Original article

Insulin resistance associated with polycystic ovary syndrome – is Pro12Ala polymorphism of the PPAR-γ gene involved?

Rezistența la insulină asociată sindromului ovarelor polichistice - este implicat polimorfismul Pro12Ala al genei PPAR-γ?

Florina Gliga[1*], Ionela Paşcanu[2], Adina Stoian[1], Theodor Nicola[3], Claudia Bănescu[4]

1. Department of Pathophysiology UMF Tîrgu-Mureş
2. Department of Endocrinology UMF Tîrgu-Mureş
3. County Emergency Clinical Hospital Tîrgu-Mureş
4. Department of Genetics UMF Tîrgu-Mureş

Abstract

Polycystic ovary syndrome (PCOS) is a complex and heterogeneous endocrine disorder with many unclear pathogenetic pathways. The aim of the present research was to investigate the potential implication of the Pro12Ala polymorphism of the PPAR-γ (peroxisome proliferator-activated receptor gamma) gene in the pathogenesis of PCOS, the possible relationship between Pro12Ala polymorphism and the hyperandrogenemia and insulin resistance. Material and methods. We carried out a case-control study involving 89 patients with 47 PCOS and 42 controls, with a mean age of 25.10±5.57 years. BMI (body mass index) was calculated. Blood samples were collected after a period of 12 hours fasting, in the early follicular phase of menstrual cycle, and serum glucose, insulin, testosterone and SHBG (sex hormone binding globulin) were measured. HOMA (homeostasis model assessment) and FAI (free androgen index) were also calculated. DNA was isolated from peripheral blood. PPAR-γ gene polymorphism was examined by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) analysis. The genotype was compared among the study groups using Fisher's test. A p-value of <0.05 was considered statistically significant. Results. The PPAR-γ genotypes and allele variants proved to be in equilibrium in both study groups. There was no difference shown in the distribution of the Pro12Ala polymorphism between PCOS and healthy controls. The insulin resistance degree and androgen indices had no significant modified values among the different genotypes of the Pro12Ala polymorphism. Conclusion. The outcome of our study does not support any association between the Pro12Ala polymorphism of the PPAR-γ gene and PCOS in Romanian women.

Keywords: *polycystic ovary syndrome, insulin resistance, Pro12Ala polymorphism, PPAR-γ gene.*

Rezumat

Sindromul ovarelor polichistice (SOPC) este o endocrinopatie heterogenă şi complexă cu multiple mecanisme patogenetice incomplet elucidate. Scopul prezentului studiu este să investigheze posibila implicare a polimorfismului Pro12Ala a genei PPAR-γ în patogeneza SOPC, precum şi relația dintre polimorfism şi hiperan-

[*]**Corresponding author:**Florina Gliga, University of Medicine and Pharmacy Tirgu-Mures
Department of Pathophysiology, 38 Gheorghe Marinescu Street Tg.Mures 540042, Romania

drogenemie şi insulinorezistenţă. Material şi metodă. Am efectuat un studiu caz-control, cu 89 de paciente din România, 47 SOPC şi 42 martori, cu vârsta medie 25.10±5.57. IMC (indicele de masă corporală) a fost înregistrat. Au fost recoltate probe de sânge după post alimentar de 12 ore, în faza foliculara a ciclului menstrual, pentru determinarea glicemiei bazale, insulinemiei bazale, testosteronului şi SHBG. HOMA (homeostasis model assessement) şi FAI (indicele androgenilor liberi) au fost calculate. ADN-ul s-a extras din sângele periferic. Polimorfismul PPARγ a fost studiat prin tehnica PCR –RFLP (amplificarea ADN cu utilizarea enzimelor de restricţie). Genotipurile au fost comparate în cele două grupuri studiate folosindu-se testul Fisher. Valorile p sub 0.05 au fost considerate semnificative statistic. Rezultate. Distribuţia populaţională a genotipurilor PPAR-γ2 a fost echilibrată în ambele grupuri de studiu; nu s-a observat nici o diferenţă în repartizarea polimorfismului Pro12Ala între lotul SOPC şi lotul martor. Gradul insulinorezistenţei şi indicele de androgeni liberi nu au prezentat diferenţe semnificative legate de variatele genotipuri ale polimorfismului PPAR-γ. Concluzie. Rezultatele prezentului studiu nu susţin asocierea între polimorfismul PPAR-γ şi PCOS.

Cuvinte cheie: *sindromul ovarelor polichistice, insulinorezistenţă, polimorfism Pro12Ala, gena PPAR-γ.*

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Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrinopathy of reproductive aged women, with a prevalence of 6-10% (1). The disorder is characterized by biochemical and/or clinical hyperandrogenism and chronic anovulation. Insulin resistance, and/or consecutive hyperinsulinemia frequently appears being associated with PCOS (up to 64%), independent of the presence of obesity, but they are not considered diagnostic criteria (2).There is a large *in vivo* and *in vitro* data base supporting the idea that insulin resistance has a major impact in the pathogenesis of PCOS; consequently many studies focused on the pathogenetic implication of insulin, including genetic background. Numerous functional candidate genes have been studied for association with PCOS phenotypes, most findings turning out negative.

PPARγ (peroxisome proliferator-activated receptor gamma) is a subtype of a nuclear hormone receptor, part of a large family of ligand-activated transcription factors that modulate insulin sensitivity, differentiation of adipocytes and lipid metabolism. There are two isoforms, PPARγ1 and PPARγ2, the second one containing 28 aminoacids more than PPARγ1 (3).

Insulin sensitivity and hyperandrogenism were improved in women with PCOS after thiazolidinedione treatment, which is a medication that activates PPAR-γ, leading to the conclusion that the PPAR-γ gene may be involved in PCOS (4)**.**

Genetic research of the PPAR-γ gene has detected several polymorphisms with possible implications in PCOS pathogenesis, but most of it has been centered on the Pro12Ala polymorphism. A frequent missense mutation (cytosine to guanine) single nucleotide polymorphism (SNP) in PPAR-γ2 exon cause a Proline to Alanine substitution at the codon 12, which modulates the transcriptional activity of the gene (5, 6). Few individual studies and meta-analyses have found an association between the Ala12 variant, enhanced insulin sensitivity and a lower risk of type 2 diabetes (7, 8), but the results regarding the correlation of the Pro12Ala polymorphism with polycystic ovary syndrome are not yet clarified.

Our study was designed to explore the relationship between the Pro12Ala genotypes and PCOS; we have also tried to find a possible link of this polymorphism with insulin resistance or androgenic status of the syndrome among Romanian women with PCOS.

Materials and methods

We performed a case-control study involving 89 Romanian women with 47 PCOS and 42 controls, with a mean age of 25.10±5.57 years. BMI (body mass index) was recorded.

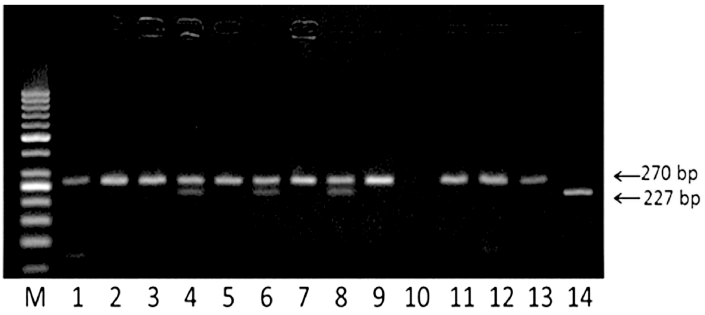

Figure 1. PCR-RFLP analysis of PPAR-γ (peroxisome proliferator-activated receptor γ) gene polymorphism. M: molecular marker 50 bp DNA ladder, lane 10: negative control, lanes 1–3, 5, 7, 9, 11-13: homozygous CC (Pro12Pro) genotype, lanes 4, 6, 8: heterozygous CG (Pro12Ala) genotype, lane 14: homozygous GG (Ala12Ala) genotype. The 43-bp fragments are not visible in the picture.

The diagnosis of PCOS was based on clinical signs of hyperandrogenemia (Ferriman–Gallwey score ≥8), oligo/amenorrhea and sonographic polycystic ovary appearance (ESHRE/ASRM 2003 criteria) (9). The control group was selected from regularly menstruating, ovulating women, with no clinical signs of hyperandrogenemia and with a similar mean age as the PCOS group.

Serum glucose was measured using the spectrophotometric method, with a glucose analyzer (Cobas Integra 400 Plus, Biochemistry Analyzer, Roche). All assays for hormonal determination (insulin, total testosterone and SHBG) were performed using electrochemiluminescence technology (Elecsys 2010, Roche Diagnostics).

Glycemia, basal insulin, testosterone and SHBG (sex hormone binding globulin) were determined from blood samples collected after overnight fasting. The degree of insulin resistance (IR) was evaluated using homeostasis model assessment (HOMA) analysis according to the formula: fasting serum insulin (μU/ml) × fasting plasma glucose (mg/dl) divided by 405. FAI (free androgen index) was calculated as follows: total testosterone (ng/ml) ×347/ SHBG (nmol/L). All women were investigated in the early follicular menstrual cycle phase (days 3-5) after a spontaneous bleeding episode; in patients with prolonged oligo-amenorrhea, blood samples were collected at the 7th day after a progesterone induced bleeding was achieved. Genomic DNA was isolated from whole blood. PPAR-γ2 (Peroxisome proliferator-activated receptor γ 2) gene polymorphism assessment was carried out using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) analysis.

DNA was extracted from whole blood containing ethylenediamine-tetraacetic acid (EDTA) as anticoagulant, by using the Genomic DNA Purification Kit (ZymoResearch). The sequences of the forward and reverse primers used were: 5'-GCCAATTCAAGCCCAGTC-3'and 5'-GATATGTTTGCAGACAGTGTATCAGTGAAGGAATCGCTTTCCG-3' (Fermentas) (10). DNA amplifications were performed with a Mastercycler Gradient Thermal Cycler (Eppendorf) with 7 minutes of denaturation at 94°C, followed by 35 cycles with denaturation for 40 s at 94°C, annealing for 40 s at 54°C, and extension for 40 s at 72°C, followed by a 10 minutes extension at 72°C. Template free water was used as negative control. The amplified products were digested with 5U Bsh1236I (BstUI) at 60 C° for 120 min (Fermentas).

The reaction products were analyzed by electrophoresis in 2% Top Vision (Fermentas) agarose gels and stained with ethidium bromide and then visualized under ultraviolet light. The genotypes were determined as follows: a single 270 bp fragment for the wild-type homozygous CC (Pro12Pro) genotype, two fragments of 227 and 43 bp for the GG (Ala12Ala) genotype and three fragments of 270, 227 and 43 bp for the CG (Pro12Ala) genotype (*Figure 1*).

All statistical analyses were performed using EpiInfo for Windows. We used independent sample t-test for mean comparison and F test for assessing the difference between variables, which were normally distributed according to the Kolmogorov–Smirnov test. For all the analyses, we chose alpha=0.05; any p

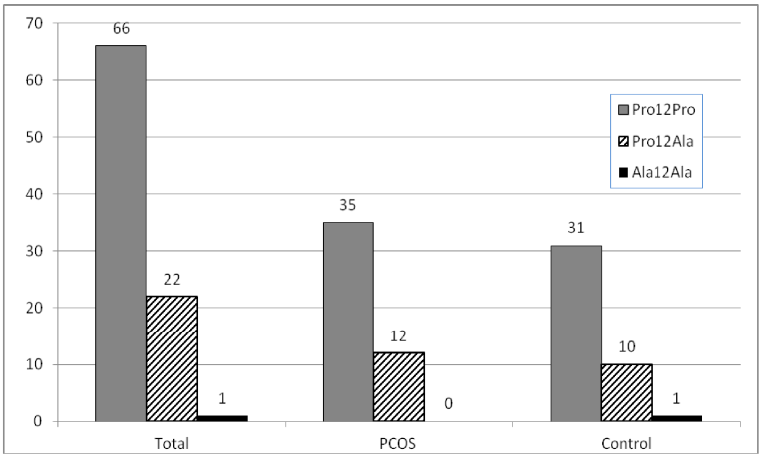

Figure 2. Distribution of PPAR-γ genotypes in the whole group

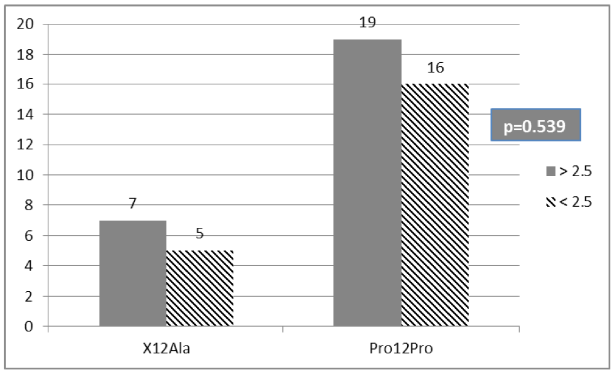

Figure 3. Distribution of PPAR-γ genotypes in PCOS group related to homeostasis model assessment for insulin resistance

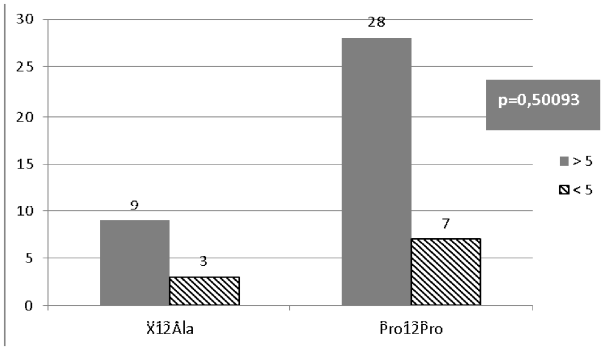

Figure 4. Distribution of PPAR-γ genotypes in PCOS group related to free androgen index

value smaller than alpha was considered to be significant. Binary data were analyzed with Fischer's exact test (11).

The experimental protocol was approved by the Research Ethics Committee of the University and informed consent was obtained from all participants.

Results

The distribution of Pro12Ala polymorphism of the PPAR-γ gene was in Hardy Weinberg equilibrium in both patients and controls (*Figure 2*). The occurrence of the Ala allele was 25.5 % (12 patients) in PCOS women and 26.2 % (11 patients) in the control group. A single case of homozygous patient for the Ala allele was diagnosed in the control group**.** Because the Ala12Ala genotype had a very low frequency, analyses were performed considering two groups of patients: the homozygous for the Pro-12 allele compared with those who had the Ala-12 allele.

Anthropometric features, metabolic and hormonal profile are presented in *Table 1*. In the PCOS group, age, BMI, insulin resistance index, fasting glucose were comparable between subjects with the Pro/Pro genotype and those with X/Ala genotype.

Also, the hormonal profile (total testosterone, SHBG and FAI) shows no statistically significant difference between the two genotype carriers.

Discussion

The actual research examined the possible correlation of polycystic ovary syndrome with the PPAR-γ Pro12Ala polymorphism,

Table 1. Phenotypic features in polycystic ovary syndrome women according to peroxisome proliferator-activated receptor γ variants. Values are mean±SD range

	Total PCOS n=47	**Pro/Pro *n*=35**	**X/Ala *n*=12**	**p value**
Age (years)	25.10±5.57	25.0±4.84	25.45±7.73	0.81
Body mass index (kg/m^2)	28.70±6.43	29.08±6.82	27.60±5.24	0.50
Fasting insulin (μIU/ml)	15.03±12.10	15.87±13.79	12.55±3.95	0.41
Fasting glucose (mg/dl)	92.83±7.95	92.55±7.57	93.63±9.30	0.69
HOMA	3.42±2.69	3.61±3.05	2.93±1.06	0.45
Testosterone (ng/ml)	0.72±0.32	0.72±0.31	0.71±0.34	0.94
FAI	9.73±5.60	10.22±5.85	8.32±4.72	0.31

and if its presence could be related to the phenotype expression in a group of Romanian women.

The findings suggested that the two studied groups (PCOS and control) show no significant statistical differences regarding the genotype and allele frequencies of the Pro12Ala polymorphism of the PPAR-γ gene. Previous reports in other populations of PCOS women sustain our conclusions (12, 13). In a larger study group Antoine et al (13) state that the PPAR-γ gene does not seem to be important in the pathogenesis of PCOS. In the same spirit, more recent papers couldn't reveal a possible link between the Pro12Ala polymorphism of the PPAR-γ gene and PCOS in Greek women (14, 15).

Korhonen et al. considered that the Ala allele of PPAR- γ gene might have a protective role in PCOS's insulin resistance pathogenesis, based on the results of their study; they found a significant higher incidence of Pro12Ala polymorphism in controls than PCOS women ($p < 0.05$) in the Finnish population. In addition, a study that supports the same conclusions was carried out on a group of Turkish women with polycystic ovary syndrome (16). A huge meta-analysis focused on the implication of PPAR-γ Pro12Ala polymorphism in PCOS, that enrolled multiple eligible studies comprising different geographic populations, suggested that Ala variant could be related to a lower risk of PCOS (17, 18).

In our study the metabolic and hormonal status seems to have no suggestive relationship with the Pro12Ala polymorphism of the PPAR-γ gene. There is no significant association between fasting insulin levels, BMI, HOMA or FAI index and the PPAR-γ genotypes (*Figures 3 and 4)*. These results are supported by previous researches (19, 20).

Nevertheless, Hara et al. support the idea that the Pro12Ala polymorphism of the PPAR-γ gene could modify the insulin resistance in Caucasian women with polycystic ovary syndrome, as patients with Pro12Ala variant were more insulin sensitive than women with Pro12Pro in an obese PCOS study (21). Tok et al. reported that both controls and PCOS women presented considerable differences in glucose metabolism related to PPAR-γ2 Pro12Ala polymorphism; the same study considers that this genotype had no substantial impact on reproductive hormones (22). Other papers also indicate that this polymorphism implies not only higher insulin sensitivity, but it is also associated with decreased androgen levels and attenuated clinical signs in PCOS women (14, 23).

The vast database obtained in all studies regarding the relationship between Pro12Ala polymorphism of the PPAR-γ gene and polycystic ovary syndrome is still contradictory. The differences may have many causes.

One of them may be due to the reduced sample size of the studies. For example, a recent original case-control study followed by systematic review and meta-analysis of existing evidence

(San-Millán et al), indicated that the individual study did not express any statistically significant association between PPAR-γ2 Pro12Ala polymorphism and PCOS; nevertheless the meta-analysis demonstrated that the carriers of the Pro12Ala variant were associated with a lower risk of developing PCOS , and that this result may correlated with a modified insulin sensitivity (19).

Other explanation for the discrepancies could be explained by different genetic backgrounds or possible interactions with other genetic variants of population enrolled in studies.

Moreover, it is well known that PCOS women have a greater chance of developing metabolic syndrome (MS), a complex disorder which implies insulin resistance. There is evidence that the syndrome has a polygenic predisposition with several minor genes involved, PPAR-γ polymorphism being considered a possible pathogenic key in both PCOS and MS. Although the contribution of insulin resistance in the pathogenesis of the MS has been largely debated, some studies suggest that the Pro12 allele of the PPAR-γ gene appears to be implicated in the hereditary predisposition of the disorder; although the presence of the Ala12 allele is related to a higher bodyweight, the presence of Ala12 allele could diminish the risk for developing the disease (24).

Additionally, environmental factors, such as nutrition habits, diet or exercise could be considered risk factors for a dysfunction at the genetic level. Actually, a potent interrelation was established between dietary polyunsaturated/saturated fatty acid ratio and the Pro12Ala polymorphism concerning the body mass index. Fatty acids originated from dietary intake or as secondary products of metabolism are natural ligands for PPAR-γ. According to Luan et al. the ponderal index and plasma insulin values were higher in Ala variant than in Pro/Pro genotypes; this condition appears in populations where the polyunsaturated fat to saturated fat nutrition ratio is low (25). Similarly, physical exercise is able to adjust the activity of PPAR-γ Ala carriers increasing the insulin sensitivity (26).

Conclusions

In conclusion, we can state that the results of this research do not support an association between the Pro12Ala polymorphism of the PPAR-γ gene and polycystic ovary syndrome in Romanian women. No significant differences in plasma glucose, insulin, homeostasis model assessment, testosterone, free androgen index or body mass index were observed between genotypes.

The Pro12Ala polymorphism does not seem to act as an important modulator of insulin sensitivity, and does not influence other PCOS's features.

Considering the fact that the results of several studies are still controversial, further studies including larger sample size are needed to define the possible involvement of PPAR-γ gene polymorphisms in PCOS's pathogenetic mechanisms.

Acknowledgement

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Abbreviation list

PPAR-γ - peroxisome proliferator-activated receptor γ
PCOS - polycystic ovary syndrome
BMI – body mass index
SHBG - sex hormone binding globulin
HOMA - homeostasis model assessment for insulin resistance
FAI - free androgen index
PCR-RFLP - polymerase chain reaction–restriction fragment length polymorphism
SNP - single nucleotide polymorphism
ESHRE/ASRM European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine
MS - metabolic syndrome

References

1. Azziz R,Woods KS, Reyna R, Key TJ, Knochenhauer ES & Yildiz BO. The prevalence and features of the polycystic ovarian syndrome in an unselected population. J Clin Endocrinol Metab. 2004;98 :2745–2749
2. DeUgarte CM, Bartolucci AA, Azziz R. May. Prevalence of insulin resistance in the polycystic ovary syndrome using the homeostasis model assessment. Fertil Steril. 2005; 83(5):1454-60
3. Vidal H. (PPAR receptors: recent data) Ann Endocrinol (Paris). 2005 Apr.; 66 (2 Pt 2):1S5-9
4. Stout DL, Fugate SE. Thiazolinediones for treatment of polycystic ovary syndrome. Pharmacotherapy, 2005; 25: 244-252
5. Deeb SS, Fajas L, Nemoto M, Pihlajamaki J, Mykkanen L, Kuusisto J, et al. A Pro12Ala substitution in PPAR-gamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. Nat Genet. 1998; 20:284-287
6. Masugi J, Tamori Y, Mori H, Koike T, Kasuga M. Inhibitory effect of a proline-to-alanine substitution at codon 12 of peroxisome proliferator-activated receptor-gamma 2 on thiazolidinedione-induced adipogenesis. Biochem Biophys Res Commun. 2000; 268:178-182
7. Ek J, Andersen G, Urhammer SA, Hansen L, Carstensen B, Borch-Johnsen K et al. Studies of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor-gamma2 (PPAR-gamma2) gene in relation to insulin sensitivity among glucose tolerant caucasians. Diabetologia. 2001;44:1170-1176
8. Altshuler D, Hirschhorn JN, Klannemark M, Lindgren CM, Vohl MC, Nemesh J et al. The common PPAR-gamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. Nat Genet. 2000; 26:76-80
9. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Fertil Steril. 2004; 81(1):19-25
10. Oh EY, Min KM, Chung JH, Min YK, Lee MS, Kim KW et al. Significance of Pro12Ala mutation in peroxisome proliferator-activated receptor-gamma2 in Korean diabetic and obese subjects. J Clin Endocrinol Metab. 2000;85(5):1801-1804.
11. Marusteri M, Bacarea V. Comparing groups for statistical differences: how to choose the right statistical test ? Biochemia medica. 2010; 20 (1), 15-32
12. Orio F Jr, Matarese G, Di Biase S, Palomba S, Labella D, Sanna V, et al. Exon 6 and 2 peroxisome proliferator-activated receptor-gamma polymorphisms in polycystic ovary syndrome. J Clin Endocrinol Metab. 2003;88: 5887-5892
13. Antoine HJ, Pall M, Trader BC, Chen YD, Azziz R, Goodarzi MO. Genetic variants in peroxisome proliferator-activated receptor gamma influence insulin resistance and testosterone levels in normal women, but not in those with polycystic ovary syndrome. Fertil Steril. 2007; 87: 862-869
14. Xita N, Lazaros L, Georgiou I, Tsatsoulis A. The Pro12Ala polymorphism of the PPAR-G gene is not associated with the polycystic ovary syndrome. Hormone. 2009; 8(4):267-272
15. Koika V, Marioli DJ, Saltamavros AD, Vervita1 V, Koufogiannis KD, Adonakis G, et al. Association of the Pro12Ala polymorphism in peroxisome proliferator-activated receptor γ2 with decreased basic metabolic rate in women with polycystic ovary syndrome. Eur J Endocrinol. 2009; 161: 317–322
16. Korhonen S, Heinonen S, Hiltunen M, Helisalmi S, Hippelainen M, Koivunen R, et al. Polymorphism in the peroxisome proliferator-activated receptor-gamma gene in women with polycystic ovary syndrome. Hum Reprod. 2003; 18:540–543
17. Yilmaz M, Ergun MA, Karakoc A, Yurtcu E, Cakir N, Arslan M. Pro12Ala polymorphism of the peroxisome proliferator-activated receptor-gamma gene in women with polycystic ovary syndrome. Gynecol Endocrinol. 2006;22: 336-342
18. He J, Wang L, Liu J, Liu F, Li X. A meta-analysis on the association between PPAR-γ Pro12Ala polymorphism and polycystic ovary syndrome. J Assist Reprod Genet. 2012 Jul; 29(7):669-77
19. San-Millán JL, Escobar-Morreale HF. The role of genetic variation in peroxisome proliferator-activated receptors in the polycystic ovary syndrome (PCOS): an original case-control study followed by systematic review and meta-analysis of existing evidence.Clin Endocrinol (Oxf). 2010 Mar; 72(3):383-92
20. Wang Y, Wu X, Cao Y, Yi L, Fan H, Chen J. Polymorphisms of the peroxisome proliferator-activated receptor-gamma and its coactivator-1alpha genes in Chinese women with polycystic ovary syndrome. Fertil Steril. 2006; 85: 1536-1540
21. Hara M, Alcoser SY, Qaadir A, Beiswenger KK, Cox NJ, Ehrmann DA. Insulin resistance is attenuated in women with polycystic ovary syndrome with the Pro12Ala polymorphism in the PPARgamma gene. J Clin Endocrinol Metab. 2002; 87: 772-775
22. Tok EC, Aktas A, Ertunc D, Erdal EM, Dilek S. Evaluation of glucose metabolism and reproductive hormones in polycystic ovary syndrome on the basis of peroxisome proliferator-activated receptor (PPAR)-gamma2 Pro12Ala genotype. Hum Reprod. 2005; 20: 1590-1595
23. Hahn S, Fingerhut A, Khomtsiv U, Khomtsiv L, Tan S, Quadbeck B, et al. The peroxisome proliferator activated receptor gamma Pro12Ala polymorphism is associated with a lower hirsutism score and increased insulin sensitivity in women with polycystic ovary syndrome. Clin Endocrinol (Oxf). 2005; 62: 573-579
24. Csép K, Dudutz G, Vitay M, Pascanu I, Bănescu C, Korányi L, et al. The relationship between the Pro12ala

polymorphism of the PPARγ2 gene and the metabolic syndrome in a population of Central Romania diagnosed according to the IDF criteria, Acta Endocrinologica (Buc). 2008; vol. IV, 3: 263-271

25. Luan J, Browne PO, Harding AH, Halsall DJ, O'Rahilly S, Chatterjee VK et al. Evidence for gene-nutrient interaction at the PPARgamma locus. Diabetes. 2001;50:686–689

26. Franks PW, Luan J, Browne PO, Harding AH, O'Rahilly S, Chatterjee VK et al. Does peroxisome proliferator-activated receptor gamma genotype (Pro12Ala) modify the association of physical activity and dietary fat with fasting insulin level? Metabolism. 2004; 53: 11-16