Int J Fertil Steril
Open Access
doi: 10.22074/ijfs.2025.2065767.1908
International Journal of Fertility & Sterility
www.ijfs.ir
Original Article
Vol 20, No 3, July-September 2026, Pages: 268-277

Phenotype-Specific Association of *LHCGR* rs13405728 Polymorphism with Anovulatory Infertility in Malay Women with Polycystic Ovary Syndrome: A Case-Control Study

Mariam Elbadie, M.Sc.[1, 2], Habibah Abdul Hamid, M.D.[3*], Zalinah Ahmad, Ph.D.[4, 5], King-Hwa Ling, Ph.D.[1, 2]

1. Department of Biomedical Science, Faculty of Medicine and Health Sciences, University Putra Malaysia, Selangor, Malaysia
2. Genetics and Regenerative Medicine Research Centre, Faculty of Medicine and Health Sciences, University Putra Malaysia, Selangor, Malaysia
3. Department of Obstetrics and Gynaecology, Faculty of Medicine and Health Sciences, University Putra Malaysia, Selangor, Malaysia
4. Department of Pathology, Faculty of Medicine and Health Sciences, University Putra Malaysia, Selangor, Malaysia
5. Laboratory of Vaccine and Biomolecules (VacBio), Institute of Bioscience, University Putra Malaysia, Selangor, Malaysia

Abstract

Background: Polycystic ovary syndrome (PCOS) is a multifactorial, polygenic endocrinopathy with an incompletely understood aetiology that accounts for approximately 80% of female anovulatory infertility. Both genetic and environmental factors contribute significantly to its pathogenesis. This study is the first to investigate the association of DENN/MADD domain-containing 1A (*DENND1A* rs2479106 and rs10986105), luteinising hormone/choriogonadotropin receptor (*LHCGR* rs13405728), follicle-stimulating hormone receptor (*FSHR* rs6166), thyroid adenoma-associated protein I (*THADA* rs13429458), and cytochrome P450 17A1 (*CYP17A1* rs743572) gene polymorphisms with PCOS-related anovulatory infertility in Malay women.

Materials and Methods: In this case-control study, 96 participants were recruited - 48 anovulatory infertile-PCOS patients and 48 infertile non-PCOS controls. Single nucleotide polymorphisms (SNPs) were detected using high-resolution melting (HRM) analysis, with sequencing performed for validation.

Results: The *LHCGR* rs13405728 polymorphism was strongly associated with an increased risk of PCOS-related anovulatory infertility, with each minor G allele conferring an odds ratio (OR) of 2.63 and 95% confidence interval (95% CI) of 1.11-6.27, P= 0.020). Conversely, the *THADA* rs7568365 T allele exhibited a protective effect, reducing the risk by 72% (OR=0.28; 95% CI: 0.08-0.90; P=0.020). No significant associations were observed for *DENND1A* rs2479106 and rs10986105, *FSHR* rs6166, *THADA* rs13429458, or *CYP17A1* rs743572. Haplotype analysis revealed that the A-T (rs13429458-rs1317772225-rs7568365) and ATA and ACA (rs13405728-rs6166-rs13429458) haplotypes were protective, with ORs of 0.13, 0.19, and 0.30, respectively (P<0.05).

Conclusion: The *LHCGR* rs13405728 polymorphism is significantly associated with the risk of PCOS-related anovulatory infertility in Malay women and may serve as a potential early prognostic biomarker for the development of PCOS-related infertility.

Keywords: Anovulation, Haplotypes, Polycystic Ovary Syndrome, Polymorphism, Receptors

Citation: Elbadie M, Abdul Hamid H, Ahmad Z, Ling KH. Phenotype-Specific Association of LHCGR rs13405728 Polymorphism with Anovulatory Infertility in Malay Women with Polycystic Ovary Syndrome: A Case-Control Study. Int J Fertil Steril. 2026; 20(3): 268-277. doi: 10.22074/ijfs.2025.2065767.1908

Introduction

Polycystic ovary syndrome (PCOS) is the most prevalent endocrinopathy among women of childbearing age, affecting 5%-20% of females aged 18-44 years worldwide (1). It is a complex systemic condition linked to numerous health issues, such as type 2 diabetes, dyslipidaemia, heart disease, increased risk of stroke, obesity, endometrial cancer, anxiety, and depression (2).

Under ultrasonography, multiple small cysts are observed in the ovaries, leading to the term "polycystic ovaries," which indicates a disruption in the normal development of follicles (3). The main characteristics associated with this

Royan Institute
International Journal of Fertility & Sterility

Received: 13/July/2025, Revised: 29/September/2025, Accepted: 22/November/2025
*Corresponding Address: Department of Obstetrics and Gynaecology, Faculty of Medicine and Health Sciences, University Putra Malaysia, Serdang, Selangor, Malaysia
Email: habib@upm.edu.my

condition are anovulatory infertility, polycystic ovaries, obesity, hyperandrogenism, and insulin resistance (4). Although the exact aetiology behind PCOS is not fully understood, it appears to be the result of an interplay of genetic, metabolic, endocrine, and environmental factors (5). Twin studies suggest that PCOS is a polygenic disorder linked to the X chromosome, with 72% of the risk attributed to genetics (6). Identifying candidate genes for PCOS has been challenging due to its uncertain causes, diverse characteristics, and clinical presentations. In Han, China, the first genome-wide association studies (GWAS) identified three susceptibility loci for PCOS: 2p16.3, 2p21, and 9q33.3. These loci are mapped to thyroid adenoma-associated protein I (*THADA*), luteinising hormone/choriogonadotropin receptor (*LHCGR*), and DENN/MADD domain-containing 1A (*DENND1A*), respectively (7).

THADA is linked to disruptions in energy metabolism that lead to a decrease in energy production and an increased vulnerability to obesity, thus impacting the risk of PCOS. The connections between genetic variations within the *THADA* gene with type 2 diabetes and insulin resistance further exacerbate PCOS symptoms (1). Ovarian theca cells, testicular Leydig cells, and adipose tissue express the 11-exon *LHCGR* gene on chromosome 2. This receptor for luteinising hormone (LH) and human chorionic gonadotropin (hCG) is involved in steroidogenesis. Ovarian steroidogenesis may be affected by numerous LHCGR gene single nucleotide polymorphisms (SNPs) (8). The *DENND1A* gene has a crucial role in the clathrin-mediated endocytosis machinery. This gene has two primary transcripts, *DENND1A* variant 1 and *DENND1A* variant 2. The latter is altered in theca cells of women with PCOS (9).

Another GWAS project suggested eight new candidate risk loci for PCOS in the Chinese population, including the follicle-stimulating hormone receptor (*FSHR*) gene (10). The cytochrome P450 17A1 (*CYP17A1*) gene encodes an enzyme called cytochrome P450 17A1, a rate-limiting enzyme in androgen production. Research has demonstrated that women with PCOS exhibit higher activity and expression of this specific enzyme in their ovarian theca cells, thus contributing to hyperandrogenaemia and insulin resistance (11).

This is the first study to explore associations between *DENND1A* rs2479106 and rs10986105, *LHCGR* rs13405728, *FSHR* rs6166, *THADA* rs13429458, and *CYP17A1* rs743572 gene polymorphisms and PCOS anovulatory infertility in Malay women. Additionally, we evaluated the phenotypic effects of the genotypes of these SNPs on clinical and endocrine parameters in infertile women with PCOS (cases) and infertile women without PCOS (controls).

Materials and Methods

Study design

The case control study was designed as an exploratory (preliminary) investigation rather than a full-scale genetic association study, as it is the first study to screen these polymorphisms in the Malay population. The effect size could only be estimated after determining the minor allele frequencies (MAFs) in this population. Consequently, different effect sizes were obtained for each SNP, resulting in different required sample sizes for each polymorphism. A total of 96 participants, 48 cases and 48 controls, were recruited from Serdang Hospital, Putrajaya Hospital, and Sultan Abdul Aziz Shah Hospital. Cases and controls were non-randomly selected and matched by ethnicity and age. Only Malay, non-lactating women aged 20-40 years were recruited for this study. Participants completed a questionnaire that described the research objectives and provided written consent. Written ethical approval and permission were granted by the Medical Research and Ethics Committee, Ministry of Health Malaysia, and Clinical Research Centre of Hospital Serdang, Putrajaya and Hospital Sultan Abdul Aziz Shah (NMRR-18-3175-44106). Written informed consent was obtained from all participants and included on the first pages of the questionnaire.

Cases

The cases were infertile women with PCOS diagnosed according to the Rotterdam criteria who attended the infertility clinics.

Controls

The controls were infertile women with regular menstrual cycles who attended infertility clinics due to male factor infertility, tubal dysfunction, or endometriosis. Those with PCOS and endocrine disorders, including Cushing's syndrome/disease, androgen-secreting tumours, hyperprolactinaemia, congenital adrenal hyperplasia, malignant disease, chronic liver disease, and thyroid disorders were excluded.

Clinical parameters

Clinical evaluation was conducted through interviews and physical examinations. Data about age, menarche, menstrual irregularities, dysmenorrhoea, menorrhagia, type and duration of infertility, ovulation induction, miscarriages, smoking, hypercholesterolaemia, and histories of diabetes and hypertension were obtained from the questionnaire. Each questionnaire was labelled with the participant's medical record number. During the physical examination, height and weight were measured to calculate body mass index (BMI) using the formula BMI=weight (kg)/height (m^2), and blood pressure was recorded for both groups. Information pertaining to PCOS features was obtained by transvaginal ultrasound, and hirsutism was obtained from the patients' medical records.

Endocrine and metabolic evaluation

Routine investigations at the infertility clinics involved obtaining early morning blood samples after at least eight hours of overnight fasting during the early follicular phase. Samples were collected to measure follicle-stimulating hormone (FSH), LH, total testosterone, oestradiol, prolactin, thyroid-stimulating hormone (TSH), free thy-

roxine (FT4), total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and fasting blood glucose (FBS). Additionally, blood samples were taken on day 21 of the menstrual cycle to measure progesterone (D21) and assess ovulation in both groups. TSH and prolactin were specifically measured to exclude infertility due to thyroid disorders and hyperprolactinaemia. The results were subsequently retrieved from the patients' medical records.

Blood sample collection and DNA processing

Five millilitres of venous blood were collected from each participant in Royal Blue K2EDTA BD Vacutainer® tubes. The tubes were transported in an ice bag for DNA extraction on the same day (using fresh blood). Genomic DNA was extracted from the whole blood of anticoagulated samples using the QIAamp® DNA Mini Kit (Qiagen, Germany).

Single nucleotide polymorphism genotyping

Six selected polymorphisms were genotyped, including the intronic variants *DENND1A* rs2479106 (*A/G*) and rs10986105 (*T/G*), *THADA* rs13429458 (*A/C*), and *LHCGR* rs13405728 (*A/G*), as well as the FSHR rs6166 (*C/T*) missense polymorphism and the *CYP17A1* rs743572 (*A/G*) polymorphism located in the 5′ UTR. Primer sequences for these six SNPs were designed using the Primer3 tool (Table S1, See Supplementary Online Information at www.ijfs.ir). The primers were synthesised by Integrated DNA Technologies, Singapore. The predicted effects of the selected SNPs were determined using the Variant effect predictor (VEP) and are presented in Table S2 (See Supplementary Online Information at www.ijfs.ir) (13). Two additional SNPs located within the targeted amplicon of *THADA* rs13429458, rs1317772225 (*C* insertion/deletion) and rs7568365 (*T/C*) were also included. High-resolution melting (HRM) analysis was utilised for genotyping, with amplification performed on the LightCycler® 480 System. HRM analysis was validated by sequencing 10% of the amplified products, which showed 100% concordance with the HRM assays the HRM assays (13).

Statistical analysis

Categorical variables are presented as percentages and analysed using the chi-square test. Biochemical data and genotype comparisons were analysed using the student's t-test and one-way ANOVA for normally distributed data and are presented as mean ± standard deviation (SD). Non-parametric tests, such as the Mann-Whitney U and Kruskal-Wallis tests, were applied for non-normally distributed data and presented as median (Q1-Q3). SNP genotypes were analysed using SNPStats software with univariate logistic regression. SPSS software (IBM Corp., Armonk, NY, USA, version 27) was used to evaluate the phenotypic effects of genotypes on clinical and biochemical parameters. The chi-square test in SPSS was used to determine whether there were significant differences in genotype and allele frequencies between the cases and controls. Additionally, linkage disequilibrium analysis of SNPs on the same chromosome was performed using SNPStats (multivariate logistic regression), generating D′ and r^2 values, as well as haplotype frequencies and their associations with PCOS-related anovulatory infertility.

Results

Clinical and endocrine characteristics of cases and controls

The clinical and endocrine characteristics of the infertile-PCOS and control groups are summarised in Table 1. Statistically significant differences were observed between the two groups in terms of age, weight, BMI, day 21 progesterone levels, testosterone levels, and the presence of dysmenorrhoea ($P<0.05$). Day 21 progesterone levels were notably higher in the control group, reflecting ovulatory cycles, whereas the infertile-PCOS group exhibited significantly lower levels, indicating anovulation. Both groups displayed borderline elevations in LDL and total cholesterol. Menstrual irregularities were observed in 88.9% of the infertile-PCOS group.

Genotype and allele frequency distribution among cases and controls

Only the *LHCGR* rs13405728 and *THADA* rs7568365 polymorphisms showed significant differences in genotype and allele frequencies between cases and controls ($P<0.002$, Table 2). For the rs13405728 SNP, 71% of cases carried the minor *G* allele versus 29% with the major A allele, whereas 45% of controls carried the G allele versus 55% with the A allele ($\chi^2=13.35$, $P<0.001$). For the rs7568365 polymorphism, the minor T allele was more frequent in controls than in cases: 30% of infertile-PCOS cases carried the T allele versus 70% with the major C allele, while 59% of controls carried the T allele and 41% the C allele ($\chi^2=16.5$, $P<0.001$).

Association of the polymorphisms with the risk of polycystic ovary syndrome-anovulatory infertility development

A significant association was found between the *LHCGR* rs13405728 and *THADA* rs7568365 polymorphisms and the development of PCOS-related anovulatory infertility under four inheritance models ($P<0.05$, Table 3). Given that BMI exacerbates PCOS symptoms and contributes to anovulation, and that age impacts ovarian function, odds ratios (OR) were adjusted for both age and BMI. After adjustment, the *LHCGR* rs13405728 polymorphism was associated with PCOS-related anovulatory infertility in the log-additive model, with an OR of 2.63 and a 95% confidence interval (95% CI) of 1.11-6.27 ($P=0.020$). In contrast, the minor T allele of the *THADA* rs7568365 polymorphism exhibited a protective effect in the log-additive model, with an OR of 0.28 (95% CI: 0.08–0.90; $P=0.020$), suggesting that each additional copy of the minor T allele was associated with a 72% reduction in the odds of developing PCOS with anovulatory infertility.

Table 1: Clinical and endocrine characteristics of cases and controls

Variables	Infertile-PCOS	n (%)	Controls	n (%)	P value
Age (Y)	31 (29-34)	38 (79.2)	32.5 (30.2-36)	36 (75)	**0.047**[*a]
Weight (kg)	75.7±19.4	32 (66.7)	62.8 ±14.7	33 (68.8)	**<0.001**[*b]
Height (cm)	157.5±4.5	31 (64.6)	157 ± 6	33 (68.8)	0.725[b]
BMI (kg/m²)	29.7 (25.4-38.2)	32 (66.7)	24.5 (20-29.4)	32 (66.7)	<0.002[*a]
Systolic BP (mmHg)	122.1±10.1	21 (43.8)	119.9 ± 11.8	19 (39.6)	0.540[b]
Diastolic BP (mmHg)	76.5±7.5	21 (43.8)	75.7 ± 8.1	19 (39.6)	0.739[b]
FSH (mIU/ml)	5.5 (4.4-6.8)	32 (66.7)	6 (5.5-8)	25 (52.1)	0.075[a]
LH (mIU/ml)	6.5 (4-9.5)	33 (68.8)	4.6 (3.3-7.2)	25 (52.1)	0.084[a]
D21 prog (nmol/L)	1.4 (0.6-17.1)	17 (35.4)	35.5 (12.8-55.9)	23 (47.0)	**<0.001**[*a]
Testosterone (nmol/L)	1.3 (0.8-2)	20 (41.7)	0.7 (0.65-1.2)	9 (18.8)	**0.048**[*a]
Oestradiol (pg/ml)	175.6 (122.6-782.4)	15 (31.3)	299.9 (178.8-786.8)	13 (27.1)	0.345[a]
Prolactin (mIU/L)	227.4 (124.7-326)	30 (62.5)	306.2 (170.4-442.1)	18 (37.5)	0.217[a]
TSH (mIU/L)	2 (1.3-3.9)	23 (47.9)	1.37 (0.7-2.1)	11 (22.9)	0.058[a]
FT4 (Pmol/L)	13.5 (11.7-16)	27 (56.3)	13.5 (12.1-18)	18 (37.5)	0.899[a]
FBS (mmol/L)	4.9 (4.1-7.1)	13 (27.1)	4.6 (4-4.8)	9 (18.8)	0.270[a]
Total cholesterol (mmol/L)	6.1 (5.1-9.3)	18 (37.5)	5.9 (3.8-7.9)	14 (29.2)	0.287[a]
Triglycerides (mmol/L)	1.4 (1-5.9)	18 (37.5)	1 (0.8-2)	16 (33.3)	0.136[a]
HDL (mmol/L)	1.6 (1.2-15)	18 (37.5)	1.7 (1.4-8.9)	9 (18.8)	0.572[a]
LDL (mmol/L)	4.3 (3.2-12.5)	18 (37.5)	3.8 (2.9-6.7)	10 (20.8)	0.291[a]
Menarche[c]	12 (12-13)	27 (56.5)	13 (12-14)	28 (58.3)	0.126
Dysmenorrhoea[c]	4 (14.8%)	27 (56.5)	13 (46.4%)	28 (58.3)	**0.011**[*]
Menorrhagia[c]	4 (14.8%)	27 (56.5)	1 (3.6%)	28 (58.3)	0.327
Primary infertility[c]	19 (70.4%)	27 (56.5)	16 (66.7%)	28 (58.3)	0.776
Infertility duration (Y)[c]	4.5 (3-7)	27 (56.5)	5 (3-8)	28 (58.3)	0.794
Ovulation induction[c]	10 (37%)	27 (56.5)	4 (14.3%)	28 (58.3)	0.053
Miscarriages[c]	5 (20%)	27 (56.5)	5 (17.9%)	28 (58.3)	0.842
Menstrual irregularities[c]	24 (88.9%)	27 (56.5)	-	28 (58.3)	-
Hirsutism[c]	2 (7%)	27 (56.5)	0	28 (58.3)	-
PCOS features on US[c]	17 (65.4%)	27 (56.5)	0	28 (58.3)	
Smoking[c]	0	27 (56.5)	0	28 (58.3)	-
Hypercholesterolemia	5 (18.5%)	27 (56.5)	1 (3.6%)	28 (58.3)	0.179
Diabetic[c]	3 (11.1%)	27 (56.5)	0	28 (58.3)	0.222
Hypertensive[c]	2 (7.4%)	27 (56.5)	0	27 (58.3)	0.455

PCOS; Polycystic ovary syndrome, BMI; Body mass index, FSH; Follicle-stimulating hormone, LH; Luteinising hormone, D21 Prog; Day 21 progesterone, TSH; Thyroid-stimulating hormone, FT4; Free thyroxine, FBS; Fasting blood sugar, HDL; High-density lipoprotein, LDL; Low-density lipoprotein, US; Ultrasound, *; Significance at P<0.05, a; Mann-Whitney test (non-parametric) data with non-normal distribution are presented by median (Q1-Q3), b; Student's t test (parametric test) data with normal distribution are presented by mean ± standard deviation (SD), and c; Pearson chi-square and Fisher's exact tests were used to measure the significant differences in categorical data.

Table 2: Genotype and allele distribution in cases and controls

Variant	Genotype	Infertile-PCOS (n=48)	Controls (n=48)	χ^2	P value	OR (95% CI)
DENND1A [a]rs2479106	AA	28 (58%)	32 (68%)	1.01	0.602	
	AG	14 (29%)	10 (21%)			
	GG	6 (12%)	5 (11%)			
	H-W test	0.074	0.019			
	alleles					
	A	70 (73%)	74 (79%)	0.87	0.350	0.72 (0.37-1.41)
	G	26 (27%)	20 (21%)			1.37 (0.70-2.68)
DENND1A rs10986105	TT	47 (98%)	48 (100%)	0.00	1.000	
	TG	1 (2%)	0			
	GG	0	0			
	H-W test	1	1			
	alleles					
	T	95 (99%)	96 (100%)	0.00	1.000	-
	G	1 (1%)	0			
LHCGR rs13405728	AA	5 (10%)	15 (31%)	12.03	**0.002***	
	AG	18 (38%)	23 (48%)			
	GG	25 (52%)	10 (21%)			
	H-W test	0.5	1			
	alleles					
	A	28 (29%)	53 (55%)	13.35	**<0.001***	0.33 (0.18-0.6)
	G	68 (71%)	43 (45%)			2.99 (1.64-5.43)
FSHR rs6166	CC	7 (15%)	10 (21%)	0.80	0.670	
	CT	18 (38%)	15 (31%)			
	TT	23 (48%)	23 (48%)			
	H-W test	0.33	0.029			
	alleles					
	C	32 (33%)	35 (36%)	0.21	0.646	0.87 (0.48-1.57)
	T	64 (67%)	61 (64%)			1.14 (0.63-2.07)
THADA rs13429458	AA	34 (71%)	37 (77%)	0.48	0.485	
	AC	14 (29%)	11 (23%)			
	CC	0	0			
	H-W test	0.57	1			
	alleles					
	A	82 (85%)	85 (89%)	0.41	0.521	0.75 (0.32-1.76)
	C	14 (15%)	11 (11%)			1.31 (0.56-3.07)
THADA rs7568365	CC	21 (44%)	5 (10%)	19.14	**<0.001***	
	CT	25 (52%)	29 (60%)			
	TT	2 (4%)	14 (29%)			
	H-W test	0.17	0.13			
	alleles					
	C	67 (70%)	39 (41%)	16.51	**<0.001***	3.37 (1.85-6.13)
	T	29 (30%)	57 (59%)			0.29 (0.16-0.53)
THADA rs1317772225	Alleles					
	-/InsC	11 (23%)	10 (21%)	0.06	0.805	1.12 (0.42-2.97)
	-/-	37 (77%)	38 (79%)			0.88 (0.33-2.33)
CYP17A1 rs743572	AA	20 (42%)	28 (58%)	2.66	0.102	
	AG	28 (58%)	20 (42%)			
	GG	0	0			
	H-W test	0.0043	0.00061			
	alleles					
	A	68 (71%)	76 (79%)	1.78	1.182	0.63 (0.33-1.23)
	G	28 (29%)	20 (21%)			1.56 (0.80-3.02_

OR; Odds ratio, 95% CI; 95% confidence interval, PCOS; Polycystic ovary syndrome, H-W test; Hardy-Weinberg test, a; One genotype from the rs2479106 control samples was excluded from the analysis due to a lack of validation, and *; Significant results were shown in bold (P<0.05).

Table 3: Association of SNPs with the risk of development of PCOS-anovulatory infertility under multiple inheritance models

Variant	Model	Genotype	Infertile-PCOS n (%)	Controls n (%)	OR (95% CI)	P value	AIC	OR (95% CI)[a]	P valuea	AIC[a]
DENND1A rs2479106	Codominant	A/A	28 (58.3%)	32 (68.1%)	1.00			1.00		
		A/G	14 (29.2%)	10 (21.3%)	1.60 (0.61-4.17)			3.37 (0.83-13.66)		
		G/G	6 (12.5%)	5 (10.6%)	1.37 (0.38-4.99)	0.600	136.7	1.01 (0.20-5.01)	0.190	76.3
	Dominant	A/A	28 (58.3%)	32 (68.1%)	1.00			1.00		
		A/G-G/G	20 (41.7%)	15 (31.9%)	1.52 (0.66-3.53)	0.320	134.7	2.12 (0.65-6.86)	0.210	76.1
	Recessive	A/A-A/G	42 (87.5%)	42 (89.4%)	1.00			1.00		
		G/G	6 (12.5%)	5 (10.6%)	1.20 (0.34-4.24)	0.780	135.6	0.66 (0.15-3.01)	0.590	77.4
	Overdominant	A/A-G/G	34 (70.8%)	37 (78.7%)	1.00			1.00		
		A/G	14 (29.2%)	10 (21.3%)	1.52 (0.60-3.88)	0.380	134.9	3.36 (0.88-12.87)	0.060	74.3
	Log-additive	-	-	-	1.28 (0.71-2.29)	0.410	135	1.24 (0.58-2.67)	0.580	77.4
	Codominant	A/A	5 (10.4%)	15 (31.2%)	1.00			1.00		
		A/G	18 (37.5%)	23 (47.9%)	2.35 (0.72-7.68)			3.17 (0.54-18.58)		
		G/G	25 (52.1%)	10 (20.8%)	**7.50 (2.15-26.17)**	**0.001***	126.6	7.40 (1.19-46.15)	0.060	75.1
	Dominant	A/A	5 (10.4%)	15 (31.2%)	1.00			1.00		
		A/G-G/G	43 (89.6%)	33 (68.8%)	**3.91 (1.29-11.85)**	**0.010***	130.5	4.71 (0.89-25.00)	0.050	74.8
	Recessive	A/A-A/G	23 (47.9%)	38 (79.2%)	1.00			1.00		
		G/G	25 (52.1%)	10 (20.8%)	**4.13 (1.68-10.14)**	**0.001***	126.7	3.21 (0.94-10.93)	0.050	74.9
	Overdominant	A/A-G/G	30 (62.5%)	25 (52.1%)	1.00			1.00		
		A/G	18 (37.5%)	23 (47.9%)	0.65 (0.29-1.47)	0.300	136	0.80 (0.25-2.53)	0.700	78.4
	Log-additive	-	-	-	**2.81 (1.52-5.19)**	**0.001***	124.7	**2.63 (1.11-6.27)**	**0.020***	73.2
FSHR rs6166										
	Codominant	C/C	7 (14.6%)	10 (20.8%)	1.00			1.00		
		C/T	18 (37.5%)	15 (31.2%)	1.71 (0.52-5.60)			0.87 (0.16-4.77)		
		T/T	23 (47.9%)	23 (47.9%)	1.43 (0.46-4.40)	0.670	138.3	2.52 (0.52-12.12)	0.250	77.8
	Dominant	C/C	7 (14.6%)	10 (20.8%)	1.00			1.00		
		C/T-T/T	41 (85.4%)	38 (79.2%)	1.54 (0.53-4.46)	0.420	136.4	1.66 (0.39-7.02)	0.490	78.1
	Recessive	C/C-C/T	25 (52.1%)	25 (52.1%)	1.00			1.00		
		T/T	23 (47.9%)	23 (47.9%)	1.00 (0.45-2.23)	1.000	137.1	2.72 (0.80-9.24)	0.090	75.8
	Overdominant	C/C-T/T	30 (62.5%)	33 (68.8%)	1.00			1.00		
		C/T	18 (37.5%)	15 (31.2%)	1.32 (0.57-3.07)	0.520	136.7	0.46 (0.13-1.70)	0.240	77.2
	Log-additive	-	-	-	1.12 (0.66-1.90)	0.680	136.9	1.72 (0.80-3.73)	0.160	76.5
THADA rs7568365										
	Codominant	C/C	21 (43.8%)	5 (10.4%)	1.00			1.00		

Table 3: Continued

Variant	Model	Genotype	Infertile-PCOS n (%)	Controls n (%)	OR (95% CI)	P value	AIC	OR (95% CI)[a]	P valuea	AIC[a]
		C/T	25 (52.1%)	29 (60.4%)	0.21 (0.07-0.62)			0.41 (0.08-1.96)		
		T/T	2 (4.2%)	14 (29.2%)	**0.03 (0.01-0.20)**	**<0.001***	118.1	0.06 (0.00-0.80)	0.050	74.7
	Dominant	C/C	21 (43.8%)	5 (10.4%)	1.00			1.00		
		C/T-T/T	27 (56.2%)	43 (89.6%)	**0.15 (0.05-0.44)**	**<0.001***	122.8	0.31 (0.07-1.48)	0.130	76.2
	Recessive	C/C-C/T	46 (95.8%)	34 (70.8%)	1.00			1.00		
		T/T	2 (4.2%)	14 (29.2%)	**0.11 (0.02-0.50)**	**<0.001***	125.2	0.12 (0.01-1.18)	0.030	74
	Overdominant	C/C-T/T	23 (47.9%)	19 (39.6%)	1.00			1.00		
		C/T	25 (52.1%)	29 (60.4%)	0.71 (0.32-1.60)	0.410	136.4	1.12 (0.34-3.72)	0.850	78.5
	Log-additive	-	-	-	**0.19 (0.08-0.43)**	**<0.001***	116.1	**0.28 (0.08-0.90)**	**0.020***	73.2

PCOS; Polycystic ovary syndrome, SNP; Single nucleotide polymorphisms, OR; Odds ratio, 95% CI; 95% confidence interval, AIC; Akaike information criteria, *; Significant results are shown in bold (P<0.05), and a; Adjusted for age and body mass index (BMI) from 31 genotypes of infertile-PCOS cases and 31 genotypes from the controls.

Linkage disequilibrium and haplotype analysis

The SNPs in the *DENND1A* gene (rs2479106 and rs10986105) showed almost complete linkage disequilibrium, with a D′ of 0.94 (Table S3, See Supplementary Online Information at www.ijfs.ir). However, the low correlation coefficient (r^2) is attributed to the rare minor allele frequency of rs10986105 in our screened samples. Haplotype frequencies were also measured in the infertile-PCOS and control groups (Table S4, See Supplementary Online Information at www.ijfs.ir). Linkage disequilibrium and haplotype analyses were further conducted for the *THADA* rs13429458 polymorphism and its two neighbouring SNPs, rs7568365 and rs1317772225 (Tables S5, 6, See Supplementary Online Information at www.ijfs.ir). The A-T haplotype was more frequent in controls than in the infertile-PCOS group (OR=0.13, 95% CI: 0.05-0.35; P=0.001), indicating a protective effect that reduces the risk of PCOS-related anovulatory infertility by 87%. The relationships among rs13405728 (2p16.3), rs6166 (2p21), and rs13429458 (2p21), all located on the short arm of chromosome 2, were assessed (Table S7, See Supplementary Online Information at www.ijfs.ir). The ATA haplotype was more frequent in controls than in infertile-PCOS cases, decreasing the risk of PCOS-related anovulatory infertility by 81% (OR=0.19, 95% CI: 0.06-0.57; P=0.003). Similarly, the ACA haplotype showed a protective effect, reducing the risk by 70% (OR=0.30, 95% CI: 0.09-0.95; P=0.044) (Table S8, See Supplementary Online Information at www.ijfs.ir).

Association of the single nucleotide polymorphisms genotypes with the clinical and endocrine parameters of cases and controls

A significant difference in triglyceride levels among rs2479106 genotypes was observed in the infertile-PCOS group (P=0.013, Table S9, See Supplementary Online Information at www.ijfs.ir). Similarly, triglyceride levels differed among rs13405728 genotypes in the control group (P=0.037, Table S10, See Supplementary Online Information at www.ijfs.ir). Diastolic blood pressure was higher in cases with the AA genotype of rs13429458 compared to those with the *AC* genotype (P=0.001, Table S11, See Supplementary Online Information at www.ijfs.ir). There were no statistically significant differences in the clinical and endocrine parameters among the genotypes of rs10986105, rs6166, and rs743572 (Tables S12, S13, and S14, See Supplementary Online Information at www.ijfs.ir).

Discussion

The wide, complex clinical spectrum in PCOS indicates the heterogeneous pathogenesis of this disease and the involvement of multiple genes. A combination of genetic interactions and environmental factors is believed to play a crucial role in its predisposition (14).

PCOS is a major cause of anovulatory infertility (15) and is estimated to account for approximately 40% of female infertility (16). The condition was first characterised in 1972 with the observation of infertile women presenting with small, shiny ovaries (17). In our replication study, we aimed to investigate the association of selected PCOS-susceptible loci in Malay women who phenotypically presented with anovulatory infertility. Our intent was to identify which polymorphisms play a crucial role in the development of infertility related to this condition. The *DENND1A* gene (also known as connecedin1) produces two main transcripts through alternative splicing: *DENND1A* variant 1 and *DENND1A* variant 2 (18). A study utilising theca cells obtained from women with normal menstrual cycles and those with PCOS provided initial evidence of a functional connection between ele-

vated *DENND1A.V2* and *CYP17A1* expression, leading to increased androgen production in PCOS theca cells (19). We investigated the association between the two most screened polymorphisms, rs2479106 and rs10986105, and found no association between these polymorphisms and PCOS-anovulatory infertility. Similarly, a study on Arab women from Bahrain found no association between the two polymorphisms (20). Another study on North African Arabs from Tunisia reported an association with rs10986105, but no association with rs2479106 (9). The frequency of the rs10986105 minor allele is 0.018 in East Asians; in our study, it was 0.01, as reported by the National Centre for Biotechnology Information, and it is 0.055 in Europeans. This suggests the need for a large sample size to find an association in Asians. Nonetheless, we did not observe any association for rs2479106, which might be attributed to the small sample size. The *DENND1A* gene may contribute to the development of PCOS through pathways related to metabolic disorders and insulin resistance, as suggested by Tian et al. (21). We observed a significant difference in infertile-PCOS cases with the homozygous mutant GG genotype of rs2479106, which had notably elevated triglyceride levels compared to the other genotypes. Elevated triglyceride levels are a key component of metabolic syndrome. Obesity, on the other hand, is an essential element in metabolic syndrome and dyslipidaemia through multiple pathways (22). Notably, patients with the GG genotype were obese with a mean BMI of 34.8 ± 7.5, which could solely lead to the high triglyceride levels observed in this genotype.

LHCGR is primarily expressed in the granulosa cells of preovulatory follicles and constitutively expressed in theca cells of the ovaries. In the later stages of follicular development, it triggers ovulation in response to the mid-cycle LH surge (23). The LH level remained within the normal range in the infertile-PCOS group, with no biochemical evidence of hyperandrogenism observed in this group. This finding supports studies where non-obese women with PCOS exhibit elevated levels of LH secreted by the pituitary gland, increased LH activity, and abundant production of androgens in the ovaries in response to LH compared to obese PCOS women (24). The *LHCGR* rs13405728 polymorphism was identified as one of the three susceptible loci associated with PCOS in the initial GWAS investigation conducted in the Han Chinese population (7). Another study on Indian ethnicity reported an association with PCOS (25). Similarly, our study found that the rs13405728 polymorphism was strongly associated with PCOS cases that presented with anovulatory infertility, with an OR of 2.63 (95% CI: 1.11-6.27; P=0.020) after adjustments for age and BMI.

In contrast to our findings, there was no observed association between the *LHCGR* rs13405728 polymorphism and Caucasian populations (26). The infertile-PCOS group had higher BMI, with a median of 29.7 kg/m² (IQR: 25.4-38.2), compared to the control group median of 24.5 kg/m² (IQR: 20-29.4). This difference was statistically significant (P<0.002). It is well-documented that women with PCOS frequently have an elevated BMI compared to those without PCOS. This observation highlights a consistent correlation between PCOS and increased BMI (27). The association between PCOS and obesity is intricate and lacks a straightforward causative relationship. Both conditions have the potential to interact and exacerbate each other's symptoms, making it challenging to pinpoint whether PCOS directly leads to obesity or vice versa (28). Furthermore, obesity exacerbates PCOS symptoms and contributes to anovulation (29), which was typically seen in our study. Accordingly, our results indicate that Malay women have a 2.6-fold increased propensity to develop PCOS, characterised by anovulatory infertility, with each copy of the minor allele G of the *LHCGR* rs13405728 polymorphism. This finding further supports the conclusions of the initial genotype-phenotype study identified by GWAS in a large cohort of Han Chinese women, which suggested that the *LHCGR* rs13405728 polymorphism may contribute to anovulation in PCOS (30). Mutations that inactivate *LHCGR* are linked to irregular menstruation and infertility in women (23). Our study is the first to highlight an association between the *LHCGR* rs13405728 polymorphism and PCOS-related infertility; however, the precise mechanism by which this polymorphism influences *LHCGR* function remains undetermined. This finding may pave the way for early screening of PCOS in Malay women, as well as for predicting the development of infertility. Despite the limitation of our small sample size, we observed a notably higher minor allele frequency of 0.71 in the infertile-PCOS group compared to 0.45 in the control group. Our findings demonstrated a statistical power of 74%, suggesting a reasonable confidence level in the results.

FSHR is regulated by FSH and plays a crucial role in promoting granulosa cell proliferation, differentiation, and the development of antral follicles (31). Two missense polymorphisms in the *FSHR* gene, Ala307Thr (rs6165) and Ser680Asn (rs6166), have been extensively studied and characterised (32). Given that PCOS is characterised by follicular growth failure, numerous studies have thoroughly examined the relationship between *FSHR* Thr307Ala or Asn680Ser coding sequence changes and PCOS, with varying and disputed findings (33). Our research shows no apparent link between the *FSHR* rs6166 missense polymorphism (Asn680Ser) and PCOS. This conclusion is in line with a study that examined Han Chinese women in northern China and found no association with the rs6166 polymorphism (34). Another study conducted on European individuals also failed to find an association with this polymorphism (35).

The *THADA* rs13429458 polymorphism, one of the first three loci identified as associated with PCOS in the initial GWAS, was investigated in our study of infertile anovulatory Malay women with PCOS. We observed no association between this gene polymorphism and PCOS-anovulatory infertility, with only two genotypes identified. Similar findings of no association were reported in various other ethnic groups and populations, including European-derived cohorts (36), Iranian (37), and Colombian women (2).

Several case-control studies have explored the potential correlation between the *CYP17A1 -34T/C* polymorphism (rs743572) and PCOS, given the crucial role of the 17α-hydroxylase/17,20-lyase enzyme in the hyperandrogenism that characterises PCOS (38). The rs743572 polymorphism, located in the promoter region of the *CYP17A1* gene, regulates gene expression and may promote increased androgen synthesis (39). Our investigation found no association between the *CYP17A1* rs743572 polymorphism and the risk of developing PCOS-related anovulatory infertility, with only two genotypes detected. The infertile-PCOS group had normal total testosterone levels; only two cases presented with hirsutism, which may explain the lack of an association. Recent research on the phenotypic classification of infertile Malay women with PCOS found that they predominantly have phenotype D, characterised by oligo-anovulation and polycystic ovarian morphology on ultrasound, but without hyperandrogenism. These findings are consistent with our results (40). A larger sample size is recommended for future research. Further categorising PCOS cases according to their phenotypes and investigating the association of the polymorphisms with each subgroup would be advantageous. Additionally, including an assessment of insulin resistance and determining the prevalence of metabolic syndrome would allow for more comprehensive comparisons.

Conclusion

This study demonstrates a significant association between the *LHCGR* rs13405728 polymorphism and the development of anovulatory infertility in Malay women with PCOS. Our finding underscores the critical role of the *LHCGR* gene in the anovulatory presentation of PCOS. This polymorphism may serve as an early predictive and prognostic biomarker for the onset of PCOS and subsequent infertility in Malay adolescent girls and married women affected by this condition. Early interventions could include lifestyle modifications, weight reduction, and dietary adjustments. Conversely, no associations were found between *DENND1A* rs2479106 and rs10986105, *FSHR* rs6166, *THADA* rs13429458, or *CYP17A1* rs743572 polymorphisms and the development of PCOS-related anovulatory infertility. Interestingly, the minor allele of the *THADA* rs7568365 polymorphism, which has not been previously reported, appeared to exert a protective effect against the development of PCOS-related anovulatory infertility.

Acknowledgements

This study was funded by Universiti Putra Malaysia through the Geran Putra Berimpak (Grant No. 9658500, 2018).

Conflict of Interest

There is no conflict of interest in this study.

Authors' Contributions

M.E.; Conceptualisation, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualisation, Roles/Writing-original draft, and Writing-review and editing. H.A.H.; Conceptualisation, Data curation, Funding acquisition, Methodology, Project administration, Resources, Supervision, Visualisation, Roles/Writing-original draft, and Writing-review and editing. Z.A.; Methodology, Supervision, Validation, Visualisation, Roles/Writing-original draft, and Writing-review and editing. K.-H.L.; Conceptualisation, Formal analysis, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualisation, Roles/Writing-original draft, and Writing-review and editing.

References

1. Park S, Liu M, Zhang T. THADA -rs13429458 minor allele increases the risk of polycystic ovary syndrome in Asian, but not in caucasian women: a systematic review and meta-analysis. Horm Metab Res. 2019; 51: 661-670. doi: 10.1055/a-0969-1872
2. Alarcón-Granados MC, Moreno-Ortíz H, Esteban-Pérez CI, Ferrebuz-Cardozo A, Camargo-Villalba GE, Forero-Castro M. Assessment of THADA gene polymorphisms in a sample of Colombian women with polycystic ovary syndrome: a pilot study. Heliyon. 2022; 8(6): e09673. doi: 10.1016/j.heliyon.2022.e09673
3. Kumar P, Clark M. Kumar & Clark's clinical medicine. Feather A, Randall D, Waterhouse M, editors. 10th ed. 2021; London; Elsevier: 2020.
4. Dadachanji R, Sawant D, Patil A, Mukherjee S. Replication study of THADA rs13429458 variant with PCOS susceptibility and its related traits in Indian women. Gynecol Endocrinol. 2021; 37(8): 716-720. doi: 10.1080/09513590.2021
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. doi: 10.1186/s12958-016-0173-x
6. Khan MJ, Ullah A, Basit S. Genetic basis of polycystic ovary syndrome (PCOS): current perspectives. Appl Clin Genet. 2019; 12: 249-260. doi: 10.2147/TACG.S200341
7. 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. doi: 10.1038/ng.732
8. Ramanathan B, Murugan J, Velayutham K. Pilot study on evaluation and determination of the prevalence of polycystic ovarian syndrome (PCOS) associated gene markers in the South Indian population. Indian J Endocrinol Metab. 20211; 25(6): 551-558.
9. Dallel M, Sarray S, Douma Z, Hachani F, Al-Ansari AK, Letaifa DB, et al. Differential association of DENND1A genetic variants with polycystic ovary syndrome in Tunisian but not Bahraini Arab women. Gene. 2018; 647: 79-84. doi: 10.1016/j.gene.2018.01.028
10. Shi Y, Zhao H, Shi Y, Cao Y, Yang D, Li Z, et al. Genome-wide association study identifies eight new risk loci for polycystic ovary syndrome. Nat Genet. 2012; 44(9): 1020-1025. doi: 10.1038/ng.2384
11. Chaudhary H, Patel J, Jain NK, Joshi R. The role of polymorphism in various potential genes on polycystic ovary syndrome susceptibility and pathogenesis. J Ovarian Res. 2021; 14(1): 125. doi: 10.1186/s13048-021-00879-w
12. McLaren W, Gil L, Hunt SE, Riat HS, Ritchie GR, Thormann A, et al. The ensembl variant effect predictor. Genome Biol. 2016; 17(1): 122. doi: 10.1186/s13059-016-0974-4
13. Elbadie M, Hamid HA, Ahmad Z, Ling KH. Validated high-resolution melting assays for efficient screening of DENND1A, LHCGR, FSHR, THADA, and CYP17A1 polycystic ovary syndrome-associated gene polymorphisms. Malaysian Journal of Medicine and Health Sciences. 2025; 21(6): 1-9. doi: 10.47836/mjmhs.v21.i6.945
14. Parker J, O'Brien C, Hawrelak J, Gersh FL. Polycystic ovary syndrome: an evolutionary adaptation to lifestyle and the environment. Int J Environ Res Public Health. 2022; 19(3): 1336. doi: 10.3390/ijerph19031336
15. Morgante G, Massaro MG, Di Sabatino A, Cappelli V, De Leo V. Therapeutic approach for metabolic disorders and infertility in women with PCOS. Gynecol Endocrinol. 2018; 34(1): 4-9. doi: 10.1080/09513590.2017.1370644

16. Krysiak R, Okopień B, Gdula-Dymek A, Herman ZS. Update on the management of polycystic ovary syndrome. Pharmacol Rep. 2006; 58(5): 614-625.
17. Insler V, Lunenfeld B. Polycystic ovarian disease: a challenge and controversy. Gynecol Endocrinol. 1990; 4(1): 51-70. doi: 10.3109/09513599009030691
18. McAllister JM, Legro RS, Modi BP, Strauss JF 3rd. Functional genomics of PCOS: from GWAS to molecular mechanisms. Trends Endocrinol Metab. 2015; 26(3): 118-124. doi: 10.1016/j.tem.2014.12.004
19. McAllister JM, Modi B, Miller BA, Biegler J, Bruggeman R, Legro RS, et al. Overexpression of a DENND1A isoform produces a polycystic ovary syndrome theca phenotype. Proc Natl Acad Sci USA. 2014; 111(15): E1519-E1527. doi: 10.1073/pnas.1400574111
20. Gammoh E, Arekat MR, Saldhana FL, Madan S, Ebrahim BH, Almawi WY. DENND1A gene variants in Bahraini Arab women with polycystic ovary syndrome. Gene. 2015; 560(1): 30-33. doi: 10.1016/j.gene.2015.01.034
21. Tian Y, Li J, Su S, Cao Y, Wang Z, Zhao S, et al. PCOS-GWAS susceptibility variants in THADA, INSR, TOX3, and DENND1A are associated with metabolic syndrome or insulin resistance in women with PCOS. Front Endocrinol (Lausanne). 2020; 11: 274. doi: 10.3389/fendo.2020.00274
22. Chait A, den Hartigh LJ. Adipose tissue distribution, inflammation and its metabolic consequences, including diabetes and cardiovascular disease. Front Cardiovasc Med. 2020; 7: 22. doi: 10.3389/fcvm.2020.00022
23. Troppmann B, Kleinau G, Krause G, Gromoll J. Structural and functional plasticity of the luteinizing hormone/choriogonadotrophin receptor. Hum Reprod Update. 2013; 19(5): 583-602. doi: 10.1093/humupd/dmt023
24. Castillo-Higuera T, Alarcón-Granados MC, Marin-Suarez J, Moreno-Ortiz H, Esteban-Pérez CI, Ferrebuz-Cardozo AJ, et al. A comprehensive overview of common polymorphic variants in genes related to polycystic ovary syndrome. Reprod Sci. 2021; 28(9): 2399-2412. doi: 10.1007/s43032-020-00375-4
25. Vishnubotla DS, Shek AP, Madireddi S. Pooled genetic analysis identifies variants that confer enhanced susceptibility to PCOS in Indian ethnicity. Gene. 2020; 752: 144760. doi: 10.1016/j.gene.2020.144760
26. Eriksen MB, Brusgaard K, Andersen M, Tan Q, Altinok ML, Gaster M, et al. Association of polycystic ovary syndrome susceptibility single nucleotide polymorphism rs2479106 and PCOS in Caucasian patients with PCOS or hirsutism as referral diagnosis. Eur J Obstet Gynecol Reprod Biol. 2012; 163(1): 39-42. doi: 10.1016/j.ejogrb.2012.03.020
27. Neubronner SA, Indran IR, Chan YH, Thu AWP, Yong EL. Effect of body mass index (BMI) on phenotypic features of polycystic ovary syndrome (PCOS) in Singapore women: a prospective cross-sectional study. BMC Womens Health. 2021; 21(1): 135. doi: 10.1186/s12905-021-01277-6
28. Barber TM, Hanson P, Weickert MO, Franks S. Obesity and polycystic ovary syndrome: implications for pathogenesis and novel management strategies. Clin Med Insights Reprod Health. 2019; 13: 1179558119874042. doi: 10.1177/1179558119874042
29. Aggarwal M, Chakole S. Prevalence of polycystic ovarian syndrome and its link to obesity in adolescent girls. Cureus. 2023; 15(9): e45405. doi: 10.7759/cureus.45405
30. Cui L, Zhao H, Zhang B, Qu Z, Liu J, Liang X, et al. Genotype-phenotype correlations of PCOS susceptibility SNPs identified by GWAS in a large cohort of Han Chinese women. Hum Reprod. 2013; 28(2): 538-544. doi: 10.1093/humrep/des424
31. Homburg R. Ovulation induction and controlled ovarian stimulation. 2nd ed. Cham: Springer International Publishing; 2014. doi: 10.1007/978-3-319-05612-8_1
32. Singhasena W, Pantasri T, Piromlertamorn W, Samchimchom S, Vutyavanich T. Follicle-stimulating hormone receptor gene polymorphism in chronic anovulatory women, with or without polycystic ovary syndrome: a cross-sectional study. Reprod Biol Endocrinol. 2014; 12: 86. doi: 10.1186/1477-7827-12-86
33. Kim JJ, Choi YM, Hong MA, Chae SJ, Hwang K, Yoon SH, et al. FSH receptor gene p. Thr307Ala and p. Asn680Ser polymorphisms are associated with the risk of polycystic ovary syndrome. J Assist Reprod Genet. 2017; 34(8): 1087-1093. doi: 10.1007/s10815-017-0953-z
34. Wu XQ, Xu SM, Liu JF, Bi XY, Wu YX, Liu J. Association between FSHR polymorphisms and polycystic ovary syndrome among Chinese women in north China. J Assist Reprod Genet. 2014; 31(3): 371-377. doi: 10.1007/s10815-013-0166-z
35. Lidaka L, Bekere L, Rota A, Isakova J, Lazdane G, Kivite-Urtane A, et al. Role of single nucleotide variants in FSHR, GNRHR, ESR2 and LHCGR genes in adolescents with polycystic ovary syndrome. Diagnostics (Basel). 2021; 11(12): 2327. doi: 10.3390/diagnostics11122327
36. 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. doi: 10.1136/jmedgenet-2011-100427
37. Naserpoor L, Jannatifar R, Roshanaei K, Khoshandam M, Kallhor N. Association of rs13429458 and rs12478601 single nucleotide polymorphisms of THADA gene with polycystic ovary syndrome. Int J Fertil Steril. 2022; 16(1): 36-41. doi: 10.22074/IJFS.2021.524795.1090.
38. Franks S, White D, Gilling-Smith C, Carey A, Waterworth D, Williamson R. Hypersecretion of androgens by polycystic ovaries: the role of genetic factors in the regulation of cytochrome P450c17 alpha. Baillieres Clin Endocrinol Metab. 1996; 10(2): 193-203. doi: 10.1016/s0950-351x(96)80057-7
39. Xu X, Hu K, Shi H, Yu Y, Xu J, Sun Y. The single-nucleotide polymorphism rs743572 of CYP17A1 shows significant association with polycystic ovary syndrome: a meta-analysis. Reprod Biomed Online. 2021; 43(5): 941-951. doi: 10.1016/j.rbmo.2021.06.012
40. Azreena N, Aziz A, Balkis Budin S, Azizuddin M, Yussof M, Rajab NF, et al. Phenotype classification and risk factors of polycystic ovary syndrome among infertility patients treated at subfertility clinic, national population and family development board Malaysia. Malaysian Journal of Medicine and Health Sciences. 2024; 20(4): 292-300.