Peroxisome Proliferator–Activated Receptor Gamma 2 Pro12Ala Gene Variant Is Strongly Associated With Larger Body Mass in the Taiwanese

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The peroxisome proliferator–activated receptor gamma 2 (PPARγ2) has been studied extensively because of its putative role in adipocyte differentiation and insulin sensitivity. The association of the Pro12Ala and Pro115Gln PPARγ2 gene variants with type 2 diabetes mellitus, the body mass index (BMI), and other diabetes-related phenotypes was examined in the Taiwanese population. Genotypes were determined by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis. Allele frequencies were compared between 280 subjects with type 2 diabetes mellitus and 310 subjects without diabetes using the chi-square test. Continuous phenotype analysis was performed by multiple logistic regression adjusting for age and BMI where appropriate. There was no significant association between the Pro12Ala gene variant and type 2 diabetes; the frequency of the Ala12 allele was 0.03 in type 2 diabetics and 0.04 in nondiabetics ($P = .40$). The Gln115 allele was not detected in any of the cases or controls. In multiple linear regression analysis of all cases and controls combined adjusted for age, sex, and diabetic status, carriers of the Ala12 allele had a mean BMI of 25.9 ± 0.5 kg/m² (mean ± SE), compared with 24.2 ± 0.1 kg/m² in Pro12 homozygotes ($P < .001$). In addition, carriers of the Ala12 allele have a 2.9 times (95% confidence interval [CI], 1.5 to 5.5) higher odds of having a BMI of at least 25 kg/m². These results suggest that in the Taiwanese, the Pro12Ala PPARγ2 gene variant may contribute to fat accumulation and a higher BMI independent of type 2 diabetes. These results need to be confirmed in future studies, as a linkage disequilibrium of this variant with other mutations cannot be ruled out.

TYPE 2 DIABETES MELLITUS has been an enigma from many standpoints, including genetic analysis. Therefore, one strategy has been to examine intermediate phenotypes that lead to type 2 diabetes including obesity and insulin resistance. Peroxisome proliferator–activated receptor gamma 2 (PPARγ2) is a transcription factor that appears to be involved in both adipocyte differentiation and insulin sensitivity.[1] The PPARγ gene, localized to chromosome 3p25,[2] encodes 2 isoforms, PPARγ1 and PPARγ2, which result from alternative promoters and differential splicing. In comparison to PPARγ1, PPARγ2 has an additional 28 amino acids at the NH2 terminus and appears more sensitive to insulin stimulation.[3] PPARγ2 also appears to be expressed exclusively in adipose tissue, in comparison to the wider distribution of PPARγ1 in adipose tissue, skeletal muscle, heart, and liver.[4]

PPARγ2 either increases or decreases adipocyte differentiation and insulin sensitivity via ligand-dependent and independent pathways.[1,5,6] On the one hand, thiazolidinedione and endogenous ligands such as fatty acids bind to PPARγ, leading to increased adipocyte differentiation and insulin sensitivity. On the other hand, in the presence of insulin and other growth factors, mitogen-activated protein kinase catalyzes the phosphorylation of a serine site in position 114 of the PPARγ2 protein, leading to decreased adipocyte differentiation and insulin sensitivity. A mutation was detected at the flanking 115 position which also appears to be involved in serine phosphorylation.[7] Therefore, mutations in the PPARγ2 gene may have varying effects depending on whether the mutation is located near ligand binding sites or the serine phosphorylation. In this study of the Taiwanese population, we examined the association of Pro12Ala and Pro115Gln PPARγ2 gene variants with type 2 diabetes, the body mass index (BMI), and other diabetes-related phenotypes.

SUBJECTS AND METHODS

Taiwanese Subjects

We studied 286 type 2 diabetic subjects randomly selected from patients at the outpatient diabetes clinic at National Taiwan University Hospital. Taiwanese individuals are primarily of Huanan descent. Type 2 diabetes was defined by physician diagnosis and at least 2 fasting glucose values higher than 7.8 mmol/L (140 mg/dL). For controls, 310 nondiabetic subjects were randomly selected from individuals who were admitted for a routine health examination at the same hospital. All subjects provided informed consent.

DNA Analysis

Genotype analysis was performed on genomic DNA isolated from peripheral whole-blood samples based on previously published methods.[8] A 270–base pair (bp) region encompassing the site of the Pro12Ala polymorphism in the PPARγ2 gene was detected by polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) analysis. PCR amplification was performed using an upstream primer (5′ GCC AAT TCA AGC CCA GTC 3′) and mutagenic downstream primer (5′ GAT ATG TTT GCA GAC AGT GTA TCA GTG AAG GAA TCG CTT TCC G 3′). The mutagenic primer introduces a *Bst*UI restriction site only when the C → G substitution at nucleotide 34 is present. PCR conditions were as follows: denaturation at 94°C for 5 minutes followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 65°C for 45 seconds, and extension at 72°C for 1 minute, with a final extension at 72°C for 10 minutes. A control without DNA was included with all PCRs to check for contamination. PCR products were digested with *Bst*UI (New England Biolabs, Beverly, MA) for 12 hours at 60°C. The digested products were then

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Submitted July 9, 1999; accepted April 9, 2000.

Supported by grants from the National Science Council of the Republic of China (NSC-87-2314-B-002-082 and NSC-88-2314-B-002-043).

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0026-0495/00/4910-0013$10.00/0

doi:10.1053/meta.2000.9517

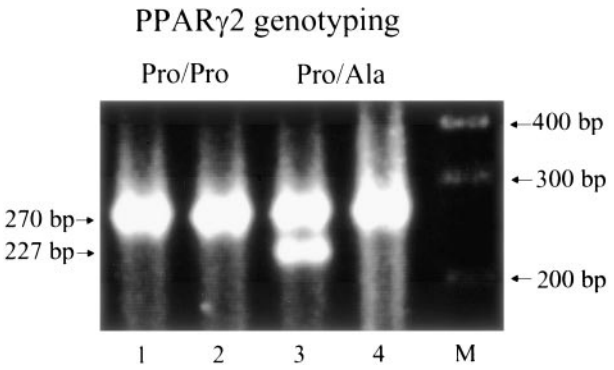

Fig 1. PCR/RFLP analysis of PPARγ2 Pro12Ala genotype. PPARγ2 genotypes were determined by the PCR products digested with *Bst*UI (lanes 1 and 3), with PCR products without digestion (lanes 2 and 4) as a control, after separation on a 2.5% agarose gel. The wild-type (Pro) allele results in 1 band of 270 bp (upper arrow), while the mutant (Ala) allele results in 2 bands of 227 bp (lower arrow) and 43 bp (not shown). M indicates the 100-bp DNA ladder.

analyzed on a 2.5% agarose gel stained with ethidium bromide. The wild-type allele results in 1 band of 270 bp, while the mutant allele results in 2 bands of 227 bp and 43 bp (Fig 1).

A 129-bp region encompassing the site of the Pro115Gln polymorphism in the PPARγ2 gene was detected by PCR-based RFLP analysis based on previously published methods.[7] PCR amplification was performed using an upstream primer (5′ TGC AAT CAA AGT GGA GCC TGC ATG TC 3′) and mutagenic downstream primer (5′ CAG AAG CTT TAT CTC CAC AGA C 3′). The PCR conditions were as follows: denaturation at 94°C for 5 minutes followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds, with a final extension at 72°C for 10 minutes. A control without DNA was included with all PCRs to check for contamination. PCR products were digested with *Hin*cII (New England Biolabs) for 12 hours at 37°C. The digested products were then analyzed on a 3.0% agarose gel stained with ethidium bromide. The wild-type allele results in 1 band of 129 bp, while the mutant allele results in 2 bands of 104 bp and 25 bp (data not shown).

Statistical Analysis

Using the World Health Organization (WHO) criteria, ordinary obesity was defined as a BMI of 25.0 kg/m^2[,9] or higher, and using the usual US criteria, extreme obesity was defined as a BMI of at least 27.8 kg/m.[2,10] Percent body fat was calculated using the following formula: % body fat = 1.2 × BMI + 0.23 × age − 10.8 × (1 if male, 0 if female) − 5.4.[11]

Multiple linear regression was used to compare quantitative phenotypic traits between subjects with and without the Ala12 allele, with results presented as the adjusted mean ± SE. Multiple linear regression was also used to analyze genotypes and data from all cases and controls, with all analyses adjusted for age, sex, and diabetic status. Multiple logistic regression was used to assess the strength of the association between the Pro12Ala PPARγ2 gene variant and type 2 diabetes along with other dichotomous phenotypes such as obesity. SAS (Version 6.12) was used for all statistical comparisons.[12]

RESULTS

Compared with the nondiabetic controls, type 2 diabetic cases tended to be older and female and to have a higher BMI, percent body fat, fasting glucose, hemoglobin A_{1c} (HbA_{1c}), triglycerides, and total cholesterol (Table 1). The overall frequency of the Ala12 allele in this Taiwanese study population was 0.04, and there was no difference in the allele frequency between type 2 diabetic cases and nondiabetic controls (0.03 *v* 0.04, $P = .40$). No subjects in our data set were homozygous for the Ala12 allele. The Gln115 allele was not detected in any of the cases and controls.

Table 1. Selected Characteristics of 596 Taiwanese Type 2 Diabetic Cases and Nondiabetic Controls

Characteristic	Type 2 Diabetic Cases (n = 286)	Nondiabetic Controls (n = 310)	*P*
Age (yr)	58.7 ± 12.4	49.1 ± 11.2	<.001
Male gender, n	144 (50.4%)	195 (62.9%)	.002
BMI (kg/m^2)	24.9 ± 3.5	23.8 ± 3.0	<.001
Body fat (%)*	32.5 ± 7.5	27.7 ± 6.1	<.001
Weight (kg)	64.2 ± 11.6	64.4 ± 10.7	.89
Height (cm)	160.5 ± 9.0	164.0 ± 8.1	<.001
Fasting glucose (mg/dL)	147.4 ± 45.9	91.3 ± 9.8	<.001
HbA_{1c} (%)	7.9 ± 1.5	5.5 ± 0.5	<.001
Triglycerides (mmol/L)	2.5 ± 2.2	1.9 ± 1.2	<.001
Total cholesterol (mmol/L)	5.2 ± 1.1	4.9 ± 1.0	.02

NOTE. Results are the mean ± SD.

*Body fat = 1.2 × BMI + 0.23 × age − 10.8 × (1 if male, 0 if female) − 5.4.

Of 43 carriers of the Ala12 allele, 18 (42%) had diabetes and 24 (56%) were men. Before adjustment for potential confounding variables, the mean age among Ala12 allele carriers was 51.4 ± 1.8 years, (mean ± SE), the mean BMI was 25.8 ± 0.6, mean body fat 31.4% ± 1.2%, and mean weight 69.3 ± 2.2 kg. The distribution of BMI values among Pro/Pro and Pro/Ala individuals was similar (Fig 2). However, in adjusted analyses of quantitative phenotypes in all cases and controls (Table 2), carriers of the Ala12 allele had a higher BMI (25.9 *v* 24.2 kg/m,^{2} $P < .001$) after adjusting for age, sex, and diabetic status. This result remained statistically significant even after log arithmically transforming the BMI values to account for the skewed data (data not shown). Ala12 carriers also had a 2.9 times (95%

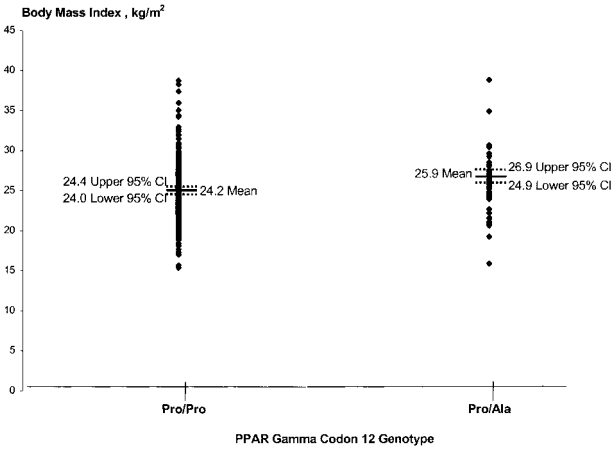

Fig 2. Scatter plot of body mass index (kg/m^2) by PPARγ2 Pro12Ala genotype. The mean values and 95% CI are shown.

Table 2. Selected Characteristics of 596 Taiwanese Subjects by PPARγ2 Pro12Ala Genotype

Trait	Pro/Pro (n = 553)	Pro/Ala (n = 43)	*P*
BMI (kg/m²)*	24.2 ± 0.1	25.9 ± 0.5	<.001
Body fat (%)*†	29.9 ± 0.2	31.9 ± 0.6	<.001
Weight (kg)‡	64.0 ± 0.4	68.8 ± 1.3	<.001
Fasting glucose (mg/dL)§	113.4 ± 1.4	114.4 ± 5.2	.85
HbA_{1c} (%)§	6.4 ± 0.05	6.4 ± 0.2	.84
Triglycerides (mmol/L)§‖	1.8 ± 1.0	1.6 ± 1.1	.21
Total cholesterol (mmol/L)§‖	4.9 ± 1.0	4.8 ± 1.0	.65

NOTE. Results are the mean ± SE from the general linear model.

*Adjusted for age, sex, and diabetic status.

†Body fat = 1.2 × BMI + 0.23 × age − 10.8 × (1 if male, 0 if female) − 5.4.

‡Adjusted for age, sex, diabetic status, and height in cm.

§Adjusted for age, sex, diabetic status, and BMI.

‖*P* for log-transformed values.

confidence interval [CI], 1.5 to 5.5) higher odds of being obese by WHO criteria and a 2.5 times (95% CI, 1.2 to 5.4) higher odds of being extremely obese by US criteria. In addition, after adjusting for age, sex, and diabetic status, carriers of the Ala12 allele had a higher percent body fat (31.9% *v* 29.9%, $P < .001$) and higher body weight even after additional adjustment for height (68.8 *v* 64.0 kg, $P < .001$). The Pro12Ala gene variant was not associated with fasting glucose, HbA_{1c}, triglycerides, or total cholesterol.

DISCUSSION

We found that in the Taiwanese population, the Pro12Ala PPARγ2 gene variant is strongly associated with larger body mass. Despite using different measurements for body size such as the BMI, percent body fat, and weight, we were able to show consistently that the Pro12Ala gene variant is associated with each. The equation that was used to measure percent body fat has been shown to be a reliable measure of body fat. Approximately 80% of the variation in body fat between individuals can be explained by this formula. Percent body fat as estimated by this equation has a standard error of about 4%.[11] We also found that individuals in the categories of ordinary obesity (BMI ≥25.0 kg/m²) and extreme obesity (BMI ≥27.0 kg/m²) tended to be carriers of the Ala12 allele.

No direct conclusion can be drawn from the present study regarding the relationship between this genetic variant and the pathogenesis of type 2 diabetes, as the study population was relatively lean and no measurement of insulin sensitivity was performed. However, the finding of an association between larger body mass and PPARγ2 is interesting and unexpected precisely because of the relative leanness of the study population. The difference in mean body mass between the group that does not carry the Ala12 allele and the group that does was small, but despite this, we were able to find a statistically significant association. The power to detect associations between the variant and body mass would have been much higher if we had a more obese population, because the phenotype would have been more extreme. However, even with the decreased power due to the leanness of the study participants, our study detected an association. And although the distribution of BMI values in both the Ala carriers and noncarriers was similar, the 95% CIS for the mean BMI of the 2 groups do not overlap.

Our relatively large sample size of 286 cases and 310 controls made it possible to detect the moderate effect of the Pro12Ala PPARγ2 gene variant on body mass. However, it is not surprising that we were not able to detect an association between the gene variant and type 2 diabetes, given our current understanding of PPARγ function. PPARγ2 may play an indirect role in increasing the risk of type 2 diabetes by increasing the risk of obesity and subsequent insulin resistance. The effects of PPARγ2 would thus be many steps removed from the onset of type 2 diabetes, and its effects on type 2 diabetes diluted along the pathway.

Studies examining the Pro12Ala polymorphism and its association with type 2 diabetes, obesity, and insulin sensitivity have had inconsistent results. Yen et al[8] identified the PPARγ2 C → G substitution at codon 12 causing alanine to be substituted for proline. In 2 Caucasian populations, one from a study of aging and one composed of very obese individuals with a mean BMI of 36.5 kg/m,[2] the Pro12Ala variant was associated with a higher BMI.[13] In contrast, the Ala12 variant is associated with a lower BMI and improved insulin sensitivity among Finns from a population-based study.[14] Likewise, the Ala12 variant is associated with lower odds of type 2 diabetes in Japanese Americans.[14] Still other studies have not shown any associations at all between the Pro12Ala PPARγ2 gene variant and diabetes-related phenotypes.[15,16] These conflicting results are most likely due to differences in selection criteria for study participants, ethnicity, sample size, and allele frequencies.

Further studies are needed to elucidate the functional and clinical significance of the Pro12Ala gene variant. Without an understanding of how the Pro12Ala mutation affects protein function, it is difficult to conclude that this specific mutation causes an increase in the BMI. It is possible that this variant is in linkage disequilibrium with a polymorphism that is the true source of the observed associations. To provide a more complete picture of the multifactorial nature of obesity, data on other gene variants, as well as environmental factors such as caloric intake, are needed. Nevertheless, our study provides an additional piece of evidence to support the role of PPARγ2 in fat accumulation independent of type 2 diabetes.

ACKNOWLEDGMENT

We thank K.C. Lee and C.L. Chao for technical assistance.

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