Scientific Contributions

Endothelial Nitric Oxide Synthase Gene Is Positively Associated With Essential Hypertension

Yoshihiro Miyamoto, Yoshihiko Saito, Noboru Kajiyama, Michihiro Yoshimura, Yukio Shimasaki, Masafumi Nakayama, Shigeaki Kamitani, Masaki Harada, Masahiro Ishikawa, Koichiro Kuwahara, Emiko Ogawa, Ichiro Hamanaka, Nobuki Takahashi, Yoshihiko Kaneshige, Hiroshi Teraoka, Takashi Akamizu, Nobuyuki Azuma, Yasunao Yoshimasa, Takaaki Yoshimasa, Hiroshi Itoh, Izuru Masuda, Hirofumi Yasue, Kazuwa Nakao

Abstract—Essential hypertension has a genetic basis. Accumulating evidence, including findings of elevation of arterial blood pressure in mice lacking the endothelial nitric oxide synthase (eNOS) gene, strongly suggests that alteration in NO metabolism is implicated in hypertension. There are, however, no reports indicating that polymorphism in the eNOS gene is associated with essential hypertension. We have identified a missense variant, Glu298Asp, in exon 7 of the eNOS gene and demonstrated that it is associated with both coronary spastic angina and myocardial infarction. To explore the genetic involvement of the eNOS gene in essential hypertension, we examined the possible association between essential hypertension and several polymorphisms including the Glu298Asp variant, variable number tandem repeats in intron 4 (eNOS4b/4a), and two polymorphisms in introns 18 and 23. We performed a large-scale study of genetic association using two independent populations from Kyoto (n=458; 240 normotensive versus 218 hypertensive subjects) and Kumamoto (n=421; 223 normotensive versus 187 hypertensive subjects), Japan. In both groups, a new coding variant, Glu298Asp, showed a strong association with essential hypertension (Kyoto: odds ratio, 2.3 [95% confidence interval, 1.4 to 3.9]; Kumamoto: odds ratio, 2.4 [95% confidence interval, 1.4 to 4.0]). The allele frequencies of 298Asp in hypertensive subjects were significantly higher than those in normotensive subjects in both groups (Kyoto: 0.103 versus 0.050, \(P<0.0017\); Kumamoto: 0.120 versus 0.058, \(P<0.0013\), respectively). No such disequilibrium between genotypes was significantly associated with any other polymorphisms we examined; the Glu298Asp variant was also not linked to any other polymorphisms. In conclusion, the Glu298Asp missense variant was significantly associated with essential hypertension, which suggests that it is a genetic susceptibility factor for essential hypertension.

(Hypertension. 1998;32:3-8.)

Key Words: genes ● nitric oxide synthase ● hypertension, essential ● polymorphism ● genetics

With a genetic contribution of from 25% to 60%, human essential hypertension has a genetic basis. Among persons younger than age 50 years, essential hypertension occurs 3.8 times more often in those having two or more first-degree relatives who developed high blood pressure before age 55.\(^1\) NO synthesis by the vascular endothelium is important for the regulation of vasodilator tone and the control of blood pressure in humans.\(^2\) A recent study using mice with disrupted eNOS gene revealed that eNOS function is required for vascular and hemodynamic responses to acetylcholine and that the disruption of the eNOS gene leads to hypertension.\(^3\) Moreover, recent reports demonstrate that whole-body NO production in patients with essential hypertension is diminished under basal conditions, as established by measurement of urinary and plasma nitrate.\(^4\) In addition, the offspring of hypertensive patients exhibit a reduced response to acetylcholine linked to a defect in the NO pathway.\(^5\) These results strongly implicate genetic alterations in the eNOS gene in the pathogenesis of human essential hypertension. On the other hand, there are no reports showing a positive association or linkage between eNOS genotype and essential hypertension. Bonnardeaux et al\(^6\) failed to demonstrate such an association or linkage in white subjects either in a case-control study using two polymorphisms in introns 18 and 23 of the eNOS gene (A to C and G to T conversion, respectively) or by an affected sib-pair analysis using CA repeat polymorphism. In other cardiovascular diseases, however, Wang et al\(^7\) examined the frequency of the 4b/4a VNTRs polymorphism (4 or 5 repeats of 27 bp, respectively) in intron 4 and found a positive association between 4a polymorphism and smoking-dependent risk of coronary artery disease.
We recently identified another variant of the eNOS gene within exon 7: G to T conversion at nucleotide position 894 of eNOS cDNA resulting in a replacement of glutamic acid by aspartic acid at codon 298 (Glu298Asp). This variant is associated with both coronary spastic angina and myocardial infarction. To further elucidate the genetic involvement of the eNOS gene in essential hypertension, we examined the possible association between the Glu298Asp variant and essential hypertension in two Japanese populations. We also analyzed 3 other polymorphisms: 27-bp tandem repeats in intron 4, A to C conversion in intron 18, and G to T conversion in intron 23. We report here a significant association of the Glu298Asp variant of the eNOS gene with essential hypertension in Japanese subjects.

Methods

Patient Population: Hypertensive and Control Subjects
A total of 218 patients (112 men, 106 women) with essential hypertension were selected from the outpatient clinics at Kyoto University Hospital and its affiliated hospitals in Kyoto according to the following criteria: (1) patient age >20 years, (2) onset of hypertension occurred at <60 years of age, (3) established hypertension defined either as long-term treatment of the disease, or in those previously untreated as systolic/diastolic blood pressures >140/90 mm Hg on two consecutive visits, and (4) absence of antihypertensive treatment. Blood pressure was measured in the supine position using a sphygmomanometer. A group of 240 normotensive control subjects (135 men, 105 women) were selected from the same clinics according to a protocol approved by the Human Study Committee of Kyoto University or Kumamoto University. At the time of recruitment, informed consent was obtained from each person according to a protocol approved by the Human Study Committee of Kyoto University or Kumamoto University.

Identification of Genotype of eNOS Gene: Enzymatic Amplification and RFLP Analysis of Glu298Asp Variant in Exon 7 of eNOS Gene
We previously reported that the missense Glu298Asp mutation is a common variant of the eNOS gene, and the specific genotype can be identified with a PCR followed by RFLP using the restriction enzymes MboI and BanII to digest mutant and wild alleles, respectively. PCR primers were generated to amplify the 248-bp fragment encompassing the missense Glu298Asp variant (sense and antisense primers were 5'-AAGGCAGGAGACAGTGATGGA-3' and 5'-CCCAGTCACCCCTTTGGTGCTCA-3', respectively). A guanine at nucleotide position 894 results in a glutamic acid at amino acid position 298, and a BanII restriction enzyme produces two fragments of 163 bp and 85 bp in length. A thymine at nucleotide position 894 results in an aspartic acid at amino acid position 298, and a MboI restriction enzyme produces two fragments of 158 bp and 90 bp in length. The restriction digest products were analyzed by electrophoresis on 2.0% agarose gels.

Detection of VNTRs in Intron 4 of eNOS Gene
VNTRs in intron 4 of the eNOS gene were determined as previously described by Wang et al.7

ASO for Polymorphisms in Introns 18 and 23 of eNOS Gene
To determine the genotype of the eNOS gene according to the polymorphism in introns 18 and 23, we performed ASO as described in the previous report by Bonnardeaux et al.6

Analysis of Differences in Clinical Parameters Between Hypertensive and Normotensive Subjects
Clinical parameters were analyzed in hypertensive and normotensive control subjects; differences in the frequencies of the following quantitative variables were tested using unpaired, two-tailed Student’s t test: age, systemic blood pressure, diastolic blood pressure, body mass index, plasma total cholesterol, HDL cholesterol, creatinine, blood urinary nitrates, plasma renin activity, plasma aldosterone concentrations, plasma ANP concentrations, plasma BNP concentrations, an amplitude of SV1+RV5 in ECG, and left ventricular mass index.

Analysis of Clinical Parameters Between Normotensive and Hypertensive Subjects
All values for clinical parameters are expressed as mean±SD. Except gender ratios, all clinical parameters were compared with Student’s t test. Gender ratios were tested by χ² test with 1 df. Values of P<0.05 were considered statistically significant.

Analysis of Genotype and Allele Frequencies for eNOS Variants
For each biallelic marker, allele frequencies were calculated from the genotypes in the hypertensive and normotensive groups. Deviation from Hardy-Weinberg equilibrium was assessed using a χ² test with 1 df. We estimated haplotype frequencies by the maximum-likelihood method.19 Differences in autosomal dominant genotype distribution between hypertensive and control subjects were tested using Fisher’s exact test. Differences in allele frequencies between hypertensive and control subjects were also tested using Fisher’s exact test. Using Bonferroni-type correction, P values of 0.0125 (= 0.05/4) or less, which correspond to corrected P values of 0.05 or less, were considered statistically significant for multiple testing. P values of 0.05 or less were considered significant for single testing. Linked disequilibrium between the two polymorphisms we examined was assessed using χ² test with 1 df, and P values of 0.0083 (= 0.05/6) or less, which correspond to corrected P values of 0.05 or less, were considered statistically significant.

Results

Clinical Characteristics of Study Subjects
The clinical characteristics of the 218 hypertensive and 240 normotensive subjects in Kyoto are summarized in Table 1. No differences between the two groups were noted with respect to age, body mass index, total serum cholesterol levels, serum HDL cholesterol levels, serum creatinine levels, plasma renin activity, or plasma aldosterone concentrations. Serum uric acid levels were significantly higher in the
hypertensive group than in the control group (P < 0.01). Plasma ANP and BNP levels in the hypertensive group were significantly higher than those in the control group (P < 0.05 and P < 0.05, respectively). Moreover, SV₁₊R V₁ in the ECG and left ventricular mass index estimated by echocardiography were also significantly higher in the hypertensive group than in the control group (P < 0.0001 and P < 0.0001, respectively). These data reflected cardiac hypertrophy associated with hypertension.

TABLE 2. Comparison of Genotype and Allele Frequencies for Several Polymorphisms of eNOS Gene in Hypertensive and Normotensive Subjects in Kyoto Group

<table>
<thead>
<tr>
<th>Variant</th>
<th>Exon 7</th>
<th>Intron 4</th>
<th>Intron 18</th>
<th>Intron 23</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NTN (n=240)</td>
<td>HTN (n=218)</td>
<td>NTN (n=240)</td>
<td>HTN (n=218)</td>
</tr>
<tr>
<td>Genotypes</td>
<td>Glu298Asp</td>
<td>eNOS4a/b</td>
<td>A27→C</td>
<td>G10→T</td>
</tr>
<tr>
<td>AA, n (%)</td>
<td>217 (90.4)</td>
<td>175 (80.3)</td>
<td>192 (98.0)</td>
<td>170 (78.0)</td>
</tr>
<tr>
<td>Aa + aa, n (%)</td>
<td>23 (9.6)</td>
<td>43 (19.7)</td>
<td>48 (2.0)</td>
<td>48 (22.0)</td>
</tr>
<tr>
<td>Significance P</td>
<td>P=0.0015</td>
<td>P=0.339</td>
<td>P=0.326</td>
<td>P=0.555</td>
</tr>
<tr>
<td>Odds ratio (95% CI)</td>
<td>2.3 (1.4–3.9)</td>
<td>1.1 (0.8–1.6)</td>
<td>1.4 (0.6–3.3)</td>
<td>1.1 (0.8–1.7)</td>
</tr>
</tbody>
</table>

Alleles
| A, n (%) | 456 (95.0) | 391 (89.7) | 427 (89.0) | 383 (87.8) | 471 (98.1) | 424 (97.2) | 281 (58.5) | 259 (59.4) |
| a, n (%) | 24 (5.0) | 45 (10.3) | 53 (11.0) | 44 (12.2) | 9 (1.9) | 12 (2.8) | 169 (41.5) | 177 (40.6) |
| Significance P | P=0.0017 | P=0.402 | P=0.253 | P=0.371 |

NTN indicates normotensive subjects; HTN, hypertensive subjects; and CI, confidence interval. Genotype and allele frequencies are indicated in absolute values and percentages in parentheses. A and a represent each allele of the polymorphisms.

*Test of comparison of autosomal dominant genotype distributions (Fisher’s exact test); †test of comparison of allele distributions (Fisher’s exact test).

Association Between Essential Hypertension and Polymorphisms of eNOS Gene

Four types of polymorphism in the eNOS gene, including Glu298Asp variant, 27-bp tandem repeats in intron 4, A to C conversion in intron 18, and G to T conversion in intron 23, were analyzed in hypertensive and normotensive subjects in Kyoto (Table 2). The genotype frequencies of all 4 polymorphisms in each group satisfied the Hardy-Weinberg equilibrium law. In the Kyoto population, only the Glu298Asp variant showed a significant effect of genotype on hypertension (P=0.0015), and its odds ratio was 2.3 (95% confidence interval, 1.4 to 3.9); there was a significant increase in the allelic frequency of the Glu298Asp variant in the hypertensive subjects (P=0.0017). The allelic frequencies of the Glu298Asp variant in normotensive and hypertensive subjects were 5.0% and 10.3%, respectively. The effect of gender on the frequency of hypertension was also considered. In both male and female populations, we observed significant differences in genotype and allele frequencies between hypertensive and normotensive subjects and control subjects (Table 3). The odds ratio in both females and males was 2.3. No significant gender-mediated preferential distribution of this susceptibility variant was noted.

We also investigated another Japanese population from Kumamoto prefecture, which is 500 miles from the Kyoto prefecture. The same genotypic and allelic disequilibrium in the Glu298Asp variant between hypertensive and normotensive subjects was observed (Table 4), and allelic frequencies of Glu298Asp variant were similar in the Kyoto and Kumamoto populations. These results demonstrate the positive association between the Glu298Asp variant and hypertension in Japanese.

VNTRs polymorphism in intron 4 of the eNOS gene (eNOS4a/b) was studied in 218 hypertensive and 240 normotensive control subjects in the Kyoto group (Table 2). The genotype frequencies for eNOS4a/b and eNOS4a/a in the normotensive population were 80.0% and 20.0%, respectively. Thus, genotype frequency for eNOS4a/b...
plus eNOS4a/a was less than in a white control population (33.4%). In the Japanese population, there was no significant effect of this genotype on the incidence of hypertension ($P = 0.339$) and no significant increase in the allelic frequency of eNOS4a in the hypertensive subjects compared with the control group ($P = 0.402$). We also analyzed the A27 to C polymorphism in intron 18 (a nucleotide substitution 27 bp downstream from exon 18) and the G10 to T polymorphism in intron 23 (a nucleotide substitution in 10 bp downstream from exon 23). Neither variant was reported to be associated with essential hypertension in whites. Similarly, there was also no significant increase of the allelic frequency of C27 or T10 in Japanese hypertensive subjects (Table 2).

To determine whether a particular linkage was present between any of these polymorphisms, we estimated eNOS haplotypes by combining two distinct polymorphisms. As shown in the Figure, disequilibrium of Glu298Asp was not linked to any other polymorphism investigated. Interestingly, disequilibrium of eNOS 4a polymorphism was strongly linked to the G10 to T polymorphism in intron 23 ($P < 0.0001$). In other words, the haplotype having both eNOS 4a and T10 in intron 23 was detected at a frequency of 8.3%, while 4a polymorphism was found at a frequency of only 11.4% (Figure). This suggests that 4a polymorphism in introns 4 and 23 may be derived from the same ancestor gene, while Glu298Asp variant in exon 7 occurred or was integrated independently.

### Discussion

In the present study, we used the candidate gene approach to explore whether the gene encoding eNOS, which is an important enzyme producing endothelial-derived relaxing factor (NO), is involved in the pathogenesis of human essential hypertension. We demonstrated that there was a

---

### Table 3. Comparison of Genotype and Allele Frequencies for Glu298Asp Variants of eNOS Gene in Each Gender Population of Kyoto Group

<table>
<thead>
<tr>
<th></th>
<th>Total (n=463)</th>
<th>Men (n=135)</th>
<th>Women (n=105)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotypes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu/Glu, n (%)</td>
<td>217 (90.5)</td>
<td>175 (80.4)</td>
<td>414 (89.4)</td>
</tr>
<tr>
<td>Glu/Asp+Asp/Asp, n (%)</td>
<td>22 (9.5)</td>
<td>40 (19.6)</td>
<td>21 (9.8)</td>
</tr>
<tr>
<td>Significance*</td>
<td>$P=0.0015$</td>
<td>$P=0.027$</td>
<td></td>
</tr>
<tr>
<td>Odds ratio (95% CI)</td>
<td>2.3 (1.4–3.9)</td>
<td>2.3 (1.1–4.7)</td>
<td>2.3 (1.1–5.2)</td>
</tr>
<tr>
<td>Alleles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu, n (%)</td>
<td>456 (95.0)</td>
<td>391 (89.6)</td>
<td>876 (94.6)</td>
</tr>
<tr>
<td>Asp, n (%)</td>
<td>24 (5.0)</td>
<td>45 (10.3)</td>
<td>90 (11.1)</td>
</tr>
<tr>
<td>Significance†</td>
<td>$P=0.0017$</td>
<td>$P=0.051$</td>
<td></td>
</tr>
</tbody>
</table>

NTN indicates normotensive subjects; HTN, hypertensive subjects; and CI, confidence interval. Genotype and allele frequencies are indicated in absolute values and percentages in parentheses.

*Test of comparison of autosomal dominant genotype distributions (Fisher’s exact test); †test of comparison of allele distributions (Fisher’s exact test).

---

### Table 4. Comparison of Genotype and Allele Frequencies for Glu298Asp Variants of eNOS Gene in Two Distinct Areas in Japan

<table>
<thead>
<tr>
<th></th>
<th>Kyoto (n=463)</th>
<th>Kumamoto (n=463)</th>
<th>Total (n=463)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotypes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu/Glu, n (%)</td>
<td>217 (90.4)</td>
<td>175 (80.3)</td>
<td>414 (89.4)</td>
</tr>
<tr>
<td>Glu/Asp+Asp/Asp, n (%)</td>
<td>23 (9.6)</td>
<td>43 (19.7)</td>
<td>21 (9.8)</td>
</tr>
<tr>
<td>Significance*</td>
<td>$P=0.0015$</td>
<td>$P=0.000005$</td>
<td></td>
</tr>
<tr>
<td>Odds ratio (95% CI)</td>
<td>2.3 (1.4–3.9)</td>
<td>2.3 (1.4–4.0)</td>
<td>2.3 (2.0–2.7)</td>
</tr>
<tr>
<td>Alleles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu, n (%)</td>
<td>456 (95.0)</td>
<td>391 (89.6)</td>
<td>876 (94.6)</td>
</tr>
<tr>
<td>Asp, n (%)</td>
<td>24 (5.0)</td>
<td>45 (10.3)</td>
<td>90 (11.1)</td>
</tr>
<tr>
<td>Significance†</td>
<td>$P=0.0017$</td>
<td>$P=0.000009$</td>
<td></td>
</tr>
</tbody>
</table>

NTN indicates normotensive subjects; HTN, hypertensive subjects; and CI, confidence interval. Genotype and allele frequencies are indicated in absolute values and percentages in parentheses.

*Test of comparison of autosomal dominant genotype distributions (Fisher’s exact test); †test of comparison of allele distributions (Fisher’s exact test).
significant association of the Glu298Asp polymorphism with essential hypertension in Japanese subjects. This is the first report of a positive association between eNOS gene polymorphism and essential hypertension.

We observed significant disequilibrium of the Glu298Asp variant of the eNOS gene in two distinct populations from Kyoto and Kumamoto, Japan. While the Japanese are thought to be racially homogeneous, association studies are nevertheless liable to be biased owing to uncontrolled stratification. However, the following results support the interpretation that the observed association is not spurious: significance was obtained in independent samples from two different populations and frequencies of Glu298Asp are remarkably similar in both populations. Bonnardetx et al.\(^6\) reported that the polymorphisms in introns 18 and 23 were not associated with essential hypertension. We therefore examined the two polymorphisms in introns 18 and 23 in addition to a Glu298Asp missense variant. We could find no association between hypertension and polymorphisms in introns 18 and 23 in this Japanese population, which is consistent with the previous study in whites reported by Bonnardetx et al.\(^6\) Although the frequencies of polymorphisms in introns 18 and 23 were quite different between Japanese and whites, an equilibrium between these alleles was similarly obtained in both hypertensive and normotensive subjects in both populations. These observations clearly indicate the validity of the present Japanese population. Because Glu298Asp variant was not linked with either polymorphism in intron 18 or intron 23, it is reasonable to suppose that polymorphisms in introns 18 and 23 were not associated with essential hypertension.

As for the case-control study of the eNOS gene on cardiovascular diseases, Wang et al.\(^7\) reported an association of VNTRs polymorphism located in intron 4 of the eNOS gene with coronary artery disease related to smoking in whites in Australia. We also examined the relation of the VNTRs polymorphism to essential hypertension in our population, but we could not find a significant association between them. Because the frequency of the 4a/298Asp haplotype is only 0.2% (Figure), the putative functional mutation related to VNTRs in intron 4 cannot be Glu298Asp variant or any unknown functional locus related to the Glu298Asp variant.

Recently, three mendelian forms of human hypertension were recognized in which a mutation in a single gene leads to elevated blood pressure in a high proportion of affected subjects: glucocorticoid-remediable aldosteronism,\(^1\) Liddle’s syndrome (pseudohypoaldosteronism),\(^2\) and the syndrome of apparent mineralocorticoid excess.\(^3\) There have been relatively few studies, however, examining the potential role of candidate genes in essential hypertension. At present, among the many candidate genes for a genetic basis of essential hypertension, the genes of the renin-angiotensin system including angiotensinogen,\(^4\) angiotensin-converting enzyme,\(^5\) angiotensin II type 1 receptor,\(^6\) and the ANP genes\(^7\) are reported to be positively associated with essential hypertension. Despite the promise of earlier research, so far, studies of some common variants of the eNOS gene have failed to clarify the nature of its involvement in essential hypertension. In this study, we used a novel missense variant of the eNOS gene to describe the association between the eNOS gene and essential hypertension for the first time.

In this case, a nucleotide substitution at the open reading frame causes amino acid substitution of glutamic acid to aspartic acid at a codon in the 298th position. Therefore, we
have only limited information about whether this missense mutation gives rise to functional alteration of eNOS enzymatic activity or is a genetic marker associated with some causal loci. The Glu298Asp variant is not located in any functional consensus sequence, but our computer analysis revealed that the Glu298Asp mutation results in a conformation change in the eNOS protein from helix to tight turn. We are now performing further studies for functional analysis in vitro as well as measurement of NO production in vivo.

In contrast to the Glu298Asp variant, polymorphism in intron 23 and VNTRs polymorphism in intron 4 were significantly linked (P<0.0001; Figure). eNOS protein is a member of the cytochrome P450 reductase-like NOS family and is composed of a heme binding region, calcium/calmodulin binding region, and NADPH-cytochrome P450 oxidoreductase. Because these compartments are conserved in the NOS family (eNOS, inducible NOS, and neural NOS), gene duplication and subsequent evolutionary divergence may have generated NOS isoforms that are highly homologous in sequence and very closely spaced on the chromosome. Glu298Asp variant in exon 7 is located in the intermediate portion of the heme binding and calcium/calmodulin binding sites, while intron 4 is next to the heme binding site and intron 23 is located in the NADPH-cytochrome P450 oxidoreductase. The degree of linkage disequilibrium among polymorphisms in intron 4, exon 7, and intron 23 may be a clue to understanding the evolutionary divergence of the eNOS gene.

In conclusion, we demonstrated that the Glu298Asp variant in the eNOS gene is significantly associated with essential hypertension in the Japanese population. These data indicate that the Glu298Asp variant of the eNOS gene may be a possible genetic susceptibility factor for essential hypertension. Further studies using sib-pair analysis or linkage study are necessary for confirming the relationship between essential hypertension and this variant.

Acknowledgments

This work was supported in part by research grants from the Japanese Ministry of Education, Science, and Culture, the Japanese Ministry of Health and Welfare, and grants from the Japanese Cardiovascular Research Foundation, Takeda Science Foundation, Smoking Research Foundation, Kanae Foundation of Research for New Medicine, and Yamanouchi Foundation for Research on Metabolic Disorders. We wish to thank Rie Ueno for her technical assistance. We are indebted to a collaborator at Shionogi & Co, Ltd, and especially to Takeshi Yoshioka and Hisanao Hiramat who provided technical information. Drs Taroh Shirakawa and Norhiro Kato kindly assisted us with the statistical analysis. The excellent secretarial work of Tomoko Okumura and Miki Yamanaka is also acknowledged.

References


Endothelial Nitric Oxide Synthase Gene Is Positively Associated With Essential Hypertension

Yoshihiro Miyamoto, Yoshihiko Saito, Noboru Kajiyama, Michihiro Yoshimura, Yukio Shimasaki, Masafumi Nakayama, Shigeki Kamitani, Masaki Harada, Masahiro Ishikawa, Koichiro Kuwahara, Emiko Ogawa, Ichiro Hamanaka, Nobuki Takahashi, Toshihiko Kaneshige, Hiroshi Teraoka, Takashi Akamizu, Nobuyuki Azuma, Yasunao Yoshimasa, Takaaki Yoshimasa, Hiroshi Itoh, Izuru Masuda, Hirofumi Yasue and Kazuwa Nakao

_Hypertension_. 1998;32:3-8
doi: 10.1161/01.HYP.32.1.3

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/32/1/3

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Hypertension_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Hypertension_ is online at:
http://hyper.ahajournals.org//subscriptions/