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Research paper

# The Pro12Ala polymorphism of the *PPAR-γ* gene is not associated with the polycystic ovary syndrome

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## ABSTRACT

**OBJECTIVE: Insulin resistance is a key factor in the pathogenesis of polycystic ovary syndrome (PCOS). Peroxisome proliferator-activated-receptor-γ (PPAR-γ) has been implicated in insulin resistance and adiposity. The aim of the study was to investigate the possible involvement of the Pro12Ala polymorphism of the *PPAR-γ* gene in the pathogenesis of PCOS. DESIGN: We studied 180 women with PCOS and 140 healthy controls. Body mass index (BMI) was recorded. Blood samples were drawn after overnight fasting and serum glucose, insulin, lipid and hormonal profiles were determined. The fasting glucose/insulin ratio and HOMA index were calculated. Moreover, 100 women with PCOS underwent a 75g oral glucose tolerance test and the area under the curve for insulin and glucose was estimated. DNA was extracted from peripheral blood leucocytes and the Pro12Ala polymorphism was genotyped. RESULTS: The *PPAR-γ* genotypes were found to be in the Hardy-Weinberg equilibrium in both study groups. No difference was found in the distribution of the Pro12Ala polymorphism between PCOS and controls. Insulin resistance indices and lipid and hormonal profile were not different among the various genotypes of the Pro12Ala polymorphism. CONCLUSIONS: The Pro12Ala polymorphism of the *PPAR-γ* gene is not involved in the pathogenesis or the phenotypic expression of PCOS.**

**Key words:** Insulin resistence, Polycystic ovary syndrome, Polymorphism, *PPAR-γ*,

## INTRODUCTION

Polycystic ovary syndrome (PCOS) is primarily a disorder of androgen excess. Insulin resistance and compensatory hyperinsulinemia is also a recognized feature of PCOS that further contributes to hyperandrogenism. Both lean and obese women with PCOS have been shown to have insulin resistance, compared to controls.[1] Insulin resistance in PCOS appears to be selective with impaired glucose uptake in peripheral tissues, whilst other intracellular actions of insulin are preserved. The reduced cellular sensitivity to insulin in PCOS is due to a post receptor defect in insulin signaling, though the exact molecular mechanisms remain unclear.[2]

Because of the improvement of insulin sensitivity

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and hyperandrogenism in women with PCOS after activation of peroxisome proliferator-activated receptor (PPAR-γ) by the administration of thiazolidinediones, the *PPAR-γ* gene has been studied in PCOS.[3] Genetic studies of the *PPAR-γ* gene have identified a number of polymorphisms, but most have been focused on the Pro12Ala polymorphism. *In vitro* studies have shown that Pro12Ala is a functional polymorphism with the Ala variant having a lower affinity to a PPAR-responsive element with modest impairment of transcriptional activation.[4]

The Pro12Ala polymorphism of the *PPAR-γ* gene has been implicated in the pathogenesis of insulin resistant conditions such as obesity and type 2 diabetes.[5] Previous studies have looked at the association of the Pro12Ala polymorphism with PCOS with conflicting results.[6-15] The aim of the present study was to examine the association of the Pro12Ala polymorphism with PCOS and the possible relationship of this polymorphism with metabolic parameters of the syndrome among Greek women with PCOS.

## MATERIALS AND METHODS

### *Subjects and study protocol*

The study population consisted of 180 Greek women (aged 16-37 years, mean age 23.7±6.4 years) with PCOS. Diagnosis of PCOS was based on the criteria proposed by the 1990 National Institute of Health-National Institute of Child Health and Human Development (NIH-NNICHD) conference on PCOS. These criteria are ovulatory dysfunction, clinical evidence of hyperandrogenism, and/or hyperandrogenemia, and exclusion of related disorders such as congenital adrenal hyperplasia, hyperprolactinemia or Cushing's syndrome.[16] Hyperandrogenism was defined by the clinical presence of hirsutism (Ferriman Gallwey score >8), acne or alopecia and/or elevated androgen levels. Menstrual dysfunction was defined by the presence of oligomenorrhea or amenorrhea. In those patients who were on medication, treatment was discontinued at least six months prior to their inclusion in the study. Women with PCOS were further divided into two subgroups based on their body mass index (BMI) values: normal-weight PCOS women (BMI<25Kg/m$^2$, n=77) and overweight-obese women with PCOS (BMI≥25Kg/m$^2$, n=103). The control group consisted of 140 healthy age-matched women, with normal body weight, regular menstrual cycles (28 to 30 days) and no signs of hyperandrogenism. In order to investigate the role of the Pro12Ala polymorphism in the pathogenesis of insulin resistance in PCOS, we compared normal weight PCOS women with normal-weight healthy women to avoid the impact of body weight on insulin resistance indices.

All women with PCOS were studied in the early follicular phase (days 3-5) of a spontaneous or progestin-induced menstrual cycle. The BMI of each patient was calculated as weight (Kg)/height$^2$ (m). Blood samples were drawn after overnight fasting for the measurement of fasting serum glucose and insulin, lipid profile, serum gonadotropins, total testosterone and Sex Hormone Binding Globulin (SHBG). The Free Androgen Index (FAI) was calculated using the formula: [total testosterone (nmol/L)/SHBG (nmol/L)] x 100. One hundred patients (33 with normal weight and 67 overweight-obese) gave their consent to undergo a 75g oral glucose tolerance test (OGTT). Blood was sampled for serum glucose and insulin concentrations before and at 30, 60, 90 and 120 min after glucose load. The fasting glucose to insulin ratio was estimated. The glucose and insulin responses to the OGTT were determined by calculating the area under the curve (AUC) for glucose (AUCglucose) and insulin (AUCinsulin) according to Tai's procedure for the metabolic curves.[17]

The study protocol was approved by the Hospital Ethics Committee and all subjects studied gave their informed consent.

### *Hormonal assays*

Serum glucose was determined by the hexokinase method using a glucose analyzer (Olympus 600, Clinical Chemistry Analyzer, Olympus Diagnostica GmbH, Ireland). Insulin was measured by Microparticle Enzyme Immunoassay on an AXSYM Immunoanalyser (Abbott Laboratory, Abbott Park. IL, USA). The CV of this method was 5%. Total testosterone and serum gonadotropins were determined by Chemiluminescent Microparticle Immunoassay on an Abbott-ARCHITECT Immunoanalyzer (Abbott Laboratory, Abbott Park. IL., USA). The CVs were 4% for total testosterone, 3.5% for LH and 4% for FSH. SHBG was measured by the Chemiluminescent Immunometric

method (IMMULITE 2000 Immunoanalyzer, DPC, CA, USA) and the CV was 5.5%. Total cholesterol, HDL cholesterol and triglycerides were determined by enzymatic methods (Olympus 600, Clinical Chemistry Analyser, Olympus Diagnostica GmbH, Ireland). LDL cholesterol was calculated by the Friedewald equation (LDL = total cholesterol (mg/L) – HDL cholesterol (mg/L) – triglycerides(mg/L)/5).[18]

### *Genotype analysis*

Genomic DNA was isolated from peripheral blood leukocytes of women with PCOS and the controls. The Pro12Ala polymorphism of the *PPAR-γ* gene was genotyped by amplification of genomic DNA using the following primers: F5'- GCCAATTCAAGCCCAGTC-3' and R5'-GATATGTTTGCAGACAGTGTATCAGTGAAGGAATCGCTTTCCG-3'. The polymorphism was typed with enzyme BstU-I and the digestion products were resolved after electrophoresis in 2.5% agarose gel and stained with ethidium bromide.

### *Statistical analysis*

Genotype and allele frequencies were compared among the study groups using the chi-square test. The Hardy Weinberg equilibrium for each polymorphism was also tested comparing observed genotype frequencies with those expected (chi-square test). Normal distribution of continuous parameters was tested via the Kolmogorov-Smirnov test. Biochemical differences between two continuous parameters were estimated with the Mann-Whitney U test. Multiple regression analysis was performed to examine the effect of the polymorphism on hormonal parameters. Continuous data are expressed as the mean ± SD. P-value of <0.05 was set as statistically significant. All analyses used the SPSS statistical package (version 15.0, SPSS Inc, Chicago, IL, USA).

## RESULTS

### *Distribution of the Pro12Ala polymorphism*

The characteristics of the study population are presented in Table 1. The Pro12Ala polymorphism of the *PPAR-γ* gene was in Hardy Weinberg equilibrium in both patients and controls (chi-square, p>0.05). The frequency of the Ala allele was 9.7% in normal-weight PCOS women, 7.3% in overweight-obese PCOS women and 6.8% in the control group. Only one woman homozygous for the Ala allele was found in the control group.

**Table 1.** Anthropometric and biochemical characteristics of PCOS women and controls (values are presented as means±SD).

| | PCOS women | Controls | p-value |
|---|---|---|---|
| Number | 180 | 140 | |
| Age (years) | 23.7±6.4 | 24.8±6.9 | ns |
| BMI (Kg/m2) | 26.6±6.9 | 20.9±1.8 | <0.001 |
| LH/FSH ratio | 1.4±1.0 | 1.0±0.4 | 0.05 |
| SHBG (nmol/L) | 37.9±26.4 | 66.8±26.8 | <0.001 |
| FAI | 13.7±10.4 | 2.2±1.1 | <0.001 |
| Total testosterone (nmol/L) | 3.4±1.5 | 1.3±0.4 | <0.001 |
| DHEAS (μmol/L) | 7.72±3.14 | 4.33±1.17 | <0.001 |
| Fasting glucose/insulin ratio | 10.3±7.4 | 15.3±4.9 | <0.001 |

LH/FSH: Luteinizing hormone/Follicle-stimulating hormone, SHBG: Sex hormone-binding globulin, FAI: Free androgen index, DHEAS: Dehydroepiandrosterone sulfate, BMI: Body mass index, ns: non-significant, PCOS: Polycystic Ovary Syndrome

Overall, there was no statistically significant difference in the genotype and allele distribution of the Pro12Ala polymorphism between PCOS and control women. No statistically significant difference was observed when normal-weight PCOS women were compared with overweight-obese PCOS women or when each of this subgroup was compared with the control group (Figure 1). No difference in the distribution

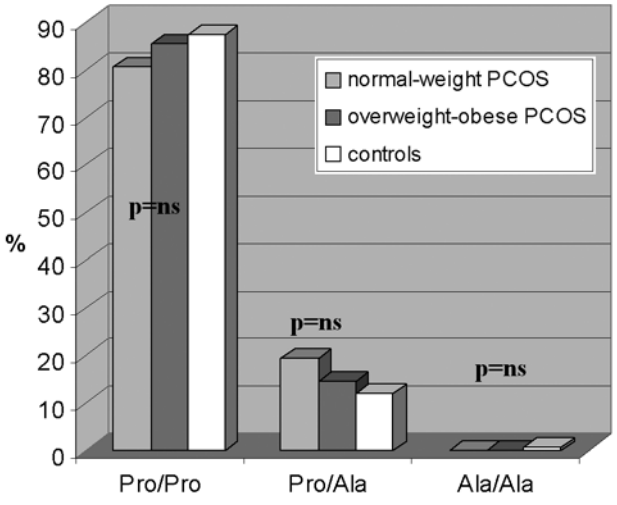


**Figure 1.** The distribution of Pro12Ala genotypes (%) in the study population. ns: non significant.

of the Pro12Ala polymorphism was observed when PCOS women with insulin resistance (fasting glucose to insulin ratio <4.5) or impaired glucose tolerance (n=49) were compared with either non-insulin resistant PCOS women or with the control group.

### *Anthropometric and biochemical differences between genotypes*

In the PCOS group, age, BMI, insulin resistance indexes and lipid profile were comparable between carriers of the Pro/Pro genotype and those with the Pro/Ala genotype (Table 2). Among patients who underwent OGTT, those with the Pro/Ala genotype appeared to have lower mean AUCinsulin compared with patients with the Pro/Pro genotype. However, the number of participants was small (14 vs 86 patients) and statistical analysis with a non-parametric test (Mann Whitney U test) revealed no significant difference in AUCinsulin levels between the genotype groups.

Regarding the hormonal profile of the two genotype groups, carriers of the Pro/Ala genotype had higher FSH (Mann-Whitney U test, p=0.05) and lower DHEAS levels (Mann-Whitney U test, p=0.01) compared with carriers of the Pro/Pro genotype (Table 2). This difference was also observed when we focused on normal-weight PCOS women. However, multiple regression analysis showed that there was no independent effect of the polymorphism on FSH and DHEAS when other parameters, especially age, were taken into account.

**Table 2.** Anthropometric and biochemical parameteres in carriers of the Pro/Pro genotype and carriers of the Pro/Ala genotype in the PCOS group (values are presented as means±SD).

| | Pro/Pro genotype | Pro/Ala genotype | p value |
|---|---|---|---|
| Number of subjects | 150 | 30 | |
| Age (years) | 22.7±5.8 | 23.7±6.4 | ns |
| BMI (Kg/m2) | 28.2±7.6 | 26.6±6.9 | ns |
| LH/FSH ratio | 1.4±1.1 | 1.4±1.0 | ns |
| LH (IU/L) | 7.5±6.0 | 7.1±3.6 | ns |
| FSH (IU/L) | 5.3±1.3 | 5.8±1.8 | 0.05 |
| DHEAS (μmol/L) | 7.95±3.19 | 6.44±2.80 | 0.01 |
| SHBG (nmol/l) | 36.6±26.6 | 43.4±23.9 | ns |
| Total testosterone (nmol/L) | 3.4±1.6 | 3.4±1.5 | ns |
| FAI | 13.7±12.1 | 11.4±10.2 | ns |
| Total cholesterol (mmol/L) | 4.63±0.85 | 4.94±0.89 | ns |
| Triglycerides (mmol/L) | 0.95±0.58 | 1.07±0.73 | ns |
| HDL-cholesterol (mmol/L) | 1.21±0.36 | 1.32±0.35 | ns |
| LDL-cholesterol (mmol/L) | 4.19±0.77 | 4.44±0.78 | ns |
| HOMA index | 3.2±2.4 | 3.3±2.2 | ns |
| Fasting glucose/insulin ratio | 10.0±7.9 | 8.6±4.6 | ns |
| Fasting insulin levels pmol/L | 99.8±70.2 | 99.8±61.3 | ns |
| AUCglucose (mg/dl) | 14920.5±3099.9 (n=86) | 14316.4±3420.7 (n=14) | ns |
| AUCinsulin (μIU/ml) | 11425.1±9876.6 (n=86) | 8554.9±3727.3 (n=14) | ns |

BMI: Body mass index, LH: Luteinizing hormone, FSH: Follicle-stimulating hormone, DHEAS: Dehydroepiandrosterone sulfate, SHBG: Sex hormone-binding globulin, FAI: Free androgen index, HDL: High density lipoprotein, LDL: Low density lipoprotein, HOMA: Homeostasis model assessment, AUC: Area under the curve, ns: non-significant

## DISCUSSION

In the present study, we investigated the association of the Pro12Ala polymorphism of the *PPAR-γ* gene with PCOS and the possible effect of this polymorphism on the phenotypic expression of PCOS in Greek women. We found that genotype and allele frequencies of the Pro12Ala polymorphism of the *PPAR-γ* gene were no different comparing PCOS women and healthy controls. This finding is in accordance with studies in other populations of PCOS women.[8-13,15] However, two studies curried out in Finnish and Turkish populations of women with PCOS, have shown that the Pro12Ala polymorphism is significantly more frequent in control subjects compared with PCOS women, indicating a protective effect of the Ala allele against the development of PCOS.[7,14] In both studies the frequency of the polymorphism was much higher compared with the observed frequency of the polymorphism in our study population.

In addition, the Pro12Ala polymorphism of the *PPAR-γ* gene does not seem to contribute to the variation of insulin resistance indices, lipid profile

or BMI and it has no apparent effect on hormonal parameters in women with PCOS, also supporting previous studies.[8,9,13,15] However, Hara et al found that the Pro12Ala polymorphism of the *PPAR-γ* gene act as a modifier of insulin resistance in Caucasian women with PCOS, since carriers of the polymorphism were more insulin sensitive than non-carriers, although only obese women with PCOS were included in this study.[6] Other studies also reported that this polymorphism is not only associated with increased insulin sensitivity but also with lower hirsutism scores and androgen levels in PCOS women.[12,14]

Discrepancies between studies on the Pro12Ala polymorphism of the *PPAR-γ* gene in PCOS may be explained by differences in the genetic background of the populations under investigation. Furthermore, possible interactions with other genetic variants as well as uncontrolled environmental factors may also explain the discrepancies between studies. In this context, a previous study indicated that the rare alleles of the P2 -689C>T and Pro12Ala polymorphisms were associated with an increased risk for the metabolic syndrome when combined with the 1431CC genotype of the *PPAR-γ* gene. When taken individually, none of the polymorphisms was associated with the metabolic syndrome.[19] On the other hand, environmental factors, such as diet and exercise, may also interact with the *PPAR-γ* gene. Fatty acids derived from nutrition or metabolism are natural ligands for PPAR-γ.[20,21] Luan et al found that BMI and insulin levels were greater in Ala carriers than in Pro/Pro homozygotes, but only if the ratio of dietary polyunsaturated fat to saturated fat was low.[22] More recent data suggest that not only diet but also the level of physical exercise can modulate the activity of PPAR-γ, with Ala carriers proving more sensitive to aerobic exercise in terms of a range of different metabolic parameters or body weight.[23,24]

Even though genetic association studies do not clearly establish any link between PCOS and *PPAR-γ* gene polymorphisms, functional investigations point to the potential role of the *PPAR-γ* gene on the development of PCOS. PPAR-γ is expressed in ovarian granulosa cells and it has been demonstrated that PPAR-γ ligands stimulate ovarian steroidogenesis.[25,26] Wood et al identified putative binding sites for PPAR-γ within the proximal promoter of several genes differentially expressed in PCOS oocytes, suggesting that PPAR-γ may regulate the expression of genes required for follicular development, ovulation and oocyte maturation.[27]

In conclusion, the findings of the present study do not support an association between the Pro12Ala polymorphism of the *PPAR-γ* gene and PCOS in Greek women. The Pro12Ala polymorphism does not appear to be a modifier of insulin resistance or of other parameters of PCOS. However, further studies are needed to clarify the role of *PPAR-γ* gene polymorphisms in the pathogenesis of PCOS in interaction with other genetic variants or environmental factors.

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