Investigation of allele frequencies of polymorphic variants in genes that are related to polycystic ovary syndrome

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SUMMARY
OBJECTIVE: Polycystic ovary syndrome is a hormonal disorder that normally affects women of reproductive age in the range of 18–44 years. This study aimed to investigate the allelic frequencies of two polymorphisms, IRS rs18012781 and INSR rs1799817, which are suspected to be involved in polycystic ovary syndrome.

METHODS: The samples were obtained from the patients admitted to the Near East University Hospital, Department of Gynecology and Obstetrics. The samples were divided into two groups: control and polycystic ovary syndrome groups. Blood samples were collected from 55 women in the control group and 65 samples from the patient group. DNA from whole blood was obtained. The allelic frequencies of single-nucleotide polymorphisms were determined using real-time PCR. Results were presented as the heterozygous and homozygous state of the single-nucleotide polymorphisms.

RESULTS: There were no significant differences in the allelic frequencies of the single-nucleotide polymorphisms between the patient and control groups. Further statistical analysis investigating the INSR Tm using the Mann-Whitney U test value revealed that there was no difference in the homozygous and heterozygous state of INSR rs1799817. The result of this study showed that there was no statistically significant difference between the allelic frequencies of IRS1 rs1801278 and INSR rs1799817 between the patient and control groups.

CONCLUSION: These single-nucleotide polymorphisms do not seem to modify the risk of polycystic ovary syndrome, and they cannot be used as a marker in clinical circumstances to evaluate the possible occurrence of polycystic ovary syndrome.

KEYWORDS: Polymorphism, genetic. Polycystic ovary syndrome. Insulin resistance. Genetic testing.
required for this study were obtained from patients of Near East University Hospital, Department of Obstetrics and Gynecology. Informed consent was obtained from each patient. Clinical information of the patient was collected, and body mass indexes were reported. The samples to be studied were divided into two groups: the control group consisting of normal ovulation and non-obese women, and the patient group involving non-obese patients with PCOS. Blood samples were collected from 55 women in the control group and from 65 women included in the patient group. DNA from whole blood was obtained. The allelic frequencies of SNPs in two genes associated with PCOS were determined using real-time PCR.

**DNA extraction from blood samples**
DNA from each sample was extracted using an Invitrogen pure link genomic DNA mini kit (Invitrogen, USA) following the manufacturer’s protocol. The concentration of the DNA was measured using NanoDrop (Thermo Scientific, Pittsburgh, USA) at a wavelength of 260 nm (OD 260). The purity and quality were evaluated by the 230/260 ratio.

**PCR amplification**
Real-time PCR was conducted in order to identify the allelic frequencies at the particular SNP sites of the polymorphic genes, \( IRS1 \) and \( INSR \), which are associated with PCOS. Primer sequences are listed in Table 1. The reaction mixture consisted of 5 \( \mu \)l of master mix (LightCycler 480 SYBR Green, Roche), 0.8 \( \mu \)l of both forward and reverse primer (Table 1, final concentration of 0.25 \( \mu \)M), 0.6 \( \mu \)l of MgCl\(_2\), and 0.8 \( \mu \)l of H\(_2\)O were included in the reaction mixture. A volume of 2 \( \mu \)l of the extracted DNA was added to each reaction. All the PCRs were set up in a laminar flow hood in order to avoid contamination. The PCR condition is shown in Table 2. The allelic frequencies of the two SNPs within two genes were analyzed using the high-resolution melting (HRM) method, and the thermal cycler software was used to obtain the cycle of threshold (Ct) and melting temperature (Tm) values.

**Statistical analysis**
Statistical packages for the social sciences (SPSS version 10, Chicago, IL, USA) were used in this study. Descriptive statistics and an independent sample test of the Mann-Whitney U test were performed. The results were considered statistically significant if \( p \leq 0.05 \).

**RESULTS**
This study was designed to investigate the allelic frequencies for the polymorphic variant genes that are associated with PCOS. A total number of 120 blood samples were collected. Of these, 65 were diagnosed with PCOS and 55 were included in the control group, who did not present any signs of PCOS. The average age was 20 years and the average body mass index for all the patients and the control group was 17. The PCOS patients were diagnosed by correcting their hormonal levels as well as by vaginal ultrasonography. For each amplification, the cycle of threshold (Ct) was recorded. Ct indicates the total amount of cycle required for the fluorescent signal to cross the threshold. Likewise, for each amplification, melting temperature (Tm) values were recorded. Tm represents the melting temperature when the DNA is 50% double-stranded and 50% single-stranded. In HRM analysis, following PCR amplification, the amplicons produced are subjected to a gradual melting analysis. This enables the emission of fluorescence that is detected by real-time PCR equipment. These melt curves have different shapes due to the differences in the Tm values.

In this study, a total of 79.3% of the patients were shown to be homozygous for \( IRS1 \) rs1801278, respectively (Figure 1, Tables 3 and 4). There was no significant

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**Table 1.** Details of primers used.

| Primer name         | Sequence (forward primer)       | Sequence (reverse primer)       |
|---------------------|---------------------------------|---------------------------------|
| IRS1 rs1801278      | GGAAGAGACTGGCACTGAGG            | CTGACGGGGACAACTCATCT            |
| INSR rs1799817      | GGTGAAGACGGTCAACGAGT            | AGAAAGGGAAGGGTCAGGAA            |

**Table 2.** PCR cycling conditions used in the amplification of IRS and INSR sites.

| PCR conditions | Denaturation | Annealing | Extension | HRM          |
|----------------|--------------|-----------|-----------|--------------|
| Temperature/time| 95 for 10 min| 95 for 10 s| 72 for 25 s| 95 for 1 h   |
| 40 for 1 h     | 65 for 1 s   | 97 for 1 s |
| Cycle          | 1            | 40        |           |              |
Polymorphic markers in polycystic ovary syndrome

Table 3. Heterozygosity status with the Ct and Tm values for IRS1 rs1801278 and INSR rs1799817 in polycystic ovary syndrome patients.

| Patients code | IRS1 Ct | IRS1 Tm | Heterozygosity | INSR Ct | INSR Tm | Heterozygosity |
|---------------|---------|---------|----------------|---------|---------|----------------|
| 1             | 27.13   | 91.5    | Homozygous     | 23.7    | 85.8    | Homozygous     |
| 2             | 25.22   | 91.4    | Homozygous     | 23.04   | 86.2    | Homozygous     |
| 3             | 25.7    | 92.1    | Homozygous     | 25.8    | 86      | Homozygous     |
| 4             | 30.98   | 78.9    | Homozygous     | 27.2    | 86      | Homozygous     |
| 5             | 31.37   | 78      | Homozygous     | 27.8    | 87.4    | Homozygous     |
| 6             | 24.99   | 91.4    | Homozygous     | 26.1    | 84.6    | Homozygous     |
| 7             | 29.44   | 83.6    | Homozygous     | 29.94   | 87.36   | homozygous     |
| 8             | 28.22   | 83.3    | Homozygous     | 36.7    | 86.3    | Heterozygous   |
| 9             | 22.8    | 91.2    | Heterozygous   | 22.5    | 85.7    | Homozygous     |
| 10            | 32.75   | 92      | Homozygous     | 28.9    | 86.7    | Homozygous     |
| 11            | 29.7    | 78.7    | Homozygous     | 24.26   | 86.4    | Homozygous     |
| 12            | 25.85   | 91.8    | Homozygous     | 23.1    | 86.1    | Homozygous     |
| 13            | 29.33   | 91.2    | Homozygous     | 23.1    | 85.4    | Homozygous     |
| 14            | 25.48   | 91.3    | Homozygous     | 23.49   | 85.8    | Homozygous     |
| 15            | 31.31   | 76.5    | Homozygous     | 22.75   | 86.1    | Homozygous     |
| 16            | 28.02   | 91      | Heterozygous   | 23.4    | 86      | Homozygous     |
| 17            | 22.27   | 92.3    | Heterozygous   | 23.58   | 85.9    | Homozygous     |
| 18            | 31.99   | 77.9    | Homozygous     | 22.1    | 90.1    | heterozygous   |
| 19            | 30.29   | 79      | Homozygous     | 26.3    | 85.8    | Homozygous     |
| 20            | 23.93   | 91.6    | Homozygous     | 24.2    | 86.5    | Homozygous     |
| 21            | 30.52   | 78.1    | Homozygous     | 24.05   | 86.1    | Homozygous     |
| 22            | 22.2    | 92.19   | Heterozygous   | 23.6    | 85.7    | Homozygous     |
| 23            | 30.47   | 79.1    | Homozygous     | 23.36   | 86.1    | Homozygous     |
| 24            | 29.36   | 78.9    | Homozygous     | 23.7    | 85.9    | Homozygous     |

Figure 1. (A) PCR-HRM image showing melting curve analysis of the PCR products of the homozygote samples for IRS1 rs1801278. (B) PCR-HRM image showing melting curve analysis of the PCR products of the homozygote samples for different alleles of IRS1 rs1801278.
Table 3. Continuation.

| Patients code | IRS1 Ct | IRS1 Tm | Heterozygosity | INSR Ct | INSR Tm | Heterozygosity |
|---------------|---------|---------|----------------|---------|---------|----------------|
| 25            | 26.5    | 92.02   | Homozygous     | 22.4    | 85.6    | Homozygous     |
| 26            | No result | No result | No result | 35.4    | 86.4    | Homozygous     |
| 27            | 31.96   | 78.4    | Homozygous     | 24.77   | 85.6    | Homozygous     |
| 28            | 29.85   | 79.1    | Homozygous     | 23.35   | 86.1    | Homozygous     |
| 29            | No result | No result | No result | 22.3    | 86      | Homozygous     |
| 30            | No result | No result | No result | 28.1    | 86.2    | Homozygous     |
| 31            | 29.48   | 78.5    | Homozygous     | 27.7    | 85.9    | Homozygous     |
| 32            | 31.8    | 78.7    | Homozygous     | 27.5    | 86      | Homozygous     |
| 33            | No result | No result | No result | 23.5    | 86      | Homozygous     |
| 34            | 30.91   | 78.5    | Homozygous     | 26.6    | 85.7    | Homozygous     |
| 35            | 29.65   | 91.7    | Homozygous     | 26.9    | 86.2    | Homozygous     |
| 36            | 32.07   | 78.4    | Homozygous     | 26.7    | 86.5    | Homozygous     |
| 37            | 31.99   | 85.62   | Homozygous     | 23      | 86.3    | Homozygous     |
| 38            | 31.68   | 86.04   | Homozygous     | 25.8    | 87.3    | Homozygous     |
| 39            | 30.42   | 91.6    | Homozygous     | 25.77   | 87.1    | Homozygous     |
| 40            | 24.47   | 83.6    | Homozygous     | 23      | 85.9    | Homozygous     |
| 41            | No result | No result | No result | 24.1    | 85.7    | Homozygous     |
| 42            | 27.2    | 91.74   | Homozygous     | 22.63   | 85.7    | Homozygous     |
| 43            | 25.85   | 91.6    | Homozygous     | 23.5    | 85.8    | Homozygous     |
| 44            | 25.15   | 91.5    | Homozygous     | 23      | 86      | Homozygous     |
| 45            | No result | No result | No result | 23.7    | 86.6    | Homozygous     |
| 46            | 25.85   | 90.7    | Heterozygous   | 23      | 84.9    | Homozygous     |
| 47            | 27.4    | 91.6    | Homozygous     | 24.3    | 84.6    | Homozygous     |
| 48            | 31.77   | 82      | Homozygous     | 29.34   | 87.1    | Homozygous     |
| 49            | 23.88   | 91      | Homozygous     | 23.3    | 84.9    | Homozygous     |
| 50            | 24.15   | 91.9    | Heterozygous   | 24.1    | 84.8    | Homozygous     |
| 51            | 26.35   | 91.6    | Homozygous     | 28.89   | 87.1    | Homozygous     |
| 52            | 24.04   | 83.8    | Homozygous     | 23.15   | 86      | Homozygous     |
| 53            | 25.7    | 91.6    | Homozygous     | 26.41   | 61.3    | Homozygous     |
| 54            | 25.98   | 91.5    | Homozygous     | 25.36   | 61.7    | Homozygous     |
| 55            | 25.83   | 91.6    | Homozygous     | 27.72   | 61.5    | Homozygous     |
| 56            | 26.6    | 91.1    | Homozygous     | 22.8    | 85.9    | Homozygous     |
| 57            | 25.65   | 92.1    | Homozygous     | 23.4    | 85.9    | Homozygous     |
| 58            | 26.36   | 92.2    | Homozygous     | 22.9    | 85.9    | Homozygous     |

difference between the homozygote and heterozygote status of IRS1 rs1801278 when the patient group was compared with the control group. Furthermore, the heterozygosity of INSR rs1799817 did not show any significant difference between the patient group and the control group (p=0.059, Tables 3 and 4).

**DISCUSSION**

PCOS is one of the most common endocrine disorders affecting women of reproductive age. PCOS is one of the leading causes of infertility. Genetic and environmental factors tend to influence the complexity of PCOS (Figure 2). A number of studies have shown that genes and proteins are overexpressed
| Patients code | IRS1 Ct  | IRS1 Tm  | Genetic conditions | INSR Ct  | INSR Tm  | Genetic conditions |
|---------------|----------|----------|--------------------|----------|----------|--------------------|
| 1             | No result| No result| No result          | 23.5     | 87.5     | Homozygous         |
| 2             | 27.72    | 83.8     | Homozygous         | 24.5     | 85.4     | Homozygous         |
| 3             | 29.94    | 82.6     | Homozygous         | 22.7     | 86.3     | Homozygous         |
| 4             | 30.74    | 78.6     | Homozygous         | 23.2     | 85.9     | Homozygous         |
| 5             | 31.1     | 78.4     | Homozygous         | 24       | 90.94    | Heterozygous       |
| 6             | 25.88    | 92.1     | Homozygous         | 29.29    | 87.23    | Homozygous         |
| 7             | 26.5     | 92.26    | Homozygous         | 23.2     | 85.9     | Homozygous         |
| 8             | 32.27    | 78.5     | Homozygous         | 24.1     | 85.9     | Homozygous         |
| 9             | 27.3     | 91.47    | Homozygous         | 22.83    | 85.8     | Homozygous         |
| 10            | 27.2     | 92       | Homozygous         | 23.01    | 85.9     | Homozygous         |
| 11            | 26.09    | 92       | Homozygous         | 33.15    | 87.3     | Homozygous         |
| 12            | 26.21    | 91.4     | Homozygous         | 28.76    | 87       | Homozygous         |
| 13            | 30.35    | 82.38    | Homozygous         | 27.1     | 87.56    | Homozygous         |
| 14            | 25.92    | 91.6     | Homozygous         | 25.69    | 62.8     | Heterozygous       |
| 15            | 21.91    | 91.4     | Homozygous         | 25       | 84.8     | Homozygous         |
| 16            | 24.4     | 91.7     | Homozygous         | 24.7     | 85       | Homozygous         |
| 17            | 25.3     | 92.4     | Homozygous         | 24.9     | 84.5     | Homozygous         |
| 18            | 24.5     | 92.2     | Homozygous         | 24.1     | 85.6     | Homozygous         |
| 19            | 24.86    | 91.9     | Homozygous         | 22.4     | 85.7     | Homozygous         |
| 20            | 35.17    | 77.9     | Homozygous         | 23       | 84.8     | Homozygous         |
| 21            | 24.67    | 91.4     | Homozygous         | 25       | 84.7     | Homozygous         |
| 22            | 23.42    | 91.3     | Homozygous         | 23.3     | 84.7     | Homozygous         |
| 23            | 25.92    | 91.4     | Homozygous         | 23.9     | 84.2     | Homozygous         |
| 24            | 28.74    | 91.6     | Homozygous         | 23.2     | 85       | Homozygous         |
| 25            | 26.01    | 91.7     | Homozygous         | 25.91    | 62.54    | Homozygous         |
| 26            | 25.02    | 91.8     | Homozygous         | 25.36    | 63.9     | Heterozygous       |
| 27            | 25.11    | 99.9     | Homozygous         | 26.7     | 63       | Homozygous         |
| 28            | 25.38    | 91.6     | Homozygous         | 24.95    | 62.9     | Heterozygous       |
| 29            | 26.8     | 91.7     | Homozygous         | 26.41    | 63.3     | Homozygous         |
| 30            | 29.35    | 83.2     | Homozygous         | 24.96    | 85.6     | Homozygous         |
| 31            | 30.33    | 83.2     | Homozygous         | 27.88    | 87.56    | Homozygous         |
| 32            | 20.99    | 87.6     | Heterozygous       | 29.41    | 86.84    | Homozygous         |
| 33            | No result| No result| No result          | 29.5     | 87.36    | Homozygous         |
| 34            | 30.13    | 83.9     | Heterozygous       | Homozygous|
| 35            | 26.8     | 91.8     | Homozygous         | 28.59    | 87.3     | Homozygous         |
| 36            | 25.85    | 91.8     | Homozygous         | 31.16    | 87.2     | Homozygous         |
| 37            | 25.46    | 91.6     | Homozygous         | 26.47    | 86.9     | Homozygous         |
| 38            | 22.55    | 91.7     | Heterozygous       | 23.61    | 82.6     | Homozygous         |
| 39            | 26.8     | 91.4     | Homozygous         | 28.17    | 86.9     | Homozygous         |
| 40            | 25.63    | 91.5     | Homozygous         | 29.22    | 86.7     | Homozygous         |
| 41            | No result| No result| No result          | No result| No result| No result          |
| 42            | 25.55    | 91.6     | Homozygous         | 29.06    | 87.1     | Homozygous         |
| 43            | 30.63    | 82.8     | Homozygous         | 28.7     | 87.62    | Homozygous         |
| 44            | 29.51    | 82.8     | Homozygous         | 23.6     | 87.2     | Heterozygous       |
| 45            | 21.49    | 84.1     | Homozygous         | 22.99    | 86       | Homozygous         |
| 46            | 29.96    | 83.3     | Homozygous         | 23.27    | 86.1     | Homozygous         |
| 47            | 22.97    | 83.9     | Homozygous         | 22.32    | 85.8     | Homozygous         |
or underexpressed in samples from PCOS patients. One of the examples of these is the androgen receptors that have been shown to be overexpressed in the endometrial samples obtained from PCOS patients. Furthermore, MKI67, BCL2/BAX, and FASLG/FAS were overexpressed in endometrium samples of PCOS patients. Moreover, these expression patterns were positively correlated with the levels of fasting insulin and negatively correlated with the levels of DHEA-S. Thus, endometrial homeostasis is associated with the levels of fasting insulin and DHEA-S that can also be correlated with the expression of the mentioned proteins. Studies have shown that PCOS patients have high resistance to insulin. Insulin receptor genes, such as IRS1 and INSR, are among the key genes that can be associated with the pathogenesis of PCOS. IRS1 and INSR polymorphisms have tendencies to participate in problems with insulin signaling. In this study, we aimed to investigate the heterozygosity status of two SNPs within IRS1 and INSR genes that may have an increased risk of PCOS.

In this study, the heterozygosity and homozygosity status of the SNPs within IRS1 and INSR genes were analyzed using Tm. The statistical differences were investigated using the Mann-Whitney U test. The results of this study showed that the heterozygosity and homozygosity status of INSR showed no significant difference between the control and patient groups. Similarly, another study reported no significant differences between the SNPs investigated within the IRS1 and INSR genes in 48 Iranian women diagnosed with PCOS and 52 women in the control group. Furthermore, the same research group reported no association of IRS1 polymorphism in PCOS patients and controls in Spain. Similarly, genome-wide association studies have failed to show the link between the polymorphism of IRS1 and PCOS in Han Chinese population. Moreover, further studies reported no association between SNPs within INS, INSR, IRS1, IRS2, PPAR-G, and CAPN10 genes and PCOS.

In contrast, one study reported significantly different frequencies of IRS1 Gly972Arg polymorphism in PCOS and the control subjects in Turkish population. Furthermore, another study was conducted to determine the frequency of polymorphism for the IRS1 at codon 972 in women with PCOS in South Italy, consisting of 65 women with PCOS and 27 age-matched healthy women. They reported that there was a significant difference in the frequencies of Gly972Arg present in PCOS patients compared to controls. Furthermore, Tang et al. performed a comprehensive meta-analysis consisting of more than 4,000 subjects and reported that the AA/AG genotype of IRS1 rs1801278 increased the susceptibility of PCOS when compared to the homozygote GG genotype. Similarly, a meta-analysis by Ruan et al. reported that the presence of the A allele significantly increased the risk of PCOS. They further recorded no significant association observed in the IRS2 Gly1057Asp polymorphism.

Further studies also investigated the genotypes of INSR rs1799817 in relation to susceptibility of PCOS in Saudi Arabia, reporting that the homozygous allele was significantly higher compared to the control group. Similarly, Chen et al. also reported a significant difference in INSR rs1799817 genotypes in nonobese patients with PCOS compared to the controls.

CONCLUSION
Allelic frequencies of genes IRS1 rs1801278 and INSR rs1799817 involved in PCOS recorded no significant difference in the study population. One of the limitations of this study was the small sample size. It is a possibility that, with the increased number of PCOS patients and control group, the heterozygosity status may have shown significant differences. Furthermore, we were able to only analyze a small number of polymorphisms. It would have been ideal to have a genome-wide investigation using next-generation sequencing (NGS) techniques. However, the results of this study are crucial to associate the insulin-related genetic polymorphisms with the PCOS patients. Furthermore, one of the most important strengths of this study was the statistical analysis techniques used. The results of this study form the basis of future studies, especially in newly developed countries where newer technologies such as NGS are out of reach.

AUTHORS’ CONTRIBUTIONS
ARA: Data curation, Formal Analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. BO:
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Conceptualization, Data curation, Formal Analysis, Resources, Software, Validation, Visualization, Writing – review & editing. **ACO:** Conceptualization, Data curation, Formal Analysis, Resources, Software, Validation, Visualization, Writing – review & editing. **PT:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

REFERENCES

1. Bani Mohammad M, Majdi Seghinsara A. Polycystic ovary syndrome (PCOS), diagnostic criteria, and AMH. Asian Pac J Cancer Prev. 2017;18(1):17-21. https://doi.org/10.22034/APCJCP.2017.18.1.17

2. Maffazioli GDN, Lopes CR, Heinrich-Oliveira V, Lobo RA, Hayashida SAY, Soares JM Jr, et al. Prevalence of metabolic disturbances among women with polycystic ovary syndrome in different regions of Brazil. Int J Gynaecol Obstet. 2020;151(13):383-91. https://doi.org/10.1002/ijgo.13374

3. Neves LPP, Marcondes RR, Maffazioli GN, Simões RS, Maciel GAR, Soares JM Jr, et al. Nutritional and dietary aspects in polycystic ovary syndrome: insights into the biology of nutritional interventions. Gynecol Endocrinol. 2020;36(12):1047-50. https://doi.org/10.1080/09513590.2020.1822797

4. Sonthalia S, Agrawal M, Sehgal VN. Topical ciclopirox olamine 1%: revisiting a unique antifungal. Indian Dermatol Online J. 2017;10(4):481-5. https://doi.org/10.4103/idoj.IDOJ_29_19

5. Khan MJ, Ullah A, Basit S. Genetic basis of polycystic ovary syndrome (PCOS): current perspectives. Appl Clin Genet. 2019;12:249-60. https://doi.org/10.2147/TACG.S200341

6. Crespo RP, Bachega TASS, Mendonça BB, Gomes LG. An update of genetic basis of PCOS pathogenesis. Arch Endocrinol Metab. 2018;62(3):352-61. https://doi.org/10.20945/2359-3997000000049

7. Giordano LA, Giordano MV, Célia Teixeira Gomes R, Dos Santos Simões R, Baracat MCP, Giordano MG, et al. Effects of clinical and metabolic variables and hormones on the expression of immune protein biomarkers in the endometrium of women with polycystic ovary syndrome and normal-cycling controls. Gynecol Endocrinol. 2022;38(6):508-15. https://doi.org/10.1080/09513590.2022.2061454

8. Thangavelu M, Godla UR, Paul Solomon FD, Maddaly R. Single-nucleotide polymorphism of INS, INSR, IRS1, IRS2, PPAR-G and CAPN10 genes in the pathogenesis of polycystic ovary syndrome. J Genet. 2017;96(1):87-96. https://doi.org/10.1007/s12041-017-0749-z

9. Rashidi B, Azizy L, Najmaddin F, Azizi E. Prevalence of the insulin receptor substrate-1 (IRS-1) Gly972Arg and the insulin receptor substrate-2 (IRS-2) Gly1057Asp polymorphisms in PCOS patients and non-diabetic healthy women. J Assist Reprod Genet. 2012;29(2):195-201. https://doi.org/10.1007/s10815-011-9693-7

10. Galusha AM. Improvement of symptoms in patients with polycystic ovarian syndrome by vitamin D and calcium supplementation. 2013. School of Physician Assistant Studies. Paper 461.

11. Thangavelu M, Godla UR, Godi S, Paul SFD, Maddaly R. A case-controlled comparative hospital-based study on the clinical, biochemical, hormonal, and gynecological parameters in polycystic ovary syndrome. Indian J Pharm Sci. 2017;79(4):608-16. https://doi.org/10.4172/pharmaceutical-sciences.1000269

12. Dilek S, Ertunc D, Tok EC, Erdal EM, Aktas A. Association of Gly972Arg variant of insulin receptor substrate-1 with metabolic features in women with polycystic ovary syndrome. Fertil Steril. 2005;84(2):407-12. https://doi.org/10.1016/j.fertnstert.2005.01.133

13. Ruan Y, Ma J, Xie X. Association of IRS-1 and IRS-2 genes polymorphisms with polycystic ovary syndrome: a meta-analysis. Endocr J. 2012;59(7):601-09. https://doi.org/10.1507/endocrj ej11-0387

14. Daghestani MH. Rs1799817 in INSR associates with susceptibility to polycystic ovary syndrome. J Med Biochem. 2019;39(2):149-59. https://doi.org/10.2478/jmembio-2019-0023

15. Chen ZJ, Shi YH, Zhao YR, Li Y, Tang R, Zhao LX, et al. Correlation between single nucleotide polymorphism of insulin receptor gene with polycystic ovary syndrome. Zhonghua Fu Chan Ke Za Zhi. 2004;39(9):582-5. PMID: 15498182