Hypoadiponectinemia Is an Independent Risk Factor for Hypertension

Yoshio Iwashima, Tomohiro Katsuya, Kazuhiro Ishikawa, Noriyuki Ouchi, Mitsuru Ohishi, Ken Sugimoto, Yuxiao Fu, Masaharu Motone, Kouichi Yamamoto, Akiko Matsuo, Koji Ohashi, Shinji Kihara, Tohru Funahashi, Hiromi Rakugi, Yuji Matsuzawa, Toshio Ogihara

Abstract—Adiponectin is one of the key molecules in the metabolic syndrome, and its concentration is decreased in obesity, type-2 diabetes, and coronary artery disease. Genetic investigation has revealed that 2 polymorphisms (I164T and G276T) are related to adiponectin concentration and diabetes. To examine whether adiponectin affects hypertension genetically or biologically, we performed a case-control study. A total of 446 diagnosed cases of hypertension (HT) in men and 312 normotensive (NT) men were enrolled in this study. Plasma adiponectin concentration was measured using an enzyme-linked immunosorbent assay system. Single nucleotide polymorphisms were determined by TaqMan polymerase chain reaction method. After adjustment for confounding factors, adiponectin concentration was significantly lower in HT (HT: 5.2±0.2 μg/mL; NT: 6.1±0.2 μg/mL; P<0.001). Furthermore, multiple regression analysis indicated that hypoadiponectinemia was an independent risk factor for hypertension (P<0.001). Blood pressure was inversely associated with adiponectin concentration in normotensives regardless of insulin resistance. In subjects carrying the TC genotype of the I164T polymorphism, adiponectin concentration was significantly lower (TC: 2.6±0.9 μg/mL; TT: 5.5±0.1 μg/mL; P<0.01), and most of them had hypertension. In contrast, the G276T polymorphism was not associated with adiponectin concentration or hypertension. In conclusion, hypoadiponectinemia is a marker for predisposition to hypertension in men. (Hypertension. 2004;43:1318-1323.)

Key Words: blood pressure ■ genetics ■ hypertension, genetic ■ men ■ mutation

Adipose tissue participates in the regulation of a variety of homeostatic processes as an endocrine organ that secretes many biologically active molecules such as leptin, tumor necrosis factor-α, and plasminogen-activator inhibitor type 1, which contribute to the development of cardiovascular disease.1–5 Furthermore, some of these molecules, such as leptin and plasminogen-activator inhibitor type 1, are known to contribute to the development of hypertension.6–8 Adiponectin is an adipose tissue-specific collagen-like factor, which is abundant in plasma, and a decrease of adiponectin is associated with obesity6 and type-2 diabetes.10 Adiponectin modulates the endothelial inflammatory response in vitro, and its concentration is decreased in patients with coronary artery disease.10–12 Furthermore, adiponectin has been reported to be associated with lipid metabolism,13,14 glucose metabolism,15 and insulin resistance.13,14,16 It was recently reported that treatment of diabetic animals with adiponectin markedly improved insulin sensitivity via reducing triglyceride accumulation in skeletal muscle.17 These results suggest that adiponectin is one of the key molecules in the metabolic syndrome.

Hypertension is a common disease that increases the risk for cardiovascular disease, and it is also a component of the metabolic syndrome, which is defined as the combination of obesity, insulin resistance, glucose intolerance, and hyperlipidemia. Hypertensive patients are known to have higher body mass index (BMI), triglyceride level, and insulin resistance compared with normotensive subjects.18 Even though an association between hypertension and serum adiponectin concentration has been reported by several groups using a small number of subjects,19–22 the obtained results were not identical. Mallamaci et al19 reported an increased plasma adiponectin concentration in hypertensive patients with renal dysfunction, but Adamczak et al20 reported decreased adiponectin in hypertensive subjects. Kazumi et al21 reported that young Japanese men with high-normal blood pressure had lower adiponectin. Recently, Furuhashi et al22 reported that only hypertensive patients with insulin resistance showed lower adiponectin concentration. Furthermore, in these studies, the association between plasma adiponectin and hypertension was evaluated without adjusting for confounding factors or without dividing the subjects by sex. It is well known that normal women have a higher adiponectin concentration than men,23 so sex is a potential confounding factor. Thus, the clinical importance of hypoadiponectinemia in hypertension has not been fully elucidated.
On the other hand, a genetic investigation revealed that subjects with the H164T polymorphism (T-to-C substitution at nucleotide 517 leading to amino acid substitution from isoleucine to threonine at position 164) more frequently had diabetes and had lower concentrations of adiponectin. It was interesting that all 9 patients with the H164T polymorphism had hypertension.25 In addition, another report showed that the G276T polymorphism in intron 2 was also associated with type-2 diabetes, partially through affecting plasma adiponectin concentration.24

To examine whether adiponectin affects blood pressure genetically or biologically, we performed a case-control study using a large number of subjects. In addition, we confirmed the hypothesis that hypoadiponectinemia is correlated with increased insulin resistance.

**Methods**

**Subjects**

A total of 758 male subjects (mean age 58.4±0.4 years, BMI 23.9±0.1 kg/m²) were selected from people who were admitted and underwent medical investigation at Osaka University Hospital or its affiliated hospitals. The numbers of normotensive subjects and hypertensive subjects were 312 and 446, respectively. Hypertension was defined as a systolic blood pressure of ≥140 mm Hg and/or a diastolic blood pressure of ≥90 mm Hg on repeated measurements, or receiving antihypertensive treatment. Diabetes was defined as fasting plasma glucose of ≥7.0 mmol/L or receiving treatment for diabetes. All subjects enrolled were Japanese, and subjects with ischemic heart disease including myocardial infarction, congestive heart failure, abnormal electrocardiogram results, valvular heart disease, atrial fibrillation, arteriosclerosis obliterans, or renal failure were excluded. The study protocol was approved by the Ethical Committee of Osaka University, and subjects gave informed consent to participate in the present study, including genetic analysis.

**Clinical Features**

Blood pressure was measured with an appropriate arm cuff and a mercury column sphygmomanometer on the left arm after a resting period of at least 10 minutes in the supine position. Blood pressure was measured by well-trained physicians who were blinded during the study, and 3 measurements at 1 visit were averaged to evaluate the systolic and diastolic blood pressures. After blood pressure measurements, venous blood sampling from all subjects was performed after fasting overnight. Height and body weight were measured, and BMI was calculated. Plasma samples for subsequent assay were stored at −80°C. Insulin sensitivity was estimated using the homeostatic model assessment (HOMA) index (ie, plasma glucose level×[plasma insulin level/22.5]). Insulin resistance was defined as HOMA ≥3. Plasma concentration of adiponectin was determined by a sandwich enzyme-linked immunosorbent assay system (adiponectin ELISA kit; Otsuka Pharmaceutical Co. Ltd.) as previously reported.8

The following parameters were also determined: total cholesterol (T-chol), triglyceride (TG), high-density lipoprotein cholesterol (HDL-chol), and serum creatinine (Cr) levels.

**Genotype Determination of Adiponectin Polymorphisms**

To investigate the association between adiponectin polymorphisms and hypertension, we selected 2 polymorphisms (H164T and G276T) that were previously reported to be related to plasma adiponectin concentration.16,24 Genomic DNA was prepared from the buffy coat using a QIAamp DNA blood kit (QIAGEN, Valencia, Calif). The genotypes of the H164T and G276T polymorphisms were determined by the TaqMan polymerase chain reaction (PCR) method.25 The following primers and probes were included in the reactions: H164T, forward primer, 5'-AAC ATT CCT GGG CTG TAC TAC TTT G-3'; reverse primer, 5'-GGG TGA CCT TCA CAT CCT TCA TA-3'; probes, 5'-FAM-CAA CAC CAC AGT CT-3', 5'-VIC-ACC ACA TCA CAG TCT A-3' ; G276T, forward primer, 5'-AGA ATG TTT CTG GCC TCT TPT ATC-3'; reverse primer, 5'-TCC TCT GTG CTT CTA GCC CCT AGT-3'; probes, 5'-FAM-AAA CTA TAT GAA GTC ATT CAT TA-3', 5'-VIC-CTA TAT GAA GTC ATT CAT TA-3'. The fluorescence level of PCR products was measured using an ABI PRISM 7900 HT Sequence Detector (Applied Biosystems).

**Statistical Analysis**

Values are expressed as mean±SE. Associations between hypertension and all other parameters were first analyzed by simple logistic regression and then by multivariate analysis. Differences in genotypes and alleles were examined by χ² analysis. The association between polymorphisms and clinical variables was examined by multivariate analysis. The quantitative effects of covariates were assessed by multiple regression analysis. P<0.05 was considered statistically significant. All calculations were performed using a standard statistical package (JMP 4.0; SAS Institute Inc).

**Results**

**Plasma Adiponectin Concentration and Hypertension**

The average length of time since the first diagnosis of hypertension was 12.5±0.6 years. Furthermore, 342 of 758 hypertensive subjects also had close relatives (parents, brothers, and sisters) who were hypertensive. To assess whether adiponectin was related to hypertension, we compared the clinical characteristics of hypertensive male subjects (HT) and normotensive male subjects (NT) (Table 1). Plasma adiponectin concentration was significantly lower in hypertensive subjects than in normotensive subjects. Age, BMI, and T-chol were also significantly higher in hypertensive men than in normotensive men. Consequently, we selected these parameters as confounding factors. After adjustment for confounding factors (age, BMI, and T-chol), adiponectin concentration was significantly lower in HT (HT: 5.2±0.2 μg/mL; NT: 6.1±0.2 μg/mL; P<0.001). Multiple regression analysis revealed that each confounding factor, age, BMI,
Characteristics

Concentration and Clinical Characteristics

Medication and normotensives without diabetes or insulin resistance significantly increased with age (in hypertensives using antihypertensive medication; the normotensive subjects were divided into 3 subgroups: with diabetes, with insulin resistance (HOMA≥3) but without diabetes, and without insulin resistance or diabetes. Thus, we compared the clinical variables among 5 subgroups (Table 3). Adiponectin concentration was significantly lower in men hypertensives using medication and normotensives without diabetes or insulin resistance, and decreased with BMI (in hypertensives using medication and normotensives without diabetes, P<0.01, respectively), and decreased with BMI (in hypertensives using medication and normotensives, P<0.01, respectively) and TG (in hypertensives using medication and normotensives with diabetes, P<0.01, respectively). Systolic blood pressure was inversely associated with adiponectin concentration in normotensive subjects without diabetes (P<0.01). Diastolic blood pressure was inversely associated with adiponectin concentration in normotensive subjects without diabetes (P<0.01). The association between plasma adiponectin concentration and blood pressure in normotensive subjects without diabetes is shown in Figure 1. However, adiponectin concentration was not associated with blood pressure in hypertensives without medication (Table 3).

Polymorphisms of Adiponectin and Hypertension

We examined the association between the I164T and G276T polymorphisms and plasma adiponectin concentration. After adjustment for confounding factors (age, BMI, TG, HDL-chol, and HOMA), plasma adiponectin concentration was significantly lower in subjects with the TC genotype of the I164T polymorphism compared with those with the TT genotype (TC: 2.6±0.9 μg/mL; TT: 5.5±0.1 μg/mL; P<0.01). No subject with the CC genotype was found in this study. The G276T polymorphism was not significantly related to plasma adiponectin concentration (GG: 5.4±0.2 μg/mL; GT: 5.8±0.2 μg/mL; TT: 4.9±0.4 μg/mL; NS) (Figure 2). We also examined the influence of these polymorphisms on the prevalence of hypertension by case-control study. The G276T polymorphism showed no association with hypertension. Table 4 shows that the TC genotype of the I164T polymorphism was significantly associated with hypertension.

Discussion

The initial finding of the present study was that plasma adiponectin concentration was significantly lower in men

**TABLE 2.** Multiple Logistic Regression Analysis for Hypertension

<table>
<thead>
<tr>
<th>Term</th>
<th>Estimate</th>
<th>SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.0497</td>
<td>0.0086</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.1144</td>
<td>0.0293</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>0.1017</td>
<td>0.0278</td>
<td>0.0003</td>
</tr>
<tr>
<td>T-chol</td>
<td>-0.0048</td>
<td>0.0023</td>
<td>0.0374</td>
</tr>
<tr>
<td>Intercept</td>
<td>3.7284</td>
<td>1.0341</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

R²=0.0754 (n=758).

**TABLE 3.** Simple Correlations Between Plasma Adiponectin Concentration and Clinical Characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Hypertensives</th>
<th>Normotensive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Medication</td>
<td>Diabetes</td>
</tr>
<tr>
<td></td>
<td>(+) (n=367)</td>
<td>(+) (n=67)</td>
</tr>
<tr>
<td></td>
<td>(-) (n=79)</td>
<td>(-) (n=93)</td>
</tr>
<tr>
<td>Age</td>
<td>0.21*</td>
<td>0.22</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.19*</td>
<td>-0.36*</td>
</tr>
<tr>
<td>T-chol</td>
<td>-0.05</td>
<td>-0.20</td>
</tr>
<tr>
<td>TG</td>
<td>-0.21*</td>
<td>-0.43*</td>
</tr>
<tr>
<td>HDL-chol</td>
<td>0.18*</td>
<td>0.11</td>
</tr>
<tr>
<td>FPG</td>
<td>-0.06</td>
<td>-0.15</td>
</tr>
<tr>
<td>HbA1C</td>
<td>-0.03</td>
<td>-0.18</td>
</tr>
<tr>
<td>HOMA</td>
<td>-0.21†</td>
<td>-0.18</td>
</tr>
<tr>
<td>Cr</td>
<td>0.15†</td>
<td>0.48†</td>
</tr>
<tr>
<td>SBP</td>
<td>-0.02</td>
<td>-0.32†</td>
</tr>
<tr>
<td>DBP</td>
<td>-0.05</td>
<td>-0.44*</td>
</tr>
</tbody>
</table>

Data indicates correlation coefficient. FPG indicates fasting plasma glucose; other definitions are defined in the text.

*P<0.01 and †P<0.05.

**Figure 1.** Correlation between plasma adiponectin concentration and blood pressure in normotensives without diabetes. ○ indicates subjects with insulin resistance (n=93); ●, subjects without insulin resistance (n=152).

**Figure 2.** Plasma adiponectin concentration in subjects with I164T and G276T polymorphisms, after adjustment for confounding factors (age, BMI, triglyceride, HDL cholesterol, and homeostatic model assessment index). Data represent mean±SEM.
with hypertension than in normotensive men and was negatively correlated with blood pressure in subjects without hypertension. Furthermore, multiple regression analysis clearly showed that hypoadiponectinemia is an independent risk factor for hypertension. Even though several studies have examined plasma adiponectin level, most of them focused on insulin resistance or diabetes and not on hypertension.

Our results were in accordance with the previous report that HOMA was significantly related to adiponectin concentration.26 Recently, Furuhashi et al22 reported that only hypertensive patients with insulin resistance showed a decreased adiponectin concentration. However, the cause–effect relationship among hypoadiponectinemia, insulin resistance, and hypertension has not been clearly elucidated. Even though the consensus has been that insulin resistance is correlated with hypertension,26,27 the association between insulin and hypertension is controversial.28–31 In fact, HOMA was not significantly different between hypertensive and normotensive subjects in the present study. As a specific finding of this study, plasma adiponectin level significantly decreased with an increase in blood pressure, even in the normotensives without insulin resistance or diabetes. These results indicate that hypoadiponectinemia may affect the pathogenesis of hypertension at a very early stage without involving insulin resistance. Recently, Lindsay et al32 reported that there were loci on chromosomes 2, 3, 9, and 10 affecting the circulating adiponectin concentration in the Pima population, suggesting the possibility of an unknown modulator of adiponectin level. However, further investigation is required to examine this hypothesis.

There are 4 possible reasons for the negative correlation between hypertension and plasma adiponectin concentration. First, as Ouchi et al33 recently reported that plasma adiponectin concentration was independently correlated with the vasodilator response to reactive hyperemia, adiponectin concentration could be an independent parameter of endothelial function. Endothelial dysfunction is an important feature of the early stage of atherosclerosis, which is related to pathogenic conditions including hypertension.34,35 Furthermore, in adiponectin-knockout mice, hypoadiponectinemia causes diet-induced hypertension. Second, an increase in sympathetic nerve activity, which is common in hypertensives,36 may inhibit adiponectin gene expression via β-adrenergic stimulation.37 Third, the reciprocal association of adiponectin and high-sensitive C-reactive protein or increased risk of arteriosclerosis suggests that a low adiponectin concentration might enhance the predisposition to hypertension via vascular injury.10,11 Fourth, activation of the renin-angiotensin system may be induced in adipose tissue by hypoadiponectinemia, resulting in an increase in fat mass and blood pressure.38,39 However, further investigation is required to examine these hypotheses.

Another important finding of this study was the positive association between plasma adiponectin concentration and age. There is a supportive report that adiponectin was decreased by sex hormones like androgens, which are suppressed with aging.23 A reduction in adiponectin clearance in older men is another possible reason for the age-related increase in adiponectin concentration. Furthermore, a previous report also suggested that age is an independent regulating factor for adiponectin concentration.40 However, it is well known that the prevalence of hypertension, insulin resistance, and diabetes increases with age. There may appear to be a discrepancy, but these results lead to the hypothesis that the implication of hypoadiponectinemia in youth is different from that in old age, and adiponectin may exert an insufficient effect without increasing sufficiently with age. The finding of a lower adiponectin concentration in elderly subjects may indicate the existence of a metabolic disorder like “adiponectin resistance.” Further investigation is required to examine these hypotheses.

The final finding of our study was related to adiponectin gene polymorphism. We examined 2 polymorphisms that were previously reported to be related to plasma adiponectin concentration in the Japanese population. Subjects with the TC genotype of the II64T polymorphism showed a significantly lower plasma adiponectin concentration, and most of the C allele carriers had hypertension. Furthermore, we also found a significant association between the TC genotype of the I164T polymorphism and hypertension. It seems to be a novel finding that >80% of C164 carriers were hypertensive in a previous study16 and in the present study. In contrast, we could not find an association between the G276T polymorphism and adiponectin concentration or hypertension. A previous study has shown an association between the G276T polymorphism and adiponectin concentration only in obese subjects (BMI ≥26.7 kg/m²).24 Because few obese subjects were included in the present study, we could not conclude a lack of association between the G276T polymorphism and adiponectin.

**Study Limitations**

This study was designed to be cross-sectional and case-controlled, but not prospective. Several important determinants of plasma adiponectin level, such as body fat content and waist circumference, were not measured in our study. Instead of these measurements, we used HOMA to evaluate insulin resistance. In addition, verification of the cause–effect relationship between hypertension and hypoadiponectinemia would require a study design with a cohort base.

It has been reported that renal function, as indicated by creatinine clearance (Ccr), is an independent regulator of adiponectin concentration in hypertensive subjects.19 In our study, also, adiponectin concentration was significantly associated with Ccr ($r = -0.38, P < 0.01$). However, the number of subjects whose Ccr was measured was small (n = 102) compared with the total number of study subjects (n = 758). The mean Ccr was almost the same in normotensive and hypertensive subjects. Therefore, Ccr was not included in the

### TABLE 4. Frequencies of Genotypes of Adiponectin Polymorphisms

<table>
<thead>
<tr>
<th>Polymorphisms</th>
<th>HT</th>
<th>NT</th>
<th>$\chi^2$</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>I164T, n</td>
<td>TT</td>
<td>433</td>
<td>311</td>
<td>6.815</td>
</tr>
<tr>
<td></td>
<td>TC</td>
<td>13</td>
<td>1</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>225</td>
<td>165</td>
<td>0.950</td>
</tr>
<tr>
<td>G276T, n</td>
<td>GT</td>
<td>180</td>
<td>124</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>41</td>
<td>23</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 4. Frequencies of Genotypes of Adiponectin Polymorphisms**

- **Polymorphisms**: Different polymorphisms of adiponectin genes.
- **HT**: Homozygous for the wild-type allele.
- **NT**: Homozygous for the variant allele.
- **$\chi^2$**: Chi-square statistic.
- **P**: P-value.

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discussion of the association between adiponectin and hypertension in this study. However, it was revealed that adiponectin concentration was significantly associated with creatinine in hypertensives using medication and normotensives with diabetes (Table 3), suggesting that hyperadiponectinemia is also involved in the progression of renal damage.

In conclusion, the present findings suggest that a lower plasma adiponectin concentration is significantly associated with hypertension. Interestingly, hyperadiponectinemia is one of the risk factors for hypertension and could be a possible target for antihypertensive treatment.

Acknowledgments
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References


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