	nr	nr	nr
87 (56/31)				
960	45.2±11.0*	26.0±4.8*	nr	nr

cles<sup>1,23</sup> provided data on 2 separate study ethnicities: whites and blacks. Thus, data were obtained from 35 studies. Data were extracted by 2 investigators (G.K. and I.S.), and disagreements were resolved after a discussion between the 3 authors. Twenty-one studies dealt with G894T,<sup>1,23-40</sup> 16 with 4a/b,<sup>1,25,27,28,35,36,41-49</sup> 7 with T786C,<sup>1,4,29,32,50,51</sup> and 4 with G23T.<sup>24,25,52,53</sup> Seven studies in 4a/b,<sup>1,25,27,28,35,36</sup> 4 studies in T786C,<sup>1,29,32</sup> and 2 studies in G23T<sup>24,25</sup> also investigated G894T. Two studies<sup>1</sup> investigated 3 polymorphisms together: G894T,

4a/b, and T786C. The design in 24 studies was case-control,\* in 7 studies was population-based cohort,<sup>4,29,31,36,43,45</sup> and in 4 studies was cross-sectional.<sup>23,37,34,47</sup> The studies were published between 1995 and 2006.

For the determination of the genetic polymorphisms of G894T, T786C, and G23T, validated genotyping methods were used in all of the studies; namely, PCR and restriction of the

\*References 1, 24-28, 30, 32, 33, 35, 38-42, 44, 46, 48-53.

PCR product with the enzyme corresponding to each polymorphism† or PCR with allele-specific probes.<sup>23,24,29,32,51,52</sup> The polymorphism 4a/b was genotyped using electrophoretic differentiation.<sup>1,25,27,28,35,36,41–49</sup> Five studies stated that the controls were age or sex matched.<sup>29,30,38,48,53</sup> Studies were conducted in various populations of racial descent: 19 involved whites,‡ 11 involved East Asians,§ 2 involved blacks,<sup>1,23</sup> 1 involved Turks,<sup>48</sup> 1 involved Arabs,<sup>40</sup> and 1 was mixed.<sup>37</sup> One study involved male cases,<sup>4</sup> 1 study involved female cases,<sup>46</sup> and 2 studies<sup>24,33</sup> provided data for males and females separately. A list of details abstracted from the studies included in the meta-analysis is provided in Tables 1 through 5.

### Summary Statistics

Overall, the studies provided 7779/10 498 cases/controls for G894T, 2216/3222 cases/controls for 4b/a, 2491/3913 cases/controls for T786C, and 833/587 cases/controls for G23T. Eleven studies<sup>23,25,28,35,47,50</sup> did not provide data for all of the genotypes separately: 5 studies<sup>23,25,28,35</sup> provided data on G894T T-carriers and non-T-carriers, 4 studies<sup>25,28,35,47</sup> provided data on 4b/a a-carriers and non-a-carriers, 1 study<sup>50</sup> provided data on T786C C-carriers and non-C-carriers, and 1 study<sup>25</sup> provided data on G23T T-carriers and non-T-carriers. Two studies provided data only for allele frequency.<sup>38,44</sup> The genotype distributions and the allele frequencies are shown in Tables 2 through 5. The distribution of genotypes in the control group deviated from HWE in 1 study for G894T<sup>24</sup> (Table 2); however, HWE deviation could not be tested for all of the studies.<sup>23,25,28,35,47,50</sup> Because lack of HWE indicates possible genotyping errors and/or population stratification, a sensitivity analysis was, therefore, carried out excluding this study. In addition, articles where HWE could not be assessed were treated as studies that deviate from HWE in the sensitivity analysis. Although the majority of studies reviewed in this meta-analysis reported on >2 of the investigated polymorphisms, only 3 of them provided analyses of haplotypes.<sup>1,32</sup> One study<sup>27</sup> reported the existence of linkage disequilibrium between G894T and 4b/a, 1 study<sup>25</sup> between 4b/a and G23T, and 2 studies between G894T and T786C,<sup>29,32</sup> whereas, 1 study<sup>50</sup> reported a lack of linkage disequilibrium between G894T and T786C.

### Main Results, Subgroup, and Sensitivity Analyses

Table 6 and Figure 2 show the results for the association between the different polymorphisms and the risk of hypertension. For the G894T polymorphism and its relationship to hypertension, the allele contrast G versus T showed nonsignificant heterogeneity among studies ( $P=0.13$ ;  $I^2=29\%$ ) and the FE and RE pooled ORs were not significant, at 1.00 (95% CI: 0.95 to 1.06) and 1.01 (95% CI: 0.93 to 1.10), respectively. In subgroup analysis, the association was not significant for whites and East Asians. The recessive and dominant models for the effect of allele G produced the same overall pattern as the allele contrast. The recessive model for blacks also showed no association.

For the 4b/a polymorphism, overall, the heterogeneity between studies was not significant ( $P=0.82$ ;  $I^2=0\%$ ), and the 4b/a allele b was associated with a 15% decreased risk of hypertension compared with the a allele (FE OR: 0.85; 95% CI: 0.74 to 0.98). In subgroup analysis, the association was marginally significant for whites (FE OR: 0.83; 95% CI: 0.69 to 0.99). Overall and in whites, the recessive model for allele b produced significant results (FE OR: 0.78; 95% CI: 0.68 to 0.90 and FE OR: 0.76; 95% CI: 0.62 to 0.92, respectively). However, for those studies involving East Asians, the RE pooled OR was not significant. The dominant model produced nonsignificant associations ( $P>0.05$ ).

Regarding the relationship between the T768C polymorphism and hypertension, the heterogeneity between studies was not significant ( $P=0.39$ ;  $I^2=4\%$ ), and the analysis did not detect an association for the allele contrast T versus C (FE OR: 0.92; 95% CI: 0.82 to 1.02). However, there is a trend for association in whites (FE OR: 0.84; 95% CI: 0.70 to 1.01). The recessive and dominant models produced nonsignificant associations overall in whites and East Asians.

Finally, in no case (ie, overall or in whites) was a statistically significant association between the G23T polymorphism and the risk of developing hypertension for the contrasts under investigation found. In addition, there is no significant heterogeneity among studies ( $P=0.17$ ;  $I^2=44\%$ ). The sensitivity analysis did not alter the pattern of results in all of the polymorphisms and contrasts.

### Potential Bias

None of the studies included in the meta-analysis stated that genotyping was performed blinded to clinical status. In cumulative meta-analysis for the recessive model, only 4b/a polymorphism showed evidence of an association as evidence accumulates (see Figure 3). In recursive cumulative meta-analysis for the recessive model, the relative change in OR for the G894T polymorphism fluctuated around the value of OR=1.0. The relative change in OR for 4b/a polymorphism showed a downward trend as evidence accumulates, whereas for T(-786)C and G23T it showed an increase (see Figure 4).

The Egger test and the Begg–Mazumdar test for the recessive model indicated that there is no differential magnitude of effect in large versus small studies for each polymorphism being investigated ( $P>0.10$ ); however, for 4b/a polymorphism there is indication of an effect: the Begg–Mazumdar test showed a significant result ( $P=0.03$ ), whereas the Egger test showed a marginal significance ( $P=0.09$ ; see Figure 5).

### Discussion

This meta-analysis examined the *eNOS* G894T, 4a/b, T786C, and G23T polymorphisms and their relationship to susceptibility for hypertension. The strength of the present analysis was based on the accumulation of published data giving greater information to detect significant differences. In the present study, the effect of allele frequency and the effects of the dominant and recessive models were estimated. In addition, the consistency of genetic effects across populations from different ethnicities was investigated. Main and subgroup analyses in whites and East Asians for the allele

†References 1, 4, 25, 27, 28, 30, 31, 33, 35–40, 50, 53.

‡References 1, 4, 23, 24, 27, 30, 32–36, 38, 39, 42, 43, 46, 51–53.

§References 25, 26, 28, 29, 31, 41, 44, 45, 47, 49, 50.

**TABLE 2. The Distribution of the G894T Genotype for Cases and Controls (in Parenthesis Are the Respective Percentages)**

All Studies, Reference	Distribution of G894T eNOS Genotype						Allele Frequency				P HWE Controls
	GG		GT		TT		G		T		
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	
24	140 (45)	35 (28)	122 (39)	67 (54)	47 (15)	21 (17)	402 (65)	137 (55)	216 (34)	109 (44)	0.02
25	492 (78)	631 (89)	131 (21)*	72 (10)*							na
26	461 (83)	433 (84)	84 (15)	76 (14)	4 (0)	4 (0)	1006 (91)	942 (91)	92 (8)	84 (8)	0.94
27	40 (43)	70 (46)	43 (47)	68 (45)	8 (8)	11 (7)	123 (67)	208 (69)	59 (32)	90 (30)	0.45
28	139 (75)	164 (84)	44 (24)*	29 (15)*							na
29	1300 (84)	2103 (83)	227 (14)	393 (15)	12 (0)	20 (0)	2827 (91)	4599 (91)	251 (8)	433 (8)	0.55
38							76 (64)	63 (74)	43 (36)	22 (26)	na
39	59 (51)	112 (51)	48 (41)	92 (41)	9 (8)	18 (8)					0.85
23, white	22 (51)	303 (45)	21 (49)*	678 (55)*							na
23, black	40 (82)	201 (80)	9 (18)*	50 (20)*							na
30	244 (48)	262 (50)	220 (43)	215 (41)	41 (8)	42 (8)	708 (70)	739 (71)	302 (29)	299 (28)	0.37
31	119 (88)	679 (86)	16 (11)	100 (12)	0 (0)	10 (1)	254 (94)	1458 (92)	16 (5)	120 (7)	0.46
32	45 (42)	38 (40)	42 (40)	36 (38)	18 (17)	20 (21)	132 (62)	112 (59)	78 (37)	76 (40)	0.15
40	4 (20)	5 (50)	10 (50)	5 (50)	6 (30)	0					0.93
33	84 (48)	93 (46)	71 (41)	88 (44)	17 (9)	19 (9)	239 (69)	274 (68)	105 (30)	126 (31)	0.73
34	1099 (49)	973 (48)	925 (41)	848 (42)	205 (9)	169 (8)	3123 (70)	2794 (70)	1335 (29)	1186 (29)	0.61
35	37 (46)	36 (35)	42 (53)*	66 (64)*							na
36	51 (54)	111 (55)	40 (43)	78 (38)	2 (2)	12 (5)	142 (76)	300 (74)	44 (23)	102 (25)	0.06
37	325 (52)	536 (55)	250 (40)	368 (38)	42 (6)	56 (5)	900 (72)	1440 (75)	334 (27)	480 (25)	0.51
1, white	48 (42)	53 (46)	54 (48)	53 (46)	10 (8)	7 (6)	150 (66)	159 (70)	74 (33)	67 (29)	0.36
1, black	57 (62)	45 (51)	31 (34)	36 (41)	3 (3)	6 (6)	145 (79)	126 (72)	37 (20)	48 (27)	0.65
Total	4633 (62)	6262 (67)	2342 (31)	2593 (28)	409 (5)	397 (4)	10151 (77)	13288 (80)	2943 (22)	3220 (19)	

The significant level (P) for HWE testing for controls is shown. na indicates not applicable.

\*Data concerned GT+TT.

**TABLE 3. The Distribution of the 4b/a Genotypes for Cases and Controls (in Parenthesis Are the Respective Percentages)**

All Studies, Reference	Distribution of 4b/a eNOS Genotype						Allele Frequency				P HWE Controls
	Bb		Ba		Aa		b		a		
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	
25	170 (77)	192 (80)	48 (22)*	48 (20)*							0.07
41	97 (78)	111 (92)	24 (19)	0 (0)	2 (1)	9 (7)	218 (88)	222 (92)	28 (11)	18 (7)	0.72
27	89 (79)	147 (79)	23 (20)	35 (19)	0 (0)	2 (1)	201 (89)	329 (89)	23 (10)	39 (10)	0.22
28	143 (78)	156 (80)	40 (21)*	37 (19)*							na
44							88 (82)	290 (80)	19 (18)	72 (20)	na
45	57 (71)	56 (75)	23 (29)	17 (23)	0	2 (2)					0.13
42	136 (62)	149 (73)	73 (33)	48 (23)	8 (3)	5 (2)	345 (79)	346 (85)	89 (20)	58 (14)	0.63
46	51 (62)	123 (65)	29 (36)	63 (34)	2 (2)	2 (1)	131 (80)	309 (82)	33 (20)	67 (18)	0.36
47	90 (82)	578 (80)	20 (18)*	144 (20)*							na
43	184 (72)	100 (71)	62 (24)	38 (27)	9 (3)	2 (1)	430 (84)	238 (85)	80 (15)	42 (15)	0.20
35	69 (63)	66 (78)	40 (36)*	18 (21)*							na
36	59 (63)	132 (65)	33 (35)	65 (32)	1 (1)	4 (1)	151 (81)	329 (81)	35 (18)	73 (18)	0.12
48	97 (75)	105 (81)	31 (24)	22 (17)	1 (1)	2 (2)					0.38
49	158 (81)	151 (83)	37 (19)	27 (15)	0	4 (2)					0.14
1, white	78 (70)	86 (81)	30 (27)	14 (13)	3 (2)	5 (4)	186 (83)	186 (88)	36 (16)	24 (11)	0.98
1, black	56 (62)	57 (67)	29 (32)	24 (28)	5 (5)	4 (4)	141 (78)	138 (81)	39 (21)	32 (18)	0.60
Total	1081 (71)	1196 (76)	402 (26)	327 (21)	28 (1)	31 (1)	1672 (83)	1788 (86)	330 (16)	286 (13)	

The significant level (P) for HWE testing for controls is shown.

\*Data concerned ba+Aa.

**TABLE 4. The Distribution of the T786C Genotype for Cases and Controls (in Parenthesis Are the Respective Percentages)**

All Studies, Reference	Distribution of T786C <i>eNOS</i> Genotype						Allele Frequency				P HWE Controls
	TT		TC		HWE		T		C		
	Cases	Controls	Controls	Controls	Cases	Controls	Cases	Controls	Cases	Controls	
50	324 (80)	362 (79)	77 (19)*	94 (20)*							na
29	1215 (78)	2023 (80)	311 (20)	462 (18)	13 (0.)	31 (1.)	2741 (89)	4508 (89)	337 (10)	524 (10)	0.43
4	34 (32)	240 (40)	48 (45)	277 (46)	24 (22)	82 (13)	116 (54)	757 (63)	96 (45)	441 (36)	0.89
51	38 (27)	16 (33)	69 (50)	22 (45)	30 (21)	10 (20)	145 (52)	54 (56)	129 (47)	42 (43)	0.65
32	26 (24)	27 (28)	50 (47)	46 (48)	29 (27)	21 (22)	102 (48)	100 (53)	108 (51)	88 (46)	0.88
1, white	37 (33)	36 (31)	62 (55)	61 (53)	13 (11)	16 (14)	136 (60)	133 (58)	88 (39)	93 (41)	0.22
1, black	49 (54)	41 (47)	36 (40)	41 (47)	5 (5)	4 (4)	134 (74)	123 (71)	46 (25)	49 (28)	0.16
Total	1723 (69)	2745 (70)	653 (26)	1003 (25)	114 (4)	164 (4)	3374 (80)	5675 (82)	804 (19)	1237 (17)	

The significant level (P) for HWE testing for controls is shown.

\*Data concerned TC+CC.

contrast; the recessive and dominant models for G894T, T786C, and G23T polymorphisms have produced nonsignificant results; and heterogeneity was ranging from none to high. There was consistency of genetic effects across different ethnicities, because they were not differentiated from the overall effect. However, the results for G23T were derived from a very small number of studies. Overall and in whites, significant results and lack of heterogeneity were observed for polymorphism 4b/a under a recessive model for allele b, conferring protection. In contrast, the association was not significant in East Asians, but the number of studies was small to draw safe conclusions. In all of the polymorphisms under investigation, the ORs for the allelic and dominant models were estimated based on fewer studies than the recessive model.

The overall lack of association between G894T, T786C, and G23T polymorphisms and hypertension and the discrepancy of results between whites and East Asians for 4b/a might be because of other unidentified functional mutations that exist in the *eNOS* gene that affect the susceptibility to hypertension.<sup>26</sup> It has been reported that the polymorphisms might be in linkage disequilibrium, and interaction of the polymorphisms within haplotypes can be a major determinant of disease susceptibility rather than the individual polymorphism. Thus, individual *eNOS* genotypes might not be reliable markers of risk for developing hypertension. Then, a meta-analysis of *eNOS* haplotypes, combinations of poly-

morphisms, may provide more reliable information than single polymorphisms.<sup>54</sup> Genome scans and genome search meta-analysis<sup>55</sup> have provided evidence for genetic linkage in hypertension; however, none of the loci with suggestive linkage included the *eNOS* gene. Discrepant results could be because of other loci that may have an effect on the genetic susceptibility to hypertension that is also in linkage disequilibrium with the examined polymorphisms of *eNOS* gene. Although there is a strong genetic contribution to hypertension,<sup>4</sup> other complex mechanisms<sup>1</sup> and environmental interactions (eg, smoking) may play a role in the pathogenesis of hypertension.<sup>56</sup> However, a meta-analysis of eNOS and ischemic heart disease involving >23 000 subjects<sup>57</sup> showed that individuals homozygous for the Asp298 and intron-4a alleles of eNOS are at moderately increased risk of ischemic heart disease.

Sampling variability and stratification in case-control study design can be a possible confounding factor on the role of genetic markers. In addition, misclassification in case-control studies might exist, because selection of participants can be based on a limited number of blood pressure measurements. The strict selection criteria ensure a clear case and control definition for meta-analysis, because if the possibility for a case to be considered as a control is minimized, then, the estimation of risk is unbiased. Prevalence of essential hypertension rises gradually with age, and it is maximized in elderly individuals.<sup>58</sup> In addition, age is independently asso-

**TABLE 5. The Distribution of the G23T Genotype for Cases and Controls (in Parenthesis Are the Respective Percentages)**

All Studies, Reference	Distribution of G23T <i>eNOS</i> Genotype						Allele Frequency				P HWE Controls
	GG		GT		TT		G		T		
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	
52	94 (47)	46 (43)	83 (41)	46 (43)	21 (10)	14 (13)	271 (68)	138 (65)	125 (31)	74 (34)	0.65
24	135 (43)	66 (53)	138 (44)	46 (37)	36 (11)	11 (8)	408 (66)	178 (72)	210 (33)	68 (27)	0.48
25	78 (35)	79 (32)	140 (64)*	161 (67)*							na
53	40 (37)	50 (42)	52 (48)	55 (46)	16 (14)	13 (11)	132 (61)	155 (65)	84 (38)	81 (34)	0.70
Total	347 (41)	241 (41)	413 (49)	308 (52)	73 (8)	38 (6)	811 (65)	471 (67)	419 (34)	223 (32)	

The significant level (P) for HWE testing for controls is shown.

\*Data concerned GT+TT.

**TABLE 6. ORs and Heterogeneity Results for the Genetic Contrasts of G894T, 4b/a, T786C, and G23T eNOS Polymorphisms for Hypertension**

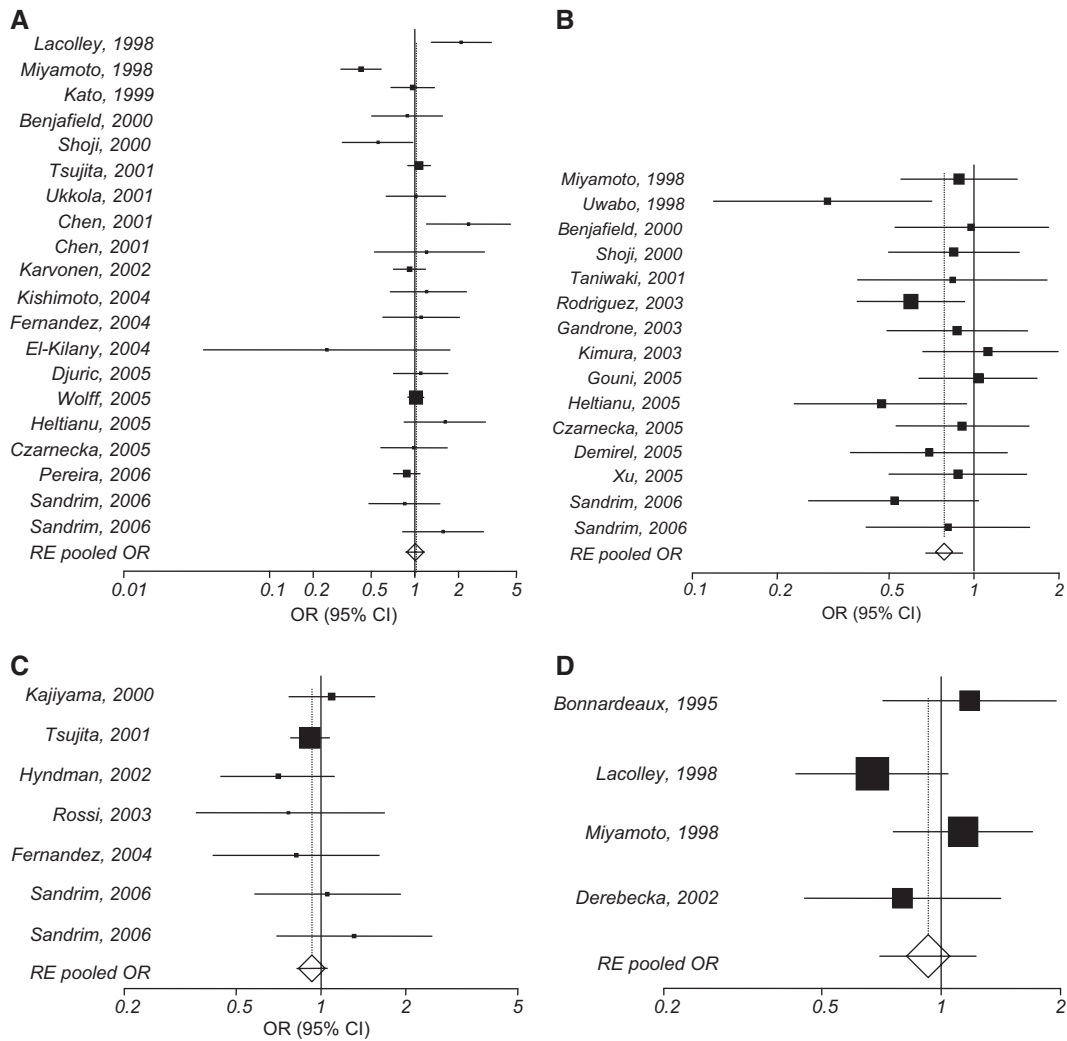
Polymorphism	Population	FE OR (95% CI)	RE OR (95% CI)	Studies	I <sup>2</sup> , %	P Q Test
<b>G894T</b>						
G vs T	All	1.00 (0.95 to 1.06)	1.01 (0.93 to 1.10)	16	29	0.13
	Sensitivity	0.99 (0.93 to 1.05)	0.99 (0.93 to 1.05)	14	0	0.52
Recessive model	Whites	1.01 (0.94 to 1.08)	1.01 (0.92 to 1.11)	10	17	0.29
	East Asians	1.06 (0.92 to 1.22)	1.06 (0.92 to 1.22)	3	0	0.65
	All	0.98 (0.92 to 1.05)	1.01 (0.87 to 1.17)	20	69	<0.01
Dominant model	Sensitivity	1.00 (0.93 to 1.08)	1.00 (0.93 to 1.08)	14	0	0.79
	Whites	1.06 (0.97 to 1.16)	1.13 (0.96 to 1.34)	11	50	0.03
	East Asians	0.86 (0.76 to 0.98)	0.78 (0.52 to 1.18)	5	87	<0.01
Dominant model	Blacks	1.38 (0.85 to 2.22)	1.38 (0.86 to 2.22)	2	0	0.49
	All	0.97 (0.84 to 1.12)	0.96 (0.83 to 1.15)	15	0	0.86
	Sensitivity	0.96 (0.82 to 1.11)	0.95 (0.82 to 1.11)	14	0	0.83
	Whites	0.98 (0.83 to 1.15)	0.97 (0.83 to 1.14)	9	0	0.86
Dominant model	East Asians	1.19 (0.64 to 2.21)	1.10 (0.59 to 2.04)	3	0	0.67
	All	0.85 (0.74 to 0.98)	0.85 (0.74 to 0.98)	12	0	0.82
<b>4b/a</b>						
b vs a	Whites	0.83 (0.69 to 0.99)	0.83 (0.69 to 0.99)	6	0	0.58
	East Asians	0.94 (0.71 to 1.24)	0.94 (0.71 to 1.25)	4	0	0.54
	All	0.78 (0.68 to 0.90)	0.78 (0.67 to 0.91)	15	12	0.32
Recessive model	Sensitivity	0.76 (0.64 to 0.90)	0.76 (0.64 to 0.91)	11	11	0.34
	Whites	0.76 (0.62 to 0.92)	0.76 (0.60 to 0.95)	7	23	0.25
	East Asians	0.83 (0.67 to 1.04)	0.82 (0.62 to 1.08)	6	33	0.19
Dominant model	All	1.38 (0.85 to 2.24)	1.28 (0.71 to 2.29)	11	14	0.31
	Whites	0.86 (0.45 to 1.61)	0.84 (0.43 to 1.64)	6	0	0.58
	East Asians	8.30 (1.86 to 36.97)	5.68 (1.62 to 19.85)	3	0	0.77
<b>T786C</b>						
T vs C	All	0.92 (0.82 to 1.02)	0.91 (0.81 to 1.03)	6	4	0.39
	Whites	0.84 (0.70 to 1.01)	0.84 (0.69 to 1.01)	4	4	0.37
Recessive model	All	0.93 (0.82 to 1.05)	0.93 (0.82 to 1.05)	7	0	0.63
	Sensitivity	0.91 (0.79 to 1.03)	0.91 (0.79 to 1.04)	6	0	0.65
	Whites	0.81 (0.62 to 1.07)	0.81 (0.62 to 1.07)	4	0	0.74
Dominant model	East Asians	0.94 (0.82 to 1.09)	0.94 (0.82 to 1.09)	2	0	0.35
	All	0.87 (0.66 to 1.16)	0.88 (0.62 to 1.24)	6	27	0.23
	Whites	0.76 (0.55 to 1.06)	0.76 (0.53 to 1.09)	4	15	0.32
<b>G23T</b>						
G vs T	Whites	0.88 (0.72 to 1.08)	0.89 (0.68 to 1.17)	3	44	0.17
Recessive model	All	0.93 (0.75 to 1.16)	0.93 (0.70 to 1.22)	4	35	0.20
	Sensitivity	0.84 (0.64 to 1.11)	0.85 (0.61 to 1.19)	3	35	0.21
Dominant model	Whites	0.84 (0.64 to 1.11)	0.85 (0.61 to 1.19)	3	35	0.21
	Whites	0.88 (0.58 to 1.35)	0.89 (0.58 to 1.36)	3	0	0.46

ciated with increased rates of hypertension. Thus, the absence of high blood pressure in young patients does not exclude the possibility of developing hypertension later. In many studies, young individuals were frequently included as controls. Therefore, a control group may include cases that are still at risk for developing hypertension. In the meta-analysis presented here, stratification because of ethnicity was taken into account, and the cases and controls were well defined with

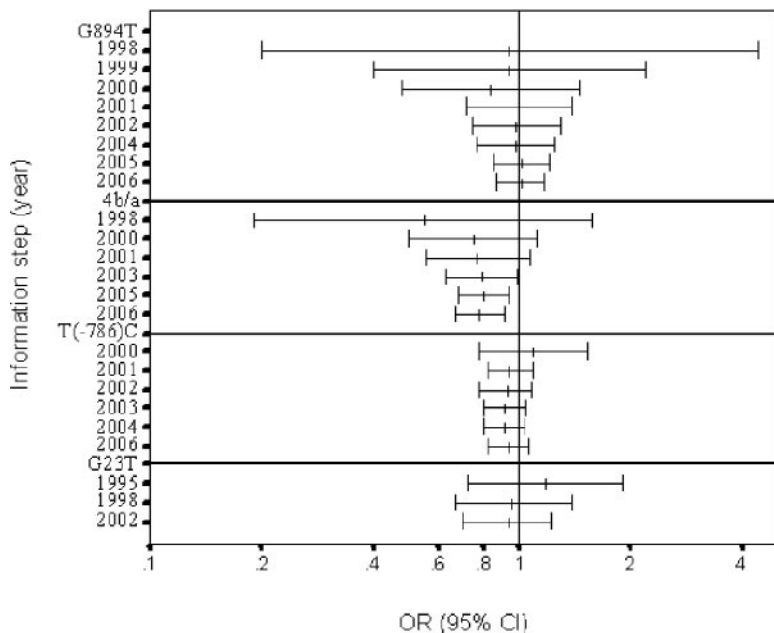
similar inclusion criteria, although they unavoidably cover a wide spectrum of disease in terms of duration, demographics, and other manifestations.

### Perspectives

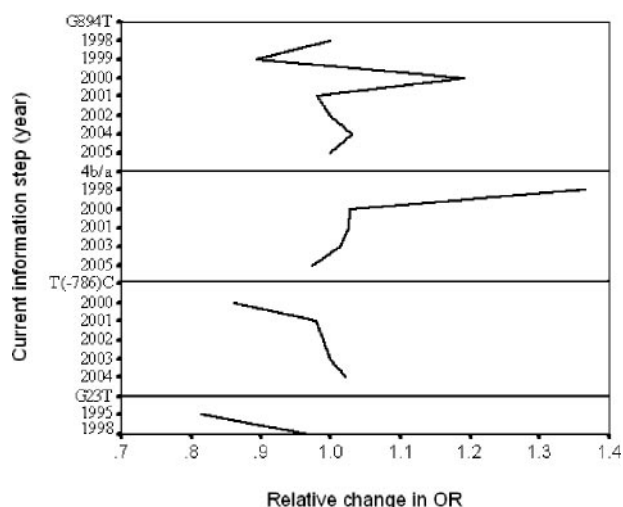
The present meta-analysis supported an association between hypertension and eNOS 4b/a polymorphism: the allele b under a recessive model provided evidence of protection,



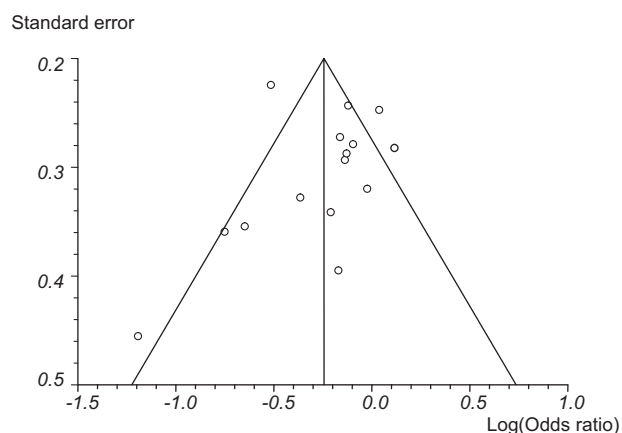
**Figure 2.** RE OR estimates with the corresponding 95% CI for the recessive models of (A) G894T allele G, (B) 4b/a allele b, (C) T786C allele T, and (D) G23T allele G and the risk of hypertension. The OR estimate of each study is marked with a ■. The size of the square represents the weight that the corresponding study exerts in the meta-analysis. The CIs of pooled estimates are displayed as a horizontal line through the diamond; this line might be contained within the diamond if the confidence interval is narrow. The horizontal axis is plotted on a log scale.



**Figure 3.** Cumulative meta-analysis: the RE-pooled OR with the corresponding 95% CI at the end of each year; information step is shown.



**Figure 4.** Recursive cumulative meta-analysis: the relative change in RE-pooled OR in each information step (OR in next year/OR in current year) is shown.



**Figure 5.** Funnel plot of SE against the log OR for 4b/a gene polymorphism.

mainly when analysis was confined to whites. There was no detectable influence of the G894T, T786C, and G23T polymorphisms. The results of the present meta-analysis should be interpreted with some degree of caution, because the numbers of studies and participants were relatively small. Wide genome scans and family-based tests for association, together with large case-control studies that investigate combinations of the polymorphisms<sup>59</sup> and gene-environment interaction,<sup>60</sup> should be performed to make conclusive claims about the genetics of hypertension.

## Disclosures

None.

## References

- Sandrim VC, Coelho EB, Nobre F, Arado GM, Lanchote VL, Tanus-Santos JE. Susceptible and protective eNOS haplotypes in hypertensive black and white subjects. *Atherosclerosis*. 2006;186:428-432.
- Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc Natl Acad Sci U S A*. 1987;84:9265-9269.
- Huang PL, Huang Z, Mashimo H, Bloch KD, Moskowitz MA, Bevan JA, Fishman MC. Hypertension in mice lacking the gene for endothelial nitric oxide synthase. *Nature*. 1995;377:239-242.
- Hyndman ME, Parsons HG, Verma S, Bridge PJ, Edworthy S, Jones C, Lonn E, Charbonneau F, Anderson TJ. The T-786->C mutation in endothelial nitric oxide synthase is associated with hypertension. *Hypertension*. 2002;39:919-922.
- Thomas GD, Zhang W, Victor RG. Nitric oxide deficiency as a cause of clinical hypertension: promising new drug targets for refractory hypertension. *JAMA*. 2001;285:2055-2057.
- Munafo MR, Flint J. Meta-analysis of genetic association studies. *Trends Genet*. 2004;20:439-444.
- Weir BS. *Genetic Data Analysis II: Methods for Discrete Population Genetic Data*. Sunderland, MA: Sinauer Associates; 1996.
- Zintzaras E, Ioannidis JP. Heterogeneity testing in meta-analysis of genome searches. *Genet Epidemiol*. 2005;28:123-137.
- Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ*. 2003;327:557-560.
- Zintzaras E, Ioannidis JP. HEGESMA: Genome search meta-analysis and heterogeneity testing. *Bioinformatics*. 2005;21:3672-3673.
- Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med*. 2002;21:1539-1558.
- DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials*. 1986;7:177-188.
- Lau J, Antman EM, Jimenez-Silva J, Kupelnick B, Mosteller F, Chalmers TC. Cumulative meta-analysis of therapeutic trials for myocardial infarction. *N Engl J Med*. 1992;327:248-254.
- Ioannidis J, Lau J. Evolution of treatment effects over time: empirical insight from recursive cumulative metaanalyses. *Proc Natl Acad Sci U S A*. 2001;98:831-836.
- Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ*. 1997;315:629-634.
- Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics*. 1994;50:1088-1101.
- Ioannidis JP, Trikalinos TA, Ntzani EE, Contopoulos-Ioannidis DG. Genetic associations in large versus small studies: an empirical assessment. *Lancet*. 2003;361:567-571.
- Zintzaras E, Hadjigeorgiou GM. Association of paraoxonase 1 gene polymorphisms with risk of Parkinson's disease: a meta-analysis. *J Hum Genet*. 2004;49:474-481.
- Ioannidis JP, Ntzani EE, Trikalinos TA. "Racial" differences in genetic effects for complex diseases. *Nat Genet*. 2004;36:1312-1318.
- Zintzaras E, Chatzoulis DZ, Karabatsas CH, Stefanidis I. The relationship between C677T methylenetetrahydrofolate reductase gene polymorphism and retinopathy in type 2 diabetes: a meta-analysis. *J Hum Genet*. 2005;50:267-275.
- Zintzaras E, Stefanidis I. Association between the GLUT1 gene polymorphism and the risk of diabetic nephropathy: a meta-analysis. *J Hum Genet*. 2005;50:84-91.
- Zintzaras E. Methylenetetrahydrofolate reductase (MTHFR) gene and susceptibility to Breast Cancer: a meta-analysis. *Clin Genetics*. 2006;69:327-336.
- Chen W, Srinivasan SR, Elkasabany A, Ellsworth DL, Boerwinkle E, Berenson GS. Combined effects of endothelial nitric oxide synthase gene polymorphism (G894T) and insulin resistance status on blood pressure and familial risk of hypertension in young adults: the Bogalusa Heart Study. *Am J Hypertens*. 2001;14:1046-1052.
- Lacolley P, Gautier S, Poirier O, Pannier B, Cambien F, Benetos A. Nitric oxide synthase gene polymorphisms, blood pressure and aortic stiffness in normotensive and hypertensive subjects. *J Hypertens*. 1998;16:31-35.
- Miyamoto Y, Saito Y, Kajiyama N, Yoshimura M, Shimasaki Y, Nakayama M, Kamitani S, Harada M, Ishikawa M, Kuwahara K, Ogawa E, Hamanaka I, Takahashi N, Kaneshige T, Teraoka H, Akamizu T, Azuma N, Yoshimasa Y, Yoshimasa T, Itoh H, Masuda I, Yasue H, Nakao K. Endothelial nitric oxide synthase gene is positively associated with essential hypertension. *Hypertension*. 1998;32:3-8.
- Kato N, Sugiyama T, Morita H, Nabika T, Kurihara H, Yamori Y, Yazaki Y. Lack of evidence for association between the endothelial nitric oxide synthase gene and hypertension. *Hypertension*. 1999;33:933-936.
- Benjafeld AV, Morris BJ. Association analyses of endothelial nitric oxide synthase gene polymorphisms in essential hypertension. *Am J Hypertens*. 2000;13:994-998.
- Shoji M, Tsutaya S, Saito R, Takamatu H, Yasujima M. Positive association of endothelial nitric oxide synthase gene polymorphism with hypertension in northern Japan. *Life Sci*. 2000;66:2557-2562.

29. Tsujita Y, Baba S, Yamauchi R, Mannami T, Kinoshita M, Yamamoto R, Katsuya T, Higaki J, Ogihara T, Ogata J, Iwai N. Association analyses between genetic polymorphisms of endothelial nitric oxide synthase gene and hypertension in Japanese: the Suita Study. *J Hypertens*. 2001;19:1941–1948.
30. Karvonen J, Kauma H, Kervinen K, Rantala M, Ikaheimo M, Paivansalo M, Savolainen MJ, Kesaniemi YA. Endothelial nitric oxide synthase gene Glu298Asp polymorphism and blood pressure, left ventricular mass and carotid artery atherosclerosis in a population-based cohort. *J Intern Med*. 2002;251:102–110.
31. Kishimoto T, Misawa Y, Kaetu A, Nagai M, Osaki Y, Okamoto M, Yoshida S, Kurosawa Y, Fukumoto S. eNOS Glu298Asp polymorphism and hypertension in a cohort study in Japanese. *Prev Med*. 2004;39:927–931.
32. Fernandez ML, Ruiz R, Gonzalez MA, Ramirez-Lorca R, Couto C, Ramos A, Gutierrez-Tous R, Rivera JM, Ruiz A, Real LM, Grilo A. Association of NOS3 gene with metabolic syndrome in hypertensive patients. *Thromb Haemost*. 2004;92:413–418.
33. Djuric T, Zivkovic M, Stankovic A, Mecanic S, Alavantic D. Endothelial NOS G894 T and MMP-3 5A/6A gene polymorphisms and hypertension in Serbian population. *J Clin Lab Anal*. 2005;19:241–246.
34. Wolff B, Grabe HJ, Schluter C, Popowski K, Volzke H, Ludemann J, John U, Felix SB, Cascorbi I. Endothelial nitric oxide synthase Glu298Asp gene polymorphism, blood pressure and hypertension in a general population sample. *J Hypertens*. 2005;23:1361–1366.
35. Heltianu C, Costache G, Gafencu A, Diaconu M, Bodeanu M, Cristea C, Azibi K, Poenaru L, Simionescu M. Relationship of eNOS gene variants to diseases that have in common an endothelial cell dysfunction. *J Cell Mol Med*. 2005;9:135–142.
36. Czarnecka C, Kawecka-Jaszcz K, Stolarz K, Olszanecka A, Dembinska-Kiec A, Kiec-Wilk B. Ambulatory blood pressure, left ventricular mass and vascular phenotypes in relation to the endothelial nitric oxide synthase gene Glu298Asp and intron 4 polymorphisms in a population-based family study. *J Hum Hypertens*. 2005;19:413–420.
37. Pereira AC, Sposito AC, Mota GF, Cunha RS, Herkenhoff FL, Mill JG, Krieger JE. Endothelial nitric oxide synthase gene variant modulates the relationship between serum cholesterol levels and blood pressure in the general population: new evidence for a direct effect of lipids in arterial blood pressure. *Atherosclerosis*. 2006;184:193–200.
38. Jachymova M, Horky K, Bultas J, Kozich V, Jindra A, Peleska J, Martasek P. Association of the Glu298Asp polymorphism in the endothelial nitric oxide synthase gene with essential hypertension resistant to conventional therapy. *Biochem Biophys Res Commun*. 2001;284:426–430.
39. Ukkola O, Erkkila PH, Savolainen MJ, Kesaniemi YA. Lack of association between polymorphisms of catalase, copper-zinc superoxide dismutase (SOD), extracellular SOD and endothelial nitric oxide synthase genes and macroangiopathy in patients with type 2 diabetes mellitus. *J Intern Med*. 2001;249:451–459.
40. Nagib El-Kilany GE, Nayel E, Hazzaa S. Nitric oxide synthase gene G298 allele Is it a marker for microvascular angina in hypertensive patients? *Cardiovasc Radiat Med*. 2004;5:113–118.
41. Uwabo J, Soma M, Nakayama T, Kanmatsuse K. Association of a variable number of tandem repeats in the endothelial constitutive nitric oxide synthase gene with essential hypertension in Japanese. *Am J Hypertens*. 1998;11:125–128.
42. Rodriguez-Esparragon F, Rodriguez-Perez J, Macias-Reyes A, Alamo-Santana F. Peroxisome proliferator-activated receptor-gamma2-Pro12Ala and endothelial nitric oxide synthase-4a/b gene polymorphisms are associated with essential hypertension. *J Hypertens*. 2003;21:1649–1655.
43. Gouni-Berthold I, Giannakidou E, Muller-Wieland D, Faust M, Kotzka J, Berthold H, Krone W. Peroxisome proliferator-activated receptor-g2 Pro12Ala and endothelial nitric oxide synthase-4a/b gene polymorphisms are not associated with hypertension in diabetes mellitus type 2. *J Hypertens*. 2005;23:301–308.
44. Yokoyama K, Tsukada T, Nakayama M, Hara S, Yamada A, Kawaguchi Y, Hosoya T. An intron 4 gene polymorphism in endothelial cell nitric oxide synthase might modulate volume-dependent hypertension in patients on hemodialysis. *Nephron*. 2000;85:232–237.
45. Taniwaki H, Ishimura E, Matsumoto N, Emoto M, Inaba M, Nishizawa Y. Relations between ACE gene and eNOS gene polymorphisms and resistive index in type 2 diabetic patients with nephropathy. *Diabetes Care*. 2001;24:1653–1660.
46. Grandone E, Colaizzo D, Martinelli P, Pavone G, Errico M, Vecchione G, Margaglione M. Does endothelial nitric oxide synthase gene variation play a role in the occurrence of hypertension in pregnancy? *Hypertens Pregnancy*. 2003;22:149–155.
47. Kimura T, Yokoyama T, Matsumura Y, Yoshiike N, Date C, Muramatsu M, Tanaka H. NOS3 genotype-dependent correlation between blood pressure and physical activity. *Hypertension*. 2003;41:355–360.
48. Demirel S, Akkaya V, Cine N, Oflaz H, Yekeler E, Ozturk S, Cleophas TJ, Fici F. Genetic polymorphisms and endothelial dysfunction in patients with essential hypertension: A cross-sectional case-control study. *Netherlands Heart J*. 2005;13:126–131.
49. Xu XJ, Wang SZ, Lin RY, Wang XF, Liang XH, Wen H, Zhang ZX. Association of episode of essential hypertension and polymorphism of endothelial nitric oxide synthase gene 27 bp VNTR in Xinjiang Kazakh population. *Chin J Clin Rehabil*. 2005;9:6–8.
50. Kajiyama N, Saito Y, Miyamoto Y, Yoshimura M, Nakayama M, Harada M, Kuwahara K, Kishimoto I, Yasue H, Nakao K. Lack of association between T-786->C mutation in the 5'-flanking region of the endothelial nitric oxide synthase gene and essential hypertension. *Hypertens Res*. 2000;23:561–565.
51. Rossi GP, Taddei S, Virdis A, Cavallin M, Ghiadoni L, Favilla S, Versari D, Sudano I, Pessina AC, Salvetti A. The T-786C and Glu298Asp polymorphisms of the endothelial nitric oxide gene affect the forearm blood flow responses of Caucasian hypertensive patients. *J Am Coll Cardiol*. 2003;41:938–945.
52. Bonnardeaux A, Nadaud S, Charru A, Jeunemaitre X, Corvol P, Soubrier F. Lack of evidence for linkage of the endothelial cell nitric oxide synthase gene to essential hypertension. *Circulation*. 1995;91:96–102.
53. Derebecka N, Hoysz M, Dankowski R, Wierchowicki M, Trzeciak W. Polymorphism in intron 23 of the endothelial nitric oxide synthase gene (NOS3) is not associated with hypertension. *Acta Biochim Pol*. 2002;49:263–268.
54. Wang J, Dudley D, Wang XL. Haplotype-specific effects on endothelial NO synthase promoter efficiency: modifiable by cigarette smoking. *Arterioscler Thromb Vasc Biol*. 2002;22:e1–e4.
55. Liu W, Zhao W, Chase GA. Genome scan meta-analysis for hypertension. *Am J Hypertens*. 2004;17:1100–1106.
56. Yoshimura M, Yasue H, Nakayama M, Shimasaki Y, Ogawa H, Kugiyama K, Saito Y, Miyamoto Y, Ogawa Y, Kaneshige T, Hiramatsu H, Yoshioka T, Kamitani S, Teraoka H, Nakao K. Genetic risk factors for coronary artery spasm: significance of endothelial nitric oxide synthase gene T-786->C and missense Glu298Asp variants. *J Investig Med*. 2000;48:367–374.
57. Casas JP, Bautista LE, Humphries SE, Hingorani AD. Endothelial nitric oxide synthase genotype and ischemic heart disease: meta-analysis of 26 studies involving 23 028 subjects. *Circulation*. 2004;109:1359–1365.
58. Hajjar I, Kotchen TA. Trends in prevalence, awareness, treatment, and control of hypertension in the United States, 1988–2000. *JAMA*. 2003;290:199–206.
59. Zintzaras E, Koufakis T, Ziakas PD, Rodopoulou P, Giannouli S, Voulgarelis M. A meta-analysis of genotypes and haplotypes of methylenetetrahydrofolate reductase gene polymorphisms in acute lymphoblastic leukaemia. *Eur J Epidemiol*. In press.
60. Clayton D, McKeigue PM. Epidemiological methods for studying genes and environmental factors in complex diseases. *Lancet*. 2001;358:1356–1360.