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Polymorphism of peroxisome proliferator-activated receptor γ (PPARγ) Pro12Ala in the Iranian population: Relation with insulin resistance and response to treatment with pioglitazone in type 2 diabetes

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ABSTRACT

The peroxisome proliferator-activated receptor γ (PPARγ) has important effects on insulin sensitivity, obesity and diabetes. Pioglitazone improves insulin sensitivity by activating PPARγ. In view of inter-individual variability in therapeutic response to pioglitazone, this study was designed to search for an association between type 2 diabetes mellitus and Pro12Ala single-nucleotide polymorphism (SNP) in PPARγ (SNP rs1801282) and to investigate whether these genetic variants affect pioglitazone response in an Iranian population. A total of 101 patients with type 2 diabetes were treated for 12 weeks with pioglitazone (15 mg/day). Paraclinical parameters were measured before and after therapy. We genotyped 128 control participants without diabetes and all patients with type 2 diabetes. The Pro12Ala polymorphism in PPARγ was detected with real-time PCR. The Ala allele was found in 7% of the control participants vs. 3% of those with type 2 diabetes ($P=0.04$). The genotypic frequencies of Pro/Ala were 14.06% in the former group vs. 5.94% in the latter ($P=0.036$). There were significant changes in some laboratory values and biochemical markers of insulin sensitivity after pioglitazone therapy. The Pro12Ala polymorphism was associated with significant changes in insulin-to-glucose ratio after treatment ($P=0.015$ and $P=0.005$). Our findings suggest that in carriers of the 12Ala variant, pioglitazone significantly reduced the risk of type 2 diabetes, and in diabetic patients with the Pro12Ala genotype, the therapeutic response to treatment was better than in patients with the Pro12Pro genotype, although the difference between groups did not reach statistical significance.

1. Introduction

Peroxisome proliferator-activated receptor (PPAR) belongs to the nuclear hormone receptor superfamily, whose members act as transcription factors in adipocyte differentiation. The PPAR has important effects on insulin sensitivity, atherosclerosis, inflammation, endothelial cell function (Desvergne and Wahli, 1999; Fajas et al., 1997; Gurnell, 2003; Spiegelman, 1998) and the pathogenesis of insulin resistance. Thiazolidinedione (TZD), by stimulating PPARγ, modulates the transcription of insulin-sensitive genes, thereby improving insulin sensitivity in muscle and adipose tissues, and reducing insulin resistance in the liver and peripheral tissues.

The synthetic PPARγ agonist, pioglitazone does not lead to the same response in all diabetic patients, and in fact 30% of the patients failed to respond to this drug in one study (Umpierrez and Dagogo-Jack, 2006). However, the molecular reasons for the different responses to TZD therapy are not fully understood. The high prevalence of Pro12Ala polymorphism makes it the most likely candidate for explaining the possible relation between PPARγ and the response to TZD treatment. Reduced transcriptional activity of PPARγ, which is seen in the Ala allele of the Pro12 Ala polymorphism, has been associated with higher insulin sensitivity and lower body mass index (Deeb et al., 1998; Jacob et al., 2000; Stumvoll et al., 2001). The Ala allele reportedly confers a 75% reduction in the risk for diabetes (Fajas et al., 1997). In PPAR, among a number of genetic variants, the highly-prevalent Pro12Ala polymorphism was first identified by Yen et al. (1997). This missense mutation in exon 6 and a CCA→GCA base exchange lead to the substitution of alanine for proline in codon 12 of exon B. This mutation in codon 12 of exon B of the *PPAR* gene encodes the NH_2-terminal residue that defines the adipocyte-specific PPAR-2 isoform, which in turn decreases the risk of insulin resistance (Schmidt et al., 1992). This polymorphism is associated with weight regulation (Pihlajamaki et al., 2004), as well as with a protective

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effect against obesity (Bluher and Paschke, 2003; Kawasaki et al., 2002), type 2 diabetes and its complications (Altshuler et al., 2000; Herrmann et al., 2002; Stumvoll and Haring, 2002; Yen et al., 1997), and myocardial infarction (Ridker et al., 2003).

In light of the potential applications of the findings to individualized treatment in the clinical management of diabetes, the aims of the present study were to investigate the effect of the Pro12 Ala polymorphism in patients with type 2 diabetes, and to evaluate the effects of this genetic variant on pioglitazone response.

2. Participants and methods

2.1. Participants

One hundred and one patients from Shiraz, Iran, with type 2 diabetes diagnosed according to the 2009 World Health Organization criteria (WHO, 2009) were enrolled. They were treated with pioglitazone (15 mg/day) for 12 weeks without changing their previous medication. They had no history of PPAR agonist use. Patients with type 1 diabetes and pregnant or lactating women were excluded from this study. One hundred and twenty-eight samples of blood from healthy volunteer donors were obtained from the Shiraz Blood Transfusion Organization as a control group.

This study was approved by the Ethics Committee of Shiraz University of Medical Sciences. All participants gave their informed consent in writing. Response to treatment was defined as a decrease in HbA_{1C} levels of more than 15% after 12 weeks of treatment in accordance with Kang et al.(2005).

2.2. Laboratory tests

Anthropometric measurements were made with standard techniques before and after treatment. Blood samples were collected and the serum was separated to measure fasting blood sugar, total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) concentrations (enzymatic assays from Man Company, Tehran, Iran). The serum insulin and C-peptide concentration were measured with a radioimmunoassay kit (Monobind, Lake Forest, CA, USA). HbA_{1C} was determined with a boronate affinity assay (Nycocard, Oslo, Norway). Insulin function was calculated with the formulas in Table 1.

2.3. Genotyping

Genomic DNA was isolated according to the Cinagen Kit dNp protocol (DNG plus DNA Extraction Kit, Cinagene Company, Tehran, Iran). To determine the presence of single-nucleotide polymorphisms (SNPs) of PPAR, we used real-time polymerase chain reaction (RT-PCR) with Taqman (MetaBion, Germany). Allelic discrimination assays were based on the procedure of Doney et al. (2002) with some modifications.

The primer and probes were:

Pro12 forward: TCCATGCTGTTATGGGTGAAACT
Pro12 reverse: CTTTACCTTGTGATATGTTTGCAGACA

Pro12 probe (Fam labeled): TCTCCTATTGACCCAGAAAGCGATTCCTT
Ala12 probe (Fam labeled): TCTCCTATTGACGCAGAAAGCGATTCCTT.

The PCR premix consisted of 1× Premix Ex Tag (Takara Bio Inc, Otsu, Shiga, Japan), 10 μM of each primer, and 10 μM Taqman probe (MetaBion, Germany). Cycling was performed in a real time thermal cycler (BioRad, CA, USA) with initial denaturation at 95 °C for 10 s, PCR at 95 °C for 5 s, and 60 °C for 20 s. This sequence was repeated 45 times.

2.4. Statistical analysis

Genotype distribution and allele frequencies were calculated and compared between groups with the chi-square (χ^2) test or Fisher's exact test. The Hardy Weinberg equilibrium was tested with Arlequin 313 software in the control group. The data are shown as the mean ± SD. Clinical characteristics before and after drug therapy were compared with paired *t* tests. Continuous variables were compared between genotypes with ANOVA. For multiple logistic regressions, the response was defined as a decrease in HbA_{1C} greater than 15%. All statistical analyses were done with SPSS software (v. 15.0, SPSS Inc., Chicago. IL, USA) and P values ≤ 0.05 were considered statistically significant.

3. Results

3.1. Clinical and laboratory characteristics of participants

A total of 101 patients with type 2 diabetes ($n = 101$; 21 men, 80 women) aged 30–70 years (mean 51.44 ± 7.7) with a mean body weight of 69.86 ± 12.40 kg were enrolled in the study. The change in insulin function after pioglitazone treatment was measured with a suite of indices that included fasting blood sugar, serum insulin concentration, C-peptide level, triglycerides, total cholesterol, LDL-C, HDL-C, HbA_{1C}, homeostasis model assessment-insulin resistance (HOMA-IR), Quicki Index, Bennetts, McAuley and revised McAuley, fasting insulin resistance index (FIRI) and insulin-to-glucose ratio (Table 2). Fasting blood sugar, insulin levels, triglyceride and HbA_{1C} values decreased significantly after 12 weeks. Insulin action measured with the HOMA-IR, insulin-to-glucose ratio, Quicki Index, FIRI, McAuley, revised McAuley and Bennetts indexes decreased significantly. Mean body weight and waist–hip ratio did not change significantly. The clinical characteristics before pioglitazone therapy are presented in Table 3.

3.2. Genotyping

The PPARγ (rs1801282) genotype and allele frequencies in both groups are shown in Table 4. Distributions of genotypes were 0.86 for Pro/Pro, 0.14 for Pro/Ala and 0.00 for Ala/Ala in control group. The allelic frequencies were 0.93 and 0.07 for Pro and Ala respectively. Distributions of genotypes in patient group were 0.94 for Pro/Pro, 0.06 for Pro/Ala and 0.00 for Ala/Ala. The allelic frequencies were 0.97 and 0.03 for Pro and Ala respectively (Table 4). The Ala substitution allele was significantly less frequent among patients with type 2

Table 1
Measures of insulin function.

	Definitions	References
Quicki Index	1/log (glucose mg/dl) + log (insulin μu/ml)	Katz et al.(2000)
HOMA-IR	(fasting insulin [micro units per milliliter] × fasting glucose [millimoles per liter])/22.5	Matthews et al.(1985)
Insulin-to-glucose ratio	Insulin (μu/ml)-to-glucose (mmol/l) ratio	Caro(1991)
McAuley	exp[2.63 − 0.28ln (insulin) − 0.31ln (triglyceride)]	McAuley et al.(2001)
Revised McAuley	exp[3.29 − 0.25ln (10) − 0.22ln (BMI) − 0.28ln (triglyceride)]	McAuley et al.(2001)
FIRI	Insulin (μu/ml) × glucose (mmol/l)/25	Frost et al.(1998)
Bennetts Index	1/log[glucose(mmol/l)] × log[insulin(μu/ml)]	Anderson and Urhammer(1995)

HOMA-IR: homeostasis model assessment-insulin resistance; FIRI: fasting insulin resistance index.