Endothelin-1 Gene Variant Associates With Blood Pressure in Obese Japanese Subjects

The Ohasama Study

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Abstract—A recent report based on the results of 2 epidemiological studies, the Etude Cas-Témoin de l’Infarctus Myocarde (ECTIM) and the Glasgow Heart Scan Study, revealed that a G/T polymorphism with an amino acid substitution (Lys→Asn) at codon 198 in the endothelin-1 gene (ET-1) is associated with blood pressure in overweight people. They suggested that G/T polymorphism of ET-1 strongly interacted with body mass index (BMI) in the determination of BP levels. To examine interaction among G/T polymorphism of ET-1, BMI, and BP, we performed an association study in a general Japanese population. Subjects (n=1250) were recruited from Ohasama, a cohort in a rural community of northern Japan. DNA was extracted from buffy coat of participants, and G/T polymorphism of ET-1 was determined by the TaqMan probe polymerase chain reaction method, a powerful tool for semiautomatic genotype determination of a large number of samples. Frequency of T (Asn 198) allele in Japanese (27%) was slightly but significantly higher than in whites (24%). Baseline characteristics (age, BMI, systolic and diastolic BP, and antihypertensive treatment) of all subjects were not significantly different according to the genotype of G/T polymorphism. However, in obese subjects (≥25 kg/m²) diastolic BPs were significantly associated with G/T polymorphism of ET-1. After adjustment for confounding factors, significant association remained; for overweight subjects, diastolic BP level in those with T allele (GT + TT) was 1.8 mm Hg (P=0.04) higher than in those with GG genotype. That similar results were obtained from subjects of different races suggests that the Lys198Asn polymorphism of ET-1 is involved in determination of BP levels in obese subjects. (Hypertension. 2001;38:1321-1324.)

Key Words: genetics ■ hypertension, essential ■ insulin resistance ■ polymorphism ■ receptors, endothelin

Endothelin (ET) originally was isolated in 1988 from a supernatant of porcine aortic endothelial cell cultures and demonstrated strong vasoconstrictive peptide. ET has 3 isoforms (ET-1, ET-2, and ET-3) translated from 3 independent genes and is produced by endothelium and many tissues. ET-1 plays a role in increasing BP, cell proliferation, and modulation of vasomotor tone; it also interacts with the pathophysiology of a variety of vascular diseases, such as hypertension, arteriosclerosis, and ischemic heart disease. Because ET-1 concentration in the blood is increased by standing and decreased by volume overload, it is considered to be modulated by body fluid volume through the renin-angiotensin-aldosterone system. Because of its vasoconstrictive actions for vessels and hypertrophic actions for heart, ET-1 has been examined as an important risk for hypertension. Plasma level of ET-1 was higher in patients with essential hypertension than in normotensive subjects and paralleled the level of target organ damage. ET receptor gene was overexpressed in arteries of hypertensive patients, which suggests that ET-1 contributes to the pathogenesis of hypertension by means of endothelial dysfunction or proliferation of vascular smooth muscle cell. An additional report that chronic treatment by use of an ET receptor antagonist for hypertensive patients reduced BP also provides supporting data for the correlation between ET-1 and hypertension. Even with these supporting data, the causal relationship between ET-1 and hypertension has yet to be clarified.

On the other hand, the full length of the human ET-1 gene (ET-1) was cloned and sequenced in 1989 and mapped on 6p24-p23. The 2026-nucleotide mRNA of ET-1, excluding the poly(A) tail, is encoded in 5 exons distributed over 6836 bases.
bp. Kurihara et al.\textsuperscript{13} reported that mice heterozygous for a knockout of \textit{ET-1} showed lower levels of \textit{ET-1} than wild-type mice and developed elevated BP, which suggests that \textit{ET-1} was genetically involved in regulation of BP. A recent report by Tiret et al.\textsuperscript{14} indicated that a G/T polymorphism with an amino acid substitution (Lys\textsuperscript{3} \textarrow{\rightarrow} Asn) at codon 198 in exon 5 of \textit{ET-1} was associated with BP in overweight European people in 2 epidemiological studies, the Etude Cas-Temoïn de l’Infarctus Myocarde (ECTIM) Study and the Glasgow Heart Scan Study. To examine whether the G/T genetic variant of \textit{ET-1} is involved in hypertension or obesity in Japanese, we performed a large genetic epidemiological study in a Japanese general population.

### Methods

#### Population

Ohasama Town is a rural community 100 km north of Sendai, the central city of northeastern Japan. The Ohasama Study began in 1987 in a cohort base, and its design was described precisely by Imai et al.\textsuperscript{15} Study protocol was approved by the Institutional Review Board of the Tohoku University School of Medicine, and subjects (n=1250) who gave informed consent for genetic analysis were recruited for the present investigation. Briefly, subjects were of age \( \geq 40 \) years and were residents of 3 of the 4 regions of Ohasama (n=2716). Informed consent to participate in our BP-measuring project was given by 1957 of 1989 eligible subjects. Casual BP was measured in 1808 of these subjects. Representativeness of the subjects was confirmed previously.\textsuperscript{16} Of the representative 1808 subjects, 1250 (69\%) gave informed consent for genetic analysis. No differences were seen in gender distribution or mean age between those who did and did not participate in the present genetic study, which suggests that selection bias was less likely in this study population.

Detailed medical histories and risk factors for cardiovascular disease were ascertained for each subject. BP was measured by nurses or technicians twice consecutively in seated subjects who had rested for \( \geq 2 \) minutes. An automatic microphone-based BP-measuring device (model USM700F, UEDA Electric Work Co Ltd) was used for measurements. The device for casual BP measurement had been calibrated previously\textsuperscript{15} and met the criteria set by the Association for the Advancement of Medical Instrumentation.\textsuperscript{17} An average of 2 measurements was used for the analysis.

#### Genotype Determination With TaqMan Polymerase Chain Reaction Method

To deal with 1250 samples, we introduced the TaqMan polymerase chain reaction (PCR) method. A fluorescent reporter dye, such as 6-carboxy-flourescein (FAM) and tetrachloro-6-carboxy-fluorescein (TAMRA) in the TaqMan polymerase chain reaction (PCR) method. A fluorescent reporter dye, such as 6-carboxy-flourescein (FAM) and tetrachloro-6-carboxy-fluorescein (TAMRA) and a T allele

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>GG (n=679)</th>
<th>GT (n=464)</th>
<th>TT (n=107)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>59.4±0.3</td>
<td>60.2±0.4</td>
<td>59.5±0.9</td>
<td>0.30</td>
</tr>
<tr>
<td>Male, %</td>
<td>35.1</td>
<td>38.4</td>
<td>42.0</td>
<td>0.27</td>
</tr>
<tr>
<td>BMI, kg/m(^2)</td>
<td>23.7±0.1</td>
<td>23.6±0.1</td>
<td>23.8±0.3</td>
<td>0.66</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>25.9</td>
<td>26.6</td>
<td>28.0</td>
<td>0.96</td>
</tr>
<tr>
<td>Hyperlipidemia, %</td>
<td>14.0</td>
<td>16.8</td>
<td>9.4</td>
<td>0.10</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>17.3</td>
<td>19.0</td>
<td>15.0</td>
<td>0.51</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>132.0±0.5</td>
<td>132.3±0.6</td>
<td>133.6±1.4</td>
<td>0.52</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>74.2±0.4</td>
<td>75.0±0.4</td>
<td>75.4±0.9</td>
<td>0.19</td>
</tr>
<tr>
<td>Antihypertensive medication, %</td>
<td>30.5</td>
<td>30.6</td>
<td>33.6</td>
<td>0.80</td>
</tr>
</tbody>
</table>

logistic regression analysis by use of computer software application JMP 3.1.5 (SAS Institute Inc). \( P<0.05 \) was accepted as statistically significant.

### Results

TaqMan PCR method clearly determined G/T nucleotide substitution of Lys198Asn \textit{ET-1} polymorphism. Genotype distribution of G/T polymorphism of \textit{ET-1} did not deviate significantly from the Hardy-Weinberg expectation and was similar between men and women. Frequency of the T allele was slightly but significantly higher in Japanese (27.1\%) than whites (24.1\%)\textsuperscript{14} (Table 1).

Each mean value of baseline characteristics, including age, gender, body mass index (BMI), smoking habit (percentage), prevalence of hyperlipidemia (percentage), diabetes (percentage), systolic and diastolic BP (SBP and DBP, respectively), and antihypertensive medication, was not significantly different among subjects with GG, GT, or TT genotype of \textit{ET-1} (Table 2). In lean subjects (BMI <25 kg/m\(^2\)), \textit{ET-1} genotype was not associated significantly with SBP, DBP, or antihypertensive medication. In contrast, by dividing the subjects into 2 groups (GG and GT+TT), significance was increased in DBP among obese subjects (BMI >25 kg/m\(^2\); \( P=0.03 \), which suggests that the T allele (Asn198) has dominant effect on the increase in BP (Table 3). After adjustment for confounding factors (age, gender, and antihypertensive treatment), in overweight subjects, DBP level for those who carry the T allele (GT+TT) was 1.8 mm Hg (\( P=0.04 \)) higher than for those with GG genotype (Figure). Moreover, a steeper increase in BP with BMI occurred in subjects with the T allele (Table 2).
Several reports suggest that an increase in ET-1 concentration with another functional mutation modulating should not be discarded that the polymorphism is in linkage disequilibrium with another genetic variant in linkage disequilibrium. These results suggest that ET-1 association between BMI and ET-1 effect of agent in vitro, any direct evidence that shows a causative effect of G/T polymorphism of ET-1 has been considered not to alter expression of ET-1. Furthermore, a recent report revealed that ET-1 and ET-3 stimulate insulin release by isolated rat pancreatic islets. In addition, hyperinsulinemia upregulates ET receptors that contribute to elevated vasoconstrictor responses to ET-1 in the Zucker rat, a model of obesity and hypertension. Identification of the mechanism of ET-1 regulation should play a key role in clarification of the correlation among ET-1, the ET<sub>2</sub> receptor, hyperinsulinemia, obesity, and hypertension. Measurement of BP also plays an important role. In the present study, we applied only casual BP measurement for analysis. The next investigation should focus on the association between ET-1 polymorphism and 24-hour ambulatory BP monitoring, home BP measurement, or the white coat effect, to clarify the actual relationship between ET-1 polymorphism and hypertension or obesity. In addition, further investigation is required to clarify the direct interaction between insulin resistance and ET-1 genotype in obese subjects. In conclusion, 2 large genetic epidemiological investigations revealed the importance of determination of Lys198Asn ET-1 polymorphism in risk estimation for hypertension among subjects with obesity.

![Chart](image)

**Diastolic BP and ET-1 genotype in obese subjects after adjustment for confounding factors (antihypertensive medication, age, and gender).**

**Table 3. BP Levels According to G/T Polymorphism of ET-1 in Lean Subjects (BMI <25 kg/m²) and Obese Subjects (BMI≥25 kg/m²)**

<table>
<thead>
<tr>
<th></th>
<th>Lean Subjects</th>
<th>Obese Subjects</th>
<th>Obese Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GG (n=448)</td>
<td>GT (n=323)</td>
<td>TT (n=71)</td>
</tr>
<tr>
<td></td>
<td>GG (n=229)</td>
<td>GT (n=143)</td>
<td>TT (n=36)</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>131.2±0.7</td>
<td>131.0±0.8</td>
<td>132.4±1.7</td>
</tr>
<tr>
<td></td>
<td>133.6±0.9</td>
<td>135.3±1.1</td>
<td>136.1±2.1</td>
</tr>
<tr>
<td></td>
<td>133.6±0.9</td>
<td>135.5±1.0</td>
<td>136.1±2.1</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>73.4±0.4</td>
<td>73.9±0.5</td>
<td>74.3±1.1</td>
</tr>
<tr>
<td></td>
<td>75.6±0.4</td>
<td>77.5±0.4</td>
<td>77.8±1.5</td>
</tr>
<tr>
<td></td>
<td>75.6±0.6</td>
<td>77.5±0.7</td>
<td>0.031</td>
</tr>
</tbody>
</table>

Antihypertensive medication, % 26.1 26.0 31.0 0.68 38.9 40.6 38.9 0.95

(DBP=0.66 BMI+60) than in those who were GG homozygous (DBP=0.49 BMI+63).

Discussion

Lys198Asn polymorphism of ET-1 significantly increased BP in obese subjects (BMI≥25 kg/m²) but not in normal to lean subjects (BMI<25 kg/m²). Results from a Japanese general population were similar to those of Tiret et al. Interaction of ET-1 genotype with BMI on BP also was observed in the Ohasama population but seemed mild. In the present study, a steeper increase in BP with BMI occurred in subjects with the T allele (DBP=0.66 BMI+60) than in those who were GG homozygous (DBP=0.49 BMI+63), but this increase was not greater as reported by Tiret et al. Moreover, direct association between BMI and ET-1 genotype was not observed (Table 2). These results suggest that ET-1 polymorphism itself or another genetic variant in linkage disequilibrium with it was involved in the pathogenesis of hypertension in obese subjects.

Although ET-1 is known to be a strong vasoconstrictive agent in vitro, any direct evidence that shows a causative effect of ET-1 for hypertension has been not been reported to date. Several reports suggest that an increase in ET-1 concentration is observed with progression of hypertension and that high BP promotes an increase in ET-1, but these results are subject to debate.

In contrast, the high insulin concentration and the state of insulin resistance strongly correlated with overexpression of the ET-1 gene. According to the previous findings, we hypothesized that insulin resistance induced by obesity enhances expression of ET-1, which results in increased BP. G/T polymorphism of ET-1 has been considered not to alter the function or structure of ET-1 protein, but the possibility should not be discarded that the polymorphism is in linkage disequilibrium with another functional mutation modulating ET-1 expression. Our present understanding is that the effect of G/T polymorphism is mild in the lean state but enhanced in the obese state, which leads to the hypothesis that the effect on BP appeared only in subjects who had both obesity and T allele of ET-1. Furthermore, a recent report revealed that ET-1 and ET-3 stimulate insulin release by isolated rat pancreatic islets. In addition, hyperinsulinemia upregulates ET receptors that contribute to elevated vasoconstrictor responses to ET-1 in the Zucker rat, a model of obesity and hypertension. Identification of the mechanism of ET-1 regulation should play a key role in clarification of the correlation among ET-1, the ET<sub>2</sub> receptor, hyperinsulinemia, obesity, and hypertension.

This work was supported by research grants from the Miyagi Prefectural Kidney Association, Mitsu Life Social Welfare, Uehara Memorial Foundation, Osaka Heart Club, and Takeda Medical Foundation; a research grant entitled “Evaluation of the Effect of Drug Treatment on Hypertension and Other Chronic Disease Conditions in the Elderly,” Rojin Hoken Jigyo (1998 and 1999), Junkanki-byou Itaku Kenkyuu 11C-5 (1999), and Rojin-hoken Jigyou-hi tou Hojokin (1999), from the Ministry of Health and Welfare; and by a research grant for scientific research (Nos. 10307018, 10470102, 12204070, and 12557063) and JSPS Research Fellow (No. 01180) from the Ministry of Education, Culture, Sports, Science and Technology of Japan. We are grateful to Dr. Kenichi Nagai, the honorary director of Ohasama Hospital, for his valuable professional assistance in the research work and to Shiori Takase for her professional assistance in the research work.

**References**


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*Hypertension*. 2001;38:1321-1324
doi: 10.1161/hy1101.095333

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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