Association of Endothelin-1 Gene Variant With Hypertension

Jing Ji Jin, Jun Nakura, Zhihong Wu, Miyuki Yamamoto, Michiko Abe, Yasuharu Tabara, Yoshikuni Yamamoto, Michiya Igase, Katsuhiko Kohara, Tetsuro Miki

Abstract—Endothelin-1 (ET-1) is a powerful vasoconstrictor peptide produced by endothelial and smooth muscle cells. Many lines of biological evidence suggest that the ET-1 gene is a candidate gene for hypertension. Moreover, recent association studies suggested that a G/T polymorphism with an amino acid substitution (Lys/Asn) at codon 198 in exon 5 of the ET-1 gene interacts with body mass index (BMI) in association with blood pressure. They suggested that T carriers are more sensitive to weight gain than GG homozygotes in association with blood pressure. However, association studies are often irreproducible, and the first study often suggests a stronger genetic effect than is found by subsequent studies. We therefore assessed the interaction in 2 large Japanese populations. The present study showed a nonsignificant but similar trend to the results of previous reports. Moreover, in line with previous reports, this study revealed a significant interaction between the ET-1 K198N (G/T) polymorphism and BMI in association with hypertension in our populations ($P=0.027$). The interaction was significant, even after adjustment for gender and age ($P=0.045$) and for all confounding factors ($P=0.044$). T carriers were more sensitive to weight gain than GG homozygotes in association with hypertension. Considering the combined impact of obesity and hypertension on the development of cardiovascular and cerebrovascular disorders, T allele carriers might represent elective targets for therapy to lower their body weight. (Hypertension. 2003;41:163-167.)

Key Words: endothelin ■ hypertension, essential ■ genetics ■ polymorphism ■ body mass index

Endothelin-1 (ET-1) is a powerful vasoconstrictor peptide produced by vascular endothelial cells. Some patients with moderate-to-severe essential hypertension, similar to some experimental rat models with severe blood pressure elevation, exhibit enhanced endothelial expression of the ET-1 gene. Plasma ET-1 concentration is elevated in hypertensive patients. An endothelin-receptor antagonist significantly lowered blood pressure in patients with essential hypertension. Given these lines of biological evidence, the ET-1 gene is a candidate responsible for hypertension.

Hypertension is a common, complex phenotype and has been intensively studied to identify susceptibility loci in humans. Nonetheless, there is no known genotypic polymorphism consistently associated with hypertension in humans, thus far. Moreover, albeit that the development of hypertension is considered to be due at least partly to gene-gene and gene-environmental interactions, fewer interaction analyses have been conducted than simple association analyses. In this regard, the ET-1 gene is an attractive candidate because, in addition to its biological function, this gene has been shown to interact with body mass index (BMI) in association with blood pressure in 3 large populations. However, association studies are often irreproducible, and the first study often suggests a stronger genetic effect than is found by subsequent studies. We therefore assessed the interaction in 2 large Japanese populations. The present study showed a significant interaction between the ET-1 K198N (G/T) polymorphism and BMI in the association with hypertension.

Methods

Subjects

The clinical characteristics of the subjects included in the study are shown in Table 1. Population 1 (n = 2466) originated from the Ehime region of Japan, and population 2 (n = 806) from the Hyogo region of Japan. The initial rate of participation in the present study was 90% and 67% for populations 1 and 2, respectively. All subjects were Japanese urban residents. Subjects in population 1 participated in medical checkups 1 to 11 times (average 6.2 times per person), and the mean values of variables in the personal health records were used in analyses. Subjects in population 2 also underwent a medical checkup, and the values of variables in the personal health records were used in analyses. All subjects gave informed consent, and the study was approved by the ethics committee of Ehime University.

Diagnostic Categories

Each subject was assigned to one of the blood pressure diagnostic categories defined by the following criteria. Hypertensive subjects were those who had a previous diagnosis of hypertension and were being treated with antihypertensive medications (7.6%) or whose systolic/diastolic blood pressure was ≥140/90 mm Hg. Normotensive subjects were those who had never been treated with medication for hypertension and whose systolic/diastolic blood pressure was <140/90 mm Hg. Blood pressure was measured with a mercury
sphygmomanometer fewer than 3 times per year in a sitting position in clinics. Obesity was defined as BMI ≥25 kg/m² (Japan Society for the Study of Obesity). Hypertriglyceremia was defined as triglyceride (TG) ≥150 mg/dL (Japanese Atherosclerosis Society).

DNA Analysis
The TaqMan chemical method was used to detect the ET-1 K198N (G/T) polymorphism. The forward primer was 5'-GCT TGA GAA ACA-3', the reverse primer was 5'-TGT GGG CCA TGA GAA ACA-3', and the G allele-specific probe was 5'-TGT GGG CCA TGA GAA ACA-3', and the T allele-specific probe was 5'-TGT GGG CCA TGA GAA ACA-3'. The person who assessed the genotype was blinded to the clinical data of the subjects from whom the samples originated. We validated the TaqMan chemical method with more standard polymerase chain reaction (PCR)/single-strand conformation polymorphism and PCR/sequencing methods in selected 57 subjects.

Statistical Analysis
Analysis of variance was used to assess differences in means and variances of continuous variables. Comparisons of categorical variables were performed using the χ² test. General linear models were used to assess whether the ET-1 K198N (G/T) polymorphism made a statistically significant contribution to prediction of blood pressure, with consideration of interactions between the polymorphism and BMI in regression models. Logistic regression models were used to assess whether the ET-1 K198N (G/T) polymorphism made a statistically significant contribution to prediction of hypertension, with consideration of interactions between the polymorphism and BMI in regression models. Logarithmically transformed plasma TG values were used in the analyses. Probability values less than 0.05 were considered statistically significant. Statistical analysis was performed with SPSS statistical software (SPSS Inc).

Results
Frequencies of Alleles and Genotypes
Table 1 presents the clinical characteristics of the participants as a function of the 3 genotypes. The relative frequencies of the GG, GT, and TT genotypes were 53%, 38%, and 8%, respectively. The allele frequencies were 72% and 28% for the G and T alleles, respectively. These results are consistent with the Hardy-Weinberg equilibrium. The frequencies of the genotypes and the alleles in Japanese were similar to, but significantly different from, those in Caucasians.

Interaction of ET-1 G/T Polymorphism With BMI in the Association With Blood Pressure and Hypertension Status
Because the Etude Cas-Témoin de l’Infarctus Myocardies (ECTIM) study has shown a strong interaction of the ET-1 K198N (G/T) polymorphism with BMI in association with blood pressure, we analyzed the interaction in our 2 populations. This analysis showed that the interaction between the polymorphism and BMI was not significant (Table 2, Figure 1). In relation to the interaction, the ECTIM study also showed that both systolic and diastolic blood pressure in T allele carriers were significantly higher than those in GG homozygotes in obese subjects but nonsignificantly lower in lean subjects. Similar analyses in our populations showed that both systolic and diastolic blood pressures in T allele carriers were nonsignificantly higher than those in GG homozygotes in obese subjects and nonsignificantly lower in lean subjects (Table 3). These results were similar when subjects on current antihypertensive treatment were excluded.

However, blood pressure readings before the start of antihypertensive treatment were not available for 248 hypertensive subjects whose values were measured under treatment. SBP indicates systolic blood pressure; DBP, diastolic blood pressure; T-Cho, total cholesterol; HDL-Cho, HDL cholesterol; and TG, triglyceride.

### Table 1. Clinical Characteristics of Participants According to ET1 G/T Polymorphism

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Population 1</th>
<th>Population 2</th>
<th>Populations 1 and 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GG (n=1283)</td>
<td>GT (n=977)</td>
<td>TT (n=206)</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>Men, %</td>
<td>87.1</td>
<td>86.8</td>
<td>83.5</td>
</tr>
<tr>
<td>Age, y</td>
<td>52.1 (0.2)</td>
<td>51.9 (0.3)</td>
<td>51.3 (0.6)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.3 (0.1)</td>
<td>23.2 (0.1)</td>
<td>23.2 (0.2)</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>131.4 (0.4)</td>
<td>131.2 (0.5)</td>
<td>129.3 (1.0)</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>76.5 (0.3)</td>
<td>76.8 (0.3)</td>
<td>75.0 (0.6)</td>
</tr>
<tr>
<td>T-Cho, mg/dL</td>
<td>198.5 (0.9)</td>
<td>198.1 (1.0)</td>
<td>197.5 (2.2)</td>
</tr>
<tr>
<td>HDL-Cho, mg/dL</td>
<td>60.3 (0.4)</td>
<td>60.6 (0.4)</td>
<td>61.2 (0.9)</td>
</tr>
<tr>
<td>TG, mg/dL</td>
<td>139.9 (2.4)</td>
<td>137.9 (2.4)</td>
<td>128.6 (4.6)</td>
</tr>
</tbody>
</table>

Data are mean (SE). P value is for GG vs GT + TT. Blood pressure readings before the start of antihypertensive medication were not available for 248 hypertensive subjects whose values were measured under treatment. SBP indicates systolic blood pressure; DBP, diastolic blood pressure; T-Cho, total cholesterol; HDL-Cho, HDL cholesterol; and TG, triglyceride.

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**Notes:**
- Hypertension was defined as a systolic blood pressure of 140 mm Hg or higher, a diastolic blood pressure of 90 mm Hg or higher, or both.
- Hypertriglyceremia was defined as triglyceride (TG) ≥150 mg/dL.
- The population sizes for each genotype were based on the number of participants with complete data for all variables.
- The statistical significance levels were determined using the Student t test, general linear models, and logistic regression analyses.
Association studies are often irreproducible. Replication studies in large populations are indispensable to establishing an association. The ECTIM study showed a strong interaction between the ET-1 K198N (G/T) polymorphism and BMI in association with both systolic and diastolic resting blood pressure levels. That is, the study has shown that T carriers are more sensitive to weight gain than GG homozygotes in association with blood pressure. Moreover, the study has shown that, in obese subjects, both systolic and diastolic resting blood pressure levels are significantly higher in T carriers than GG homozygotes.

However, other studies have shown similar but not the same results. The Glasgow Heart Scan study showed a similar strong interaction between the polymorphism and BMI in association with the maximum blood pressure achieved during a treadmill exercise test, but not with the resting blood pressure. The Ohasama study did not assess the interaction, but has shown a significant association, that is, in obese subjects, casual diastolic blood pressure level is significantly higher in T carriers than in GG homozygotes. The study has also shown a similar trend in the relation of the polymorphism with casual systolic blood pressure in obese subjects.

Finally, the present study showed a similar trend that T carriers are more sensitive to weight gain than GG homozygotes in association with blood pressure. This study also showed a similar trend that, in obese subjects, both systolic and diastolic resting blood pressure were higher in T carriers than in GG homozygotes. Thus, the trends were all similar to those in previous reports. Moreover, in line with previous reports, the present study revealed a significant interaction between the ET-1 K198N (G/T) polymorphism and BMI in the association with hypertension in population 1, and a similar trend was shown in population 2.

Taken together, all of the studies have shown a similar trend, that is, that T allele carriers are more sensitive to weight gain than GG homozygotes in association with blood pressure. This study also showed a similar trend that, in obese subjects, both systolic and diastolic resting blood pressure were higher in T carriers than in GG homozygotes. Thus, the trends were all similar to those in previous reports. Moreover, in line with previous reports, the present study revealed a significant interaction between the ET-1 K198N (G/T) polymorphism and BMI in the association with hypertension in population 1, and a similar trend was shown in population 2.

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Taken together, all of the studies have shown a similar trend, that is, T allele carriers are more sensitive to weight gain than GG homozygotes in association with blood pressure and with hypertension (chance occurrence <0.063), providing evidence in favor of interaction between the polymorphism and BMI in association with hypertension. Consequently, the ET-1 gene may be a promising candidate responsible for hypertension. However, the first study often suggests a stronger genetic effect than is found by subsequent studies, as in the case of the ET-1 polymorphism. Therefore, further studies in large populations and a systematic meta-analytical approach are needed to accurately assess the genetic effect of the ET-1 gene. Moreover, haplotype studies, as well as examinations of variants in linkage disequilibrium with the polymorphism, are also needed.

To establish an association, it is also important that reported associations make biological sense and that associated alleles affect the gene product in a physiologically meaningful way. In this context, plasma ET-1 levels were

**Discussion**

**TABLE 2. General Linear Model for Regression of BMI in Association With Blood Pressure According to Genotype**

<table>
<thead>
<tr>
<th>Blood Pressure</th>
<th>Subjects</th>
<th>Genotype (n)</th>
<th>Coefficient</th>
<th>Constant</th>
<th>(P) (Regression)</th>
<th>Determination Coefficient</th>
<th>(P) (Interaction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure</td>
<td>Population 1</td>
<td>GG (1283)</td>
<td>1.58</td>
<td>94.5</td>
<td>&lt;0.0001</td>
<td>0.091</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>GT + TT (1183)</td>
<td>1.86</td>
<td>88.6</td>
<td>&lt;0.0001</td>
<td>0.121</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>Population 2</td>
<td>GG (421)</td>
<td>1.52</td>
<td>90.1</td>
<td>&lt;0.0001</td>
<td>0.046</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>GT + TT (385)</td>
<td>1.93</td>
<td>80.1</td>
<td>&lt;0.0001</td>
<td>0.076</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>Populations 1 and 2</td>
<td>GG (1704)</td>
<td>1.61</td>
<td>92.5</td>
<td>&lt;0.0001</td>
<td>0.077</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>GT + TT (1568)</td>
<td>1.92</td>
<td>85.5</td>
<td>&lt;0.0001</td>
<td>0.108</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>ECTIM study*</td>
<td>GG (450)</td>
<td>0.75</td>
<td>111.9</td>
<td>0.002</td>
<td>0.022</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>GT + TT (306)</td>
<td>1.99</td>
<td>80.9</td>
<td>&lt;0.0001</td>
<td>0.133</td>
<td>&lt;0.001</td>
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<tr>
<td>Diastolic blood pressure</td>
<td>Population 1</td>
<td>GG (1283)</td>
<td>1.05</td>
<td>51.9</td>
<td>&lt;0.0001</td>
<td>0.110</td>
<td></td>
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<tr>
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<td></td>
<td>GT + TT (1183)</td>
<td>1.15</td>
<td>49.9</td>
<td>&lt;0.0001</td>
<td>0.126</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>Population 2</td>
<td>GG (421)</td>
<td>1.16</td>
<td>51.1</td>
<td>&lt;0.0001</td>
<td>0.067</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>GT + TT (385)</td>
<td>1.18</td>
<td>50.8</td>
<td>&lt;0.0001</td>
<td>0.066</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>Populations 1 and 2</td>
<td>GG (1704)</td>
<td>1.07</td>
<td>52.0</td>
<td>&lt;0.0001</td>
<td>0.093</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>GT + TT (1568)</td>
<td>1.14</td>
<td>50.4</td>
<td>&lt;0.0001</td>
<td>0.103</td>
<td>0.50</td>
</tr>
</tbody>
</table>

*See Tiret et al.*

**Figure 1.** Genotype-specific regression slopes of systolic blood pressure on BMI in populations 1 and 2. Solid line indicates GG genotype; dotted line indicates GT and TT genotypes.

**Figure 2.** General linear model for regression of BMI in association with blood pressure according to genotype.
shown to be significantly higher in obese normotensives than in lean normotensives, suggesting an influence of obesity on plasma ET-1 level.\textsuperscript{12,13} Indeed, weight loss significantly decreased the plasma ET-1 level in both obese normotensives and obese hypertensives.\textsuperscript{12,14} Thus, the interaction between the ET-1 polymorphism and BMI may make biological sense. However, thus far, there is no evidence showing that the ET-1 K198N (G/T) polymorphism affects the gene product in a physiologically meaningful way, although the polymorphism changes the corresponding amino acid (Lys/Asn). Therefore, it is required to investigate a possible biological change of the gene product by the K198N (G/T) polymorphism or another variant in linkage disequilibrium with it.

In conclusion, the present study revealed a significant interaction between the ET-1 K198N (G/T) polymorphism and BMI in association with hypertension in large Japanese populations. This result is in line with biological evidence on ET-1 and with the results of 3 previous association studies. Considering the combined impact of obesity and hypertension on the development of cardiovascular and cerebrovascular disease, T allele carriers might represent elective targets for therapy to lower their body weight.

**Perspectives**

Thus far, the interaction between the ET-1 K198N (G/T) polymorphism and BMI in association with blood pressure and hypertension was assessed in 5 large populations including ours. Although these populations were studied in different design and differed in characteristics including BMI and race, all of the results showed a similar trend, suggesting the presence of the interaction. Consequently, together with several lines of biological evidence, the ET-1 gene may be a promising candidate gene for hypertension. Meanwhile, this study may have a broad implication, the importance of categorical analyses of blood pressure. The present study showed a significant interaction between the ET-1 K198N (G/T) polymorphism and BMI in association with hypertension, but a nonsignificant trend in association with blood pressure. This is possibly due to the unstable nature of blood pressure and to the presence of treated hypertensive subjects. Thus, in some populations, blood pressure may have much information content but less correct information than categorical hypertension status.

**Acknowledgments**

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**Figure 2.** Genotypic variations in relationship between BMI and hypertension. a, In population 1, the regression between BMI and the probability of having hypertension in subjects with GG genotype was represented by the equation \( y = \exp(0.16 \times -4.53x + 0.18 \times -4.70) \). The equation was \( y = \exp(0.21 \times -5.73x + 0.18 \times -4.70) \) in subjects with GT and TT genotypes. Subjects with GT and TT genotypes show a steeper slope than those with GG genotype \( P=0.035 \). b, In population 2, the regression between BMI and the probability of having hypertension in subjects with GG genotype was represented by the equation \( y = \exp(0.16 \times -4.47x + 0.18 \times -4.70) \). The equation was \( y = \exp(0.22 \times -5.97x + 0.18 \times -4.79) \) in subjects with GT and TT genotypes. Subjects with GT and TT genotypes show a steeper slope than those with GG genotype \( P=0.027 \).
References


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