



Association Between Single Nucleotide Polymorphisms (rs1484215 and rs6495096) in CYP11A1 Gene in Iranian Women with Polycystic Ovary Syndrome

Parry Fathy¹, Ebrahim Cheraghi^{2*}, Seyed Mohsen Miresmaeili¹

1- Department of Biology, Science and Arts University, Yazd, Iran

2- Department of Biology, Faculty of Science, University of Qom, Qom, Iran

Abstract

Background: Genetic factors significantly have important role in the etiology of the polycystic ovary syndrome (PCOS). This study examined the possible relation of rs1484215 and rs6495096 polymorphisms of CYP11A1 gene in Iranian women with PCOS.

Methods: The population of the case-control research included 100 women presenting with PCOS and 100 women as a control group who were referred to Infertility Center in Qom, Iran. The genotypes of rs1484215 and rs6495096 polymorphisms in CYP11A1 gene were detected with the tetra-ARMS PCR method. The independent segregation of alleles was tested for the Hardy–Weinberg equilibrium (HWE). Differences in quantitative traits were assessed between each group with a single PCOS feature and control group using Mann–Whitney U test for categorical variables and student's t-test for continuous variables. Statistical analysis of allele and genotype frequencies between women with PCOS and control was performed using the chi-square test. Significance level was defined as $p < 0.05$.

Results: There was a significant association of C with G alleles in rs6495096 polymorphism and susceptibility to PCOS ($p = 0.001$), but no significant relation was found between C and T alleles in rs1484215 polymorphism and susceptibility to PCOS. Also, GG genotype of rs6495096 was significantly associated with the waist-to-hip ratio (WHR) and infertility duration compared to CG and CC ($p < 0.01$). However, rs1484215 showed no association with these variables.

Conclusion: The results from the research indicated that rs6495096 polymorphism of CYP11A1 gene is related to the PCOS risk in Iranians women.

Keywords: Cholesterol side-chain cleavage enzyme, Infertility, Polycystic ovary syndrome, Polymorphism, Single nucleotide.

To cite this article: Fathy P, Cheraghi E, Miresmaeili SM. Association Between Single Nucleotide Polymorphisms (rs1484215 and rs6495096) in CYP11A1 Gene in Iranian Women with Polycystic Ovary Syndrome. *J Reprod Infertil.* 2023;24(1):18-25. <https://doi.org/10.18502/jri.v24i1.11905>.

Introduction

Polycystic ovary syndrome (PCOS) is characterized by a wide range of clinical, biochemical, and sonographic characteristics; there is often ultrasound evidence for polycystic ovaries, and clinical signs such as oligomenorrhea, hyperandrogenism, hirsutism, and anovulation can also be categorized as biochemical hallmarks of PCOS

(1). Other important complications of polycystic ovary syndrome include infertility, obesity, dark brown or black patches on the neck, male pattern baldness, and its long-term effects include an enhanced risk of type 2 diabetes, insulin resistance, hypertension, dyspepsia, lipidemia, and cardiovascular diseases (2). It has been shown that

PCOS has a prevalence of 5–10% (1).

One of the main physio-pathological features of PCOS is the overproduction of ovarian androgens (3). Previous studies showed various genes, including CYP11A1, CYP17, HSD17B6, PPAR γ , INSR along with CYP19 encoding necessary enzymes in androgen biosynthesis pathways (4-10). However, the findings from studies about the association of these genes are inconsistent.

CYP11A1 gene is located on chromosome 15 (15q23-24). It has 10 exons and 9 introns with an approximate length of 30 kb (11). It encodes the cholesterol side-chain cleavage enzyme, P450 *scc*, a member of the cytochrome P450 superfamily of enzymes, which resides in the mitochondrial inner membrane and catalyzes the conversion of cholesterol to pregnenolone, the first rate-limiting stage in steroid hormones synthesis (12). This is mainly expressed in steroidogenic tissues, including the adrenal cortex, gonads, and placenta (13). Genetic variants of CYP11A1 change its expression, causing definite hormone-related diseases, including breast cancer, prostate cancer, endometrial cancer, and polycystic ovary syndrome (14-17). Polymorphisms in this gene are proposed to have an essential role in regulating CYP11A1 expression via transcriptional up- or down- regulation resulting in enhanced or reduced androgen production (3).

A research in 2013 demonstrated a significant relation between rs6495096 polymorphism and PCOS (18). In another study performed by Zhang et al. in 2012, a relation between SNP rs4077582 in CYP11A1 and susceptibility to PCOS was reported. It changes testosterone levels by regulating LH in various genotypes in Chinese women but no association was found between rs11632698 and PCOS (19). Gao et al. showed that CYP3A4 and CYP11A1 genes might have an important impact on the stroke risk in Han Chinese women (20). Although CYP11A1 gene's role in PCOS patients is not yet fully understood, the expression of CYP11A1 gene in human ovaries has been demonstrated (21). There has been no study regarding the genotype distribution of SNP rs1484215 and rs6495096 in CYP11A1 in Iranian women (PCOS vs. normal); thus, the current research was performed to investigate the relation of CYP1A1 polymorphism and development of PCOS in Iranian women.

Methods

Study cases: In this case-control study, the population included 200 Iranian women (20-40 years)

referred to infertility treatment center of ACECR of Qom in December 2019 to December 2021 (100 women with PCOS, and 100 aged-matched non-PCOS women as control with normal menstrual cycles between 28-32 days, without hirsutism, severe acne, hair loss or insulin resistance signs). Subjects suffering from PCOS were enrolled according to 2003 Rotterdam criteria which determine the disorder based on symptoms of clinical, biochemical hyperandrogenism, ovulatory dysfunction, and polycystic ovary morphology (22). The exclusion criteria of the study included infertility with male factor, endometriosis, diabetes mellitus, hypertension, acromegaly, congenital adrenal hyperplasia, androgen-secreting tumors, Cushing's syndrome, hyperthyroidism, hyperprolactinemia, and ovarian tumor or women on oral contraceptives for the last 6 months, and smokers. In this study, people referred to this IVF selection center for sex and family balancing determination with healthy children and lack of any disorders were considered as the control group. The study was confirmed by the Institutional Review Board and its protocol was confirmed by the ethical committee of the University of Qom (Ethics code number: IR.Qom.REC.1398.018). Moreover, all of participant in this study completed an informed consent form.

Assessment of demographics and endocrine profiles: Demographic information was registered, including height and body weight. Body mass index (BMI) was evaluated as weight (kg) divided by height (m^2). For both groups, on the morning of the second or third day of the menstrual cycle after overnight fasting of at least 12 hr, blood samples were taken. The peripheral blood sample was instantly centrifuged for 10 min at 3000 rpm (Hettich, UK). The serum levels of follicle-stimulating hormone (FSH; Cat. N. DE1288), luteinizing hormone (LH; Cat. N. DE1289), prolactin (PRL; Cat. N. DE1291), estradiol (E2; Cat. N. LOT 1007843740), anti-mullerian hormone (AMH; Cat. N. LOT 001-50-N488), and total testosterone (TT; Cat. N. DE1559) of subjects were evaluated by ELISA (Demeditec Diagnostics GmbH, Germany) for the hormonal profile. The electrochemiluminescence immunoassay kit with a Roche Cobase 411 auto-analyzer (Roche Diagnostics, Germany) was utilized to evaluate the serum level of fasting blood sugar (FBS, mg/dl).

Genotyping of SNP rs1484215 and rs6495096: The data on the human CYP11A1 gene were gathered

from Entrez Gene on National Center for Biotechnology Information (NCBI) website. The SNP ID-info of this gene was received from the NCBI's single-nucleotide polymorphism database (dbSNP) at <http://www.ncbi.nlm.nih.gov/snp/>. SNPs of the CYP11A1 gene with minor allele frequency (MAF) >0.05 in the HapMap Asian population were obtained from previously published polymorphism studies related to PCOS and were applied to an initial screening. Genomic DNA was extracted from peripheral leukocytes via salting out procedure (23). For DNA extraction, 3 ml of peripheral blood were obtained from the coagulant in sterile tubes with EDTA anticoagulants and stored at -70°C.

Polymerase chain reaction (PCR): The DNA extraction process was done by the GeneAll Exgene™ kit (105-101) and according to the relevant instructions. DNA purity along with quantity was assessed by NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA) at 260/280 nm. The quality of bands from DNA derived by agarose electrophoresis was studied. Genotypes were determined by the amplification-refractory mutation system (ARMS) technique. Tetra-primer amplification refractory mutation system-polymerase chain (T-ARMS-PCR) was optimized to find rapid, efficient, and economic laboratory method for detecting SNPs or mutations. The reaction of T-ARMS-PCR was run in a single tube and in one PCR step, and genotype variation from SNPs of interest could be visualized directly using common agarose gel electrophoresis (24). To amplify the gene in specific SNPs, specific primers were designed using primer design for tetra-primer ARMS-PCR (PRIMER1). The characteristics of the primers along with the length of the reproduced piece are given in table 1.

Initial denaturation at 94°C for 5 min was per-

formed by 35 cycles at 94°C for 60 s, at 56°C for 40 s and extension at 72°C for 40 s continued with a final extension at 72°C for 10 min. Subsequently, the PCR products were separated by 2% agarose gel electrophoresis and visualized under UV illuminator following ethidium staining (Alpha-Imager™ Gel Imaging System, Alpha Innotech, USA) (Figure 1).

Statistical analysis: Mann–Whitney U test and Chi-square test for categorical variables and the student's t-test for continuous variables were utilized to measure the differences in demographic characteristics of both groups and for analysis of the genotype distribution in our case–control study using SPSS software vs. 23.0 (SPSS Inc., USA). All p-values were two-tailed, and those less than 0.05 were determined statistically significant. Moreover, 95% confidence intervals (CIs) and odds ratios (ORs) were measured. Genotype frequencies were checked for Hardy–Weinberg equilibrium in both PCOS and control groups.

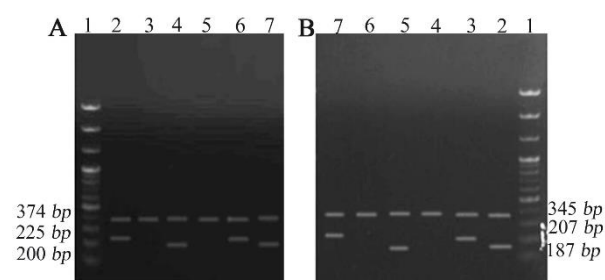


Figure 1. Electrophoresis of tetra-ARMS PCR products to determine the genotype of rs1484215 and rs6495096 in CYP11A1 gene. A: (rs6495096): Lane 1: DNA ladder (100 bp); Lane 2 and 3: homozygous genotype (GG); Lane 4 and 5: homozygous genotype (CC); Lane 6 and 7: heterozygous genotypes (GC) B: (rs1484215): Lane 1: DNA ladder (100 bp); Lane 2 and 3: heterozygous genotypes (TC); Lane 4 and 5: homozygous genotype (TT); Lane 6 and 7: homozygous genotype (CC)

Table 1. Primer sequences of SNPs (rs1484215 and rs6495096) of CYP11A1 gene

SNPs	Primer sequences (5'-3')	Product size (bp)
rs1484215		
Forward inner primer (C allele)	TTTGTTTTAGGGAGGCAGGAAAATTAC	207
Reverse inner primer (T allele)	AGGGCCTCTAGGTGAATCCACA	187
Forward outer primer	AGTAAGGTGGGACTCAGTGAGCAAA	345
Reverse outer primer	GGTCGCCTATCACCAGTATTACCAG	345
rs6495096		
Forward inner primer (G allele)	GACCTTTGTAAACATTCTACAAGTCAG	225
Reverse inner primer (C allele)	CAGCAACAGTGATCATAAAGGTG	200
Forward outer primer	TCACAGTCCACAACCTTGCTG	374
Reverse outer primer	ACTCTAGCAGTGACATGAAAGTGTG	374

Results

Characteristics of the study participants: The demographic, biochemical, and hormonal parameters in both groups were compared (Table 2). BMI, the waist-to-hip ratio (WHR), and infertility duration significantly differed in both groups ($p < 0.01$). Also, hormonal as well as biochemical parameters (LH, LH/FSH ratio, total testosterone, fasting blood sugar (FBS), prolactin, AMH, and estradiol) illustrated that both groups had significant differences ($p < 0.01$). Nonetheless, FSH and age did not significantly differ in both groups.

Association of SNPs with PCOS: Table 3 shows the genotype distribution of SNPs rs1484215 along with rs6495096 of CYP11A1 gene and allele

frequencies. The genotype "CG" of rs6495096 was associated with enhanced PCOS risk (OR=0.336, 95%CI=0.171-0.66, $p=0.005$) as analyzed by the co-dominant model (Table 3). No difference was found in rs1484215 (Table 3).

Distributions of the genotypic frequencies satisfied Hardy-Weinberg equilibrium in both groups. According to the Hardy-Weinberg test, the frequency of genotypes in rs1484215 and rs6495096 is not in balance and varies from generation to generation ($p < 0.001$). The frequency distributions of CYP11A1 rs6495096 alleles in PCOS were 1.14 for C and 0.86 for G compared with 1.34, 0.66 for C and G in the control group, respectively ($p=0.05$) (OR=0.653, 95% CI=0.435-0.98). The

Table 2. Basal characteristics of the control and women with PCOS

Variables/groups	PCOS (n=100)	Control (n=100)	p-value
Age (year)	30.04±4.52	31.56±4.74	0.107
BMI (kg/m^2)	27.89±5.11	25.51±3.28	0.001
Waist-to-hip ratio (WHR)	0.843±0.031	0.745±0.021	0.001
Infertility duration (year)	4.1±0.2	1.1±0.2	0.01
FBS (mg/dl)	97.9±12.02	89.29±7.17	0.01
FSH (IU/ml)	4.9±1.69	5.16±2.07	0.1
LH (IU/ml)	9.33±2.61	4.93±1.59	0.001
LH/FSH	1.9±0.57	0.95±0.29	0.01
AMH (ng/ml)	7.13±3.26	2.41±0.53	0.001
Prolactin (ng/ml)	69.72±15.57	35.39±12.12	0.001
Total testosterone (T; ng/dl)	58.61±12.5	36.86±5.86	0.001
Estradiol (E2; pg/ml)	54.2±12.4	50.13±15.19	0.01

Values are expressed as mean ± SD (t-test and Mann-Whitney test).

Bold values denote statistical significance. BMI: body mass index; FSH: follicle-stimulating hormone, LH: luteinizing hormone, AMH: anti-mullerian hormone; FBS: fasting blood sugar

Table 3. The frequency distributions (genotype and alleles) and Hardy-Weinberg equilibrium test in SNPs rs1484215 and rs6495096 in PCOS and controls

SNP	Model	Genotype	PCOS (n=100)	Control (n=100)	HWE P	OR (95% CI)	p-value
rs1484215	Co-dominant	TT	11	21		0.44 (0.197-0.982)	0.116
		TC	75	63		1.00	
		CC	14	16	0.008	0.735 (0.333-1.622)	
	Allele	T	97	105		1	0.484
		C	103	95		0.852 (0.576-1.261)	
rs6495096	Co-dominant	CC	16	36		1.00	0.005
		CG	82	62		0.336 (0.171-0.66)	
		GG	2	2	0.0005	0.756 (0.104-5.518)	
	Allele	C	114	134		1	0.05
		G	86	66		0.653 (0.435-0.98)	

Bold values denote statistical significance.

OR: Odds Ratio; CI: Confidence Interval; SNP: Single Nucleotide Polymorphism; HWE: Hardy-Weinberg Equilibrium

frequency distribution of CYP11A1 rs1484215 alleles in PCOS were 0.97 for T and 1.03 for C compared with 1.05, 0.95 for T and C in the control group, respectively ($p=0.484$) ($OR=0.852$, $95\%CI=0.576-1.261$) (Table 3).

Associations between different demographic, biochemical, hormonal, and sonographic characteristics with different genotypes among 100 women with PCO were studied. The waist-to-hip ratio (WHR) and infertility duration were significantly higher among women with genotype GG of rs6495096 compared to genotypes CC and CG ($p<0.01$). Other biochemical and hormonal parameters showed insignificant differences among different genotypes of rs6495096 (Table 4). As for rs1484215, no difference was identified in all

biochemical and hormonal parameters among the three genotypes (Table 4).

Receiver operating characteristic (ROC) analysis was used as a scale to measure the ability to separate a model with the largest area that indicates better predictive ability. It was applied to compare the performance of models. In this study, the value of the area under the ROC curve for the conditional logistic regression (95% , $CI:0.96-1$) was obtained with a sensitivity of 99% and a specificity of 97% . The results of the analysis of the conditional logistic regression showed that among all the variables in table 2, the level of testosterone, FSH, and AMH variables demonstrated the greatest effect (Figure 2).

Table 4. Association of different demographic and hormonal profiles with different genotypes among women with PCOS

Variables	rs6495096			p-value
	CC n=16	CG n=82	GG n=2	
Age	30.44±4.258	29.98±4.589	29.5±6.364	0.92
BMI	28.05±5.27	27.88±5.132	26.92±5.996	0.957
Waist-to-hip ratio (WHR)	0.843±0.028 ^a	0.847±0.039 ^a	0.932±0.012 ^b	0.0001
Duration of infertility	5.46±1.865 ^a	5.86±1.711 ^a	7.94±1.692 ^b	0.003
LH	7.54±2.632	7.31±2.637	6.85±1.626	0.915
FSH	6.11±1.974	5.91±1.608	7.9±2.546	0.248
LH/FSH	1.36±0.665	1.31±0.563	0.88±0.078	0.543
AMH	7.07±3.126	7.21±3.317	4.25±0.071	0.452
Prolactin	71.18±13.483	69.58±16.159	63.7±1.838	0.803
Estradiol	58.34±14.389	53.37±11.908	55.1±19.658	0.345
Testosterone	60.56±15.148	58.02±13.018	66.5±12.02	0.51
FBS	100.75±15.614	97.29±11.263	103.5±12.02	0.467

Variables	rs1484215			p-value
	CC n=14	CT n=75	TT n=11	
Age	30.64±5.597	29.99±4.196	29.64±5.519	0.843
BMI	28.17±4.85	27.71±4.947	28.79±6.774	0.79
Waist-to-hip ratio (WHR)	0.843±0.032	0.848±0.033	0.836±0.031	0.367
Duration of infertility	6.68±1.968	6.25±1.483	4.5±0.707	0.213
LH	7.52±2.798	7.21±2.507	7.97±3.133	0.641
FSH	6.11±1.36	6.01±1.814	5.67±1.161	0.793
LH/FSH	1.29±0.508	1.3±0.589	1.45±0.589	0.694
AMH	6.96±3.331	6.97±3.107	8.35±4.217	0.42
Prolactin	70.21±15.355	69.11±15.822	73.2±14.957	0.717
Estradiol	48.44±9.393	55.03±13.033	55.9±10.224	0.171
Testosterone	57.71±10.586	59.59±13.33	53±6.826	0.256
FBS	96.64±8.889	99.09±13.038	92±4.266	0.171

Values are expressed as mean ± SD (statistical analyses were performed by ANOVA). Means without a common letter are significantly different ($p<0.05$). BMI: body mass index; FSH: follicle-stimulating hormone, LH: luteinizing hormone, AMH: anti-mullerian hormone; FBS: fast blood sugar

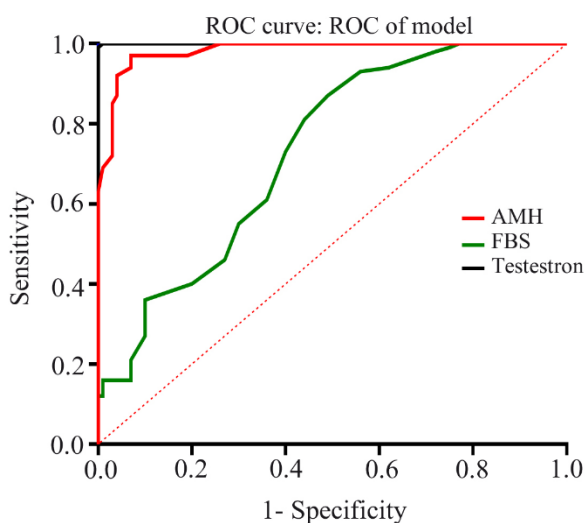


Figure 2. ROC curve and corresponding variables for PCOS

Discussion

The complex nature of polycystic ovary syndrome (PCOS) generates a need to investigate genetic and environmental factors contributing to its progression in Iranian women. In the present study, rs6495096 was found to be associated with infertility duration and waist-to-hip ratio (WHR) showed significant association with PCOS, indicating that rs6495096 may affect the P450scc enzyme activity and thus androgen production. The statistical results of the distribution of genotypes of rs6495096 indicate that individuals with CG genotype are at higher risk of PCOS when compared to CC and GG genotype groups; however, in control group, carriers of CC and GG consist the majority of cases in the group. Consequently, "C" allele of rs6495096 in PCOS cases was less frequent than the control group. Therefore, carriers of allele "G" are more susceptible to developing PCOS. Also, the current study showed that waist-to-hip ratio (WHR) and infertility duration were higher among women with PCOS who carried the genotype GG in comparison to genotypes CG and CC.

While PCOS is considered a heterogeneous disorder, its genetic base is supported via accumulated evidence from twin concordance and familial aggregation (3). In the light of lacking universally accepted criteria for PCOS, researchers could not propose a pathogenic gene for triggering PCOS, although earlier studies showed the potential influence of SNPs of CYP11A1 gene in different populations (13, 25, 26). This study investigated the potential pathogenic role of CYP11A1, espe-

cially SNPs rs1484215 and rs6495096, and the association of several genetic variants of rs1484215 and rs6495096 with PCOS predisposition in Iranian women. Results from the study indicated that significant genotypic and allelic frequencies for SNP rs6495096 significantly differ in both groups.

In line with our results, Shan et al. discovered four SNPs (rs12917295, rs11632698, rs1484215, and rs6495096) which constructed four haplotypes "(CACG, CATC, CACC, GGCC)" in the CYP11A1. There was no significant relation between haplotypes and the risk of PCOS (13). In Chinese women, four-, six-, and eight-repeat alleles are related to breast cancer (27) and polycystic ovary syndrome (28) are the key variants. The mechanism was different while CYP11A1 polymorphism was related to breast cancer and PCOS. Estrogen is associated with breast cancer. Androgen is related to PCOS (13).

Earlier researches on the relation of CYP11A1 with PCOS dealt with (tttta)_n microsatellite polymorphism in the CYP11A1 promoter (9, 14, 29). In a research by Zhang et al., rs4077582 was related to PCOS pathogenesis, showing that rs4077582 can influence the P450scc enzyme activity and affect androgen production (19). P450 scc catalyzes the first stage of androgen and estrogen biosynthesis and changes cholesterol into pregnenolone, an androgen precursor (30). Thecal cells cultured from PCOS samples indicate enhanced androgen production or enhanced CYP11A mRNA expression compared to samples from control women (31). In particular, PCOS individuals with the GG genotype of rs6495096 showed a significantly higher waist-to-hip ratio (WHR) and infertility duration compared to that of CC and CG genotypes, though no difference was found in the waist-to-hip ratio (WHR) and infertility duration in the CC and CG genotype. These bits of evidence demonstrated that normal women with GG genotype of rs6495096 were more likely to experience PCOS, which was supported by the fact that PCOS patients had a greater proportion of GG genotype as compared to controls.

This research is the first to report the relation of rs6495096 in CYP11A1 gene with PCOS in Iranian population. Some limitations have constrained the replication of the current research. Firstly, the sample size was relatively small, and further studies are required to identify the possible relation of CYP11A1 gene with PCOS. Secondly, only two SNPs causing incomplete coverage of the gene

variations were selected. Large and more genome-wide association researches among PCOS cases will be essential to determine new candidate genes as well as proteins responsible for PCOS risk.

Conclusion

Based on the findings of this study, it was revealed that polymorphism rs6495096 of CYP11A1 gene may increase the risk of PCOS in Iranian women. However, PCOS cannot be associated with a single factor of this gene, and more research should be conducted to discover other risk factors for PCOS; moreover, the capacity of CYP11A1 gene must be studied in future research.

Acknowledgement

We appreciate the assistance of members of the Infertility Research Center at ACECR, Qom.

Funding: This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

1. Fox R, Corrigan E, Thomas PA, Hull MG. The diagnosis of polycystic ovaries in women with oligo-amenorrhoea: predictive power of endocrine tests. *Clin Endocrinol (Oxf)*. 1991;34(2):127-31.
2. Payne AH, Hales DB. Overview of steroidogenic enzymes in the pathway from cholesterol to active steroid hormones. *Endocr Rev*. 2004;25(6):947-70.
3. Dadachanji R, Shaikh N, Mukherjee S. Genetic variants associated with hyperandrogenemia in PCOS pathophysiology. *Genet Res Int*. 2018;2018:7624932.
4. Petry CJ, Ong KK, Michelmore KF, Artigas S, Wingate DL, Balen AH, et al. Association of aromatase (CYP 19) gene variation with features of hyperandrogenism in two populations of young women. *Hum Reprod*. 2005;20(7):1837-43.
5. Gaasenbeek M, Powell BL, Sovio U, Haddad L, Gharani N, Bennett A, et al. Large-scale analysis of the relationship between CYP11A promoter variation, polycystic ovarian syndrome, and serum testosterone. *J Clin Endocrinol Metab*. 2004;89(5):2408-13.
6. Jones MR, Italiano L, Wilson SG, Mullin BH, Mead R, Dudbridge F, et al. Polymorphism in HSD17B6 is associated with key features of polycystic ovary syndrome. *Fertil Steril*. 2006;86(5):1438-46.
7. Prapas N, Karkanaki A, Prapas I, Kalogiannidis I, Katsikis I, Panidis D. Genetics of polycystic ovary syndrome. *Hippokratia*. 2009;13(4):216-23.
8. Araki T, Elias R, Rosenwaks Z, Poretsky L. Achieving a successful pregnancy in women with polycystic ovary syndrome. *Endocrinol Metab Clin North Am*. 2011;40(4):865-94.
9. Unluturk U, Harmanci A, Kocafe C, Yildiz BO. The genetic basis of the polycystic ovary syndrome: a literature review including discussion of PPAR- γ . *PPAR Res*. 2007;2007:49109.
10. Kaur R, Kaur T, Sudhir N, Kaur A. Association analysis of CYP11A1 variants with polycystic ovary syndrome: a case-control study from north India. *Reprod Sci*. 2021;28(10):2951-60.
11. Akgül S, Derman O, Alikasıfoğlu M, Aktaş D. CYP1A1 polymorphism in adolescents with polycystic ovary syndrome. *Int J Gynecol Obstet*. 2011;112(1):8-10.
12. Ashraf S, Nabi M, Rashid F, Amin S. Hyperandrogenism in polycystic ovarian syndrome and role of CYP gene variants: a review. *Egypt J Med Hum Genet*. 2019;20(1):1-10.
13. Shan B, Zhou L, Yang S, Yan M, Wang Z, Ouyang Y, et al. Li Z. Association between polycystic ovary syndrome (PCOS) and CYP11A1 polymorphism in Hainan, China: a case-control study. *Int J Clin Exp Pathol*. 2016;9(1):230-6.
14. Celhar T, Gersak K, Ovcak Z, Sedmak B, Mlinaric-Rascan I. The presence of the CYP11A1 (TTTTA) 6 allele increases the risk of biochemical relapse in organ confined and low-grade prostate cancer. *Cancer Genet Cytogenet*. 2008;187(1):28-33.
15. Setiawan VW, Cheng I, Stram DO, Giorgi E, Pike MC, Van Den Berg D, et al. A systematic assessment of common genetic variation in CYP11A and risk of breast cancer. *Cancer Res*. 2006;66(24):12019-25.
16. Zheng W, Gao YT, Shu XO, Wen W, Cai Q, Dai Q, et al. Population-based case-control study of CYP11A gene polymorphism and breast cancer risk. *Cancer Epidemiol Biomark Prev*. 2004;13(5):709-14.
17. Terry K, McGrath M, Lee IM, Buring J, De Vivo I. Genetic variation in CYP11A1 and StAR in relation to endometrial cancer risk. *Gynecol Oncol*. 2010;117(2):255-9.
18. Pau C, Saxena R, Welt C K. Evaluating reported candidate gene associations with polycystic ovary syndrome. *Fertil Steril*. 2013;99(6):1774-8.
19. Zhang CW, Zhang XL, Xia YJ, Cao YX, Wang WJ, Xu P, et al. Association between polymor-

- phisms of the CYP11A1 gene and polycystic ovary syndrome in Chinese women. *Mol Biol Rep.* 2012; 39(8):8379-85.
20. Gao N, Tang H, Gao L, Tu G, Luo H, Xia Y. CYP3A4 and CYP11A1 variants are risk factors for ischemic stroke: a case control study. *BMC Neurol.* 2020;20(1):77.
 21. Hong SH, Hong YS, Jeong K, Chung H, Lee H, Sung YA. Relationship between the characteristic traits of polycystic ovary syndrome and susceptibility genes. *Sci Rep.* 2020;10(1):10479.
 22. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril.* 2004;81(1):19-25.
 23. Miller SM, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* 1988;16(3):1215.
 24. Ahlawat S, Sharma R, Maitra A, Roy M, Tantia M. Designing, optimization and validation of tetra-primer ARMS PCR protocol for genotyping mutations in caprine Fec genes. *Meta Gene.* 2014;2: 439-49.
 25. Bayoumy N, El-Shabrawi M, Younes S, Atwa K. CYP1A1 gene (6235T<C) polymorphism as a risk factor for polycystic ovarian syndrome among Egyptian women. *Hum Fertil (Camb).* 2020;23(2): 142-7.
 26. Abdel-Mageed W S, Dabous E, Gerguis A. Association between polymorphisms of the CYP11A1 gene and polycystic ovary syndrome in Egyptian Female. *Res J Appl Biotechnol.* 2016;2(1):19-28.
 27. Sakoda LC, Blackston C, Doherty JA, Ray RM, Lin MG, Stalsberg H, et al. Polymorphisms in steroid hormone biosynthesis genes and risk of breast cancer and fibrocystic breast conditions in Chinese women. *Cancer Epidemiol Biomark Prev.* 2008;17(5):1066-73.
 28. Gao GH, Cao YX, Yi L, Wei ZL, Xu YP, Yang C. [Polymorphism of CYP11A1 gene in Chinese patients with polycystic ovary syndrome]. *Zhonghua Fu Chan Ke Za Zhi.* 2010;45(3):191-6. Chinese.
 29. Wang Y, Wu X, Cao Y, Yi L, Zou Y, Qu J, et al. [Microsatellite polymorphism of (tttta)_n in the promoter of CYP11a gene in Chinese women with polycystic ovary syndrome]. *Zhonghua Yi Xue Za Zhi.* 2005;85(48):3396-400. Chinese.
 30. Guo C, Hu MC, Chung BC. Transcriptional regulation of CYP11A1. *J Biomed Sci.* 2003;10(6 Pt 1):593-8.
 31. Gilling-Smith C, Story H, Rogers V, Franks S. Evidence for a primary abnormality of thecal cell steroidogenesis in the polycystic ovary syndrome. *Clin Endocrinol (Oxf).* 1997;47(1):93-9.