Evaluating the impact of *LHCGR* gene polymorphism on polycystic ovary syndrome: a comprehensive meta-analysis and power assessment

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Abstract

Objective: Polycystic ovary syndrome (PCOS) is prevalent among reproductive-aged women and is categorized by hormonal imbalances, irregular menstrual cycles, and challenges with fertility. PCOS affects approximately 3.6% of women globally, with prevalence varying by region. The luteinizing hormone/choriogonadotropin receptor *(LHCGR)* gene, which encodes the LHCGR, has been implicated in PCOS pathophysiology. This study investigated the association between the *LHCGR* gene polymorphism rs2293275 and PCOS through a meta-analysis.

Material and Methods: An extensive literature review was carried out using Embase, PubMed, and Google Scholar databases to identify research studies exploring the association between *LHCGR* gene variants and PCOS. The review was conducted based on the PRISMA checklist. Eligible case-control studies from 2016 to 2024 were chosen based on predefined criteria. Quantitative data analysis was performed using MetaGenyo software, employing a significance threshold of $p<0.05$. Odds ratios (OR) and confidence intervals (CI) were calculated to evaluate the relationships. G*Power 3.1 software was employed for statistical power analysis to assess the study's strength. The meta-analysis explored the link between *LHCGR* gene variant rs2293275 and PCOS across diverse ethnic groups and genetic models.

Results: Analyzing data from 10 studies involving 1,431 PCOS cases and 1,317 controls, the findings revealed no significant associations in most genetic models: allele (OR: 0.89, 95% CI: 0.54-1.49), dominant (OR: 0.74, 95% CI: 0.47-1.18), recessive (OR: 0.80, 95% CI: 0.41-1.57), and over-dominant (OR: 1.13, 95% CI: 0.69-1.85). Subgroup analyses by ethnicity (Arabs, Asians, Caucasians) consistently showed no significant correlations, except a protective effect in Caucasians (OR: 0.57, 95% CI: 0.34-0.95) in the AA vs. aa comparison. Sensitivity analyses confirmed robustness, and there was no indication of publication bias. Power analysis validated adequate sample sizes, and protein-protein interaction networks underscored biological relevance.

Conclusion: The meta-analysis concluded that no significant connection was observed between the *LHCGR* gene variant rs2293275 and the risk of PCOS among different populations. This suggests a complexity in PCOS etiology and indicating that *LHCGR* may not be a significant genetic marker for PCOS. Future research should explore other genetic and environmental factors contributing to PCOS, emphasizing the importance of genetic and ethnic variability in such studies. (J Turk Ger Gynecol Assoc. 2024; 25: 207-18)

Keywords: Polycystic ovary syndrome, *LHCGR* gene, genetic polymorphism, reproductive health and susceptibility

Received: 10 July, 2024 **Accepted:** 11 October, 2024

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DOI: 10.4274/jtgga.galenos.2024.2024-6-10

Introduction

Polycystic ovary syndrome (PCOS) is commonly acknowledged as a predominant endocrine disorder and was initially described by Stein and Leventhal in 1935 (1). Mostly women of reproductive age (18-44 years) are affected by this very prevalent endocrine (2), metabolic, and reproduction condition. PCOS is mainly associated with hormonal disturbances that alter the menstrual cycle, thereby causing irregular monthly cycles and eventually affecting the fertility profile of women (3).

According to the World Health Organization estimates, 116 million women, approximately 3.6% of women globally, are in the reproductive age range and have PCOS. The global incidence of PCOS ranges from 2.2% to 26% (1). In India, the prevalence of PCOS varies between 3.7% and 22.5%, according to population studies and diagnostic standards (4). Based on the Rotterdam criteria, PCOS is identified as a diverse syndrome characterized by the occurrence of at least two of these criteria: persistent lack of ovulation or irregular ovulation; clinical or biochemical signs of excess androgens; and polycystic ovarian morphology which may be observed using ultrasound (5). This morhology is illustrated in Figure 1. Hyperandrogenism may halt folliculogenesis, leading to multifollicular morphology, which disrupts the menstrual cycle and causes anovulatory infertility (6).

The imbalance in female sex hormones causes the development of a cyst in the ovarian antral follicle. The cyst, an egg-containing sac filled with fluid, is ordinarily released during fertilization. The transformation of the egg into a functional cyst usually prevents the ovulation process. As a result of ovulation inhibition, the development of several cysts takes place, leading to amenorrhea (7). PCOS is often characterized by increased levels of testosterone and luteinizing hormone (LH) in the blood. In addition, individuals with PCOS commonly experience metabolic disruptions, including as insulin resistance, high levels of insulin in the blood, and irregularities in energy expenditure. A recent study indicated a 2 to 3 times higher likelihood of developing cardiovascular diseases and type 2 diabetes in PCOS-affected individuals in the later stages of their lives (8). The etiology and progression of type 2 diabetes mellitus are complex and include several environmental variables, including but not limited to physical inactivity, excessive dietary glucose intake, poor eating habits, smoking, alcohol use, obesity, and hereditary factors (9). A strong association between PCOS and obesity is known in the affected population of reproductive-aged females (10).

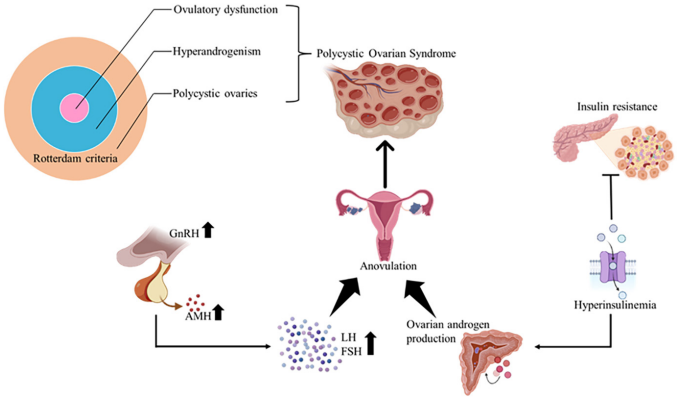

Figure 1. Pathophysiology of PCOS and the Rotterdam criteria
PCOS: Polycystic ovary syndrome, GnRH: Gonadotropin-releasing hormone, AMH: Anti-Mullerian hormone, LH: Luteinizing hormone, FSH: Follicle stimulating hormone

The underlying mechanisms of PCOS involve ovarian dysfunction influenced by external factors, such as the hypothalamic-pituitary-ovarian axis and hyperinsulinemia. Elevated levels of gonadotropin-releasing hormone lead to increased secretion of LH, affecting androgen production and ovulation. Insufficient feedback communication between the ovaries and the hypothalamic-pituitary unit exacerbates the suppression of gonadotropin secretion. Insulin resistance, induced by compensatory hyperinsulinemia, is identified as a major contributor to the development of symptoms of PCOS. Hyperinsulinemia promotes androgen production and reduces levels of sex hormone-binding globulin, resulting in hyperandrogenism and its associated clinical symptoms (11). This leads to an increased risk for the development of several gynecological cancers, including an elevated incidence rate of endometrial cancer (12). Among the gynecological cancers, cervical neoplasia is the second most common malignancy threatening women and is also associated with fertility issues among reproductive-aged women (13).

The luteinizing hormone/choriogonadotropin receptor *(LHCGR)* gene codes for the LH/choriogonadotropin receptor and is situated on chromosome 2p16.3, spanning over 70 kbp with 11 exons. This gene is mainly active in granulosa cells during the final phases of preovulatory follicles. Its primary role is to generate receptors for human chorionic gonadotropin (hCG) and LH (14). LH and hCG receptor functions allow the body to respond to these hormones functionally and structurally appropriately. In males, hCG supports Leydig cell growth in the testes, while LH prompts these cells to produce androgens. Androgens, like testosterone, are crucial for regulating male sexual development and reproductive processes (15).

The LHCGR protein functions as a receptor for glycoprotein hormones and is found in granulosa cells in the late stages of preovulatory follicles. LH stimulates the ovarian theca cells to produce testosterone, which is converted into estrogen. The expression of *LHCGR* during granulosa cell maturation allows the pre-ovulatory follicle to respond to the mid-cycle LH surge, leading to ovulation and the release of the mature egg cell (16).

Irregular *LHCGR* expression correlates with elevated LH levels, enlarged ovaries, infrequent menstrual cycles, and resistance to LH and/or hCG, all of which contribute to infertility. During pregnancy, women produce hCG, which promotes the continuation of the pregnancy (17).

The expression of *LHCGR* mainly occurs in the granulosa cells and theca of the ovary, and studies have shown increased expression in individuals with PCOS. When LH binds to its receptor LHCGR, a structural change activates the receptor, leading to signaling cascades involving cAMP and specific kinases. These signals regulate the expression of genes essential for steroid production. Furthermore, LH-induced activation of LHCGR plays a crucial role in follicle development and ovulation. Therefore, any genetic variation affecting the structure or function of the protein product of *LHCGR* could directly impact ovarian function and related conditions, such as PCOS (18).

Various studies have demonstrated that the *LHCGR* single nucleotide polymorphism (SNP) rs2293275 (p. Ser312Asn polymorphism) is significantly associated with women with PCOS. The fertility profile was also examined, revealing a notable correlation with infertile women with PCOS. In the Bulgarian population, a significant association was explicitly observed with infertile women (19). The Egyptian population also showed a significant association between PCOS and several anthropometric and biochemical characteristics, including an elevated free androgen index and hirsutism index (20).

The correlation of the rs2293275 variation with many cases of PCOS and the evident connection between *LHCGR* and reproductive signaling pathways indicates that it might constitute a fundamental and common mechanism contributing to the development of PCOS. Determining the practical implications of the *LHCGR* rs2293275 variation may clarify a typical reproductive phenotype that connects the etiology of various reproductive issues. Consequently, an extensive meta-analysis was conducted to determine the relationship pattern between *LHCGR* and PCOS.

Material and Methods

The study adhered to the PRISMA checklist, a recognized guideline for conducting meta-analyses and systematic reviews. Furthermore, the study's prospective review protocol (ID: 559449) was registered with the International Prospective Register of Systematic Reviews (PROSPERO), confirming the study's validity.

Literature analysis

The literature search for this study was conducted from January 1, 2014, to December 31, 2024, using the Google Scholar, Embase, and PubMed databases. The search focused on identifying relevant studies on the *LHCGR* gene, PCOS, polymorphisms, SNPs, rs2293275, and genetic variations. MeSH terms and commonly used keywords were combined with Boolean logic operators (AND, OR) to refine the search results. For instance, in PubMed, the search string included terms such as "*LHCGR* gene," "PCOS," "rs2293275," and "genetic variations." Filters were applied to include only studies published in English within the specified timeframe. The extensive search and exploration of the research articles were independently conducted by [Author 1 (Thomas)], and Author 2 (Veerabathiran) who independently screened the included articles, and any disagreements were resolved through discussion and consensus between the authors. The inclusion criteria focused on studies published in English that investigated the association between *LHCGR* gene polymorphisms, particularly rs2293275, and PCOS. Studies that did not meet these criteria or were published outside the designated search period were excluded. The complete search strategy for PubMed, including the detailed search string and filters used, is provided in the supplementary materials.

Criteria for inclusion and exclusion

A thorough assessment of the inclusion criteria ensured that the selected papers met the criteria for this meta-analysis. Specifically, studies employing case-control or cohort study design to investigate the association among the *LHCGR* genetic variants and PCOS and offering allelic and genotypic frequency data for determining 95% confidence intervals (CI) and odds ratios (OR), with corresponding p values were among the critical prerequisites for study selection. Female candidates aged 18-40 years were included in the studies, with a focus on their fertility profiles and hormonal ranges for data analysis. Infertile females and those with a family history of PCOS were also considered. The research considered hormonal profiles, with elevated hormone levels and irregular menstrual cycles indicative of the condition. The studies that reported adjusted estimates from the multivariable analysis were also included in this study to ensure the robustness of the findings.

Excluded studies included inadequate data or did not meet the following criteria. Using predetermined inclusion and exclusion criteria, we assessed the publications. For an article to be accepted, it had to fulfil two requirements: the study required using a case-control research design and the presentation of genotype frequencies for both cases and controls. Exclusion criteria included: studies using animal models or cell lines; case reports; no control group; or there was inadequate data.

Extraction of data

Based on predetermined criteria, the data were chosen from relevant publications, and the required data was then

meticulously acquired as described. A thorough analysis of the gathered articles was conducted to extract information regarding the genotypic and allelic frequencies among individuals in the case and control groups. The research was deemed ineligible if it failed to submit complete genotypic information, including allelic frequencies, or could not gather the information required from the patient and control groups. Every study used several data, including the Hardy-Weinberg equilibrium (HWE) value, language, initial author name, publication year, ethnicity, sample size, and PubMed ID.

Methodological quality evaluation using Hardy-Weinberg equilibrium and Newcastle Ottawa Scale scoring

The criteria employed to evaluate the quality of the chosen analysis were the Newcastle Ottawa Scale (NOS) and the HWE. A control genotype assignment was necessary to meet HWE (>0.05). The NOS rating, which has a maximum possible score of nine, considers three factors: relevance, equivalency, and selection. Studies with a score of six or above were included in this meta-analysis.

Statistical analysis

MetaGenyo software was used to set a statistical significance criterion of $p<0.05$ for all genomic changes during the data analysis. Specific protocols and resources are essential for conducting thorough meta-analyses on genetic interactions, evaluating genetic variations for potential therapeutic applications, ensuring rigorous significance testing in large-scale genetic studies, and maximizing statistical power. Previous research has used the Q statistic test, based on chi-square analysis, to interpret the heterogeneity assumption, as indicated by the I^2 metric. If the I^2 value was below 50, a fixed-effect model was applied to determine the OR and the 95% CI. Conversely, a random-effect model was employed if the I^2 value exceeded 50. The HWE technique used chi-square testing. We performed a subgroup analysis on the entire population to explore our analysis further. The sensitivity plot was also examined to determine the effects of leaving out individual studies, especially those where the controls deviated from the HWE. Egger's regression technique was applied to identify any possible publication bias.

Power analysis

Power analysis was performed on the metadata, using a 0.05 α error and a 95% CI. For the designated genes, the power of the sample size in each study-which included both case and control groups-was assessed separately. G*Power 3.1 was the program used to calculate power.

Protein-protein interactions

For the discovered SNPs linked with PCOS, the STRING (v11.0) online search tool database can predict functional proteins and protein-protein interactions (PPIs) with a minimum score of ≥0.4. STRING applies a minimum score criterion of ≥0.4 to signify the confidence level of projected interactions and aggregates large-scale experimental data, text mining, and computational predictions to gather information on protein interactions. STRING facilitates the visualization, interpretation, and analysis of intricate biological relationships by building networks of interacting proteins and offering functional annotations for proteins. Based on the predicted PPIs, it supports the identification of essential proteins, pathways, or molecular mechanisms associated with, in this case, PCOS through integrating various data sources and providing visualization tools, ultimately facilitating a deeper understanding of the condition's underlying biology.

Results

Search results

The literature search identified ten studies that examined the *LHCGR* gene, including information from 1,431 PCOS patients and 1,317 control participants. Once the articles were gathered, they were scrutinized to select the ones pertinent to this research and with important information. Figure 2 displays the research approach for *LHCGR*. Ten research studies examined the relationship between the collected data and PCOS severity, with ten of them focusing on the SNP rs2293275 polymorphism.

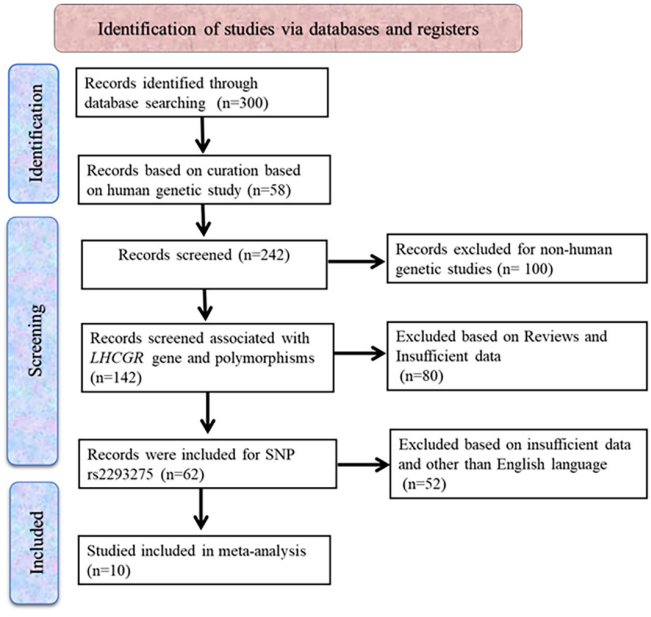

Figure 2. A flow diagram shows the overview of the study selection
LHCGR: Luteinizing hormone/choriogonadotropin receptor, SNP: Single nucleotide polymorphism

Table 1 presents the details of all the studies we looked at, including the traits of the controls and patients, to explore the connection between PCOS condition susceptibility and *LHCGR* polymorphisms. Participants in the 10 study projects were from various ethnic backgrounds (21-30).

Assessment of methodological quality

The methodological quality of the findings included in this meta-analysis was evaluated using the NOS and HWE criteria, including papers with a NOS score of six or higher guaranteed the selection of high-caliber research using dependable methods, lowering the possibility of bias. The NOS ratings primarily evaluate the research quality by reflecting varying degrees of methodological correctness among the included studies. Two studies out of the ten that were reviewed had an NOS score of six, indicating a moderate quality rating, and four studies received an NOS score of seven, indicating a somewhat superior methodological quality. Four research publications obtained an 8 on the NOS scale, signifying outstanding methodological excellence. These scores indicate that most of the studies meet the requirements in a good to exceptional manner for comparison, research group selection, and outcome evaluation. It is feasible to thoroughly examine the body of evidence and pinpoint the advantages and disadvantages of the research to this distribution. To preserve the accuracy of the genetic data and prevent biases, we only included and displayed in Table 1 studies whose control genotype distributions fulfilled HWE ($p>0.05$). These strict standards will improve the validity and robustness of the meta-analysis findings.

Quantitative data analysis

Ten studies were assessed to investigate the link between the rs2293275 polymorphism in the *LHCGR* gene and the propensity for PCOS. The results revealed no significant connection between PCOS risk and *LHCGR* polymorphisms. Based on the I^2 value, the models were obtained from random effect values. These models are the following: the allele model, G vs. A ($I^2=93\%$), with an OR of 0.89, 95% CI: 0.54-1.49, and $p=0.81$ and is depicted in Figure 3; the dominant model, GG + GA vs. AA ($I^2=55\%$), with an OR of 0.74, 95% CI: 0.47-1.18, and $p=0.03$ which is depicted in Figure 4; the recessive model, GG vs. GA + AA ($I^2=93\%$), with an OR of 0.80, 95% CI: 0.41-1.57, and $p=0.81$, shown in Figure 5; and the over-dominant model, GA vs. GG + AA ($I^2=86\%$), with an OR of 1.13, 95% CI: 0.69-1.85, and $p>0.05$, depicted in Figure 6.

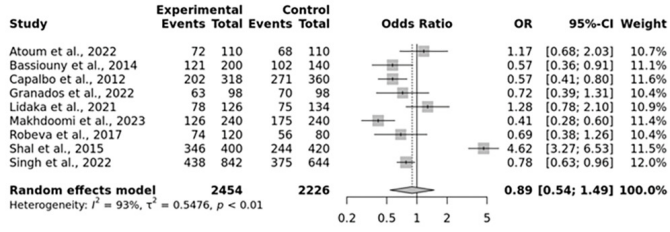

Figure 3. The forest plot showing an association between *LHCGR* gene polymorphism and PCOS in the allele model
LHCGR: Luteinizing hormone/choriogonadotropin receptor, PCOS: Polycystic ovary syndrome, OR: Odds ratio, CI: Confidence interval

Table 1. The characteristics of selected case-control studies of *LHCGR* (rs2293275) gene polymorphism and PCOS and HWE score

Author &year	Genotypic frequency						Allele frequency				Sample size		Ethnicity	NOS scoring	HWE
	Case			Control			Case		Control						
	GG	GA	AA	GG	GA	AA	G	A	G	A	Case	Control			
Atoum et al. (21), 2022	26	20	9	23	22	10	72	38	68	42	55	55	Arab	7	0.2575
Bassiouny et al. (22), 2014	47	27	26	42	18	10	121	79	102	38	100	70	Arab	8	0.0034
Capalbo et al. (23), 2012	63	76	20	103	65	12	202	116	271	89	159	180	Caucasian	8	0.6891
Alarcón-Granados et al. (24), 2022	20	23	6	23	24	2	63	35	70	28	49	49	Caucasian	7	0.1615
Lidaka et al. (25), 2021	26	26	11	22	31	14	78	48	75	59	63	67	Caucasian	7	0.6161
Makhdoomi et al. (26), 2023	10	106	4	58	59	3	126	114	175	65	120	120	Asian	8	0.0080
Robeva et al. (27), 2017	27	20	13	21	14	5	74	46	56	24	60	40	Caucasian	6	0.2918
El-Shal et al. (28), 2015	146	54	0	75	94	41	346	54	244	176	200	210	Arab	7	0.2424
Singh et al. (29), 2022	138	162	121	120	135	67	438	404	375	269	421	322	Asian	8	0.0131
Thathapudi et al. (30), 2015	59	124	21	22	155	27	242	166	199	209	204	204	Asian	6	0

LHCGR: Luteinizing hormone/choriogonadotropin receptor, PCOS: Polycystic ovary syndrome, HWE: Hardy-Weinberg equilibrium, NOS: Newcastle Ottawa Scale

Subgroup analysis

The results of the meta-analysis provide a complex relationship between genetic variations and the desired outcome for various genetic models across several ethnic populations. The results for the allele comparison model (A vs. a) in the ten selected studies showed no substantial correlation, with an OR of 0.9476 (95% CI: 0.6025-1.4904, p=0.8158). Furthermore, non-significant relationships were found in subgroup analyses by ethnicity, with ORs for Arabs (1.4681, 95% CI: 0.3839-5.6145, p=0.575), Asians (0.7967-95% CI: 0.4170-1.5222, p=0.49), and Caucasians (0.7639, 95% CI: 0.5236-1.1146, p=0.16) all showing non-significant connections. Likewise, no significant correlations were seen overall (OR: 0.9249, 95% CI: 0.4804-1.7804, p=0.815) or among subgroups in the recessive model (AA vs. Aa + aa).

The analysis for Caucasians neared significance (OR: 0.6362, 95% CI: 0.3914-1.0340, p=0.0680); however, no substantial relationships were detected in subgroups in the dominant model (AA + Aa vs. aa). The total OR in the model was 0.8077 (95% CI: 0.5239-1.2454, p=0.33). An overall OR of 1.0306 (95% CI: 0.6502-1.6336, p=0.9) for the overdominant model (Aa vs. AA + aa) indicated no significant connection.

Except for the comparison of AA vs. aa in Caucasians, which revealed a substantial protective effect (OR: 0.5656, 95% CI: 0.3376-0.9474, p=0.0304), pairwise comparisons (AA vs. aa, AA vs. Aa, and Aa vs. aa) likewise generally showed no significant relationships within subgroups or overall. In many situations, heterogeneity was negligible across studies (I^2=0%), and no discernible publication bias was found (p>0.05 for the Egger's test). Therefore, the meta-analysis across different genetic models and ethnic subgroups did not reveal a substantial link between the genetic variants and the result, except for an essential protective finding in the pairwise comparison of AA vs. aa in the Caucasian subgroup. These results underscore the importance of considering genetic and ethnic heterogeneity in genetic association studies. Table 2 depicts the data included in the subgroup analysis.

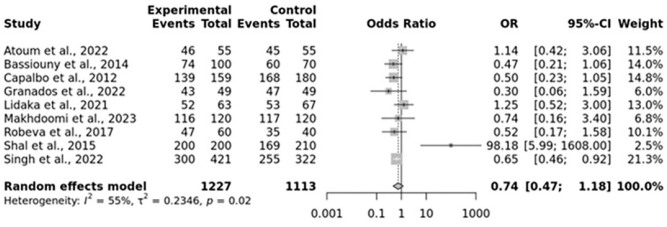

Figure 4. The forest plot showing an association between *LHCGR* gene polymorphism and PCOS in the dominant model
LHCGR: Luteinizing hormone/choriogonadotropin receptor, PCOS: Polycystic ovary syndrome, OR: Odds ratio, CI: Confidence interval

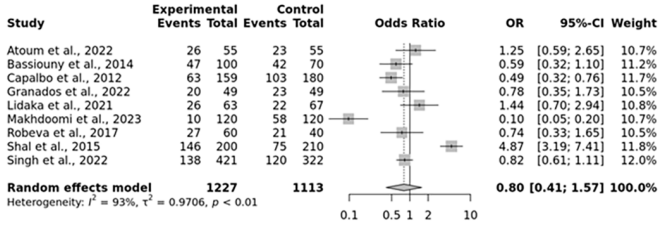

Figure 5. The forest plot showing an association between *LHCGR* gene polymorphism and PCOS in the recessive model
LHCGR: Luteinizing hormone/choriogonadotropin receptor, PCOS: Polycystic ovary syndrome, OR: Odds ratio, CI: Confidence interval

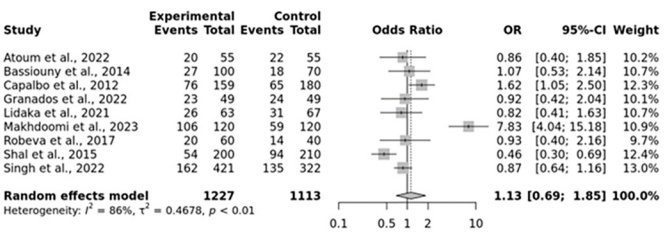

Figure 6. The forest plot showing an association between *LHCGR* gene polymorphism and PCOS in the over-dominant model
LHCGR: Luteinizing hormone/choriogonadotropin receptor, PCOS: Polycystic ovary syndrome, OR: Odds ratio, CI: Confidence interval

An examination of sensitivity analysis and publication bias

A sensitivity analysis examined the inconsistent findings from several investigations, particularly concerning departures from HWE. Research with intervention changes or non-compliance with HWE criteria was omitted from the analysis. As Figure 7 shows, removing these studies had no discernible impact on the final p-value. In addition, a funnel plot was used to detect publication bias and validate the results, and showed no evident bias, as illustrated in Figure 8.

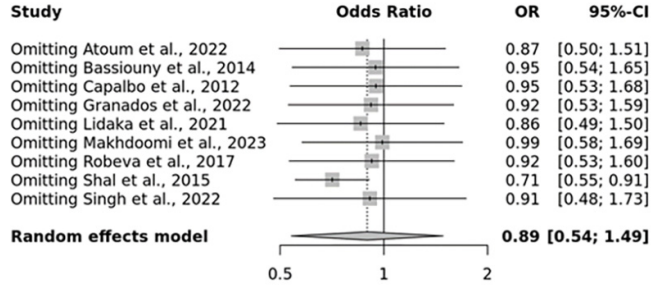

Figure 7. Sensitivity analysis was performed for *LHCGR* rs2293275 gene polymorphism among PCOS cases and controls
LHCGR: Luteinizing hormone/choriogonadotropin receptor, PCOS: Polycystic ovary syndrome, OR: Odds ratio, CI: Confidence interval

Power analysis and PPI interaction evaluation

A power analysis was conducted to assess the significance level of each research study related to the chosen SNPs. After conducting an inquiry, we discovered that the sample sizes in the examined literature, with an α error probability of 0.05, satisfied the necessary significance level. The outcomes of a two-tailed hypothesis test are displayed in the power analysis graph. The graph indicates that the hypothesis test is conducted at a significance level (α) of approximately 0.05, corresponding to a 95% confidence level, with a critical z-value of 1.95996. Thus, the graph underscores the need for careful study design to balance the risks of type 1 and type 2 errors, ensuring that the study has sufficient power to detect true associations while minimizing the risk of false positives. This sort of analysis (Figure 9) evaluates the probability of finding an effect of a certain magnitude under specific variables, such as sample size, effect size, and significance level. Table 3 provides specifics of the power analysis.

Twenty-one nodes and 131 edges in the PPI network were built using the STRING database. Its low PPI enrichment p-value was less than 1.0e-16, and its clustering coefficient was 0.814. Its average node degree was 12.5. This suggests that the proteins interact more than one could anticipate from a randomly chosen protein group of comparable size and distribution from the genome. Figure 10 shows the network of other genes and proteins associated with the *LHCGR* gene. Such enrichment suggests a degree of biological interconnectedness among the proteins. These findings also suggest a tightly integrated

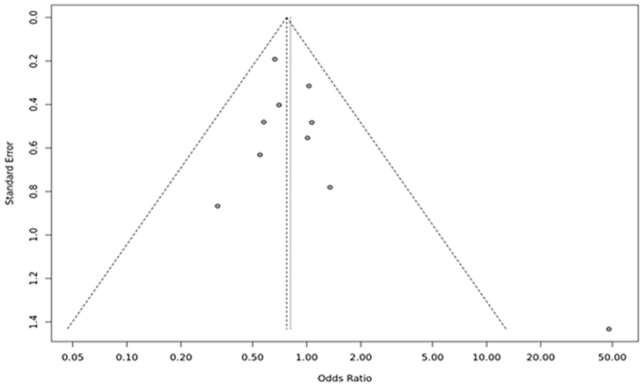

Figure 8. Publication bias in association between *LHCGR* gene polymorphism and PCOS in all models
LHCGR: Luteinizing hormone/choriogonadotropin receptor, PCOS: Polycystic ovary syndrome

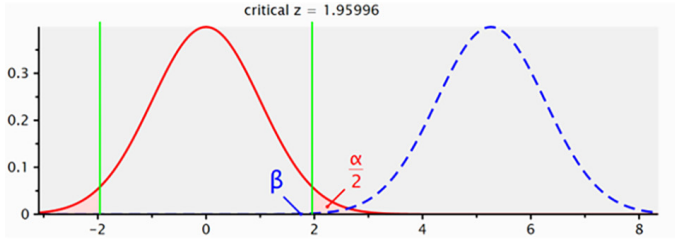

Figure 9. The graphical representation of a power analysis plot depicts how statistical power is affected by either the sample size or effect size in a two-tailed hypothesis test for the *LHCGR* rs2293275 gene polymorphisms
LHCGR: Luteinizing hormone/choriogonadotropin receptor, ß: Beta

Table 2. Subgroup meta-analysis of the association between *LHCGR* (rs2293275) gene polymorphism with PCOS susceptibility

Model	Ethnicity	Number of studies	Test of association			Test of heterogeneity			Publication bias
			OR	95% CI	p value	Model	p	I^2 value	p value (Egger's test)
Allele contrast (A vs. a)	Overall	10	0.9476	(0.6025; 1.4904)	0.815807	Random	0	0.9313	0.9062
	Arab	3	1.4681	(0.3839; 5.6145)	0.574787	Random	0	0.9627	0.3915
	Asia	3	0.7967	(0.4170; 1.5222)	0.491407	Random	0	0.9376	0.8021
	Caucasian	4	0.7639	(0.5236; 1.1146)	0.162334	Random	0.0709	0.5734	0.5142
Recessive model (AA vs. Aa + aa)	Overall	10	0.9249	(0.4804; 1.7804)	0.815211	Random	0	0.9301	0.6288
	Arab	3	1.5565	(0.3921; 6.1785)	0.529334	Random	0	0.9397	0.3813
	Asia	3	0.6606	(0.1370; 3.1859)	0.60547	Random	0	0.9657	0.8215
	Caucasian	4	0.7597	(0.4640; 1.2438)	0.274576	Random	0.0883	0.541	0.2612
Dominant model (AA + Aa vs. aa)	Overall	10	0.8077	(0.5239; 1.2454)	0.333777	Random	0.0099	0.5851	0.3368
	Arab	3	2.1265	(0.3296; 13.7177)	0.42765	Random	0.0012	0.8511	0.1027
	Asia	3	0.7726	(0.5768; 1.0349)	0.08365	Fixed	0.1331	0.5041	0.7065
	Caucasian	4	0.6362	(0.3914; 1.0340)	0.067985	Fixed	0.3103	0.1625	0.6232

Table 2. Continued

Model	Ethnicity	Number of studies	Test of association			Test of heterogeneity			Publication bias
			OR	95% CI	p value	Model	p	I^2 value	p value (Egger's test)
Overdominant (Aa vs. AA + aa)	Overall	10	1.0306	(0.6502; 1.6336)	0.898061	Random	0	0.8646	0.4151
	Arab	3	0.701	(0.3967; 1.2386)	0.221271	Random	0.0766	0.6107	0.2146
	Asia	3	1.4433	(0.4159; 5.0084)	0.563231	Random	0	0.9588	0.5195
	Caucasian	4	1.2005	(0.8798; 1.6380)	0.249145	Fixed	0.2846	0.2093	0.0776
pairw1 (AA vs. aa)	Overall	10	0.8412	(0.4311; 1.6416)	0.612205	Random	0	0.7975	0.5944
	Arab	3	2.6908	(0.3116; 23.2387)	0.368201	Random	0.0003	0.8787	0.0931
	Asia	3	0.7636	(0.1768; 3.2984)	0.717866	Random	0	0.9026	0.9837
	Caucasian	4	0.5656	(0.3376; 0.9474)	0.03036	Fixed	0.1272	0.4737	0.917
pairw2 (AA vs. Aa)	Overall	10	0.9623	(0.5251; 1.7635)	0.901082	Random	0	0.9063	0.5542
	Arab	3	1.5197	(0.5619; 4.1098)	0.409698	Random	0.0009	0.8566	0.2739
	Asia	3	0.6918	(0.1387; 3.4495)	0.653102	Random	0	0.9651	0.7459
	Caucasian	4	0.7368	(0.5300; 1.0243)	0.069237	Fixed	0.1496	0.4364	0.1519
pairw3 (Aa vs. aa)	Overall	10	0.7736	(0.6023; 0.9936)	0.044424	Fixed	0.1774	0.2904	0.2043
	Arab	3	1.7294	(0.3339; 8.9584)	0.513906	Random	0.0138	0.7667	0.0671
	Asia	3	0.7666	(0.5599; 1.0496)	0.097305	Fixed	0.3774	0	0.3608
	Caucasian	4	0.7085	(0.4220; 1.1895)	0.192387	Fixed	0.6308	0	0.287

LHCGR: Luteinizing hormone/choriogonadotropin receptor, PCOS: Polycystic ovary syndrome, OR: Odds ratio, CI: Confidence interval

Table 3. Power analysis for *LHCGR* (rs2293275) gene polymorphism and PCOS

Gene	SNP	No. of studies	Cases	Controls	A- err prob	Power (1β err prob)
LHCGR	rs2293275	10	1431	1317	0.05	0.99955

LHCGR: Luteinizing hormone/choriogonadotropin receptor, PCOS: Polycystic ovary syndrome, SNP: Single nucleotide polymorphism, β: Beta

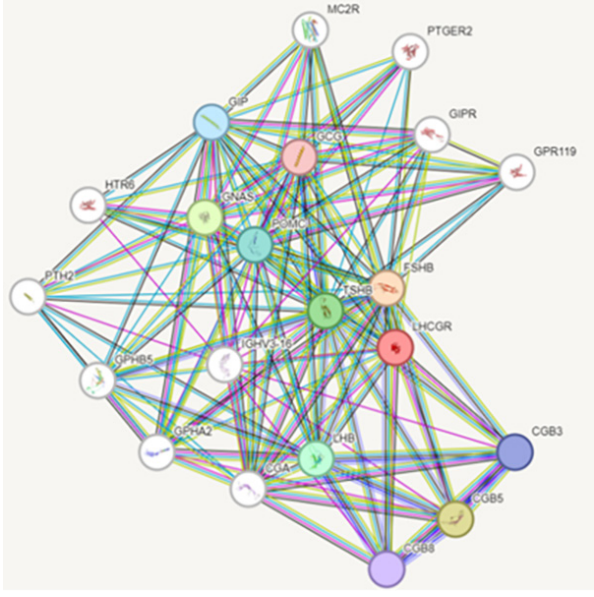

Figure 10. The protein-protein interaction network of differentially expressed genes of the selected gene associated with PCOS
PCOS: Polycystic ovary syndrome

network of proteins, indicating that the proteins in this network interact more extensively and are more interconnected than would be anticipated by chance. The PPI network reveals a substantial level of biological interrelationships among the associated proteins, underscoring the importance and complexity of their interactions and potential functional relevance in the context of the *LHCGR* gene.

Discussion

The *LHCGR* gene produces a G protein-coupled receptor that binds to both LH and hCG. In the ovary, the expression of *LHCGR* is central to the interaction of pre-ovulatory follicles and the mid-cycle LH surge, facilitating ovulation during the differentiation of granulosa cells. While women with inactivating *LHCGR* mutations may experience elevated LH levels and enlarged ovaries with irregular menstrual cycles, they do not typically exhibit significant reproductive issues, as seen in males with similar mutations who present with early puberty (31). Studies have associated *LHCGR* variations, particularly the rs2293275 SNP in exon 10, causing an S312N

amino acid change with PCOS. Although this SNP does not seem to affect glycosylation, its functional implications in PCOS remain unclear. Interestingly, unlike in males, where activating LHCGR mutations trigger early puberty and increased testosterone production, these mutations are not linked to ovarian hyperandrogenemia in women. This challenges the assumption that "hyper-responsive" *LHCGR* isoforms are associated with the dysfunction of theca cells (27).

There is strong evidence suggesting that PCOS has a genetic basis despite its diverse manifestations. Recent genome-wide association studies (GWAS) have identified multiple genetic regions associated with PCOS, strengthening the hypothesis of a likely oligogenic or polygenic model, although evidence suggests autosomal dominant inheritance. Environmental factors, incomplete penetrance, and epigenetic modifications require intricate efforts to understand the inheritance patterns fully. Despite advances in genetic research, the identification of definitive PCOS susceptibility genes remains limited. Though much research has been done on candidate gene connections, only a few have shown consistently reproduced statistically significant relationships (32).

The study conducted by Branavan et al. (33) showed no substantial link between the *LHCGR* gene and PCOS patients. This study was conducted in Sri Lankan women of 16-19 years of age, and genotype associations were analyzed using amplification-refractory mutation system - polymerase chain reaction. Although this study did not show any association between genetic polymorphisms and the *LHCGR* gene, in contrast, various other studies have shown a substantial correlation between the *LHCGR* genetic variant and PCOS, some reporting that the *LHCGR* gene was strongly associated with PCOS, thereby playing an essential part in the pathophysiology of PCOS (33). Capalbo et al. (23) reported a substantial correlation between Sardinian PCOS patients and the *LHCGR* gene. This study identified the *LHCGR* gene as a functional solid candidate for susceptibility to PCOS, showing the highest relative risk among specific genotypes. The findings suggest that assessing the *LHCGR* genotype, particularly the 312N allele, in PCOS patients and their family members could be valuable (23).

Based on the studies conducted, an association between the *LHCGR* gene and PCOS was demonstrated through GWAS analysis (34,35). The initial report found that granulosa and theca cells from PCOS patients expressed higher levels of *LHCGR* compared to normal control cells. In a subsequent study, using data from the Gene Expression Omnibus (GEO) database to confirm their findings, the authors identified an increased expression of *LHCGR* in cumulus cells from women with PCOS, regardless of obesity status. Women diagnosed with PCOS, especially those who were not overweight, demonstrated heightened secretion of LH from the pituitary gland, enhanced bioactivity of LH, and increased androgen production in response to LH stimulation. The researchers proposed that increased receptors due to LHCGR overexpression led to heightened androgen production from the theca cells, thereby increasing ovarian sensitivity to LH. Moreover, in non-obese women with PCOS, there was evidence of reduced methylation and increased expression of *LHCGR* in adipose tissue, suggesting a consistent gene regulatory profile across different tissues. To validate their finding of *LHCGR* overexpression as a unique feature of PCOS, the researchers analyzed GEO datasets that allowed stratification, based on traits such as obesity and insulin sensitivity. They found no differences in *LHCGR* expression between lean and obese subjects in three adipose GEO datasets stratified by obesity or in three datasets stratified by insulin sensitivity. These findings suggest that the observed variations in *LHCGR* expression are specific to PCOS and not merely a result of metabolic differences within the cohort.

LHCGR genetic polymorphisms and PCOS did not correlate in the current investigation. The I^2 value surpassed 50%, indicating no substantial linkages at the allele level, recessive relationships, over-dominant associations, or dominant associations between the *LHCGR* genetic variant and PCOS. Therefore, these results suggest that the *LHCGR* gene polymorphism and PCOS symptoms are unrelated. A NOS rating was used to select studies with high-quality methodologies and guarantee high-quality research to minimize the possibility of bias. The study concluded that the *LHCGR* gene polymorphism was congruent with the HWE value principle. Both Egger's test and a funnel plot were used to evaluate publication bias, but neither revealed any bias. The PPI network indicates that the proteins show more interactions than one would anticipate from a haphazardly chosen set of proteins with comparable sizes and genomic distributions. The degree of biological interconnectedness between the proteins and the network of other genes and proteins that share the *LHCGR* gene is shown by this enrichment.

This meta-analysis examined various genetic models and ethnic subgroups, revealing no significant association between genetic variants and outcomes, except for a notable finding in comparing AA vs. aa within the Caucasian subgroup. These findings underscore the importance of accounting for genetic and ethnic diversity in genetic association studies. PCOS susceptibility may be influenced by the *LHCGR* polymorphism across different populations, where its impact could vary due to diverse genetic backgrounds, environmental exposures, and lifestyles. These factors, serving as confounding variables across broader populations, may weaken the observed associations. However, the link becomes more apparent and

detectable in more homogeneous subgroups with reduced variability in these factors. In addition, interactions with other genetic or hormonal factors prevalent in these subgroups could enhance the biological significance of the *LHCGR* polymorphism. The subgroup analysis highlighted a significant relationship, emphasizing the need for further investigation into how specific factors, such as genetic diversity, environmental influences, hormone levels, and lifestyle choices amplify the impact of the *LHCGR* polymorphism on PCOS susceptibility. The statistical data strongly supports these conclusions, with rigorous data extraction and analysis methods ensuring reliable study outcomes.

This study advances our understanding of the genetic underpinnings of PCOS, potentially contributing to improved diagnosis and treatment strategies in the future. Despite inconsistent results addressing the minor relationship between *LHCGR* gene polymorphisms and PCOS risk due to the inadequate and short sample size, our investigation emphasized the need to investigate the process of *LHCGR* genetic variation. Recognizing genetic indicators could aid in early detection, personalized treatment, and risk assessment. Ultimately, we believe our research contributes to elucidating the connection between the risk of PCOS and *LHCGR* gene polymorphisms, highlighting the growing importance of addressing these challenges.

Study limitations

This study is subject to several limitations. Variations in different populations may impact outcomes differently. First, various populations may have distinct genetic origins, and although ethnicity-based subgroup analysis was attempted, it could not include all ethnic groups. This restriction emphasized how important it is to carry out more inclusive research encompassing a more comprehensive range of genetic varieties. Furthermore, publication bias is a risk, which might distort the overall results since research with unfavorable outcomes can be under-represented. Therefore, the subgroup analysis based on ethnicity could not encompass all ethnic groups. Another significant limitation was the relatively small sample sizes in some of the included studies, which may reduce the statistical power and limit the generalizability of the findings. Small sample sizes can also contribute to more significant variability and uncertainty in the results. Furthermore, the reported relationships between *LHCGR* gene variants and PCOS may have been impacted by confounding factors, which may not have been adequately controlled across investigations. The study's emphasis on specific demographics further restricts the generalizability of findings. Subsequent investigations must bridge these gaps by executing extensive, multi-ethnic investigations encompassing heterogeneous populations and accommodating plausible confounding factors. Understanding the temporal link between *LHCGR* gene variants and the onset of PCOS might also benefit from longitudinal research. Furthermore, investigating gene-environment interactions and broadening the study scope to encompass a greater variety of genetic variants may contribute to a more thorough knowledge of the genetic foundation of PCOS. Recognizing these constraints underscores the necessity for more extensive research with larger sample sizes to enhance our understanding of the role of *LHCGR* gene variations in PCOS.

The implications of this study on the correlation between *LHCGR* gene polymorphisms and PCOS have significant ramifications, particularly in genetics and personalized medicine. Understanding how *LHCGR* variations contribute to susceptibility to PCOS may lead to the development of more precise diagnostic techniques, enabling early detection of high-risk individuals. Furthermore, a more detailed study could pave the way for tailored treatments that address the genetic aspects of PCOS, potentially improving therapeutic outcomes. By identifying these correlations, healthcare professionals can adopt a more personalized approach to managing PCOS, tailoring prevention and treatment strategies to each patient's unique genetic profile. Future studies need to be conducted to explore the genetic underpinnings of PCOS, fostering advances in precision medicine and improving patient outcomes.

Conclusion

This study used a thorough meta-analysis spanning many ethnic backgrounds to investigate the relationship between a single *LHCGR* gene polymorphism, rs2293275, and PCOS. Except for a small finding in the Caucasian subgroup, our study revealed no substantial correlation between this polymorphism and PCOS across various genetic models and ethnic groupings, despite several reports pointing to a connection. The robustness of the conclusions was underscored by adherence to strict methodological criteria. The results demonstrated the complexity and variety of PCOS, indicating that more research is required to identify additional genetic and environmental variables contributing to the syndrome, even though the *LHCGR* gene may not be a significant marker for PCOS risk. This work adds to the continuing efforts to understand and manage PCOS better and highlighted the need to take genetic and ethnic heterogeneity into account in genetic association studies.

Acknowledgments: *The authors thank the Chettinad Academy of Research Education for their constant support and encouragement.*

Ethics Committee Approval: *Not applicable.*

Informed Consent: *Not applicable.*

Author Contributions: *Surgical and Medical Practices: S.M.T., R.V.; Concept: S.M.T., R.V.; Design: S.M.T., R.V.; Data Collection or Processing: S.M.T., R.V.; Analysis or Interpretation: S.M.T., R.V.; Literature Search: S.M.T., R.V.; Writing: S.M.T., R.V.*

Conflict of Interest: *No conflict of interest is declared by the authors.*

Financial Disclosure: *The authors declared that this study received no financial support.*

References

1. 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: 125.
2. Goussalya D, Jancy MS, Jemi AA, Soundarya R, Varghese S, Nalini AP, et al. Association of interleukin 6 and insulin resistance gene polymorphism with polycystic ovarian syndrome: a meta-analysis. Meta Gene. 2020; 24: 100675.
3. Rizwana A, Rajkumar SA, Anuradha CR. Pregnancy and perinatal outcomes in women with polycystic ovarian syndrome. Int J Reprod Contracept Obstet Gynecol. 2021; 10: 4232-6.
4. Nirupama AY, John JB, Chellaiyan DV. Polycystic ovary syndrome: current perspectives and recent advances. Curr Med Issues. 2022; 20: 89-94.
5. Laven JSE. Follicle stimulating hormone receptor (FSHR) polymorphisms and polycystic ovary syndrome (PCOS). Front Endocrinol (Lausanne). 2019; 10: 23.
6. Deswal R, Nanda S, Dang AS. Association of luteinizing hormone and LH receptor gene polymorphism with susceptibility of polycystic ovary syndrome. Syst Biol Reprod Med. 2019; 65: 400-8.
7. Patel S. Polycystic ovary syndrome (PCOS), an inflammatory, systemic, lifestyle endocrinopathy. J Steroid Biochem Mol Biol. 2018; 182: 27-36.
8. Ding H, Zhang J, Zhang F, Zhang S, Chen X, Liang W, et al. Resistance to the insulin and elevated level of androgen: A major cause of polycystic ovary syndrome. Front Endocrinol (Lausanne). 2021; 12: 741764.
9. Viji D, Aswathi P, Charmine PP, Husain RA, Ameen SN, Ahmed SS, et al. Genetic association of ABCC8 rs757110 polymorphism with type 2 diabetes mellitus risk: a case-control study in South India and a meta-analysis. Gene Rep. 2018; 13: 220-8.
10. Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, et al.; International PCOS Network. Erratum. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Hum Reprod. 2019; 34: 388.
11. Jamshidi M, Mohammadi Pour S, Bahadoram M, Mahmoudian-Sani MR, Saeedi Boroujeni A. Genetic polymorphisms associated with polycystic ovary syndrome among Iranian women. Int J Gynaecol Obstet. 2021; 153: 33-44.
12. Shetty C, Rizvi SMHA, Sharaf J, Williams KD, Tariq M, Acharekar MV, et al. Risk of gynecological cancers in women with polycystic ovary syndrome and the pathophysiology of association. Cureus. 2023; 15: e37266.
13. Husain RS, Ramakrishnan V. Global variation of human papillomavirus genotypes and selected genes involved in cervical malignancies. Ann Glob Health. 2015; 81: 675-83.
14. 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: 2399-412.
15. Casarini L, Santi D, Brigante G, Simoni M. Two hormones for one receptor: evolution, biochemistry, actions, and pathophysiology of LH and hCG. Endocr Rev. 2018; 39: 549-92.
16. Kawai T, Richards JS, Shimada M. The Cell type-specific expression of LHCGR in mouse ovarian cells: Evidence for a DNA-demethylation-dependent mechanism. Endocrinology. 2018; 159: 2062-74.
17. Lu X, Yan Z, Cai R, Khor S, Wu L, Sun L, et al. Pregnancy and live birth in women with pathogenic LHCGR variants using their own oocytes. J Clin Endocrinol Metab. 2019; 104: 5877-92.
18. Zhang JH, Zhan L, Zhao MY, Wang JJ, Xie FF, Xu ZY, et al. Role of EGFR expressed on the granulosa cells in the pathogenesis of polycystic ovarian syndrome. Front Endocrinol (Lausanne). 2022; 13: 971564.
19. Robeva R, Andonova S, Tomova A, Kumanov P, Savov A. LHCG receptor polymorphisms in PCOS patients. Biotechnology & Biotechnological Equipment. 2018; 32: 427-32.
20. Abouzeid SA, Abou Khadr NA, Gaafar SS, Eldeeb ME. Relation of polycystic ovarian syndrome phenotypes with cutaneous and biochemical hyperandrogenism: a case–control study. J Egypt Women Dermatol Soc. 2022; 19: 160-8.
21. Atoum MF, Alajlouni MM, Alzoughool F. A case-control study of the luteinizing hormone level in luteinizing hormone receptor gene (rs2293275) polymorphism in polycystic ovarian syndrome females. Public Health Genomics. 2022; 29: 1-9.
22. Bassiouny YA, Rabie WA, Hassan AA, Darwish RK. Association of the luteinizing hormone/choriogonadotropin receptor gene polymorphism with polycystic ovary syndrome. Gynecol Endocrinol. 2014; 30: 428-30.
23. Capalbo A, Sagnella F, Apa R, Fulghesu AM, Lanzone A, Morciano A, et al. The 312N variant of the luteinizing hormone/choriogonadotropin receptor gene (LHCGR) confers up to 2·7-fold increased risk of polycystic ovary syndrome in a Sardinian population. Clin Endocrinol (Oxf). 2012; 77: 113-9.
24. Alarcón-Granados MC, Moreno-Ortíz H, Rondón-Lagos M, Camargo-Villalba GE, Forero-Castro M. Study of LHCGR gene variants in a sample of colombian women with polycystic ovarian syndrome: A pilot study. J King Saud Univ Sci. 2022; 34: 102202.
25. 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: 2327.
26. Makhdoomi MJ, Shah IA, Rashid R, Rashid A, Singh S, Shah ZA, et al. Effect modification of LHCGR gene variant (rs2293275) on clinico-biochemical profile, and levels of luteinizing hormone in polycystic ovary syndrome patients. Biochem Genet. 2023; 61: 1418-32.
27. Robeva R, Andonova S, Tomova A, Kumanov P, Savov A. LHCG receptor polymorphisms in PCOS patients. Biotechnology & Biotechnological Equipment. 2018; 32: 427-32.
28. El-Shal AS, Zidan HE, Rashad NM, Abdelaziz AM, Harira MM. Association between genes encoding components of the leutinizing hormone/luteinizing hormone-choriogonadotrophin receptor pathway and polycystic ovary syndrome in Egyptian women. IUBMB Life. 2016; 68: 23-36.
29. Singh S, Kaur M, Kaur R, Beri A, Kaur A. Association analysis of LHCGR variants and polycystic ovary syndrome in Punjab: a case-control approach. BMC Endocr Disord. 2022; 22: 335.
30. Thathapudi S, Kodati V, Erukkambattu J, Addepally U, Qurratulain H. Association of luteinizing hormone chorionic gonadotropin

receptor gene polymorphism (rs2293275) with polycystic ovarian syndrome. Genet Test Mol Biomarkers. 2015; 19: 128-32.

31. Mann ON, Kong CS, Lucas ES, Brosens JJ, Hanyaloglu AC, Brighton PJ. Expression and function of the luteinizing hormone choriogonadotropin receptor in human endometrial stromal cells. Sci Rep. 2022; 12: 8624.
32. Khan MJ, Ullah A, Basit S. Genetic basis of polycystic ovary syndrome (PCOS): current perspectives. Appl Clin Genet. 2019: 12: 249-60.
33. Branavan U, Muneeswaran K, Wijesundera S, Jayakody S, Chandrasekharan V, Wijeyaratne C. Identification of selected genetic polymorphisms in polycystic ovary syndrome in Sri Lankan women using low cost genotyping techniques. PLoS One. 2018; 13: e0209830.
34. Waterbury JS, Teves ME, Gaynor A, Han AX, Mavodza G, Newell J, et al. The PCOS GWAS candidate gene ZNF217 influences theca cell expression of DENND1A.V2, CYP17A1, and androgen production. J Endocr Soc. 2022; 6: bvac078.
35. Idicula-Thomas S, Gawde U, Bhaye S, Pokar K, Bader GD. Meta-analysis of gene expression profiles of lean and obese PCOS to identify differentially regulated pathways and risk of comorbidities. Comput Struct Biotechnol J. 2020; 18: 1735-45.