Genetic Association Between Polycystic Ovary Syndrome and the *APOA5* rs662799 and *PLIN1* rs894160 Metabolic Variants in the Western Saudi Population: A Case-Control Study

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ABSTRACT

BACKGROUND: Polycystic ovary syndrome (PCOS) is a common endocrinological condition affecting women of reproductive age, associated with insulin resistance and obesity. PCOS pathogenesis is complex and multifactorial, involving genetic and environmental factors.

OBJECTIVES: This study aimed to determine and compare genotype and allele frequencies of single nucleotide polymorphisms (SNPs) in the apolipoprotein A5 (*APOA5*; rs662799) and perilipin 1 (*PLIN1*; rs894160, rs1052700 and rs6496589) genes in Western Saudi women to investigate their association with PCOS and its clinical characteristics.

DESIGN AND METHODS: This was a case-control study conducted on women with ($n = 104$) and without ($n = 87$) PCOS. The SNPs were genotyped using TaqMan genotyping assays.

RESULTS: Significant and direct associations were detected between PCOS susceptibility and *APOA5* SNP rs662799 and *PLIN1* SNP rs894160 ($P < .001$). For *APOA5* SNP rs662799, women with the A allele were more likely to have PCOS (relative risk [RR] = 1.348, odds ratio [OR] = 2.313, $P < .001$) and hypertriglyceridaemia (OR = 17.0, $P = .5$) than women with the G allele. For *PLIN1* SNP rs894160, women with the T allele were more likely to have PCOS than women with the C allele (RR = 8.043, OR = 7.427, $P < .001$). For *PLIN1* SNP rs1052700, women with the TT genotype were more likely to have hyperandrogenism (OR = 29.75, $P = .02$) and an irregular period (OR = 0.07, $P = .040$) than women with the AT genotype.

CONCLUSION: We identified novel alleles and genotypes contributing to the genetic risk of PCOS in the Western Saudi population.

KEYWORDS: Genotype, polycystic ovary syndrome, single nucleotide polymorphism, apolipoprotein A5, perilipin 1

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Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinological condition in women of reproductive age.[1] The prevalence of PCOS globally is approximately 4% to 6.6% following the 1990 National Institutes of Health (NIH) criteria and approximately 4% to 21% following the 2003 Rotterdam criteria.[1] In the Middle East, the prevalence was reported as 6.1% under the 1990 NIH criteria and 16.0% under the 2003 Rotterdam criteria.[2] PCOS is characterised by excessive androgen secretion, persistent anovulation and polycystic ovarian morphology (PCOM).[3-5] The Rotterdam criteria define PCOS by the presence of at least 2 out of the following 3 criteria: irregular period, clinical or biochemical hyperandrogenism (HA), and PCOM.[6] PCOS pathogenesis is complex and multifactorial, involving genetic and environmental factors. PCOS results from unbalanced hypothalamus–pituitary–ovarian axis signalling, promoting ovarian and adrenal HA. It is complicated by insulin resistance exacerbated by HA-related adipose tissue accumulation and dysfunction. Therefore, it is accompanied by lipotoxicity and oxidative stress.[7] Moreover, a strong association exists between PCOS and increased body weight, with PCOS affecting up to 14% of women with a body mass index (BMI) of at least 30 kg/m.[2,8]

Multiple studies have associated PCOS with a myriad of comorbidities, including glucose intolerance, dyslipidaemia, type 2 diabetes mellitus (T2DM), hypertension, endometrial cancer, obesity, depression, anxiety, non-alcoholic fatty liver disease, sleep apnoea, eating disorders and infertility.[9,10] Among these, dyslipidaemia is among the most common in patients with PCOS.[5] PCOS patients show altered lipid levels, including decreased high-density lipoprotein-cholesterol (HDL-C) levels and elevated triglyceride (TG), total cholesterol (TC) and low-density lipoprotein-cholesterol (LDL-C) levels.[11,12] One recent study found that mild hypercholesterolaemia was frequently observed in patients with PCOS.[13] Dyslipidaemia in PCOS is associated with frequent insulin resistance, although

not all patients with PCOS have insulin resistance.[14] The association between dyslipidaemia and insulin resistance is explained by hyperinsulinaemia inhibiting lipolysis and increasing esterified acids. Therefore, increased levels of non-esterified fatty acids may increase TGs and decrease HDL-C levels.[15]

Apolipoprotein A5 (*APOA5*) is located in the *APOA1*/C3/A4 gene cluster on chromosome 11q23. *APOA5* is mainly found in hepatocytes and secreted into the blood. It is involved in TG synthesis and removal. Increased *APOA5* levels correlated with decreased serum TG levels.[16] Defective APOA5 structure or biosynthesis may result in disorders of the plasma lipid transport system and the development of coronary artery disease (CAD).[17] *APOA5* SNP rs662799 was associated with increased TG levels in Asian Indians.[18] Additionally, *APOA5* SNP rs662799 was associated with high TG levels and myocardial infarction (MI) in Italians.[19] A significant correlation also existed between *APOA5* SNP rs662799 and CAD in Japanese and Chinese people.[16,20]

Perilipins (*PLIN*) are phosphorylated proteins found on the surface of lipid droplets in adipocyte, steroid-producing, liver, heart and muscle cells.[21] The *PLIN1* gene is located on chromosome 15q26, a region previously associated with obesity, hypertriglyceridaemia and diabetes. The role of these proteins is to produce fat droplets, store TGs and perform lipolysis.[21,22] The most frequently studied *PLIN1* single nucleotide polymorphisms (SNPs) associated with obesity are rs894160, rs1052700, rs2289487 and rs2304795.[23] Previous studies have shown that *PLIN1* SNPs are associated with impaired glucose tolerance and increased LDL in individuals with PCOS, especially rs1052700, which is also seen in those without PCOS.[24] *PLIN1* SNPs rs2289487 and rs894160 appeared protective against lipidosis. In contrast, *PLIN1* SNPs rs1052700 and rs2304795 were associated with an increased risk of obesity.[23]

SNPs in the *APOA5* and *PLIN1* genes have been previously associated with human lipid levels. Therefore, we aimed to determine the effect of *APOA5* SNP rs662799 and *PLIN1* SNPs rs894160, rs1052700 and rs6496589 on PCOS risk in Western Saudi women. Our findings will fill a crucial knowledge gap concerning the contribution of these SNPs to genetic risk for PCOS among women in the western region of Saudi Arabia.

Materials and Methods

Study design

This case-control observational study was conducted between 2016 and 2018 in the Obstetrics and Gynaecology Clinics at the King Abdulaziz University (KAU) Hospital and Center of Innovation in Personalised Medicine (CIPM), KAU, in Jeddah, Saudi Arabia. It included 104 women of reproductive age, between 18 and 38 years old, with PCOS diagnosed according to the Rotterdam criteria, as well as 87 women with normal ovulation (controls). Women with 1 or more of the following criteria were excluded:

1. A condition with reproductive symptoms similar to PCOS, such as congenital adrenal hyperplasia, Cushing syndrome, hyperprolactinemia, thyroid disease and androgen-secreting tumours.
2. Chronic diseases, such as diabetes and cardiovascular disease.
3. Any other female infertility issue.

The sample size was calculated using Raosoft (www. raosoft.com), as previously described.[25] The research was reported according to the STROBE guidelines.

All participants completed a questionnaire that collected their demographic data, family history and treatments. An abdominal ultrasound was performed on days 2 to 4 of the menstrual cycle, using a SonixTouch machine (Ultrasonix Medical Corporation; Richmond, BC, Canada). Serum levels of luteinising hormone (LH) and follicle-stimulating hormone (FSH) were obtained using an automated multi-analysis system with electrochemiluminescence immunoassay (ECLIA) kits (Roche, Basel, Switzerland). Serum anti-Müllerian hormone (AMH) levels were measured with an enzyme-linked immunosorbent assay using Ultra-Sensitive Anti-Müllerian Hormone/Müllerian Inhibiting Substance (US AMH/MIS Kit, AnshLabs, Webster, TX, USA) according to the manufacturer's guidelines. Non-fasting serum TC and TG levels were measured using a Beckman Coulter Kit (Beckman Coulter, Inc., CA, USA) since previous studies showed no significant differences between lipid profiles obtained with fasting and non-fasting blood samples.[26-30]

PCOS patients were diagnosed by the presence of 2 out of the following 3 criteria: PCOM ($\geq$12 follicles with a diameter of 2-9 mm in 1 ovary), irregular period ($\leq$8 menstrual cycles in 12 months or a menstrual interval of more than 35 days) or HA (hirsutism, acne or androgenic alopecia). BMI was calculated as the individual's weight (in kilograms) divided by their squared height (in m^2).

The study was approved by the Biomedical Ethics Unit of the Faculty of Medicine at KAU (approval number 407-15). Written informed consent was obtained from participants before sample collection. The study was conducted following the Declaration of Helsinki.

Genotyping

DNA was isolated from peripheral blood using the QIAamp DNA Mini Blood Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The SNPs were genotyped using TaqMan SNP genotyping assays (Thermo Fisher Scientific, Waltham, MA, USA) with 1 to 20 ng/µL of DNA: *APOA5* SNP rs662799 (assay ID: C__2310403_10) and *PLIN1* SNPs rs894160 (assay ID: C__8722593_10), rs1052700

Table 1. Clinical characteristics of PCOS patients and controls.

VARIABLE	CONTROL (*N* = 87)	PCOS (*N* = 104)	*P*-VALUE
Age (years)	21.0 (3.0)	24.5 (10.0)	<.001
BMI (kg/m^2)	23.1 (6.6)	24.4 (8.7)	.012
Acne (Yes/No)	21/66	46/56	.003
Hirsutism (Yes/No)	4/83	40/62	<.001
Hair loss (Yes/No)	16/71	58/43	<.001
HA (Yes/No)	32/55	73/29	<.001
Regular period (Yes/No)	87/0	39/63	<.001
PCOM (Yes/No)	0/87	66/18	<.001
LH (IU/mL)	5.6 (5.5)	8.3 (6.6)	<.001
FSH (IU/mL)	4.5 (27.0)	5.0 (2.6)	.033
LH/FSH ratio	1.4 (1.5)	1.8 (1.5)	.014
AMH (ng/mL)	2.3 (1.4)	6.4 (6.0)	<.001
TC (mmol/L)	3.9 (0.8)	4.4 (1.1)	<.001
TG (mmol/L)	0.8 (0.5)	0.9 (0.7)	.187

Abbreviations: AMH, anti-Müllerian hormone; BMI, body mass index; FSH, follicle-stimulating hormone; HA, hyperandrogenism; LH, luteinising hormone; PCOM, polycystic ovarian morphology; TC, total cholesterol; TG, triglyceride.
Quantitative data are presented as median (interquartile range), and qualitative data are presented as frequencies. The *P*-values were calculated using Chi-squared tests for categorical variables and Mann–Whitney *U* tests for continuous variables.

(assay ID: C__8722587_10) and rs6496589 (assay ID: C__30373512_10). Allelic PCR products were analysed using the QuantStudio 12K Flex Real-Time PCR System (Thermo Fisher Scientific).

Statistical analyses

A normality test using the Shapiro–Wilk test showed that the data did not follow a normal distribution. Therefore, quantitative data are presented as the median (interquartile range [IQR]), and qualitative data are presented as the frequency (percentage). Associations between the study groups (PCOS and control) and qualitative variables (acne, hirsutism, hair loss, HA, regular period, PCOM and the examined SNP genotypes) were assessed using Chi-square tests. Similarly, associations between the study groups and the examined SNP alleles were assessed using Chi-square tests, with the odds ratio (OR) and relative risk (RR) values used to determine the risk alleles for each SNP. Differences in quantitative variables (age, BMI, and serum LH, FSH, LH/FSH ratio, AMH, TC and TG levels) between the study groups were assessed using Mann–Whitney *U* tests.

Associations between the genotypes of the examined SNPs and the qualitative variables were assessed using Chi-square tests. Because *APOA5* SNP rs662799 and *PLIN1* SNP rs1052700 were multiallelic, genotype-based differences in quantitative variables were assessed using Kruskal–Wallis tests, followed by pairwise comparisons (Mann–Whitney test) to identify which genotypes differed. Genotype-based differences in quantitative variables for biallelic *PLIN1* SNPs rs894160 and rs6496589 were assessed using Mann–Whitney tests. The associations between the studied SNPs and PCOS symptoms were then assessed using multinomial logistic regression tests.

Data analyses were performed using IBM SPSS software (version 25; SPSS Inc., Armonk, NY, USA). $P < .05$ was considered statistically significant.

Results

Associations of APOA5 and PLIN1 SNPs with PCOS

The clinical characteristics of PCOS patients and controls are summarised in Table 1. The genotype frequencies of *APOA5* SNP rs662799 and *PLIN1* SNPs rs894160, rs1052700 and rs6496589 and their associations with PCOS are provided in Table 2. Highly significant and direct associations existed between PCOS and *APOA5* SNP rs662799 and *PLIN1* SNP rs894160 ($P < .001$). *APOA5* SNP rs662799 genotype (AA, AG and GG) frequencies were 54.8%, 37.5% and 7.7% in the PCOS group, respectively, and 27.6%, 54.0% and 18.4% in the control group, respectively. *PLIN1* SNP rs894160 genotype (CC and CT) frequencies were 81.6% and 18.4% in the PCOS

Table 2. Genotype frequencies for *APOA5* and *PLIN1* SNPs and their associations with PCOS.

SNP	GENOTYPE	PCOS (*N* = 104) (%)	CONTROL (*N* = 87) (%)	*P*-VALUE
APOA5 rs662799	A/A	57 (54.8)	24 (27.6)	<.001
	A/G	39 (37.5)	47 (54)	
	G/G	8 (7.7)	16 (18.4)	
PLIN1 rs894160	C/C	84 (81.6)	83 (97.6)	<.001
	C/T	19 (18.4)	2 (2.4)	
PLIN1 rs1052700	A/A	47 (45.2)	41 (47.7)	.793
	A/T	47 (45.2)	35 (40.7)	
	T/T	10 (9.6)	10 (11.6)	
PLIN1 rs6496589	C/C	83 (79.8)	64 (73.6)	.593
	C/G	20 (19.2)	22 (25.3)	
	G/G	1 (1)	1 (1.1)	

Data are presented as frequencies (percentages). All *P*-values were calculated using Chi-squared tests.

Table 3. Relative risk of PCOS for each *APOA5* and *PLIN1* SNP allele.

SNP	ALLELE	ALLELE FREQUENCY PCOS (*N* = 104) (%)	ALLELE FREQUENCY CONTROL (*N* = 87) (%)	RR	OR	*P*-VALUE
APOA5 rs662799	A	153 (73.6)	95 (54.6)	1.347	2.313	<.001
	G	55 (26.4)	79 (45.4)			
PLIN1 rs894160	C	187 (90.8)	168 (98.8)	8.043	7.427	<.001
	T	19 (9.2)	2 (1.2)			
PLIN1 rs1052700	A	141 (67.8)	117 (68)	0.997	0.989	.961
	T	67 (32.2)	55 (32)			
PLIN1 rs6496589	C	186 (89.4)	150 (86.2)	1.016	1.137	.684
	G	22 (10.6)	24 (13.8)			

Abbreviations: OR, odds ratio; RR, relative risk.
Data are presented as frequencies (percentages). The *P*-values were calculated using Chi-squared tests.

group, respectively, and 97.6% and 2.4% in the control group, respectively. However, no significant associations were detected between PCOS and *PLIN1* SNPs rs1052700 and rs6496589.

Relative risks of PCOS for the APOA5 and PLIN1 SNP alleles

The allele frequencies of *APOA5* SNP rs662799 and *PLIN1* SNPs rs894160, rs1052700 and rs6496589 are provided in Table 3. The RR and OR were calculated for each gene to determine the risk alleles for each SNP. For *APOA5* SNP rs662799, women with the A allele were more likely to have PCOS than women with the G allele (RR = 1.348, OR = 2.313, $P < .001$), indicating that the A allele could be a risk allele for PCOS. For *PLIN1* SNP rs894160, women with the T allele were more likely to have PCOS than women with the C allele (RR = 8.043, OR = 7.427, $P < .001$).

Associations between PCOS clinical characteristics and APOA5 SNP rs662799 and PLIN1 SNPs rs894160, rs1052700 and rs6496589

A significant association existed between irregular periods and *APOA5* SNP rs662799 genotypes in the PCOS group ($P = .027$). Additionally, TG levels differed significantly by *APOA5* SNP rs662799 genotype in the PCOS group ($P = .044$), specifically between the A/A (0.9 [0.7] mmol/L) and G/G (0.6 [0.3] mmol/L) genotypes ($P = .039$). However, the other clinical characteristics (BMI, acne, HA, hirsutism, PCOM, AMH, LH, FSH and TC) did not differ significantly by *APOA5* SNP rs662799 genotype (Table 4).

A significant association existed between *PLIN1* SNP rs894160 genotypes and PCOM in the PCOS group ($P = .005$). However, the other clinical characteristics (BMI, acne, HA, hirsutism, regular period, AMH, LH, FSH, TG and TC) did

Table 4. Associations between PCOS clinical characteristics and *APOA5* SNP rs662799 and *PLIN1* SNP rs894160 genotypes.

APOA5 SNP RS662799								
VARIABLE	PCOS			*P*-VALUE	CONTROL			*P*-VALUE
	A/A	A/G	G/G		A/A	A/G	G/G	
Age (years)	27.0 (10.3)	24.0 (7.5)	21.0 (2.3)	0.002	21.0 (4.8)	21.0 (2.8)	21.0 (2.8)	.261
BMI (kg/m^2)	25.8 (6.8)	24.1 (9.9)	22.7 (5.5)	0.587	24.0 (8.6)	23.0 (6.7)	19.6 (3.0)	.374
Acne (Yes/No)	27/30	17/20	2/6	0.488	6/18	12/35	3/13	.855
Hirsutism (Yes/No)	24/33	15/22	1/7	0.270	2/22	2/45	0/16	.461
Hair loss (Yes/No)	36/21	20/16	2/6	0.119	1/23	13/34	2/14	-
HA (Yes/No)	42/15	28/9	3/5	0.082	8/16	20/27	4/12	.417
Regular period (Yes/No)	28/29	8/29	3/5	0.027	24/0	47/0	16/0	-
PCOM (Yes/No)	35/8	25/8	6/2	0.811	0/24	0/47	0/16	-
LH (IU/mL)	8.2 (6.8)	8.1 (5.8)	13.3 (6.5)	0.096	4.4 (3.6)	6.5 (6.2)	6.5 (5.5)	.373
FSH (IU/mL)	4.7 (2.6)	5.3 (2.1)	4.5 (2.0)	0.616	4.8 (3.4)	4.6 (1.6)	2.7 (4.4)	.364
LH/FSH ratio	1.8 (1.2)	1.4 (1.7)	2.9 (1.8)	0.178	1.0 (1.7)	1.4 (1.4)	1.6 (3.0)	.395
AMH (ng/mL)	6.6 (6.0)	5.7 (5.4)	6.9 (6.0)	0.888	2.3 (2.4)	2.3 (0.9)	2.5 (1.3)	.856
TC (mmol/L)	4.4 (1.3)	4.5 (1.3)	4.4 (1.0)	0.953	3.9 (0.9)	4.0 (0.8)	3.7 (1.2)	.415
TG (mmol/L)	0.9 (0.7)	1.0 (0.7)	0.6 (0.3)	0.044	0.8 (0.4)	0.8 (0.5)	0.8 (0.5)	.896

(Continued)

Table 4. (Continued)

PLIN1 SNP RS894160								
VARIABLE	PCOS			*P*-VALUE	CONTROL			*P*-VALUE
	C/C	C/T	T/T		C/C	C/T	T/T	
Age (years)	23.0 (9.5)	29.0 (8.5)	-	0.017	21.0 (3.0)	-	-	-
BMI (kg/m^2)	25.1 (8.6)	24.1 (8.8)	-	0.603	22.0 (7.0)	-	-	-
Acne (Yes/No)	35/47	10/9	-	0.432	20/63	1/1	-	-
Hirsutism (Yes/No)	32/50	7/12	-	0.860	4/79	0/2	-	-
Hair loss (Yes/No)	43/38	14/5	-	0.103	14/69	2/0	-	-
HA (Yes/No)	58/24	14/5	-	0.798	30/53	2/0	-	-
Regular period (Yes/No)	29/53	10/9	-	0.164	83/0	2/0	-	-
PCOM (Yes/No)	60/12	5/6	-	0.005	0/83	0/2	-	-
LH (IU/mL)	8.6 (6.3)	7.2 (5.9)	-	0.321	5.7 (5.9)	-	-	-
FSH (IU/mL)	4.7 (2.5)	5.6 (2.3)	-	0.274	4.5 (3.0)	-	-	-
LH/FSH ratio	1.9 (1.5)	1.4 (1.0)	-	0.075	1.4 (1.5)	-	-	-
AMH (ng/mL)	6.3 (5.7)	6.7 (6.2)	-	0.786	2.3 (1.4)	-	-	-
TC (mmol/L)	4.4 (1.2)	4.6 (1.4)	-	0.574	4.0 (0.7)	-	-	-
TG (mmol/L)	0.9 (0.7)	0.7 (0.9)	-	0.338	0.8 (0.4)	-	-	-

Abbreviations: AMH, anti-Müllerian hormone; BMI, body mass index; FSH, follicle-stimulating hormone; HA, hyperandrogenism; LH, luteinising hormone; PCOM, polycystic ovarian morphology; TC, total cholesterol; TG, triglyceride.
Qualitative data are presented as frequencies and compared using Chi-squared tests. Quantitative data are presented as median (interquartile range) and compared using Kruskal–Wallis or Mann–Whitney tests.

Table 5. Multinomial logistic regressions between PCOS clinical characteristics and genotypes of the examined SNPs.

SNP	GENOTYPE	VARIABLE	*B*	*P*-VALUE	OR	95% CI	
APOA5 rs662799[a]	AA	Age	.30	.040	1.35	1.01	1.80
		TG (mmol/L)	2.83	.050	17.0	0.97	296.51
	AG	BMI (kg/m^2)	.21	.030	1.24	1.02	1.50
		FSH (IU/mL)	.75	.040	2.12	1.04	4.35
PLIN1 rs1052700[b]	TT	HA (Yes)	3.39	.020	29.75	1.60	553.72
		Regular period (Yes)	–2.68	.040	0.07	0.01	0.93

Abbreviations: B, β coefficient; BMI, body mass index; CI, confidence interval; FSH, follicle-stimulating hormone; HA, hyperandrogenism; TG, triglyceride.
[a]The reference genotype was GG.
[b]The reference genotype was AT.

not significantly differ by *PLIN1* SNP rs894160 genotype (Table 4). None of the clinical characteristics differed significantly between the genotypes of the other examined *PLIN1* SNPs.

Patients with PCOS carrying the AA genotype of the *APOA5* SNP rs662799 were 17 times more likely to have hypertriglyceridaemia than those with the GG genotype ($P = .05$). Additionally, those with the AG genotype for the *APOA5* SNP rs662799 were more likely to have a high BMI (OR = 1.24, $P = .030$) or a high FSH level (OR = 2.12, $P = .040$) than those with the GG genotype (Table 5).

Patients with PCOS carrying the TT genotype for *PLIN1* SNP rs1052700 were more likely to have HA (OR = 29.75, $P = .020$) or an irregular period (OR = 0.07, $P = .040$) than those with the AT genotype (Table 5).

Discussion

In this study, we assessed the impact of SNPs in the *PLIN1* and *APOA5* genes on the susceptibility to PCOS, selecting each SNP based on its association with hypertriglyceridaemia.[31,32] To our knowledge, this is the first study to report associations between these SNPs and PCOS in Saudis. Dyslipidaemia is 1 of the most common metabolic defects among patients with PCOS.[33,34] PCOS patients have an atherogenic lipid profile characterised by lower HDL-C and higher TG and LDL-C levels.[33] Therefore, people with PCOS are more susceptible to cardiovascular disease (CVD) due to the risk of abdominal obesity and dyslipidaemia.[35]

In our cohort, we found direct associations between *APOA5* SNP rs662799 and PCOS susceptibility and hypertriglyceridaemia among patients with PCOS. This confirms previous findings of an association between *APOA5* SNP rs662799 and elevated plasma TG levels.[36,37] An association between PCOS and metabolic syndrome has been detected previously.[38] PCOS patients have features of metabolic syndrome, including insulin resistance, hyperinsulinaemia and visceral obesity.[39] Several studies have reported a significant correlation between *APOA5* SNP rs662799 and different CVDs, such as coronary heart disease, ischaemic stroke, CAD, atherosclerosis and MI, in various populations, including Moroccan,[40] Chinese,[41] Japanese[20] and Italian people.[19] However, no strong correlations were observed between *APOA5* SNP rs662799 and CVDs in North Africans,[42] Canadians,[43] Pakistanis,[44] Costa Ricans[45] or British people.[46] A North African study showed a significant association between the *APOA5* SNP rs662799 and metabolic syndrome.[42] In our study, the frequency of the major A allele for *APOA5* SNP rs662799 was 0.74 in the PCOS group compared to 0.26 for the G allele, indicating that A was the risk allele, highly associated with the incidence of PCOS and raised TG. However, this is contrary to other populations where G was detected as the risk allele for hypertriglyceridaemia and hypercholesterolaemia.[47,48] This discrepancy could be attributed to differences in ethnicity and environmental stimuli since gene expression can be influenced by individuals' internal and external factors.[49,50] To our knowledge, no study has been performed on the association of *APOA5* SNP rs662799 with PCOS.

The second studied gene, *PLIN1*, has been associated with various PCOS risk factors such as diabetes, obesity, weight gain, insulin resistance and hypertension.[23,51-53] In our cohort, a significant correlation was detected between *PLIN1* SNP rs894160 and PCOS susceptibility, with the T allele considered the potential risk allele for PCOS. A previous study on non-Hispanic white women with PCOS living in Chicago and St. Louis found no association between *PLIN1* SNP rs894160 and PCOS.[24] This difference between studies might reflect differences in lifestyle since *PLIN1* expression has been previously shown to be reduced by 80% in women with PCOS after exercise training.[54,55]

Our study revealed no direct association between *PLIN1* SNP rs1052700 and PCOS, confirmed by another study on non-Hispanic white women that also reported no association.[24] However, that study found the A allele of rs1052700 to be significantly associated with T2DM in women with or without PCOS.[24] This is contrary to our findings that showed an association between the T allele and some PCOS clinical

characteristics (HA and irregular period), potentially reflecting ethnic differences. However, the T allele in the rs1052700 variant has been associated with central obesity and diabetes among Chinese, Malaysian and Indian populations.[56,57]

Our study found no significant association between *PLIN1* SNP rs6496589 and PCOS and its clinical characteristics, consistent with a study on non-Hispanic white women.[24] However, another study in the Western Saudi population reported that *PLIN1* SNP rs6496589 was associated with T2DM.[58] This difference from our study might reflect that study's larger sample size of 406 participants. The limitation of our study is its small sample size. Thus, we recommend conducting similar multicentre research with a larger sample size throughout several Saudi Arabian regions.

Conclusion

Our case-control study demonstrated that the frequencies of the A allele of *APOA5* SNP rs662799 and the T allele of *PLIN1* SNP rs894160 were significantly higher among patients with PCOS than among the controls. Thus, given also their previously established roles in metabolic disorders and obesity, we believe that these SNPs have a substantial role in the development of PCOS in the Western Saudi population.

Declarations

Ethical Approval and Consent to Participate

The study was conducted following the Declaration of Helsinki and approved by the Biomedical Ethics Unit of the Faculty of Medicine at KAU (approval number 407-15). Written informed consent was obtained from all participants involved in this study.

Consent for Publication

Not applicable.

Author Contributions

Sherin Bakhashab: Conceptualisation; Methodology; Formal analysis; Funding acquisition; Writing - original draft; Writing - review & editing. **Asma A Batarfi**: Methodology; Writing - original draft; Formal analysis. **Mahinar M Alhartani**: Methodology; Writing - original draft. **Rola Turki**: Methodology. **Wessam Mady**: Methodology.

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Competing Interests

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Availability of Data and Materials

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