Pro12Ala Substitution in Peroxisome Proliferator-Activated Receptorγ2 Is Associated With Low Adiponectin Concentrations in Young Japanese Men

Nobuki Takata, Takuya Awata, Kouichi Inukai, Masaki Watanabe, Tomoko Ohkubo, Susumu Kurihara, Munemichi Inaba, and Shigehiro Katayama

Peroxisome proliferator-activated receptor gamma (PPARγ) has been shown to play an important role in adipocyte differentiation. A Pro12Ala substitution in PPARγ2 has been reported to decrease receptor activity in vitro and to be associated with a decreased risk of type 2 diabetes in the general population. Recently, a PPAR response element (PPRE) was identified in the adiponectin promoter, suggesting that decreased PPARγ activity may lead to lower adiponectin levels. In the present study, serum adiponectin concentrations and the PPARγ Pro12Ala polymorphism were analyzed to determine whether this polymorphism is associated with lower serum adiponectin concentrations in young healthy Japanese subjects. Serum adiponectin concentrations were significantly lower in men with than in those without the Ala12 allele, whereas body mass index (BMI), homeostasis model assessment (HOMA)-β, HOMA-IR, the insulin sensitivity index during oral glucose tolerance test (ISI [composite]), and serum leptin did not differ significantly between subjects with and without the Ala12 allele. Stepwise regression demonstrated BMI and the Ala12 allele to be independent predictors of serum adiponectin concentrations in men. In conclusion, the Pro12Ala substitution in PPARγ2 may reduce serum adiponectin concentrations in young Japanese men.

PEROXISOME PROLIFERATOR-activated receptor gamma (PPARγ), the molecular target for thiazolidinediones, plays a pivotal role in adipogenesis, and heterozygous PPARγ-deficient mice are reportedly partially protected from high-fat diet-induced obesity and insulin resistance.[1] It has also been reported that the Pro12Ala substitution in PPARγ2[2] decreased receptor activity and was associated with lower body mass index (BMI) and higher insulin sensitivity in Finns.[3] In the Japanese population, the frequency of Ala12 was significantly lower in diabetic than in nondiabetic subjects.[4,5] A relationship between the Ala12 allele and insulin sensitivity in overweight or obese Japanese subjects has also been described.[4]

Adiponectin, an adipocyte-derived hormone, may play an important role in the regulation of insulin sensitivity. Plasma adiponectin concentrations are reportedly reduced in individuals with obesity,[6] insulin resistance,[7] type 2 diabetes,[8] or coronary heart disease.[9] Meanwhile, adiponectin reportedly increases in response to body weight reduction[10] or treatment with PPARγ agonists, thiazolidinediones.[11] A functional PPAR response element (PPRE) has been identified in the adiponectin promoter,[12] suggesting that PPARγ regulates adiponectin expression. However, the mechanism underlying the decrease in adipocyte-derived hormone levels in obese subjects remains unclear.

Recently, an association of the PPARγ Ala12 allele with low adiponectin concentrations was reported in Japanese,[13] but not in healthy Caucasians.[14] The aim of this study was to determine whether PPARγ Pro12Ala substitution is associated with low serum adiponectin concentrations in young healthy adults who are considered to be a relatively homogenous population in terms of nongenetic factors, which may influence serum adiponectin levels.

MATERIALS AND METHODS

Subjects

A total of 247 (146 men, 101 women) young (aged 21 to 29 years, mean ± SD; 24.2 ± 1.6 years) healthy Japanese were studied. None had any significant physical illness. Informed consent was obtained from all individuals. This study was approved by the ethics committee of Saitama Medical School.

Measurements

After a 10-hour fast, all subjects underwent a 75-g oral glucose tolerance test (OGTT); plasma glucose and serum insulin concentrations were measured at 0, 30, 60, and 120 minutes. In addition, to assess β-cell function and insulin resistance, homeostasis model assessment (HOMA)-β, HOMA-IR and the insulin sensitivity index during OGTT (ISI [composite]) were calculated: (HOMA-β) = 20 × fasting insulin (μU/mL)/(fasting glucose [mmol/L] − 3.5), (HOMA-IR) = fasting insulin (μU/mL) × glucose (mmol/L)/22.5 and ISI (composite) was calculated as previously reported.[15] Plasma glucose was measured by the glucose oxidase method. Serum insulin concentrations were measured by radioimmunoassay (RIA) using a commercially available kit (Eiken Chemical, Tokyo, Japan). Fasting serum adiponectin concentrations were measured by enzyme-linked immunosorbent assay (Otsuka Pharmaceutical, Tokyo, Japan) and fasting serum leptin concentrations were measured by RIA (Linco Research, St Charles, MO).

Analysis of Pro12Ala Polymorphism

Genomic DNA was isolated from human leukocytes with a Wizard Genomic DNA purification kit (Promega, Madison, WI). The Pro12Ala polymorphism was detected by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The genomic DNA was amplified by PCR using a forward primer (5′-TCTGGGAGATTCTCCTATTGGC-3′) containing a single nucleotide mismatch and a reverse primer (5′-CTGGAAGACAACTACAAGAG-3′). The PCR conditions were: denaturation at 94°C for 11 minutes

From the Division of Endocrinology and Diabetes, Department of Medicine, Saitama Medical School, Saitama, Japan.

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Address reprint requests to Takuya Awata, MD, PhD, Division of Endocrinology and Diabetes, Department of Medicine, Saitama Medical School, 38 Morohongou, Moroyama-cho, Iruma-gun, Saitama 350-0495, Japan.

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Table 1. Clinical Characteristics of Study Subjects

	Total (n = 247)	Men (n = 146)	Women (n = 101)	*P*
Age (yr)	24.2 ± 1.6	24.5 ± 1.7	23.8 ± 1.4	<.001
BMI (kg/m^2)	21.6 ± 3.1	22.9 ± 3.2	19.8 ± 1.9	<.0001
Fasting glucose (mmol/L)	4.6 ± 0.4	4.7 ± 0.3	4.5 ± 0.4	<.0001
30-min glucose (mmol/L)	7.3 ± 1.4	7.7 ± 1.4	6.7 ± 1.2	<.0001
60-min glucose (mmol/L)	6.5 ± 1.8	6.9 ± 1.9	5.9 ± 1.5	<.0001
120-min glucose (mmol/L)	5.4 ± 1.1	5.6 ± 1.1	5.1 ± 0.9	.0005
Fasting insulin (μU/mL)	8.0 ± 4.7	8.5 ± 5.0	7.3 ± 4.2	NS
30-min insulin (μU/mL)	68.9 ± 38.2	69.4 ± 42.8	68.2 ± 30.7	NS
60-min insulin (μU/mL)	66.6 ± 36.0	67.7 ± 37.5	65.0 ± 33.8	NS
120-min insulin (μU/mL)	50.6 ± 32.4	49.9 ± 36.1	51.6 ± 26.5	NS
Adiponectin (μg/mL)	8.6 ± 3.9	6.8 ± 2.7	11.2 ± 4.0	<.0001
Leptin (ng/mL)	5.8 ± 4.2	3.9 ± 2.9	8.5 ± 4.3	<.0001
HOMA-β	146 ± 93	143 ± 81	151 ± 109	NS
HOMA-IR	1.7 ± 1.0	1.8 ± 1.1	1.5 ± 0.9	.03
ISI (composite)	6.4 ± 3.2	6.0 ± 3.1	6.8 ± 3.3	.02

Note: Values are expressed as means ± SD. *P* values are men *v* women.
Abbreviation: NS, not significant.

followed by 44 cycles of denaturation for 60 seconds, annealing at 52°C for 30 seconds, extension at 72°C for 30 seconds. The mutation created a new digestion site for HhaI, cutting the PCR product, a 154-bp segment, into fragments of 132 and 22 bp.

Statistical Analysis

All statistical analyses were performed with Stat View for Windows 5.0 (SAS, Cary, NC). We assessed the normality of the distributions of variables and used the log-transformed data of skewed variables in all analyses. Differences in variables between groups were analyzed using the unpaired *t* test. The relationships between serum adiponectin concentrations and other study variables were examined by calculating Pearson's correlation coefficients. Stepwise regression analysis was used to examine the relationships between genotypes and variables. Data are given as mean ± SD, and $P < .05$ was considered statistically significant.

RESULTS

Characteristics of Study Subjects

Characteristics of the subjects are presented in Table 1. Age, BMI, plasma glucose during OGTT and HOMA-IR were significantly higher, and serum adiponectin, leptin, and ISI (composite) significantly lower in men than in women. Among the 247 subjects, 4 (all men) showed impaired glucose tolerance, but no subject was diabetic by the 1999 OGTT criteria of the World Health Organization (WHO). Because of these differences in characteristics, men and women were analyzed separately. In both men and women, serum adiponectin was inversely correlated with BMI ($r = -0.35$, $P < .0001$ and $r = -0.33$, $P = .001$, respectively), serum leptin ($r = -0.28$, $P = .0005$ and $r = -0.34$, $P = .0004$, respectively), HOMA-β ($r = -0.21$, $P = .01$ and $r = -0.22$, $P = .03$, respectively) and HOMA-IR ($r = -0.24$, $P = .003$ and $r = -0.29$, $P = .003$, respectively), and positively correlated with ISI (composite) ($r = 0.28$, $P = .0006$ and $r = 0.29$, $P = .004$, respectively).

Relationships Between PPARγPro12Ala Substitution and Study Variables

The allelic frequency of PPARγ Ala12 was 0.036 in the entire group of subjects, similar to previous studies in Japanese populations.[4,5] There were no homozygous carriers of the Ala12 allele. In men, but not in women, serum adiponectin concentrations were significantly lower in subjects with than in those without the Ala12 allele (Table 2). Plasma glucose, serum insulin during OGTT (data not shown), BMI, HOMA-β, HOMA-IR, ISI (composite), and serum leptin did not differ between subjects with and those without the Ala12 allele in

Table 2. Clinical Characteristics of Study Subjects According to PPARγ Genotype

	Men			Women		
	Pro/Pro	Pro/Ala	*P*	Pro/Pro	Pro/Ala	*P*
N	139	7		90	11	
BMI (kg/m^2)	22.9 ± 3.2	22.7 ± 3.1	NS	19.8 ± 1.9	19.1 ± 1.4	NS
Fasting glucose (mmol/L)	4.7 ± 0.3	4.8 ± 0.4	NS	4.6 ± 0.4	4.5 ± 0.2	NS
Fasting insulin (μU/mL)	8.5 ± 5.0	7.5 ± 4.2	NS	7.4 ± 4.3	6.9 ± 2.7	NS
HOMA-β	144 ± 83	116 ± 51	NS	151 ± 114	146 ± 47	NS
HOMA-IR	1.8 ± 1.1	1.6 ± 1.0	NS	1.5 ± 0.9	1.4 ± 0.6	NS
ISI (composite)	6.0 ± 3.0	6.9 ± 4.0	NS	6.8 ± 3.4	6.7 ± 2.1	NS
Adiponectin (μg/mL)	6.9 ± 2.7	4.5 ± 1.9	<.01	11.1 ± 4.1	12.3 ± 2.2	NS
Leptin (ng/mL)	3.9 ± 3.0	3.7 ± 1.3	NS	8.5 ± 4.4	8.3 ± 3.9	NS

Note: Values are expressed as means ± SD.

Table 3. Stepwise Regression Analysis With Adiponectin as Dependent Variable (men only)

Independent Variable	β	SE	F	P
BMI	−0.35	0.25	21.19	<.0001
PPARγA1a12 allele	−0.22	0.07	8.53	.004

Note: The model includes BMI, PPARγ Pro12A1a genotype, serum leptin, HOMA-β, HOMA-IR, and ISI (composite).

both sexes. Stepwise regression analysis demonstrated BMI and the Ala12 allele (presence of the Ala12 allele) to be independent predictors of serum adiponectin levels in men (Table 3).

DISCUSSION

Previous studies have shown that PPARγ agonists increase the plasma adiponectin level,[11,16-18] and PPRE has recently been identified in the adiponectin promoter.[12] The PPARγ Pro12Ala substitution was shown to reduce the ability to transactivate responsive promoters.[3] Taking these observations together, it may be hypothesized that serum adiponectin levels are reduced in subjects with the PPARγ Pro12Ala substitution. Yamamoto et al[13] reported the relationship between the PPARγ Ala12 allele and low serum adiponectin concentrations in healthy Japanese, 30 to 65 years of age. However, Thamer et al[14] reported that there was no relationship between the PPARγ Pro12Ala substitution and serum adiponectin levels in nondiabetic Caucasians (aged 36 ± 1 year). To determine whether the PPARγ Pro12Ala polymorphism is associated with serum adiponectin concentrations, we studied a young healthy population considered to be essentially unaffected by aging, visceral fat accumulation, and the metabolic syndrome. We demonstrated an association between the PPARγ Ala12 and low adiponectin concentrations only in men, whereas the previous study[13] showed such an association in both sexes. Androgens decrease plasma adiponectin in men[19] and, in the present study, women had low BMI (19.8 ± 1.9 kg/m^2) as well as low glucose concentrations during OGTT (Table 1). These factors, which would presumably increase serum adiponectin levels in women, might mask the effect of PPARγ Ala12 on adiponectin concentrations. The discrepancy between the present and previous[13] results may arise from the difference in subject age. Age-related factors, such as sex hormone status (especially in women), visceral fat volume, muscle volume, glucose tolerance, physical activity, and so on may affect serum adiponectin levels.

The Pro12Ala substitution in PPARγ is reportedly associated with resistance to type 2 diabetes.[3-5,20] However, among subjects with type 2 diabetes, this substitution has been associated with reduced insulin secretion and a tendency for higher glycosylated hemoglobin (HbA_{1C}) levels.[5] The present and previous[13] studies have demonstrated subjects with the Ala12 allele to have decreased adiponectin levels. Is this substitution associated with resistance or susceptibility to diabetes? Hererozygous PPARγ-deficient mice were partially protected from high-fat diet-induced obesity and insulin resistance,[1] and adiponectin knockout mice showed high-fat/high-sucrose diet-induced severe insulin resistance.[21] However, with a normal diet, the insulin sensitivities of both murine models were similar to those of wild-type mice.[1,21] As to humans, we can raise the following hypothesis. During young adulthood, decreased PPARγ activity and adiponectin levels may not markedly affect insulin sensitivity. In middle age, with normal glucose tolerance, decreased PPARγ activity can promote beneficial effects, such as resistance to obesity and diabetes. However, after the development of impaired glucose tolerance with aging, visceral fat accumulation, decreased physical activity, reduced muscle volume, chronic metabolic disease, and so on, the beneficial effect of the reduced PPARγ activity may be outweighed by unfavorable effects, such as lowering adiponectin concentrations and suppressing insulin secretion. Long-term prospective studies may be necessary to assess our hypothesis.

In conclusion, the Pro12Ala substitution in PPARγ2 was associated with low serum adiponectin concentrations in young Japanese men. We suggest that low adiponectin concentrations reflect decreased receptor activity due to a Pro12Ala substitution in the PPARγ2 gene.

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