PPARγ2 Gene Pro12Ala Polymorphism May Influence Serum Level of an Adipocyte-Derived Protein, Adiponectin, in the Japanese Population

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Adiponectin is an adipocyte-derived protein, which possesses an anti-atherosclerotic action and improves insulin sensitivity. Peroxisome proliferator-activated receptor gamma (PPARγ) regulates the transcription of many adipocyte-specific genes. A Pro12Ala polymorphism has been detected in the PPARγ2 gene, and this substitution has been reported to reduce transactivation activity in vitro. We hypothesized that individuals possessing this Ala12 allele may have a lower serum adiponectin level, because of the observation that PPARγ agonists increase the plasma adiponectin level in humans. To test this hypothesis, we investigated the effects of the PPARγ2 Pro12Ala polymorphism on anthropometric and metabolic parameters, including serum adiponectin level, in 478 Japanese men and 117 women aged 30 to 65 years. There were no homozygous subjects for the Ala12 allele of the PPARγ2 gene in this study. Plasma adiponectin levels were significantly lower in subjects with the Ala12 allele in the Japanese population of both sexes, although body mass index (BMI), plasma glucose, serum lipids, and insulin resistance index were not significantly different between subjects with and without this polymorphism. It is suggested that the Pro12Ala polymorphism of the PPARγ2 gene may reduce serum adiponectin level in the Japanese population.

ADIPONECTIN IS A plasma protein secreted specifically by adipose tissue.[1] It has an anti-atherosclerotic action through the suppression of tumor necrosis factor-alpha (TNF-α)–induced adhesion molecule expression in vascular endothelial cells and of TNF-α production itself by macrophages.[2-4] It was also reported in mouse models that adiponectin improves insulin sensitivity.[5,6] In humans, the plasma concentration of adiponectin in obese subjects was paradoxically lower than that in non-obese subjects,[7] despite the fact that adiponectin is secreted only by adipose tissue. It is, however, unclear how plasma adiponectin levels are controlled.

Peroxisome proliferator-activated receptor gamma 2 (PPARγ2) is mainly expressed in adipocytes and regulates the transcription of many adipocyte-specific genes. A Pro12Ala substitution has been detected in the PPARγ2 gene.[8] The association of this polymorphism with body mass index (BMI), insulin sensitivity, and diabetes mellitus has been studied. The Pro12Ala substitution was associated with higher BMI in 2 independent Caucasian populations[9] and in Taiwanese.[10] In contrast, the Pro12Ala substitution was associated with lower BMI in the Finnish population.[11] In carriers of the Ala12 allele, increased insulin sensitivity was reported for Japanese,[12] Japanese Americans,[11] and Caucasian subjects[13-15]. The frequency of the Ala12 allele was significantly lower in the diabetic subjects in Japanese[12,16] and Caucasian subjects.[17]

We[18] and others[19] have found a marked increase in plasma adiponectin level in subjects treated with synthetic PPARγ ligands, thiazolidinediones. It was reported that PPARγ2 with the Ala12 allele had reduced transactivation activity.[11]

We hypothesized that subjects with the Ala12 allele may have a lower serum adiponectin level because of the fact that PPARγ agonists increase the plasma adiponectin level. To test this hypothesis, we investigated the effects of the PPARγ2 Pro12Ala polymorphism on anthropometric and metabolic parameters including serum adiponectin level.

MATERIALS AND METHODS

Subjects

This study included 478 Japanese men and 117 women, aged 30 to 65 years, who received an annual health check-up. Subjects with endocrine disease, or significant renal or hepatic disease, and those receiving antidiabetic or lipid-lowering medication were excluded. The present study was conducted according to the principles expressed in the Declaration of Helsinki. Informed consent was obtained from each subject after full explanation of the purpose, nature, and risk of all procedures used. The protocol was approved by the Ethical Review Committee of the Department of Internal Medicine, Keio University School of Medicine, Tokyo, Japan.

Measurements

Height, weight, fasting plasma glucose, serum insulin, adiponectin, total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and uric acid levels were measured in the morning after an overnight fast. Plasma glucose, lipids, and uric acid were assayed by routine automated laboratory methods, as described previously.[18,20,21] Serum insulin concentration was measured by an enzyme immunoassay, using a commercially available kit (Tosoh, Tokyo, Japan). Insulin resistance index was calculated based on homeostasis model assessment (HOMA-IR).[22,23] Serum adiponectin level was measured by enzyme-linked immunosorbent assay (ELISA) without a denaturing step, as described previously.[18]

Determination of Pro12Ala Polymorphism

Pro12Ala polymorphism was determined by TaqMan (Applied Biosystems, Tokyo, Japan) polymerase chain reaction method.[24-26] The following primers and probes were included in the reaction: forward primer, 5'-GTT ATG GGT GAA ACT CTG GGA GAT-3'; reverse

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primer, 5'-TGT TTG CAG ACA GTG TAT CAG TGA A-3'; Pro-allele–specific probe, 5'-Vic-CTA TTG ACC CAG AAA G-MGB-3'; and Ala-allele–specific probe, 5'-Fam-CTA TTG ACG CAG AAA G-MGB-3'. Polymerase chain reaction (PCR) was performed with ABI Prism 7700 (Applied Biosystems) under the following conditions: initial denaturation at 95°C for 10 minutes, followed by 35 cycles of 92°C for 15 seconds, and 60°C for 60 seconds.

Statistical Analysis

All statistical analyses were performed using the StatView program for Windows (version 5.0-J, SAS Institute, Cary, NC). The relationships between PPARγ2 Pro12Ala polymorphism and clinical variables were examined by the Mann-Whitney *U* test. All data are expressed as mean ± SD, and *P* values less than .05 were considered statistically significant.

RESULTS

There were no homozygous subjects for the Ala12 allele of the PPARγ2 gene in this study. BMI, plasma glucose, serum insulin, lipids, uric acid, and HOMA-IR were not different between subjects with and without the Ala12 allele.

However, serum adiponectin level was significantly lower in subjects with the Ala12 allele than in those without, in both men and women (Tables 1 and 2). When the subjects were divided into 2 groups according to the 50th percentile of BMI, only the difference in adiponectin level was more significant in male subjects with higher BMI (>23.0 kg/m^2) ($P = .017$).

DISCUSSION

Although the relationships between the PPARγ2 Pro12Ala polymorphism and lipids, plasma glucose and serum insulin have been reported,[9-16,27,28] this is the first study to show a relationship between this polymorphism and plasma adiponectin level. Studies on the relationship between this polymorphism and plasma lipids have yielded discrepant results.[11,12,16,27,28] In this study, we did not find any difference in plasma lipids between subjects with the Ala12 allele and those without. No significant association could be detected between this polymorphism and fasting plasma glucose either, as reported previously.[9-16,27] Some studies found a significant association between the polymorphism and fasting insulin,[11-13] but others found no association[10,14-16,27] in agreement with our result. Several studies have suggested that insulin sensitivity is higher in subjects with Ala12.[11-15] In this study, HOMA-IR was not different between subjects with the Ala12 allele and those without. The reason for this discrepancy is not clear, but we consider that it may be because the subjects in this study were from the normal population and had a relatively low BMI. In the Japanese population, a significant difference in insulin resistance was observed only in an overweight or obese group.[12] In this study, however, even when the subjects were divided into 2 groups according to the 50th percentile of BMI, only the difference in adiponectin level was significant in male subjects with higher BMI (>23.0 kg/m^2). Unfortunately, we were not able to observe clinical outcome such as atherosclerosis, because this study was based on annual health check-up and most subjects were healthy individuals.

To our knowledge, this is the first study to suggest that subjects with the PPARγ2 Ala12 allele in the normal population have lower plasma adiponectin levels. In vitro studies have revealed that human PPARγ2 with Ala12 had reduced transactivation activity,[11] and that the promoter activity of adiponectin was markedly enhanced by PPARγ ligands in 3T3L1 adipocytes, although a putative PPARγ-responsive element (PPRE) in this region was not identified.[19] Taking these reports into account, it is possible that subjects with the Ala12 allele have lower adiponectin promoter activity, resulting in a lower plasma adiponectin level. In the present study, the difference in adiponectin level was more significant in male subjects with higher BMI. There is a possibility that environmental or genetic factors causing obesity may interact with the PPARγ2 gene and/or adiponectin gene, resulting in a greater difference in adiponectin level.

Table 1. Relationship Between the PPARγ2 Pro12Ala Genotype and Subject Profile and Metabolic Variables in 478 Men Aged 30 to 65 Years

Parameter	PPARγ2 Pro12Ala Genotype		*P**
	Pro/Pro	Pro/Ala	
No. of subjects	454 (95%)	24 (5%)	
Age (yr)	48.5 ± 9.4	45.8 ± 10.6	NS
Body mass index (kg/m^2)	23.2 ± 2.6	23.7 ± 3.7	NS
Glucose (mg/dL)	94.2 ± 9.7	91.0 ± 7.1	NS
Insulin (μU/mL)	5.4 ± 3.4	6.1 ± 5.3	NS
HOMA-IR	1.27 ± 0.84	1.38 ± 1.13	NS
Adiponectin (μg/mL)	7.3 ± 4.6	5.5 ± 3.0	.038
Total cholesterol (mg/dL)	200 ± 27.0	206 ± 24.7	NS
Triglycerides (mg/dL)	124 ± 95.8	134 ± 76.6	NS
HDL cholesterol (mg/dL)	54.4 ± 13.3	51.0 ± 9.2	NS
LDL cholesterol (mg/dL)	125 ± 26.7	133 ± 23.4	NS
Uric acid (mg/dL)	6.3 ± 1.2	6.3 ± 1.1	NS

NOTE. Values are the mean ± SD.
Abbreviation: NS, not significant ($P < .05$).
*Mann-Whitney *U* test.

Table 2. Relationship Between the PPARγ2 Pro12Ala Genotype and Subject Profile and Metabolic Variables in 117 Women Aged 30 to 65 Years

Parameter	PPARγ2 Pro12Ala genotype		*P**
	Pro/Pro	Pro/Ala	
No. of subjects	109 (93.2%)	8 (6.8%)	
Age (yr)	46.5 ± 8.9	44.1 ± 6.1	NS
Body mass index (kg/m^2)	20.8 ± 2.9	19.8 ± 2.6	NS
Glucose (mg/dL)	90.9 ± 9.3	91.9 ± 7.0	NS
Insulin (μU/mL)	4.5 ± 2.3	4.5 ± 1.9	NS
HOMA-IR	1.04 ± 0.58	1.04 ± 0.53	NS
Adiponectin (μg/mL)	13.6 ± 7.3	9.0 ± 3.9	.042
Total cholesterol (mg/dL)	202 ± 33.3	188 ± 26.5	NS
Triglycerides (mg/dL)	70.3 ± 33.7	74.1 ± 17.4	NS
HDL cholesterol (mg/dL)	68.5 ± 14.3	65.4 ± 14.2	NS
LDL cholesterol (mg/dL)	119 ± 28.5	110 ± 30.5	NS
Uric acid (mg/dL)	4.5 ± 1.0	4.2 ± 1.0	NS

NOTE. Values are the mean ± SD.
*Mann-Whitney *U* test.

To summarize, we found that plasma adiponectin level was significantly lower in subjects with the Ala12 allele of the PPARγ2 gene, although BMI, plasma glucose, serum lipids, and HOMA-IR were not different between subjects with and without this polymorphism. It is suggested that genetic variation in the PPARγ2 gene, together with other genes, may influence the serum level of adiponectin, which is considered to prevent atherosclerosis.

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