PPARγ Gene Polymorphism Is Associated With Exercise-Mediated Changes of Insulin Resistance in Healthy Men

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Exercise training improves insulin sensitivity, but individual responses vary greatly. Peroxisome proliferator-activated receptor-γ (PPARγ) is a regulator of adipose cell differentiation and plays an important role in systemic insulin action. We investigated whether *PPARγ* gene polymorphism affects insulin resistance in response to exercise in Japanese healthy men. The exercise program at an individual intensity of 50% of the maximal heart rate was performed for 20 to 60 min/d, and 2 to 3 days per week to attain a level of physical activity of 700 kcal/wk. The program was conducted for 3 months without any dietary intervention, and the clinical and metabolic characteristics were examined before and after the exercise program. Body mass index (BMI) did not change significantly after the exercise program, whereas percentage of body fat (% body fat), fasting plasma glucose (FPG), and serum leptin levels decreased significantly. Pro12Ala polymorphism in *PPARγ* gene was performed on genomic DNA isolated from human leukocytes and examined with polymerase chain reaction (PCR) and subsequent restriction enzyme analysis using BstU-I. In this study, the Ala allele did not correlate with fasting immunoreactive insulin (IRI) and homeostasis model assessment–insulin resistance index (HOMA-R) at baseline, but did so with the changes in IRI and HOMA-R after exercise (ΔIRI, Pro/Pro 0.55 ± 3.49 μU/mL *v* Pro/Ala −2.83 ± 1.47 μU/mL, *P* < .05; ΔHOMA-R, Pro/Pro 0.09 ± 0.86 v Pro/Ala −0.61 ± 0.32, *P* < .05). This result suggests that the Ala allele is associated with improvement in insulin resistance after exercise. We conclude that *PPARγ* gene polymorphism may be a reliable indicator of whether exercise will have a beneficial effect as part of the treatment of insulin resistance syndrome.

REGULAR PHYSICAL ACTIVITY directly and indirectly results in an increase in muscle glucose uptake and insulin sensitivity,[1] and plays an important role in the treatment of metabolic disorders such as type 2 diabetes. However, the effect of exercise varies for each individual and may be affected not only by environmental conditions but also by genetic background. Peroxisome proliferator-activated receptor-γ (*PPARγ*) belongs to the nuclear hormone receptor superfamily and is a central regulator of adipose cell differentiation.[2] PPARγ mRNA expression increases in adipose tissue of obese subjects, and insulin stimulates transcriptional activity of PPARγ.[3] PPARγ has also been identified as the nuclear receptor for the thiazolidinedione class of insulin-sensitizing drugs.[4] Transfection studies have shown that the Pro12Ala substitution in the *PPARγ* gene results in reduced receptor activity, suggesting that the Ala allele leads to less efficient expression of *PPARγ*-target genes.[5] Therefore, this polymorphism may be associated with lower accumulation of adipose tissue and the subsequent improvement of insulin resistance. Recently, it has been reported that a significantly improved insulin sensitivity index measured with a hyperinsulinemic-euglycemic clamp is associated with Pro12Ala polymorphism in *PPARγ* in normal glucose-tolerant Swedish men.[6] However, it remains unclear whether this polymorphism affects the response of insulin sensitivity to interventions such as diet restriction, exercise, and medication. For the study presented here, we prospectively examined the association of this gene polymorphism in Japanese healthy men with changes in insulin resistance after intervention with an exercise program.

MATERIALS AND METHODS

The subjects were 123 Japanese men aged 21 to 69 years (mean age ± SD, 45.2 ± 11.6 years), who were classified as having normal glucose tolerance on the basis of World Health Organization criteria (1998), and who had not used prescription medicine for diabetes mellitus, hyperlipidemia, or hypertension. Informed consent was obtained from all subjects. The exercise program at an individual intensity of 50% of the maximal heart rate was performed for 20 to 60 minutes a day, and 2 to 3 days each week to attain a level of physical activity of 700 kcal/wk (mainly brisk walking). Since weight reduction was not the aim of this program, it did not include any specific dietary intervention. The program was conducted for 3 months at the Hokuriku Institute of Wellness and Sports Science in Ishikawa, Japan. Blood samples were obtained in the morning after an overnight fast.

The clinical and metabolic characteristics, such as body weight, percentage of body fat (% body fat), systolic and diastolic blood pressure, serum total cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, fasting serum leptin, fructosamine, fasting plasma glucose (FPG), and fasting immunoreactive insulin (IRI) were examined before and after the exercise program. Percent body fat was calculated with the equation given by Brozek.[7] Serum leptin concentration was measured by radioimmunoassay (Linco Research Inc, St Charles, MO). Fructosamine was measured with a commercial enzymatic test (Enzymotest; Hoffman-La Roche, Basel, Switzerland), and IRI with a double-antibody radioimmunoassay for human insulin (Kabi Pharmacia Diagnostics, Uppsala, Sweden). Obesity was assessed in terms of body mass index (BMI = body weight [kg]/height [m]^2), and insulin resistance with the homeostasis model assessment–insulin resistance index (HOMA-R = FPG [mmol/L] × IRI [μU/mL]/22.5). Genotyping was performed on genomic DNA extracted from peripheral blood leukocytes with a QIAamp DNA Blood Mini kit (QIAGEN K.K., Tokyo, Japan). A 270-bp fragment of the *PPARγ* gene encompassing

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Table 1. Association of *PPARγ* Gene Polymorphism With Clinical and Metabolic Characteristics at Baseline (N = 123)

Variable	Pro/Pro (n = 117)	Pro/Ala (n = 6)	*P* Value
Body mass index (kg/m^2)	23.4 ± 2.9	22.7 ± 2.1	.548
% Body fat (%)	19.6 ± 7.5	23.5 ± 5.6	.219
Systolic blood pressure (mm Hg)	127.9 ± 15.2	132.0 ± 30.0	.542
Diastolic blood pressure (mm Hg)	79.3 ± 11.7	84.3 ± 16.7	.312
Total cholesterol (mmol/L)	5.2 ± 0.8	5.6 ± 0.8	.257
Triglyceride (mmol/L)	1.4 ± 0.8	1.2 ± 0.7	.656
HDL-cholesterol (mmol/L)	1.4 ± 0.4	1.4 ± 0.3	.999
Serum leptin (μg/L)	4.2 ± 2.7	2.9 ± 1.1	.238
Fructosamine (μmol/L)	263.0 ± 18.9	250.7 ± 17.1	.121
Fasting plasma glucose (mmol/L)	5.1 ± 0.4	5.0 ± 0.6	.629
Fasting immunoreactive insulin (μU/mL)	5.7 ± 3.8	6.8 ± 1.5	.453
HOMA insulin resistance index	1.3 ± 0.9	1.5 ± 0.5	.503

NOTE. Data are the mean ± SD.

the site of the polymorphism was generated from genomic DNA by means of polymerase chain reaction (PCR) using the primers 5′-GCCAATTCAAGCCCAGTC-3′ (forward) and 5′-GATATGTTTG-CAGACAGTGTATCAGTGAAGGAATCGCTTTCCG-3′ (reverse). The PCR products were digested with BstU-I, electrophoresed on a 2.5% agarose gel, and stained with ethidium bromide.[8] The expected products after digestion with BstU-I were 270 bp for normal homozygotes, 227 bp and 43 bp for Pro12Ala homozygotes, and 270 bp, 227 bp, and 43 bp for heterozygotes.

Statistical Analysis

All data are expressed as the mean ± SD. Statistical analyses were performed with the StatView V statistical package for Macintosh (Abacus Concepts, Berkeley, CA). Differences between genotypes and means were tested with the Bonferroni *t* test after justification by 1-way analysis of variance (ANOVA). The chi-square test was used to compare frequencies. A *P* value less than .05 indicated statistical significance.

RESULTS

After the 3-month exercise program, the levels of % body fat, serum leptin, and FPG significantly decreased (% body fat: 19.8% ± 7.4% to 19.0% ± 7.2%, $P < .01$; serum leptin: 4.1 ± 2.7 μg/L to 3.7 ± 1.8 μg/L, $P < .05$; FPG: 5.08 ± 0.42 mmol/L to 4.94 ± 0.47 mmol/L, $P < .01$), whereas the other clinical or metabolic characteristics showed no significant differences. The frequency of the Pro12Ala allele was 0.024 in our study (Pro/Pro: 117 subjects; Pro/Ala: 6 subjects). As shown in Table 1, there was no correlation between *PPARγ* gene polymorphism and clinical and metabolic characteristics at baseline. Association of *PPARγ* gene polymorphism with changes in clinical and metabolic characteristics after exercise, as shown in Table 2, demonstrates that this polymorphism significantly correlates with the exercise-mediated changes in IRI and HOMA-R. Subjects with the Ala allele improved more in IRI and HOMA-R than those without it (Fig 1).

DISCUSSION

Insulin resistance is a key factor in the pathogenesis of type 2 diabetes, and is caused by such factors as obesity and physical inactivity. A thiazolidinedione class of insulin-sensitizing agents acts as high-affinity ligands for PPARγ, and reduces expression or concentration of adipocyte-derived factors such as free fatty acid, tumor necros factor-alpha, and leptin, which are known to cause peripheral insulin resistance.[9-11] Because Pro12Ala polymorphism is associated with reduced transcriptional activity of *PPARγ* in vitro,[5] it may influence the adipocyte-mediated insulin resistance status. It has been reported that the polymorphism of *PPARγ* is associated with lower BMI and IRI levels, and that the Ala allele frequency is lower in type 2 diabetes than in control subjects.[5,12,13] On the other hand, other studies including ours have been unable to replicate these

Table 2. Association of *PPARγ* Gene Polymorphism With Changes in Clinical and Metabolic Characteristics After Exercise (N = 123)

Variable	Pro/Pro (n = 117)	Pro/Ala (n = 6)	*P* Value
Δ Body mass index (kg/m^2)	0.01 ± 0.50	0.20 ± 0.47	.375
Δ % Body fat (%)	−0.87 ± 2.42	−0.82 ± 1.75	.958
Δ Systolic blood pressure (mm Hg)	1.08 ± 12.57	8.83 ± 9.58	.140
Δ Diastolic blood pressure (mm Hg)	−0.58 ± 9.96	0.33 ± 9.85	.827
Δ Total cholesterol (mmol/L)	0.005 ± 0.51	−0.04 ± 0.34	.836
Δ Triglyceride (mmol/L)	0.05 ± 0.60	0.04 ± 0.34	.957
Δ HDL-cholesterol (mmol/L)	0.007 ± 0.15	−0.02 ± 0.09	.653
Δ Serum leptin (μg/L)	−0.46 ± 2.14	−0.27 ± 0.75	.823
Δ Fructosamine (μmol/L)	0.11 ± 14.14	−2.67 ± 7.63	.635
Δ Fasting plasma glucose (mmol/L)	−0.15 ± 0.48	0.14 ± 0.44	.146
Δ Fasting immunoreactive insulin (μU/mL)	0.55 ± 3.49	−2.83 ± 1.47	.020
Δ HOMA insulin resistance index	0.09 ± 0.86	−0.61 ± 0.32	.050

NOTE. Data are the mean ± SD.

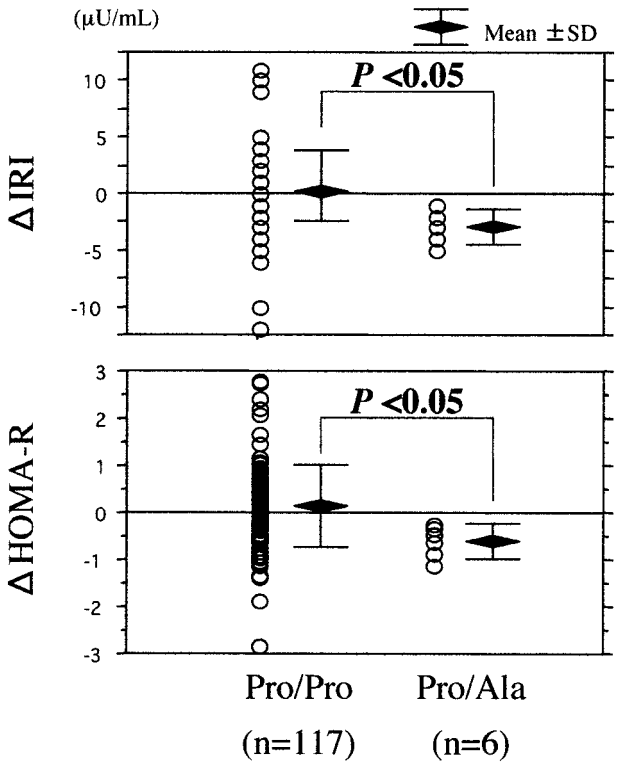

Fig 1. Individual subject data of Pro12Ala polymorphism in PPARγ for the 2 parameters correlating with exercise-mediated changes.

results.[14-17] The allele frequency of the Pro12Ala polymorphism in *PPARγ* gene was 0.12 in Caucasian Americans, 0.10 in Mexican Americans,[8] 0.15 in the United Kingdom, 0.12 in Central Europe, and 0.07 in Southern Europe.[18] The allele frequency of the Pro12Ala polymorphism in *PPARγ* gene was 0.024 in our study, which was similar to that reported in a previous study of Japanese subjects.[13,14] The Ala allele frequency for Japanese is rather lower than for Americans or Europeans. The discrepancies among these studies may therefore be due to racial differences in the Ala allele frequency or distribution in BMI.

Hara et al found that neither fasting plasma insulin level nor HOMA-R was affected by the Ala allele in lean and normal-weight groups, and that both fasting plasma insulin level and HOMA-R were lower in subjects with the Ala allele than those without it in an overweight group.[13] Since the *PPARγ* gene polymorphism may be associated with the genetic or environmental factors causing obesity, it is important to investigate whether this polymorphism affects response to some interventions. Only in mice, it has been reported that heterozygous of *PPARγ* resulted in protection from the development of insulin resistance due to adipocyte hypertrophy during administration of a high-fat diet.[19] However, it still remains unclear whether this polymorphism affects the response of metabolic markers to physical activity, as we have demonstrated here in healthy Japanese men. In our study, *PPARγ* gene polymorphism correlated with the improved insulin resistance after exercise in healthy Japanese men, although it did not do so with insulin resistance at baseline. These findings suggest that the Ala allele of *PPARγ* contributed to the reduction in insulin resistance after exercise. This result seemed to be independent of the change in % body fat after exercise because there were no significant differences in the change in % body fat between the genotypes. Muscle and liver play a critical role in insulin resistance. Although these tissues express only trace amounts of PPARγ under basal conditions, these minute quantities of PPARγ might be sufficient to account for the improvement in insulin resistance after exercise. How PPARγ activity affects the responses of insulin resistance in muscle and liver to exercise needs to be clarified.

This study may provide a basis for selecting the most suitable exercise prescription for the prevention and treatment of insulin resistance syndrome related disorders such as type 2 diabetes and cardiovascular disease. It would be worth examining whether a more intensive exercise program can improve insulin sensitivity in subjects without the Ala allele in *PPARγ* gene and whether mutations in other insulin resistance–associated genes can also serve as predictors of the effect of exercise on metabolic abnormality. Patients with insulin resistance or type 2 diabetes who are genetically poor responders to exercise may need to be educated and intensively cared for through extensive exercise programs.

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