A Common 34C>G Variant at the Peroxisome Proliferator-Activated Receptor-γ2 Gene in Chilean Women with Polycystic Ovary Syndrome and Controls

Polimorfismo 34C>G del Gen del Receptor Activado por Proliferadores Peroxisomales-γ2 en Mujeres Chilenas con Síndrome de Ovario Poliquístico y Controles


SUMMARY: The 34C>G (Pro12Ala) polymorphic variant of the peroxisome proliferator-activated receptor-γ2 (PPAR-γ2) gene has been associated with polycystic ovary syndrome (PCOS). However, the results between populations are contradictory. Thus, in the present study was investigated the possible association between 34C>G polymorphism at the PPAR-γ2 gene and PCOS in Chilean women. A total of 50 unrelated women (29.1 ± 8.1 years) with diagnosis of PCOS and 75 healthy controls (29.3 ± 9.3 years) were included in this study. Serum lipids, glucose and uric acid levels were determined by enzymatic-colorimetric methods. The 34C>G variant in the PPAR-γ2 gene was analyzed by PCR-RFLP. Women with PCOS exhibited a higher levels of glucose, total cholesterol, triglycerides, LDL-C and uric acid, and lower HDL-C levels than controls (p <0.05). The frequency of the 34G allele was 9% in PCOS patients and 12% in control women (p = 0.589). The odds ratio for PCOS associated with 34G allele was 0.73 (95% CI = 0.31 - 1.69) confirming the absence of association. We conclude that the 34C>G polymorphism of the PPAR-γ2 gene is not related to PCOS in Chilean women.

KEY WORDS: Polycystic ovary syndrome; Peroxime proliferator-activated receptor; Pro12Ala polymorphism.

INTRODUCTION

Polycystic Ovary Syndrome (PCOS) constitutes an important health public problem in young women, with a prevalence of 5-10% depending on their ethnic background. This disorder involves the combination of chronic anovulation, clinical and endocrinological signs of hyperandrogenism, evident hyperinsulinemia and polycystic ovaries (Norman et al., 2007). In addition, several studies have been associated this disorder with metabolic abnormalities as dyslipidemia, diabetes, hypertension, obesity and cardiovascular diseases (Cussons et al., 2006, 2007).

Although the inheritance mode of PCOS is still uncertain, multiple genetic factors including mutations and polymorphisms to several genes have been associated with PCOS and its phenotypic traits (Diamanti-Kandarakis & Piperi, 2005; Haap et al., 2005; Luque-Ramirez et al., 2006; Unluturk et al., 2007); specially, the genes involved in the energy homeostasis as the peroxisome proliferator activated receptor-γ (PPARγ).

The PPARγ gene is located on 3p25, comprises nine exons and extends over more than 100 kb of genomic DNA (Fajas et al., 1997). Three different PPARγ mRNAs have been characterized in humans generated by alternative splicing. Among these, the PPARγ 2 is a protein predominantly expressed in adipose tissue, and has been considerate a regulator of adipocyte differentiation and glucose homeostasis (Beaven & Tontonoz, 2006).

Several genetic variants have been described in the PPAR-γ2 gene (Unluturk et al.). However, there are two
PPAR-γ2 gene polymorphisms that have been systematically investigated in various populations. The first is the silent 478C>T substitution which resides in the exon 6 and the second is the missense 34C>G mutation resulting in a change of proline by alanine at codon 12 (Pro12Ala) of the exon 2 (Yen et al., 1997; Meirhaeghe & Amouyel, 2004).

Numerous studies have been conducted with the objective to evaluate the possible relationships between 34C>G mutation (Pro12Ala) of the PPAR-γ2 gene and dyslipidemia (Zietz et al., 2002; Tai et al., 2004), obesity (Masud & Ye, 2003), type 2 diabetes (Hara et al., 2000) and insulin sensitivity (Ek et al., 2001; Meshkani et al., 2007). Since PCOS and type 2 diabetes share certain phenotypic features such as obesity and insulin resistance, several studies have been also investigated the association between PCOS and 34C>G variant at the PPAR-γ2 gene (Hara et al., 2002; Korhonen et al., 2003; Orio et al., 2003; San Millán et al., 2004; Hahn et al., 2005; Tok et al., 2005; Wang et al., 2006; Antoine et al., 2007). However, these studies have yielded conflicting results.

Considering a significant interethnic allele frequency variation for 34C>G polymorphism across populations, the aim of the present study was to investigate the frequency and possible association between 34C>G variant at the PPAR-γ2 gene and the presence of PCOS in Chilean women.

**MATERIAL AND METHOD**

**Subjects.** A total of 125 unrelated Chilean women were studied. Fifty were patients with PCOS (16 - 43 years old) and 75 were non-PCOS women (controls, 20 – 50 years old) with normal menstrual cycles (< 32 days) without hirsutism, acne, or male-type alopecia, and not taking hormonal medications. All women were recruited from the Obstetrics and Gynecology Service of the Hernán Henríquez Hospital of Temuco city, Chile.

The diagnosis of PCOS was assigned using the 1990 National Institute of Health criteria, which define PCOS as ovulatory dysfunction plus hirsutism and/or hyperandrogenemia, with exclusion of other disorders (Zawadzki & Dunaif, 1992). Polycystic ovary syndrome was diagnosed after exclusion of androgen-producing tumors, nonclassic 21-hydroxylase-deficient adrenal hyperplasia, hyperprolactinemia, active thyroid disease, and Cushing’s syndrome. Ovulatory dysfunction was defined as menstrual cycles ≥45 days in length, or a progesterone level <2 ng/mL on days 22–24 of the menstrual cycle, in conjunction with a monophasic basal body temperature chart.

Demographic data and history of hypertension, diabetes mellitus, and hypercholesterolemia were assessed in each subject. Subjects with a history of diabetes or basal glycemia ≥126 mg/dl were defined as diabetic. We calculated the BMI [body weight (kg) divided by square of height (m)] to assess obesity.

The study protocol was approved by the Ethics Committee of the University of La Frontera, and all subjects gave written informed consents according basic principle of biomedical investigation enumerated in the Helsinki Declaration.

**Laboratory measurements.** Biochemical measurements were determined from blood sample collected after overnight (>12h) fast. Triglycerides (TG) and total cholesterol (TC) levels were assayed by enzymatic colorimetric methods (Fossati & Prencipe, 1982; Fossati & Medici, 1987). High-density lipoprotein cholesterol (HDL-C) concentrations were measured by enzymatic assay after phosphotungstic acid and magnesium precipitation (Burstein et al., 1970). Low density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation when the triglyceride concentrations did not exceed 4.8 mmol/l (Friedewald et al., 1972). Serum glucose and uric acid levels were also determined by enzymatic methods (Barham & Trinder, 1972; Fossati et al., 1980).

**DNA analysis.** Genomic DNA was extracted from blood leukocytes by salting out procedure optimized by Salazar et al. (1998). The PPAR-γ2 34C>G polymorphism was identified according to conditions described by Tavares et al. (2005). A 244-bp fragment was amplified by PCR in a final volume of 50 µl containing 50 ng of genomic DNA, 100 nM of each primer, 200 mM of each dNTP, 1 unit of Taq DNA polymerase and PCR buffer (KCl 50 mM, 2 mM MgCl2, 20 mM (NH4)2SO4, 75 mM Tris–HCl pH 9.0). After initial denaturation at 98°C for 3 min, the amplification was performed in 30 cycles consisting of 1 min at 94°C, 1 min at 62°C and 1 min at 72°C. A final extension of 10 min at 72°C completed the reaction.

PCR products were submitted to FnuDII cleavage (5U) in a total reaction volume of 20µl. Enzymatic digestions were carried out at 37°C over night. The fragments were separated on 3% agarose gel for about 60 min at 100V and stained with 0.5mg/dl of ethidium bromide, and visualized on a UV transilluminator. The correct assessment of genotype for 34C>G (Pro12Ala) polymorphism at the PPAR γ2 gene was evaluated using a homozygous sample for restriction site as a positive control. In addition, all gels were reread blindly by two persons without any change, and 10% of the analyses were randomly repeated.
Table I. Clinical and metabolic characteristics of the study population.  

<table>
<thead>
<tr>
<th></th>
<th>PCOS (50)</th>
<th>Controls (75)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>29.1 ± 8.1</td>
<td>29.3 ± 9.3</td>
<td>0.919</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>33.0 ± 8.2</td>
<td>23.3 ± 2.6</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>117.8 ± 9.9</td>
<td>110.4 ± 11.0</td>
<td>0.049</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>76.0 ± 9.7</td>
<td>69.8 ± 9.8</td>
<td>0.066</td>
</tr>
<tr>
<td>Fasting glucose, mg/dl</td>
<td>103 ± 18</td>
<td>90 ± 16</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>201 ± 36</td>
<td>180 ± 32</td>
<td>&lt; 0.040</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>153 ± 73</td>
<td>82 ± 43</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>HDL-C, mg/dl</td>
<td>43 ± 7.5</td>
<td>54 ± 11.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>LDL-C, mg/dl</td>
<td>127 ± 41</td>
<td>108 ± 32</td>
<td>0.002</td>
</tr>
<tr>
<td>Uric acid, mg/dl</td>
<td>4.7 ± 1.0</td>
<td>3.7 ± 0.6</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Number of individuals in parenthesis; BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; PCOS, polycystic ovary syndrome; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.  * Student’s t-test.

Table II. Genotype distribution and relative allele frequencies of 34C>G (Pro12Ala) polymorphism at the PPAR-γ² gene in Chilean women with PCOS and controls.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>PCOS</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>84%</td>
<td>79%</td>
</tr>
<tr>
<td>CG</td>
<td>14%</td>
<td>19%</td>
</tr>
<tr>
<td>GG</td>
<td>2%</td>
<td>2%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Alleles</th>
<th>PCOS</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.91</td>
<td>0.88</td>
</tr>
<tr>
<td>G</td>
<td>0.09</td>
<td>0.12</td>
</tr>
</tbody>
</table>

χ² = 0.26; 1 df; p=0.610*  
χ² = 0.29; 1 df; p=0.589

Table III. Clinical and laboratory characteristics (mean ± SD) of Chilean women with PCOS and controls according to different genotypes of 34C>G (Pro12Ala) polymorphism of the PPAR-γ² gene.

<table>
<thead>
<tr>
<th></th>
<th>PCOS</th>
<th>Controls</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>26 ± 7</td>
<td>32 ± 6</td>
<td>0.238</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>32 ± 7</td>
<td>34 ± 6</td>
<td>0.606</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>124 ± 8</td>
<td>116 ± 8</td>
<td>0.192</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>78 ± 4</td>
<td>77 ± 6</td>
<td>0.725</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>104 ± 19</td>
<td>96 ± 11</td>
<td>0.283</td>
</tr>
<tr>
<td>TC, mg/dl</td>
<td>206 ± 43</td>
<td>173 ± 19</td>
<td>0.076</td>
</tr>
<tr>
<td>HDL-C, mg/dl</td>
<td>43 ± 8</td>
<td>44 ± 7</td>
<td>0.695</td>
</tr>
<tr>
<td>LDL-C, mg/dl</td>
<td>131 ± 43</td>
<td>104 ± 13</td>
<td>0.125</td>
</tr>
<tr>
<td>TG, mg/dl</td>
<td>158 ± 73</td>
<td>128 ± 74</td>
<td>0.362</td>
</tr>
<tr>
<td>Uric acid, mg/dl</td>
<td>4.7 ± 1.0</td>
<td>4.9 ± 1.4</td>
<td>0.703</td>
</tr>
</tbody>
</table>

Number of individuals in parenthesis; BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; PCOS, polycystic ovary syndrome; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.  *P values from Student’s t test.

RESULTS

The clinical characteristics of women enrolled in the study are summarized in Table I. The serum total cholesterol, triglycerides, LDL-C, glucose and uric acid concentrations were higher in the PCOS women (P<0.05). In addition, PCOS patients presented a lower HDL-C levels (P <0.05) and higher systolic blood pressure (P=0.049) and BMI values (P< 0.001) when compared to control women.

The genotype distribution and the relative allele frequencies of the Pro12Ala polymorphism at the PPAR-γ² gene in PCOS patients and controls was also calculated. Statistical significance was at P<0.05.

RESUMEN: El polimorfismo 34C>G del gen del receptor activado por proliferadores peroxisomales-γ2 (PPAR-γ2) ha sido relacionado con el síndrome de ovario poliquístico (SOP). Sin embargo, los resultados obtenidos entre poblaciones son contradictorios. En el presente estudio se investigó la posible asociación entre el polimorfismo 34C>G del gen PPAR-γ2 y SOP, en mujeres chilenas. Fueron analizadas 50 mujeres no relacionadas con diagnóstico de SOP (29.1 ± 8.1 años) y 75 mujeres controles (29.3 ± 9.3 años). Se evaluaron las concentraciones séricas de lípidos, glucosa y ácido úrico mediante métodos enzimáticos-colorimétricos. La genotipificación de la variante 34C>G del gen PPAR-γ2 fue realizada mediante la técnica de PCR-RFLP. Los datos muestran que las mujeres con SOP presentan elevados niveles de glucosa, colesterol total, triglicéridos, LDL-C y ácido úrico, y bajos niveles de HDL-C al ser comparadas con las mujeres controles (p<0.05). La frecuencia del alelo mutado 34G fue 9% en las mujeres con SOP y 12% en las mujeres controles (p=0.589). La odds ratio para PCOS asociada al alelo 34G fue 0.73 (IC 95% = 0.31 – 1.69) confirmando la ausencia de asociación. En conclusión, nuestros datos sugieren que el polimorfismo 34C>G del gen PPAR-γ2 no está relacionado a SOP, en mujeres chilenas.

PALABRAS CLAVE: Síndrome de ovario poliquístico; Receptor activado por proliferadores peroxisomales; Polimorfismo Pro12Ala.
REFERENCES


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