Polycystic ovary syndrome: Pro12Ala polymorphism, hormonal and metabolic profiles

Wafaa Ghoneim Shousha¹, Moushira Erfan Zakí², Hala T. El Bassyouni³, Sara Mohamed Abdo¹, Salwa Mahmoud Mohamed Ali⁴

¹Biochemistry division, chemistry department, faculty of science, Helwan University, Cairo, Egypt.
²Biological Anthropology Department, National Research Centre, Cairo, Egypt.
³Clinical Genetics Department, National Research Centre, Cairo, Egypt.
⁴Private Medical Laboratory, Cairo, Egypt.

*Correspondence: s_mhistory@yahoo.com Accepted: 02 Aug, 2018 Published online: 30 Sep, 2018

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy affecting women and a leading cause of female infertility worldwide. This study aimed to delineate the association between the peroxisome proliferator-activated receptor gamma (PPARG) and gonadotrophin hormonal disturbance and energy metabolism and its impact on patients with PCOS in Egyptian women. A total of 90 adult women of childbearing age, 40 women were diagnosed by means of ultrasound to be suffering from PCOS, compared to 50 age-matched normal controls. All subjects were examined per PPARG2-Pro12Ala polymorphism, gonadotrophin hormones and metabolic parameters. There was a significant difference with PPARG2-Pro12Ala between Egyptian women with PCOS and controls. Some metabolic parameters were significantly increased in the PCOS patients with Pro12Ala. In conclusion, the PPARG2-Pro12Ala was associated with hormonal disturbed PCOS patients leading to the complications of metabolic syndrome.

Keywords: Polycystic ovary syndrome; peroxisome proliferator-activated receptor-gamma; hyper androgenemia; metabolic syndrome.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is observed as a heterogeneous androgen excess disorder with a variable degree of hormonal and metabolic abnormalities (Ding et al., 2017). Evidence showed that PCOS is a complex heterogeneous syndrome in which both genetic and environmental influences play an important role in its manifestation (Diamanti-Kandarakis and Piperi, 2005; Yang et al., 2013). PCOS represents a state of hormonal dysregulation, disrupted ovarian follicle dynamics, and subsequent oligo- or anovulation (Panidis et al., 2013). The syndrome’s prevalence is attributed, at least partly, to a well-established association with obesity and insulin resistance (IR). Indeed, the presence of severe PCOS in human genetic obesity and IR syndromes supports a causal role for IR in the pathogenesis of PCOS (Huang-Doran and Franks, 2016). 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 (Hart et al., 2004). Insulin resistance (IR) and hyperinsulinemia are considered the most important cause of reproductive and metabolic abnormalities in PCOS (Froment et al., 2006). Several women with PCOS are susceptible to increased weight and showing the phenotype of metabolic abnormalities (Hara et al., 2002;
Korhonen et al., 2003; Yilmaz et al., 2006; Koika et al., 2009). Consequently, the responsibility of genes such as PPARG2 (PPARG), which is a candidate gene involved in energy metabolism, insulin function, and adiposity, are also connected to the pathogenesis of PCOS (Deeb et al., 1998; Urbanek et al., 1999). The most widely studied genetic polymorphism is the proline (Pro) to alanine (Ala) variant at codon 12 in exon 2 of PPARG. The PPARG Pro12Ala allele lowered the risk of type 2 diabetes with a protective effect on the adipocyte and metabolic dysfunction (Deeb et al., 1998; Shaikh et al., 2013; Shaikh et al., 2014).

Several studies have evaluated the association of this variant with PCOS risk as well as obesity and insulin resistance parameters. Increased insulin sensitivity, decreased fasting insulin levels, Homeostatic model assessment (HOMA-IR), and basal metabolic rate, were observed in women with PCOS who were carriers of Ala alleles (Hara et al., 2002; Korhonen et al., 2003; Yilmaz et al., 2006; Koika et al., 2009). Shaikh et al., 2013 observed that Indian women carriers of polymorphic Pro12Ala genotype had significantly lower 2 hr glucose levels. Also, a study by Baldani et al., 2014 demonstrated that Pro12Ala was significantly associated with insulin sensitivity in lean PCOS women of Croatian population. On the contrary, studies in German, Chinese, Caucasian, and Greek population showed no association of this polymorphism with PCOS (Hahn et al., 2005; Wang et al., 2006; Antoine et al., 2007; Koika et al., 2009; Xita et al., 2009; Christopoulos et al., 2010; San-Millán and Escobar-Morreale, 2010). A meta-analysis indicated Ala alleles reduce the probability of having PCOS in European populations but not in Asians, which included only Chinese and Korean studies (He et al., 2012; Zhang et al., 2012). Overall these association studies imply PPARG to be an important gene associated with PCOS and its related traits (Shaikh et al., 2014).

MATERIALS AND METHODS

Study participants

This study was carried out on 90 adult women of childbearing age. Forty women were diagnosed by means of ultrasound to be suffering from a polycystic ovarian syndrome, compared with 50 women in normal weight and normal blood pressure were diagnosed with ultrasound that they did not suffer from PCOS. Anthropometric measurements were recorded to calculate the body mass index (BMI). Systolic/diastolic blood pressure was also recorded. Fasting blood samples were collected and centrifuged at 3000xg for 10 minutes to obtain sera which were stored at (-70 °C to -80°C) until assayed. Blood samples were collected on ethylene diaminetetra acetic acid (EDTA) tubes which were stored as a whole blood at (-20 °C).

Methods

Fasting blood glucose determination

Fasting blood glucose (FBG) was determined by GOD-PAP enzymic colorimetric method according to (Tietz, 1995). Glucose was determined after enzymatic oxidation in the presence of glucose oxidase (GOD). The formed hydrogen peroxide reacts under catalysis of peroxidase (PAP) with phenol and 4-aminoantipyrine to form a red violet quinoneimine dye as indicator.

Fasting blood insulin determination

Fasting blood insulin (FBI) was determined by means of Enzyme-linked Immunosorbent Assay (ELISA) technique (Olsson and Carlsson, 2005). Then Homeostasis Model Insulin Resistance (HOMA-IR) was calculated using Turner et al., equation (Turner et al., 1993). \[ HOMA-IR = \frac{glucose \times insulin}{405} \]

Lipid profile measurement

Lipid profile including total cholesterol (TC) (Ellelsson and Caraway, 1976), triglycerides (TG) (Bucolo and David, 1997), high-density lipoprotein cholesterol (HDL-C) (Friedewald et al., 1972) and low-density lipoprotein cholesterol (LDL-C) (Okada et al., 1998) were measured by the direct enzymatic colorimetric method.

FSH and LH estimation

ELISA technique was used for quantitative determination of Follicle Stimulating Hormone (FSH)(Odell et al., 1968) and Luteinizing hormone (LH) (Kosasa, 1981).

Determination of Pro2Ala

Genomic DNA was isolated from peripheral blood leucocytes by using the standard Phenol-chloroform extraction method (Kambalachenu et al., 2013). 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 mmol/L of each primer of forward 5'- CCA ATT CAA GCC
CAG TCC TTT C- 3’ and reverse 5’: GCA GAC AGT GTA TCA GTG AAG GAA TCG CTT CTC G-3’ and master mix (Promega) USA in a final volume of 25 mL(Jacob et al., 2016).

Consent form and ethical issues
This study protocol was approved by the ethical committee board of the National Research Centre of Egypt (No.16361), in accordance with the World Medicine Association Declaration of Helsinki. A written informed consent was obtained from each participant.

Statistical analysis
Data were performed using Statistical Package for the Social Science (SPSS) version 16 software on a DELL laptop computer (SPSS; SPSS Inc., Chicago, Illinois, USA). The results were expressed as mean values ± standard deviation (SD) by one-way analysis of variance, where P value <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Allele and genotype frequencies of PPARG gene in PCOS
Cui et al. demonstrated the direct correlation between genotypes and PCOS features. Each PCOS feature has a specific genetic association and etiological mechanisms. The increased frequency of PCOS-risk alleles in women with a single PCOS feature suggests that these patients have a greater risk of developing the syndrome (Cui et al., 2015).

In our study, we found that Ala alleles of PPARG in PCOS patients (15%) were lower than those in controls (16%), while the pro alleles in PCOS patients (85%) were higher than those in controls (84%) (Table 1).

Merged results of several case-control studies confirmed our findings and revealed that the carriers of the polymorphic Ala allele have a lower risk of developing PCOS. The Pro allele would increase the risk of PCOS and that the populations without Ala allele may need to pay more attention to the early prevention and diagnosis of this disease. Whereas the Ala variant would be protective against this disease (San-Millán and Escobar-Morreale, 2010; He et al., 2012; Zhang et al., 2012), which agreed with our results.

The genotypes (pro-ala)+(ala-ala) were significantly higher by 1.08 folds in PCOS group (30%)+(2.5%) compared to controls group (28%)+(2%), while the genotypes (pro-pro)+(pro-ala) in PCOS group (67.5%)+(30%) were non significantly lower than those in controls group (70%)+(28%) (Table 1).

Jacob et al. noted that PPARG gene Pro12Ala polymorphism was supposed to be susceptible genes in PCOS, this is inconsistent with our study(Jacob et al., 2016).

Association of the PPARG Pro12Ala polymorphism with metabolic parameters in PCOS
In our study, we observed some significant changes in anthropometric measurements and biochemical parameters due to PCOS genotypes. PPARG Pro12Ala polymorphism was associated with metabolic parameters in PCOS in women during the reproductive age. Obese PCOS women with high BMI carrying this polymorphism were suffering from diabetes mellitus due to high insulin resistance that was expressed as high HOMA-IR.

We found that PCOS patients group caring the genotype (Pro-Ala)+(Ala-Ala) showed a significant increase in BMI compared to both the controls group caring the same genotype (33.1 kg/m² versus 23.1 kg/m², P=0.001) and the PCOS patients group caring the genotype (Pro-Pro) (33.1 kg/m² versus 25.2 kg/m², P=0.03). Regarding the metabolic parameters, FBG and HOMA-IR in (Pro-Ala)+(Ala-Ala) PCOS patients were significantly higher than the (Pro-Ala)+(Ala-Ala) controls group (149 mg/dl versus 80 mg/dl, 6.7 versus 2.6, P=0.005, 0.006, respectively), FBI and HOMA-IR in (Pro-Ala)+(Ala-Ala) PCOS patients were significantly higher than the (Pro-Pro) PCOS patients (18.20 µU/ml versus 8.20 µU/ml, P=0.05, 6.7 versus 2.88, P=0.04 respectively) and HDL-C was significantly decreased in comparison of the phenotype (Pro-Ala)+(Ala-Ala) in PCOS patients with the same phenotype in controls group (P= 0.001) and with (Pro-Pro) PCOS patients (P= 0.01) (Tables 2 and 4).

There was a distinct relationship between the metabolism in adipose tissues and PPARG genotypes. PCOS patients with PPARG-Pro12Ala polymorphism showed a significant increase in FBG and HOMA-IR. While a significant decrease in HDL-C when compared to both the control carriers of PPARG-Pro12Ala polymorphism and to the PCOS carriers of PPARG-Pro-Pro polymorphism. On the contrary, the genotype (Pro-Pro) in PCOS patients revealed no effect on the metabolism (Table 3).

Metabolic syndrome was regarded as a set of
abnormalities, increasing the risk of serious functioning disorders. It can developed as a result of genetic predisposition (Szkup et al., 2018). Entezari et al., 2018 reported that, PPARG controls body weight, glucose homeostasis, adipocyte differentiation and is a valuable candidate gene for IR.

Table 1: Percentages of alleles and genotypes of PPARG gene in PCOS patients group and controls group.

<table>
<thead>
<tr>
<th></th>
<th>Alleles</th>
<th>Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pro</td>
<td>Ala</td>
</tr>
<tr>
<td>PCOS (n=40)</td>
<td>85%</td>
<td>15%</td>
</tr>
<tr>
<td>Controls (n=50)</td>
<td>84%</td>
<td>16%</td>
</tr>
</tbody>
</table>

Table 2: Anthropometric, clinical and biochemical parameters in carriers of the (pro-ala)+(ala-ala) genotype among the PCOS patients group compared to the control group.

<table>
<thead>
<tr>
<th></th>
<th>Control (pro-ala)+(ala-ala)</th>
<th>PCOS (pro-ala)+(ala-ala)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>23 ± 2.3</td>
<td>23 ± 2.8</td>
<td>0.1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.1 ± 7.8</td>
<td>33.1 ± 2.8</td>
<td>0.001</td>
</tr>
<tr>
<td>FBI (µU/ml)</td>
<td>10.2 ± 0.75</td>
<td>18.2 ± 0.65</td>
<td>0.1</td>
</tr>
<tr>
<td>Systolic/diastolic BP (mmHg)</td>
<td>(119±4)/(76±5)</td>
<td>(154±5)/(91±4)</td>
<td>0.3/0.7</td>
</tr>
<tr>
<td>FBG (mg/dL)</td>
<td>80 ± 5</td>
<td>149 ± 2</td>
<td>0.005</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.6 ± 0.4</td>
<td>6.7 ± 0.5</td>
<td>0.006</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>119 ± 3</td>
<td>195 ± 7</td>
<td>0.2</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>116 ± 5</td>
<td>150 ± 4</td>
<td>0.8</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>59 ± 16</td>
<td>40 ± 8</td>
<td>0.001</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>113 ± 21</td>
<td>163 ± 21</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Data are expressed as Mean ± SD. *: Values statistically significant (at P<0.05) in comparison with the control group. BMI: body mass index, FBI: fasting blood insulin, BP: blood pressure, FBG: fasting blood glucose, HOMA-IR: homeostatic model assessment insulin resistance, TC: total cholesterol, TG: triglycerides, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol

Table 3: Anthropometric, clinical and biochemical parameters in carriers of the (pro-pro) genotype in the PCOS patients group compared to the control group.

<table>
<thead>
<tr>
<th></th>
<th>Control (pro-pro)</th>
<th>PCOS (pro-pro)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>24±2.5</td>
<td>22±2.6</td>
<td>0.9</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.2±8.9</td>
<td>25.2 ± 2.9</td>
<td>0.3</td>
</tr>
<tr>
<td>FBI (µU/ml)</td>
<td>8.20±0.80</td>
<td>8.20 ± 0.85</td>
<td>0.49</td>
</tr>
<tr>
<td>Systolic/diastolic BP (mmHg)</td>
<td>(116±9)/(74±11)</td>
<td>(116±9)/(74±11)</td>
<td>0.13</td>
</tr>
<tr>
<td>FBG (mg/dL)</td>
<td>97±6</td>
<td>97±8</td>
<td>0.14</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.88±1.01</td>
<td>2.88±1.29</td>
<td>0.13</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>120±10</td>
<td>125±11</td>
<td>0.84</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>116±12</td>
<td>116±21</td>
<td>1.5</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>61±9</td>
<td>58±17</td>
<td>0.63</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>114±26</td>
<td>114±26</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Data are expressed as Mean ± SD.(NS): Values statistically insignificant (at P>0.05 ) in comparison with the control group. BMI: body mass index, FBI: fasting blood insulin, BP: blood pressure, FBG: fasting blood glucose, HOMA-IR: homeostatic model assessment insulin resistance, TC: total cholesterol, TG: triglycerides, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol
In a recent study by Xia et al., hyperinsulinemia played an important role in the subsequent development of gestational diabetes mellitus (GDM) in PCOS patients and it might be a variable predictor in PCOS women (Xia et al., 2017). De Wilde et al., detected that both insulin levels and HOMA-IR were significantly higher in women with PCOS who developed GDM. The risk of developing gestational diabetes in PCOS women is approximately three times greater, as compared to non-PCOS women (De Wilde et al., 2015). T2DM is associated with both environmental and genetic factors and several metabolic abnormalities are implicated in its pathogenesis, such as obesity and other environmental factors. Lv et al., 2017 investigated the impact of additional PPARG gene polymorphism on T2DM risk.

The genotype pro-pro showed insignificant changes between PCOS patient group and the control group regarding BMI, FBI and the metabolic parameters measured (Table 3).

Association between metabolism and gonadotropines in the pathogenesis of PCOS
We emphasized in this study an increase in the gonadotropines in the pathogenesis of PCOS. According to our data, the gonadotropines level was significantly different according to the BMI which inconsistent with our study as we found a high significant increase in LH and BMI in PCOS Patients. Moreover, Shim et al., 2011 reported that the HDL level was significantly different according to the LH/FSH ratio which confirms our results. Thus, Accordingly, the association between obesity and IR, jointly with LH determines the pathogenesis of PCOS. Some metabolic parameters may predict the pathogenesis of PCOS. We found that luteinizing hormone levels were significantly increased (8.43 mIU/mL versus 7 mIU/mL, \( P=0.02 \)) in the PCOS patients group compared to the controls group while the FSH hormone showed insignificant changes. However, the ratio of the two hormones (LH/FSH) showed a significant increase (\( P=0.001 \)) in PCOS patients group compared to the controls group (Table 5) Yao et al., 2017 reported that the higher LH/FSH ratio is a good prognostic biomarker for the onset of PCOS in women. Kumar et al. 2016 found a predominance of IR, dyslipidemia, and increased LH/FSH ratio in women with PCOS compared to control women and they reported that the LH/FSH ratio was perceived to be the gold standard for the diagnosis of PCOS which agreed with our results, but they revealed that LH is inversely proportional to BMI which inconsistent with our study as we found a high significant increase in LH and BMI in PCOS Patients.

Table 4: Anthropometric, clinical and biochemical parameters in PCOS patients carrying the (pro-ala)+(ala-ala) genotype to another PCOS patients carrying the (pro-pro) genotype.

<table>
<thead>
<tr>
<th></th>
<th>PCOS (pro-pro)</th>
<th>PCOS (pro-ala)+(ala-ala)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>22 ± 2.6</td>
<td>23 ± 2.8</td>
<td>0.1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.2 ± 2.9</td>
<td>33.1 ± 2.8</td>
<td>0.03</td>
</tr>
<tr>
<td>FBI (µU/ml)</td>
<td>8.20 ± 0.85</td>
<td>18.2 ± 0.65</td>
<td>0.05</td>
</tr>
<tr>
<td>Systolic/diastolic BP (mmHg)</td>
<td>116±9/(74±11)</td>
<td>(154±5)/(91±4)</td>
<td>0.3/0.7</td>
</tr>
<tr>
<td>FBG (mg/dL)</td>
<td>97± 8</td>
<td>149 ± 2</td>
<td>0.08</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.88 ± 1.29</td>
<td>6.7± 0.5</td>
<td>0.004</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>125±11</td>
<td>195 ± 7</td>
<td>0.6</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>116 ± 21</td>
<td>150 ± 4</td>
<td>0.7</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>58 ± 17</td>
<td>40 ± 8</td>
<td>0.01</td>
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<tr>
<td>LDL-C (mg/dL)</td>
<td>114 ± 26</td>
<td>163 ± 21</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Data are expressed as Mean ± SD. *: Values statistically significant (at \( P<0.05 \)) in comparison with groups. BMI: body mass index, FBI: fasting blood insulin, BP: blood pressure, FBG: fasting blood glucose, HOMA-IR: homeostatic model assessment insulin resistance, TC: total cholesterol, TG: triglycerides, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol.

Table 5: The gonadotropins in the PCOS group compared to the control group.

<table>
<thead>
<tr>
<th></th>
<th>FSH(mIU/mL)</th>
<th>LH(mIU/mL)</th>
<th>LH/FSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control(Mean±SE)</td>
<td>5.20 ± 1.31</td>
<td>7.0 ± 2.48</td>
<td>1.43 ± 0.49</td>
</tr>
<tr>
<td>PCOS(Mean±S)</td>
<td>4.94 ± 1.37</td>
<td>8.43 ± 2.43</td>
<td>1.81 ± 0.61</td>
</tr>
<tr>
<td>P value</td>
<td>0.371</td>
<td>0.024</td>
<td>0.001</td>
</tr>
</tbody>
</table>

LH: luteinizing hormone, FSH: follicle stimulating hormone. SE: standard error. *: Values statistically significant (at \( P<0.05 \)) in comparison with the control group.
CONCLUSION
The Pro12Ala polymorphism of PPARG may be closely associated with the PCOS among Egyptian women. The association between obesity, IR, and diabetes mellitus jointly with LH regulates the pathogenesis of PCOS. These findings could postulate evidence for personalized management in PCOS patients.

CONFLICT OF INTEREST
The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEDGEMENT
Biochemistry division, chemistry department, faculty of science, Helwan University, Cairo, National Research Centre, Cairo, Egypt.

AUTHOR CONTRIBUTIONS
Wafaa Ghoneim Shousha Designed for the study and supervises the work, Moushira Erfan Zaki Conceived the idea, planned for the study, did the statistical analysis, Hala T. El Bassyouni Clinical evaluation, Sara Mohamed Abdo Collecting data Salwa Mahmoud Mohamed Ali Did the biochemical analyses and writing paper.

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