A novel link between *KCNQ1* genetic variants and polycystic ovary syndrome susceptibility

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Abstract. Genome-wide association studies (GWAS) have identified the potassium voltage-gated channel subfamily Q member 1 (*KCNQ1*) gene, as a potential contributor to the pathogenesis of type 2 diabetes (T2D). Given the known genetic overlap between T2D and polycystic ovary syndrome (PCOS), the present study aimed to investigate the potential association between *KCNQ1* gene variants and PCOS susceptibility in a population of Tunisian women. A total of 230 patients and 230 healthy controls were recruited for this case control study. The Rotterdam consensus criteria were used to diagnose patients with PCOS. Genotyping of three *KCNQ1* variants (rs231361, rs151290 and rs2237895), was performed using allelic discrimination (real-time PCR). After excluding false positive associations using the false discovery rate adjustment and ensuring statistical power >80%, the present results suggested that the *KCNQ1* gene may play a role in PCOS susceptibility. Specifically, the rs231361 variant showed a significant association with an increased risk of PCOS through multiple genetic inheritance models. Additionally, the A/A genotype of the rs231361 variant displayed a correlation with increased levels of triglycerides compared with those with the G/G wild-type and the G/A heterozygous genotypes. To the best of our knowledge, this is the first study to identify the *KCNQ1* rs231361 variant as a potential genetic risk factor for PCOS. These findings have important implications for risk assessment and the development of personalized treatment approaches for affected women.

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Introduction

Polycystic ovary syndrome (PCOS) is the most prevalent hormonal and metabolic disorder that significantly impacts women during their reproductive years (1), with a global prevalence estimated to range from 4-20% (2). Women with PCOS often experience ovulatory irregularities, leading to infertility, and face an increased risk of adverse pregnancy outcomes (3,4). The management of PCOS-related infertility typically involves first-line therapies such as lifestyle modifications and pharmacological interventions aimed at inducing ovulation (5,6). However, for women who are resistant to these treatments, surgical interventions such as laparoscopic ovarian drilling, have proven to be effective strategies (7).

The pathophysiology of PCOS is complex, characterized by hormonal imbalances, neuroendocrine disruptions, alterations in adipocytes function, changes in gut microbiota, insulin resistance and hyperandrogenism which impair folliculogenesis and increase the risk of related comorbidities, including endometrial cancer and type 2 diabetes (T2D) (8,9). PCOS is also recognized as a complex polygenic disease influenced by various factors such as gene variants, epigenetic and ethnicity (10). A previous genetic study identified various potential genes associated with single nucleotide polymorphisms (SNPs) or mutations which are linked to a range of PCOS symptoms (11).

KCNQ1 are potassium voltage-gated channels that play pivotal role in various physiological processes, including cell homeostasis, electrical signalling in cardiac myocytes, gastric acid secretion, sperm motility, glucose metabolism and insulin secretion (12-19). The activity of KCNQ1 is primarily dependent on its interactions with KCNE (Potassium Voltage-Gated Channel Subfamily E Regulatory) family proteins, particularly KCNE1, and is further regulated by cellular factors such as calmodulin, PIP2, PKA and post-translational modifications (13).

The *KCNQ1* gene is located on chromosome 11p15.5 and spanning over 400 kb (17) and mutations in this gene can lead to channel dysfunction or loss of function, resulting in multiple diseases (18-22).

SNPs within the *KCNQ1* gene have been identified and linked to T2D risk with evidence across diverse populations including, Chinese, Singaporean, Indian and Euro-Caucasian individuals (23-28).

Given the well-established intrinsic connection and shared pathophysiological mechanisms between PCOS and T2D (29-31), several genes associated with T2D, have been identified as potential candidates for the development of PCOS (32-39), yet the potential role of *KCNQ1* in PCOS remains unexplored.

In the present study, a critical knowledge gap in the field of PCOS research was addressed by investigating the potential relationship between three *KCNQ1* variants (rs151290, rs231361 and rs2237895) and PCOS in the Tunisian Arab population. The study sought to provide valuable insights into the genetic basis of PCOS, ultimately leading to the development of improved diagnostic and therapeutic strategies and enhancing the overall health and well-being of women affected by this syndrome.

Materials and methods

Study subjects. The present retrospective case-control study enrolled a total of 460 women, comprising 230 patients diagnosed with PCOS and 230 ethnically matching control individuals. The age range of the PCOS participants was 28-37 years (average, 32.5 years), while the control subjects had an age range of 27-33.25 years (average, 30 years). Participants were recruited from the outpatient obstetrics, gynaecology and endocrinology clinics at Farhat Hached Hospital in Tunisia between December 2023 and June 2024.

The three diagnostic criteria outlined for PCOS encompass the identification and quantification of its classical features, namely hyperandrogenism, ovulatory dysfunction and polycystic ovarian morphology (40,41). While these criteria are essential for a definitive diagnosis, it is crucial to consider that relying on any single criterion may not provide a conclusive result.

Other endocrine disorders, such as hyperprolactinemia, thyroid disease and non-classical congenital adrenal hyperplasia, can present with similar symptoms and manifestations as PCOS. Therefore, to ensure a homogeneous study population and accurate diagnosis, the study excluded women with these potential disorders.

The control group comprised women examined during the follicular phase (day 2-5) of their menstrual cycle, meeting the following criteria: i) Regular menstrual cycles; ii) androgen and hormonal levels within range; iii) and ovaries that were free of any cysts on an ultrasound examination by the study gynaecologist.

To ensure the integrity of the study, participants were carefully screened to exclude those who had been on hormonal therapy, or any medication known to influence carbohydrate metabolism or endocrine parameters for at least 6 months prior to their inclusion in the study. This exclusion criterion was implemented to minimize the potential confounding effects of these interventions on the study outcomes.

Data collection. The present study was conducted in adherence with the principles outlined in the Helsinki Declaration (2014) (42), and received ethical approval from the local research and ethics committee of Farhat Hached Hospital (approval no. 35220228; Sousse, Tunisia). All patients who met the eligibility criteria were recruited and their voluntary participation was confirmed through signed informed consent.

The collected data encompassed demographic information such as age and body mass index (BMI). Additionally, lipide profiles, sexual hormone levels were collected from all participants.

Biochemical and hormonal analysis. During the early follicular phase of the menstrual cycle (days 2-5), venous blood samples were collected from control subjects or any day for women with PCOS after an overnight fast. Blood was collected into two types of tubes: Plain tubes for serum collection and EDTA-containing tubes for plasma and DNA preparation.

Serum samples underwent analysis for fasting blood glucose using the hexokinase method in fluoride oxalate tube, with the Cobas Integra 800 from Roche Diagnostics GmbH. Immunofluorometric assay or radioimmunoassay were employed to measure follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone with both intra- and inter-assay coefficients of variation <10%. Serum lipid levels, including total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides, were assessed using an enzymatic colorimetric method on the Integra 800 from Roche.

Genomic DNA extraction and genotyping. Genomic DNA was extracted from peripheral blood leukocytes using Qiagen QIAamp DNA blood Mini Kit (Qiagen GmbH). Quantification and purity assessment of the extracted DNA were conducted using an ND-2000 Nanodrop spectrophotometer (Thermo Fisher Scientific, Inc.). The purity of the DNA was considered satisfactory with an A260/A280 ratio of ~1.8.

The potential association between *KCNQ1* variants and PCOS susceptibility has not yet been explored and given the known genetic overlap between PCOS and T2D, the selection of the three *KCNQ1* variants was based on their established association with T2D on prior research studies as well as their frequency in Caucasians with a minor allele frequency (MAF) >5%. The National Center for Biotechnology Information Gene SNP Geneview data base was utilized for this purpose (www.ncbi.nlm.nih.gov/snp).

Genotyping of the variants was performed using the allelic discrimination method on StepOne real-time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). Commercially available primers from the assay-on-demand system were utilized and well-defined genotype clusters were obtained. Specific TaqMan assay IDs were used to target each variant: C_3075843_1_ (rs231361), C_3075727_1_(rs151290) and C_16171034_10 (rs2237895). Replicated blinded quality control samples were included to assess reproducibility, which indicated concordance >99%.

Statistical analysis. The sample size and power analysis for detecting an association between *KCNQ1* variants and PCOS were calculated using the G*power calculator, version 3.1.9.7 (Heinrich Heine University). The parameters considered included the number of study subjects (230 cases and 230 controls), genotypic odds ratio (OR) for heterozygotes and

Table I. Clinical and biochemical characteristics of study subjects.

Characteristic	Polycystic ovary syndrome[a] (n=230)	Controls[a] (n=230)	P-value[b]	FDR P-value[c]
Age, years	32.5 (28-37)	30 (27-33.25)	0.001	0.011
Body mass index (kg/m^2)	26 (23-29)	27 (24-31)	0.018	0.099
High-density lipoprotein (mmol/l)	1.38 (1.24-1.42)	1.36 (1.26-1.42)	0.876	0.938
Low-density lipoprotein (mmol/l)	2.97 (2.58-3.03)	2.92 (2.38-3.08)	0.402	0.816
Triglycerides (mmol/l)	1.51 (1.03-1.62)	1.58 (1.42-2.09)	0.055	0.201
Cholesterol (mmol/l)	5.80 (5.25-5.94)	5.91 (5.20-6.32)	0.478	0.816
Luteinizing hormone levels (mlU/mol)	2.99 (0.49-5.86)	2.94 (0.48-4.51)	0.633	0.816
Follicle-stimulating hormone levels (mlU/mol)	4.67 (3.82-6.52)	4.45 (3.34-5.01)	0.215	0.591
Progesterone (mlU/mol)	0.97 (0.92-1.02)	0.94 (0.92-1)	0.619	0.816
Testosterone (nmol/l)	2.75 (1.59-3.91)	2.97 (1.89-3.84)	0.668	0.816
Prolactin (ng/ml)	114.87 (84.74-135.41)	99.95 (77.38-130.37)	0.938	0.938

[a]Values presented as median (25-75th percentile); [b]Mann-Whitney U-test; [c]Benjamini-Hochberg adjusted P-value. FDR, False discovery rate.

mutant homozygotes, MAF for PCOS cases and controls across the three tested variants, and a 12% prevalence of PCOS in the general population according to the Rotterdam criteria (43). Under these assumptions, the overall power was calculated as the average power of the tested variants and was found to be 80%.

Statistical analysis for the study was performed using SPSS Statistics for Windows, Version 28 (IBM Corp.). The clinical characteristics were presented as median (25-75th percentile). To compare quantitative variables, Mann-Whitney U (Wilcoxon) was used.

Allelic and genotypic frequencies were determined using the gene-counting method implemented through SNPassoc R package (https://github.com/isglobal-brge/SNPassoc). The Hardy-Weinberg equilibrium (HWE) for each variant, was assessed using a chi-squared test, also performed with the SNPassoc R package.

The differences in PCOS traits levels between the three genotypes (wild homozygous, heterozygous and mutant homozygous) of the three tested variants were assessed with the non-parametric Kruskal Wallis test.

Correlation between covariate traits and the three genotypes, was estimated by Spearman coefficient. A positive correlation is considered when the coefficient ranges between 0 to 1. Conversely, a negative correlation corresponds to a coefficient ranging between 0 to -1.

The differences in allele and genotype frequencies between cases and controls were calculated for each variant, utilizing the gene-counting method using the SNPassoc R package.

Genetic association analysis was performed using binary logistic regression under four genetic models (additive, codominant, dominant and recessive). The control group served as the reference for calculating ORs. Suppose (A) is the ancestral allele and (a) is the altered allele. The codominant model compared heterozygous (Aa) and homozygous variant (aa) genotypes to the ancestral homozygous (AA) genotype. The dominant model compared carriers of the altered allele (Aa + aa) to non-carriers (AA), while the recessive model compared homozygous variant (aa) individuals with those with the (AA + Aa) genotype. The additive model assumed a proportional increase in risk with each additional altered allele (a).

SHEsisPlus software (http://shesisplus.bio-x.cn/SHEsis.html) was used for binary variant interactions and haplotype association analysis. The multifactor dimensionality reduction (MDR) method was applied to analyse epistasis (44) which helps identify genetic variant combinations that influence disease susceptibility, by reducing the data's dimensionality and evaluating the predictive power of these combinations.

Corrected P-values for multiple hypothesis testing for analysis was performed with false discovery rate (FDR) method by Benjamini-Hochberg. P<0.05 was considered to indicate a statistically significant difference.

Results

Study subjects. The demographic and biochemical parameters of all the study participants are presented in Table I. A significant difference in age was revealed at examination between PCOS and controls subjects after applying the Benjamini-Hochberg adjustment. However, no substantial disparities were observed in BMI, fasting glucose, total testosterone, LDL, triglycerides, total cholesterol, FSH or LH in women with PCOS compared with controls (P>0.05).

Correlation between clinical, biochemical and hormonal parameters. The correlation between the different quantitative parameters including BMI, cholesterol, triglycerides, HDL, LDL, FSH, LH, progesterone, testosterone and prolactin was assessed by Spearman's coefficient. The results showed a positive correlation between LDL and cholesterol (P=0.034) consistent with the well-established relationship between these lipids, as well as between FSH and LH (P=0.021) (Fig. 1). Additionally, a weak negative correlation was detected

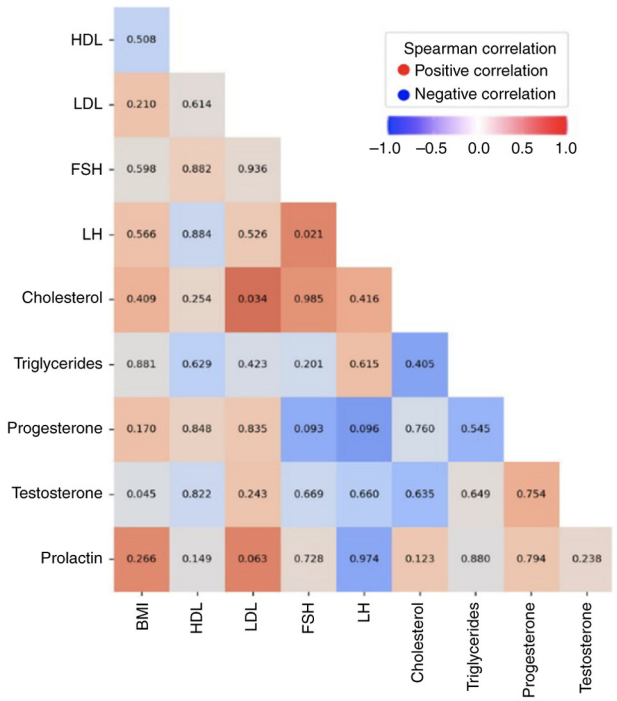

Figure 1. Heatmap of Spearman correlation coefficient matrix among quantitative parameters in PCOS cases. The colours represent the correlation with red being more positive and, blue more negative. A positive correlation is considered when the r coefficient ranges between 0 to 1. Conversely a negative correlation corresponds to a r coefficient ranging between 0 to -1. Spearman P-values appear in the squares between different covariates. A significant correlation is considered when the Spearman P<0.05. PCOS, polycystic ovary syndrome; BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein; FSH, follicle-stimulating hormone; LH, luteinizing hormone.

between testosterone and BMI (P=0.045). No other statistically significant differences were observed between the other parameters (Fig. 1).

Variants association analysis. The HWE and MAF for the three *KCNQ1* variants (rs231361, rs151290 and rs2237895) in both case and control subjects are summarized in Table II. All variants were in HWE (P>0.05), indicating that the genotype distributions follow the expected pattern, and no population stratification was detected.

The subsequent genetic analysis examined the relationship between PCOS susceptibility and these variants using various genetic models, including additive, codominant, dominant and recessive models. The results of the data analysis, presented in Table III, revealed that only the rs231361 variant was significantly associated with an increased risk of PCOS across multiple genetic models.

The additive model showed an OR of 1.59, with an FDR P-value of 0.011 and a power of 84.7%. Similarly, the codominant and recessive models also demonstrated significant associations, with ORs of 4.93 and 4.76, respectively, and FDR P-values of 0.002, along with high statistical power (>95%). However, while the rs151290 and rs2237895 variants showed association with an increased risk of PCOS, these associations were not considered statistically significant as the statistical power did not exceed the threshold of 80%.

Table II. Genotype and risk allele frequencies of *KCNQ1* gene variants in PCOS cases and controls.

Variant	PCOS, n (%)	Controls, n (%)	HWE-P-value[a]
rs231361			0.063
G/G	130 (56.5)	148 (64.3)	
G/A	74 (32.2)	76 (33.0)	
A/A	26 (11.3)	6 (2.6)	
RA (A)	126 (27.4)	88 (19.1)	
rs151290			0.297
C/C	112 (48.7)	132 (57.4)	
C/A	108 (47.0)	80 (34.8)	
A/A	10 (4.3)	18 (7.8)	
RA (A)	128 (27.8)	116 (25.2)	
rs2237895			0.132
A/A	70 (30.4)	94 (40.9)	
A/C	106 (46.1)	102 (44.3)	
C/C	54 (23.5)	34 (14.8)	
RA (C)	214 (46.5)	170 (36.9)	

[a]Hardy-Weinberg Equilibrium P-value (Pearson square test). *KCNQ1*, potassium voltage-gated channel subfamily Q member 1; PCOS, polycystic ovary syndrome; RA, risk allele.

Correlation between KCNQ1 variants and PCOS-associated features. The correlation between *KCNQ1* tested variants and PCOS-associated metabolic and endocrine traits was assessed. One of the key findings was a positive correlation between the rs231361 variant and increased triglyceride levels (Table IV). Furthermore, when comparing different genotypes of the rs231361 variant, individuals carrying the A/A genotype displayed a statistically significant correlation with higher triglyceride levels compared with those with the wild-type G/G genotype (P=0.002) and the heterozygous G/A genotype (P=0.014) (Fig. 2). This indicates that the individuals carrying A/A genotype may be more prone to elevated triglyceride levels, which is a metabolic parameter often associated with PCOS. This genotype-specific association highlights the importance of considering genetic variations when studying the metabolic disturbances in PCOS and the importance of personalized medicine in PCOS management. No statistically significant differences were observed between the other *KCNQ1* variants (rs151290 and rs2237895) and any of the metabolic or endocrine parameters tested (Table IV), indicating that these variants may not have a major impact on PCOS-related traits.

Variants interaction analysis. The binary variant interaction analysis aimed to investigate whether specific combinations of *KCNQ1* variants (rs231361, rs151290 and rs2237895) were associated with PCOS susceptibility. However, the results in Table V indicate that none of the variant interactions showed statistically significant differences between individuals with PCOS and controls. Both the nominal P-values and the more stringent FDR P-values were above the 0.05 threshold

Table III. PCOS association for *KCNQ1* gene variants in the Tunisian study samples.

Variant	Model	P-value[a]	FDR P-value[b]	FDR P-value[c]	OR (95% CI)	Power
rs231361	Additive: (A vs. G)	0.003	0.004	0.011	1.59 (1.17-2.17)	0.847
	Codominant: (G/A vs. G/G)	0.611	0.611	0.611	1.11 (0.73-1.64)	
	Codominant: (A/A vs. G/G)	$2.2x10^{-4}$	$6.6x10^{-4}$	0.002	4.93 (1.35-18.8)	0.959
	Dominant: (G/A+A/A vs. G/G)	0.087	0.087	0.130	1.39 (0.95-2.02)	
	Recessive: (A/A vs. G/A+G/G)	$2.47x10^{-4}$	$7.4x10^{-4}$	0.002	4.76 (1.92-11.79)	0.958
rs151290	Additive: (A vs. C)	0.526	0.526	0.564	1.14 (0.85-1.53)	
	Codominant: (C/A vs. C/C)	0.017	0.051	0.036	1.59 (1.08-2.34)	0.760
	Codominant: (A/A vs. C/C)	0.305	0.305	0.352	0.65 (0.29-1.48)	
	Dominant: (C/A+A/A vs. C/C)	0.061	0.087	0.102	1.42 (0.98-2.05)	
	Recessive: (A/A vs. C/A+C/C)	0.119	0.119	0.149	0.54 (0.24-1.19)	
rs2237895	Additive: (C vs. A)	0.003	0.004	0.011	1.48 (1.14-1.93)	0.551
	Codominant: (A/C vs. A/A)	0.112	0.168	0.149	1.40 (0.92-2.11)	
	Codominant: (C/C vs. A/A)	0.005	0.007	0.015	2.13 (1.26-3.62)	0.660
	Dominant: (A/C+C/C vs. A/A)	0.019	0.057	0.036	1.58 (1.08-2.32)	0.662
	Recessive: (C/C vs. A/C+A/A)	0.018	0.027	0.036	1.77 (1.10-2.84)	0.551

[a]Pearson square test; [b]Benjamini-Hochberg adjusted P-value for each inheritance model; [c]Benjamini-Hochberg adjusted P-value for all inheritance models. PCOS, polycystic ovary syndrome; *KCNQ1*, potassium voltage-gated channel subfamily Q member 1; FDR, false discovery rate; OR, odds ratio; CI, confidence interval.

Table IV. Correlation between *KCNQ1* gene variants with PCOS features.

Trait	Variant	Risk allele	P-value[a]	r^b
Body mass index	rs231361	A	0.942	0.008
	rs151290	A	0.464	0.078
	rs2237895	C	0.167	0.146
Cholesterol	rs231361	A	0.173	0.467
	rs151290	A	0.314	-0.355
	rs2237895	C	0.155	0.485
Triglycerides	rs231361	A	$1.5x10^{-5}$	0.676
	rs151290	A	0.600	0.108
	rs2237895	C	0.929	0.018
High-density lipoprotein	rs231361	A	0.639	0.074
	rs151290	A	0.133	0.233
	rs2237895	C	0.182	-0.207
Low-density lipoprotein	rs231361	A	0.539	-0.096
	rs151290	A	0.983	0.003
	rs2237895	C	0.336	0.150
Follicle-stimulating hormone	rs231361	A	0.302	0.250
	rs151290	A	0.638	-0.115
	rs2237895	C	0.709	0.092
Luteinizing hormone	rs231361	A	0.761	0.083
	rs151290	A	0.548	-0.150
	rs2237895	C	0.107	-0.418
Progesterone	rs231361	A	0.704	0.107
	rs151290	A	0.491	0.193
	rs2237895	C	0.975	-0.009
Testosterone	rs231361	A	0.554	-0.077
	rs151290	A	0.582	-0.072
	rs2237895	C	0.826	-0.029

Table IV. Continued.

Trait	Variant	Risk allele	P-value[a]	r^b
Prolactin	rs231361	A	0.150	0.405
	rs151290	A	0.659	0.129
	rs2237895	C	0.062	0.590

[a]Spearman correlation P-value (2-tailed); [b]Spearman correlation coefficient (2-tailed). *KCNQ1*, potassium voltage-gated channel subfamily Q member 1; PCOS, polycystic ovary syndrome.

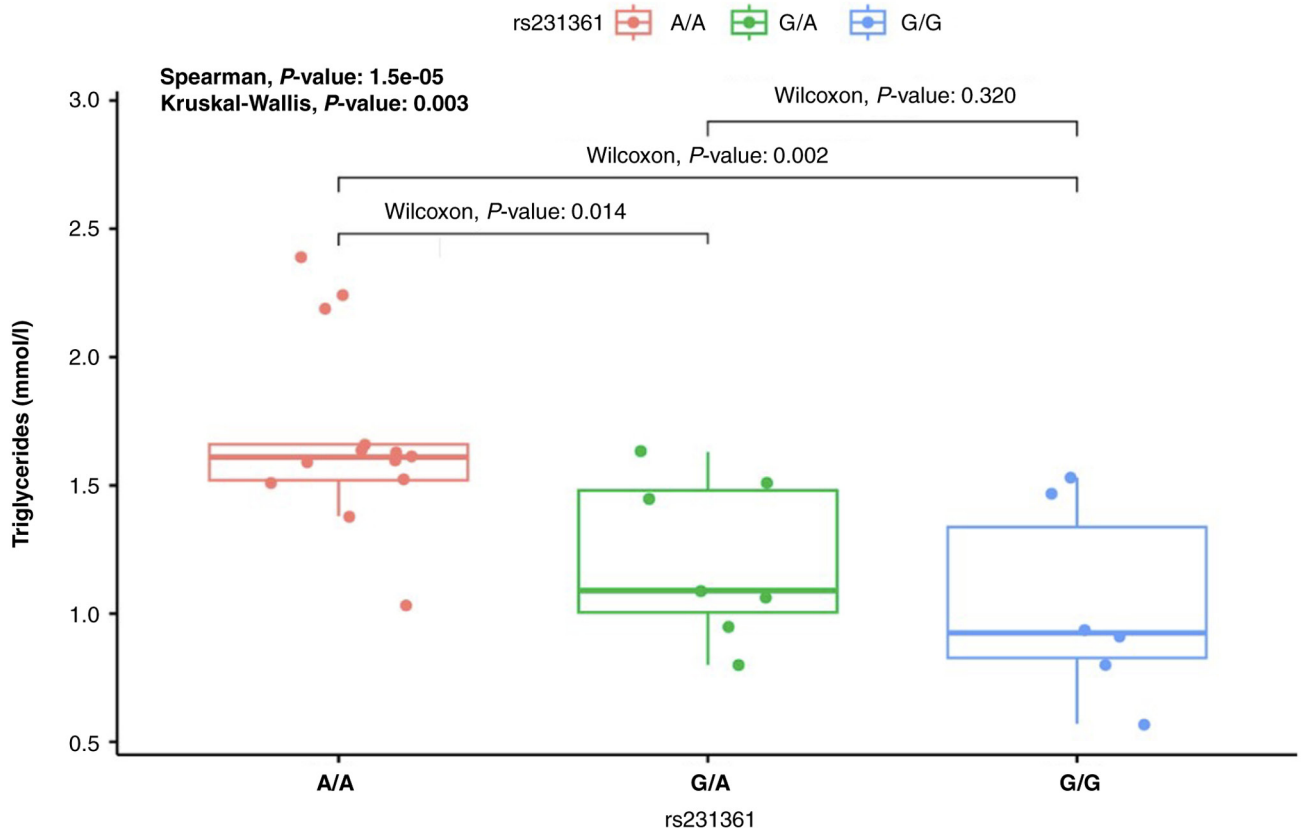

Figure 2. Comparison of triglycerides levels across the three genotypes of rs231361.

(Table V), suggesting that variant interactions may not contribute to PCOS susceptibility in this study population.

MDR simulation for the effect of KCNQ1 interaction on PCOS risk. To further explore the epistatic interactions of *KCNQ1* variants, a MDR simulation was conducted. A total of two visualization methods were employed: A dendrogram illustrating variant interaction patterns and a Fruchterman-Reingold network.

The visualizations in Fig. 3A (dendogram) and Fig. 3B (Fruchterman-Rheingold) suggest a moderate synergy between rs231361 and rs2237895 variants, forming a cluster indicative of a combined, amplified effect. This indicates that these two variants may interact in a positively synergistic manner, influencing PCOS risk. By contrast, the rs151290 variant showed weak and antagonistic interactions with both rs231361 and rs2237895 indicating that its presence may counteract the effects of the aforementioned variants on PCOS risk.

The best models of *KCNQ1* variant interactions and their potential association with PCOS susceptibility are reported in Table VI. Although multiple models were identified, none achieved statistical significance. Future studies using larger sample sizes are warranted to validate potential interactions.

Haplotype analysis. The haplotype analysis provides further insights into the potential associations between *KCNQ1* gene variants and PCOS susceptibility. Linkage disequilibrium (LD) analysis, which refers to the random association of three alleles, revealed limited LD between the three tested *KCNQ1* variants (Fig. 4), suggesting that these variants act independently rather than as a tightly linked block. Several haplotypes were identified in the study population. The frequencies of these haplotypes differed between cases and control subjects as shown in Table VII.

Table V. Binary variant interactions analysis.

KCNQ1 variants	PCOS[a]	Controls[b]	Difference	P-value[c]	FDR P-value[b]
rs231361-rs151290	-0.009	-0.014	0.005	0.309	0.309
rs231361-rs2237895	-0.011	-0.024	0.012	0.189	0.309
rs151290-rs2237895	-0.005	-0.016	0.011	0.249	0.309

[a]PCOS *KCNQ1* variants interaction; [b]Controls *KCNQ1* variants interaction; [c]Pearson square test. *KCNQ1*, potassium voltage-gated channel subfamily Q member 1; PCOS, polycystic ovary syndrome; FDR, false discovery rate.

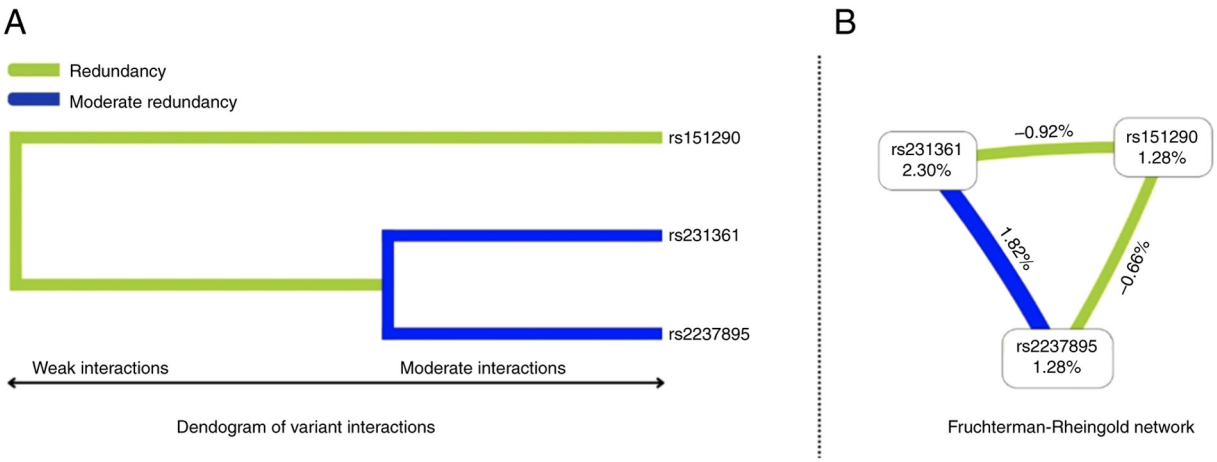

Figure 3. Dendrogram and Fruchterman-Rheingold network of *KCNQ1* variant interaction for PCOS risk. (A) Elements that interact strongly with each other appear close together in the leaves of the tree, while elements that interact weakly appear far from each other. (B) A negative value for the two-locus entropy indicates that it is an antagonistic effect, and a positive value indicates that it is a synergistic effect.

Taking the GCA haplotype as a common (reference), two haplotypes showed positive associations with PCOS risk. The GAC haplotype had an OR of 2.51, indicating that individuals carrying this haplotype were ~2.5 times more likely to develop PCOS compared with those with the reference haplotype. Similarly, the ACC haplotype had an OR of 2.12, suggesting a slightly lower but still significant association with PCOS risk (Table VII). However, despite these observed associations, the statistical power did not reach the threshold of >80%, thus none of these haplotypes are considered associated with PCOS susceptibility.

Discussion

The present study provides new insights into the potential role of the *KCNQ1* gene in the genetic predisposition to PCOS. Although the *KCNQ1* gene is well-recognized as a risk factor for T2D in multiple ethnic populations (24-28), its association with PCOS has remained unexplored. To the best of our knowledge, the present study is the first to investigate the potential relationship between three *KCNQ1* variants (rs231361, rs151290 and rs2237895) and the risk of developing PCOS, offering a new perspective on the shared genetic mechanisms underlying these complex disorders. Nevertheless, given the absence of prior research on the association between PCOS and the *KCNQ1* gene, direct comparisons are currently challenging.

One of the key outcomes of the present study is the identification of the *KCNQ1* rs21361 variant as a novel risk factor for PCOS, with statistical analyses demonstrating its strong association across multiple genetic models. Interestingly, this variant was also found to be significantly correlated with increased triglycerides levels in affected women, suggesting a potential mechanistic link between *KCNQ1* genetic variation and metabolic dysfunction in PCOS.

Additionally, previous research has indicated that the rs231361 variant does not contribute to T2D susceptibility in Tunisian Arab populations (45), suggesting a unique role in PCOS development, independent of its relationship with T2D in this ethnic group. This specificity suggests that the rs231361 variant may act through distinct molecular pathways independent of those involved in T2D, highlighting the need for further functional studies to elucidate the precise mechanisms through which this variant contributes to PCOS pathogenesis.

The analysis of the remaining *KCNQ1* variants, including rs151290 and rs2237895, did not reveal statistically significant associations with PCOS in the studied population, indicating their unlikely involvement in the pathogenesis of PCOS in Tunisian Arabs. Interestingly, previous studies have observed the absence of association between these variants and T2D in the Tunisian Arab population (45), contrasting with findings in other ethnic groups (25,46). This highlights the potential influence of ethnic-specific genetic factors on disease susceptibility. It also indicates that these *KCNQ1* variants may have

Table VI. Best models for variant interactions of the *KCNQ1* gene and the predisposition to PCOS.

Models	Testing balance accuracy	Cross validation consistency	OR (95% CI)	P-value[a]	FDR P-value[b]
rs231361	0.448	6/10	1.68 (0.69-4.07)	0.250	0.337
rs231361-rs2237895	0.583	10/10	2.02 (0.37-11.17)	0.189	0.337
rs231361-rs151290-rs2237895	0.483	10/10	2.55 (0.35-18.47)	0.337	0.337

[a]Pearson square test; [b]Benjamini-Hochberg adjusted P-value. *KCNQ1*, potassium voltage-gated channel subfamily Q member 1; PCOS, polycystic ovary syndrome; OR, odds ratio; CI, confidence interval.

Table VII. Haplotype association analysis of *KCNQ1* gene variants with PCOS.

Haplotype[a]	PCOS, n (%)[b]	Controls, n (%)[b]	P-value[c]	FDR P-value[d]	OR (95% CI)	Power
GCA	112 (24.3)	158 (34.3)	-	-	Reference	
G**A**A	82 (17.8)	92 (20.0)	0.242	0.242	1.26 (0.86-1.85)	
GC**C**	108 (23.4)	104 (22.6)	0.038	0.057	1.46 (1.02-2.10)	
G**AC**	32 (6.9)	18 (3.9)	0.003	0.009	2.51 (1.34-4.69)	0.521
ACA	40 (8.6)	34 (7.3)	0.054	0.065	1.66 (0.99-2.78)	
ACC	72 (15.6)	48 (10.4)	7.22×10^{-4}	0.004	2.12 (1.37-3.29)	0.650
AAA	12 (12.6)	6 (1.3)	0.037	0.057	2.82 (1.03-7.74)	

Boldface and underlined indicates minor allele. [a]*KCNQ1* haplotypes are composed of rs231361, rs151290 and rs2237895; [b]Count represents number of chromosomes assigned a particular haplotype by the expectation maximization algorithm; [c]Pearson square test; [d]Benjamini-Hochberg adjusted P-value. PCOS, polycystic ovary syndrome; FDR, false discovery rate; OR, odds ratio; CI, confidence interval.

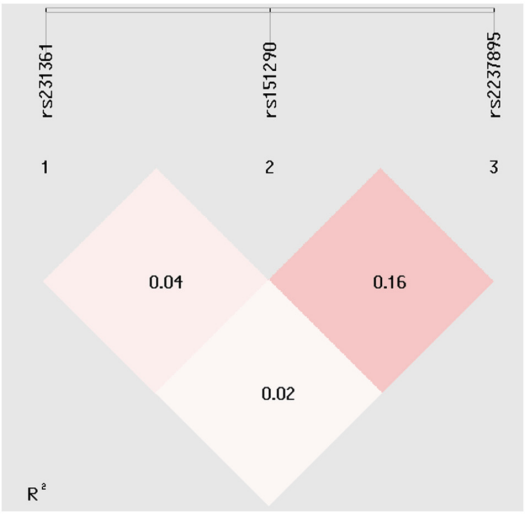

Figure 4. LD plot for the three *KCNQ1* variants in the study of Tunisian women. The degree of LD between different variant pairs is presented in both r^2 values and progressive pink color scale. The r^2 is a measure of correlation of alleles for two genetic variants. r^2 values range from 0 to 1, with low LD indicated by r^2 values between 0 to 0.2. The pairwise r^2 values are shown in diamonds. The LD plot was generated using the SHEsisPlus software. LD, linkage disequilibrium.

a limited role in the genetic susceptibility to both PCOS and T2D within the Tunisian population. This emphasizes the significance of investigating diverse populations to gain a more comprehensive understanding of the genetic complexities underlying these disorders.

Beyond identifying specific genetic associations, the present study also explored the phenotypic correlations between *KCNQ1* variants with PCOS-related metabolic features. A significant association was observed between the risk allele (A) of the rs231361 variant and elevated triglyceride levels in women with PCOS, underscoring the potential role of *KCNQ1* variants in lipid metabolism and their potential contribution to the metabolic disturbances commonly observed in PCOS. The aforementioned results align with previous research on other *KCNQ1* variants such as r2283228 and rs2237892, which have been linked to higher triglycerides levels, and lower HDL levels (47). The clinical implications of the current findings are particularly relevant for the development of personalized treatment strategies for individuals with PCOS. Given the association between *KCNQ1* variants and lipid metabolism, targeted interventions aimed at modulating triglyceride levels may prove beneficial for patients with PCOS carrying the rs231361 variant. Furthermore, pharmacogenomic studies could further explore how genetic variants influence individual responses to lipid-lowering agents, such as statins or fibrates, in PCOS management.

The MDR analysis revealed epistatic interactions between *KCNQ1* variants and their collective impact on PCOS risk. Specifically, the rs231361 and rs2237895 variants exhibited a moderate synergistic effect, increasing susceptibility to PCOS. By contrast, the rs151290 variant displayed

weaker, antagonistic interactions with both rs231361 and rs2237895 variants. These findings suggest a complex interplay between *KCNQ1* genetic variants in modulating PCOS risk, emphasizing the importance of considering epistatic effects in pharmacogenomic strategies, rather than solely focusing on the independent contributions of individual variants. Furthermore, given the association of rs231361 with elevated triglyceride levels, and its synergistic effect with rs2237895, patients carrying these variants may benefit from personalized lipid-modulating interventions, such as statins, fibrates, or novel lipid-lowering agents. This opens avenues for gene-based therapeutic strategies, including the potential for selective potassium channel modulators to regulate metabolic imbalances in PCOS. Furthermore, the antagonistic effect of rs151290 suggests that its presence may influence drug response in patients with PCOS, warranting further investigation. These findings highlight the necessity of integrating pharmacogenomic screening into PCOS treatment protocols, to ensure that genetic variations are accounted for when prescribing metabolic and hormonal therapies.

The haplotype analysis identified two haplotypes (GAC and AAC) which exhibited positive associations with PCOS risk. However, despite the observed associations, statistical power limitations of the study prevent us from drawing definitive conclusions regarding their contribution to disease susceptibility. Larger-scale studies are warranted to validate these findings and determine whether specific *KCNQ1* haplotypes indeed increase the risk of developing PCOS.

While the present study possesses several strengths that contribute to its validity, it is essential to acknowledge its limitations. The homogenous ethnic origin (Tunisian Arabs) of the present study population reduces confounding effects from genetic admixture but may limit the generalizability of our findings to other populations. Additionally, the present study focused on only three *KCNQ1* variants, potentially overlooking other variants implicated in PCOS pathogenesis. The retrospective nature of our case-control study design, further limits causal inference. Future research should encompass these limitations by incorporating larger, multi-ethnic cohorts, performing comprehensive genomic analyses, and utilizing longitudinal study designs to establish causal relationships.

In conclusion, the current findings pave the way for a deeper understanding of the complex genetic architecture of PCOS, identifying *KCNQ1* rs21361 variant as an important factor in disease susceptibility. The observed associations between this variant and triglyceride levels underscore the potential metabolic implications of *KCNQ1* variation, opening avenues for targeted pharmaceutical interventions. Future studies, integrating genomics, pharmacogenomics and larger-scale genetic analyses will be crucial for elucidating the biological mechanisms linking *KCNQ1* to PCOS and optimizing treatment strategies for affected individuals.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

ABS designed the study, collected samples and selected data. IE collected samples and selected data. HBA contributed to data acquisition and critically reviewed the manuscript. NM analysed and interpreted data and critically reviewed the manuscript. SS was the project leader, interpretated data, and wrote and reviewed the manuscript. All authors read and approved the final version of the manuscript. ABS and NM confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was conducted in adherence with the principles outlined in the Helsinki Declaration (2014) and received ethical approval from the local research and ethics committee of Farhat Hached Hospital (approval no. 35220228; Sousse, Tunisia). Written informed consent was provided by all participants before the start of the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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