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Association of Luteinizing Hormone/Choriogonadotropin Receptor rs2293275 Polymorphism with Polycystic Ovary Syndrome Susceptibility and Reproductive Outcomes in an Iraqi Population

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Abstract

Background

Polycystic ovary syndrome (PCOS) is a complex endocrine disorder with a polygenic etiology. Although luteinizing hormone/chorionic gonadotropin receptor (LHCGR) gene variants influence PCOS susceptibility globally, data on the Iraqi population remain absent.

Objective

To evaluate the association between the LHCGR rs2293275 polymorphism and PCOS clinical symptoms, including hirsutism, menstrual irregularities, and reproductive hormones, in Iraqi women.

Methodology

: A case-control study included 100 PCOS women (Rotterdam 2003 criteria) and 50 age-matched healthy controls. Clinical, anthropometric, and hormonal (FSH, LH, PRL, E2, testosterone, AMH) assessments were performed. Genomic DNA was extracted, and the rs2293275 variant was genotyped using PCR and Sanger sequencing in a subset (32 patients, 31 controls).

Results

PCOS patients exhibited significantly higher BMI, testosterone, AMH, and LH/FSH ratios compared to controls ($p < 0.05$). The rs2293275 GG genotype was significantly more frequent in PCOS patients (50%) than controls (23%), increasing PCOS risk 7.5-fold (OR = 7.5, 95% CI: 1.78–31.04; $p = 0.004$). The G allele was an independent risk factor (OR = 3.3, $p = 0.002$). Furthermore, the GG genotype was associated with significantly elevated AMH levels ($p = 0.002$), which strongly predicted menstrual irregularity ($p < 0.001$). A novel synonymous variant (c.805T > C) was also identified upstream of rs2293275.

Conclusion

The LHCGR rs2293275 GG genotype and G allele are significant risk factors for PCOS in Iraqi women, strongly correlating with elevated AMH levels. This genetic variant may mechanistically contribute to the follicular arrest and anovulatory phenotypes observed in this population, highlighting its potential as a molecular risk marker.

1 Introduction

Polycystic ovarian syndrome (PCOS, Stein-Leventhal syndrome) is one of the most common endocrine disorders in women of reproductive age due to hyperandrogenism, and gene-gene interaction and environmental factor interaction[1]. The global prevalence of PCOS is said to be 4%–21%, depending on the criteria for diagnosis, and most guidelines indicate that up to 40%–70% of patients with PCOS are asymptomatic/go untreated according to WHO reports[2, 3]. PCOS is not only a disease of the reproductive system, but also implications for general reproductive health and metabolic, as well as immunological dysfunction. It is linked to long-term complications including metabolic and psychological issues, infertility, and a higher incidence of type 2 diabetes, cardiovascular disease, and some cancers [4, 5]. It has been difficult to elucidate the etiology of PCOS, largely due to both genetic heterogeneity and polygenic origin [6].

Recent studies have identified several genes associated with PCOS susceptibility, implicating genes involved in gonadotropin signaling, steroidogenesis, and insulin regulation [7, 8]. Among these, the Luteinizing Hormone/Choriogonadotropin Receptor (LHCGR) gene has emerged as a particularly compelling candidate due to its critical role in ovarian function and reproductive hormone signaling. The LHCGR gene encodes the receptor for luteinizing hormone (LH) and human chorionic gonadotropin (hCG), both of which are pivotal in follicular maturation and ovulation. Alterations in LHCGR function or expression may therefore contribute to the aberrant hormonal milieu and follicular dynamics observed in PCOS [9, 10]. The single-nucleotide polymorphism (SNP) rs2293275 is a genetic variant located within the LHCGR gene, preliminarily linked to PCOS and hypothesized to affect the ovarian response to LH and influence the severity or presentation of PCOS phenotypes [11, 12].

However, despite increasing evidence linking LHCGR variants to PCOS susceptibility across various populations, no prior study has investigated this association in Iraqi women. Despite a growing body of evidence across diverse ethnic groups, data on the distribution and potential implications of this variant in the Iraqi population remain unavailable. This lack of population-specific data represents a significant knowledge gap. It underscores the need for localized genetic studies to enhance the generalizability and applicability of genomic findings across populations, potentially paving the way for genotype-based diagnostic or therapeutic strategies. Therefore, the present study aimed to evaluate the relationship between LHCGR gene polymorphisms (rs2293275) and PCOS, with particular emphasis on hirsutism, menstrual pattern, reproductive history, and hormonal profiles in Iraqi women of reproductive age.

2 Methodology

Study design and participants

A case-control investigation was designed and carried out on a total of 150 married women. The study population comprised 100 women confirmed to have PCOS according to the 2003 Rotterdam diagnostic guidelines [13], and 50 age-matched women who were clinically healthy and served as controls. Recruitment took place at the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Baghdad, Iraq, during the period from January through December

2024. The primary objective was to explore the immunological and genetic determinants underlying PCOS through a comparative analysis between affected and unaffected women.

2.1.1 Inclusion and Exclusion Criteria
Inclusion criteria: Participation was restricted to married women within the age range of 18–35 years who were referred to the Infertility Treatment Center and met the established Rotterdam diagnostic criteria for PCOS [13, 14]. Healthy married women of comparable age who showed no signs of PCOS constituted the control group.

Exclusion criteria: Women with other endocrine disorders (e.g., thyroid dysfunction, hyperprolactinemia, or adrenal disorders), autoimmune or chronic inflammatory diseases, or metabolic conditions that could confound the results were excluded. Women who were pregnant or lactating were excluded, as pregnancy can significantly alter hormonal and inflammatory profiles.
2.1.2 Sample Size and Genetic Subset.
The study initially screened 250 participants according to the eligibility criteria. A total of 100 patients with PCOS and 50 control women (a 2:1 ratio) were enrolled in the comparative analyses, and the statistical power for the key variables exceeded 80% ($\alpha = 0.05$), as validated using G*Power 3.1 [15, 16]. Genetic analysis was performed on a subset of 32 patients and 31 controls, selected based on the availability of high-quality DNA and the completeness of clinical and laboratory records. Post hoc power analysis using the pwr package in R [17] confirmed 81.2% statistical power for detecting genetic associations ($Chi^2 = 8.316$, $N = 63$), exceeding the acceptable threshold. Hardy–Weinberg equilibrium was also confirmed in both groups (PCOS: $p = 0.472$; Control: $p = 0.141$), supporting the representativeness of the subsample with respect to the studied population.

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Clinical and Hormonal Assessment

PCOS was diagnosed by a specialist gynecologist in compliance with the Rotterdam diagnostic criteria established in 2003 [13]. A detailed clinical examination was conducted for all 150 study participants, covering body measurements (height, weight, and BMI calculation), degree of hirsutism as scored by the modified Ferriman-Gallwey method [18], patterns of menstrual cycle disturbances, family history pertaining to PCOS, and reproductive history.

Following an overnight fast of no less than 8 hours, peripheral venous blood (7 mL) was drawn from each participant between days 2 and 5 of the menstrual cycle for biochemical analysis. Serum separation was achieved by allowing the blood to clot, followed by centrifugation at 3000 rpm for a duration of 10 minutes. Serum hormone levels were measured by ELFA)using the Mini VIDAS® system (bioMérieux, France) [19]. The hormonal panel comprised FSH, LH, E_2, total testosterone, PRL, and AMH. The ratio of LH to FSH was calculated thereafter.

Genomic DNA Extraction and Quantification

DNA was extracted from peripheral blood samples using the EasyPure® Blood Genomic DNA Extraction Kit (TransGen, China; Cat. No: EE101-01), strictly following the protocol provided by the manufacturer. Quantification and quality assessment of the isolated DNA were performed on a NanoDrop OneC spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Only DNA samples that demonstrated OD_{260}/OD_{280} ratios ranging from 1.8 to 2.0 were regarded as suitable for use in further molecular procedures.

2.1.3 Primer Design and PCR Amplification

The target region of the LHCGR gene encompassing the SNP rs2293275 was amplified using primers designed with Primer3Plus(v. 0.4.0) software [20] aligned and confirmed against the reference sequence GRCh38.p14 (NC_000002.12) at genomic position chr2:48,694,236. The designed primers yielded a 675 bp amplicon flanking rs2293275 (Forward: 5'-TGCCCACACAAAATGCTTAG-3'; Reverse: 5'-TGTGGAATGTTGACCATGTG-3').

Primer specificity was validated against the human reference LHCGR gene sequence (NCBI RefSeqGene ID: NG_008193.2) using NCBI BLAST. The alignment demonstrated a bit score of 635, an E-value of 2×10^{-176}, 94% sequence identity, and a 1% gap (Fig. 2). In silico validation using Geneious Prime (Biomatters Ltd, Auckland, New Zealand) confirmed acceptable parameters: amplicon length of 600–1200 bp, melting temperature (Tm) of 54–58°C, and a GC content of 45–60%.

The target gene was amplified by polymerase chain reaction (PCR) as previously described [6]. The reaction was prepared in a total volume of 27 µL, comprising 12.5 µL of 2× EasyTaq® SuperMix (TransGen Biotech, Beijing, China; Cat. No: AS111-01), 1.5 µL each of the forward and reverse primers (10 µM), 8 µL of template genomic DNA (25–50 ng), and the volume was adjusted to 27 µL with 3.5 µL of nuclease-free water.

Thermal cycling was performed on a QIAamplifier 96 (QIAGEN /Germany), and conditions included an initial denaturation at 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 30 s, with a final extension at 72°C for 5 min. PCR products were resolved on 2% agarose gel electrophoresis (run at 70 V for 70 min) and stained with ethidium bromide for visualization under ultraviolet (UV) transillumination (Fig. 1).

2.3.2 Sanger Sequencing and Variant Identification

Successfully amplified PCR products were purified and subjected to bidirectional Sanger sequencing (Macrogen Inc., Seoul, South Korea) using an ABI 3730XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Using Geneious Prime software, the obtained sequence chromatograms were assembled, aligned, and cross-referenced with corresponding reference sequences downloaded from the NCBI GenBank database[21]. Variants were identified by comparison with the reference sequence and confirmed by visual inspection of chromatogram peaks.

The representative genetic sequence obtained from this study was deposited in the GenBank (NCBI) and DDBJ international databases under accession number LC894597.1. Sequencing analysis revealed two

nucleotide variants in exon 10 of the LHCGR gene: the targeted SNP rs2293275 (c.935G > A, p.Ser312Asn), and a novel variant at the genomic locus chr2:48,694,106 (GRCh38) in the sequence context 5'-CTATTAAAAG[T/C]TGCTTTGCAA-3'. This latter variant has not been previously recorded in the dbSNP, gnomAD, or ClinVar databases and is reported here for the first time in the Iraqi population

Figure 1: *LHCGR* 675 bp PCR product for representative samples on a 2% agarose gel for 70 min at 70 volts.

Figure 2: Alignment of the LHCGR gene obtained from the Gene Bank. The query represents samples, and the subject represents the database of Nucleotide BLAST

Statistical Analyses:

All the statistical analyses were carried out by using the SPSS v.26 (IBM Corp, Armonk, NY, USA) software and R v.4.3.0 (R Foundation for Statistical Computing, Vienna, Austria) [22, 23]. Normality of continuous variables was tested with the Shapiro-Wilk test before deciding on a parametric or non-parametric test. Categorical variables were compared by the Chi-square and Fisher's exact test (when the expected cell count was < 5); continuous variables were evaluated by Student's t-test (if normally distributed) or by Mann-Whitney U test (if not normally distributed). The odds ratios (OR) with 95% confidence intervals (CI) were estimated under the codominant and dominant inheritance models for the genetic analyses. Hardy-Weinberg equilibrium (HWE) was tested by using the Chi-square goodness-of-fit test. Statistical power was calculated using G*Power 3.1[24] and the pwr package in R [17]. In all analyses, two-tailed $p < 0.05$ was considered statistically significant.

Ethical Considerations

The study was carried out following the principles of the Declaration of Helsinki (2013 revision) and was reviewed and approved by the Institutional Ethics Committee of the College of Science, Mustansiriyah University, Baghdad, Iraq (Protocol No. BCSMU/0101/0048Z; approval date: [2024-01-1]). Written informed consents were obtained from all participants before recruitment. All data were pseudonymized, and this manuscript contains no information that could reveal the identity of any patient.

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3 Result

Anthropometric characteristics

Table 1 lists the anthropometric data of the 150 subjects included in this study. The age of the two groups did not differ significantly (27.1 ± 4.9, 28.5 ± 5.3, $p = 0.12$). The PCOS group had a significantly higher BMI than the control group (29.57 ± 5.36 kg/m² vs. 26.10 ± 3.55 kg/m²; $p < 0.001$). There were 42

(42%) with obesity in the PCOS group compared to 14 (18%) in the control group. However, normal weight was more prevalent in the control group (44% vs 19%; $\chi^2 = 14.41$; $p < 0.001$).

Table 1
Anthropometric characteristics between PCOS patients and healthy controls.

Characteristic	PCOS (n = 100) Mean ± SD or n(%)	Control (n = 50) Mean ± SD or n(%)	p-value
Age (years)	27.1 ± 4.9	28.5 ± 5.3	0.123 NS[a]
BMI (kg/m²)	29.57 ± 5.36	26.10 ± 3.55	< 0.001***[a]
BMI categories, n (%)			
Normal weight (< 25 kg/m²)	19 (19%)	22 (44%)	< 0.001***[b]
Overweight (25–29.9 kg/m²)	36 (36%)	19 (38%)	—
Obese (≥ 30 kg/m²)	42 (42%)	9 (18%)	—

Data are expressed as mean ± standard deviation (SD) for continuous variables and as n (%) for categorical variables. An independent−samples t−test was performed. a;b Chi−square test. NS signifies $p \geq 0.05$. BMI, body mass index.

Clinical and reproductive assessments

The clinical and reproductive assessments, including family history, menstrual disorder, pregnancy rate, and hirsutism, are presented in Table 2. A positive family history of PCOS was reported in 47% of patients compared to none of the controls (Fisher's exact test, $p < 0.001$), supporting a hereditary component of the condition (Table 2 and Figs. 3). Menstrual cycle irregularity was substantially more prevalent in PCOS patients: 61% had irregular cycles compared with only 2% of controls, which included 98% women with regular cycles ($\chi^2 = 45.45$; $p < 0.001$; Table 2, Fig. 4). Reproductive outcomes differed markedly between groups Table 2, where most of the PCOS patients 67% reported never having achieved pregnancy, compared with only 10% of controls. Successful pregnancy with live birth was reported in 78% of controls versus 22% of patients, and failed pregnancy (> 1 year without success) occurred in 11% of patients versus 8% of controls. The overall difference was highly significant ($\chi^2 = 49.20$; $p < 0.001$; Fig. 5). Hirsutism, on the other hand, was significantly higher in the PCOS group (65%, $\chi^2=47.75$, $p < 0.001$)

Table 2
Clinical and reproductive features of PCOS patients and healthy controls.

Characteristic	PCOS (n = 100) n (%)	Control (n = 50) n (%)	p-value
Family history of PCOS			
Positive	47 (47%)	0 (0%)	< 0.001***
Negative	53 (53%)	50 (100%)	—
Menstrual disorder			
Regular cycles	39 (39%)	49 (98%)	< 0.001***
Irregular cycles	61 (61%)	1 (2%)	-
Overall $\chi^2 = 45.45$	-	-	< 0.001***
Pregnancy history			
Never been pregnant	67 (67%)	5 (10%)	< 0.001***
Achieved childbearing	22 (22%)	39 (78%)	-
Unsuccessful pregnancy (> 1 year)	11 (11%)	2 (4%)	-
Overall $\chi^2 = 49.20$	-	-	< 0.001***
Hirsutism			
Present	65 (65%)	2 (4%)	—
Absent	35 (35%)	48 (96%)	< 0.001***
Overall (χ^2=47.75)	—	—	< 0.001***
Data presented as n (%). ***p < 0.001 (chi-square test).			

*Data presented as n (%). Chi-square (χ^2) test used for menstrual pattern, pregnancy history, and hirsutism. Fisher's exact test applied for family history (zero events in controls). ***p < 0.001. PCOS, polycystic ovary syndrome.*

Hormonal and genetic results

Serum hormonal parameters are shown in Table 3. Both testosterone and AMH are highly significantly increased in the PCOS group compared with control (testosterone: 0.48 ± 0.33 vs 0.19 ± 0.10 ng/μL, AMH 5.29 ± 2.22 vs 2.06 ± 0.91 ng/μL, both p < 0.001). FSH was significantly decreased in PCOS versus control (5.92 ± 1.8 vs 7.14 ± 2.69 mIU/mL, p = 0.001) in opposition to LH that was significantly increased in PCOS (5.59 ± 4.20 vs 4.41 ± 1.37 mIU/mL; p = 0.046). LH/FSH ratio reveals significant difference between the groups (0.94 ± 0.57 vs 0.68 ± 0.43; p = 0.009).

Table 3
Hormonal parameters of PCOS patients and controls (n = 150).

Parameter	Mean Level ± SD		*p-value*
	Patients	**Control**	
Total Testosterone (ng/µl)	0.48 ± 0.32	0.19 ± 0.10	< 0.001
AMH ng/µl	5.29 ± 2.22	2.06 ± 0.96	< 0.001
FSH (mIU/mL)	5.92 ± 1.8	7.14 ± 2.69	0.001
LH (mIU/mL)	5.59 ± 4.20	4.41 ± 1.37	0.046
LH/FSH	0.94 ± 0.57	0.68 ± 0.43	0.009

Data are expressed as mean ± standard deviation (SD). Statistical significance was set at $p < 0.05$. NS signifies $p \geq 0.05$.

The association between hormones and clinical characteristics in PCOS women was shown in Table 4. Women with irregular cycles had significantly elevated AMH (5.952 ± 2.236 vs 4.254 ± 1.787; p < 0.001), LH (5.886 ± 3.670 vs 5.147 ± 5.015; p = 0.027), and LH/FSH (0.998 ± 0.530 vs 0.850 ± 0.645; p = 0.031) than women with regular cycles.

In terms of hirsutism, the LH/FSH ratio was significantly higher among women with hirsutism than those without (1.010 ± 0.592 vs 0.810 ± 0.540; p = 0.027). Conversely, FSH was markedly lower in patients with hirsutism (5.671 ± 1.841 vs 7.309 ± 5.555; p = 0.017).

Table 4
Association between hormonal parameters and clinical features in PCOS patients (n = 100).

Parameter	Clinical feature		p-value
	Menstrual irregularity		
	regular	Irregular	
AMH (ng/µl)	4.254 ± 1.787	5.952 ± 2.236	< 0.001***
LH (mIU/mL)	5.147 ± 5.015	5.886 ± 3.670	0.027
LH/FSH ratio	0.850 ± 0.645	0.998 ± 0.530	0.031*
	Hirsutism		
	present	Absent	
LH/FSH ratio	1.010 ± 0.592	0.810 ± 0.540	0.027*
FSH (mIU/mL)	7.309 ± 5.555	5.671 ± 1.841	0.017*

Data are expressed as mean ± standard deviation (SD); Only statistically significant associations at $p < 0.05$.

The frequencies of LHCGR genotypes and alleles in PCOS patients and controls are shown in Table 5. Beginning with the rs2293275 polymorphism, we found a statistically significant difference in genotype distribution among the two groups (χ^2=8.95, p = 0.015). Three genotypes were observed: the AA homozygous genotype was found in 13% of the PCOS patients as compared with 42% of the control group and the AG heterozygous genotype was seen in 37% of the PCOS patients vs 35% in the control group OR = 3.55 (95% CI: 0.89–14.20, p = 0.068),GG genotype from 50% of the PCOS patients vs 23% of the controls with OR = 7.43 (95% CI: 1.78–31.04, p = 0.004). The G allele frequency was higher 68.8% in the patient compared to 40.3% in the control group, and an OR of 3.3 (1.75–6.05, P = 0.0026). The Hardy-Weinberg equation revealed no significant deviation from the expected genotype distributions for controls (p = 0.5725) or patients (p = 0.8483), indicating that both groups were in equilibrium, which supports the reliability of the data.

There was no significant difference in frequency distribution between the PCOS and control groups (χ^2 = 0.579, p = 0.093) for the novel variant at the position 48694106. Three genotypes were observed: the wild-type homozygous TT genotype in 48.4% of the PCOS patients and 53.1% in controls, the heterozygous TC genotype in 29.0% in of PCOS patient compared to 34.4% in n controls group with OR 1.08 (0.38–3.05, χ^2= 0.017, p = 0.895), and the homozygous mutant CC genotype in 22.6% of pcos and 12.5% in control with (OR 95% CI: 0.62 (0.16–2.43; χ^2= 0.093; p = 0.337) none of the genotypes shows significance results. In PCOS, the frequency of the T allele was 62.9%, whereas in controls it was 70.3%, and the frequency of the C allele was 37.1% in PCOS compared with 40.3% in controls (OR = 0.72; 95% CI: 0.38–1.37; p = 0.750), indicating that there is no statistically significant association between PCOS and the novel variant at this site.

Table 5
Genotype and allele frequencies of *LHCGR* (rs2293275, new position 48694106) in PCOS patients and controls.

Genotype frequency of the rs2293275 variant							
Genotype	**Patients n = 32(%)**	**Control n = 31(%)**	**OR (95% CI)**	**X2**	**P-value**	**HWE pcos**	**HWE Control**
AA	4 (13%)	13 (42%)	1.00(Ref)	-	-		
AG	12 (37%)	11 (35%)	3.55 (0.89–14.20)	5.41	0.068	p = 0.84	p = 0.57
GG	16 (50%)	7 (23%)	7.5 (1.78–31.04)	4.44	0.004**		
Overall	-	-	-	8.95	0.015*		
Allele frequency							
Allele	n = 62	n = 64					
A	20 (31.3%)	37 (59.7%)	1.00(Ref)	-	-		
G	44 (68.8%)	25 (40.3%)	3.3 (1.75–6.05)	10.45	0.002**		
Genotype frequency of the new position 48694106							
TT	15(48.4%)	17 (53.1%)	1.00 (Ref)	-	-		
TC	9 (29.0%)	11 (34.4%)	1.08 (0.38–3.05)	0.017	0.895	p = 0.77	P = 0.46
CC	7 (22.6%)	4 (12.5%)	0.62 (0.16–2.43)	0.093	0.337		
Overall			-	0.579	0.093		
Allele frequency							
Allele	n = 62	n = 64					
T	39 (62.9%)	45 (70.3%)	1.00 (Ref)				
C	23 (37.1%)	25 (40.3%)	0.72 (0.38–1.37)	0.78	0.750 NS		

*p < 0.05; **p < 0.01. HWE p > 0.05 indicates equilibrium.

Table 6 identified two statistically significant associations between the rs2293275 genetic variant and the parameters studied. The GG genotype was associated with significantly higher AMH concentrations in PCOS patients (12.32 ± 4.33 ng/μL) compared to the AG genotype (2.91 ± 0.95 ng/μL; p = 0.002). All other hormonal, immunological, and clinical parameters, including LH, FSH, and the LH LH/FSH ratio, showed no significant association with the rs2293275 genotype in either study population.

Table 6
Association between LHCGR rs2293275 genotype and hormonal parameters in the genotyped subset (n = 63).

Parameter	Group	AA (n = 4/13)	AG (n = 12/11)	GG (n = 16/7)	p-value
FSH (mIU/mL)	Control	6.46 ± 2.55	7.15 ± 2.08	5.93 ± 1.19	0.73
	PCOS	5.66 ± 0.68	5.73 ± 1.35	5.64 ± 1.69	0.99
LH (mIU/mL)	Control	4.56 ± 1.47	4.74 ± 1.99	4.28 ± 1.11	0.84
	PCOS	4.01 ± 2.28	5.40 ± 2.46	5.76 ± 2.47	0.82
AMH (ng/μL)	Control	—	—	—	—
	PCOS	intermediate	12.32 ± 4.33†	2.91 ± 0.95	0.002**

4 Discussion

The present study provides novel insights into the genetic and endocrine architecture of PCOS within the Iraqi population, representing the first investigation of the LHCGR rs2293275 polymorphism in this demographic. The findings of the present study demonstrate a profound hormonal dysregulation in women with PCOS, characterised by significant elevations in total testosterone, AMH, and LH, alongside a markedly increased LH/FSH ratio and diminished FSH levels. These endocrine alterations reflect the hallmark hyperandrogenic state and follicular arrest inherent to PCOS pathogenesis [25]. Critically, the genetic analysis revealed a robust association between the LHCGR rs2293275 GG genotype and increased susceptibility to PCOS, conferring a 7.5-fold higher risk compared to controls (OR = 7.5, 95% CI: 1.78–31.04; p = 0.004). Furthermore, the G allele was identified as an independent risk factor, augmenting the risk by 3.3-fold (OR = 3.3, p = 0.002). These findings underscore the potential clinical utility of LHCGR genotyping as a molecular risk stratification tool in this population.

The LHCGR gene, located on chromosome 2p16.3, has been established as a critical susceptibility locus for PCOS since the seminal genome-wide association study (GWAS) by Chen et al. in the Han Chinese population[10]. However, the association of the specific rs2293275 variant with PCOS risk has yielded highly heterogeneous results globally, underscoring the profound influence of ethnic and geographical variations on genetic penetrance. The observation of a strong positive association aligns with findings from a South Indian cohort by Thathapudi et al., who reported that the G allele and GG genotype conferred a significant risk for PCOS development, alongside elevated LH levels and insulin resistance[26]. In the Egyptian population, Bassiouny et al. similarly demonstrated that the LHCGR

rs2293275 polymorphism was significantly associated with PCOS, with homozygous AA individuals exhibiting a higher risk of developing the syndrome (OR: 1.80; 95% CI: 1.54–2.09; $p < 0.001$), further supporting the relevance of this variant in North African and Middle Eastern populations [27]. Similarly, a recent study among Jordanian females a population with closer ethnic and geographical proximity to the present cohort also demonstrated a significant association between the LHCGR rs2293275 polymorphism and PCOS, particularly noting higher LH levels and LH/FSH ratios in patients harbouring the GG and AG genotypes[28]. Interestingly, however, Almawi et al. found no significant association between rs2293275 and PCOS in Bahraini Arab women (allele frequency 0.34 in both groups), although other LHCGR variants (rs4953616, rs7371084) were significantly associated, demonstrating that even within Arab populations, the genetic architecture of PCOS susceptibility varies considerably [29]. Conversely, extensive meta-analyses, including a comprehensive 2024 assessment by Thomas and Veerabathiran encompassing diverse ethnic groups, found no significant overarching connection between the rs2293275 variant and PCOS risk globally, highlighting a potential protective effect only within Caucasian subgroups[30]. Another updated meta-analysis by Ibrahim et al. similarly concluded a lack of cumulative association across different ethnicities[31]. The stark contrast between the robust positive findings of the present study in Iraqi women and the null associations reported in broader meta-analyses emphasises that the genetic architecture of PCOS susceptibility is highly population-specific. It is hypothesised that the LHCGR rs2293275 variant may interact with unique environmental exposures, epigenetic modifications, or epistatic gene–gene interactions specific to Middle Eastern populations, thereby amplifying its pathogenic penetrance. Notably, the prevalence of consanguineous marriages in Iraq ranges from 44% to 61% in central and southern regions [32], which may further concentrate homozygosity for risk alleles such as the LHCGR G allele, contributing to the stronger effect sizes observed in the present cohort compared to outbred populations.

A particularly compelling observation in the present study is the genotype–phenotype correlation, specifically the significant association between the GG genotype and elevated AMH concentrations ($p = 0.0017$). AMH, predominantly secreted by the granulosa cells of pre-antral and small antral follicles, plays a pivotal inhibitory role in follicular recruitment and development by attenuating FSH-mediated follicular selection[33]. In PCOS, the characteristic accumulation of these small follicles leads to profound AMH hypersecretion, which subsequently blunts the sensitivity of granulosa cells to FSH, thereby perpetuating follicular arrest and chronic anovulation[34]. The LHCGR rs2293275 polymorphism, which involves an amino acid substitution (p.Asn312Ser) in the extracellular domain of the receptor, may alter the receptor's binding affinity, signal transduction efficiency, or protein stability. This substitution occurs in close proximity to a glycosylation site within the receptor ectodomain, and recent pharmacogenomic evidence indicates that the 312Asn (N) variant is associated with increased receptor potency, resulting in approximately two-fold enhanced LH and hCG signalling compared to the wild-type 312Ser variant [35]. Furthermore, Robeva et al. demonstrated that the rs2293275 polymorphism was significantly associated with elevated insulin levels in obese PCOS women and with a higher frequency of infertility (40% vs. 12.5%, $p = 0.022$) in heterozygous carriers, suggesting that this variant modulates both reproductive and metabolic phenotypes [36]. It is postulated that the GG genotype enhances

LHCGR signalling sensitivity to LH, creating a state of relative receptor hypersensitivity. This heightened responsiveness, even in the context of the relative LH excess characteristic of PCOS, could prematurely stimulate granulosa cells, exacerbating premature luteinisation and overproduction of AMH. This genotype-specific increase in AMH provides a crucial mechanistic link connecting the LHCGR variant status to the clinical manifestations of follicular arrest and anovulatory infertility observed in the present cohort, where 67% of PCOS patients reported never having achieved a pregnancy. This observation is further corroborated by Javadi-Arjmand et al., who reported that the GG genotype of rs2293275 was a significant susceptibility factor for IVF failure in Iranian women (OR = 3.88, p = 0.003), while the AA genotype conferred a protective effect [37]. Collectively, these findings support the emerging paradigm that LH receptor hypersensitivity, rather than LH hypersecretion alone, may be the primary driver of ovarian dysfunction in a genetically defined subset of PCOS patients [38].

The clinical phenotype of the present PCOS cohort was notably severe, with 65% presenting with hirsutism and 61% experiencing menstrual irregularities. The prevalence of obesity (42%) and overweight status (36%) in the present PCOS group was considerably high, aligning with the growing burden of obesity in the Middle East and North Africa (MENA) region, which is known to exacerbate both the metabolic and reproductive phenotypes of PCOS through insulin resistance-mediated pathways[39]. Importantly, a profound familial aggregation was identified, with 47% of PCOS patients reporting a positive family history compared to none in the control group. This strong heritability supports previous assertions that genetic factors contribute to up to 70% of PCOS pathogenesis[40, 41], further validating the necessity of investigating candidate genes such as LHCGR within consanguineous or genetically distinct populations. A recent comprehensive review by Hawi et al. documented that consanguinity rates in Iraq remain among the highest globally, with first-cousin marriages representing the predominant type of consanguineous union, particularly in central and southern provinces where rates exceed 50% [32]. This high degree of endogamy increases genome-wide homozygosity and may amplify the phenotypic expression of risk variants through autozygosity mapping, which may partly explain the pronounced clinical severity and strong genetic associations observed in the present cohort.

In addition to the targeted rs2293275 polymorphism, the sequencing analysis identified a novel synonymous variant at genomic locus chr2:48,694,106 (c.805T > C). Although this variant did not exhibit a statistically significant association with PCOS susceptibility in the relatively small genetic subset, its discovery delineates the unique genomic landscape of the Iraqi population and contributes to the expanding catalogue of LHCGR sequence diversity. Synonymous mutations, once considered ‘silent’, are increasingly recognised for their potential to alter mRNA secondary structure, splicing regulatory elements, codon usage bias, or translation kinetics, thereby influencing protein folding and expression levels [42]. Given the proximity of this novel variant to rs2293275 within exon 10, it is plausible that these variants may exist in linkage disequilibrium or exert combinatorial effects on LHCGR transcript processing.

This study has several notable strengths, including the stringent application of the Rotterdam criteria for PCOS diagnosis, comprehensive clinical and hormonal profiling, confirmation of Hardy–Weinberg

equilibrium in both groups, and the novelty of being the first to map the LHCGR genetic landscape in Iraqi PCOS patients. The use of bidirectional Sanger sequencing with deposition of representative sequences in international databases further enhances the reproducibility and transparency of the present findings. However, certain limitations are acknowledged that should be considered when interpreting these results. First, the sample size for the genetic subset (n = 63) was relatively modest, although post hoc power analysis confirmed it was sufficient (81.2%) to detect the robust effect sizes observed. Second, the lack of extensive metabolic parameters, such as detailed lipid profiles, fasting insulin levels, across the entire cohort, limits the ability to fully explore the metabolic implications of the LHCGR variants and their potential interaction with insulin signaling pathways. Third, the cross-sectional design precludes causal inference regarding the temporal relationship between genotype and phenotype development. Finally, the present study was conducted at a single tertiary referral center, which may introduce selection bias toward more severe PCOS phenotypes. Given the emerging pharmacogenomic evidence that LHCGR variants influence ovarian response to gonadotropin stimulation during assisted reproduction [35, 37], future studies should also evaluate whether Iraqi women carrying the GG genotype exhibit differential responses to ovulation induction protocols, which could have direct implications for personalised fertility treatment in this population. Future prospective multicenter studies incorporating larger and more ethnically diverse Middle Eastern cohorts, alongside functional genomic analyses, are essential to validate these associations and to delineate the precise molecular mechanisms through which LHCGR variants influence PCOS pathophysiology.

5 Conclusions

The LHCGR rs2293275 polymorphism constitutes a significant genetic determinant of PCOS susceptibility among Iraqi women, with the GG genotype conferring a 7.5-fold increased risk and the G allele acting as an independent predisposing factor. Beyond mere statistical association, the genotype-specific elevation in AMH concentrations implicates a mechanistic pathway whereby enhanced LH receptor signaling promotes excessive follicular recruitment, granulosa cell proliferation, and subsequent follicular arrest, collectively driving the anovulatory and hyperandrogenic phenotype characteristic of PCOS. These findings position rs2293275 as a candidate molecular marker for early risk stratification in Middle Eastern populations, where population-specific genetic data have been notably lacking. Clinically, incorporating LHCGR genotyping into diagnostic algorithms may facilitate personalized ovulation-induction protocols and targeted therapeutic interventions. Larger, multicenter studies across diverse Middle Eastern cohorts are warranted to validate these associations and to explore potential gene environment interactions that may modulate the penetrance of this variant.

Declarations

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Conflicts of interest

The authors have nothing to disclose.

Declaration of AI Use:

During the preparation of this work, the authors utilized artificial intelligence-assisted technologies (Paperpal) to improve sentence structure and language clarity.

Availability of data and materials:

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' Contributions

HAA conceived and designed the study, supervised the overall research project, and critically revised the manuscript for important intellectual content. KHH participated in the study design, performed the data collection, and contributed to the interpretation of results. FGL conducted the statistical analysis, prepared the figures and tables, and drafted the initial manuscript. RDAA contributed to data acquisition, assisted in the methodology development, and reviewed the manuscript. AMM participated in the experimental work, contributed to the literature review, and provided critical feedback on the final version of the manuscript. All authors read and approved the final manuscript.

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Figures

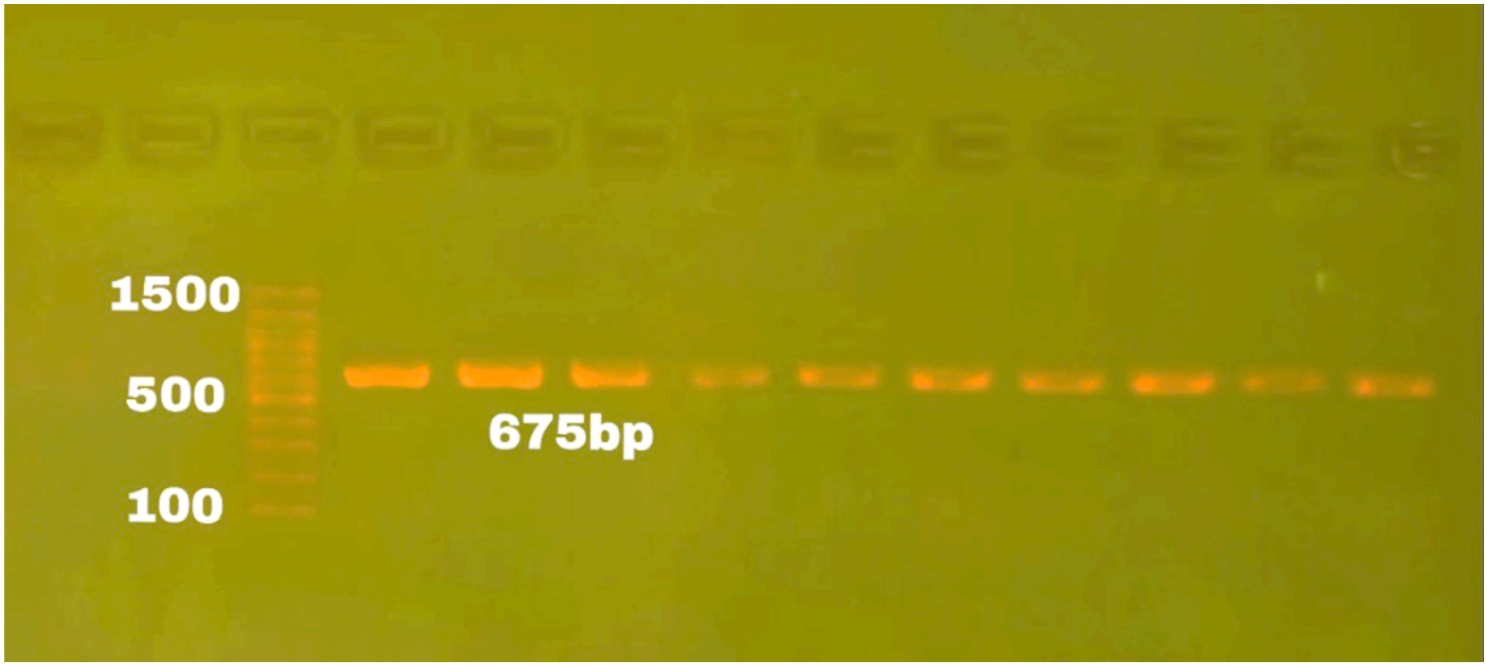


Figure 1

Download GenBank Graphics

Homo sapiens luteinizing hormone/choriogonadotropin receptor (LHCGR), RefSeqGene on chromosome 2

Sequence ID: NG_008193.2 Length: 75968 Number of Matches: 1

Range 1: 66266 to 66678 GenBank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
634 bits(343)	2e-176	394/418(94%)	5/418(1%)	Plus/Plus

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Query  105    TGCTGGTGTAATAGGTAAAGAATTTGCATGAACAAAGCATAAATAGACGCACAGTCAGGT  164
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Query  165    TTAGCCTGAAGTCCAAAAGCTCAAATGCttttttAACCTCTTCTCTTTCAGACAGAATT  224
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Figure 2

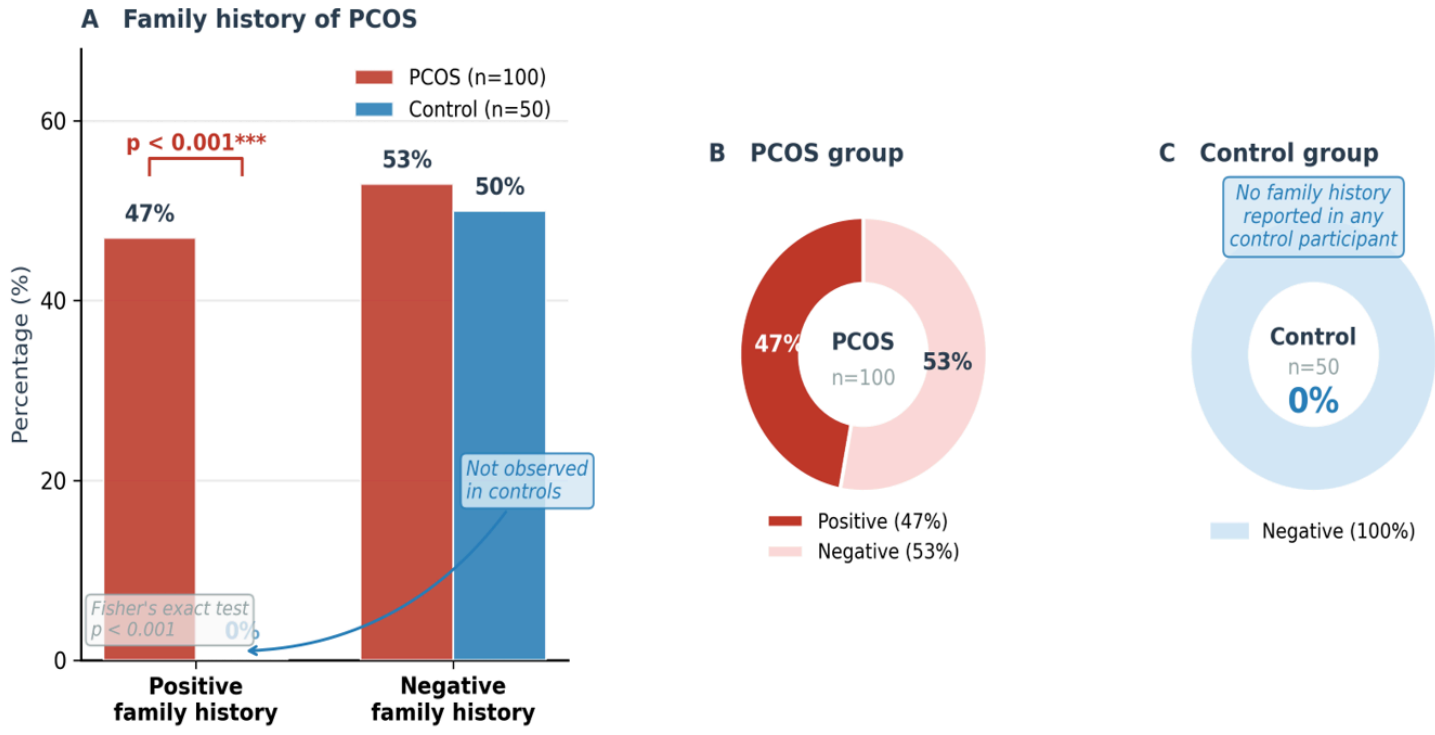


Figure 3

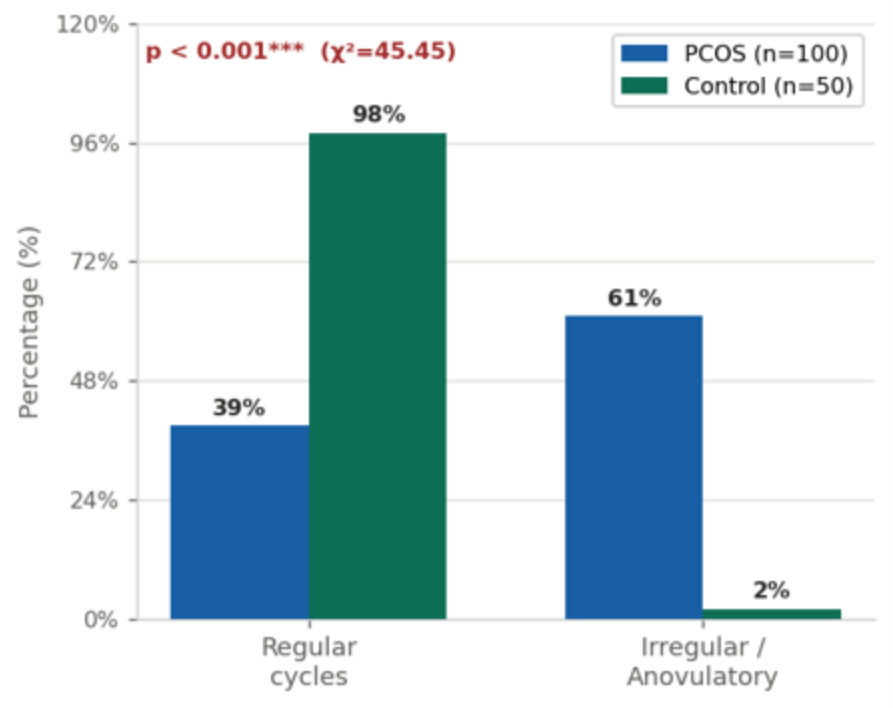


Figure 4

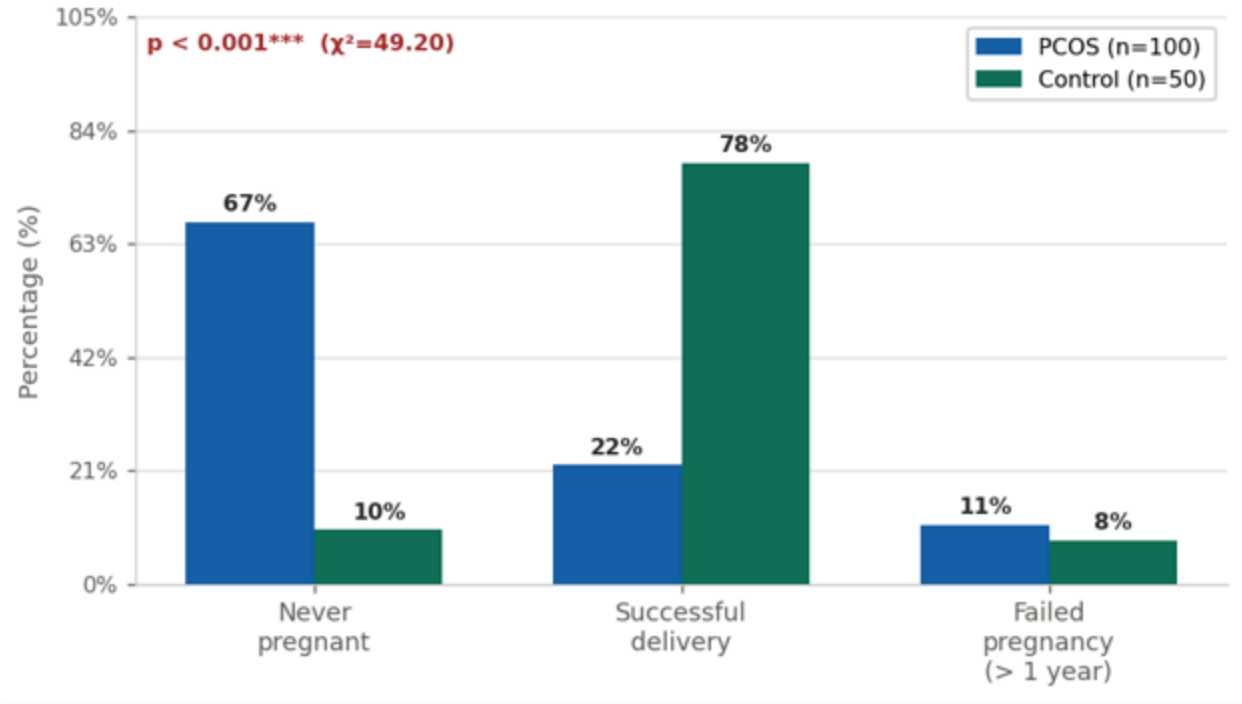


Figure 5


Contig View
Annotations
Lengths Graph
Text View
Lineage
Info
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R.C.
Translate
Add Annotation
Allow Editing
Annotate & Predict
Save
Consensus
Sequence L...
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2R_FR.ab...
3R_FR.ab...
4R_FR.ab...
5R_FR.ab...
6R_FR.ab...
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Figure 6