Brief Genetics Report

Pro12Ala Substitution in the Peroxisome Proliferator-Activated Receptor-γ2 Is Not Associated With Type 2 Diabetes

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Peroxisome proliferator-activated receptor (PPAR)-γ is a major regulator of adipogenesis and insulin sensitivity. The PPAR-γ gene generates two isoforms through alternative splicing, PPAR-γ1 and -γ2, the latter having an additional stretch of 28 amino acids at its NH_2-terminus in the ligand-independent activation domain. This extension renders PPAR-γ2 more sensitive to insulin action. Since there is a Pro12Ala substitution in this domain, we tested whether it is related to type 2 diabetes or insulin resistance. Therefore, 131 type 2 diabetic patients and 312 normoglycemic control subjects were screened for the presence of the mutation and for major clinical and metabolic features. The frequency of the mutation did not differ significantly between diabetic patients and control subjects. BMI, insulin, and other metabolic and anthropometric variables were also not associated with the mutation. Although the study was carried out on a sufficiently large sample, the conclusions do not support a major role for the Pro12Ala substitution of the PPAR-γ gene in the etiology of type 2 diabetes. *Diabetes* 48:1466–1468, 1999

The connections among glucose, lipid, and energy metabolisms are becoming better understood at the molecular level as the multiple functions of the peroxisome proliferator-activated receptors (PPARs) are being elucidated (1–4). PPARs are ligand-dependent transcription factors that belong to the superfamily of nuclear receptors. The three isoforms identified so far, PPAR-α, -β, and -γ, have quite divergent ligand-binding domains that allow recognition of different ligands, resulting in different biological activities (3).

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PCR, polymerase chain reaction; PPAR, peroxisome proliferator-activated receptor.

PPAR-γ is a major regulator of adipogenesis, adipocyte gene expression, and insulin sensitivity, although it may be responsible for other still unknown functions (5–7). The PPAR-γ activators identified so far include some inflammatory activators, such as a prostaglandin J2 metabolite (15-deoxy-Δ12,14-PGJ2), and the antidiabetic drugs thiazolidinediones. The latter compounds reduce *ob* gene expression in rodents through activation of PPAR-γ (8) and act as insulin sensitizers (9). In addition, the potency of thiazolidinediones to stimulate PPAR-γ is correlated with their antidiabetic action (10).

A cytosine-to-guanine substitution, causing a proline-to-alanine replacement at codon 12 in exon B, unique to PPAR-γ2, has recently been reported (11,12). The mutation is very close to the NH_2-terminus of the protein in the ligand-independent activation domain, whose activity is potentiated by insulin through phosphorylation (13). Because proline prevents, while alanine favors, α-helix, this amino acid change could have profound effects on the structure and, consequently, on the function of the protein.

We argued that the Pro12Ala variant could be associated with type 2 diabetes. To test this hypothesis, we examined 131 patients with type 2 diabetes and 312 normoglycemic control subjects randomly selected from among those eligible from the same large population of Italian-Caucasian men. Of the 312 control subjects, 174 were weight-matched with the diabetic patients and 138 were normal-weight individuals. They were genotyped for the presence of the Pro12Ala mutation and tested for other clinical and metabolic factors. By assuming a frequency of the Pro12Ala mutation of 12% in the general population, the sample size studied has a 80% power of detecting a relative risk of 2 for the presence of the mutation in the diabetic versus the normoglycemic group with a significance level of 0.05 (two-sided).

The main result of this study is that the frequency of the Pro12Ala mutation of the PPAR-γ gene did not differ significantly between diabetic patients and normoglycemic control subjects (13.0 vs. 18.3%, $\chi^2 = 1.5$, $P = 0.22$), as reported in Table 1. The odds ratio for the presence of the mutation in diabetic patients versus control subjects was 0.66 (95% CI 0.36–1.24). The frequency of the mutation did not differ significantly between diabetic patients and control subjects or when diabetic patients were compared separately with the weight-matched or the normal-weight control subjects (Table 1). Only three homozygous carriers of the Ala substitution were

TABLE 1
Prevalence of the Pro12Ala mutation in the PPAR-γ gene in diabetic individuals and different control groups

	Total n	Age (years)	BMI (kg/m^2)	Pro12Ala mutation n	%	χ^2	P
Diabetic individuals	131	50.1 ± 7.5	27.3 ± 3.5	17	13.0	—	—
Weight-matched control subjects	174	45.6 ± 6.2*	27.3 ± 3.2	30	17.2	0.74	0.4
Normal-weight control subjects	138	44.7 ± 6.1*	23.4 ± 1.5†	27	19.6	1.67	0.2
Combined control groups	312	45.2 ± 6.2*	25.6 ± 3.3†	57	18.3	1.28	0.3

Data are means ± SD, unless otherwise indicated. χ^2 scores are versus diabetic patients. *$P < 0.01$ vs. diabetic patients; †$P < 0.001$ vs. diabetic patients.

identified, and all of them were in the control group. The metabolic and biochemical parameters tested were not significantly different from those in the rest of the population.

Since PPAR-γ2 is an important regulator of adipogenesis, it was also relevant to verify whether the mutation was associated with overweight in normal individuals. The frequency of the mutation did not, however, differ significantly between overweight and normal-weight control subjects (17.4 vs. 19.0%, $\chi^2 = 0.059$, $P = 0.8$). No significant differences in insulinemia, glycemia, HDL cholesterolemia, triglyceridemia, BMI, waist circumference, or systolic and diastolic blood pressure were observed between individuals with or without the mutation in either diabetic patients or control subjects (Table 2).

The relationship between the Pro12Ala variant of the PPAR-γ gene and type 2 diabetes has been analyzed in a single report (14). In contrast with our results, the Ala allele was shown to be significantly less frequent in diabetic patients than in control subjects. That study was not population-based, however: the control group was quite small, and few biochemical and metabolic parameters were characterized. In addition, the ethnic background of that group was different from that of our group. Recent reports have also analyzed the relationship of the Pro12Ala mutation with obesity or insulin sensitivity. The conclusions are very discordant: In fact, both a positive (15) and a negative (14) relationship with BMI have been reported. In a third series, no relation was found (16), which coincides with our study. The reasons for these discrepancies are not clear, and may be partially due to differences in the degree of obesity among the patients studied. On the basis of the available evidence, it may be hypothesized that the Pro12Ala variant does not play a major role in regulating body weight in the normal population, while it might contribute to the development of morbid obesity.

The frequency of the Ala allele appears to vary according to the genetic background of the populations under investigation. In our series of normoglycemic Italian-Caucasians, the frequency is, in fact, similar (0.096) to those reported in two other groups of Caucasians (0.089 and 0.108) (15) and slightly lower than that reported in Finns (0.122) (14). On the contrary, it is threefold higher than that reported in a Japanese group living in the homeland (0.030) (16), although comparable to that of Japanese-Americans (0.093) (14).

In conclusion, our findings, based on a sufficiently large sample, do not support the hypothesis that the Pro12Ala mutation of the PPAR-γ gene plays a major role in the development of type 2 diabetes. As a secondary end point of this study, the association with obesity was also checked, and no correlation was found. Because of the important role of PPAR-γ2 in insulin sensitivity and/or body weight, however, it is still possible that the same mutation in different ethnic groups or other mutations in the gene may be associated with or be responsible for abnormalities in glucose/energy metabolism.

TABLE 2
Major plasma and clinical variables linked to insulin resistance in diabetic individuals and normoglycemic control subjects with or without the Pro12Ala mutation in the PPAR-γ gene

	n	Age (years)	Insulin (μU/ml)	Glucose (mg/dl)	Triglycerides (mg/dl)	HDL cholesterol (mg/dl)	BMI (kg/m^2)	Waist circumference (cm)	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)
Diabetic individuals										
With mutation	17	50.3 ± 8.4	11.8 ± 5.8	174.9 ± 46.9	207.9 ± 109.8	46.6 ± 14.8	27.5 ± 2.9	97.5 ± 7.4	145.8 ± 24.0	87.5 ± 11.8
Without mutation	114	50.1 ± 7.4	11.8 ± 5.4	178.3 ± 54.1	191.6 ± 114.6	45.5 ± 12.3	27.3 ± 3.6	97.0 ± 9.4	143.7 ± 20.1	89.4 ± 11.5
P value (with vs. without)		0.9	1.0	0.8	0.6	0.7	0.8	0.8	0.7	0.5
Normoglycemic control subjects										
With mutation	57	45.3 ± 6.3	7.0 ± 3.1	95.4 ± 10.6	141.7 ± 88.8	48.0 ± 14.9	25.6 ± 3.1	92.7 ± 1.0	136.7 ± 16.9	86.4 ± 11.1
Without mutation	255	45.2 ± 6.1	7.8 ± 4.0	96.4 ± 8.3	142.5 ± 69.7	44.2 ± 12.1	25.6 ± 3.3	91.7 ± 0.8	136.4 ± 17.5	87.9 ± 12.0
P value (with vs. without)		0.6	0.12	0.4	0.9	0.08	0.9	0.6	0.9	0.4

Data are means ± SD. Normoglycemic control subjects with the mutation included 54 heterozygous and 3 homozygous subjects. Differences between subjects with and without the mutation were analyzed by unpaired t test.

RESEARCH DESIGN AND METHODS

Subjects. Participants were chosen from a large unselected population of 3,596 male employees of the Italian telephone company aged 35–65 years who were undergoing routine health screening. A total of 145 individuals were found to have diabetes (fasting glucose 140 mg/dl or treatment): of these, 8 individuals, in the age range 36–42 years, were on insulin treatment and were excluded from the study because the diagnosis of type 1 diabetes could not be ruled out with certainty. For six additional diabetic individuals, blood was not available. Therefore, 131 diabetic patients entered the study. From the same population, 312 individuals with a fasting blood glucose <110 mg/dl were randomly enrolled in the study as normoglycemic control subjects. Out of these, 174 were selected and matched with diabetic patients for BMI, and 138 were selected for being of normal weight (BMI <25). The second control group was included to evaluate whether any association between the Pro12Ala variant and diabetes was due to the confounding influence of obesity; moreover, it provided a sufficiently large number of normal weight individuals to explore the relationship between the PPAR-γ variant and body weight.

Plasma assays. Fasting glucose, HDL cholesterol, and triglycerides were measured on fresh plasma by dry chemistry methods using an Ektachem DT-60 analyzer (Eastman Kodak, Rochester, NY) (17). Plasma insulin concentrations were determined by radioimmunoassay with a nonspecific double antibody, solid phase method (18).

DNA analysis. Genotyping was carried out on genomic DNA extracted from peripheral blood leukocytes (19). DNA (100 ng) was amplified by polymerase chain reaction (PCR) and subsequently digested for 2 h at 60°C with the restriction endonuclease BstUI. The resulting fragments were separated by electrophoresis on a 2% agarose gel and visualized by ethidium bromide staining. The PCR primers, reaction conditions, and restriction digestion products are described elsewhere (12), except for the Mg^{2+} concentration in the PCR reaction (3.0 mmol/l).

Statistical methods. Unpaired *t* test was used to compare means. Nonparametric statistics was used for variables not normally distributed (BMI). Proportions were compared by χ^2 analysis. Odds ratios and 95% CIs were calculated according to standard methods (20). The study protocol was approved by the local Ethics Committee and performed after written informed consent was obtained from all participants.

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