Research article

Is there a relationship between polycystic ovary syndrome and the FABP1 gene rs2197076 single nucleotide polymorphism?

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ABSTRACT

Aim: Polycystic ovary syndrome (PCOS) is a multifactorial, endocrine, and metabolic disorder seen in 10%-20% of women of reproductive age. Due to the close relationship observed between the increased risk of type 2 diabetes and insulin resistance and the polymorphism of the fatty acid binding protein 1 (FABP1) gene rs2197076 single nucleotide polymorphism (SNP), we investigated the frequency of the FABP1 gene rs2197076 SNP in patients with PCOS.
Methods: This is a prospective case-control study. The study included 151 women—75 patients with PCOS and 76 healthy women. A real-time polymerase chain reaction was performed for the FABP1 rs2197076 polymorphism. Additionally, biochemical and hormonal levels of the patients were studied.
Results: Menstrual irregularities, the body mass index (BMI), hirsutism scores, the luteinizing hormone / follicular stimulating hormone ratio, dehydroepiandrosterone sulfate and testosterone levels were significantly higher in the PCOS group than in the control. There was no significant difference between the PCOS and control groups in terms of FABP1 rs2197076 genotype distribution and FABP1 rs2197076 allele frequency distribution.
Conclusion: There was no increase in the genotype distribution and allelic frequency of the FABP1 gene rs2197076 SNP in PCOS patients. Further studies are needed on this subject.
Keywords: Polycystic ovary syndrome; FABP1 gene polymorphism; rs2197076 polymorphism.

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Introduction

Polycystic ovary syndrome (PCOS) is a multifactorial, endocrine, and metabolic disorder seen in 10%–20% of women of reproductive age [1]. It is characterized by

obesity, hirsutism / hyperandrogenism, oligo-anovulation, and polycystic ovaries identified by ultrasound [2].

PCOS is associated with type 2 diabetes mellitus, dyslipidemia, metabolic syndrome, and an increased risk of cardiovascular disease [3]. Insulin resistance, hypothalamic-pituitary-ovarian axial dysfunction, ovarian steroidogenesis dysregulation, and genetic factors are thought to play a role in the etiopathogenesis of PCOS [4,5,6]. Studies have shown that single nucleotide polymorphisms (SNPs) of various genes are closely related to PCOS [7].

Fatty acid binding proteins (FABPs) are a family of lipid-binding proteins that play a role in lipid metabolism and signal transduction [8]. They act as molecular chaperones and are regarded as critical agents of metabolic and inflammatory processes both locally and systemically [9]. Liver fatty acid binding protein (L-FABP, also known as FABP1) is a protein found in the cytoplasm of hepatocytes that may also be present in intestinal cells and to a lesser extent in the core and outer mitochondrial membrane. Unlike the other members of the FABP family, FABP1 contains two long-chain fatty acid (LCFA) molecules. FABP1 mediates transcriptional programs by delivering lipids to nuclear receptors [10,11]. FABP1 plays an important role as a cryoprotectant by controlling the presence of LCFAs and oxidative metabolites [12]. FABP1 SNP rs2197076 polymorphism is reported closely related to type 2 diabetes and insulin resistance [13]. Due to the close relationship observed between the increased risk of type 2 diabetes and insulin resistance and the polymorphism of the FABP1 gene rs 219076, we investigated whether there is a link between PCOS etiopathogenesis and the FABP1 SNP rs2197076 polymorphism.

Methods

This prospective case-control study was carried out with the approval of the ethics committee of the Faculty of Medicine of Bolu Abant Izzet Baysal University (06 /04 /2016, document number 63). The study included 151 women, 75 patients with PCOS and 76 healthy women who were admitted to the Gynecology and Obstetrics Department. The diagnosis of PCOS was based on the Rotterdam criteria [2]: 1- clinical and/or biochemical hyperandrogenism (hirsutism or elevated serum total/free testosterone levels); 2- oligo/amenorrhea and anovulation; and 3- polycystic ovarian morphology upon ultrasound examination (presence of at least 12 antral follicles up to 12 mm in diameter and/or ovarian volume ≥ 10 cm^3 and increased stromal echogenicity). PCOS was diagnosed if two of these three criteria were met. Those who read and signed the volunteer form were included in the study. Patients with thyroid disease, hyperprolactinemia, history of malignancy, Cushing's syndrome, or congenital adrenal hyperplasia or who were taking hormonal drugs, oral antidiabetics, antidepressants, oral contraceptives, glucocorticoids, or antiandrogenic / antihypertensive drugs were excluded from the study.

Insulin resistance (IR) was defined as homeostasis model assessment of insülin resistance (HOMA-IR) formula greater or equal to 2.6: HOMA-IR = fasting insulin (µU/mL) × fasting blood glucose (mg/dL) / 405.

Venous blood samples were collected from the participants when at rest following one night of fasting for biochemical tests. Fasting venous blood samples were taken on the second or third day of menstruation to perform hormonal tests. Blood samples were collected in two separate fractions using BD Vacutainer®

EDTA tubes and BD Vacutainer® serum tubes. BD Vacutainer® serum tubes were centrifuged at 8000 rpm for 20 minutes at 4 ° C. Luteinizing hormone (LH), follicular stimulating hormone (FSH), prolactin (PRL), serum insulin, thyroid stimulating hormone (TSH) and total testosterone (T) were measured by Abbott Architect i2000SR system(Abbott Laboratories, IL, USA) using Chemiluminescent assay. Glucose, Cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) levels in fasting blood samples were measured by the Architect c8000 analyzer system (Abbott Laboratories, IL, USA) using kinetic, colorimetric and enzymatic methods. In addition, 3 ml blood samples were collected in EDTA tubes for DNA isolation.

The DNA was isolated with Roche High Pure

PCR Template Preparation Kit (DNA extraction kit). FABP1 gene rs2197076 polymorphism was genotyped using real-time polymerase chain reaction (RT-PCR) with Light Cycler 480-II RT PCR. At the end of the RT-PCR study, homozygous (wt: wild type), homozygous mutant, and heterozygous genotypes were differentiated according to the melting point of the peaks formed in the melt curve analysis. Heterozygous genotypes have two peaks at two different melting points (Figure 1), while homozygous mutants and homozygous wild genotypes have single peaks at the same melting point (Figure 2 and 3). In the FABP1 rs2197076 A/G polymorphism, the Tm (melting point) of the A allele was 57.39 ° C, and the Tm of the G allele was 51.39 ° C. AA genotype is homozygous wild, GG genotype is homozygous mutant, AG genotype is heterozygous mutant genotypes.
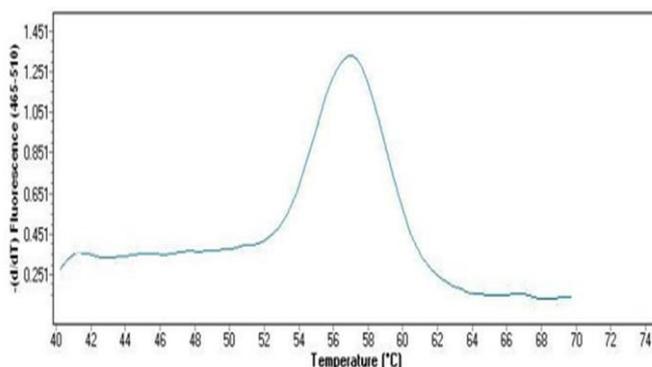
Figure 1. AG Genotype (Heterozygous mutant).
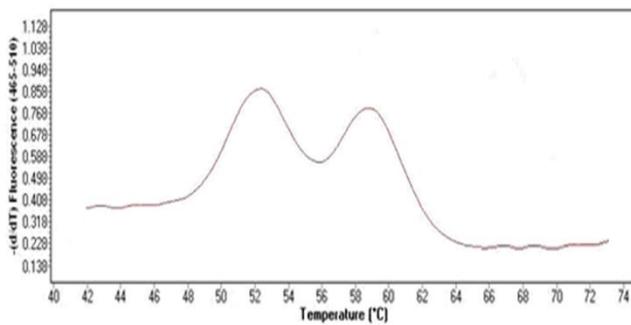
Figure 2. GG Genotype (Homozygous mutant).
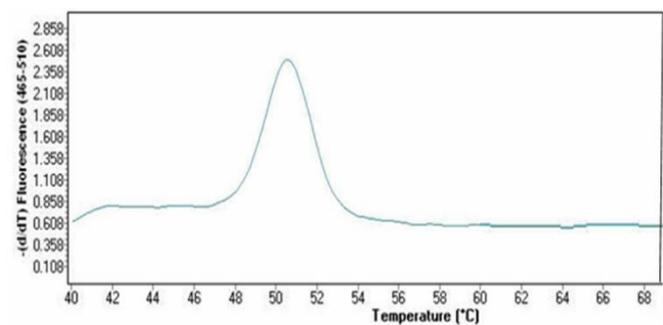
Figure 3. AA Genotype (Homozygous wild).

Statistical Analysis

SPSS 17.0 was used for the statistical analysis. Categorical measurements were taken as number and percentage. Continuous measurements were evaluated as means and standard deviations. Chi square tests or Fisher test statistics were used to compare categorical variables. In comparing continuous measurements between groups, a Student's t test was used when parametric test

assumptions were provided, and a Mann–Whitney U test was used when parametric test assumptions were not provided. The data obtained from the genotype analysis were calculated using the Epi Info 3.5.3 statistical program. P <0.05 was considered statistically significant.

Results

The study group consisted of 75 women with PCOS, and the control group consisted of 76 healthy women. In the PCOS group, the mean age was 23.0 ± 4.9 years, the mean body weight was 65.9 kg ± 13.8 kg, the mean body mass index (BMI) was 24.9kg/m^2 ± 5.8 kg/m^2, the mean stature was 161.9 ± 5.6 Centimeters. The mean waist circumference (WC) was 81.3 cm ± 12.9 cm, the mean hip circumference (HC) was 102.6 cm ± 11.1 cm, and the mean WC/HC ratio was 0.79 ± 0.09. In the control group, the mean age was 25.3 ± 5 years, the mean body weight was 57.3 kg ± 7.3 kg, the mean BMI was 21.1 kg/m^2 ± 2 kg/m^2, the mean stature was 162.8 ± 6.5 cm, the mean WC was 72.3 cm ± 8 cm, the mean HC was 97.8 cm ± 6.8 cm, and the mean WC/HC ratio was 0.75 ±0.06. Body weight, BMI, WC, HC, and WC/HC were found to be significantly higher in the PCOS group compared to the control group (p<0.01, p<0.01, p<0.01, p<0.01, and p<0.01 respectively). No significant difference was found between patients' stature (p=0.316). The mean age was significantly higher in the control group compared to the PCOS group (p<0.01)

In the PCOS group, there were 70 patients with oligo-amenorrhea (93.3%), 50 patients with hirsutism (66.6%), 75 patients with polycystic ovaries (100%), and 15 patients with a history of smoking (20%). In the control group, there were 2 women with oligo-amenorrhea (2.6%), 74 women with regular menstruation (97.4%),

6 women with hirsutism (7.9%), 18 women with polycystic ovaries (23.6%), and 15 women with a history of smoking (19.7%). There were significantly more instances of oligo-amenorrhea, hirsutism, and polycystic ovaries in the PCOS group (p <0.001, p<0.001, and p<0.001, respectively). No significant difference was found between the two groups in terms of smoking (p=0.608). There were significantly more instances of regular menstrual cycles in the control group (p<0.001).

Table 1. Biochemical and hormonal findings of the patients.

Parameters	Control group (n=76)	PCOS group (n=75)	*P*
Age (years)	25.3 ± 5	23.0 ± 4.9	<0.001
BMI (kg/m^2)	21.1 ± 2	24.9 ± 5.8	<0.001
FBG (mg/ Dl)	87 ± 5.31	89 ± 4.76	0.007
Fasting insulin (µU/mL)	1,9 ± 1.3	4 ± 2.42	<0.001
HOMA-IR	0.44 ± 0.12	0.87 ± 0.15	0.09
Total cholesterol (mg/dL)	160 ± 5.71	163 ± 7.32	0.300
Triglycerides (mg/dL)	69 ± 6.42	84 ± 6.21	<0.001
HDL (mg/dL)	51 ± 3.45	50 ± 2.83	0.198
LDL (mg/dL)	94 ± 9.37	94,5 ± 8.62	0.879
VLDL (mg/dL)	14 ± 1.34	17 ± 1.63	<0.001
LH / FSH	0.76 ± 0.84	1.22 ± 1.04	<0.001
Estradiol (pg/mL)	39 ± 4.31	43 ± 2.12	0.085
Prolactin (ng/L)	13.6 ±1.21	13.3± 0.81	0.360
DHEAS (µg/ dL)	180 ± 4.23	220 ± 2.34	<0.001
Testosterone (ng/dL)	19 ± 1.21	34 ± 3.12	<0.001
TSH (µIU/ mL)	2.1 ± 1.12	2.4 ± 0.96	0.320

Data are expressed as mean ± SD. P<0.05 was considered significant. PCOS: Polycystic ovary syndrome, BMI: Body mass index, FBG: Fasting blood glocose, HOMA-IR: Homeostasis model assessment of insulin resistance, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, T: testosterone, DHEA: dehydroepiandrostenedion sulfate, TSH: Thyroid stimulating hormone.

The biochemical and hormonal findings are shown in Table 1. Fasting blood glucose levels and insulin levels were significantly higher in the PCOS group than in the control group. In

both groups, HOMA-IR was calculated for insulin resistance and none of them had 2.6 or higher HOMA-IR results. The mean HOMA-IR calculation was 0.87 ± 0.15 in the PCOS group and 0.44 ± 0.12 in the control group and no significant difference was found between the groups. Triglycerides and VLDL levels were found to be significantly higher in PCOS patients. In the PCOS patient group, FSH was found to be significantly lower, and the LH/FSH ratio, dehydroepiandrosterone sulfate, and testosterone values were significantly higher.

Table 2. FABP1 rs2197076 polymorphism genotype and allele distribution of working groups.

		PCOS group (n= 75)	Control Group (n= 76)	*P*
FABP1 Genotype	GG	39 (% 52)	38 (% 50)	*P=0.362*
	AG	32 (% 42.6)	33 (% 43.5)	
	AA	4 (% 5.4)	5 (% 6.5)	
Allele Frequency	G	110 (%73.3)	109 (% 71.7)	*p=0.376*
	A	40 (%26.7)	43 (% 28.3)	

The data are given as number and percentage. P<0.05 was considered significant. PCOS: polycystic ovary syndrome, FABP1: Fatty acid binding protein 1.

The genotype and allele distribution of the rs2197076 polymorphism region of the FABP gene for the PCOS and control groups is shown in Table 2. In the PCOS group, FABP1 genotypes were found to be wild type homozygous in 4 cases, mutant type homozygous in 39 cases, and heterozygous genotype in 32 cases. FABP1 genotypes of the control group were found to be wild type homozygous in 5 cases, mutant type homozygous in 38 cases, and heterozygous genotype in 33 cases (Figure 4). There was no significant difference between the PCOS and control groups in terms of homozygous wild (AA) and homozygous mutant (GG) FABP1 genotype distribution (p=0.362). The FABP1

rs2197076 allele frequency distribution is shown in Figure 5. There was no statistically significant difference in the frequency of alleles between the PCOS and control groups (p=0.376).

Figure 4. Genotype distribution for the FABP1 rs2197076 polymorphism.
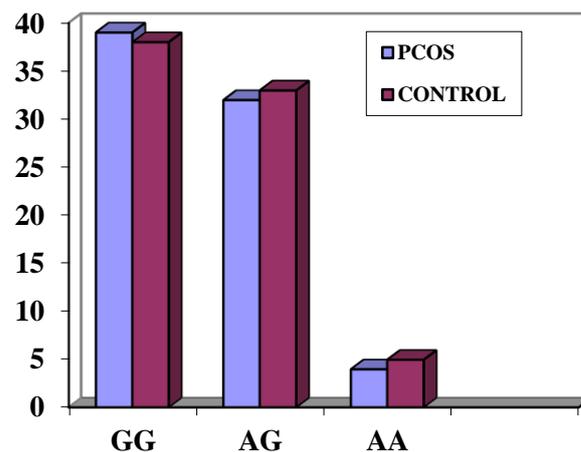
Figure 5. Allele distribution in PCOS and the control groups for the FABP1 rs2197076 polymorphism.
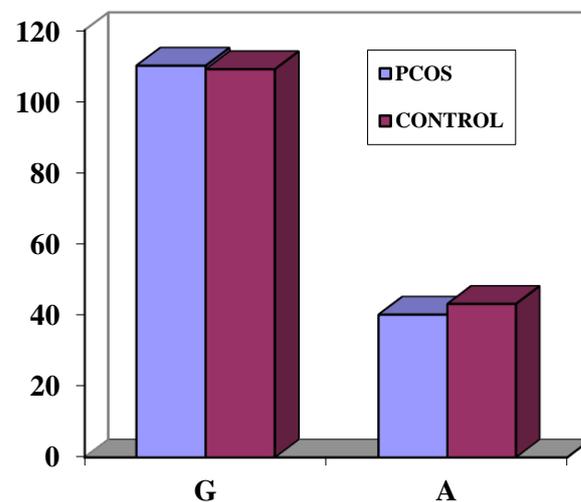
Discussion

PCOS is a heterogeneous and multifactorial disease. Women with PCOS have an increased risk of metabolic diseases, type 2 diabetes, insulin resistance, endometrial carcinoma, and cardiovascular disease. The FABP1 gene

rs2197076 SNP is associated with an increased risk of cardiovascular disease, insulin resistance, and metabolic syndrome. Approximately 65% of the risk of having PCOS is thought to be genetic [14]. There has been a tendency for candidate gene association studies in PCOS genetics studies. Candidate gene studies on PCOS have been performed in China and Korea [15, 16]. In these studies, 12 genetic risk loci have been determined for PCOS, and these loci have increased in number in other ethnic groups [17,18]. Candidate gene studies seem to be a powerful method for screening sensitive genes associated with complex diseases. However, these studies mainly focus on individual SNPs and neglect the interaction of genes. Some defined SNPs seem to lack a relationship that can be explained by genetic inheritance.

Regarding the FABP1 gene, Xue et al. investigated the relationship between SNP rs2197076 and rs2241883 and PCOS. In their study of 221 PCOS women and 198 normal women, the authors found a close association with rs 2197076 and rs 2241883 polymorphisms in women with PCOS but no association with BMI [19]. In a study of 95 PCOS women and 45 healthy women, Rashid et al. found that the allele and genotype frequency of IL-1β, IL-1Ra, and FABP1 gene polymorphisms did not differ between the PCOS and control groups. However, the IL-1β C [-511] T variant, IL-1Ra A allele II intron 2, and the A/G variant of the FABP1 (rs2197076) A allele showed a significant correlation with many metabolic features associated with PCOS [20]. In our study, we found no difference in the distribution of FABP1 gene rs2197076 SNP alleles among PCOS women and healthy women. Although the average BMI of the patient group was higher than that of the control group, no significant difference was found between the groups in terms of FABP1 gene distribution.

Cui et al. investigated the incidence of SNP allele in 15 women with polycystic ovary syndrome, 746 women with oligo-anovulation, 278 women with hyperandrogenism, and 536 women with polycystic ovaries identified by ultrasound [21]. In this study, rs13405728, rs2059807, and rs4385527 allele frequency were found to be significant in oligo-anovulatory women, rs4385527 allele frequency was found to be significant in women with hyperandrogenism, rs13429458, rs12478601, rs10818854 and rs4385527 allele frequency in women with polycystic ovaries were found to be significant, independent of BMI and age. In addition, the frequency of the SNP rs4385527 allele was found to be related to all three criteria. Lee et al. [16] studied 862 women with PCOS and 860 healthy women. Seven of the PCOS-associated 11 SNP loci (LHCGR, THADA, FSHR, KHDRBS3, YAP1, RAB5B and TOX3) were found to have a significant relationship with PCOS after Bonferroni correction. We did not examine these gene loci in our study.

We thought that this difference is caused by the ethnic diversity and use of different genotyping techniques among studies. The BMI of the women in our study was generally below 30. The absence of metabolic diseases and of excessive obesity in all women included in the study ensured that the comparison between PCOS and healthy patients was independent of metabolic events and obesity. Although the low number of patients in our study was seen as a limitation of our study, the fact that both groups were composed of individuals without insulin resistance and without metabolic diseases may have enabled us to reveal the relationship with PCOS more accurately.

In conclusion, no relation was found between genotype distribution and allele frequency of the FABP1 gene rs2197076 polymorphism in patients with PCOS. Further studies are needed on this subject.

References

[1] Azziz R, Woods KS, Reyna R, Key TJ, Knochenhauer ES, Yildiz BO. The prevalence and features of the polycystic ovary syndrome in an unselected population. J Clin Endocrinol Metab. 2004;89(6): 2745–49.

[2] The Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. Consensus on women's health aspects of polycystic ovary syndrome (PCOS) Hum Reprod. 2012;27(1):14–24.

[3] Luque-Ramírez M, Escobar-Morreale HF. Polycystic ovary syndrome as a paradigm for prehypertension, prediabetes, and preobesity. Curr Hypertens Rep. 2014;16(12):500.

[4] Franks S, Mc Carthy M, Hardy K. Development of polycystic ovary syndrome: involvement of genetic and environmental factors. Int J Androl. 2006;29(1):278–85.

[5] De Leo V, Musacchio MC, Cappelli V, Massaro MG, Morgante G, Petraglia F. Genetic, hormonal and metabolic aspects of PCOS: an update. Reprod Biol Endocrinol. 2016;14(1):38.

[6] Xita N, Georgiou I, Tsatsoulis A. The genetic basis of polycystic ovary syndrome. Eur J Endocrinol. 2002;147(69:717–25.

[7] Chen L, Hu LM, Wang YF, Yang HY, Huang XY, Zhou W, Sun HX. Genome-wide association study for SNPs associated with PCOS in human patients. Exp Ther Med. 2017;14(5):4896-4900.

[8] Zimmerman AW, Veerkamp JH. New insights into the structure and function of fatty acid-binding proteins. Cell Mol Life Sci. 2002;59(7):1096–116.

[9] Binas B, Erol E. FABPs as determinants of myocellular and hepatic fuel metabolism. Mol Cell Biochem. 2007;299(1-2):75-84.

[10] Chmurzyńska A. The multigene family of fatty acid-binding proteins (FABPs): function, structure and polymorphism. J Appl Genet. 2006;47(1):39-48.

[11] Haunerland NH, Spener F. Fatty acid-binding proteins--insights from genetic manipulations. Prog Lipid Res. 2004;43(4):328-49.

[12] Huang H, McIntosh AL, Martin GG, Landrock D, Chung S, Landrock KK, et al. FABP1: A Novel Hepatic Endocannabinoid and Cannabinoid Binding Protein. Biochemistry. 2016;55(37):5243-55.

[13] Mansego ML, Martínez F, Martínez-Larrad MT, Zabena C, Rojo G, Morcillo S, et al. Common variants of the liver fatty acid binding protein gene influence the risk of type 2 diabetes and insulin resistance in Spanish population. PLoS One. 2012;7: e31853.

[14] Kosova G, Urbanek M. Genetics of the polycystic ovary syndrome. Mol cell endocrinol. 2013;373(1-2):29-38.

[15] Chen ZJ, Zhao H, He L, Shi Y, Qin Y, Shi Y et al. Genome-wide association study identifies susceptibility loci for polycystic ovary syndrome on chromosome 2p16.3, 2p21 and 9q33.3. Nat Genet. 2011;43(1):55-59.

[16] Lee H, Oh JY, Sung YA, Chung HW. A genetic risk score is associated with polycystic ovary syndrome-related traits. Hum Reprod. 2016;31(1):209-15.

[17] Lerchbaum E, Trummer O, Giuliani A, Gruber HJ, Pieber TR, Obermayer-Pietsch B. Susceptibility loci for polycystic ovary syndrome on chromosome 2p16.3, 2p21, and 9q33.3 in a cohort of Caucasian women. Horm Metab Res. 2011;43(11):743-47.

[18] Goodarzi MO, Jones MR, Li X, Chua AK, Garcia OA, Chen YD et al. Replication of association of DENND1A and THADA variants with polycystic ovary syndrome in European cohorts. J Med Genet. 2012;49(2):90-95.

[19] Xue H, Zhao H, Liu X, Zhao YR, Chen ZJ, Ma J. Association of single-nucleotide polymorphisms rs2197076 and rs2241883 of FABP1 gene with polycystic ovary syndrome. J Assist Reprod Genet. 2016;33(1):75-83.

[20] Rashid N1, Nigam A, Saxena P, Jain SK, Wajid S. Association of IL-1β, IL-1Ra and FABP1 gene polymorphisms with the metabolic features of polycystic ovary syndrome. Inflamm Res. 2017;66(7):621-36.

[21] Cui L, Li G, Zhong W, Bian Y, Su S, Sheng Y et al. Polycystic ovary syndrome susceptibility single nucleotide polymorphisms in women with a single PCOS clinical feature. Hum Reprod. 2015;30(3):732-36.