NO SYNTHASE GENE AND AMBULATORY BP

The relationship between the nitric oxide synthase gene and the risk of hypertension defined according to ambulatory blood pressures

Geoffrey Candy, Danelle Badenhorst, Elena Libhaber, Pinhas Sareli, Gavin R. Norton, Richard Brooksbank and Angela J. Woodiwiss

Cardiovascular Pathophysiology and Genomics Research Unit, School of Physiology (GC, DB, PS, GRN, RB, AJW) and Medicine (GC, EL), Faculty of Health Sciences, University of the Witwatersrand, Johannesburg

Address for correspondence:
Angela Woodiwiss
Cardiovascular Pathophysiology and Genomics Research Unit
School of Physiology
University of the Witwatersrand Medical School
7 York Road
Parktown
2193
South Africa

Email:
angela.woodiwiss@wits.ac.za

ABSTRACT

Although nitric oxide (NO) plays an important role in blood pressure (BP) control, whether variation of genes involved in regulating the synthesis of NO influences BP is uncertain. As the heritability of BP is stronger for ambulatory than it is for conventional BP, we assessed the independent association of the well described functional exon 7 Glu298Asp variant of the eNOS gene with the presence of hypertension in 511 randomly selected normotensive control participants and 503 hypertensives with a diagnosis of hypertension confirmed with 24-hour ambulatory BP profiles whilst off therapy. We also assessed the relationship between eNOS genotype and 24 hour ambulatory BP. Comparisons of genotype and allele frequencies indicated a lack of association of the exon 7 Glu298Asp gene variant with hypertension (Odds ratio of genotype predicting the presence of hypertension=0.97, confidence interval=0.70-1.30, p=0.92). However, patients with the Glu/Glu genotype of the Glu298Asp variant (n=424) had increased 24-hour systolic and diastolic blood pressures (152±1/97±1 mm Hg) in comparison to patients heterozygous for the Glu298Asp variant or homozygous for the 298Asp allele (n=79) (145±1/94±1 mm Hg, p<0.005 for systolic BP and p<0.001 for diastolic BP after multiple adjustments including age, gender, body mass index and the presence of diabetes mellitus). Differences in systolic and diastolic BP between genotype groups were noted during the day as well as at night. The association of eNOS genotype with ambulatory BP translated into an increased risk of more severe grades of hypertension in patients with the Glu/Glu genotype (grade II and III vs. grade I, Odds ratio=2.20, confidence interval=1.34-3.59, p<0.0002). In conclusion, a functional gene variant (Glu298Asp) at the eNOS locus contributes ~1.4-2.5% to the variation in ambulatory blood pressure within hypertensives, but is not associated with the presence of hypertension in patients in whom the diagnosis has been confirmed by 24-hour ambulatory BP values. The relationship between eNOS genotype and 24-hour ambulatory BP and the severity of hypertension warrants further study. SAHeart 2009; 6:148-153

INTRODUCTION

A high proportion of the risk for cardiovascular events is attributed to hypertension.[1] Despite the risk associated with hypertension, apart from age and body size, the factors that account for most of the variation in blood pressure (BP) at a population level have remained elusive. Nevertheless, familial aggregation and heritability analyses indicate that a significant proportion of BP variability can be attributed to genetic effects.[2-4] However, in this regard, there has been an inability to replicate genetic outcomes of even large studies evaluating genome-wide associations.[5,6]

The reasons for inconsistencies in the scientific literature with regards to the role of genetic loci in contributing to variations in BP are potentially numerous. However, one confounding factor that has been overlooked is that in the absence of repeated BP measurements obtained over considerable time periods,[6] 24-hour ambulatory as opposed to office BP measurements may be required to increase the ability to show familial aggregation or the heritability of BP.[2-4] Nevertheless few studies assessing the impact of specific genetic loci on BP have employed 24-hour BP measurements in large study samples. Indeed, our group is one of the few that has recently employed this approach to assess

the role of genes that influence the renin-angiotensin-aldosterone system in hypertension.[7,8] In addition, a study to determine the impact of leptin gene variants on blood pressure used ambulatory monitoring to assess blood pressure.[9] To extend these studies, in the present study we assessed the ability to detect a relationship between a common variant of the endothelial nitric oxide synthase (eNOS) gene and the presence of hypertension confirmed with 24-hour BP measurements in a large case-control study. In this regard, in studies employing conventional BP measurements to identify the presence of hypertension or the variability of BP, the role of the eNOS gene in contributing to hypertension or BP variability has produced inconsistent outcomes.[10-21]

METHODS

Study groups

Five hundred and three consecutive hypertensive patients of African ancestry initially screened at district clinics in suburban areas of Johannesburg and referred to tertiary care centres (Johannesburg and Chris Hani-Baragwanath Hospital) for more thorough clinical assessments were recruited if they had mean daytime ambulatory diastolic BPs (DBPs) >90 mm Hg (SpaceLabs model 90207) off medication. Ambulatory BP measurements were performed on patients with auscultatory BPs <200/115 mm Hg after at least 2 weeks off medication. A minority of patients (6%) with either first auscultatory BPs ≥200/115 mm Hg; or with either target organ damage; or two or more additional risk factors for cardiovascular disease had 24-hour BP monitoring performed within a shorter period off medication (4 days). To reduce the possibility of population stratification only patients of the Nguni, Sotho and Venda chiefdoms of South Africa were selected. In addition, patients with type I diabetes mellitus, uncontrolled type II diabetes mellitus (defined as a HbA1c> 10%), renal and endocrine disease and clinically important cardiac pathology (clinically significant arrhythmias, heart failure, valvular disease, ischaemic heart disease, previous myocardial infarction, and unstable angina) were excluded. Five hundred and eleven control participants of African descent without a family history of hypertension were recruited from suburban areas of Johannesburg and were considered to be normotensive if the mean of 3 auscultatory and the mean of 3 oscillometric (SpaceLabs model 90207) DBPs were <90 mm Hg after 5 minutes of rest in the seated position and if the subjects had been residents of an urban area for at least 2 years.

Ambulatory blood pressures and grading of hypertension

Ambulatory BP measurements were performed at least every half hour during the day (06:00 to 22:00 hours) and hourly during the night (22:00 to 06:00). Ambulatory monitors were calibrated using standard techniques. All patients were advised not to smoke, drink alcohol, or ingest caffeine during this period. Five hundred and three patients had >90% and the remaining >85% of intended ambulatory BP recordings obtained. The grading of hypertension was based on the mean values obtained for daytime ambulatory BP and the categories (I, II and II) were according to those described in guidelines for the diagnosis and management of hypertension.[1]

Genotyping

Deoxyribonucleic acid was extracted from whole blood by lysing red blood cells and digesting the remaining white cell pellet with proteinase K. A functional variant[22] in exon 7 of the eNOS gene was studied, where a substitution of a thymidine for a guanine nucleotide at position 894 results in aspartate (Asp) replacing glutamate (Glu) in amino acid position 298 of the protein.[10-15,18-21] For the detection of the Glu298Asp variant, polymerase chain reaction-restriction fragment length polymorphism-based techniques were employed. A 248 base pair fragment was amplified from the primers 5'-AAG GCA GGA GAC AGT GGA TGG A-3' (sense) and 5'-CCC AGT CAA TCC CTT TGG TGC TCA-3' (antisense).[11] The restriction enzyme BanII produced 163 and 85 base pair fragments in the presence of a guanine at nucleotide position 894 (Glu). The restriction enzyme NdeI produced 158 and 90 base pair fragments in the presence of a thymidine at nucleotide position 894.

Data analysis

To test for Hardy Weinberg equilibrium, the expected genotype numbers were calculated from the allele frequencies and deviation from the observed genotype numbers was determined using a X^2 test. Effects of genotypes on the presence of hypertension or the severity of hypertension (grade II and II versus grade I) was evaluated using logistic regression analysis with age, gender and

body mass index (BMI) and history of smoking or alcohol consumption included as covariates. The impact of genotype on ambulatory BP and pulse pressure was evaluated by ANCOVA with age, gender, body mass index, duration of hypertension, history of smoking or alcohol consumption, previous drug therapy, and the presence or absence of type II diabetes mellitus included as covariates.

RESULTS

Demographic and clinical characteristics

Demographic and clinical data for the study groups are shown in Table 1. Both the case and the control groups had more females than males and a mean body mass index that reflects a high prevalence of obesity. The high proportion of females in both groups reflects the gender distribution of patients attending district clinics rather than a greater incidence of hypertension in women as compared to men. Except for a higher BMI in the case group, the case and control groups were matched for all other demographic features. However, the eNOS Glu/Glu genotype group in the hypertensives consisted of more females ($p<0.05$) and had a trend for a higher mean BMI ($p<0.05$) than the patients with the Glu/Asp and Asp/Asp genotypes.

Genotype effects on the risk of hypertension

The genotype frequencies of the eNOS gene variant in the case and the control groups are given in Table 2. The genotype frequencies were in Hardy Weinberg equilibrium for both the case and the control groups. The eNOS gene variant was however not independently associated with the presence of hypertension (Table 2).

Genotype effects on ambulatory blood pressure

Figure 1 shows the 24-hour ambulatory systolic and diastolic blood pressures and Figure 2 shows the mean 24-hour, day and night ambulatory blood pressure profiles of hypertensive patients grouped according to eNOS gene Glu298Asp polymorphism genotypes. Patients with the Glu/Glu genotype had higher 24-hour, day and night systolic and diastolic BP values as compared to patients with the other genotypes. Indeed patients with the Glu/Glu genotype had a 4.5% (~7 mm Hg) greater 24-hour systolic BP and a 3.5% (~3 mm Hg) greater 24-hour diastolic BP compared to patients with the other genotypes. The eNOS genotype contributed ~1.4-2.5% to the variation in ambulatory blood pressure within hypertensive patients. Pulse pressure did not differ according to eNOS gene Glu298Asp polymorphism genotypes (Glu/Glu: 54±1 mm Hg, Glu/Asp+Asp/Asp: 53±1 mm Hg, $p=0.17$).

Genotype effects on the severity of daytime ambulatory blood pressure

Table 2, lower panel shows the proportion of patients with grade II and III hypertension as compared to those with grade I hypertension in the different eNOS genotype groups. Patients with the Glu/Glu genotype had a higher proportion of patients with grade II and III hypertension as compared to grade I hypertension.

TABLE 1: Demographic and clinical characteristics of hypertensives and control participants

	Control (n=511)	Hypertensives (n=503)
Age (years)	52.1±0.4	51.3±0.6
Gender (Female/male, (% female))	358/153 (70)	372/131 (74)
Body mass index (kg/m^2)	29.2±0.30	30.6±0.30*
Duration of hypertension (years)	0	2.72±0.10*
Type II diabetes mellitus	2.7	5.0
Smoking (%)	20	18
Alcohol consumption (%)	18	17

** p<0.05 versus the control group*

TABLE 2: Comparison of genotype frequencies of the Glu298Asp variant of the endothelial nitric oxide gene between hypertensive and control participants of African ancestry and between different grades of hypertension (grade I versus grade II and III) within the hypertensive participants.

	Glu/Glu	Glu/Asp + Asp/Asp
Hypertensives	424 (84.3%)	74+5 (15.7%)
Controls	429 (84.0%)	81+1 (16.0%)
Grade I Hypertension	145 (34.2%)	42 (53.2%)
Grade II+III Hypertension	279 (65.8%)	37 (46.8%)

Odds ratio for Glu/Glu genotype associated with hypertension = 0.97, confidence interval=0.70-1.30, Not significant.
Odds ratio for Glu/Glu genotype associated with grade II and III hypertension as compared to grade I hypertension = 2.20, confidence interval=1.34-3.59, p<0.0002.

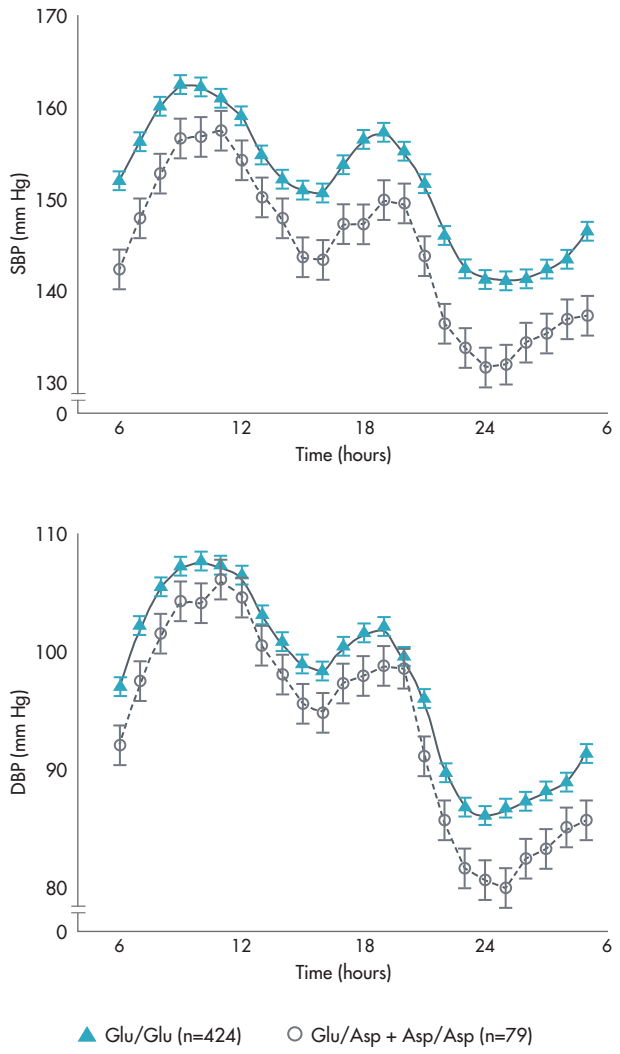

FIGURE 1: 24-Hour ambulatory systolic (SBP) and diastolic (DBP) blood pressure profiles of hypertensive patients grouped according to eNOS gene Glu298Asp polymorphism genotypes. Glu/Glu, patients homozygous for the Glu298 variant; Asp/Asp, patients homozygous for the 298Asp variant; Glu/Asp, patients heterozygous for the Glu298Asp variant. Comparisons of blood pressures are made in Figure 2.

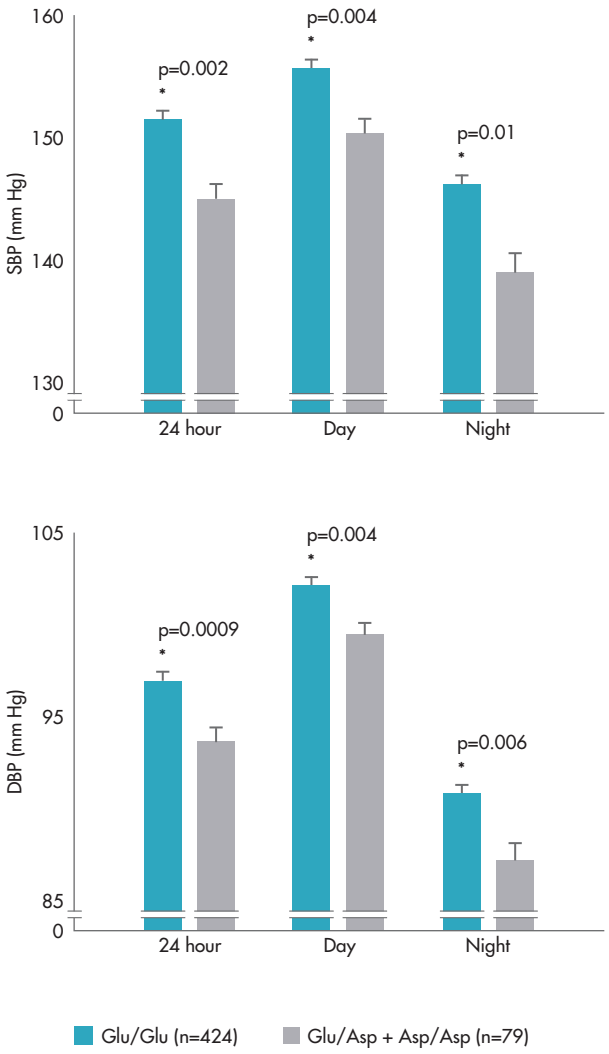

FIGURE 2: Mean 24-hour, day and night ambulatory systolic (SBP) and diastolic (DBP) blood pressure profiles of hypertensive patients grouped according to eNOS gene Glu298Asp polymorphism genotypes. Glu/Glu, patients homozygous for the Glu298 variant; Asp/Asp, patients homozygous for the 298Asp variant; Glu/Asp, patients heterozygous for the Glu298Asp variant. Probability values indicate comparisons between genotype groups.

DISCUSSION

The main findings of the present study are that in a large study sample (n=1014), in which hypertension in the case group was confirmed with ambulatory BP monitoring, the Glu298Asp polymorphism of the eNOS gene is not associated with the presence of hypertension. However, within the hypertensive group, patients with the Glu/Glu genotype had a ~3-7 mm Hg greater ambulatory BP than patients with other eNOS genotypes, and the eNOS genotype was associated with the severity of hypertension and contributed ~1.4-2.5% to the variation in ambulatory blood pressure within hypertensive patients.

The present study is the first to evaluate the relationship between the well described functional[21] Glu298Asp variant of the eNOS gene and the presence of hypertension in patients in whom the diagnosis was confirmed with ambulatory BP monitoring whilst off medication. Previous studies have evaluated the relationships between eNOS gene variants and the presence

of hypertension diagnosed according to guidelines, but without a confirmation of elevated BP values from 24-hour measurements.[10-21] In this regard there is considerable uncertainty as to the role of the Glu298Asp variant in determining the presence of hypertension or the variability of office BP, with some studies demonstrating a relationship between the Asp/Asp and Glu/Asp genotypes and the presence of hypertension,[12,13,15] another a relationship between the Glu/Glu genotype and hypertension,[10] whilst in further studies a lack of relationship between the Glu298Asp genotype and the presence of hypertension was noted.[18-21] In this regard, the strongest evidence, obtained in large study samples (n=1165-4055),[18,20] indicates that the Glu298Asp variant is indeed not associated with hypertension. Moreover, our study, also conducted in a large study sample (n=1014) and with the additional benefit of the presence of diastolic hypertension confirmed off medication using ambulatory BP monitoring, concurs with the outcomes of previous studies conducted in large study samples[18,20] indicating that the Glu298 Asp variant is indeed not associated with hypertension.

Although in the present study we were unable to show a relationship between Glu298Asp genotype and the presence of hypertension, we were able to show a fairly robust relationship between this genotype and ambulatory BP in hypertensives not receiving medication at the time of assessment. These data would suggest that the eNOS gene may be associated with BP, but that the sensitivity to detect an effect is enhanced by considering BP as a continuous rather than a dichotomous trait and employing ambulatory BP to assess these effects. This is indeed consistent with the notion that ambulatory BP measurements enhance the ability to detect a genetic effect as previously proposed.[2-4]

It may be argued that the genetic association described in the present study with ambulatory BP in hypertensives is of little clinical relevance if ambulatory BP is required to detect a difference in BP between genotype groups. Importantly, however, the differences in ambulatory BP detected in patients with the Glu/Glu as compared to those with the Glu/Asp and Asp/Asp genotypes were of clinical significance in that they translated into an association with different grades of severity of hypertension defined according to mean daytime BP measurements. In this regard, grade II and III hypertension carries a significantly greater risk of a cardiovascular event than grade I hypertension.[1]

An explanation for the relationship between the Glu/Glu genotype of the Glu298Asp variant and an increased ambulatory BP was not explored in the present study. In this regard, the Asp298 allele is associated with the production of an eNOS protein that is more susceptible to cleavage than the 298Glu allele[22] an effect that is likely to reduce NO production and consequently increase and not decrease BP as reported on by our group. Hence, in the present study two explanations for the relationships between genotype and ambulatory BP need to be considered. First, the Glu298Asp variant may be in linkage disequilibrium with an alternative functional eNOS gene variant. Second, the association noted may be explained by an increased mortality in hypertensive participants with the Asp298 allele with more severe forms of hypertension. In the latter case the remaining patients with the Asp298 allele would have lower BP values as reported on by us. Only prospective studies will be able to evaluate this hypothesis.

The strengths of the present study include the relatively large study sample evaluated (n=1014) and the use of ambulatory BP in 503 hypertensives to assess the outcomes. The limitations of the present study include the lack of prospective analysis. Thus the outcomes of the present study may represent an epiphenomenon. Moreover, the relationship between genotype and ambulatory BP was assessed in hypertensives rather than in a cross-section of the population. In this regard, further work is being conducted to assess the relationship between eNOS genotype and ambulatory BP in ~750-1000 participants derived from a cross-section of the population.

In conclusion, in the present study conducted in a large cohort (n=1014) in which hypertension in the cases was confirmed using 24-hour ambulatory BP monitoring, we show that the functional Glu298Asp gene variant of the eNOS gene is not associated with hypertension. However, within the hypertensive group, a robust relationship between eNOS genotype and variations in ambulatory BP was observed. The latter important relationship observed in the hypertensives requires further investigation.

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REFERENCES

1. Mancia G, de Backer G, Dominiczak A, et al. Management of Arterial Hypertension of the European Society of Hypertension: European Society of Cardiology. 2007 Guidelines for the Management of Arterial Hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). J Hypertens. 2007;6:1105-1187.
2. Fava C, Burri P, Almgren P, et al. Heritability of ambulatory and office blood pressure phenotypes in Swedish families. J Hypertens. 2004;22:1717-1721.
3. Kotchen TA, Kotchen JM, Grim CE, et al. Genetic determinants of hypertension: identification of candidate phenotypes. Hypertension. 2000;36:7-13.
4. Bochud M, Bovet P, Elston RC, et al. High heritability of ambulatory blood pressure in families of East African descent. Hypertension. 2005;45:445-450.
5. Ehret GB, Morrison AC, O'Connor AA, et al. Replication of the Wellcome Trust genome-wide association study of essential hypertension: the Family Blood Pressure Program. Eur J Hum Genet. 2008;16:1507-1511.
6. Levy D, Larson M, Benjamin EJ, et al. Framingham Heart Study 100K Project: genome-wide associations for blood pressure and arterial stiffness. BMC Medical Genetics. 2007;8:S3.
7. Tiago AD, Samani NJ, Candy GP, et al. Angiotensinogen gene promoter region variant modifies body-size-ambulatory blood pressure relations in hypertension. Circulation. 2002;106:1483-1487.
8. Tiago AD, Badenhorst D, Nkeh B, et al. Impact of renin-angiotensin-aldosterone system gene variants on the severity of hypertension in newly diagnosed patients. Am J Hypertens. 2003;16:1006-1010.
9. Gaukrodger N, Mayosi BM, Imrie H, et al. A rare variant of the leptin gene has large effects on blood pressure and carotid-intima-medial thickness: a study of 1 428 individuals in 248 families. J Med Genet. 2005;42:474-478.
10. Lacolley P, Gautier S, Poirier O, et al. Nitric oxide synthase gene polymorphisms, blood pressure and aortic stiffness in normotensive and hypertensive subjects. J Hypertens. 1998;16:31-35.
11. Hyndman ME, Parsons HG, Verma S, et al. The T-786-C mutation in endothelial nitric oxide synthase is associated with hypertension. Hypertension. 2002;39:919-922.
12. Miyamoto Y, Saito Y, Kajiyama N, et al. Endothelial nitric oxide synthase gene is positively associated with essential hypertension. Hypertension. 1998;32:3-8.
13. Shoji M, Tsutaya S, Saito R, et al. Positive association of endothelial nitric oxide synthase gene polymorphism with hypertension in northern Japan. Life Sci. 2000;66:2557-2562.
14. Uwabo J, Soma M, Nakayama T, et al. 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.
15. Jachymova M, Horky K, Bultas J, et al. 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.
16. Bonnardeaux A, Nadaud S, Charru A, et al. Lack of evidence of linkage of the endothelial cell nitric oxide synthase gene to essential hypertension. Circulation. 1995;91:96-102.
17. Hunt SC, Williams CS, Sharma AM, et al. Lack of linkage between the endothelial nitric oxide synthase gene and hypertension. J Hum Hypertens. 1996;10:27-30.
18. Kato N, Sugiyama T, Morita H, et al. Lack of evidence for the association between the endothelial nitric oxide synthase gene and hypertension. Hypertension. 1999;33:933-936.
19. Benjafield AV, Morris BJ. Association analyses of endothelial nitric oxide synthase gene polymorphisms in essential hypertension. Am J Hypertens. 2000;13:994-998.
20. Tsujita Y, Baba S, Yamauchi R, et al. Association analyses between genetic polymorphisms of the endothelial nitric oxide synthase gene and hypertension in Japanese. The Suita Study. J Hypertens. 2001;19:1941-1948.
21. Sandrim VC, de Syllos RWC, Lisboa HRK, et al. Endothelial nitric oxide synthase haplotypes affect the susceptibility to hypertension in patients with type 2 diabetes mellitus. Atherosclerosis. 2006;189:241-246.
22. Tesauro M, Thompson WC, Rogliani P, et al. Intracellular processing of endothelial nitric oxide synthase isoforms associated with differences in severity of cardiopulmonary disease: cleavage of proteins with aspartate versus glutamate at position 298. Proc Natl Acad Sci USA. 2000;97:2832-2835.