CLINICAL STUDY

Effect of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor γ-2 gene on adiposity, insulin sensitivity and lipid profile in the Spanish population

J L González Sánchez, M Serrano Ríos, C Fernández Pérez, M Laakso¹ and M T Martínez Larrad

Department of Internal Medicine II, Hospital Universitario Clínico San Carlos, Madrid, Spain and ¹Department of Medicine, University of Kuopio, Kuopio, Finland

(Correspondence should be addressed to M Serrano Ríos, Cea Bermúdez 66, 5ºG 28003 Madrid, Spain; Email: uinvest7@hcsc.insalud.es or mserrano@hcsc.insalud.es)

Abstract

Objective: To investigate the role of the Pro12Ala peroxisome proliferator-activated receptor (PPAR) γ-2 polymorphism in the susceptibility to the insulin resistance syndrome and its metabolic complications in a population-based nationwide multicenter study in Spain.

Design: 464 unrelated adults (45.3% men and 54.7% women) aged between 35 and 64 years were randomly chosen from a nationwide population-based survey of obesity and related conditions including insulin resistance and cardiovascular risk factors.

Methods: Anthropometric determinations included: body mass index (BMI), waist-to-hip ratio, sagittal abdominal diameter; biochemical determinations included: fasting plasma glucose concentration and concentration 2 h after an oral glucose tolerance test (OGTT), total cholesterol, high and low density lipoprotein-cholesterol, triglycerides, leptin and insulin. Systolic and diastolic blood pressure were also measured. Genotyping of the PPARγ-2 Pro12Ala polymorphism was determined by polymerase chain reaction and single strand conformation polymorphism analysis.

Results: The Ala12 allele frequency was higher in obese men than in lean men (0.15 vs 0.08, P = 0.03). Men carriers of the Ala12 allele had a higher BMI than non-carriers (38.9% vs 21.3%; adjusted odds ratio 2.36, 95% confidence interval 1.10 – 5.05, P = 0.03). However, despite higher BMI obese men carriers of the Ala12 allele had lower sagittal abdominal diameter than Pro12 homozygotes (24.1±3.2 vs 26.3±2.5 cm, P = 0.01). The Ala12 allele was associated with lower total triglycerides levels in the overall population and it was also associated with lower fasting insulin levels and a higher insulin sensitivity by homeostasis model assessment (HOMA) in women.

Conclusions: Our results suggest that the Pro12Ala polymorphism of the PPARγ-2 gene promotes peripheral deposition of adipose tissue and increased insulin sensitivity for a given BMI. The results in women might be due to their different adipose tissue distribution.

European Journal of Endocrinology 147 495–501

Introduction

The peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor subfamily of transcription factors. Three subtypes of PPARs (α, β, γ) have been described (1). The PPARγ plays an important role in adipocyte differentiation and gene expression (2). Alternative use of promoters and differential splicing of the human form of PPARγ result in two different isoforms: PPARγ-1 and PPARγ-2 (3, 4). PPARγ-2 differs from PPARγ-1 since it contains 28 additional amino acids at its NH₂-terminus, encoded by exon B (4). PPARγ-1 is expressed in diverse tissues including adipose, skeletal muscle, heart, and liver, while PPARγ-2 is almost exclusively expressed in adipose tissues (1, 5). PPARγ is activated by natural ligands (fatty acids and prostanoids) and by synthetic ligands such as the antidiabetic drugs, thiazolidinediones (6), that bind with high-affinity to PPARγ (7, 8), stimulating adipocyte differentiation and improving insulin sensitivity (8–10) in vitro. In overexpression studies in cultured fibroblasts it was shown that the PPARγ is the predominant receptor regulating adipogenesis (11). Also, mice heterozygous for inactivation of the PPARγ gene fed a high fat diet showed improved insulin sensitivity when compared with wild-type mice, suggesting that a reduced PPARγ expression improved insulin sensitivity (12). Interestingly, human studies have shown that thiazolidinediones decrease insulin resistance and hypertriglyceridemia through their
interaction with the PPARγ receptor (13, 14). Overall, these findings suggest that the PPARγ gene is one of the potential candidate genes predisposing to the insulin resistance syndrome including central type obesity, high fasting insulin, high total triglycerides and low high density lipoprotein (HDL)-cholesterol plasma levels.

The aim of our study was to investigate for the first time in the Spanish population the relationship between the Pro12Ala PPARγ-2 gene polymorphism, adipose tissue distribution and biochemical parameters of insulin sensitivity in obese and non-obese individuals identified in a population-based nationwide multicenter study.

**Subjects and methods**

**Population**

Genomic DNA was available from 464 non-related Caucasian men (n = 210, 45.3%) and women (n = 254, 54.7%) randomly chosen from a nationwide population-based survey of obesity-associated complications, insulin resistance and cardiovascular risk factors. The age of this population ranged between 35 and 64 years (mean age was 49 years ± 8 years with a body mass index (BMI) of 28.0 ± 4.4 kg/m² (range 18.3–46.5 kg/m²). Subjects with previous diagnosis of type 1 diabetes were excluded from the study. All study subjects were unrelated and they gave their written consent to participate in the study after being informed of its nature. The study protocol was approved by the Ethics Committee of the Hospital Clínico San Carlos of Madrid.

**Methods**

Anthropometric measurements included BMI (kg/m²), waist-to-hip ratio (WHR), and sagittal abdominal diameter (SAD, cm). Systolic and diastolic blood pressures were measured to the nearest even digit by use of a random-zero sphygmomanometer. For laboratory studies, 20 ml blood were obtained after a >10 h overnight fasting period from an antecubital vein without compression. Blood samples were collected in EDTA-coated tubes and immediately centrifuged at room temperature. Plasma was aliquoted for future use.

Plasma glucose, cholesterol, HDL-cholesterol and triglyceride (TG) determinations. Plasma glucose was determined by an autoanalyzer (Hitachi 704, Boehringer Mannheim, Mannheim, Germany). Total cholesterol, triglycerides and HDL-cholesterol were determined by enzymatic methods using commercial kits (Boehringer Mannheim, Mannheim, Germany). Low-density lipoprotein (LDL)-cholesterol was calculated by the Friedewald formula. Plasma insulin concentrations were determined by RIA (Human Insulin Specific RIA Kit, Linco Research Inc., St Louis, MO, USA). Plasma proinsulin levels were also determined by RIA (Human Proinsulin Specific RIA Kit, Linco Research Inc., St Louis, MO, USA). Insulin resistance (IR) was estimated according to the homeostasis model assessment (HOMA-IR) method from fasting glucose and insulin concentrations, according to the formula: insulin (µIU/ml) × glucose (mmol/l)/22.5 (15). Total leptin levels were measured with a highly sensitive RIA kit (Human Leptin RIA Kit, Linco Research Inc., St Louis, MO, USA).

**Carbohydrate profile**

An oral glucose tolerance test (OGTT) using 75 g glucose according to the WHO recommendations was performed. After glucose administration, blood samples were obtained after 1 and 2 h for determination of glucose, insulin and proinsulin plasma levels. According to the Expert Committee criteria on the diagnosis and classification of diabetes mellitus (16), subjects were classified as having normoglycemia, impaired fasting glucose, impaired glucose tolerance or type 2 diabetes.

**Screening of Pro12Ala mutation in exon B of the PPARγ-2 gene**

DNA was prepared from peripheral blood leukocytes by the proteinase K-phenol-chloroform extraction method. Exon B of the PPARγ gene was amplified by PCR with specific primers, and variants were detected by single strand conformation polymorphism analysis as previously described (17).

**Statistical analysis**

Genotypic and allelic distributions were compared using the chi-squared test. Hardy-Weinberg equilibrium was computed to the expected genotype distribution. The Student’s t-test and ANOVA were used to compare continuous variables — expressed as means and standard deviation (s.d.) — while categorical variables were compared using the chi-squared test. Multiple linear regression was performed to investigate the effect of the exon B polymorphism with fasting insulin (log-transformed) and HOMA-IR. Adjusted odds ratios (adjOR) and their 95% confidence intervals (95% CI) were calculated. The existence of interactions was evaluated. The null hypothesis was rejected in each statistical test when P < 0.05. Analysis was performed using Windows SPSS version 10.0 software.

**Results**

Data on BMI in both obese (BMI ≥ 30) and non-obese (BMI < 30) individuals by sex are included in Table 1. According to the results of the OGTT normal glucose tolerance occurred in 354 (81.8%) individuals,
impaired fasting glucose occurred in 35 (8.1%) individuals, impaired glucose tolerance occurred in 36 (8.3%) subjects and clinical type 2 diabetes mellitus occurred in 8 individuals (1.8%), 4 of which were obese.

The genotype distribution of the Pro12Ala PPARγ-2 polymorphism in the whole population (n = 464) was 83.0% (n = 385), 16.1% (n = 75) and 0.9% (n = 4) for the Pro12Pro12, Pro12Ala12 and Ala12Ala12 genotypes respectively; the Ala12 allele frequency was 0.09 and it was in Hardy-Weinberg equilibrium. Because there were only four Ala12 homozygous subjects, these individuals were combined with the Pro12Ala heterozygous subjects and were compared with Pro12Pro subjects in all statistical analyses.

Both genders had similar genotype distribution in the whole population (Table 2). However, when analyzing exclusively the obese group gender differences were noticed. Obese men had higher Ala12 allele frequency than lean men (0.15 vs 0.08, P = 0.03) while there were no such differences in women (0.06 vs 0.10, P = 0.15). Genotype distribution according to BMI and gender indicated that the prevalence of obesity was higher in men with the Ala12 allele than in men who were non-carriers of the Ala12 allele (38.9 vs 21.3%; adjOR: 2.36; 95% CI 1.10–5.05, P = 0.03) (data not shown). Genotype distribution was not influenced by the degree of glucose tolerance in the whole population.

Tables 3, 4 and 5 show the results of the anthropometrical and biochemical variables according to genotypes. Statistical analysis by ANOVA did not show significant differences in anthropometrical variables between the genotypes when the whole group was analyzed (data not shown). As regards the biochemical parameters (Table 4) the Ala12 allele was associated with slightly lower fasting insulin levels than in non-mutated individuals (67.8 ± 28.2 vs 79.2 ± 68.4 pmol/l, P = 0.079) and with lower total triglycerides levels compared with the wild-type in the overall population (1.13 ± 0.56 vs 1.38 ± 0.96 mmol/l, P = 0.028). All women carrying the Ala12 allele had significantly lower total triglycerides levels, lower fasting insulin levels and a higher insulin sensitivity by HOMA-IR. No significant differences were found between the genotype groups regarding plasma levels of fasting and 2 h glucose, proinsulin, leptin or in blood pressure measurements.

### Table 1 Anthropometrical parameters of the study groups. Results are means±s.d. (n)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Obese</th>
<th>P</th>
<th>Non-obese</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>51.9 ± 8.7 (51)</td>
<td>0.94</td>
<td>48.0 ± 8.5 (157)</td>
<td>0.08</td>
</tr>
<tr>
<td>Women</td>
<td>52.1 ± 8.3 (94)</td>
<td></td>
<td>46.3 ± 8.2 (157)</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>32.5 ± 2.3 (51)</td>
<td>0.08</td>
<td>25.8 ± 2.5 (159)</td>
<td>0.28</td>
</tr>
<tr>
<td>Women</td>
<td>33.5 ± 3.5 (94)</td>
<td></td>
<td>25.5 ± 2.6 (158)</td>
<td></td>
</tr>
<tr>
<td>WHR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>1.01 ± 0.04 (51)</td>
<td>&lt;0.001</td>
<td>0.99 ± 0.08 (158)</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>0.96 ± 0.06 (94)</td>
<td></td>
<td>0.92 ± 0.07 (158)</td>
<td></td>
</tr>
<tr>
<td>SAD (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>25.7 ± 2.8 (51)</td>
<td>0.09</td>
<td>21.4 ± 2.7 (159)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Women</td>
<td>24.7 ± 3.4 (94)</td>
<td></td>
<td>19.9 ± 2.9 (158)</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2 Genotype and allele frequencies of the Pro12Ala PPARγ-2 polymorphism according to obesity (BMI ≥ 30). Data are n (%) for genotypes and n (frequency) for alleles.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>All subjects</th>
<th></th>
<th>All subjects</th>
<th></th>
<th>Men</th>
<th></th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pro12Pro12</td>
<td>174 (82.9%)</td>
<td>211 (83.1%)</td>
<td>119 (82.1%)</td>
<td>264 (83.9%)</td>
<td>37 (72.5%)</td>
<td>137 (86.2%)</td>
<td>82 (87.2%)</td>
</tr>
<tr>
<td>Pro12Ala12</td>
<td>33 (15.7%)</td>
<td>42 (16.5%)</td>
<td>25 (17.2%)</td>
<td>50 (15.8%)</td>
<td>13 (25.5%)</td>
<td>20 (12.6%)</td>
<td>12 (12.8%)</td>
</tr>
<tr>
<td>Ala12Ala12</td>
<td>3 (1.4%)</td>
<td>1 (0.4%)</td>
<td>0.47</td>
<td>1 (0.7%)</td>
<td>0.89 (0.9%)</td>
<td>1 (2.0%)</td>
<td>0.09 (1.3%)</td>
</tr>
<tr>
<td>Allele frequencies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pro12</td>
<td>381 (0.91%)</td>
<td>464 (0.91%)</td>
<td>263 (0.91%)</td>
<td>578 (0.91%)</td>
<td>87 (0.85)</td>
<td>294 (0.92)</td>
<td>176 (0.94)</td>
</tr>
<tr>
<td>Ala12</td>
<td>39 (0.09)</td>
<td>44 (0.09)</td>
<td>0.74</td>
<td>27 (0.09)</td>
<td>0.81 (0.09)</td>
<td>15 (0.15)</td>
<td>24 (0.08)</td>
</tr>
</tbody>
</table>

*Obese subjects vs non-obese subjects. Values were compared by χ² analysis.

www.eje.org
On the other hand, all obese subjects with the Ala12 allele, regardless of gender, tended to have lower fasting insulin levels than did non-carriers (68.4 ± 27.6 vs 94.8 ± 67.8 pmol/l, P = 0.057) as well as slightly better insulin sensitivity (2.8 ± 1.4 vs 3.8 ± 2.5, P = 0.07). Furthermore, in all individuals with BMI < 30 the Ala12 allele was associated with lower triglycerides levels (1.02 ± 0.49 vs 1.32 ± 0.90 mmol/l, P = 0.020). After adjustment for age, sex, BMI and WHR, carriers of the Ala12 still had a lower fasting insulin than did non-carriers (P < 0.05). Furthermore, in all individuals with BMI < 30 the Ala12 allele was associated with lower triglycerides levels (1.02 ± 0.49 vs 1.32 ± 0.90 mmol/l, P = 0.020). After adjustment for age, sex, BMI and WHR, carriers of the Ala12 still had a lower fasting insulin than did non-carriers (P < 0.05).
individuals who were homozygous for the Pro12 allele (adjOR: 0.14, 95% CI 0.05–0.38, \( P = 0.05 \)). Stratification by sex and the degree of obesity showed that obese men carrying the Ala12 allele also had a significantly lower sagittal abdominal diameter (24.1 ± 3.2 vs 26.3 ± 2.5 cm, \( P = 0.013 \)) (Table 3) and they tended to have lower levels of fasting insulin than did Pro12 homozygous subjects (Table 4). As regards non-obese women, total triglycerides levels were lower in Ala12 carriers than in Pro12 homozygous individuals (\( P = 0.027 \)) (data not shown). There were no differences between the genotype groups in obese women and non-obese men.

**Discussion**

Specific mutations in the ligand binding domain of PPAR\( \gamma \)-2 are the genetic proof that PPAR\( \gamma \)-2 is key for glucose, lipid and blood pressure homeostasis (18). Detailed descriptions of several mutations such as the Pro12Ala and the Pro115Gln have been reported (19). This Pro115Gln mutation resulted in a permanently active PPAR\( \gamma \) since it inhibited phosphorylation of the protein at Ser114 and led to increased adipocyte differentiation capacity in vitro. Some epidemiological studies have suggested an association between the Pro12Ala PPAR\( \gamma \)-2 gene polymorphism, obesity and other related metabolic disorders but results are not always concordant. The frequency of the Ala12 allele in our population was similar to that reported for other Caucasian populations (20–22) such as the Italian population (20) but was slightly lower than that reported in Finnish (17, 23), Danish (24) and German populations (25) and higher than in Japanese (26) and Korean populations (27). As in other studies (23, 28), we found an association between Ala12 carriers, lower total triglycerides levels, lower fasting insulin levels and a higher insulin sensitivity by HOMA-IR, together with gender differences since these findings were present only in women. Ek et al. (29) have recently reported also an association between the Ala12 allele and improved whole body insulin sensitivity among glucose-tolerant Swedish Caucasian individuals, even if it did not quite reach the level of conventional statistical significance (\( P = 0.07 \)). Similarly, Koch et al. (30) (\( n = 108 \)) reported an association of the Pro12Ala polymorphism with improved insulin sensitivity estimated by the euglycemic hyperinsulinemic glucose clamp in obese subjects. Also, in non-diabetic overweight or obese Japanese subjects Hara et al. (31) found an association between the Ala12 allele and lower levels of fasting plasma insulin and higher insulin sensitivity by HOMA-IR than in non-carriers.

Ethnic differences in the strength of these associations have also been described. Deeb et al. (23) reported an association of the Pro12Ala polymorphism in 333 randomly chosen middle-aged non-diabetic Finns, suggesting that the carriers of the Ala12 allele had a significantly lower BMI, lower fasting insulin levels and higher insulin sensitivity. On the other hand, in another Finnish study on obese women (17) (\( n = 141 \)) the Ala12Ala genotype was associated with increased BMI, fat mass and waist and hip circumferences compared with those obese women with the Pro12Ala or Pro12Pro genotypes. Discrepancies between lean and obese subjects could indicate a variable interaction of the Ala12 allele with other genetic and/or environmental factors (32). In two other Caucasian populations from the USA the Ala12 allele was also associated with increased body weight and BMI (22). Also, in three Japanese populations, the frequency of the Ala12 allele was shown to be significantly lower in individuals with type 2 diabetes than in normal subjects (23, 30, 33). Altshuler et al. (34) also showed a significant decrease in diabetes risk associated with the Ala12 allele in a Caucasian population. Mori et al. (33) in the Japanese population (2201 with type 2 diabetes and 1212 normal control subjects) found that among diabetic subjects, carriers of the Ala12 allele had a higher concentration of total cholesterol than those without the allele. The Ala12 variant of the PPAR\( \gamma \)-2 gene has also been associated with protection against type 2 diabetes in Finnish subjects (35). In diabetic subjects (\( n = 522 \)), the presence of the variant was associated with greater weight change after 20 years of age and lower serum triglycerides levels. In some of the few prospective studies (24, 36) the Ala12 allele was associated with a tendency to gain weight over time. Lindi et al. (36) also found a better insulin sensitivity in subjects with the Ala12Ala12 genotype.

To our knowledge the present study is the first population-based survey conducted in Spain and the third reported from Southern European populations (20, 21). In a French study (\( n = 839 \)) the Ala12 allele was associated with increased body weight, BMI, waist circumference and an atherogenic lipid profile (21). In the Italian study, the authors failed to find an association between this polymorphism and anthropometrical and biochemical parameters among diabetic and normoglycemic subjects (20). The reasons for discrepancies in association studies could be related to ethnic differences, study design and effects of gender and BMI. Differences in PPAR\( \gamma \)-2 expression between subcutaneous and visceral fat have been reported by Vidal (37). Since women have a different fat distribution and because PPAR\( \gamma \)-2 mRNA expression is increased in subcutaneous adipose tissue (38) it would suggest a possible explanation of the differences found in the frequency of the Ala12 allele between men and women. Our results suggest that the Pro12Ala polymorphism of the PPAR\( \gamma \)-2 gene may promote peripheral deposition of adipose tissue and insulin sensitivity and might potentially modify the response to PPAR\( \gamma \).
agonists used for therapeutic purposes such as the thiazolidinediones. In fact, activation of PPARγ receptors by thiazolidinediones promotes adipocyte differentiation and regulation of a number of genes that regulate lipid metabolism. These mechanisms would increase fat deposition in adipose tissue due to increased insulin sensitivity. This effect of thiazolidinediones on insulin sensitivity through its action on PPARγ mechanisms is less marked in the intra-abdominal fat than in other insulin-sensitive tissues (39) and subcutaneouspreadipocytes are more sensitive to the differentiating effect of thiazolidinediones to promote PPARγ than are intra-abdominal cells (40). Since PPARγ is present in β-cells (41) PPARγ agonists may also have a direct effect on the pancreas, and preclinical studies with PPARγ agonists such as rosiglitazone show that this drug increases pancreatic islet area, density and insulin content (42). These effects of rosiglitazone may be mediated by the reported ability of this agent to reduce net β-cell death (43). In addition, since genes coding for proteins involved in the intracellular insulin signaling pathway, such as IRS-2 (44) and p85αPI-3K (45), are target genes of PPARγ in human adipocytes the effects of PPARγ insulin sensitivity could be mediated, at least in part, by a direct effect of PPARγ on insulin secretion. In summary, women but not men carriers of the Ala12 allele are more insulin sensitive and have better lipid profiles than the subjects with the Pro12Pro genotype in the Spanish population. The role of other polymorphisms particularly if found in a location near to the Pro12Ala is still to be defined.

Acknowledgements

Thanks are due to members of the Spanish Insulin Resistance Study Group: Juan Cabello López, Hospital Universitario de Alicante; Isabel Esteva, Hospital Civil de Guadalajara; Javier Muñiz, Hospital de La Coruña; Manel Pladevall Vila, Hospital General de Vic; Amelia Porres, Fundación Jiménez Díaz (Lipid Laboratory) Madrid; Pedro Horcajo Aranda, Hospital General Universitario de Guadalajara; Javier Muñiz, Hospital de La Coruña; Manel Pladevall Vila, Hospital General de Vic; Amelia Porres, Fundación Jiménez Díaz (Lipid Laboratory) Madrid; Pedro Suenz de Aranzabu, Hospital de Mèrida; Antonio Segura Fragosa, Hospital de Talavera: Federico Soriguer Escoté, Hospital Civil de Málaga; and Saturio Vega Quiroga, Hospital de Arévalo.

We wish to thank Dr Antonio J Vidal-Puig (Addenbrooke’s Hospital, Cambridge, UK) for suggestions and the critical reading of the manuscript. We acknowledge Milagros Pérez Barba for dedicated and careful technical assistance.

This work was supported by grants FISS 98/0857 from Fondo de Investigaciones Sanitarias. Thanks are also given to the Consejería de Educación de la Comunidad Autónoma de Madrid.

References

29 Koch M, Pfeiffer E, Volk A, Haist K, Deninger M et al. The PPARγ2 amino acid polymorphism Pro12Ala is prevalent in offspring of type II diabetic patients and is associated to increased insulin sensitivity in a subgroup of obese subjects. *Diabetologia* 1999 42 758–762.

Received 22 November 2001
Accepted 14 May 2002