Association of CYP21 Gene Polymorphism rs13405728, CYP11A1 Gene Polymorphism rs4077582 and Insulin Receptor Gene rs2059806 in Polycystic Ovarian Syndrome Patients in Egypt: A Pilot Study

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Abstract

Background: **Polycystic Ovary Syndrome (PCOS) is a prevalent endocrine disorder characterized by hyperandrogenism, insulin resistance, and ovulatory dysfunction. Genetic factors, including polymorphisms in steroidogenic (*CYP21*, *CYP11A1*) and insulin signaling (*INSR*) genes, may contribute to PCOS pathogenesis.** ***Aim:*** **This study investigated the association of *CYP21* rs13405728, *CYP11A1* rs4077582, and *INSR* rs2059806 polymorphisms with PCOS in Egyptian women.** ***Subjects/Materials and Methods:*** **A case-control study included 50 PCOS patients and 50 age-matched controls. Anthropometric, hormonal (LH, FSH, HOMA-IR), and genotypic analyses were performed. SNPs were genotyped using TaqMan assays. Statistical analyses included Hardy-Weinberg equilibrium, logistic regression, and ROC curves.** ***Results:*** **PCOS patients had higher BMI, waist circumference, LH/FSH ratio, and HOMA-IR ($p < 0.001$). The *INSR* rs2059806 AG/GG genotypes (OR: 3.08–5.25), *CYP21* rs13405728 CT/TT (OR: 3.41–6.42), and *CYP11A1* rs4077582 CT/TT (OR: 3.46–6.31) were significantly associated with PCOS ($p < 0.01$). Risk alleles (G for *INSR*, T for CYP21/CYP11A1) were more frequent in cases ($p < 0.001$). These SNPs correlated with elevated LH/FSH and HOMA-IR in PCOS patients. ROC analysis showed LH, LH/FSH ratio, and HOMA-IR had excellent diagnostic accuracy (AUC = 0.998–1.0).** ***Conclusions:*** ***CYP21* rs13405728, *CYP11A1* rs4077582, and *INSR* rs2059806 polymorphisms are significantly associated with PCOS in Egyptian women, influencing hormonal and metabolic disturbances. These SNPs may serve as potential biomarkers for PCOS risk stratification.**

Keywords: PCOS, genetic polymorphisms, insulin resistance, SNPs, Egypt.

Introduction

Polycystic Ovary Syndrome (PCOS) is a common endocrine disorder affecting women of reproductive age with a significant global impact that continues to grow. It is characterized by a combination of reproductive, metabolic, and hormonal abnormalities [(1)]. The Rotterdam criteria for diagnosing Polycystic Ovary Syndrome require at least two of the following three

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conditions after excluding other causes: oligo-ovulation or anovulation (irregular/absent menstrual cycles), clinical/biochemical signs of hyperandrogenism (hirsutism, acne, elevated androgens), and polycystic ovaries on ultrasound (≥12 follicles or ovarian volume >10 mL) [2]. PCOS constitutes a principal cause of anovulatory infertility, furthermore, individuals diagnosed with PCOS are at an increased risk of progressing to impaired glucose tolerance, type 2 diabetes mellitus, metabolic syndrome, and cardiovascular diseases as they age [3].

Polycystic Ovary Syndrome is a complex condition with a multifactorial etiology, involving genetic, hormonal, and environmental factors, genetic predisposition plays a significant role [4]. Insulin resistance in PCOS impairs glucose uptake, leading to hyperinsulinemia, androgen excess, and increased risk of metabolic complications like type 2 diabetes and cardiovascular disease [5]. Reduced insulin sensitivity is frequently associated PCOS in women [6]. Upon insulin binding to the α-subunit of the insulin receptor (INSR), the receptor's intrinsic tyrosine kinase activity is initiated, leading to downstream signaling cascade activation [7]. The INSR gene plays a vital role in insulin signaling and glucose metabolism by encoding the insulin receptor. The rs2059806 SNP is a genetic variation within Insulin Receptor gene, characterized by a G-to-A substitution [8]. The rs2059806 SNP of INSR can disrupt insulin receptor function, leading to increased insulin levels. This hyperinsulinemia can stimulate ovarian androgen production, worsening PCOS symptoms such as hyperandrogenism and ovulatory dysfunction [9].

CYP21 gene (Cytochrome P450 21-hydroxylase) is involved in the biosynthesis of steroid hormones. Variants in this gene may alter adrenal androgen production, which could contribute to hyperandrogenism, a key feature of PCOS. The rs13405728 polymorphism is a single nucleotide change located in the regulatory region of the *CYP21* gene, has been associated with alterations in gene expression and enzyme activity and alterations in this SNP have been linked to changes in steroid hormone production [10]. CYP11A1 (Cytochrome P450 11A1) is crucial for converting cholesterol into pregnenolone, the precursor to steroid hormones. Changes in this gene can affect the synthesis of adrenal and gonadal steroids, which play a role in the endocrine imbalances observed in PCOS. The rs4077582 SNP within the CYP11A1 gene has been studied for its impact on ovarian steroid production and insulin sensitivity, both of which are important in the development of PCOS [11].

Elucidating the molecular basis and genetic factors underlying PCOS may offer crucial insights into the etiology of the condition and is essential for improving diagnostic precision and tailoring treatment strategies to individual needs, ultimately enhancing patient outcomes [12], therefore, the objective of this study is to study the CYP21 gene polymorphism rs13405728, CYP11A1 gene polymorphism rs4077582 and Insulin Receptor Gene rs2059806 and their association with polycystic ovary syndrome patients from Egypt.

Research Design and Methods:

A comparative cross-sectional analytical study was conducted on a cohort of Egyptian female patients diagnosed with polycystic ovaries, who had attended the

obstetrics and gynecology clinics at both Suez Canal University Hospital and Fayed Hospital. The study included 50 young adult Egyptian women with PCO and 50 age- and sex-matched healthy controls. The inclusion criteria comprised female patients aged 18 to 50 years who had been diagnosed with PCO. Laboratory analyses were performed at the Clinical Pathology Department. Written informed consent was obtained from all participants.

Group A – Study Group:

Inclusion Criteria:

- Adult female patients aged between 18 and 50 years.
- Diagnosis of PCO was based on the following:

Clinical diagnosis was determined through medical history and gynecological examination.

▪ All patients in the case group fulfilled the Rotterdam criteria [13], which require the presence of at least two of the following: oligo- or anovulation, clinical and/or biochemical signs of hyperandrogenism, and the presence of polycystic ovaries on ultrasonography.

Both ovaries were examined using ultrasonography (USG) for diagnostic criteria of PCOS. Diagnosis was established if one or both ovaries exhibited 12 or more follicles measuring 2–9 mm in diameter. The presence of just one ovary meeting of this condition was deemed sufficient for diagnosis. Ultrasound imaging was performed during the early follicular phase (days 3–5 of the menstrual cycle) [2].

Exclusion Criteria:

Participants were excluded if they were taking oral contraceptive pills, oral steroids, undergoing hormone replacement therapy, or receiving any medication that could influence endocrine parameters or lipid profiles. Additionally, individuals with a history of alcohol consumption, smoking, pregnancy, hypertension, diabetes mellitus, dyslipidemia, thyroid disorders, hyperprolactinemia, or ovarian tumors (assessed via blood tests and USG in both cases and controls) were also excluded. Critically ill patients and those with a body mass index (BMI) above 25 kg/m² were excluded as well.

Group B – Control Group:

This group included apparently healthy individuals who matched the case group by age and sex. Blood samples for the control group were collected from healthy donors at the blood bank of Suez Canal University Hospital.

Sample Size:

The sample size was calculated using the following formula [14]:

Where n = sample size,

$Z\ \alpha/2 = 1.96$ (standard value corresponding to a 95% confidence interval),

$Z\beta = 0.84$ (standard value corresponding to 80% power),

$p1$ = prevalence of rs13405728 polymorphism in the PCO group = 53% [15],

$p2$ = prevalence of rs13405728 polymorphism in the control group = 22%,

$q = 1 - P$ [15]

Based on these values, the required sample size was 45 patients and 45 controls. Allowing a 10% dropout rate, the final sample included 50 patients and 50 controls.

Methods and Data Collection:

The following procedures were performed for all participants: comprehensive medical history, clinical examination, complete blood count, measurement of weight, waist circumference (WC), Body Mass Index (BMI), serum levels of LH, FSH and LH/FSH by using automated

electrochemiluminescence immunoassay (Cobas 6000) module Cobas e 601, Glucose was measured by Hexokinase method on Cobas 6000 module Cobas e 501. Insulin was measured by Invitrogen human insulin ELIZA kit (catalogue No. KAQ1251) and Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was calculated by using the following formula (Fasting Insulin [μU/mL] × Fasting Glucose [mg/dL]) / 405, with a reference ranges as follows: a value less than 1.0 suggests insulin sensitivity; values between 1.0 and 2.0 are considered within the normal range; values from 2.0 to 2.9 indicate early insulin resistance; and values equal to or greater than 3.0 suggest significant insulin resistance [16].

SNP Selection and Genotyping:

Three tagging single nucleotide polymorphisms in the Insulin receptor gene(rs2059806), *CYP21gene(rs13405728)*, and *CYP11A1gene* (rs4077582) were selected based on a minor allele frequency greater than 5% in African populations, according to the 1000 Genomes Project database (https://www.internationalgenome.org/) . These SNPs were subsequently validated using the dbSNP database (https://www.ncbi.nlm.nih.gov/snp/) and the Ensembl genome browser version 107 (https://www.ensembl.org/index.html).

Sample Collection and Genomic DNA Extraction:

A 3 mL sample of peripheral venous blood was drawn via venipuncture into EDTA-containing tubes from each participant in both the case and control groups. Genomic DNA was extracted using the DNeasy Blood Kit Cat. No. 69504: For 50 preps - includes 50DNeasy Mini Spin Columns, Proteinase K, Buffers, and Collection Tubes (2 ml) (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Extracted DNA was dissolved in 100 μL of TE buffer. The DNA quality was evaluated using a Nanodrop spectrophotometer.

Genotyping of Insulin Receptor, CYP21, and CYP11A1 Polymorphisms:

Genotyping was conducted via real-time PCR (RT-PCR) using TaqMan allelic discrimination assays, in accordance with standard protocols from Applied Biosystems (Carlsbad, USA). Primers and probes for rs2059806 in the Insulin receptor gene, rs13405728 in *CYP21*, and rs4077582 in *CYP11A1* were designed using the Assays-by-Design service (Applied Biosystems, Foster City, California, USA), and are listed in Table 1. The probes were labeled with FAM™ for the first allele and VIC™ for the second allele at the 5′-end, and with quenchers at the 3′-end. These primers and probes were combined with TaqMan Universal PCR Master Mix and dispensed into 96-well microtiter plates. PCR amplification was performed using an Applied Biosystems thermal cycler, and genotype detection was analyzed with SDS 2.1 software (Applied Biosystems). PCR was carried out according to manufacturer instructions, and detection of the different genotypes was done using the Bio-Rad CFX96 Real-Time Detection System C1000 Thermal Cycler (Bio-Rad Laboratories, Hercules ,USA). Data were processed using the CFX Maestro Software for CFX Real-Time PCR Instruments (Bio-Rad Laboratories, Hercules, USA)

Table 1: the Primer Sequences of the 3 SNP under study:		
SNP	Forward primer	Reverse primer
rs13405728 (CYP21 gene)	5′-GTGGTTCTTACTCTAGCACAATGAT-3′	5′-CCATCCACATACTCACTTCAATATC-3′
rs4077582 (CYP11A1 gene)	5′-GCCAGTCAGACAAGGGCACAG-3′	5′-GTGGCCGACTATGTAAACCAG-3′
rs2059806 (Insulin Receptor Gene)	5′-CGGTCTTGTAAGGGTAACTG-3′	5′-GAATTCACATTCCCAAGACA-3′

Data Management:

The data was analyzed using IBM Corp's Statistical Package for Social Science (released in 2017). IBM SPSS Statistics for Windows, Version 25.0 (Armonk, NY: IBM Corp.). The Mann-Whitney test was used to determine the statistical significance of the difference in a nonparametric variable between two research groups. The Kruskal-Walli's test was used to determine the statistical significance of differences between more than two research groups' non-parametric variables. The degree of confidence between observed and expected genotype frequencies was used to calculate deviations from Hardy-Weinberg equilibrium expectations. The degree of link between two quantitative variables was evaluated using Spearman's correlation. A helpful method for assessing the sensitivity and specificity of quantitative diagnostic measures that divide cases into two groups is to utilize the receiver operating characteristic, or ROC curve. The cutoff point that maximized the AUC value was considered the ideal one. Risk variables were predicted using logistic regression analysis, by reducing a huge set of variables into a smaller one that yet retains the majority of the information in the larger set, principal component analysis was utilized to reduce the dimensionality of large data sets. P values below 0.05 are regarded as significant.

Ethical Considerations:

The ethical guidelines established by the Ethics Committee of the Faculty of Medicine, Suez Canal University, were strictly adhered to throughout the study. Approval of the study was granted by the Research Ethics Committee of the Faculty of Medicine, Suez Canal University, under reference number Research (6073#). Informed consent was obtained from all participants or from their legal guardians in cases where participants were younger than 21 years—prior to any data collection or investigation.

Results

The study was conducted among 50 Egyptian women with polycystic ovaries and 50 healthy controls, attending g to obstetrics and gynecology clinic in both Suez Canal university hospital and fayed hospital in Egypt. The study aimed to compare and analyze various factors between the two groups.

Table 2 revealed significant differences between PCO patients and controls, including higher BMI and waist circumference, elevated luteinizing hormone (LH) and LH/FSH ratio, lower follicle-stimulating hormone (FSH) levels, and substantial insulin resistance as indicated by higher HOMA-IR scores in PCO patients.

Table 2. Association of age, anthropometric and laboratory parameters between the studied groups.

		Control	PCO	*P*
		N=50	N=50	
Age (years)	mean±SD	30.98±6.48	29.68±6.76	0.267
	Median (min-max)	32(21-44)	29(20-43)	
BMI (kg/m²)	mean±SD	21.86±4.18	28.62±4.33	<0.001*
	Median (min-max)	22(2-32)	29(20-35)	
WC (cm)	mean±SD	80.92±7.89	101.1±7.25	<0.001*
	Median (min-max)	79(70-101)	101(88-113)	
LH (mIU/µl)	mean±SD	2.62±0.63	9.27±1.27	<0.001*
	Median (min-max)	2.65(1.3-3.88)	8.9(7.6-12.3)	
FSH (mIU/ml)	mean±SD	5.18±1.06	4.01±0.96	<0.001*
	Median (min-max)	5(3.3-8.3)	4.3(1.5-5.6)	
LH/FSH ratio	mean±SD	0.53±0.17	2.5±0.92	<0.001*
	Median (min-max)	0.5(0.25-0.87)	2.27(1.52-5.8)	
HOMA-IR	mean±SD	1.2±0.39	3.6±0.86	<0.001*
	Median (min-max)	1(0.5-2.5)	3.5(2-5.5)	

N, number; SD, standard deviation; min, minimum; max, maximum. PCO, polycystic ovary syndrome; BMI, body mass index; WC, waist circumference; LH, luteinizing hormone; FSH, follicle-stimulating hormone; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; p<0.05 is considered significant.

Table 3 presents a comprehensive analysis of the association between specific genetic polymorphisms rs2059806 in the Insulin receptor gene, rs13405728 in *CYP21*, and rs4077582 in *CYP11A1* and the occurrence of PCO. Genotype distributions for all three variants were in Hardy-Weinberg equilibrium, suggesting genetic stability within the study groups. Significant differences in genotype frequencies were observed between cases and controls, with the AG and GG genotypes of rs2059806 showing notably increased odds ratios (ORs of 3.081 and 5.254), indicating a strong link to PCO susceptibility. Similarly, the CT and TT genotypes of both rs13405728 and rs4077582 were significantly associated with elevated risk, with ORs of 3.409 and 6.421 for rs13405728, and 3.456 and 6.31 for rs4077582. These associations were further supported by dominant, recessive, and codominant inheritance models. Moreover, allele frequency analysis revealed that the G allele of rs2059806 and the T alleles of rs13405728 and rs4077582 were significantly more prevalent in the PCO group, with p-values < 0.001 and ORs exceeding 1, highlighting their potential role as genetic risk factors.

Table 3. Association of studied polymorphisms among the studied groups.							
		Control		PCO		*P*	OR (95% CI)
		N=50		N=50			
		N	%	N	%		
rs2059806							
Genotypes	AA	44	88.0	24	48.0		Reference
	AG	5	10.0	17	34.0	0.001*	3.081 (1.599-5.938)
	GG	1	2.0	9	18.0	0.003*	5.254 (1.744-15.826)
Dominant model	AA	44	88.0	24	48.0		Reference
	AG+GG	6	12.0	26	52.0	<0.001*	3.541 (1.967-6.377)
Recessive model	AA+AG	49	98.0	41	82.0		Reference
	GG	1	2.0	9	18.0	0.012*	4.028 (1.353-11.989)
Over-dominant model	AA+GG	45	90.0	33	66.0		Reference
	AG	5	10.0	17	34.0	0.004*	2.565 (1.346-4.887)
Alleles	A	93	93.0	65	65.0		Reference
	G	7	7.0	35	35.0	<0.001*	3.292 (2.012-5.386)
HW-*p*		0.101		0.074			
rs13405728							
Genotypes	CC	42	84.0	18	36.0		Reference
	CT	7	14.0	22	44.0	<0.001*	3.409 (1.87-6.214)
	TT	1	2.0	10	20.0	0.001*	6.421 (2.158-19.11)
Dominant model	CC	42	84.0	18	36.0		Reference
	CT+TT	8	16.0	32	64.0	<0.001*	3.92 (2.252-6.823)
Recessive model	CC+CT	49	98.0	40	80.0		Reference
	TT	1	2.0	10	20.0	0.007*	4.316 (1.479-12.591)
Over-dominant model	CC+TT	43	86.0	28	56.0		Reference
	CT	7	14.0	22	44.0	0.001*	2.637 (1.476-4.712)
Alleles	C	91	91.0	58	58.0		Reference
	T	9	9.0	42	42.0	<0.001*	3.354 (2.133-5.273)
HW-*p*		0.304		0.493			
rs4077582							
Genotypes	CC	44	88.0	21	42.0		Reference
	CT	5	10.0	18	36.0	<0.001*	3.456 (1.795-6.653)
	TT	1	2.0	11	22.0	0.001*	6.31 (2.169-18.359)
Dominant model	CC	44	88.0	21	42.0		Reference
	CT+TT	6	12.0	29	58.0	<0.001*	4.086

							(2.279-7.328)
Recessive model	CC+CT	49	98.0	39	78.0		Reference
	TT	1	2.0	11	22.0	0.005*	4.599 (1.604-13.187)
Over-dominant model	CC+TT	45	90.0	32	64.0		Reference
	CT	5	10.0	18	36.0	0.002*	2.703 (1.427-5.12)
Alleles	C	93	93.0	60	60.0		Reference
	T	7	7.0	40	40.0	<0.001*	3.724 (2.298-6.033)
HW-*p*		0.101		0.077			
OR, odds ratio; CI, confidence interval; HW-p, Hardy Weinberg equation p value							

Table 4 demonstrates that within the control group, the rs2059806 genotypes do not significantly influence BMI, waist circumference (WC), LH, FSH, the LH/FSH ratio, or HOMA-IR, as indicated by p-values above 0.05. In contrast, the PCO group shows pronounced increases in BMI and WC among individuals with AG and GG genotypes compared to those with the AA genotype ($p < 0.001$). Additionally, the LH/FSH ratio is significantly higher in AG and GG carriers ($p = 0.043$), pointing to a potential link between these genotypes and hormonal imbalance. HOMA-IR values are also markedly elevated in the AG and GG genotypes ($p < 0.001$), indicating a stronger association with insulin resistance in the PCO group.

Table 5 highlights the relationship between rs13405728 genotypes and various clinical and hormonal parameters. Among the control group, no significant differences are observed in BMI, waist circumference (WC), LH levels, LH/FSH ratio, or HOMA-IR across genotypes ($p > 0.05$). However, in the PCO group, the CT and TT genotypes are associated with significantly higher BMI ($p = 0.003$), WC ($p = 0.005$), LH/FSH ratio ($p = 0.002$), and HOMA-IR ($p = 0.005$), indicating a potential link to increased metabolic and hormonal disturbances. In contrast, FSH levels are significantly lower in individuals with these genotypes ($p = 0.001$), suggesting that the rs13405728 polymorphism may contribute to hormonal dysregulation in PCO.

Table 4. Association of rs2059806 genotypes with different parameters among PCO and control groups.

rs2059806		Control			*p*	PCO			*p*
		AA	AG	GG		AA	AG	GG	
		N=44	N=5	N=1		N=24	N=17	N=9	
BMI (kg/m²)	mean± SD	22±4.42	20.4±0.89	23	0.199	24.96±2.77	31±2.09	33.89±0.6	<0.001*
	Median (min-max)	22(2-32)	20(20-22)	23(23-23)		25(20-30)	32(27-33)	34(33-35)	
WC (cm)	mean± SD	81.23±7.94	78.4±8.68	80	0.785	96±5.64	103.53±4.47	109.56±4.48	<0.001*
	Median (min-max)	79(70-101)	76(70-89)	80(80-80)		94(88-109)	102(98-110)	111(99-113)	
LH (mIU/µl)	mean± SD	2.65±0.62	2.52±0.82	2.1	0.602	8.94±1.07	9.69±1.32	9.33±1.55	0.186
	Median (min-max)	2.7(1.3-3.88)	2.2(1.8-3.4)	2.1(2.1-2.1)		8.7(7.7-11.8)	9.4(7.6-11.5)	8.7(7.9-12.3)	
FSH (mIU/ml)	mean± SD	5.24±1.09	4.86±0.71	4.3	0.506	4.27±0.74	3.74±1.07	3.8±1.15	0.216
	Median (min-max)	5(3.3-8.3)	5.3(3.9-5.4)	4.3(4.3-4.3)		4.4(2.3-5.6)	3.9(1.9-5.3)	4.1(1.5-5.3)	
LH/FSH ratio	mean± SD	0.52±0.16	0.55±0.26	0.49	0.974	2.15±0.45	2.85±1.1	2.73±1.21	0.043*
	Median (min-max)	0.5(0.25-0.87)	0.41(0.33-0.87)	0.49(0.49-0.49)		2.13(1.52-3.43)	2.67(1.74-5.38)	2.32(1.76-5.8)	
HOMA-IR	mean± SD	1.22±0.41	1.1±0.22	1	0.767	2.9±0.33	3.9±0.53	4.91±0.28	<0.001*
	Median (min-max)	1(0.5-2.5)	1(1-1.5)	1(1-1)		3(2-3.5)	4(3.5-5.5)	4.9(4.5-5.3)	

N, number; SD, standard deviation; min, minimum; max, maximum. PCO, polycystic ovary syndrome; BMI, body mass index; WC, waist circumference; LH, luteinizing hormone; FSH, follicle-stimulating hormone; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; p<0.05 is considered significant

Table 5 highlights the relationship between rs13405728 genotypes and various clinical and hormonal parameters. Among the control group, no significant differences are observed in BMI, waist circumference (WC), LH levels, LH/FSH ratio, or HOMA-IR across genotypes ($p > 0.05$). However, in the

PCO group, the CT and TT genotypes are associated with significantly higher BMI (p = 0.003), WC (p = 0.005), LH/FSH ratio (p = 0.002), and HOMA-IR (p = 0.005), indicating a potential link to increased metabolic and hormonal disturbances. In contrast, FSH levels are significantly lower in individuals with these genotypes (p = 0.001), suggesting that the rs13405728 polymorphism may contribute to hormonal dysregulation in PCO.

Table 5. Association of rs13405728 genotypes with different parameters among PCO and control groups

rs13405728		Control				PCO			
		CC	CT	TT	*p*	CC	CT	TT	*p*
		N=42	N=7	N=1		N=18	N=22	N=10	
BMI (kg/m²)	mean± SD	21.1±3.58	25±4.73	32	0.083	26.28±4.71	28.82±3.53	32.4±2.01	0.003*
	Median (min-max)	21.5(2-28)	28(20-30)	32(32-32)		25(20-34)	30(23-34)	33(29-35)	
WC (cm)	mean± SD	80.24±7.68	85.29±8.9	79	0.381	97.24±7.64	101.86±6.32	106±5.21	0.005*
	Median (min-max)	79(70-99)	86(76-101)	79(79-79)		97(88-112)	101(90-113)	106.5(99-113)	
LH (mIU/µl)	mean± SD	2.63±0.62	2.39±0.55	3.88	0.151	9.08±1.35	9.36±1.35	9.4±1	0.475
	Median (min-max)	2.65(1.3-3.8)	2.2(1.8-3.3)	3.88(3.88-3.88)		8.65(7.8-12.3)	8.94(7.6-11.8)	9.25(7.9-11.3)	
FSH (mIU/ml)	mean± SD	5.1±1.06	5.36±0.79	7.3	0.181	4.54±0.79	4.06±0.55	2.93±1.13	0.001*
	Median (min-max)	4.9(3.3-8.3)	5.4(3.8-6.4)	7.3(7.3-7.3)		4.75(2.9-5.6)	4.3(2.5-4.7)	2.6(1.5-4.5)	
LH/FSH ratio	mean± SD	0.54±0.17	0.47±0.19	0.53	0.420	2.05±0.38	2.33±0.38	3.67±1.42	0.002*
	Median (min-max)	0.51(0.25-0.87)	0.41(0.33-0.87)	0.53(0.53-0.53)		2.19(1.52-2.69)	2.42(1.79-3.16)	3.53(2.09-5.8)	
HOMA-IR	mean± SD	1.18±0.4	1.36±0.38	1	0.239	3.4±1.02	3.42±0.6	4.35±0.68	0.005*
	Median (min-max)	1(0.5-2.5)	1.5(1-2)	1(1-1)		3(2-5.5)	3(3-4.9)	4.15(3.5-5.3)	

N, number; SD, standard deviation; min, minimum; max, maximum. PCO, polycystic ovary syndrome; BMI, body mass index; WC, waist circumference; LH, luteinizing hormone; FSH, follicle-stimulating hormone; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; p<0.05 is considered significant.

Table 6 explores the association between rs4077582 genotypes and various clinical and hormonal parameters. In the control group, no significant differences were observed in BMI, WC, LH, FSH, LH/FSH ratio, or

HOMA-IR across genotypes ($p > 0.05$). In contrast, the PCO group showed significant increases in BMI ($p = 0.029$), LH levels ($p = 0.006$), LH/FSH ratio ($p < 0.001$), and HOMA-IR ($p = 0.023$), particularly in individuals with CT and TT genotypes. Additionally, FSH levels were significantly reduced in these genotypes ($p < 0.001$), suggesting a strong association between rs4077582 and hormonal imbalance in PCO.

Table 6. Association of rs4077582 genotypes with different parameters among PCO and control groups

rs4077582		Control				PCO			
		CC	CT	TT	*p*	CC	CT	TT	*P*
		N=44	N=5	N=1		N=21	N=18	N=11	
BMI (kg/m^2)	mean± SD	21.55±4.18	24.8±3.77	21	0.227	26.81±4.41	29.22±4.04	31.09±3.3	0.029*
	Median (min-max)	22(2-32)	25(21-29)	21(21-21)		25(20-34)	28.5(23-34)	32(24-35)	
WC (cm)	mean± SD	80.75±7.57	83.4±11.55	76	0.596	100±8.41	101.39±6.89	102.64±5.66	0.570
	Median (min-max)	79(70-99)	80(70-101)	76(76-76)		100.5(88-113)	101(89-112)	102(93-113)	
LH (mIU/µl)	mean± SD	2.62±0.65	2.6±0.51	2.8	0.930	8.58±0.61	10.02±1.47	9.35±1.19	0.006*
	Median (min-max)	2.6(1.3-3.88)	2.7(1.9-3.3)	2.8(2.8-2.8)		8.7(7.6-9.5)	9.55(7.8-12.3)	9.7(7.8-11.3)	
FSH (mIU/ml)	mean± SD	5.24±1.08	4.88±0.8	4.1±.	0.385	4.62±0.48	4.13±0.6	2.65±0.75	<0.001*
	Median (min-max)	5.15(3.3-8.3)	4.7(3.8-5.9)	4.1(4.1-4.1)		4.5(3.7-5.6)	4.05(3.2-5.3)	2.5(1.5-3.7)	
LH/FSH ratio	mean± SD	0.52±0.17	0.55±0.18	0.68	0.516	1.87±0.19	2.43±0.18	3.79±1.16	<0.001*
	Median (min-max)	0.5(0.25-0.87)	0.5(0.41-0.87)	0.68(0.68-0.68)		1.93(1.52-2.12)	2.42(2.17-2.68)	3.43(2.68-5.8)	
HOMA-IR	mean± SD	1.18±0.37	1.4±0.55	1	0.563	3.3±0.83	3.59±0.77	4.18±0.84	0.023*
	Median (min-max)	1(0.5-2.5)	1(1-2)	1(1-1)		3(2-5.3)	3.25(2.5-4.9)	4(3-5.5)	

N, number; SD, standard deviation; min, minimum; max, maximum. PCO, polycystic ovary syndrome; BMI, body mass index; WC, waist circumference; LH, luteinizing hormone; FSH, follicle-stimulating hormone; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; $p<0.05$ is considered significant.

Table 7 presents the correlations among various clinical and hormonal parameters in both control subjects and individuals with PCOS. In the control group, the LH/FSH ratio is positively correlated with LH and negatively correlated with FSH, both significantly. In the PCOS group, stronger and more extensive correlations are observed, with BMI, waist circumference, LH, LH/FSH ratio, and HOMA-IR all showing significant positive associations with one another. Conversely, FSH demonstrates significant negative correlations with these parameters, suggesting a distinct pattern of hormonal and metabolic interplay in PCOS.

Table 7. Correlations of the parameters studied among PCO and control groups.

Group			BMI	WC	LH	FSH	LH/FSH	HOMA-IR
Control	Age	*rs*	0.045	-0.108	0.100	0.130	0.003	0.215
		p	0.754	0.455	0.488	0.370	0.982	0.126
	BMI	*rs*		0.277	0.069	0.107	-0.019	0.221
		p		0.052	0.635	0.460	0.896	0.124
	WC	*rs*			-0.037	0.175	-0.162	0.022
		p			0.797	0.225	0.262	0.880
	LH	*rs*				-0.009	0.802	0.062
		p				0.948	<0.001*	0.670
	FSH	*rs*					-0.565	-0.126
		p					<0.001*	0.383
	LH/FSH	*rs*						0.163
		p						0.259
PCO	Age	*rs*	0.219	0.241	-0.017	-0.251	0.165	0.128
		p	0.127	0.095	0.907	0.079	0.253	0.375
	BMI	*rs*		0.822	0.301	-0.482	0.589	0.908
		p		<0.001*	0.034*	<0.001*	<0.001*	<0.001*
	WC	*rs*			0.171	-0.330	0.384	0.741
		p			0.239	0.021*	0.007*	<0.001*
	LH	*rs*				0.131	0.405	0.189
		p				0.366	0.003*	0.189
	FSH	*rs*					-0.803	-0.453
		p					<0.001*	0.001*
	LH/FSH	*rs*						0.546
		p						<0.001*

rs, correlation coefficient; p<0.05 is considered significant.

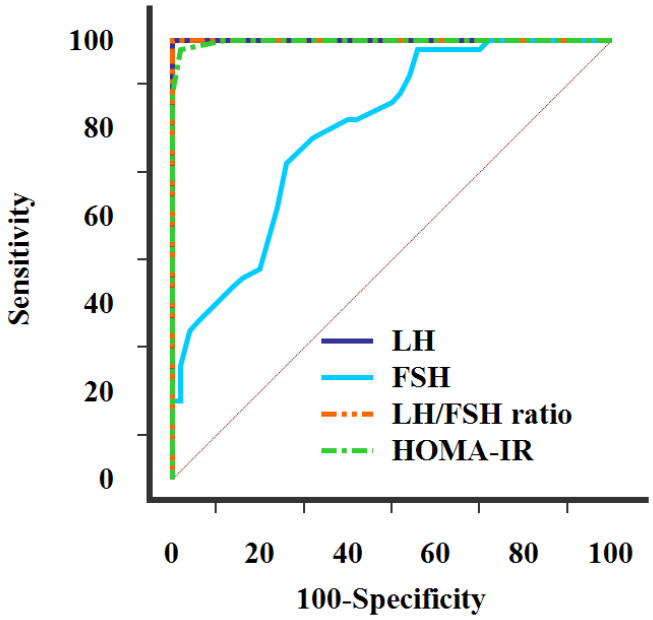

Figure 1: ROC curve of LH, FSH, LH/FSH and HOMA-IR for prediction of PCO risk.

Figure 1: evaluated the efficacy of LH, FSH, LH/FSH ratio, and HOMA-IR in forecasting PCO risk using ROC curve analysis. LH and LH/FSH ratio attained optimal AUC scores of 1.0, indicating exceptional ability to differentiate between PCO and control groups, with 100% sensitivity and specificity at cut-off values exceeding 3.88 for LH and 0.87 for LH/FSH ratio. FSH had a moderate AUC of 0.796, with a cut-off of ≤4.5 yielding 72% sensitivity and 74% specificity. HOMA-IR had an AUC of 0.998, indicating excellent predictive accuracy, with a cut-off value of >2 delivering 98% sensitivity and specificity, highlighting the importance of LH, LH/FSH ratio, and HOMA-IR as reliable biomarkers for assessing PCO risk.

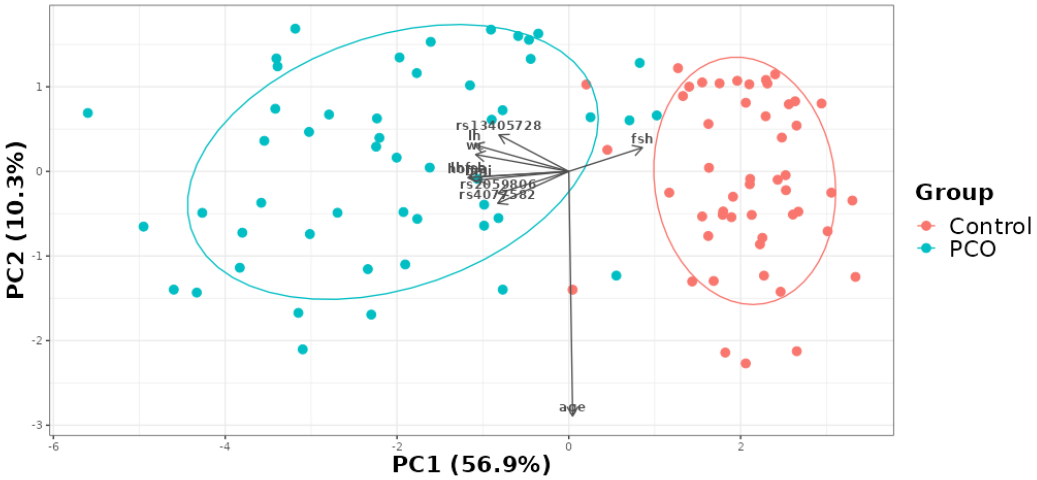

Figure 2: PCA for studying covariates between PCO and control groups.

In figure 2, Principal component analysis was used to reduce the dataset dimensions and visualize the covariates across two principal components. PC1,

represented on the horizontal axis, accounts for 56.9% of the total variance, while PC2, on the vertical axis, explains 10.3%. The samples were categorized into two groups: PCO and Control. The plot shows a distinct separation between the groups. Variables such as BMI, waist circumference (WC), luteinizing hormone (LH), the LH/FSH ratio, HOMA-IR, and the analyzed genetic variants (rs13405728, rs10859804, rs4076828) appear more closely associated with the PCO group. In contrast, FSH levels are more elevated in the Control group. Age contributes minimally to the variation between the two groups based on the directions of the vectors.

Discussion

Polycystic Ovary Syndrome is a prevalent endocrine disorder among women of reproductive age, characterized by chronic anovulation, hyperandrogenism, and polycystic ovarian morphology, and is a major contributor to anovulatory infertility. Its multifactorial etiology involves genetic, hormonal, and environmental components, with insulin resistance playing a central role in metabolic dysfunction and excess androgen production. Key genetic polymorphisms, including rs2059806 in the insulin receptor (INSR) gene, rs13405728 in the CYP21 gene, and rs4077582 in the CYP11A1 gene, have been implicated in altering insulin signaling and steroid hormone synthesis, contributing to PCOS pathogenesis. Our study was carried out among 50 Egyptian women diagnosed with polycystic ovaries and 50 healthy controls, all of whom were recruited from selected hospitals in Egypt. The primary objective was to compare and evaluate specific clinical, biochemical, and genetic factors between the two groups to identify potential associations relevant to PCOS pathogenesis.

Our study demonstrated several statistically significant differences between patients with polycystic ovary syndrome and healthy controls. Notably, PCO patients exhibited higher BMI and waist circumference, indicating a greater degree of central obesity, which is a known contributor to metabolic dysfunction. Furthermore, elevated luteinizing hormone (LH) levels and an increased LH/FSH ratio, along with reduced FSH, are consistent with the classical endocrine features of PCO. Additionally, the PCO patients in our study showed marked insulin resistance, evidenced by significantly higher HOMA-IR scores. This supports the hypothesis that insulin resistance is a key pathophysiological component of PCO. Recent researches supports our findings, showing that insulin resistance is a core feature of PCOS, independent of obesity status, and is associated with reproductive dysfunction [17,18]. Additionally, other studies confirm that PCOS patients often have elevated LH and LH/FSH ratios, decreased FSH levels [17,19], and higher BMI and waist circumference [20,21], which are linked to hormonal disturbances and insulin resistance. These findings support a growing body of evidence that PCOS is a heterogeneous syndrome with both metabolic and reproductive components. Elevated insulin resistance, hormonal imbalance, and central obesity appear to reinforce each other in a cyclical manner. Effective clinical management of PCOS should therefore aim to target both hormonal dysregulation and metabolic dysfunction, with special emphasis on improving insulin sensitivity and reducing central adiposity.

Our study found significant associations between three SNPs (rs2059806 in the INSR gene, rs13405728 in the CYP21 gene, and rs4077582 in the CYP11A1 gene) and PCO susceptibility. Specific genotypes (AG and GG for rs2059806, CT and TT for rs13405728 and rs4077582) increased PCO risk. The G allele (rs2059806) and T alleles (rs13405728 and rs4077582) were more frequent in PCO patients, suggesting that they may act as genetic risk factors. Our findings on rs2059806 (INSR gene) are supported by another study which found a significant association between this SNP and insulin resistance and PCOS risk, particularly in South Asian women and the G allele was linked to impaired insulin receptor function and metabolic disruption, consistent with our results showing strong associations between AG and GG genotypes and PCO [22]. The association between rs2059806 and PCOS may be population-specific, as a 2023 study found no association in certain European cohorts, highlighting genetic variability across different populations [23]. Recent studies support our findings, showing associations between rs13405728 (CYP21 gene) and androgen excess/PCOS risk, particularly with elevated LH/FSH ratios [24], and between rs4077582 (CYP11A1 gene) and PCOS in women with hyperandrogenemia, potentially enhancing androgen synthesis [25]. These findings align with our results, suggesting these SNPs contribute to abnormal steroidogenesis and elevated androgen levels in PCOS. The SNPs identified may be valuable targets for early detection, personalized treatment, and genetic counseling in high-risk women. However, population-specific genetic screening and larger multiethnic cohort studies are needed to validate findings and account for genetic variability across different populations.

Our results revealed that the genetic polymorphisms rs2059806 (INSR), rs13405728 (CYP21), and rs4077582 (CYP11A1) are significantly associated with metabolic and hormonal disturbances in PCO patients but not in healthy controls, highlighting gene–environment interactions specific to the disease state. In PCO patients, AG and GG genotypes of rs2059806 were linked to higher BMI, waist circumference, LH/FSH ratio, and HOMA-IR, suggesting a role in insulin resistance and hormonal imbalance. Similarly, CT and TT genotypes of rs13405728 and rs4077582 were associated with increased BMI, LH/FSH ratio, HOMA-IR, and decreased FSH levels, indicating disrupted steroidogenesis and pituitary function. These associations were not observed in the control group, emphasizing that the impact of these polymorphisms manifests primarily under the physiological stress of PCOS. Recent studies support these genotype-specific effects, reinforcing the role of these SNPs as potential markers of disease severity and therapeutic targets in PCOS management [22-25].

Our study's ROC analysis results strongly support the diagnostic utility of LH, LH/FSH ratio, and HOMA-IR in predicting PCO, with exceptional discriminatory power (AUC = 1.0 for LH and LH/FSH ratio, and 0.998 for HOMA-IR). These findings align with recent studies [26], although some variability in AUCs is noted across different populations and studies [27]. The results suggest combining LH, LH/FSH ratio, and HOMA-IR as a reliable biomarker panel for early PCO risk assessment, with limited reliance on FSH alone.

The principal component analysis in our study showed a clear separation

between PCO and control groups, driven by metabolic, hormonal, and genetic variables. Key factors such as BMI, waist circumference, LH, LH/FSH ratio, HOMA-IR, and specific genetic variants were closely associated with the PCO group, highlighting the role of insulin resistance and androgen excess in PCOS. **Liu et al.** identified a strong genetic link between increased BMI, central obesity, and PCOS, suggesting shared genetic influences [28], this aligns with our PCA results, where BMI and waist circumference significantly contributed to group differences. The elevated LH levels and higher LH/FSH ratio observed in the PCO group are consistent with findings in a Chinese cohort, where the rs13405728 variant in the *CYP21A2* gene was associated with increased LH and disrupted gonadotropin regulation [29], this supports the variant's role in disturbing the hypothalamic-pituitary-ovarian axis, central to PCOS development.

The presence of the *INSR* gene variant rs2059806 also corresponds with prior studies linking *INSR* mutations to insulin resistance in PCOS, especially among non-obese women [30]. Our finding of HOMA-IR clustering with PCO further highlights insulin resistance as a key factor.

Conversely, the higher FSH levels in the Control group reflect the hormonal imbalance typical in PCOS, where elevated LH suppresses FSH, impairing follicular growth and ovulation [31]. Interestingly, age showed little effect on group separation, differing from studies like Teede et al. which found age influenced PCOS traits in older womenlikely due to the narrow age range in our sample [32].

Conclusion:

This study demonstrated a significant association between polymorphisms in the CYP21 gene (rs13405728), CYP11A1 gene (rs4077582), and Insulin Receptor gene (rs2059806) and the susceptibility to polycystic ovary syndrome in Egyptian women. Patients with PCOS exhibited higher frequencies of the risk alleles and genotypes in these polymorphisms compared to healthy controls. These genetic variations correlated with increased BMI, waist circumference, hormonal imbalances (elevated LH and LH/FSH ratio, decreased FSH), and higher insulin resistance (HOMA-IR), suggesting these SNPs contribute to the pathogenesis of PCOS through effects on steroidogenesis and insulin signaling. The findings support the role of genetic predisposition involving steroidogenic enzymes and insulin receptor function in the etiology of PCOS.

Recommendations:

The study recommends genetic screening for specific SNPs (rs13405728, rs4077582, rs2059806) in Egyptian women at risk for PCOS to improve early diagnosis and personalized management. It also suggests exploring targeted therapies for altered steroidogenesis and insulin signaling pathways, conducting larger multi-center and multi-ethnic studies to validate associations, and emphasizing lifestyle modifications focusing on weight management and metabolic health for PCOS patients carrying risk alleles.

Limitations of the Study:

The study's limitations include a small sample size from a specific geographic area in Egypt, a cross-sectional design that can't establish causality, limited SNP selection potentially overlooking other

genetic variants, lack of control for environmental and lifestyle factors, exclusion of obese individuals, absence of functional studies to assess the polymorphisms' effects, and single-center recruitment introducing potential selection bias

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