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Letters

Mutations of the peroxisome proliferator-activated receptor γ (*PPARγ*) gene in a Japanese population : the *Pro12Ala* mutation in *PPARγ2* is associated with lower concentrations of serum total and non-HDL cholesterol

To the Editor: High-fat diet is a major cause of increasing obesity in Japan as well as in western countries. Obesity, which is associated with insulin resistance, has been suggested to cause diabetes, hypertension, hyperlipidaemia or cardiovascular disease. Peroxisome proliferator-activated receptor γ (PPARγ the major functional receptor for the thiazolidinedione class of insulin-sensitizing agents, was reported to be a key regulator of adipocyte differentiation [1]. In addition, PPARγ could play a role as a mediator of adipocyte hypertrophy probably causing insulin resistance under a high-fat diet in animal models. Heterozygous *PPARγ*-deficient mice were shown to be protected from the development of insulin resistance [2]. Moreover, a previous study indicated the association of the *Pro12A1a PPARγ2* substitution with reduced receptor activity resulting in lower BMI and improved insulin sensitivity [3]. Because it is known that insulin resistance is related to glucose and/or lipid metabolic disorder, these studies suggest that it might be possible to examine the relation between genetic mutations involved in diseases such as diabetes and their variables with respect to plasma glucose or lipid concentration. On the other hand, two dominant negative mutations of the *PPARγ* gene, *Val290Met* and *Pro467Leu*, were recently found in subjects with severe insulin resistance, diabetes mellitus and hypertension [4]. These mutations might be rare but the relation between the genotypes and the phenotypes has been uncertain yet. Besides, hypertension is not a rare feature of Type II diabetic patients. And no study of these mutations in Japanese subjects can be found at present. The aim of this study was to examine the association between carriers of *PPARγ* mutations such as *Val290Met*, *Pro467Leu* or *Pro12Ala* and clinical characteristics, particularly involved in blood pressure, plasma glucose or lipid concentration, in a Japanese cohort with Type II diabetes mellitus.

The study subjects consisted of 106 Type II (non-insulin-dependent) diabetic patients (age; 33–80 years, mean 61.6 years, SEM 1.0 years: BMI; 17.7–36.8 kg/m^2, mean 23.4 kg/m^2, SEM 0.4 kg/m^2) who were attended or admitted to NTT West Osaka Hospital. All subjects gave their informed consent to participation in this study. Screening for all three mutations was done using the PCR-RFLP method. We carried out PCR amplification of the segment that should include the *Val290Met* mutation located in exon 5 of the *PPARγ* gene, using the restriction enzyme *Nco* I (Takara, Shiga, Japan). As the restriction enzyme cuts the mutant allele, we always included a control sample in each run. We carried out the same procedure as described above to detect the *Pro467Leu* variation located in exon 6, using the restriction enzyme *Aci* I (New England BioLabs, Mass., USA) that cuts the wild-type *Pro467* allele. Analysis of the *Pro12Ala* polymorphism was based on the previous report [5] with some modifications. In statistical analyses, nine subjects were excluded due to a few non-determined variables. Statistical differences between the subjects with and without the polymorphism were evaluated by nonparametrical analysis (Mann-Whitney's U test) and a p value of less than 0.05 was regarded as statistically significant.

Although the hypertensives with either more than 140 mmHg systolic or more than 90 mmHg diastolic blood pressure (World Health Organization International Society of Hypertention Guidelines, 1999) were 53 % ($n = 56$), neither *Val290Met* nor *Pro467Leu* mutation of the *PPARγ* gene was found in this study. Concerning the *Pro12Ala* mutation, the number of subjects homozygous for the *Pro12* allele was 97 (92 %), and the heterozygous for the *Pro12* and *Ala12* allele was 9 (8 %). There were no subjects homozygous for the *Ala12* allele. Consequently, the allelic frequency of the *Ala12* allele was 0.04.

Statistical analyses showed no association of the genotypes with the clinical parameters, that is, age, BMI, HbA_{1c}, fasting blood glucose, triglyceride, total cholesterol, HDL cholesterol, LDL cholesterol, systolic blood pressure and diastolic blood pressure (data not shown). As PPARγ appears to regulate adipocyte differentiation and hypertrophy, it could be involved in obesity. Therefore, we subdivided the population into two groups – one with a BMI of less than 23 kg/m^2 and one with a BMI of 23 kg/m^2 or more, because BMI = 23 kg/m^2 was the mean value. In the BMI ≥ 23 kg/m^2 group, the subjects with the *Ala12* allele had lower concentrations of total cholesterol ($p = 0.033$), LDL cholesterol (calculated) ($p = 0.023$, data not shown), and non-HDL cholesterol (total cholesterol–HDL cholesterol) ($p = 0.028$). That is, with regard to total cholester-

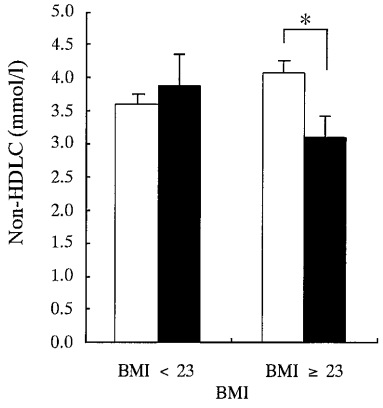

Fig. 1. Influence of the *Pro12Ala PPARγ2* polymorphism on non-HDL cholesterol concentrations. Means ± SEM are shown. *Pro/Ala* genotype (■); *Pro/Pro* genotype (□). Non-HDL cholesterol was calculated by subtracting HDL cholesterol from total cholesterol. BMI ≥ 23: *Pro/Pro*, $n = 45$; *Pro/Ala*, $n = 4$. BMI < 23: *Pro/Pro*, $n = 43$; *Pro/Ala*, $n = 5$. *$p < 0.05$

ol levels in the BMI ≥ 23 kg/m^2 group, the subjects with the *Ala12* allele ($n = 4$) had a lower value, 4.4 ± 0.3 mmol/l (means ± SEM), compared with the subjects without ($n = 45$), 5.5 ± 0.1 mmol/l ($p = 0.033$). In contrast, in the BMI < 23 kg/m^2 group, the subjects with the *Ala12* allele ($n = 5$) had a similar total cholesterol value, 5.5 ± 0.4 mmol/l, to the subjects without ($n = 43$), 5.1 ± 0.1 mmol/l ($p = 0.25$). Concerning the non-HDL cholesterol concentration in the BMI ≥ 23 kg/m^2 group, the subjects with the *Ala12* allele ($n = 4$) had a lower value, 3.1 ± 0.3 mmol/l, compared with the subjects without ($n = 45$), 4.1 ± 0.2 mmol/l (p = 0.028). On the other hand, in the BMI < 23 kg/m^2 group, the subjects with the *Ala12* allele ($n = 5$) had a similar non-HDL cholesterol value, 3.9 ± 0.5 mmol/l, to the subjects without ($n = 43$), 3.6 ± 0.2 mmol/l ($p = 0.42$) (Fig. 1).

The present study showed different results on the total cholesterol concentration among Type II diabetic patients from that of the previous study [6], where no effect of the *Ala12* allele was found on the total cholesterol concentration in Japanese non-diabetics. As in lipid metabolic index, we used non-HDL rather than LDL cholesterol concentrations, based on the recent report [7]. That is, non-HDL cholesterol consists of both total cholesterol and HDL cholesterol measured directly, in contrast to LDL cholesterol calculated by the Friedewald equation. In addition, non-HDL cholesterol values reflect the sum of serum cholesterol carried by LDL, VLDL and remnant lipoproteins that have influences on latent atherogenesis. In conclusion, the *Pro12Ala PPARγ2* polymorphism might have some effect on lipid metabolism.

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