Peroxisome proliferator activated receptor gamma gene variants influence susceptibility and insulin related traits in Indian women with polycystic ovary syndrome

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Abstract

Purpose Peroxisome proliferator activated receptor gamma (*PPAR* γ), a transcription factor involved in glucose and lipid metabolism is one of the candidate genes associated with polycystic ovary syndrome (PCOS). We investigated individual and combined associations of Pro12Ala and His447His polymorphisms of *PPAR* γ with PCOS susceptibility and its related traits (hyperinsulinemia, hyperandrogenemia and lipid parameters) in Indian women.

Capsule Pro12Ala but not His447His polymorphism of *PPAR* γ shows significant association with PCOS susceptibility. However both polymorphisms influence insulin related traits in Indian women with PCOS.

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Method Genotyping of *PPAR* γ polymorphisms in this case–control study was performed in PCOS ($n=450$) and age-matched controls ($n=300$) by direct sequencing. Clinical, anthropometric, hormonal and metabolic parameters were estimated in 275 women with PCOS and 169 controls. Chi-square test was used to compare the categorical data while regression analysis was used to evaluate association of genotypes with PCOS as well as its related phenotypes.

Results The frequencies of CC and CG+GG genotypes of Pro12Ala ($\chi^2=15.3$, $p<0.0001$) and CC and CT+TT genotypes of His447His ($\chi^2=12.7$, $p=0.0004$) polymorphisms were significantly different between PCOS and controls. Logistic regression analysis revealed a significant association of PCOS with Pro12Ala but not the His447His polymorphism. Carriers of variant genotypes at both *PPAR* γ loci showed significantly reduced 2 h glucose levels while carriers of variant His447His genotype showed lower fasting insulin and HOMA-IR levels in PCOS women.

Conclusions Pro12Ala polymorphism of *PPAR* γ showed significant association with decreased PCOS susceptibility. Both polymorphisms influenced insulin related traits (2 h glucose, fasting insulin and HOMA-IR) and improved glucose metabolism in these women. This is the first report to establish that variations in *PPAR* γ gene influence the insulin resistance pathophysiology in Indian women with PCOS.

Keywords Peroxisome proliferator activated receptor gamma (*PPAR* γ) · Polycystic ovary syndrome (PCOS) · Polymorphism · Insulin resistance · Genetic

Introduction

Polycystic ovary syndrome (PCOS) is a complex heterogeneous disorder in women of child bearing age characterized

by irregular menses, altered gonadotropin secretions, hyperandrogenemia, polycystic ovarian morphology and metabolic abnormalities such as hyperinsulinemia and obesity [7]. Genetic predisposition to PCOS has been established but the relative contribution of genetic and environmental entities underlying this multifactorial disorder remains elusive [8]. Many women with PCOS have insulin resistance (IR), which is now considered a major contributor to the pathogenesis of this disorder [29]. Hyperinsulinemia induces excess ovarian androgen production that affects follicular development leading to chronic anovulation and compromised fertility [2]. Visceral obesity, further adds to the prevailing intrinsic IR, placing them at a higher risk of developing impaired glucose tolerance and type 2 diabetes mellitus (T2DM) at an early age. Insulin sensitizers such as thiazolidinediones (TZDs), synthetic agonists of peroxisome proliferator activated receptor gamma ($PPAR\gamma$) ameliorate glucose intolerance, decrease insulin levels and consequently improve ovulation in women with PCOS [31].

$PPAR\gamma$, a nuclear transcription factor plays an important role in influencing insulin sensitivity through regulation of genes related to carbohydrate and lipid metabolism. Moreover, it is involved in adipocyte differentiation, fat deposition in adipose tissue, improving insulin sensitivity [24] and regulating ovarian steroidogenesis [25]. Polymorphisms of $PPAR\gamma$ gene have been strongly implicated in the etiology of IR, T2DM [11] and obesity [18]. Considering these crucial roles played by $PPAR\gamma$, it stands out as a logical candidate gene to be investigated for its genetic contribution towards many features of PCOS like altered insulin sensitivity, obesity, dyslipidemia and hyperandrogenism.

Studies exploring association of Pro12Ala C/G (*rs1801282*; exon-B) and/or His447His C/T (*rs3856806*; exon-6) SNPs of $PPAR\gamma$ with PCOS susceptibility and its related traits in different populations have yielded contradictory results [1, 4–6, 12–14, 16, 17, 20, 21, 23, 30, 32, 33]. Recent meta-analyses on Pro12Ala polymorphism of $PPAR\gamma$ have indicated that there are ethnic differences in association of this polymorphism with PCOS. The protective role of Pro12Ala against development of PCOS has been confirmed in European but not the Asian population [15, 35] which include studies from China [32] and Korea [4]. A recent study in South Indian women showed probable protective effects of Pro12Ala on PCOS, emphasizing the investigative potential of this polymorphism in Indian women [6]. No other report exploring association of $PPAR\gamma$ polymorphisms in Indian women with PCOS and its related traits is available. Furthermore the associations of both polymorphisms together with PCOS related traits have not yet been explored extensively in any population.

Therefore, the current investigation is a case–control study in Indian women with PCOS to evaluate the genetic influence of both Pro12Ala and His447His polymorphisms

of $PPAR\gamma$ individually and in combination with the risk of PCOS and its related traits of IR, dyslipidemia and hyperandrogenemia and to determine whether these polymorphisms can be a predictor of these phenotypes.

Methods

Study subjects

The study population consisted of 450 Indian women with PCOS (aged 18–38 years) recruited from the Infertility Clinic of National Institute for Research in Reproductive Health and Endocrinology Clinic of Seth GS Medical College and KEM Hospital, Mumbai (India). The women with PCOS were selected on the basis of European Society of Human Reproduction and Embryology (ESHRE)/American Society for Reproductive Medicine (ASRM) Rotterdam consensus diagnosis criteria [22]. The control subjects included 300 healthy, age-matched, regularly menstruating women with normal ovaries on ultrasound examination and no clinical or biochemical signs of hyperandrogenism. None of the participants had received medications which could affect their carbohydrate and lipid metabolism or alter endocrine parameters for the previous 4 months or had any history of diabetes. Ethical approval was obtained from both the above-mentioned Institutes and informed consent was obtained from all participants. Biological materials were collected according to the guidelines of the Human Ethics Committee. Complete phenotyping was performed in 275 women with PCOS and 169 controls whose fasting serum samples were available whereas genotyping was conducted in all participants. Anthropometric data including height, weight, waist and hip circumferences, waist/hip ratio (WHR) and body mass index (BMI) were obtained from all the subjects. Except for amenorrheic women with PCOS the remaining subjects were studied in the early follicular phase (days 3–7) of their menstrual cycle. Fasting blood samples from amenorrheic PCOS women were collected on any day as and when available. After an overnight fast, each subject underwent an oral glucose tolerance test (OGTT). The obtained fasting serums were stored at -80°C until assayed and were used for measuring hormones and other biochemical parameters.

Biochemical estimations

Glucose levels at fasting and 2 h after 75 g of glucose load were measured enzymatically by glucose oxidase method. Follicle stimulating hormone (FSH), luteinizing hormone (LH), thyroid stimulating hormone (TSH) and total testosterone were measured by chemiluminescence immunoassay while serum insulin and sex hormone binding globulin (SHBG) levels were estimated by radioimmunoassay method

as stated earlier [19]. Complete lipid profiling including triglycerides (TG), cholesterol, high-density lipoprotein-cholesterol (HDL-C), apolipoprotein A-1 (Apo A-1) and apolipoprotein B (Apo B) was performed by enzymatic and immunoturbidimetric methods on an automated biochemistry analyzer (Erba 200, Mannheim, Germany) using commercial kits (Randox laboratories Ltd., Llanberis, UK). Indices of IR including homeostatic model assessment of insulin resistance (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI); hyperandrogenic parameters such as free testosterone, bioavailable testosterone and free androgen index (FAI) were estimated as reported earlier [19]. Further, homeostatic model assessment of steady-state β -cell function (HOMA-B %) was calculated as $[20 \times \text{Fasting insulin } (\mu\text{IU/ml})]/[\text{fasting glucose (mmol/l)}-3.5]$ [27] while LDL values were determined using $\text{LDL (mg/dl)} = \text{Cholesterol (mg/dl)} - \text{HDL-C (mg/dl)} - [\text{TG (mg/dl)}/5]$ formula [10].

Genotyping

Genomic DNA was extracted from peripheral whole blood using QIAamp DNA Blood Mini Kit (QIAGEN GmbH, Hilden, Germany) for genotyping Pro12Ala and His447His polymorphisms of *PPAR* γ . Published primer sequences were used for amplification of Pro12Ala [17] whereas His447His polymorphism primers were designed using Primer-3 (v.3.0.0) as follows: i) Forward primer: 5'-GACTGAACCCCTG TTGTGT-3'; ii) Reverse primer: 5'-TGGAAGAAGGGA AATGTTGG-3'. Amplifications were performed using 0.5 μg of genomic DNA in a total reaction of 50 μl containing buffer, 1.5 mM MgCl_2 , 200 μM of dNTP mix, 10 pmols of each primer and 0.5 U of Taq Polymerase (MBI Fermentas, USA). PCR conditions for Pro12Ala amplification, which gave an amplicon of 181 bp consisted of initial denaturation of 5 min at 94 °C followed by 35 cycles each of denaturation at 94 °C for 30 s, annealing at 59.5 °C for 30 s, extension at 72 °C for 1 min 30 s and a final extension of 10 min at 72 °C. For the His447His amplification which gave an amplicon of 329 bp, the PCR conditions consisted of initial denaturation of 5 min at 94 °C followed by 35 cycles each of denaturation at 94 °C for 30 s, annealing at 55.2 °C for 30 s, extension at 72 °C for 45 s and a final extension of 10 min at 72 °C. Amplicons were purified by QIAquick Gel Extraction Kit, (QIAGEN GmbH, Hilden, Germany) and subjected to direct sequencing using 3100 Avant Genetic Analyzer (Applied Biosystems, Foster City, USA) with Big Dye Terminator Chemistry (v.3.1). Seqscape analysis software (Applied Biosystems) enabled screening of known and/or novel variants.

Statistical analysis

Univariate analysis for comparing means of all the variables between the PCOS and control group was performed by

Student's *t*-test. Chi square tests were used to assess the allelic and genotypic frequency distributions of polymorphisms. Logistic regression analysis was used to evaluate association of genotypes with PCOS risk after adjusting for different covariates in an additive model. Further genotype-phenotype relationship was evaluated by linear regression model to find whether the two *PPAR* γ polymorphisms have an effect on the PCOS related phenotypes. All tests were carried out at level of significance ($p < 0.05$) using R statistical software.

Results

Clinical and biochemical characteristics of study subjects

Clinical and biochemical estimations were carried out in 275 women with PCOS and 169 control subjects (Table 1) which showed significantly higher BMI, WHR, fasting insulin, fasting blood sugar, 2 h glucose, TG and LDL levels and lower HDL-C and Apo A-1 levels in PCOS women. The IR index, HOMA-IR, HOMA-B% were higher ($p < 0.0001$) while insulin sensitivity index QUICKI was lower ($p < 0.0001$) in PCOS relative to controls. Insulin resistant status as assessed by increased HOMA-IR (≥ 2.59 , 80th percentile of controls used as cutoff value) was present in 53.0 % of PCOS women in our study. Hyperandrogenemia was evident in PCOS group with significantly higher total, free and bioavailable testosterone, FAI and low SHBG ($p < 0.0001$) levels compared to controls.

*Allelic and genotypic frequencies of *PPAR* γ polymorphisms*

All three genotypes of the two polymorphic loci of *PPAR* γ , Pro12Ala (genotypes CC, CG and GG) and His447His (genotypes CC, CT and TT), were present among the study subjects. The genotype frequencies for Pro12Ala and His447His were in Hardy-Weinberg equilibrium among controls ($p = 0.622$, $p = 0.146$) as well as in the PCOS ($p = 0.511$, $p = 0.387$) groups respectively. Due to low frequencies of the minor allele homozygotes at these two loci, the heterozygotes and the minor allele homozygotes were combined together as "variant genotypes". The genotypic as well as allelic frequency distributions of both Pro12Ala and His447His polymorphism differed significantly between control and PCOS groups (Table 2).

*Association of *PPAR* γ polymorphisms with PCOS susceptibility*

PCOS is a complex disorder where multiple phenotypic features contribute to overall PCOS status. Therefore to investigate the influence of *PPAR* γ polymorphisms towards genetic susceptibility for PCOS, logistic regression analysis

Table 1 Characteristics of the study participants

Variable	Controls (<i>n</i> =169)	PCOS (<i>n</i> =275)	<i>p</i> ^a	<i>p</i> ^b
Age (years)	25.78±0.46	24.8±0.30	0.13	0.146
Weight (kg)	54.69±0.93	59.27±0.88	0.0006	0.001
BMI (kg/m ²)	22.79±0.38	25.32±0.35	1.61×10 ⁻⁶	3.47×10 ⁻⁶
Waist (cm)	76.29±0.83	80.14±0.74	0.0008	0.0012
Hip (cm)	96.86±0.89	98.92±0.73	0.074	0.086
WHR	0.79±0.004	0.81±0.003	0.0002	0.0003
FBS (mg/dl)	86.04±0.54	88.52±0.61	0.0049	0.0069
2 h glucose (mg/dl)	92.22±1.23	102.72±1.23	6.984×10 ⁻⁹	1.63×10 ⁻⁸
Fasting insulin (μIU/ml)	9.94±0.40	14.8±0.56	1.859×10 ⁻⁹	6.51×10 ⁻⁹
HOMA-IR	2.11±0.09	3.31±0.14	4.426×10 ⁻⁶	1.13×10 ⁻⁸
HOMA-B (%)	175.41±9.94	232.01±10.20	2.654×10 ⁻⁵	7.43×10 ⁻⁹
QUICKI	0.35±0.002	0.33±0.002	2.208×10 ⁻⁹	6.87×10 ⁻⁹
FSH (μU/ml)	7.22±0.18	6.46±0.12	0.0007	0.0011
LH (μU/ml)	4.88±0.16	10.64±0.34	<2.2×10 ⁻¹⁶	1.03×10 ⁻¹⁵
LH:FSH	0.72±0.03	1.76±0.06	<2.2×10 ⁻¹⁶	1.03×10 ⁻¹⁵
TSH (μU/ml)	2.34±0.14	2.37±0.09	0.419	0.434
TT (ng/dl)	43±1.50	68.41±2.27	<2.2×10 ⁻¹⁶	1.03×10 ⁻¹⁵
SHBG (nmol/l)	85.21±3.19	59.77±2.41	2.28×10 ⁻¹³	9.12×10 ⁻¹³
FAI	2.17±0.11	5.6±0.29	<2.2×10 ⁻¹⁶	1.03×10 ⁻¹⁵
FT (pmol/l)	15.42±0.62	33.78±1.38	<2.2×10 ⁻¹⁶	1.03×10 ⁻¹⁵
Bio-T (nmol/l)	0.36±0.02	0.8±0.03	<2.2×10 ⁻¹⁶	1.03×10 ⁻¹⁵
Cholesterol (mg/dl)	149.24±2.16	149.95±1.84	0.911	0.911
HDL-C (mg/dl)	50.68±1.30	43.93±1.04	6.431×10 ⁻⁵	0.0001
TG (mg/dl)	83.87±2.54	94.22±2.57	0.019	0.024
LDL (mg/dl)	81.39±1.83	86.92±1.51	0.039	0.0472
Apo A-1 (mg/dl)	109.39±3.10	97.42±2.31	0.0002	0.0004
Apo B (mg/dl)	62.31±1.40	62.57±1.52	0.308	0.332
Apo B:ApoA-1	0.63±0.02	0.73±0.02	0.015	0.021
Amenorrhea	0 (0)	152 (55)	–	–
Oligomenorrhea	0 (0)	92 (34)	–	–
Regular menstrual cycle	169 (100)	31 (11)	–	–
Hirsutism	0 (0)	159 (57.82)	–	–
Acne	2 (1)	169 (61.45)	–	–
Acanthosis Nigricans	0 (0)	112 (40.73)	–	–
Impaired fasting glucose (IFG)	0 (0)	29 (10.36)	–	–
Impaired glucose tolerance (IGT)	0 (0)	12 (4.29)	–	–

Data are given as mean ± S.E or n (%). *p*^a values obtained by comparison of variables between controls and PCOS by Student's *t* test. *p*^b values are obtained after adjusting for multiple testing. The statistical significance was defined as *p*<0.05 for both *p*^a and *p*^b

BMI body mass index, *WHR* waist to hip ratio, *FBS* fasting glucose, *HOMA-IR* homeostasis model assessment for insulin resistance, *HOMA-B* homeostasis model assessment for beta cell function, *QUICKI* quantitative insulin sensitivity check index, *TT* total testosterone, *FT* free testosterone, *Bio-T* bioavailable testosterone, *SHBG* sex hormone binding globulin, *FAI* free androgen index, *HDL-C* High density lipoprotein cholesterol, *TG* Triglycerides, *LDL* low density lipoprotein, *ApoA-1* Apolipoprotein A-1, *Apo B* Apolipoprotein B

was performed. The PCOS associated phenotypes had been estimated in 275 women with PCOS and 169 controls. A stepwise forward regression was conducted with all variables to determine the essential covariates which were identified as BMI, WHR, TG, QUICKI, fasting insulin and LDL, and included in the model for further analysis. Association between

PCOS-control status and *PPAR*γ genotypes were analysed by logistic regression with “logit” link function where wild type homozygotes (CC) were recoded as 0 and the minor allele homozygotes/heterozygotes as 1. Two separate logistic regressions were fitted for investigating association of the both polymorphisms individually, among PCOS and control groups. At

Table 2 Genotype and allele frequencies of *PPAR* γ polymorphisms in controls and women with PCOS

<i>PPAR</i> γ Genotype/Allele	Subjects		χ ² (<i>p</i>)	O.R (95 % C.I)
	Controls (<i>n</i> =300)	PCOS (<i>n</i> =450)		
Pro12Ala C/G (<i>rs1801282</i>)				
Genotype	Controls [n (%)]	PCOS [n (%)]	15.3 (<0.0001)	0.490 (0.34–0.70)
CC	219 (73.0)	381 (84.67)		
CG+CG	81 (27.0)	69 (15.33)		
Allele	Controls [n (%)]	PCOS [n (%)]	12.7 (0.0004)	0.55 (0.39–0.77)
C	517 (86.17)	827 (91.89)		
G	83 (13.83)	73 (8.11)		
His447His C/T (<i>rs3856806</i>)				
Genotype	Controls [n (%)]	PCOS [n (%)]	6.24 (0.0125)	0.66 (0.48–0.92)
CC	198 (66.0)	335 (74.44)		
CT+TT	102 (34.0)	115 (25.56)		
Allele	Controls [n (%)]	PCOS [n (%)]	5.78 (0.0163)	0.71(0.53–0.94)
C	492 (82.0)	779 (86.56)		
T	108 (18.0)	121 (13.44)		

χ^2 test, $p < 0.05$ is considered significant

the Pro12Ala locus, frequency of variant genotype (CG+GG) was significantly lower ($p=0.008$) among PCOS women than controls but not at the His447His locus (Table 3). Log odds of being affected with PCOS decreased by 0.667 among women with CG/GG genotypes compared to those with CC genotype of Pro12Ala SNP.

Further, the combined influence of both polymorphisms on PCOS susceptibility was investigated by logistic regression model. Categorical variable (combination) with three groups were defined according to the number of CC genotypes present in the combination genotype of the two SNPs: 0 if both the SNPs have CC genotype; 1 if one of the two SNPs has CC genotype; 2 if none of the SNPs has CC genotype. Combinations of wild CC genotypes of both polymorphisms showed significant association with the risk of developing PCOS among the study subjects ($p=0.023$) (Table 3). As the number of CC genotype decreases in the combination, the log odds of being affected decreases by 0.312. Combination of both *PPAR* γ variant genotypes was protective for PCOS susceptibility but the protection was remarkably less as compared to that of Pro12Ala alone (Table 3).

Table 3 Logistic regression with “logit” link function results for association for the two *PPAR* γ polymorphisms independently as well as in combination among the control and PCOS groups

Polymorphism	Estimate	Standard error	<i>p</i>
Pro12Ala	−0.667	0.252	0.008
His447His	−0.313	0.228	0.169
Combination	−0.312	0.137	0.023

The final logit model is $\log(\pi/(1-\pi)) = \beta_0 + \beta_1 \text{ BMI} + \beta_2 \text{ WHR} + \beta_3 \text{ TG} + \beta_4 \text{ QUICKI} + \beta_5 \text{ Fasting Insulin} + \beta_6 \text{ LDL}$; where π is the probability of being affected. $p < 0.05$ is considered significant

Genetic influence of *PPAR* γ polymorphisms on PCOS associated traits

To further explore the potential influence of these polymorphisms independently and in combination on PCOS related phenotypes, linear regression analysis was carried out separately in the control and PCOS groups (Tables 4 and 5). Both polymorphisms individually and in combination were significantly associated with insulin related traits among women with PCOS but not in controls. Carriers of variant (CG+GG) genotypes of Pro12Ala and variant (CT+TT) genotypes of His447His loci independently; and in combination showed significant association with 2 h glucose levels. The direction of the association was negative i.e. individuals with variant genotypes have a lower level of 2 h glucose relative to carriers of wild CC homozygotes. Presence of both variant genotypes together showed significantly lowered 2 h glucose levels relative to their independent occurrence in PCOS women. Interestingly, His447His polymorphism showed significant association with reduced fasting insulin and HOMA-IR levels in these women. Neither of the polymorphisms individually or in combination showed any association with hyperandrogenemic or lipid parameters in PCOS group nor with any phenotypes in the control subjects.

Discussion

Evidence suggests that the association of *PPAR* γ polymorphisms with PCOS and its related traits in different populations are controversial due to differences in lifestyle, environmental factors as well as the sample size studied [26]. Despite these, importance still remains to investigate in a given population, the role of a candidate gene entailed in

Table 4 Linear regression analysis to find the association of the two *PPAR* γ polymorphisms and their combination with different quantitative phenotypes among controls

Variable	Pro12Ala <i>p</i> (direction) [95%CI]	His447His <i>p</i> (direction) [95%CI]	Combination <i>p</i> (direction) [95%CI]
BMI (kg/m ²)	0.712 (–) [–0.082, 0.056]	0.308 (+) [–0.032, 0.01]	0.702 (+) [–0.031, 0.046]
HOMA-IR	0.536 (–) [–0.210, 0.109]	0.52 (+) [–0.103, 0.203]	0.978 (+) [–0.087, 0.090]
LH:FSH	0.837 (+) [–0.139, 0.171]	0.34 (–) [–0.22, 0.076]	0.663 (–) [–0.104, 0.07]
TT (ng/dl)	0.893 (–) [–0.165, 0.144]	0.68 (–) [–0.18, 0.117]	0.755 (–) [–0.099, 0.072]
LDL (mg/dl)	0.185 (–) [–0.190, 0.037]	0.606 (–) [–0.138, 0.081]	0.302 (–) [–0.096, 0.03]
HDL-C (mg/dl)	0.169 (+) [–0.04, 0.224]	0.312 (–) [–0.192, 0.062]	0.86 (+) [–0.067, 0.08]
Fasting Insulin (μ IU/ml)	0.402 (–) [–0.224, 0.09]	0.601 (+) [–0.111, 0.191]	0.871 (–) [–0.095, 0.080]
QUICKI	0.541 (+) [–0.017, 0.032]	0.492 (–) [–0.031, 0.015]	0.954 (–) [–0.014, 0.013]
2 h glucose (mg/dl)	0.545 (–) [–0.075, 0.034]	0.539 (+) [–0.038, 0.072]	0.985 (+) [–0.032, 0.032]

The *p*-value for testing the significance of the beta coefficients along with their directions are given in the table. The directions of the beta coefficients as well as the 95 % C.I are given in parenthesis. *t*-test was used to test the significance of the beta coefficient. *p*<0.05 is considered significant

contributing towards pathogenesis of a multigenic complex disorder like PCOS. Here, we have not only explored the association of Pro12Ala and His447His polymorphisms of *PPAR* γ individually and in combination with PCOS susceptibility, but also with its related traits of hyperinsulinemia, hyperandrogenemia, dyslipidemia and hormones in a cohort of Indian women who were phenotypically well characterized for all the above mentioned PCOS related traits.

Association of *PPAR* γ Pro12Ala C/G polymorphism with PCOS and its related traits

A significantly lower occurrence of variant genotypes and lower Ala allele frequency of Pro12Ala polymorphism among PCOS women in our study cohort and its significant association with reduced risk of PCOS is in agreement with previous findings in Finnish [17], Turkish [34] and Korean [12] women. Recently, a study from South India showed marginally significant difference in allelic (*p*=0.05) but not

in the genotypic frequency (*p*=0.23) of this polymorphism in PCOS women (*n*=243) as compared to controls (*n*=281) [6]. However, in our study with increased sample size of PCOS women (*n*=450) and controls (*n*=300), a significant difference in both allelic and genotypic frequency were found; and further logistic regression analysis also revealed a stronger association of Pro12Ala polymorphism with reduced susceptibility to PCOS. Other studies with Italian [20, 21], German [13], Chinese [32], Caucasian [1], Korean [4], Greek [5, 33], Spanish [23] and Polish [3] women with PCOS failed to find any such association. It is well established that Ala allele, enhances insulin sensitivity and protects against development of T2DM [28]. Increased insulin sensitivity, decreased fasting insulin levels, HOMA-IR, [13, 14, 17, 30, 34] basal metabolic rate [16] and increased HDL-C levels [4] were observed in women with PCOS who were carriers of Ala alleles. Consistently we too observed that the variant genotype carriers had significantly lower 2 h glucose levels than wild genotype carriers in PCOS group but not in controls.

Table 5 Linear regression analysis to find the association of the two *PPAR* γ polymorphisms and their combination with different quantitative phenotypes among women with PCOS

Variable	Pro12Ala <i>p</i> (direction) [95%CI]	His447His <i>p</i> (direction) [95%CI]	Combination <i>p</i> (direction) [95%CI]
BMI (kg/m ²)	0.417 (+) [–0.042, 0.100]	0.692 (–) [–0.074, 0.049]	0.848 (+) [–0.035, 0.042]
HOMA-IR	0.857 (+) [–0.194, 0.233]	0.047 (–) [–0.37, –0.002]	0.255 (–) [–0.182, 0.049]
LH:FSH	0.292 (–) [–0.296, 0.090]	0.177 (–) [–0.281, 0.052]	0.158 (–) [–0.179, 0.029]
TT (ng/dl)	0.801 (+) [–0.151, 0.195]	0.778 (+) [–0.128, 0.171]	0.755 (+) [–0.079, 0.108]
LDL (mg/dl)	0.253 (–) [–0.161, 0.043]	0.647 (+) [–0.068, 0.109]	0.74 (–) [–0.065, 0.046]
HDL-C (mg/dl)	0.961 (–) [–0.145, 0.138]	0.847 (–) [–0.135, 0.111]	0.884 (–) [–0.082, 0.071]
Fasting Insulin (μ IU/ml)	0.796 (+) [–0.174, 0.226]	0.044 (–) [–0.348, –0.004]	0.267 (–) [–0.169, 0.047]
QUICKI	0.951 (+) [–0.031, 0.033]	0.1 (+) [–0.005, 0.051]	0.29 (+) [–0.008, 0.027]
2 h glucose (mg/dl)	0.016 (–) [–0.135, –0.014]	0.033 (–) [–0.11, –0.005]	0.008 (–) [–0.077, –0.011]

The *p*-value for testing the significance of the beta coefficients along with their directions are given in the table. The directions of the beta coefficients as well as the 95 % C.I are given in parenthesis. *t*-test was used to test the significance of the beta coefficient. *p*<0.05 is considered significant

Recent meta-analyses reported the association of Pro12Ala with PCOS only in European but not in Asian populations [15, 35] and further no association with HOMA-IR in PCOS women [35]. However, our findings highlight a significant association of Pro12Ala polymorphism with reduced susceptibility to PCOS as well as its association with reduced 2 h glucose levels in these Indian women. Our results corroborate the role of Ala allele in enhancing insulin sensitivity and facilitating increased glucose uptake. The Ala variant, which has been reported to be associated with reduced transcriptional activity of *PPAR* γ , may play a role in modifying the prevailing IR. It enhances insulin action towards suppression of lipolysis thereby reducing the production of free fatty acids (FFAs) and facilitates their storage in adipocytes. Reduced activity of *PPAR* γ may alter expression of genes, such as *GLUT-4* and adiponectin (having putative *PPAR* response elements), which results in improved utilization of glucose by skeletal muscles, inhibition of hepatic glucose production and storage of FFAs in adipose tissues [28]. Overall, these metabolic activities are likely to decrease IR, which in turn may confer better glucose tolerance observed among women with variant Pro12Ala genotypes.

Relationship of *PPAR* γ His447HisC/T polymorphism with PCOS and its associated traits

The second polymorphism, His447His of *PPAR* γ is a synonymous SNP, reported to be associated with PCOS risk in Italian [21], Korean [12] and South Indian women [6] but not in Caucasian [1] and Greek [5] women. The association of this polymorphism with PCOS phenotypes too has been ambiguous. His447His has been shown to be related to obesity, higher leptin levels [20] and decreased testosterone levels [5] in PCOS women. Conversely, decreased levels of testosterone, insulin and IR were associated with this polymorphism in healthy controls but not women with PCOS in Caucasian population [1]. Allelic ($p=0.03$) but not genotypic ($p=0.18$) association of His447His polymorphism was reported with PCOS risk in South Indian women [6] however, we observed significant difference in both allelic and genotypic frequency between PCOS and controls, though not corroborated by logistic regression analysis. Genotype-phenotype association showed that variant genotype carriers of this polymorphism had better insulin related traits such as lower fasting insulin, HOMA-IR and 2 h glucose levels relative to wild genotype carriers only among PCOS subjects but not in controls. Although His447His polymorphism has no influence on PCOS status in Indian women, those with variant genotype carriers of this polymorphism had better insulin sensitivity than with wild genotypes in the PCOS group which has not yet been reported in any population. As this is a synonymous SNP it could be in linkage disequilibrium with some other functional *PPAR* γ SNP to execute this effect.

Combined influence of both Pro12Ala and His447His polymorphisms of *PPAR* γ on PCOS and its related traits

To understand the effect of both *PPAR* γ polymorphisms together on PCOS and its related traits, a combination analysis was carried out instead of haplotype analysis as these two SNPs are located 82.4 kb apart. When present together, these polymorphisms showed significant association with reduced PCOS susceptibility but analysis revealed that this protective effect was mainly exerted by Pro12Ala. Interestingly among PCOS women, a cumulative effect of both polymorphisms was observed towards reducing 2 h glucose levels that is likely to contribute to better glucose tolerance in these women. Better glucose metabolism could contribute towards lowering IR of PCOS, which in turn reduces other pathologic components like hyperandrogenemia, dyslipidemia and ameliorate abnormal hormonal levels in these women. Our study establishes that Pro12Ala polymorphism of *PPAR* γ gene exerts a strong protection against PCOS risk while His447His has no such contribution. Both SNPs influenced insulin related traits and improved glucose metabolism in Indian women with PCOS.

PCOS women are often obese with higher BMI and elevated lipid levels [9]. As *PPAR* γ is involved in energy regulation and fat deposition, it has been recognized as an important gene contributing towards obesity, obesity induced IR and dyslipidemia. Studies in Italian [21] as well as in German [13] women with PCOS found no significant difference in adiponectin, HDL-C, LDL and TG levels between carriers of wild and variant genotypes of Pro12Ala polymorphism. On the contrary, significantly increased HDL-C levels were observed in variant genotype carriers of Pro12Ala in PCOS women from Korea [4]. In our study, we failed to observe any effect of both these polymorphisms on lipid or lipoprotein levels as well as on BMI among the study subjects. Further, neither of these polymorphisms individually or in combination showed any association with the hyperandrogenemic and hormonal parameters; however PCOS women with variant genotypes had better lipid and hormonal profiles than wild CC carriers.

Summary

This study in Indian women showed significant association of Pro12Ala polymorphism with reduced risk of occurrence of PCOS, while His447His showed no such association. In combination analysis, carriers of both variant genotypes showed protection against development of PCOS which is mainly determined by Pro12Ala. Further, these polymorphisms showed association with insulin related traits preserving insulin sensitivity and better glucose metabolism in PCOS women while no association was observed with any of the hyperandrogenic indices, hormones or lipid and

lipoprotein parameters. The findings of our study strongly suggest *PPAR* γ to be one of the genetic predisposing factors influencing PCOS susceptibility as well as insulin sensitivity in Asian Indian women. Additional studies are required from both Indian as well as Asian populations to confirm such associations and clarify the role of *PPAR* γ in IR pathophysiology of PCOS.

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