CORRESPONDENCE

Association of *eNOS* gene polymorphism (rs3918166) with blood pressure in adult Japanese

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Hypertension studies have been prominent among researchers for a long time because it is a silent killer and a major risk factor for cardiovascular disease and stroke in developed countries. The etiology of hypertension is multifactorial but the genetic element constitutes a high contribution (30–50%).¹ The most extensively studied system is the renin–angiotensin–aldosterone (RAA) system and the three common gene polymorphism enzymes include angiotensinogen (AGT Met235Thr), angiotensin-converting enzyme (ACE I/D) and aldosterone synthase (CYP11B2-344T/C).² However, because of the inconsistent findings on the associations between these gene polymorphisms with hypertension, genome-wide association studies are widely conducted to identify new genes and significant associations for the prevention and treatment of hypertension.^{3,4}

An important enzyme in relation to blood pressure regulation is endothelial nitric oxide synthase (*eNOS*) as it secretes a potent vasodilator, nitric oxide (NO).⁵ Previous association studies of the three common *eNOS* gene polymorphisms (Glu298Asp, intron 4 and –786T/C) in relation to hypertension have produced conflicting results with no definite association.⁶ This has prompted us to search for other suitable *eNOS* gene SNPs in the gene database provided by The National Center for Biotechnology Information (NCBI). Our criteria of selection were SNPs that produce a missense mutation and with heterozygosity of >0.5. There are a total of 20 SNPs with missense mutation of the *eNOS* gene in the NCBI gene database, but rs3918166 is the only SNP with heterozygosity close to 0.5 in the African–American population (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=3918166). Hence, in this study we examined *eNOS* gene polymorphism (rs3918166) in relation to blood pressure in adult Japanese.

The subjects were adults aged 25–58-years old ($n=58$; 31 (53%) males; 27 (47%) females) residing in Kamigoto island of Nagasaki Prefecture. Demographic data of the subjects such as age and gender were obtained using a standard questionnaire and blood pressure data were obtained from an annual health check-up. Normotensive, pre-hypertension, Stage-1 hypertension and Stage-2 hypertension are defined by JNC 7.⁷ There were 30 (52%) subjects with normal blood pressure and 28 (48%) subjects with pre-hypertension to Stage-1 hypertension. The percentage of subjects with normal blood pressure was consistent with the statistics of overall Japanese population in the respective age group based on the National Health and Nutrition Survey 2006.⁸ This study was approved by the Research Ethics Committee of University of Nagasaki and written informed consent was obtained from all subjects. DNAs were extracted from buccal mucosal cells with polyester fiber-tipped applicator swab (Falcon, Becton Dickinson and Company, Sparks, MD, USA) and were purified in buffer, QIAamp DNA Blood Mini kit (Qiagen, Germantown, MD, USA). PCR amplification was done using the set of primers: forward, 5'-CCTGTCCTGACCTTTGCACT-3' and reverse, 5'-CGGGAAGCTGT CACCTCTTA-3' (Sigma Genosys, Tokyo, Japan), mixed into PCR Premix kit (Bioneer Corporation, Daejeon, Korea) containing each DNA samples and subjected to a thermal cycler (Astec PC818, Fukuoka, Japan) for 45 cycles at 95 °C for 30 s; 59 °C for 30 s and 72 °C for 90 s. After digestion with *Sfi*I (New England Biolabs, Boston, MA, USA), the PCR products were separated onto 3% agarose gel electrophoresis and stained with SYBR Safe (Invitrogen, Eugene, OR, USA). Genotyping using RFLP was performed using a lumino-image analyzer LAS-1000 plus (Fuji-film, Tokyo, Japan) (Figure 1). Statistical analysis

was performed using Statistical Package for Social Sciences (SPSS v.11J for Windows, SPSS Inc., Tokyo, Japan). A *P*-value of <0.05 was considered statistically significant.

The allele frequencies were 0.56 for major G allele and 0.44 for minor A allele, which are in contrast with the African–American population (major G allele, 0.917 and minor A allele, 0.083) and the Caucasians who did not have any polymorphism (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=3918166). To date, there is no published data for comparison in Japanese population. The majority of the major G allele homozygotes (6 out of 7 subjects) were normotensive, whereas more than half (53%) of the heterozygote group were in the pre- to Stage-1 hypertension category. The means for both systolic and diastolic blood pressure were also significantly ($P<0.05$) different between the two groups of the *eNOS* genotype with no significant differences in mean age and body mass index (BMI) (Table 1).

In this study, we have provided new evidence on the relation of rs3918166 SNP of the *eNOS* gene with blood pressure in adult Japanese and our finding is further supported by a recent report of a study, which revealed that there were more minor allele A carriers of the *eNOS* SNP (rs3918166) with migraine aura and stroke among Black women.⁹ The enzyme activity of *eNOS* is highly regulated by Ca^{2+} sensor protein calmodulin (CaM) through the caveolin–CaM cycle whereby when endothelial cells are activated, *eNOS* will dissociate from its predominant location, caveolin-1 and binds to CaM for the production of NO.¹⁰ It is intriguing for us to speculate that a missense mutation in the *eNOS* gene at rs3918166 SNP site with a substitution of arginine to glutamine residue at codon 112 in exon 4 may cause changes in the regulation of the enzyme activity through interaction with caveolin

Figure 1 Electrophoretic profile of PCR-RFLP for *eNOS* gene polymorphism (rs3918166). Lane 1, DNA ladder marker, pUC19 DNA/MspI (*Hpa*II); Marker, 23 (Fermentas, Ontario, Canada); lane 2 and 3, *Sf*cl fragments from A/G heterozygote yielding 163 bp, 87 bp, 76 bp and 31 bp; lane 4, fragments from G/G homozygote yielding 87 bp, 76 bp and 31 bp, and no A/A homozygote in this study.

Table 1 Characteristics of age, BMI, blood pressure and categories of hypertension of subjects according to *eNOS* genotype

<i>eNOS</i> genotype	G/G (n=7)	A/G (n=51)	P-value
Age (years) ^a	40.1 ± 8.8	43.0 ± 9.5	0.455
BMI (kg m ⁻²) ^a	22.3 ± 2.5	22.8 ± 2.7	0.638
Systolic BP (mm Hg) ^a	107.1 ± 10.5	120.5 ± 15.2	0.029
Diastolic BP (mm Hg) ^a	66.0 ± 5.3	75.6 ± 12.6	0.049
Normotensive ^b	6 (85.7)	24 (47.1)	—
Pre-hypertension ^b	1 (14.3)	22 (43.1)	—
Stage-1 hypertension ^b	0 (0)	5 (9.8)	—

Abbreviations: BMI, body mass index; *eNOS*, endothelial nitric oxide synthase.

Normotensive (SBP <120 mm Hg and DBP <80 mm Hg).

Pre-hypertension (SBP 120–139 mm Hg or DBP 80–89 mm Hg).

Stage-1 hypertension (SBP 140–159 mm Hg or DBP 90–99 mm Hg).

^aValues are expressed as means ± s.d. Statistical analysis was carried out using *t*-test.

^bValues are expressed as n=total of subjects (percentage).

resulting in a defect in the physiological regulation of NO production.

This study strongly suggests that the major G allele of the *eNOS* gene polymorphism (rs3918166) may have an antihypertensive effect, whereas minor A allele may have a higher risk for hypertension. We definitely know that further investigation is certainly required with a larger sample size for in-depth statistical analysis involving regression model to confirm our findings. We also

recommend incorporating the analysis of gene-environment interactions as it is recommended for complex diseases such as hypertension.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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