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Association of the Pro12Ala Polymorphism with the Metabolic Parameters in Women with Polycystic Ovary Syndrome

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Abstract

AIM: To investigate the association of peroxisome proliferator-activated receptor gamma (*PPARG*) Pro12Ala polymorphism with polycystic ovary syndrome (PCOS) and its effect on the metabolic parameters in PCOS women.

METHODS: The study used PCR to identify the presence of the *PPARG* Pro12Ala polymorphism in 100 PCOS women and 120 age-matched healthy women. All participants were subjected to anthropometry, biochemical and metabolic evaluation.

RESULTS: Significant difference in the genotypes distributions of *PPARG* Pro12Ala polymorphism was observed among PCOS women and controls ($p = 0.03$). The frequency of the polymorphic allele Ala was significantly higher in PCOS cases than that in the controls (OR = 2.01, $p = 0.01$). The carries of the variant allele Ala in PCOS women showed significant higher values in body mass index (BMI), systolic and diastolic blood pressure, waist circumference, waist to hip ratio, sum of skin folds, fasting blood glucose, fasting blood insulin, HOMA-IR, fasting triglycerides, total cholesterol and low-density lipoprotein than non-carriers.

CONCLUSION: The *PPARG* Pro12Ala polymorphism might contribute to the risk of PCOS and abnormal metabolic parameters and could be considered as a biomarker for early diagnosis and clinic prediction of metabolic complications.

Introduction

Insulin sensitivity might be regulated by the peroxisome proliferator-activated receptor gamma (PPARG). The *PPARG* gene product regulates glucose and lipid metabolism as it plays a substantial role in the energy storage management and insulin sensitivity [1]. It is expressed in adipose tissue, macrophages, intestines and ovaries and supposed to be involved in polycystic ovary syndrome (PCOS) risk [2].

It is known that the prevalence of PCOS is about 5–10 % among women in the reproductive age and cases are frequently diagnosed during adolescence [3]. PCOS is associated with different degrees of hormonal and metabolic disturbances [4]. In addition, high prevalence of women with PCOS are susceptible to gaining weight and showing phenotype with metabolic abnormalities such as insulin resistance, compensatory hyperinsulinemia [5], risk for diabetes mellitus type 2 (T2DM) [6] and cardiovascular diseases (CVD) [7] (Hosoya et al., 2000). The development of PCOS is due to interactions of multiple genetic and environmental factors that are involved in its occurrence and complications [5].

However, the exact pattern of inheritance still needs to be more clarified. The *PPARG* gene plays a role in the regulation of insulin sensitivity and lipid metabolism [8]. It might be involved in insulin action and secretion, energy metabolism, adiposity [1] and also implicated in the pathogenesis of PCOS.

Our goal in this study was to explore the possible association that might present between the *PPARG* Pro12Ala polymorphism and PCOS risk and abnormal metabolic parameters.

Patients and Methods

This study was designed as a case-control study including 100 women with PCOS between 20-30 years of age and 120 age-matched healthy female controls. All the procedures used in this study were by the guidelines of the Helsinki Declaration on Human Experimentations. The study was approved by local ethics committee of the National Research Centre (No: 13176); the purpose of the protocol was explained to the women, and written informed consent was obtained from them before beginning the study. They were referred from different centres to the National Research Centre obesity clinic between 2013 and 2014. Polycystic ovaries were diagnosed by pelvic or transvaginal sonography according to the Rotterdam Conference criteria [9].

DNA extraction and genotyping

From each participant, 5 mL of venous blood sample was taken. To examine the Pro12Ala polymorphism in exon 2 of PPAR-c, genomic DNA was extracted from peripheral whole-blood samples (QIAamp DNA Blood Mini Kit; (QIAGen GMbH Germany, Dusseldorf, Germany).

Each reaction mixture was 25 µL in volume, comprising (100 ng/µL) DNA, 2 U Taq polymerase (Thermo Scientific Fermetas, USA), 0.2 mmol/L dNTPs, 0.5 mmol/L of each primer, PCR buffer (containing 1 mmol/L Tris HCL and 50 mmol/LKCL) and 1.5 mmol/L $MgCl_2$. The primers sequence were 5'-GCCAATTCAAGCCCAGTC-3' for the forward and 5'-GATATGTTTGCAGACAGTGTATCAGTGAAGGAA TCGCTTTCCG-3' for the mutagenic reverse primer as described by Yilmaz et al. 2006 [10].

PCR was performed with an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s annealing at 65°C for 45 s and extension 72°C for 10 min. The amplified fragment of 270 bp was treated with the restriction enzyme BstUI, which resulted in digestion of the PCR product into two fragments of 227 and 43 bp in length if the polymorphic site had the G allele (Ala), while the PCR amplicon remained uncut in the presence of the C allele (Pro). The digested product was separated on a 3% agarose gel stained with ethidium bromide. The wild-type allele resulted in a fragment of 270 bp while the mutant allele resulted in two fragments of 227 bp and 43 bp.

Anthropometry and blood pressure

Body weight, height, mid-upper arm circumference, waist and hip circumferences and skin fold thickness biceps, triceps and subscapular, supra iliac and abdominal skin fold thickness were measured. All measurements were taken 3 times on the left side of the body, and the mean of the 3 values was used. Body weight was measured to the nearest 0.1 kg and height was measured to the nearest 0.1 cm. Height was measured with the patients standing with their backs leaning against the stadiometer of the same scale. Body mass index (BMI) was calculated as weight in kilogrammes divided by height in meters square (kg/m^2). Mid-upper arm circumference (MUAC) was measured using a flexible tape at the midway between the olecranon and acromial process on the upper right arm with the elbow flexed 90 degrees. Waist circumference (WC) and hip circumference (HC) were measured in cm using a plastic, non-stretchable tape. WC was measured with light clothing at a level midway between the lower rib margin and the iliac crest standing and breathing normally. HC was measured at the level at the widest circumference over the buttocks (at the greater trochanter). Waist to hip ratio (WHR) was calculated. Skin-fold thickness was measured to the nearest mm, except for low values (usually 5 mm or less) when it was taken to the nearest 0.5 mm. These readings were made at six sites on all subjects, at the biceps, triceps, subscapular and supra-iliac areas, using Holtain calliper (Ltd, Bryberian, Crymmych, Pembrokeshire). The subscapular skinfold was measured below the lower angle of the left scapula at a diagonal in the natural cleavage of the skin. Biceps was measured at the level of the midpoint between the acromion (lateral edge of the acromion process) and the radius (proximal and lateral border of the radius bone) on the mid-line of the anterior surface of the arm, triceps (vertical fold, midway between acromion and olecranon processes on the posterior surface of the arm), and the position of the supra iliac skinfold was the diagonal fold just above the iliac crest even with the anterior axillary line, abdominal skin fold was at 5 cm adjacent to the umbilicus to the right side. Subsequently, sum of skin folds was calculated. Anthropometric measurements were obtained according to standardized equipment and following the recommendations of the International Biological Program [11]. Systolic and diastolic blood pressures (SBP and DBP) were measured twice in the right arm in a sitting position after a 10 min rest period; using a mercury sphygmomanometer the average of the two measurements was used for analysis. Blood pressure was measured according to a standardized operating procedure using a calibrated sphygmomanometer and brachial inflation cuff (HEM-7200 M3, Omron Healthcare, Kyoto, Japan).

Laboratory measurements

Venous blood samples were collected by direct venipuncture after an overnight fast (minimum 12 h). Fasting plasma glucose and serum lipids (total cholesterol, high-density lipoprotein cholesterol (HDL-C) triglycerides (TG) were measured by enzymatic

colorimetric methods using a Hitachi autoanalyzer 704 (Roche Diagnostics. Switzerland) [12].

Low-density lipoprotein cholesterol (LDL-C) was calculated according to certain equation (LDL-C= Total cholesterol –Triglycerides/5+ HDL-C) [13]. Serum insulin concentration was analysed by chemiluminescent immunoassay (Immulite2000, Siemens, Germany [14]. Insulin resistance was determined by the Homeostasis Model Insulin Resistance (HOMA-IR) is calculated as the product of the fasting plasma insulin level (IU/mL) and the fasting plasma glucose level (mmol/L), divided by 22.5 [15].

Statistical analysis

Statistical presentation and analysis of the results were carried out using SPSS software version 17, SPSS Inc., Chicago, IL, USA. Statistical tests included chi-square test, and Student's t-tests were used. P<0.05 was considered to indicate a statistically significant difference.

The Hardy-Weinberg equilibrium of polymorphism was obtained by Fisher's exact test, and the distribution of the genotypes among participant groups were tested by the Chi-square test or Fisher's exact test. All continuous variables, including age anthropometric and metabolic indices, were expressed as means (standard deviation).

Due to the low frequency of the homozygous Ala/Ala genotype, we divided genotypes into two categories: Pro/Pro and combined genotypes group (Pro/Ala+Ala/Ala). For calculation of risk, Odd ratio (OR) and 95% confidence interval (CI) was calculated.

Results

Table 1 shows the clinical characteristics and biochemical variables for the women with PCOS and controls. There were significant increase in BMI, WHR, systolic blood pressure (SBP), diastolic blood pressure (DBP), LH/FSH, fasting insulin, HOMA-IR, TG, TC and LDL-C in PCOS group as compared with control group (P<.05 for all)

The allele and genotype distributions of the *PPARG* Pro12Ala polymorphism for the PCOS and the control groups are presented in Table 2. They were consistent with the Hardy-Weinberg equilibrium. The distribution of the *PPARG* Pro12Ala alleles and genotypes showed statistically significant difference between PCOS and controls (p = 0.03). The frequency of polymorphic allele Ala was significantly higher in PCOS than controls (OR = 2.01, p = 0.01).

Table 1: Clinical and biochemical characteristics in PCOS patients and controls

Variable	PCOS	Controls	*P*
Age (years)	24 ± 2.3	23 ± 2.5	0.78
BMI (kg/m^2)	30.12 ± 4.8	23.2 ± 5.9	0.02
WHR	0.99 ± 0.07	0.76 ± 0.09	0.03
SBP (mmHg)	138.5 ± 7.3	110.2 ± 6.2	0.05
DBP(mmHg)	89.6 ± 9.2	74 ± 11.3	0.04
LH/FSH	1.61 ± 0.83	0.71 ± 0.96	0.03
FBI (µU/ml)	11.20 ± 0.45	8.20 ± 0.80	0.04
HOMA-IR	3.9 ± 0.99	2.81 ± 1.01	0.05
Triglycerides (mg/dl)	120.61 ± 8.29	113.65 ± 9.69	0.03
Total cholesterol (mg/ dl)	134.71 ± 7.81	90.71 ± 6.45	0.04
LDL-C (mg/dl)	130.61 ± 11.32	100.71 ± 15.81	.002

LH, luteinizing hormone/ FSH, stimulating follicle hormone, Values are mean (standard deviation).

Table 3 shows clinical, biochemical characteristics and anthropometric parameters in controls according to *PPARG* Pro12Ala polymorphism. No significant differences were found in all biochemical and anthropometric parameters between carriers and non-carriers of Ala polymorphic allele.

Table 2: Distribution of *PPARG* Pro12Ala polymorphism in PCOS patients and controls

Groups	Allele (%)		P	OR	Genotype (%)			P
	Pro N (%)	Ala N (%)		95% CI	Pro/Pro N (%)	Pro/Ala N (%)	Ala/Ala N (%)	
PCOS (n=100)	162 (81)	38(19)	0. 01	2.01	68 (70)	26 (26)	6 (6)	0.03
Controls (n=120)	215 (89.58)	25 (10.14)		(1.17-3.47)	96 (80)	23 (19.66)	1 (0.8)	

[a]χ^2 test, *PPARG*, peroxisome proliferator-activated receptor gamma gene; PCOS, polycystic ovary syndrome.

In PCOS group the carriers of variant allele (Pro/Ala + Ala/Ala) showed significantly higher values of systolic and diastolic blood pressure, waist circumference, waist to hip ratio, sum of skin folds, fasting blood glucose, fasting blood insulin, HOMA-IR, triglycerides, total cholesterol, low-density lipoprotein compared to the carriers of the wild type Pro alleles (Table 4).

Table 3: Clinical, biochemical characteristics and anthropometric parameters in control group according to *PPARG* Pro12Ala polymorphism

	Pro/Ala + Ala/Ala Mean (± SD)	Pro/Pro Mean (± SD)
Age (years)	23 ± 2.3	22 ± 2.5
BMI (kg/m^2)	23.12 ± 7.8	24.2 ± 8.9
Systolic BP (mmHg)	125.3 ± 10.3	116.2 ± 9.2
Diastolic BP(mmHg)	76.6 ± 9.2	74 ± 11.3
MUAC (cm)	26.9 ± 5.3	26.5 ± 2.4
WC (cm)	90.1 ± 9.5	85.39 ± 7.7
Hip circumference (cm)	121.59 ± 9.8	120.33 ± 8.5
WHR	0.81 ± 0.07	0.86 ± 0.09
Sum SF (mm)	98.6 ± 11.9	99.19 ± 12.4
FBG (mg/dl)	105.52 ± 5.22	96.52 ± 6. 22
FBI (µU/ml)	10.20 ± 0.75	8.20 ± 0.80
HOMA-IR	2.3 ± .99	2.88 ± 1.01
Triglycerides (mg/dl)	114.41 ± 10.29	115.65 ± 11.61
Total cholesterol (mg/ dl)	114.71 ± 11.81	119.71 ± 10.45
LDL-C (mg/dl)	112.61 ± 21.38	113.71 ± 25.89

BMI: Body mass index; WHR: Waist to hip ratio; (HOMA-IR): Homeostasis Model Insulin Resistance; Sum SF: the sum of skin folds, FBG: fasting blood glucose; FBI: fasting blood insulin, Values are mean (standard deviation).

Discussion

The discrepancy has been found between numerous studies that have investigated the

association between *PPARG* Pro12Ala polymorphism and PCOS [2, 16, 17]. In our study, a significant association was found between PCOS and *PPARG* Pro12Ala polymorphism which is in agreement with the previous studies supposed its predisposing role in PCOS etiopathogenesis [10, 17-19]. Moreover, ethnicity differences in the involvement of this polymorphism have also been suggested by He et al., 2012 [2].

Table 4: Clinical, biochemical and anthropometric characteristics in women with PCOS patients according to *PPARG* Pro12Ala polymorphism

	Pro/Ala + Ala/Ala Mean (± SD)	Pro/Pro Mean (± SD)
Age (years)	23 ± 2.8	22 ± 2.6
BMI (kg/m^2)	29.12 ± 2.8	30.1 ± 2.9
Systolic BP (mmHg)	150.3 ± 10.3**	116.2 ± 9.2
Diastolic BP (mmHg)	96.6 ± 9.2*	74 ± 8.3
MUAC (cm)	31.9 ± 5.3	30.5 ± 2.4
WC (cm)	113.1 ± 15.5**	74.39 ± 7.7
Hip circumference (cm)	123.59 ± 9.8	120.33 ± 8.5
WHR	0.91 ± 0.07 **	0.76 ± 0.09
Sum SF (mm)	156.6 ± 11.9**	89.19 ± 14.4
FBG (mg/dl)	145.52 ± 5.22 **	96.52 ± 8. 22
FBI (µU/ml)	18.20 ± 0.65 ***	8.20 ± 0.85
LH/FSH	1.61 ± 0.53	1.51 ± 0.46
HOMA-IR	6.3 ± 0.99 ***	2.88 ± 1.29
Triglycerides (mg/dl)	144.41 ± 13.29 ***	115.65 ± 20.61
Total cholesterol (mg/ dl)	184.71 ± 13.81 **	124.71 ± 10.81
LDL-C (mg/dl)	162.61 ± 21.381 **	113.71 ± 25.89

BMI: Body mass index; WHR: Waist to hip ratio; Sum SF: the sum of skin folds; HOMA-IR: Model Insulin Resistance. *P<0.05, ** P<0.01***, P <.001 (P value for t-test).

Obesity and insulin resistance are very common features in PCOS [20]. The *PPARG* Pro12Ala polymorphism was examined for its potential effect on obesity in PCOS cases as it is substantially expressed in adipose tissue. The data of the present study showed that carriers of Ala allele had statistically significant higher values of BMI waist circumference, waist to hip ratio and sum of skin folds than on- carriers in PCOS group.

The *PPARG* plays an important role in adipose tissue metabolism, and Pro12Ala polymorphism has been reported to be associated with abdominal obesity in patients with metabolic dysfunction [21]. A positive correlation between polymorphism and BMI has been found in European and Asian populations [22]. In meta-analysis study [23], the correlation between this polymorphism and BMI has been found only in the subgroup of BMI ≥ 27 kg/m^2 where Ala carriers had higher BMI than non-carriers. Previous studies reported no apparent effect of the *PPARG* Pro12Ala genotype on BMI in PCOS patients [18, 24-27].

Furthermore, in the present study, we investigated the possible association of the PPARG Pro12Ala polymorphism with different metabolic parameters in PCOS women. We observed significantly higher values of blood pressure, fasting blood glucose, fasting blood insulin, and HOMA-IR and lipid parameters in the carriers of variant allele than cases with the wild type.

PPARG is vital in the manifestation of main genes tangled in adipose tissue, lipid and glucose metabolism, insulin sensitization, adipokine construction and inflammation [28]. Some studies reported the association of Pro12Ala polymorphism and fasting insulin levels [16, 29, 30] However, others [18, 26, 27, 31, 32] reported no associations. Moreover, other study showed lower insulin levels in carriers of a polymorphic allele in both PCOS cases and controls and this effect was not pronounced in investigating the PCOS patients alone. Similarly, some studies reported that the Pro12Ala polymorphism could cause lower insulin levels but showing no effect on HOMA-IR in PCOS patients [17, 29].

Some factors have been proposed to explain such a conflict between studies such as the variations in the genetic background of the population studied and gene-gene interaction plus the gene-environmental interactions [30, 33, 34]. Luan et al., 2006 demonstrated that the consumption of a diet with a low polyunsaturated to saturated fat ratio was associated with lower insulin levels and BMI in polymorphic allele carriers compared to those with the wild genotype [34]. An intersection between the genetic basis of PCOS and T2D in addition to the epidemiological evidence suggested that genes involved in gonadotropin secretion and insulin signalling may act in a common pathway or network leading to the PCOS phenotype.

The previous studies derived from European population reported that the metabolic parameters seemed to be different in PCOS patients with different ethnic descents [35-38]. The present study observed significantly higher abnormal metabolic components in the Ala carries compared to the Pro/Pro carriers, confirming the genetic effects of Pro12Ala on metabolic parameters in Egyptian PCOS women. Therefore, *PPARG* Pro12Ala gene polymorphism can be used as the biomarkers for early diagnosis and clinic prediction of metabolic risk in PCOS. Some studies reported that improvement in insulin sensitivity is predominantly observed only in lean subjects [23, 33, 39-42]. It appears that obesity may mask the protective effect of the Ala allele [42]. Free fatty acids that are generated in the process of nutrition and metabolism are natural ligands for PPARG. The high frequency of the *PPARG* Pro12Ala polymorphism may explain why the PCOS exhibited high levels of fasting insulin and HOMA-IR as well as other abnormal metabolic risk parameters.

In conclusion, the *PPARG* Pro12Ala polymorphism might contribute to increased risk of PCOS in Egyptian women, and it could be considered as a biomarker for early diagnosis and clinic prediction of related abnormal metabolic parameters.

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