BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **251,** 195–198 (1998)
ARTICLE NO. RC989421

Effect of the Pro12Ala Variant of the Human Peroxisome Proliferator-Activated Receptor γ2 Gene on Adiposity, Fat Distribution, and Insulin Sensitivity in Japanese Men

Yasumichi Mori,*,† Hoon Kim-Motoyama,* Tomiyoshi Katakura,‡ Kazuki Yasuda,†,§ Hiroko Kadowaki,† Brock A. Beamer,¶ Alan R. Shuldiner,‖ Yasuo Akanuma,† Yoshio Yazaki,* and Takashi Kadowaki*,[1]

**Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Tokyo, Japan; †Institute of Diabetes Care and Research, Asahi Life Foundation, Tokyo, Japan; ‡Nippon Express Health Insurance Society, Tokyo, Japan; §Department of Complex Diseases, Faculty of Medicine, University of Chiba, Chiba, Japan; ¶Division of Geriatric Medicine and Gerontology, Johns Hopkins University, Baltimore, Maryland 21205; and ‖Division of Diabetes, Obesity and Nutrition, University of Maryland School of Medicine, Baltimore, Maryland 21201*

Received September 3, 1998

To examine the role of the Pro12Ala variant of the human PPARγ2 gene on adiposity and insulin resistance, we studied the effect of the variant on fat distribution assessed by CT scan, plasma glucose, and insulin levels during a 75g oral glucose load in 215 non-diabetic Japanese men. The allele frequency of the variant was 0.03 in this population. There were no differences in body mass index (BMI), subcutaneous fat area (S), visceral fat area (V), V/S ratio, fasting plasma insulin levels, or insulin resistance index in homeostatic model assessment between 203 subjects who were homozygous for the wild-type Pro12 allele and 12 subjects with the variant Ala12 allele (11 heterozygotes and one homozygote). These data suggest that the Pro12Ala variant is not a major contributor to adiposity, fat distribution, or insulin resistance in Japanese men.

Obesity contributes to many health disorders, such as type 2 diabetes, hypertension, cardiovascular and respiratory diseases. To understand the mechanism of these obesity-related diseases, not only the quantity of fat but also the distribution of adipose tissue should be considered [1].

Peroxisome proliferator activated receptor-gamma (PPARγ) is a nuclear receptor that plays a pivotal role in the regulation of adipocyte differentiation [2,3]. PPARγ is also the target of thiazolidinediones, synthetic ligands that improve insulin resistance of diabetic subjects [4-6]. Thiazolidinediones promote differentiation of preadipocytes into mature adipocytes presumably via PPARγ binding and activation [7], and increase the number of small adipocytes in white adipose tissue in Zucker fatty rats [8]. Of the two human PPARγ isoforms, γ2 is relatively more adipose-specific than is γ1 [9]. Molecular scanning of the human PPARγ gene was performed [10-12], and a Pro12Ala missense mutation was detected in the adipocyte-specific γ2 exon B [10,12]. Although this Pro12Ala mutation was not identified in subjects with lipoatrophic diabetes [10-12], it is relatively common in Caucasians and Mexican Americans [10]. Although one study showed preliminary data in 57 subjects with type 2 diabetes suggesting that body mass index in those subjects with or without this variant were not different [12], another study has shown association with increased BMI in two different Caucasian populations [13]. In contrast, a recent abstract showed that the variant Ala12 allele was associated with lower BMI [14].

Two studies showed that the role of PPARγ is likely to be different between subcutaneous fat tissue and visceral fat tissue [15,16]. One study showed that thiazolidinediones promote differentiation of adipocytes more intensely in subcutaneous fat than in visceral fat [15]. The other study showed that chronic administration of the thiazolidinedione, troglitazone in diabetic patients led to subcutaneous fat accumulation and a decrease in visceral fat [16]. These studies raise the possibility that functional alteration of PPARγ may affect fat distribution in humans.

[1] To whom correspondence should be addressed at Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113, Japan. Fax: +81-3-5689-7209. E-mail: kadowaki-3im@h.u-tokyo.ac.jp.

0006-291X/98 $25.00

Activation of PPARγ is known to be associated with increased insulin sensitivity [4-6, 8]. It is thus possible that the Pro12Ala PPARγ2 variant in humans may lead to altered insulin sensitivity. Indeed, a recent abstract showed that the variant Ala12 allele exhibited decreased binding affinity to the cognate response element as well as reduced ability to cause transactivation, *in vitro*, yet the Ala12 allele was associated with increased insulin sensitivity [14]. These results suggest the possible contribution of Pro12Ala variant of the PPARγ2 gene to human obesity and insulin sensitivity. We now report results of characterization of the Pro12Ala PPARγ2 variant in Japanese non-diabetic subjects, with respect to adiposity, fat distribution and insulin resistance.

METHODS

Study subjects. The subjects were 215 Japanese men (age: 21- 65 years, mean 51.0 years, SD 8.9 years; BMI: 17.1-41.0 kg/m^2, mean 24.4 kg/m^2, SD 3.3 kg/m^2) who underwent a medical check-up in a company based clinic. They included 151 subjects with normal glucose tolerance, and 64 subjects with impaired glucose tolerance (IGT) using the World Health Organization (WHO) criteria [17].

Study protocol. Informed consent was obtained from all subjects. Following a 12 h fast, venous blood samples were obtained. Body height, body weight, plasma glucose and insulin levels during a 75g oral glucose load, waist to hip ratio (W/H), serum total cholesterol, triglycerides, systolic and diastolic blood pressures, and CT scan for visceral and subcutaneous fat at the level of the umbilicus were studied on the same day [18]. Using the homeostasis model assessment (HOMA), indices of insulin resistance (fasting insulin × fasting glucose/405 μU × g/10 × L^2) were calculated [19,20].

Detection of the Pro12Ala PPARγ2 missense mutation. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis was performed as previously described [10]. Briefly, a 270-base-pair (bp) fragment of the human PPARγ2 gene encompassing the site of the polymorphism was generated from genomic DNA by PCR using upstream primer 5′-GCCAATTCAAGCCCAGTC-3′ and mutagenic downstream primer 5′-GATATGTTTGCAGACAGTGTATCAGTGAAGGAATCGCTTTCCG-3′ which introduces a BstU-I restriction site (CG$\underline{C}$) only when the C to G substitution at nucleotide 34 is present. The PCR products were digested with BstU-I, electrophoresed on a 3% agarose gel and stained with ethidium bromide.

Statistical analysis. Unpaired t-test was used to estimate the differences between the subjects with and without the variant. Differences in allele frequencies between groups were assessed by chi square analysis. StatView-J4.11 software (Abacus Concepts, Inc, California) was used.

RESULTS

Identification of Pro12Ala variant in Japanese. The Pro12Ala variant was detected in Japanese by the PCR-RFLP method previously reported [10]. As predicted, digestion of the 270 bp PCR product with BstU-I produced three distinct patterns. A single 270 bp product defined Pro12 homozygotes; 270, 227, and 43 bp products defined Pro12/Ala12 heterozygotes; and 227 and 43 bp products defined Ala12 homozygotes (Fig 1).

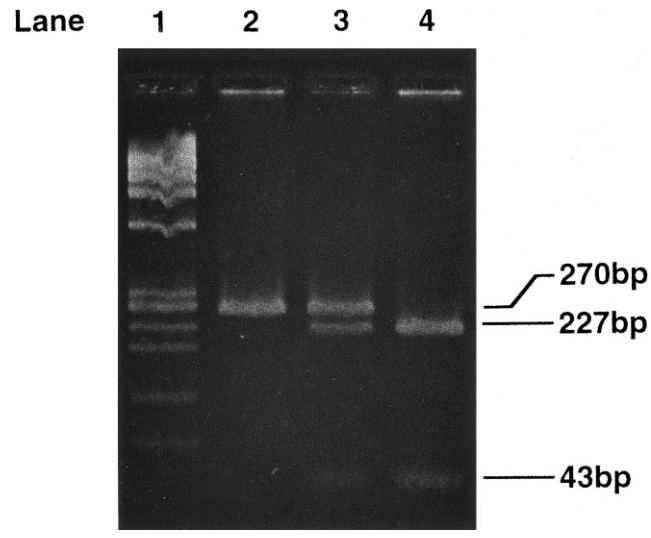

FIG. 1. PCR-RFLP detection of the Pro12Ala PPARγ2 variant. PCR followed by digestion with BstU-I, 3% agarose gel electrophoresis, ethidium bromide staining and UV transillumination was performed. Lane 1 contains a ϕ×-Hae III digest DNA size standard. Lane 2-4 each contains normal homozygote (270bp), Pro12Ala heterozygote (270bp, 227bp and 43bp), and Pro12Ala homozygote (227bp and 43bp), respectively.

Frequency of the Pro12Ala PPARγ2 variant in Japanese men. In the 215 subjects, 203 Pro12 homozygotes, 11 Pro12/Ala12 heterozygotes, and 1 Ala12 homozygote were detected. The allele frequency of Ala12 was 0.03 in Japanese men. Because there was only one Ala12 homozygote, this individual was combined with the Pro12/Ala12 heterozygotes for all analyses.

The Pro12 Ala variant and adiposity. The subjects with and without the Pro12Ala variant had a BMI (mean ± SD) of 24.0 ± 3.0 kg/m^2 and 24.4 ± 3.3 kg/m^2 (n.s.), W/H ratio of 0.87 ± 0.07 and 0.88 ± 0.05 (n.s.), subcutaneous fat area of 12642 ± 1653 mm^2 and 13106 ± 6765 mm^2 (n.s.), visceral fat area of 8211 ± 4418 mm^2 and 8711 ± 3988 mm^2 (n.s.), and V/S ratio of 0.64 ± 0.20 and 0.71 ± 0.27 (n.s.), respectively (Table 1).

The Pro12Ala variant and the plasma fasting insulin level and insulin resistance index. Subjects with the Pro12Ala variant had slightly higher fasting blood glucose than subjects without the variant, however, there was no difference in blood glucose at 2 hours after an oral glucose load (Table 1).

The subjects with and without the Pro12Ala mutation had a fasting insulin level (mean ± SD) of 6.5 ± 2.9 μU/ml and 6.5 ± 3.2 μU/ml (n.s.), and insulin resistance index by HOMA of 1.77 ± 0.74 and 1.67 ± 0.85 (n.s.), respectively (Table 1).

The Pro12Ala and serum lipid and blood pressure. The subjects with and without the Pro12Ala variant had a fasting serum total cholesterol (mean ± SD) of 5.3 ± 1.3 mmol/L and 5.4 ± 0.8 mmol/L (n.s.), fasting

TABLE 1

Characteristics of the Pro12Ala PPARγ2 Variant in Japanese Men

	with Ala12 allele(s) (n = 12)	without Ala12 allele (n = 203)	p value of unpaired t-test
parameter (mean ± SD)			
age (year)	51.4 ± 5.8	50.9 ± 9.1	0.86
BMI (kg/m^2)	24.0 ± 3.0	24.4 ± 3.3	0.65
W/H ratio	0.87 ± 0.07	0.88 ± 0.05	0.36
S (mm^2)	12642 ± 5726	13106 ± 6765	0.82
V (mm^2)	8212 ± 4418	8711 ± 3989	0.68
V/S ratio	0.64 ± 0.20	0.71 ± 0.27	0.38
fasting blood glucose (mmol/l)	6.1 ± 0.6	5.7 ± 0.6	0.02
2 hr blood glucose (mmol/l)	7.0 ± 0.9	6.8 ± 1.8	0.69
fasting insulin (μU/ml)	6.5 ± 2.9	6.5 ± 3.2	0.98
2hr insulin (μU/ml)	29.5 ± 10.1	34.8 ± 25.3	0.47
insulin resistance index	1.77 ± 0.74	1.67 ± 0.85	0.69
systolic blood pressure (mmHg)	128.3 ± 20.7	125.3 ± 17.5	0.56
diastolic blood pressure (mmHg)	84.2 ± 12.5	81.0 ± 12.3	0.39
total cholesterol (mmol/l)	5.3 ± 1.3	5.4 ± 0.8	0.63
triglycerides (mmol/l)	1.9 ± 2.8	1.5 ± 1.0	0.19

serum triglyceride (mean ± SD) of 1.9 ± 2.8 mmol/L and 1.5 ± 1.0 mmol/L (n.s.), systolic blood pressure of 128 ± 21 mmHg and 125 ± 18 mmHg (n.s.), and diastolic blood pressure of 84 ± 12 mmHg and 81 ± 12 mmHg (n.s.), respectively (Table 1).

Frequency of the Pro12Ala variant according to BMI or V/S ratio. The subjects were subdivided into 3 groups according to BMI. Normal subjects (BMI $<$ 22 kg/m^2), moderately obese subjects (22 kg/m^2 $\leq$ BMI $<$ 26.4 kg/m^2), and obese subjects (BMI $\geq$ 26.4 kg/m^2) had the Ala12 allele frequency of 0.04, 0.03, and 0.03 (n.s.), respectively (Table 2).

The subjects were subdivided into two groups according to the V/S ratio. Subjects with lower V/S ratio (V/S $<$ 0.6) and subjects with higher V/S ratio (V/S $\geq$ 0.6) had the Ala12 variant allele frequency of 0.03 and 0.03 (n.s.), respectively (Table 2).

DISCUSSION

The allele frequency of Ala12 PPARγ2 in Japanese men without diabetes (0.03) is relatively low, as compared to Caucasian Americans (0.12), Mexican Americans (0.10), and approximately equal to African American (0.03), Nauruans (0.02), and Chinese (0.01) as previously reported [10].

In the present study, there was no difference in BMI between subjects with and without the variant. This study is the first to investigate the influence of the Pro12Ala PPARγ variant on body fat distribution, however, the Pro12Ala variant did not affect either subcutaneous fat area, visceral fat area or body fat distribution estimated by the V/S ratio. Thus, the Pro12Ala variant does not appear to alter the quantity or distribution of fat accumulation in this group of Japanese men.

Since the frequency of the Pro12Ala variant was relatively low, we identified only 12 subjects with the variant. Thus, this study may not have adequate power to detect modest differences in the traits that were measured. For example, given our sample size, we would be able to detect a difference in the BMI between genotypes of about 2.7 kg/m^2 with 80% power at the p=0.05 significance level. Thus we can not rule out the possibility that the Pro12Ala variant may be a modest contributor to obesity-related phenotypes in Japanese

TABLE 2

The Allele Frequency of the Ala12 Variant According to BMI, V/S Ratio

	Ala12 allele frequency
Normal subjects (BMI $<$ 22)	
Pro/Pro n = 42	
Pro/Ala n = 4	0.04
Ala/Ala n = 0	
Moderately obese (22 $\leq$ BMI $<$ 26.4)	
Pro/Pro n = 114	
Pro/Ala n = 4	0.03
Ala/Ala n = 1	
Obese subjects (BMI $\geq$ 26.4)	
Pro/Pro n = 47	
Pro/Ala n = 3	0.03
Ala/Ala n = 0	
Subjects with lower V/S ratio (V/S ratio $<$ 0.6)	
Pro/Pro n = 68	
Pro/Ala n = 4	0.03
Ala/Ala n = 0	
Subjects with higher V/S ratio (V/S ratio $\geq$ 0.6)	
Pro/Pro n = 121	
Pro/Ala n = 7	0.03
Ala/Ala n = 1	

men. Similarly, we can not rule out the possibility that homozygotes for the Pro12Ala variant are affected (e.g., recessive inheritance) since only one of our subjects was homozygous. This individual was 44 years of age, and had a BMI = 25.4 kg/m^2, V/S = 0.70, fasting insulin = 6.7 μU/ml, fasting glucose = 6.0 mmol/L, 2 hr glucose = 7.9 mmol/L, 2 hr insulin = 24.0 μU/ml, total cholesterol = 4.2 mmol/L, triglycerides = 1.1 mmol/L; all within 1.5 SD of the group studied.

In the present study, we failed to observe differences in fasting plasma insulin levels and insulin sensitivity index by HOMA model between subjects with and without the variant, and contrary to the previous report that the Ala 12 allele was associated with improved insulin sensitivity [14]. More sensitive indicators of insulin sensitivity, such as the glucose disposal rate as determined by the hyperinsulinemic euglycemic clamp, is needed to more fully assess insulin sensitivity.

The human PPARγ2 isoform differs from the PPARγ1 isoform by the addition of 28 amino acids at the amino terminus. The proline to alanine substitution is present in the exon that encodes these additional 28 amino acids, and therefore is present only in the γ2 isoform. The importance of the amino terminus of PPARγ is controversial. Deletion of the amino terminal 129 amino acids did not diminish ligand-stimulated adipogenic potency of PPARγ in 3T3 fibroblasts [7]. It has been reported, however, that the amino terminus of PPARγ has a ligand-independent activation domain that is potentiated by insulin [21], which may be influenced by the Pro12Ala substitution. Indeed, a previous study showed that the Pro12Ala variant is associated with altered receptor function [14]. In human adipose tissue, both γ1 and γ2 isoforms exist. The expression of γ2 mRNA is increased with human obesity, while γ1 mRNA is not affected, and the ratio of γ2/γ1 mRNA expression was positively correlated with BMI [22]. These results suggest that other populations with higher BMI than our studied subjects may manifest the effect of the Pro12Ala variant of the γ2 isoform. Indeed, a study of Beamer and coworkers showed association of the Pro12Ala variant with increased BMI in two different Caucasian cohorts, and the association was more marked in the more obese cohort than in the leaner cohort [13]. Furthermore, the association in that study tended to be more marked in females than males. Our study contained no women. Thus, further studies will be needed to determine if phenotypic expression of the Pro12Ala PPARγ2 variant may be influenced by interactive effects of ethnic (genetic) background, environmental influences, and gender.

ACKNOWLEDGMENTS

This study was supported by a Grant-in-Aid for Creative Basic Research (10NP0201) from the Ministry of Education, Science, Sports, and Culture of Japan.We thank K. Kimura and R. Ohtsuka for their help.

REFERENCES

1. Zamboni, M., Armellini, F., Cominacini, L., Turcato, E., Todesco, T., Bissoli, L., Micciolo, R., Bergamo-Andreis, I. A., and Bossello, O. (1994) *Am. J. Clin. Nutr.* **60,** 682–687.
2. Tontonoz, P., Graves, R. A., Budavari, A. I., Erdjument-Bromage, H., Lui, M., Tempst, P., and Spiegelman, B. M. (1994) *Nuc. Acids Res.* **22,** 5628–5634.
3. Tontonoz, P., Hu, E., and Spiegelman, B. M. (1994) *Cell* **79,** 1147–1156.
4. Lehmann, J. M., Moore, L. B., Smith-Oliver, T. A., Wilkison, W. O., Willson, T. M., Kliewer, S. A. (1995) *J. Biol.Chem.* **270,** 12953–12956.
5. Forman, B. M., Tontonoz, P., Chen, J.,Brun, R. P., Spiegelman, B. M., and Evans, R. M. (1995) *Cell* **83,** 803–812.
6. Spiegelman, B. M. (1998) *Diabetes* **47,** 507–514.
7. Sandouk, T., Reda, D., and Hofmann, C. (1993) *Am. J. Physiol.* **264,** C1600–1608.
8. Okuno, A., Tamemoto, H., Tobe. K., Ueki, K., Mori, Y.,Iwamoto, K., Umesono, K., Akanuma, Y., Fujiwara, T., Horikoshi, H., Yazaki, Y., and Kadowaki, T. (1998) *J. Clin. Invest.* **101,** 1354–1361.
9. Elbrecht, A., Chen, Y., Cullinan, C. A., Hayes, N., Leibowitz, M. D., Moller, D. E., and Berger, J. (1996) *Biochem. and Biophys. Res. Commun.* **224,** 431–437.
10. Yen, C. J., Beamer, B. A., Negri, C., Silver, K., Brown, K. A., Yarnall, D. P., Burns, D. K., Roth, J., and Shuldiner, A. R. (1997) *Biochem. and Biophys. Res. Commun.* **241,** 270–274.
11. Okazawa, H., Mori, H., Tamori, Y., Araki, S., Niki, T., Masugi, J., Kawanishi, M., Kubota, T., Shinoda, H., and Kasuga, M. (1997) *Diabetes* **46,** 1904–1906.
12. Vigouroux, C., Fajas, L., Khallouf, E., Meier, M., Gyapay, G., Lascols, O., Auwerx, J., Weissenbach, J., Capeau, J., and Magre, J.(1998) *Diabetes* **47,** 490–492.
13. Beamer, B. A., Yen, C. J., Andersen, R. E., Muller, D., Elahi, D., Cheskin, L. J., Andres, R., Roth, J., and Shuldiner, A. R. (1998) *Diabetes* (in press).
14. Deeb, S S.,Fajas, L., Nemoto, M., Laakso, M., Fujimoto, W., Auwerx, J.,A (1997) *9th International Center for Biotechnology (CBT) Symposium on Steroid and Orphan Nuclear Receptors* (abstract), Sweden.
15. Adams, M., Montague, C. T., Prins, J. B., Holder, J. C., Smith, S. A., Sanders, L., Digby, J. E., Sewter, C. P., Lazar, M. A., Chatterjee, K. K., and O'Rahilly, S. O. (1997) *J. Clin. Invest.* **100,** 3149–3153.
16. Mori, Y., Murakawa, Y., Yokoyama, J., Tajima, N., and Ikeda, Y. (1998) *Diabetes* (supplement) **A18,** 0069.
17. Diabetes Mellitus (1985) Report of a WHO study group, *World Health Organ. Tech. Res. Series* **727,** 7–133.
18. Kim-Motoyama, H., Yasuda, K., Yamaguchi, T., Yamada, N., Katakura, T., and Shuldiner, A. R. et al.(1997) *Diabetologia* **40,** 469–472.
19. Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Tracher, D. F., and Turner, R. C. (1985) *Diabetologia* **28,** 412–419.
20. Haffner, S. M., Kennedy, E., Gonzalez, C., Stern, M. P., and Miettinen, H. (1996) *Diabetes Care* **19,** 1138–1141.
21. Werman, A., Hollenberg, A., Solanes, G., Bjorbaek, C., Vidal-Puig, A. J., and Flier, J. S. (1997) *J. Biol. Chem.* **272,** 20230–20235.
22. Vidal-Puig, A. J., Considine. R. V., Jimenez-Liñan, M., Werman, A., Walter, J. P., Caro, J. F., and Flier, J. S. (1997) *J. Clin. Invest.* **99,** 2416–2422.