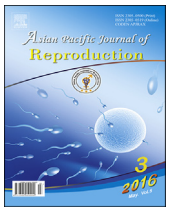
Contents lists available at ScienceDirect

Asian Pacific Journal of Reproduction

journal homepage: www.apjr.net



Original research http://dx.doi.org/10.1016/j.apjr.2016.04.002

Peroxisome proliferator activated receptor gamma polymorphism Pro12Ala in polycystic ovary syndrome (PCOS) of South Indian Population

Raichel Jacob[1*], Chandirasekar Ramachandran[1], Calistus Jude[2], Uthayakumar Venkatachalam[1], Sasikala Keshav Rao[1]

[1]*Division of Human Genetics, Department of Zoology, Bharathiar University, Coimbatore, India*

[2]*Department of Life Sciences, Kristu Jayanti College, Bangalore, India*

ARTICLE INFO

Article history:
Received 30 Aug 2015
Received in revised form 19 Dec 2015
Accepted 28 Dec 2015
Available online 16 Apr 2016

Keywords:
PPAR gamma
Polymorphism
Hormones
PCOS

ABSTRACT

Objective: To investigate the frequency of Pro12Ala polymorphism in PPAR gamma gene among PCOS of South Indian Population. Understanding this polymorphism may help us in better diagnosis prevention and therapeutic approaches towards management of PCOS.

Method: A total of 75 women with the diagnosis of PCOS and 75 healthy controls were included in this study. The Pro12Ala variant in the PPAR gamma gene was analysed by PCR-RFLP.

Results: Genotypic frequencies for PPAR gamma gene Pro12Ala polymorphism showed the frequency of Pro/Pro genotypes was 77% in PCOS and 81% in controls. The odds ratio was 0.71 (95% *CI* 0.31–1.63) and 2.66 (95% *CI* 0.21–32.9) and alleles expressed 0.87 (95% *CI* 0.42–1.79). Regarding hormone levels, there were significant differences between PCOS and non PCOS.

Conclusion: PPARγ2 gene Pro12Ala polymorphism was supposed to be susceptible genes in PCOS. The present study demonstrated that there is a statistical difference between the distributions of PPAR gamma Pro12Ala polymorphism in South Indian Population.

1. Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting up to 7% of women of reproductive age [1]. The disorder is characterised mainly by menstrual irregularities acne, hyperandrogenism central obesity, type 2 diabetes [2]. The pathogenesis of PCOS is unknown. Recent reports show that it remains as a complex multigenic disorder characterised by abnormal gonadotropin release and dysregulation of steroidogenesis [3]. PCOS is a familial condition with autosomal dominant mode of inheritance [4]. It is a combination of genetic abnormalities combined with environmental factors such as nutrition and body weight, which mainly affect the PCOS [5].

Recent studies show that initiation of pathological mechanisms occurs mainly during the foetal life, with high maternal serum levels of androgen/insulin may influence the genetic programme which result in both reproductive and metabolic consequences of PCOS [6]. The peroxisome proliferators activated receptor gamma PPAR gamma gene is located at chromosomal region 3p25 and is mainly expressed in adipose tissue which promotes the differentiation of pre-adipocytes into adipocytes and belong to nuclear hormone receptor family [7]. Peroxisome proliferators-activated receptor-gamma (PPARγ) is a candidate gene involved in glucose homoeostasis, lipid metabolism and adipocyte differentiation also implicated in the pathogenesis of PCOS [8].

*Corresponding author: Raichel Jacob, Department of Zoology, Bharathiar University, Coimbatore, Tamil Nadu, India.
Tel: +91 9442413152
E-mail: raichelshaju@yahoo.co.in

Peer review under responsibility of Hainan Medical College.
Foundation project: This study was supported by DST funding agency (grant No. WOS-A/LS-21/2010).

PPARγ is a nuclear receptor which combines with the retinoid X receptors to form heterodimers which controls transcription of genes involved in free fatty acid uptake and lipogenesis, and plays an important role in regulation of insulin sensitivity and adipose tissue metabolism, carcinogenesis and inflammation [9]. A functional polymorphism in exon 2 of PPARγ2 gene produces a Pro–Ala substitution at the codon 12 causing the Pro12Ala polymorphism (rs1801282). This CCA-GCA change causes a conformational change in the protein thus affecting the activity [10]. The Pro12Ala polymorphism of PPAR gamma gene has been associated with reduced transcriptional activity of PPAR gamma and presence of Ala isoform has been linked to higher insulin sensitivity and lower body mass index [11].

According to reports of Zargar *et al.* [12] significant number of PCOS patients show impaired glucose tolerance and are in potential risk of developing type 2 diabetes. The objective of the study was to determine the genetic frequency of Pro12Ala polymorphism in PPAR gamma gene in South Indian PCOS population. The association between the Pro12Ala polymorphism and increased insulin sensitivity in PCOS has been observed in reports of Hahn *et al.* [13]. PPAR gamma gene associated with the risk of colorectal adenoma was reported in a study [14]. PCOS were diagnosed based on the Rotterdam criteria 2003 [15] which includes polycystic ovarian with >10 small peripheral cysts, hyperandrogenism, anovulation and hyperinsulinemia. Subjects were considered to have oligomenorrhea if they had less than 8 cycles per year and amenorrhea in absence of menses for6 months or more [16]. Clinical hyperandrogenism was diagnosed by hirsutism assessment using F–G scoring if seen with 8 or more [17]. Biochemical hyperandrogenism was diagnosed by elevated levels of serum free testosterone as it is considered the single most predictive evidence of hyperandrogenemia [18].

2. Materials and methods

2.1. Collection of blood samples

Peripheral blood samples of 3 mL was collected from the subjects using a heparinised needle and transported to the laboratory with the help of portable coolers. The subjects were recruited in the study from the women population attending the gynaecology clinic of a tertiary care hospital (n = 75) were served as experimental and were included in the study. Anthropometric details and general clinical characteristics and history of disease were obtained by a standardized health questionnaire. Age matched healthy controls were included in the study. The inclusion criteria of the PCOS were based on the Rotterdam diagnostic criteria. The subjects have not received hormonal therapy for at least 3 months before hormone assays. All the study evaluations and procedures were conducted in accordance with the guidelines of Helsinki Declaration on human experimentation. An ethical committee approval was obtained for the study and written informed consent was obtained from the subjects involved in the study.

2.2. Hormone assays

The level of serum follicle stimulating hormone FSH luteinizing hormone LH total testosterone T were determined using commercially available human Elisa kits. The intra and inter assay coefficient of variation of all the assays were less than 10%.

2.3. Genotyping

Genomic DNA was isolated from peripheral blood leucocytes by using the standard Phenol-chloroform extraction method [19]. Pro12Ala polymorphism was determined using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) method. The PCR reaction mixture contained 50 ng of genomic DNA 0.5 μmol/L of each primer of forward 5′CCA ATT CAA GCC CAG TCC TTT C3′ and reverse 5′GCA GAC AGT GTA TCA GTG AAG GAA TCG CTT TCC G and master mix (Promega) USA in a final volume of 25 μL. The reaction mixture was subjected to denaturation at 95 °C for 2 min followed by 35 cycles at 95 °C for 30 s, 52 °C for 30 s, 72 °C for 45 s then by a final extension of 72 °C for 7 min. The PCR products were 237 bp and were digested with BstUI restriction enzyme at 37 °C for 18 h. After the digested products were electrophoresed on a 2.5% agarose gel and visualised by ethidium bromide staining, the C and the G alleles could be distinguished as bands 217 bp and 20 bp respectively.

2.4. Statistical analysis

The standard χ^2 tests were used to compare genotype frequencies among PCOS and control groups. The results were in Hardy–Weinberg equilibrium of genotype frequencies. The results of continuous data were reported as mean and ±SD. Differences in serum hormone levels between PCOS and control groups were assessed by students t test $P < 0.05$ was considered significant for all tests. Pearson bivariate correlation was studied for hormones among controls and PCOS. Statistical analysis was performed using the SPSS Version 16 (Statistical Package for the Social Sciences, USA).

3. Results

Table 1 depicts the anthropometric data and hormone levels between PCOS and control groups. There was a significant difference in age, FSH, LH, testosterone hormone levels between the two groups. The subjects were grouped into three based on their age as G I, G II and G III and they were significant at $P < 0.05$ levels. However, the level of testosterone hormone was significantly higher in the PCOS group when compared to the control subjects. Pearson bivariate correlation was studied among hormones. Correlation was observed among the Group I as $r = 0.459$, $P = 0.006$ significant at $P < 0.001$ level. A negative correlation was observed among Group II and Group III individuals with ($r = 0.39$ $P = 0.19$, $r = 0.43$ $P = 0.15$).

Table 1
The hormonal concentrations of PCOS and controls.

Groups	N	Age	FSH (mIU/mL)	LH (mIU/mL)	Testosterone (ng/mL)
Controls	G I 35	23.25 ± 2.70	9.12 ± 1.80	9.65 ± 2.48	0.60 ± 0.26
	G II 28	33.00 ± 2.56	9.07 ± 2.17	9.53 ± 1.96	0.78 ± 0.24
	G III 12	40.50 ± 2.80	8.80 ± 2.63	10.90 ± 2.02	0.76 ± 0.49
PCOS	G I 35	24.40 ± 1.80	9.30 ± 2.88**	11.30 ± 2.85**	0.85 ± 0.30*
	G II 28	32.60 ± 2.60*	9.51 ± 2.69	12.30 ± 2.37	0.90 ± 0.26*
	G III 12	42.50 ± 3.14*	11.5 ± 2.00	11.90 ± 2.78	0.48 ± 0.27*

Data were expressed as (Mean ± SD), G I-(18–27) years, G II-(28–37) years, G III-(38–47) years. *Statistically significant values between controls and PCOS at $P < 0.05$ levels. **Statistically significant values between Group I of controls and PCOS at $P < 0.001$ levels.

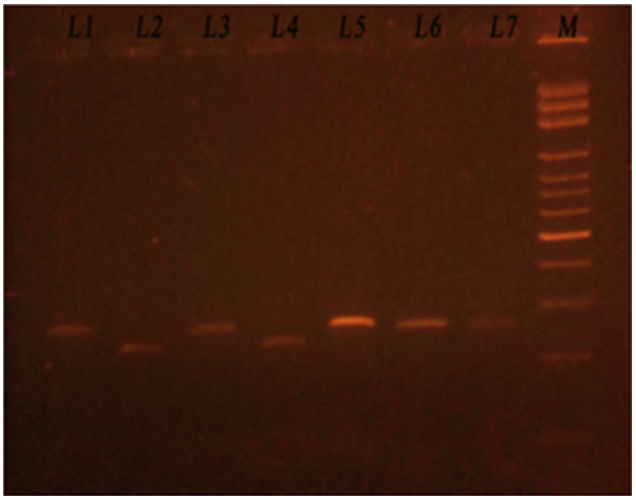


Figure 1. Electrophoresis of PCR product shows Pro12Ala polymorphism in the PPAR gamma gene among the PCOS samples.
Lane-(1, 3, 5, 6 and 7) Ala12Ala homozygote; Lane-(2 and 4) Pro12Pro homozygote; M-100 bp DNA Marker.

Table 2
Genotype frequency distribution of Pro12Ala polymorphism in women with PCOS and controls.

Genotypes	Experimental (*n* %)	Controls (*n* %)	*P*-value	Odds ratio 95% CI
Allelic frequency				
Pro/Pro	58.0 (77.30)	61.0 (81.30)	1.00	1.0
Pro/Ala	16.0 (21.00)	12.0 (16.00)	0.64	0.7 (0.3–1.63)
Ala/Ala	1.0 (1.30)	2.0 (2.60)	0.62	2.6 (0.21–32.9)
Allelic frequency				
Pro	132.0 (0.88)	134.0 (0.89)	–	–
Ala	18.0 (0.12)	16.0 (0.10)	0.13	0.87 (0.42–1.79)

$\chi 2$ – Chi-square values or Odds ratio at 95% confidence level.

The allele and genotype frequency distribution (Figure 1) for both PCOS and control groups were consistent with the Hardy–Weinberg equilibrium and are shown in Table 2. Frequencies of the G allele was (0.12) and C allele (0.88) among PCOS and (0.10) and (0.89) in controls. Genotype frequencies was 0.71 *CI* (0.31–1.63) and 2.66 *CI* (0.21–32.9) and alleles expressed 0.87 *CI* (0.42–1.79). Statistically significant values were obtained.

4. Discussion

Polycystic ovary syndrome is a metabolic disorder resulting from the interaction of genetic predisposition and environmental risk factors. The familial tendency of PCOS and the increased levels of hormones play a pathogenic role in the disease. In the current study, we investigated the possible association between Pro12Ala polymorphism at PPAR gamma gene and the occurrence of PCOS.

The metabolic syndrome is evident at an early age in women with PCOS irrespective of race and ethnicity. Due to high impact of PPAR gamma gene as an insulin-sensitising transcription factor involved in adipogenesis and lipogenesis, the occurrence of single nucleotide polymorphisms (SNPs) in the PPARγ gene was addressed by numerous reports on studying subjects with insulin resistance, type 2 diabetes, arteriosclerosis, and hypertension [20].

Hyperinsulinemia acts as a central factor for critical link between PCOS and metabolic syndrome. Reports [10] showed abdominal adipose was increased in Ala 12 carriers with diabetes and obesity. Metabolic dysfunction in lipid profile and abdominal obesity is regarded as an indicator of CVD cardiovascular disease in the Ala genotype women with PCOS [21]. Studies [22] have shown that Ala allele is associated with high BMI and dyslipidemia in elderly subjects. This may indicate that the functional role of Pro12 Ala substitution differs depending on the degree of obesity. Pro12Ala polymorphism showed an association with PCOS susceptibility in Korean women [23]. Cardona *et al.* [24] reported Pro12Ala polymorphism is associated with high risk of hyper triglycerides. Kalra *et al.* [25] found that obese carriers of Pro12Ala polymorphism have a risk for hyperlipidemia and concluded that IR is associated with dyslipidemia in women with PCOS, independent of obesity. Studies of Yang *et al.* [21] reported Ala allele frequency has a positive association with PCOS. Several studies have described ethnic allelic variation of PPAR gamma with a higher allelic frequency in Caucasians and minor frequency in Asian subjects.

The present study may have some limitations which include relatively small size of the sample, PCOS and non PCOS (controls) were not matched for body weight and this factor may limit the influence of BMI on the hormonal secretion of PCOS group. Mainly from the animal studies data it is seen that the testosterone hormone has both facilitatory and inhibitory effect on LH secretion and suppressive effect on progesterone. Most of the PCOS have higher levels of free testosterone. In general, the testosterone hormone has both negative and positive action on female gonadotropins via modulating the gene expression of LH and FSH.

In conclusion, the study showed close association between the testosterone levels and Pro12Ala polymorphism in PCOS women. They posed partially metabolic dysfunction in PCOS.

Relationship between Pro12Ala polymorphism and metabolic dysfunction in PCOS may provide evidence for the individualised treatment and early diagnosis of disorder.

Apart from conventional candidate gene approach, genomic wide scans might be promising. Further cDNA technology would shed light on functional aspect of candidate genes for understanding the genetic aetiology of PCOS. Ala allele of PPAR gamma may be advantageous against the development of PCOS when gene-environment interactions are considered. These results imply that the Pro allele may be a susceptibility factor for PCOS. In conclusion, a significant association was found between the single polymorphism located in the coding region of the PPAR gamma and PCOS.

Conflict of interest statement

The authors declare that we have no conflict of interest.

Acknowledgments

The authors are thankful to the authorities of Bharathiar University for the research facilities. We also gratefully acknowledge DST (WOS-A/LS-21/2010) funding agency for timely funding.

References

[1] Diamanti-Kandarakis E, Argyrakopoulou G, Economou F, Kandaraki E, Koutsilieris M. Defects in insulin signalling pathways in ovarian steroidogenesis and other tissues in polycystic ovary syndrome (PCOS). *J Steroid Biochem Mol Biol* 2008; **109**: 242-246.

[2] Norman RJ, Hickey T, Moran L, Boyl J, Wang M. Polycystic ovary syndrome-diagnosis and aetiology. *Int Congr Ser* 2004; **1266**: 225-232.

[3] Balen AH. *Polycystic ovary syndrome and secondary amenorrhoea. Dewhurst textbook of obstetrics and gynaecology*. 7th ed. USA: Wiley Blackwell; 2007, p. 377-397.

[4] Franks S, McCarthy M. Genetics of ovarian disorders: polycystic ovary syndrome. *Rev Endocr Metab Disord* 2004; **5**: 69-76.

[5] Unluturk U, Harmanci A, Kocaefe C, Yildiz BO. The genetic basis of the polycystic ovary syndrome: a literature review including discussion of ppar-gamma. *PPAR Res* 2007; **49**: 109.

[6] Dasgupta S, Reddy BM. Present status of understanding on the genetic etiology of polycystic ovary syndrome. *J Postgrad Med* 2008; **54**: 115-125.

[7] Michalik L, Auwerx J, Berger JP, Chatterjee VK, Glass CK, Gonzalez FJ, et al. International Union of Pharmacology. *LXI*. Peroxisome proliferators-activated receptors. *Pharmacol Rev* 2006; **58**(4): 726-741.

[8] Beaven S, Tontonoz P. Nuclear receptors in lipid metabolism: targeting the heart of dyslipidemia. *Annu Rev Med* 2006; **57**: 313-329.

[9] Gouda HN, Sagoo GS, Harding AH, Yates J, Sandhu MS, Higgins JP. The association between the peroxisome proliferators-activated receptor-γ2 (*PPARG2*) Pro12Ala gene variant and type 2 diabetes mellitus: a huge review and meta-analysis. *Am J Epidemiol* 2010; **171**(6): 645-655.

[10] 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 proliferators-activated receptor (PPAR)-gamma2 Pro12Ala genotype. *Hum Reprod* 2005; **20**(6): 1590-1595.

[11] Korhonen S, Heinonen S, Hiltunen M, Helisalmi S, Hippelainen M, Koivunen R, et al. Polymorphism in the peroxisome proliferators-activated receptor-gamma gene in women with polycystic ovary syndrome. *Hum Reprod* 2003; **18**: 540-543.

[12] Zargar AH, Gupta VK, Wani AI. Prevalence of ultrasonography proved polycystic ovaries in North Indian women with type 2 diabetes mellitus. *Reprod Biol Endocrinol* 2005; **3**: 35.

[13] Hahn S, Fingerhut A, Khomtsiv U, Khomtsiv L, Tan S, Quadbeck B, et al. The peroxisome proliferators 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**(5): 573-579.

[14] Gong Z, Xie D, Deng Z, Bostick RM, Muga SJ, Hurley TG, et al. The PPAR gamma Pro12Ala polymorphism and risk for incident sporadic colorectal adenomas. *Carcinogenesis* 2005; **26**(3): 579-585.

[15] Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril* 2004; **81**: 19-25.

[16] Chang WY, Knochenhauer ES, Bartolucci AA, Azziz R. Phenotypic spectrum of polycystic ovary syndrome: clinical and biochemical characterization of the three major clinical subgroups. *Fertil Steril* 2005; **83**(6): 1717-1723.

[17] Yildiz BO, Bolour S, Woods K, Moore A, Azziz R. Visually scoring hirsutism. *Hum Reprod Update* 2010; **16**: 51-64.

[18] Fauser B. Revised 2003 consensus on diagnostic criteria and long term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod* 2004; **19**: 41-47.

[19] Kambalachenu HR, Durairaj Paul SF, Nellepalli SR, Venkatachalam P. Study of follicle stimulating receptor gene polymorphisms in South Indian women with polycystic ovary. *Am Med J* 2013; **4**(2): 160-167.

[20] Savage DB. PPAR gamma as a metabolic regulator: insights from genomics and pharmacology. *Expert Rev Mol Med* 2005; **7**: 1-16.

[21] Yang J, Gong H, Tao T. The association of Pro12Ala polymorphism in the peroxisome proliferators-activated receptor gamma gene with the metabolic characteristics in Chinese women with polycystic ovary syndrome. *Int J Clin Exp Pathol* 2013; **6**(9): 1894-1902.

[22] Tai ES, Corella D, Deurenberg-Yap M, Adiconis X, Chew SK, Tan CE, et al. Differential effects of the C1431T and Pro12AlaPPARgamma gene variants on plasma lipids and diabetes risk in an Asian population. *J Lipid Res* 2005; **45**(4): 674-685.

[23] Gu BH, Baek KH. Pro12Ala and His447His polymorphisms of PPAR-gamma are associated with polycystic ovary syndrome. *Reprod Biomed Online* 2009; **18**: 644-650.

[24] Cardona F, Morcillo S, Gonzalo-Marin M, Garrido-Sanchez L, Macias Gonzalez M. Pro12 Ala sequence variant of the PPARG gene is associated with post prandial hypertriglyceridemia in patients with the metabolic syndrome. *Clin Chem* 2006; **52**: 1920-1925.

[25] Kalra A, Nair S, Rai L. Association of obesity and insulin resistance with dyslipidemia in Indian women with polycystic ovarian syndrome. *Indian J Med Sci* 2006; **60**: 447-453.