Pharmacogenomics

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.

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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 PPARy, 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→CCA 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 NH2-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...
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 HbA1C 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 level, triglycerides, total cholesterol, LDL-C, HDL-C, HbA1C, 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 HbA1C 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 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 HbA1C 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, HbA1C, 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 HbA1C 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 allele 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 allele 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
diabetes. The Ala phenotype was negatively associated with diabetes, with an odds ratio of 0.4048 (95% confidence interval: 0.1576–1.0395) (Table 5).

Three indexes of insulin function—FIRI index, HOMA-IR and insulin-to-glucose ratio—differed significantly between participants with the Pro/Pro and Pro12Ala genotype, as shown in Table 6. No significant relation was found between the Pro12 Ala variant and waist circumference, waist–hip ratio, body mass index (BMI), fasting blood sugar, insulin, C-peptide level, triglyceride, cholesterol, LDL. HOMA-IR, Quicki Index, Bennetts, McAuley or the revised McAuley indexes (Table 6). However, patients with the Ala allele had lower BMI, fasting blood sugar and LDL although these differences in comparison to patients with the Pro/Pro genotype did not reach statistical significance. In Pro12Ala, there were significant differences in insulin-to-glucose ratio before and after pioglitazone treatment with the Pro/Pro and Pro/Ala + Ala/Ala genotypes (1.28 ± 0.78 vs. 2.12 ± 1.15, P = 0.015 and 1.22 ± 0.58 vs. 1.98 ± 1.25, P = 0.005, Table 6).

Multiple logistic regression analysis to evaluate the associations between age, gender, BMI, baseline fasting blood sugar, HOMA-IR and Pro12Ala variants with the response to pioglitazone detected no statistically significant associations (Table 7).

In a sample of the Iranian population, response as defined by a 15% decrease in HbA1C level was achieved in 31.7% of the patients after 12 weeks of treatment with pioglitazone (Fig. 1). There was no association between the Pro/Pro and Pro/Ala variants in the PPARγ gene and response to pioglitazone treatment.

### 4. Discussion

The importance of PPARγ in humans was first reported in 1995 when it was identified as the receptor for TZD insulin-sensitizing drugs (Lehmann et al., 1995). Three potent, highly PPARγ-selective TZDs as the first new class of insulin-sensitizing agents (Nissen and Wolski, 2007; Nissen et al., 2007) have been used in large-scale clinical practice to date. The effects of different genetic variants in the PPARγ gene on responses to the drug in vitro were reported earlier (Masugi et al., 1999). These variations may cause differences in the efficiency of TZD therapy in clinical practice, and may represent a molecular explanation of good or poor response to TZD treatment.

To make clinical use of a polymorphism, it is crucial to recognize in which context the genotype affects the phenotype. Several genetic variants in the PPARγ gene have been identified. Among them, the highly-prevalent Pro12Ala polymorphism in PPARγ is the result of a CCA-to-GCA missense mutation in codon 12 of exon B of the PPARγ gene. The contrasting physical properties of proline and alanine residues suggest that this amino acid exchange (Proline to Alanin) has functional consequences (Snitker et al., 2004). Several studies have given some valuable information about the possible mechanism by which the Pro12Ala polymorphism decreases the risk for type 2 diabetes. This variant also makes a major candidate as a possible pharmacogenetic determinant of TZD response. Furthermore, Pro12Ala genotype is a determinant of insulin sensitivity and susceptibility to diabetes (Altshuler et al., 2000; Celi and Shuldiner, 2002; Mori et al., 2001; Stumvoll and Haring, 2002; Stumvoll et al., 2001). In vitro studies show reduced binding of the Ala12 protein to the PPAR responsive element of several genes and decreased transactivation in the presence of increasing concentrations of a TZD (Deeb et al., 1998; Masugi et al., 2000). The same polymorphism may be associated with decreased insulin resistance and a decreased risk of type 2 diabetes. Because today’s lifestyle is characterized by a diet rich in carbohydrates and fats and poor in fiber, these genetic factors have now become detrimental, leading to an increase in the risk of chronic diseases such as type 2 diabetes. The PPAR variant gene is now known to be of 20 loci identified recently as important in the development of type 2 diabetes (Altshuler et al., 2000).

### Table 2
Clinical characteristics of the patients before and after pioglitazone treatment.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Before</th>
<th>After</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg m⁻²)</td>
<td>27.45 ± 4.23</td>
<td>25.51 ± 2.98</td>
<td>0.271</td>
</tr>
<tr>
<td>Waist–hip ratio</td>
<td>0.89 ± 0.053</td>
<td>0.90 ± 0.02</td>
<td>0.672</td>
</tr>
<tr>
<td>Fasting blood sugar (mg/dl)</td>
<td>196.47 ± 66.92</td>
<td>184.67 ± 40.82</td>
<td>0.671</td>
</tr>
</tbody>
</table>

### Table 3
Baseline characteristics of patients with type 2 diabetes before pioglitazone treatment.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Genotypes</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg m⁻²)</td>
<td>Pro/Pro</td>
<td>Pro/Ala + Ala/Ala</td>
</tr>
<tr>
<td></td>
<td>27.45 ± 4.23</td>
<td>25.51 ± 2.98</td>
</tr>
<tr>
<td>Waist–hip ratio</td>
<td>0.89 ± 0.053</td>
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<td>196.47 ± 66.92</td>
<td>184.67 ± 40.82</td>
</tr>
</tbody>
</table>

### Table 4
Genotypes and allele frequencies of PPARγ in patients with diabetes and healthy volunteers.

<table>
<thead>
<tr>
<th>Genotype/allele</th>
<th>Patients (n = 101)</th>
<th>Control (n = 128)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
<td></td>
</tr>
<tr>
<td>Genotypes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pro/pro</td>
<td>95 (94.06)</td>
<td>110 (85.94)</td>
<td></td>
</tr>
<tr>
<td>Pro/Ala</td>
<td>6 (5.94)</td>
<td>18 (14.06)</td>
<td>0.036</td>
</tr>
<tr>
<td>Ala/Ala</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td></td>
</tr>
<tr>
<td>Alleles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pro</td>
<td>196 (97.02)</td>
<td>238 (92.97)</td>
<td>0.040</td>
</tr>
<tr>
<td>Ala</td>
<td>6 (2.97)</td>
<td>18 (7.03)</td>
<td></td>
</tr>
</tbody>
</table>

12 weeks of treatment with pioglitazone (Fig. 1). There was no association between the Pro/Pro and Pro/Ala variants in the PPARγ gene and response to pioglitazone treatment.

### Table 5
Association of the Pro12Ala variant with type 2 diabetes mellitus.

<table>
<thead>
<tr>
<th></th>
<th>Diabetic patients (n = 97)</th>
<th>Controls (n = 128)</th>
<th>P value (A)</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pro</td>
<td>6 (2.97)</td>
<td>18 (7.03)</td>
<td>0.040</td>
<td>2.4706 (0.5621–6.3443)</td>
</tr>
</tbody>
</table>

CI: confidence interval.
Our work confirmed previous studies (Andersen et al., 2001; Hara et al., 2003; Lohmueller et al., 2003; Parikh and Groop, 2004; Vardarli, 2007) of the association between the PPARγ Pro12Ala polymorphism and type 2 diabetes. The first study in 1998 reported that carriers of the 12A variant had a 75% lower risk of type 2 diabetes (Michalik et al., 2006). We found that the common Pro allele increased the risk of type 2 diabetes, whereas the Ala variant was protective against the disease. The protective effect of this functional variant against type 2 diabetes was probably due to its ability to reduce transcriptional activity of the PPARγ gene. Evidence for this was the fact that insulin enhances the transcriptional effect mediated by PPARγ2 by activating a ligand-independent domain in the N terminal of the molecule (Werman et al., 1998). The location of the Pro12Ala amino acid substitution in the N-terminal region indicates its involvement in reducing transcription, and is a further indication that the mutation is associated with increased insulin sensitivity.

In our study, there are zero people with Ala/Ala genotype. The Ala allele is not lethal, because there have been Europeans, Asians and even French Canadians reported to be Ala/Ala, although the frequency is ~0.02 in these populations. In our previously-published work (Namvaran et al., 2011), we have compared the frequency of Pro12Ala polymorphism in Iranian population with other populations. We have reported many studies in Asia that the frequency for Pro12Ala polymorphism is ~0.02 in these populations. In our previously-published work (Namvaran et al., 2011), we have compared the frequency of Pro12Ala polymorphism in Iranian population with other populations. We have reported many studies in Asia that the frequency for Pro12Ala polymorphism is ~0.02 in these populations. Our work confirms previous studies (Andersen et al., 2001; Hara et al., 2003; Lohmueller et al., 2003; Parikh and Groop, 2004; Vardarli, 2007) of the association between the PPARγ Pro12Ala polymorphism and type 2 diabetes. The first study in 1998 reported that carriers of the 12A variant had a 75% lower risk of type 2 diabetes (Michalik et al., 2006). We found that the common Pro allele increased the risk of type 2 diabetes, whereas the Ala variant was protective against the disease. The protective effect of this functional variant against type 2 diabetes was probably due to its ability to reduce transcriptional activity of the PPARγ gene. Evidence for this was the fact that insulin enhances the transcriptional effect mediated by PPARγ by activating a ligand-independent domain in the N terminal of the molecule (Werman et al., 1998). The location of the Pro12Ala amino acid substitution in the N-terminal region indicates its involvement in reducing transcription, and is a further indication that the mutation is associated with increased insulin sensitivity.

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We hypothesized that patients with the Ala allele would show an enhanced response to pioglitazone therapy, and therefore investigated the possible association between the PPARγ polymorphism and pioglitazone response in 101 patients with type 2 diabetes. Our response was defined as at least 15% decrease in HbA1c. Of course, we accept that these results would be more reliable if we could study on a larger sample.

We hypothesized that patients with the Ala allele would show an enhanced response to pioglitazone therapy, and therefore investigated the possible association between the PPARγ polymorphism and pioglitazone response in 101 patients with type 2 diabetes. Our results suggest that genetic variations in PPARγ may be related to difference in the clinical effect of pioglitazone. However, our multiple logistic regression results showed that the SNP we targeted for pioglitazone response was defined as at least 15% decrease in HbA1C.
study was not associated with the response to pioglitazone (Table 7). There was no significant relationship between the frequency of response to pioglitazone treatment and the presence of the Pro/Pro or Pro/Ala variant (Fig. 1). However, the significant differences we observed in insulin-to-glucose ratio before and after pioglitazone therapy suggest that the Ala allele has some protective effect. Moreover, we found that fasting blood sugar, serum insulin, C-peptide, HOMA-IR, Quicki Index, and the MacCauley, FIRI and Bennett indexes showed larger (albeit statistically non-significant) improvements after drug therapy in patients with the Ala allele.

Like two previous reports (Blüher et al., 2003; Matthias et al., 2003), which could not establish a statistically-significant association between genotype and the response rate to pioglitazone therapy, we found no evidence of an association between response to this drug and the PPARγ genotype in a sample of the Iranian population. However, Kang et al. (2005) did find such an association for another member of the TZD drug family, so the question of the relationship between the Pro/Ala polymorphism and clinical response to pioglitazone remains open. We suggest that this should be tested in larger samples from different populations, including individuals of different ethnic origin.

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References


