NOS3 Genotype–Dependent Correlation Between Blood Pressure and Physical Activity

Tomomi Kimura, Tetsuji Yokoyama, Yasuhiro Matsumura, Nobuo Yoshiike, Chigusa Date, Masaaki Muramatsu, Heizo Tanaka

Abstract—Endothelium-dependent vasorelaxation plays an important role in reduction of blood pressure and is mediated through release of nitric oxide (NO), which is generated by constitutively expressed endothelial nitric oxide synthase (NOS3). Exercise also augments NO release and has been recommended for primary prevention and improvement of hypertension, but individual responses are highly variable. We therefore postulated that genetic polymorphisms of NOS3 might interact with physical activity level to differentially influence blood pressure level. We genotyped 832 healthy Japanese (mean age of 54.4±8.6 years, 372 men and 460 women) for a polymorphism of NOS3 in intron 4 (ecNOS4a/b), using the polymerase chain reaction method, and scored their habitual physical activity level by using the rate of energy expenditure per resting metabolic rate through an interview according to a semiquantitative assessment method. Only in the subjects who had the rarer a allele (aa + ba type), systolic blood pressure was found to be inversely correlated with physical activity level (P for linear trend=0.0496, for interaction=0.0071). Eventually, this polymorphism was significantly associated with the prevalence of systolic hypertension only in the subjects who were in the lowest tertile of physical activity level (OR=2.4, 95% CI, 1.1 to 5.6, P for interaction=0.0474). In the present study, we found a significant interaction between the genotype and physical activity level on systolic blood pressure. These results might allow a better understanding of the mechanism to improve hypertension by exercise and to thereby reduce the risk of cardiovascular disease. (Hypertension. 2003;41:355-360.)

Key Words: nitric oxide synthase ■ polymorphism ■ blood pressure ■ exercise ■ genes ■ lifestyle ■ cross-sectional study

Hypertension is one of the established risk factors of cardiovascular disease. The pathological, physiological, and biological mechanisms of hypertension have not yet been totally clarified, but nitric oxide (NO) release followed by endothelium-dependent vasorelaxation is considered to play an important role in reducing blood pressure.

NO is generated in vivo by NO synthase (NOS). Constitutively expressed endothelial NOS (NOS3) is one of three NOS genes known, and accumulating data suggest an association between this NOS3 gene and hypertension: NOS3 knockout mice grow to be hypertensive,1,2 whereas induction of NOS3 cDNA reduces blood pressure.3 An NOS3 polymorphism, which represents 4 or 5 times the 27-basepair variable number of tandem repeats in intron 4 (ecNOS4a/b), was first reported to be associated with smoking-dependent coronary artery disease in white subjects.4 More recently, Yoshimura and his colleagues5–7 found that the shorter a allele (4 repeats) is in linkage disequilibrium with the T(-786)C substitution8 that is associated with coronary spasm and reduces endothelial NO production. However, the relation between this polymorphism in intron 4 and hypertension remains controversial.4,8–12

Physical exercise stimulates NOS3 activity and increases NO release through the augmentation of sheer stress13,14 and thereby is considered generally to lower the blood pressure. However, individual responses for the reduction of blood pressure are highly variable, and it currently is impossible to predict which person will reduce his or her blood pressure with exercise. In the HERITAGE family study, an NOS3 genotype has been reported to associate with the training-induced reduction in blood pressure.15 Therefore, we postulated that a genetic polymorphism of NOS3 might interact with daily physical activity level to differentially influence blood pressure level.

Methods

Subjects

The present cross-sectional study was carried out as part of a Japan Lifestyle Monitoring Study,16 in Shioso County of Hyogo Prefecture and Shibata city of Niigata Prefecture, Japan (1999–2000). A total of
TABLE 1. NOS3 Polymorphism in Intron 4 (ecNOS4a/b) Distribution and Allele Frequency in Japanese General Population

<table>
<thead>
<tr>
<th>Population</th>
<th>Genotype, % (n)</th>
<th>a Allele Frequency</th>
<th>Total, n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>aa</td>
<td>ba</td>
<td>bb</td>
</tr>
<tr>
<td>Total</td>
<td>1.3 (11)</td>
<td>18.4 (153)</td>
<td>80.3 (668)</td>
</tr>
<tr>
<td>Male</td>
<td>1.3 (5)</td>
<td>18.3 (68)</td>
<td>80.4 (299)</td>
</tr>
<tr>
<td>Female</td>
<td>1.3 (6)</td>
<td>18.5 (85)</td>
<td>80.2 (369)</td>
</tr>
</tbody>
</table>

The genotype frequency did not deviate from Hardy-Weinberg equilibrium. No difference was found between sexes.

871 Japanese (403 men and 468 women), 40 to 69 years of age, were selected from all the residents by stratified random sampling based on gender and decade of age. After excluding those with a missing value, 832 subjects (mean age of 54.4±8.6 years, 372 men and 460 women) were recruited for the current analyses. We obtained written informed consent from all the subjects and all procedures followed were in accordance with institutional guidelines. Ethical approval for the conduct of the present study was given by the Ethics Review Committee, Medical Research Institute, Tokyo Medical and Dental University.

Genotyping for NOS3 Gene Polymorphisms

DNA was extracted from white blood cells with the use of the PUREGENE DNA Isolation Kit (Gentra Systems, Inc), and the genotyping for NOS3 intron4 a/b alleles was done by polymerase chain reaction methods as described previously, with some minor modifications.4,17

Assessment of Physical Activity Level

Habitual physical activity level was determined by metabolic equivalent (MET) scores18 as previously described.19,20 The subjects were classified into 3 groups (low, middle, and high level groups) according to their physical activity level as follows; low level group: 1.40 to 1.79 METs, middle level group: 1.80 to 2.02 METs, and high level group: 2.03 to 3.84 METs. The physical activity level represented in this score includes those of working hours and leisure time exercises. For further analysis, subjects were first divided into 3 groups (according to their age) or 2 groups (according to their gender); each group was then subdivided into 3 subgroups according to the level of physical activity.

Blood Pressure and Other Measurements

Blood pressure was measured by following the standardized protocol,21 and the mean of the first and second values was used for the study. Data on alcohol consumption, smoking, and personal history of diseases were collected with the use of a standardized questionnaire. The frequency of drinking per week and quantity in each occasion of consuming Sake, beer, whisky, Shochu (70-proof Japanese spirits), and wine were recorded. We regarded one “drink” as 12 g of pure ethanol, and the total alcohol consumption per day was computed by using the unit “drink.” Smoking status was categorized as nonsmokers, ex-smokers, moderate smokers (<20 cigarettes per day), and heavy smokers (≥20 cigarettes per day). Body height and weight were measured, and body mass index (BMI) was calculated as weight (kg)/height (m)². Serum nitrate/nitrate (NOx) level was measured by means of the Griess method as described,22 with some modifications.

Statistical Analysis

NOS3 allele frequencies were calculated using a gene-counting method, and the Hardy-Weinberg equilibrium was confirmed by means of the exact test. Comparison of gender ratio, the portion of subjects with a smoking habit, a history of diabetes, and antihypertensive medication between the NOS3 genotype groups (for classification, see Results section) were made by means of the Fisher exact test. Differences in the mean values of age, clinical characteristics, alcohol consumption, smoking status, and physical activity levels between the genotype groups were compared by means of the t test. Triglycerides and glucose levels only in fasting subjects (n=695) were log-transformed, and the mean values were calculated because significant differences in values between fasting and nonfasting states were observed (t test, P<0.05). Comparison between two NOS3 genotype groups and among three groups classified according to physical activity level was done by using ANCOVA adjusted for age, gender, BMI, alcohol consumption, smoking status, antihypertensive medication, population area, and history of diabetes for the null hypothesis that the NOS3 genotype and the physical activity level had no effect on blood pressure levels. Interaction between the genotype and the effect of physical activity level on blood pressure was assessed with a multivariate linear regression model. In this assessment, log-transformed continuous physical activity levels were used. Odds ratios and 95% CIs were calculated by means of a logistic regression model. Probability value for interaction was calculated with an interaction term of the genotype group multiplied by continuous log-physical activity level. A 2-sided probability value of <0.05 was considered to be statistically significant. The Bonferroni-Holm multiple comparison adjustment was used where appropriate. Serum NOx levels were log-transformed and compared between two genotype groups by using ANCOVA adjusted for age, gender, and smoking status. The log-transformed levels are shown as their exponentials in the Results section. All the analyses were performed with the use of SAS version 6.2 (SAS Institute Inc).

Results

Distribution of NOS3 Gene Polymorphism in Intron 4 (ecNOS4a/b)

We genotyped 832 Japanese subjects for 27 base pair tandem repeat polymorphism in intron 4 of NOS3. Table 1 shows the frequency of the NOS3 genotypes. No significant difference in the frequency of this polymorphism was found between genders and decades of age (data not shown). Frequencies of each genotype did not deviate from the Hardy-Weinberg equilibrium.

Clinical Characteristics and Lifestyle Factors According to NOS3 Genotype Groups

Because of the low frequency of aa homozygous, we could not analyze recessive and/or additive effects of the a allele effectively. Alternatively, we combined aa and ba genotypes (aa+ba group) and compared them with bb-type subjects (bb group) to elucidate the a allele dominant effect. The mean values of clinical characteristics and lifestyle factors in aa-type subjects were similar to those in ba-type subjects (data not shown). No significant differences were found between aa+ba and bb groups in clinical characteristics or in lifestyle factors (Table 2). Among these, age, gender, BMI, alcohol consumption, smoking status, antihypertensive medication, and history of diabetes mellitus were
TABLE 2. Clinical Parameters in Each NOS3 Genotype Group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NOS3 Genotype Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>aa + ba (n=164)</td>
</tr>
<tr>
<td>Gender, M/F</td>
<td>73/91</td>
</tr>
<tr>
<td>Age, y</td>
<td>54.0±8.9</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>23.0±3.1</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>206.1±34.3</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>59.0±15.0</td>
</tr>
<tr>
<td>Triglycerides, mg/dL*</td>
<td>109.2±55.6</td>
</tr>
<tr>
<td>Glucose, mg/dL*</td>
<td>97.9±14.0</td>
</tr>
<tr>
<td>Alcohol consumption, drinks/day</td>
<td>1.3±2.1</td>
</tr>
<tr>
<td>Physical activity intensity index (METs)</td>
<td>2.0±0.4</td>
</tr>
<tr>
<td>Antihypertensive Medication, % (n)</td>
<td>12.2 (20)</td>
</tr>
<tr>
<td>History of diabetes mellitus, % (n)</td>
<td>4.3 (7)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Smoking habit, % (n)</th>
<th>Male</th>
<th>Female</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonsmoker</td>
<td>16.4 (12)</td>
<td>94.5 (86)</td>
<td>22.1 (66)</td>
<td>93.2 (344)</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>30.1 (22)</td>
<td>11.1 (1)</td>
<td>21.4 (64)</td>
<td>2.7 (10)</td>
</tr>
<tr>
<td>Moderate smoker†</td>
<td>16.4 (12)</td>
<td>2.2 (2)</td>
<td>12.0 (36)</td>
<td>3.5 (13)</td>
</tr>
<tr>
<td>Heavy smoker†</td>
<td>37.0 (27)</td>
<td>2.2 (2)</td>
<td>44.5 (133)</td>
<td>0.5 (2)</td>
</tr>
</tbody>
</table>

Data are shown as the mean±SD or as otherwise indicated. No significant difference in parameters was found between the genotype groups.

*Data collected only from fasting subjects (n=696).
†Moderate and heavy smokers were defined as smoking<20 or ≥20 cigarettes/day, respectively.

Interaction Between NOS3 Genotype and Physical Activity Level on Blood Pressure

When the subjects in all levels of physical activity were included in analysis, there was no significant difference in blood pressure levels between aa+ba and bb groups (aa+ba versus bb: systolic blood pressure [SBP], 131.2±1.5 versus 129.5±0.7 mm Hg [least-squares mean±SEM], P=0.3170; diastolic blood pressure [DBP], 79.2±0.8 versus 78.0±0.4 mm Hg, P=0.1689, respectively). However, in the low level group, SBP level was significantly higher by 8.9 mm Hg in the aa+ba group than in the bb group (135.6±2.8 versus 126.7±1.3 mm Hg, corrected P=0.0108, Figure 1). DBP was also slightly higher in the aa+ba group than in the bb group (80.7±1.5 versus 77.2±0.7 mm Hg, corrected P=0.1059), although the difference was not significant. No such difference between the aa+ba and bb groups was found in the middle level group (aa+ba versus bb: SBP, 130.4±2.5 versus 130.4±1.3 mm Hg; DBP, 78.8±1.4 versus 78.3±0.7 mm Hg, respectively) and in the high level group (aa+ba versus bb: SBP, 128.5±2.5 versus 131.4±1.3 mm Hg; DBP, 78.5±1.3 versus 78.4±0.7 mm Hg, respectively).

Figure 2 shows the adjusted regression lines of SBP on physical activity level (in logarithm of METs; log physical activity level) in each genotype group. There was a significant inverse relation between the NOS3 genotype and physical activity level in the aa+ba group (Figure 2A, β regression coefficient=−18.47, P for β=0.0496), whereas a positive relation was found in the bb group (Figure 2B, β=9.70, P for β=0.0352). Interestingly, the slopes of regression lines were significantly different between these two groups (Figure 2C, P=0.0071 for difference in β). This demonstrated a notable interaction between the NOS3 genotype and physical activity level on SBP. Other significant covariates in this model were age, BMI, alcohol consumption, antihypertensive medication, and population area (P<0.0001 for each covariate). There were no significant interactions between these factors and NOS3 genotype on SBP (data not shown). DBP was not associated with physical activity level in either genotype group.

Such an interaction was prominent in elderly people (age ≥60 years); an inverse relation of SBP with physical activity level was found in the aa+ba group (β=−35.44, P for β=0.0376), whereas a significant positive relation was found in the bb group (β=31.18, P for β=0.0011, P for difference in β=0.0006). On the other hand, there was no significant interaction.
relation between physical activity levels and blood pressure levels in younger people (age <60 years, data not shown).

Odds Ratio of Hypertension According to NOS3 Genotype Groups

For the purpose of comparing our results with those of previous case-control studies, we calculated odds ratios of severe hypertension (defined as SBP ≥160 mm Hg and/or DBP ≥95 mm Hg) for aa+ba-type subjects compared with bb-type subjects in the high, middle, and low level groups. The odds ratio of systolic hypertension (SBP ≥160 mm Hg) in the low, middle, and high level groups was 2.4 (95% CI, 1.1 to 5.6), 1.4 (0.6 to 3.2), and 0.6 (0.2 to 1.6), respectively, indicating that the odds ratio was larger when physical activity level was lower (P for interaction=0.0474, Table 3). Notably, 27.7% of aa+ba subjects had systolic hypertension in the low level group (10.2% in the high level group) (Table 3).

In the subjects not taking antihypertensive medication, the physical activity level-dependent association between NOS3 genotype and systolic hypertension was much more prominent: odds ratio in the low, middle, and high level groups was 6.7 (95% CI, 2.0 to 22.9), 1.4 (0.3 to 6.2), and 0.2 (0.03 to 1.9), respectively (P for interaction=0.0087, Table 3).

Serum NOx Level

We measured serum NOx levels in 375 fasting subjects and found no significant differences between the 2 genotype groups (aa+ba [n=85] versus bb [n=290]: 29.1±1.0 versus 30.6±1.1 μmol/L, respectively, P=0.4776).

Discussion

In this study, we tested the hypothesis that one of the polymorphic variations at NOS3 gene loci would participate in determining blood pressure levels, and we demonstrated that the NOS3 polymorphism in intron 4 certainly influenced the inverse correlation between physical activity level and SBP. It was also noteworthy that only in low level group, the prevalence of systolic hypertension (SBP ≥160 mm Hg) was significantly higher in the aa+ba group than in the bb group (OR=2.4; 95% CI, 1.1 to 5.6) (Table 3). In other words, aa+ba-type subjects had a higher risk to be hypertensive than did bb-type subjects when their physical activity level was low. Possibly, a few subjects with extremely high SBP (≥180 mm Hg) brought the strong relation between physical activity level and SBP in the aa+ba group. However, since consistent results were obtained even through a categoric analysis with a logistic regression model that is robust for these outliers, this relation is unlikely to result from these outliers. Beneficial effects of exercise for reduction of blood pressure have been reported and supported also by meta-analyses of randomized controlled trials.23,24 Our result that the bb genotype group has a positive relation between physical activity level and SBP may seem inconsistent with previous studies. However, physical activity in this study is defined as the sum of the work time and leisure time activities, and thus our result may be in the same line with a previous report showing the highest prevalence rates of hypertension among men doing heavy physical work and having no sporting leisure activities.25 More detailed analysis discriminating occupational and leisure time activities will be needed in the future study.

Correlation between hypertension and NOS3 gene loci or its genotypes is still controversial.26–32 The NOS3 polymorphism in intron 4 is first reported to be one of the risk factors of coronary artery disease in currently smoking and ex-smoking men,4 but in the Japanese population, no association between this genotype and myocardial infarction is found in either smokers or nonsmokers.7 The shorter a allele...
frequency in the Japanese population,7–9,29,30 including ours, is much lower than that in whites in Australia and Germany4,11 (P < 0.001), suggesting a different distribution of this genotype among races.

The molecular biological involvement of this polymorphism in determining the mechanism of blood pressure levels is unclear. This genotype might be in the condition of further linkage disequilibrium with some other functional variants that lay in or out of the NOS3 gene. T(-786)C substitution is one of those candidates3 and this may account for the functional characteristics of the polymorphism in intron 4 in controlling blood pressure level. T(-786)C substitution has been reported to provide a new binding site for the repressor protein RPA1 and thereby downregulates the NOS3 gene.7 However, correlation between NOx level and intron 4 a allele or T(-786)C is still been controversial.7,33–35 In this study, we could not find significant difference in serum NOx level between aa + ba and bb groups. Therefore the current effect of NOS3 genotype may not be simply mediated by steady-state NOx level. Further investigation on the mechanism by which NOS3 gene controls the blood pressure is needed.

In previous case-control studies, neither NOS3 variation in intron 4,8–12,29,30 nor T(-786)C substitution3,12 is associated with blood pressure levels, but the physical activity levels are not taken into consideration in these studies. Similarly, in our study, before the subjects were divided into three physical activity level groups, the prevalence of systolic hypertension was not significantly different between aa + ba and bb groups (OR = 1.2; 95% CI, 0.8 to 2.0). The association between the genotype and blood pressure might be veiled by the interaction effect of physical activity level; hence the assessment of physical activity level should be incorporated into future studies.

Our findings showed that the inverse correlation between blood pressure and physical activity is affected by NOS3 genotype in a rural Japanese population. This suggests but does not prove that there is a gene-lifestyle interaction between NOS3 genotype and physical activity. Thus, it is important to study whether this result can be replicated in different populations, preferably with larger sample size. If this repeatedly holds true, then we may speculate that more efficient reduction of blood pressure can be expected for the NOS3 aa + ba group than for the bb group. However, intervention type of study is necessary before we can use this genetic information for prescribing physical therapy for the individuals. In this context, it is noteworthy that even for hypertensive subjects under medication, only in the aa + ba group but not in the bb group, physical activity level also inversely correlated with SBP level (r = 0.388, P = 0.0396, data not shown). It is tempting to speculate that NOS3 genotyping might be applied for prescribing physical exercise in addition to the antihypertensive drug therapy. Again, this should be confirmed by more intense studies in the future.

In conclusion, a 27 base pair tandem repeat polymorphism in intron 4 of NOS3 was associated with the differential response of blood pressure to physical activity level. The physical activity level seems to be a modulate factor relating NOS3 and blood pressure. This result may shed light on the mechanisms by which NOS3 and physical exercise influence blood pressure.

### Perspectives

The NOS3 27 base pair tandem repeat polymorphism in intron 4 might be applied to identify the hypertensive patients whose blood pressure is likely to be reduced more effectively through physical activity. Genotype-dependent responses to lifestyles would allow better understanding for the mechanism by which blood pressure is controlled in the individuals.
Consequently, we would be able to optimize the preventive programs individually to improve blood pressure and thereby reduce cardiovascular disease risk.

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