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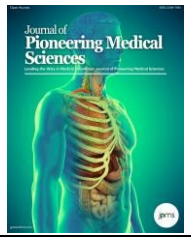

Research Article

Evaluating The Relationship between Women's Risk Of Polycystic Ovarian Syndrome and Genetic Variations in The CYP2C8 Gene in the Diyala Governorate of Iraq

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Abstract: Polycystic ovarian syndrome (PCOS) is the most prevalent endocrine and metabolic disorder among women of reproductive age, characterized by complex polygenic influences. This study aimed to assess the role of genetic polymorphisms in the CYP2C8 gene, focusing on the exon region (rs10509681 variant) and the 3-UTR region (rs1058932 variant), in relation to PCOS risk. Blood samples were collected from 24 women with PCOS and 24 healthy controls (ages 20-40) at Al-Batool Teaching Hospital, Diyala Governorate, Iraq. Sequencing analysis identified three genotypes (GG, GA, AA) at the rs1058932 variant, with odds ratios of 0.82, 1.50, and 0.32, respectively. The GA genotype was identified as a potential risk factor for PCOS, while GG and AA were protective. In the exon region (rs10509681), two genotypes (TT, TC) were found, with the TT genotype showing an odds ratio of 3.29, suggesting an increased risk, while the TC genotype had an odds ratio of 0.32, indicating a protective role. Overall, the distribution of genotypes and allele frequencies showed no significant differences between groups according to Fisher's probability and Hardy-Weinberg equilibrium. The study highlights the potential involvement of CYP2C8 polymorphisms in PCOS risk, emphasizing the need for further large-scale investigations.

Keywords: PCOS, Exon, 3-UTR, Genetic polymorphisms, Cytochrome P450

INTRODUCTION

The most prevalent endocrine and metabolic condition affecting women of reproductive age is polycystic ovarian syndrome, or PCOS. As of right now, there are four identified PCOS phenotypes: polycystic ovary, hyperandrogenism, and absence of ovulation. All of these conditions have long-term health and metabolic implications [1] Infertility, amenorrhea, oligomenorrhea, and disruptions in the ovulation process are among the hormonal abnormalities associated with polycystic ovarian syndrome [2] According to [1] women who have PCOS are more likely to experience mental and psychological issues such as low fertility, metabolic syndrome, intolerance of glucose, type 2 diabetes, and delivery difficulties. According to [3] PCOS is among the most prevalent manifestations of endometrium, pregnancies that begin early loss, and postpartum problems, all of which reduce a woman's fertility. Diabetes type 2, impaired glucose tolerance, cardiovascular disorders, metabolic disorders, stress related to psychology, and vitamin D insufficiency are among the long-term adverse impacts of PCOS patients [4].The markers of inflammation—IL-8, IL-6, IL18, CRP, and TNF-∝—are all consistently high in PCOS women. PCOS is a chronically low-grade inflammatory condition [5]. According to [1], PCOS is a multifactorial, multigenetic, and very complicated genetic condition. Along with other genes like *PGR*, *ACE*, and *TP* [6],there are many genes with genetic polymorphisms leading to polycystic ovarian syndrome, such as the cytochrome *CYP19* and *CYP17A1* genes [7].Several genetic research have explored the relations among genetic variants that are acquired from studies of a genome-wide genetic association and some significant clinical properties of PCOS few years ago [8,9] Investigating the process whereby genetic polymorphisms (SNPs) change the etiology of the PCOS pathological state is vital for enhancing the therapy. Polycystic ovarian syndrome is linked to differences in many genes, such as the *NSR*, *LHCGR*, *AMHR2*, *THADA*, *TOX3*, and *DENNDA1* genes. Based on the studies [9,10], these genes are involved in numerous pathways, such as those interrelated to insulin resistance, androgen control, gonadal function, metabolic diseases, and polycystic ovarian syndrome. If female's family has one or more PCOS-afflicted members, specifically her mother or sister, she will be like other females to acquire PCOS [11] This suggests that genetics may play a significant role in the development of Prosperous research indicates that at least 100 genes are related to the development of PCOS.*CYP19*, *CYP11A*, *CYP1*, *CYP2C8*, and *CYP21*

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belong to the genes responsible for producing steroid hormones. Additional genes associated with chronic inflammation include *TPA*, *PT-1*, and *TNF-α*, which are enzymes implicated in insulin resistance. *IR* genes include *FSHR*, *INSR*, and *IL-6* [12].The current research aims to investigate the genetic polymorphisms of the *CYP2C8* gene in the exon region at variant site 10509681 and the 3-UTR region at variant site rs1058932. These are transion and transversion point mutations that cause polycystic ovarian disorder in women.

MARTIAL AND METHODS

The current study was carried out in the molecular genetics laboratory at Diyala University's College of Education for Pure Sciences in Iraq. The current study concentrated on healthy women in the Diyala Governorate as well as patients with polycystic ovarian syndrome who were admitted to Al-Batoul Teaching Hospital. Between October 2022 and May 2023, blood samples were drawn from both healthy and polycystic ovarian syndrome-afflicted women. There are 48 study samples in all; 24 of these samples are from healthy women, while the remaining 24 are from women who have polycystic ovarian syndrome. Participants were recruited from Al-Batool Teaching Hospital using convenience sampling due to practical constraints. While randomization was not implemented, efforts were made to match controls and cases by age to reduce selection bias. The study was conducted using a single-blind approach, where laboratory staff conducting genetic testing were unaware of the participants' PCOS status to minimize detection bias.

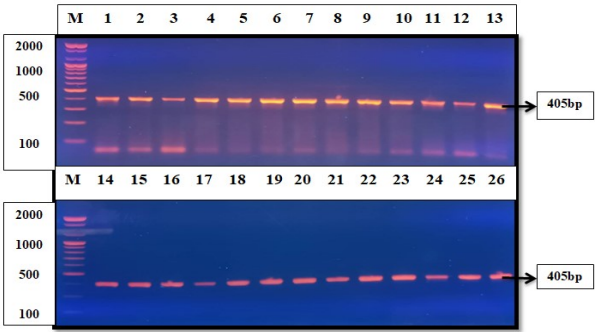
DNA was extracted using the System gDNAMiniprep Blood ReliaPr extraction kit, which was provided by Bioneers in South Korea. To amplify the CYP2C8 gene in the 3-UTR region at the variant site rs1058932 using the forward specific primer (3-TGTGCAGTGACCTGAACAACT-5) , reverse specific primer (3-CACAGTCACAGTTAAACACAAGGA-5) and in the exon region at the variant site rs10509681 using the forward specific primer (3-CCATGGCCAGAGACTTAGCA-5),reverse specific primer (3-ATTACTGTGTCTGGCTGGACC-5).1.5 µl of forward primer, 1.5 µl of reverse primer, 3 µl of DNA, 5 µl of master mix, and 14 µl of free nuclease water constituents of the PCR mixture. The total volume of the reaction product for each sample was 25 µl. Next, the polymerase chain reaction device's samples of healthy women and patients with polycystic ovarian syndrome were mixed with the reaction mixture. The following reaction conditions were programmed into the apparatus: five minutes at 94°C for initial denaturation, thirty seconds at 94°C for denaturation, thirty seconds at 63°C for primer annealing, five minutes at 72°C for extension, and five minutes at 72°C for final extension. This was carried out due to a total of 35 cycles involving primer annealing, denaturation, and extension. Following the completion of the PCR gene amplification procedure, the samples were electrophoresed on a 1% agarose gel for 1.5 hours at 90 V. After that, the amplification product was sent to Macrogen Company in South Korea so that the *CYP2C8* gene could be nucleotide sequenced using the Sanger method. The equation of Hardy-Weinberg was adopted to determine which genotype is said to be a protecting factor and which genotype is assumed to be a causal factor depending on analysis of nucleotide sequence data by making use of the Genius program. [13]

Quality control measures included running each PCR reaction in duplicate to ensure consistency. Positive and negative controls were used to validate results, and sequencing quality was assessed using chromatogram analysis. Ethical approval for this study was obtained from the Institutional Ethics Committee (IEC) of the University of Diyala, Iraq. Informed consent was obtained from all participants, and the study adhered to the Declaration of Helsinki guidelines."

RESULTS

Figure 1. The sample members in the *CYP2C8* gene amplification for the 3-UTR area, that contains the rs1058932 G/A/C variation, were woman with polycystic ovarian syndrome and healthy controls from the Diyala community. The samples migrated on an agarose gel at a concentration of 1.5 percent, during 1:30 hour, and with an electrical potential of 90 volts.

The CYP2C8 gene is augmented in those having polycystic ovarian syndrome and healthy females in the 3-UTR area, in which the rs1058932 G/A/C variant exists.



After staining with ethidium bromide dye and being photographed under ultraviolet light, the results of amplifying a portion of the *CYP2C8* gene for the 3-UTR non-coding region at the rs1058932 variant site revealed that the molecular weight of the resulting fragment is 405 base pairs for both female with polycystic ovarian syndrome and healthy female

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samples. Numbers from 1 to 13 are samples of female patients and numbers from 14 to 26 are samples of healthy female.

Nucleotide sequence of the gene*CYP2C8* in the 3-UTR region at the rs1058932 variant location compared to the GenBank sample with study samples consisting of women with polycystic ovarian syndrome and healthy women

The current research revealed the presence of a genetic mutation of the transion type in samples of patients with polycystic ovarian syndrome at position 509 of the nucleotide sequence of the gene. This was demonstrated by the results in Figure 2, which compared the nucleotide sequence of the *CYP2C8* gene in the 3-UTR at the rs1058932 variant location of study samples consisting of women with polycystic ovarian syndrome and healthy women with the GenBank sample.

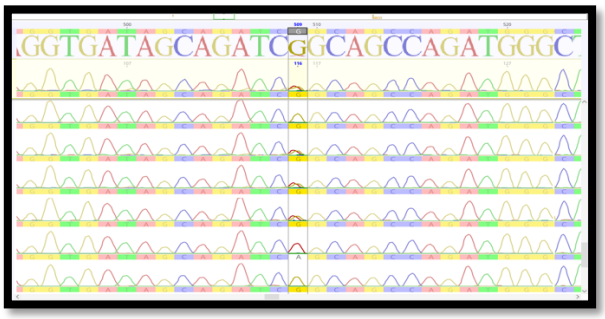

Figure 2. compares the nitrogenous base alignment of the *CYP2C8* gene between the research samples, which comprised women with polycystic ovarian syndrome and healthy women, with the GenBank sample indicating the type of mutation and location of the rs1058932 G/A/C variant.

Genotypes of *CYP2C8* gene in the 3′-UTR region at rs1934953 variant site in healthy and polycystic ovary syndrome women

Table 1 results show that the *CYP2C8* gene contains three genotypes: GG, GA, and AA. These genotypes are important to consider when assessing the relationship between *CYP2C8* genotypes and alleles in the 3′-UTR region at the rs1058932 G/A/C heterozygous location in PCOS patients and healthy controls. Fisher's probability values of 0.767, 0.546, and 0.500, respectively, supported the statistical analysis that found no statistically significant differences between PCOS patients and healthy women.

Table 1. Genotype distribution and allele frequency of *CYP2C8*rs1058932 G/A/CSNPs

Genotype // rs1058932 G/A/C	**Patients No. (%)**	**Control No. (%)**	**Fisher's/P-value**	**O.R. (C.I.)**
GG	16 (66.66%)	17 (70.83%)	0.767 NS	0.82(0.23 - 2.89)
GA	8 (33.33%)	6(25%)	0.546 NS	1.50(0.41 - 5.53)
AA	0 (0.00%)	1 (4.16%)	0.500 NS	0.32 (0.01- 7.72)
Total	24 (100%)	24 (100%)		
Allele	*Frequency*			
G	40 (83.33%)	40 (83.33%)	O.R. (C.I.) = 1.00 (0.33 - 3.02)	
A	8 (16.67%)	8(16.67%)	O.R. (C.I.) = 1.00 (0.33 - 3.02)	
NS: Non-Significant.				

The distribution of allelic frequencies and genotypes of the *CYP2C8* gene in the 3′-UTR regions at the rs1934953 variant location has been studied between PCOS patients and healthy controls in accordance with the Hardy-Weinberg law. Based on the Hardy probability values reaching 0.3272 and 0.6242, respectively, the results in Table 2 show that there are no statistically significant differences between the observed and expected values for the group of polycystic ovary syndrome patients and healthy women.

Table 2. Hardy-Weinberg equilibrium-based predicted genotype and allele frequencies of the *CYP2C8* gene's 3'UTR region at the rs1058932 G/A/C variant location

Hardy P-values	**Allele frequencies**			**Genotype // rs1058932 G/A/C**				**Groupe**
0.3272 NS	**A**	**G**	**AA**	**GA**	**GG**	**No.**	**Observed**	**Patients**
	8	40	0	8	16			
	16.67	83.33	0.00	33.33	66.66	%		

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	Not diagnosed		0.67	6.67	16.67	No.	**Expected**	
			2.78	27.78	69.44	%		
0.6242 NS	8	40	1	6	17	No.	**Observed**	**Contro 24**
	16.67	83.33	4.16	25	70.83	%		
	Not diagnosed		0.67	6.67	16.67	No.	**Expected**	
			2.78	27.78	69.44	%		

NS: Non-Significant.

Amplified of the CYP2C8 gene in both healthy Woman and patients woman with polycystic ovarian syndrome in the Exon region harboring the rs10509681 T/C variation

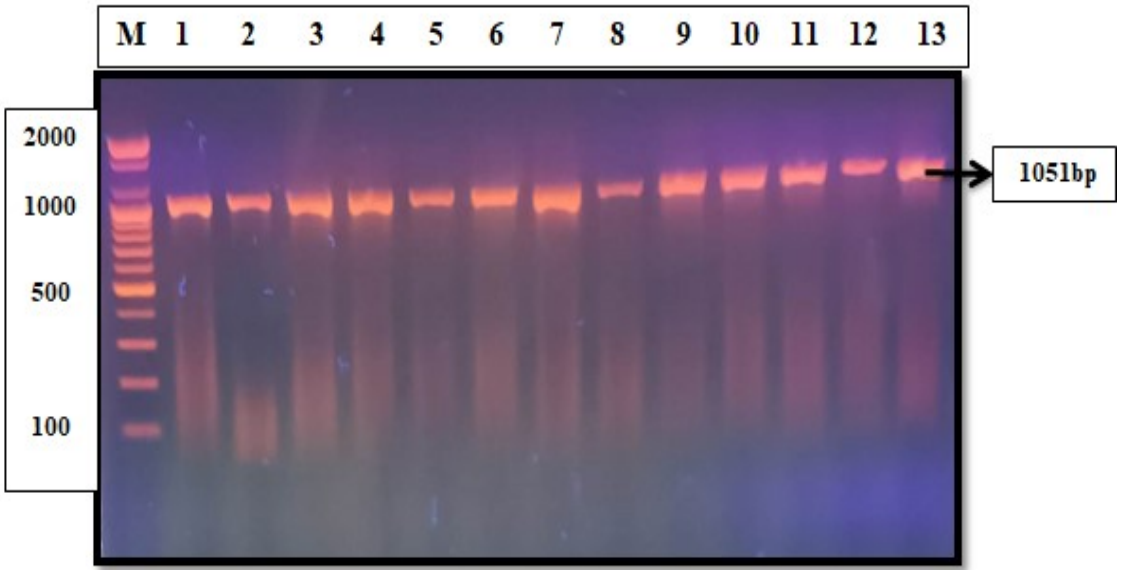

Figure 3. The polycystic ovarian syndrome patients as well as healthy females from the Diyala Governorate community were the subject's amplification of the *CYP2C8* gene for the Exon region, which includes the rs10509681 T/Cvariant. The samples migrated on an agarose gel at a concentration of 1.5 percent, during 1:30 hour, and with an electrical potential of 90 volts. After staining with ethidium bromide dye and being photographed under ultraviolet light, the results of amplifying a portion of the *CYP2C8* gene for the Exon region at the rs10509681 T/C variant site revealed that the molecular weight of the resulting fragment is 1051 base pairs for both female with polycystic ovarian syndrome and healthy female samples. Numbers from 1 to 13 are samples of female patients.

Nucleotide sequence of the gene*CYP2C8* in Exon region at the rs10509681 T/Cvariant location compared to the GenBank sample with study samples consisting of women with polycystic ovarian syndrome and healthy women

The current research revealed the presence of a genetic mutation of the transion type in samples of patients with polycystic ovarian syndrome at position 866 of the nucleotide sequence of the gene. This was demonstrated by the results in Figure 4, which compared the nucleotide sequence of the *CYP2C8* gene in the Exon at the rs10509681 T/C variant location of study samples consisting of women with polycystic ovarian syndrome and healthy women with the GenBank sample.

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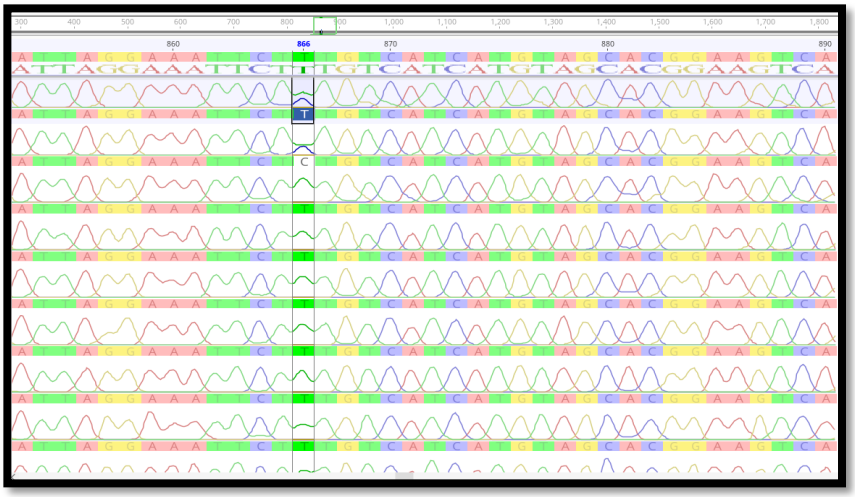

Figure 4. compares the nitrogenous base alignment of the *CYP2C8* gene between the research samples, which comprised women with polycystic ovarian syndrome and healthy women, with the GenBank sample indicating the type of mutation and location of the rs10509681 T/C variant.

Genotypes of *CYP2C8* gene in the Exon region at rs10509681 T/C variant site in healthy and polycystic ovary syndrome women

Table 3 results show that the *CYP2C8* gene contains three genotypes: TT, TC, and CC. These genotypes are important to consider when assessing the relationship between *CYP2C8* genotypes and alleles in the Exon region at the rs10509681 T/Cvariant location in PCOS patients and healthy controls. Fisher's probability values of 0.359, 0.359, and 0.000, respectively, supported the statistical analysis that found no statistically significant differences between PCOS patients and healthy women.

Table 3. Genotype distribution and allele frequency of *CYP2C8*rs10509681 T/CSNPs

Genotype // rs10509681 T/C	**Patients No. (%)**	**Control No. (%)**	**Fisher's/P-value**	**O.R. (C.I.)**
TT	23(95.83%)	21 (87.5%)	0.359 NS	3.29(0.32 - 89.99)
TC	1 (4.16%)	3(12.5%)	0.359 NS	0.30(0.01 - 3.15)
CC	0 (0.00%)	0 (0.00%)	0.000 NS	0.00(0.00 – 0.00)
Total	24 (100%)	24(100%)		
Allele	*Frequency*			
T	47(97.92%)	45(93.75%)	O.R. (C.I.) = 3.13 (0.32 - 84.12)	
C	1 (2.08%)	3 (6.25%)	O.R. (C.I.) = 0.32 (0.01 - 3.14)	
NS: Non-Significant. ,* (P≤0.05)				

The distribution of allelic frequencies and genotypes of the *CYP2C8* gene in the Exon region at the rs10509681 T/C variant location has been studied between PCOS patients and healthy controls in accordance with the Hardy-Weinberg law. Based on the Hardy probability values reaching 0.917 and 0.744, respectively, the results in Table 4 show that there are no statistically significant differences between the observed and expected values for the group of polycystic ovary syndrome patients and healthy women.

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Table 4. Hardy-Weinberg equilibrium-based predicted genotype and allele frequencies of the *CYP2C8* gene's Exon region at rs10509681 T/C variant location

Hardy P-values	Allele frequencies			Genotype // rs10509681 T/C				Groupe
0.917 N.S	C	T	CC	TC	TT	No.	Observed	Patient 24
	1	47	0	1	23			
	2.08	97.92	0.00	4.16	95.83	%		
	Not diagnosed		0.01	0.98	23.01	No.	Expected	
			0.04	4.08	95.88	%		
0.744 N.S	3	45	0	3	21	No.	Observed	Control 24
	6.25	93.75	0.00	12.5	87.5	%		
	Not diagnosed		0.09	2.81	21.09	No.	Expected	
			0.39	11.72	87.89	%		

NS: Non-Significant.

DISCUSSION

As seen in Figures 2 and 4, the sequences of all 48 samples—24 belonging to patients with polycystic ovarian syndrome and 24 representing the control group—were compared with one another in one chat in order to investigate genetic polymorphisms found in the 3-UTR segment at the rs1058932 G/A/C variant site. For the *CYP2C8* gene on the tenth chromosome, the exon segment at the location of the variant rs10509681 T/C has a molecular weight of 1051 and has a molecular weight of 404, respectively. This is referred to as the nucleotide sequence alignment of the gene. The Genius program was utilized for this purpose. The alignment method is to identify any nucleotide variation and where it occurs within the sequence, then compare those sequences with each other and with the reference DNA sequence—that is, the original DNA, which is the DNA that has been documented. The National Center of Biotechnology in Formation (NCBI) website, by comparing the DNA of those samples to the sequence, the findings revealed the presence of point mutations of the transion type in the both segments 3-UTR and Exon of both variants, rs1058932 and rs10509681 of the *CYP2C8* gene, respectively in the samples of female patients with polycystic ovarian syndrome and healthy woman with the DNA reference.

According to Table 1's results, there are 40 patients with the G allele and 16 individuals with the homozygous genotype GG. In comparison to the control group, which reached 17 with a percentage of 70.83 and 40 with a percentage of 83.33, there was a modest drop in the group of patients with cystic ovary syndrome, with percentages of 66.66 and 83.33, respectively. Fisher's probability P = 0.767 indicates that there are no notable distinctions between the control group and the female patients. Given that the odds ratio values for the G allele and the GG genotype were 1.00 and 0.82, respectively, these factors are therefore thought to be protective factors against the disease. While the results showed that the observed number of female patients carrying the heterozygous genotype GA was 8, and the allele was 8, there was an increase in the group of female patients, as the percentages reached 33.33 and 16.67, respectively, compared to the control group, which reached 6 with a percentage of 25 and 8 with a percentage of, respectively, according to Fisher's probability P = 0.546 There are no significant differences between the patient group and the control group, so the GA genotype is considered a causative factor for the disease according to the odds ratio values, which reached 1.50 and 1.00, respectively. The results showed that the homozygous genotype AA and the A allele did not appear in the female patients, as it reached 0, with a percentage of 0.00, and 8, with a percentage of 16.67, respectively, and a significant decrease was recorded in the control group, which was 1, with a percentage of 4.16. According to Fisher's probability P = 0.500, there are no significant differences between the female patients and the control group, so the AA genotype is a protective factor for the disease according to odds values of 0.32 and 1.00.

The results in Table 2 showed that the distribution of the three genotypes, namely GA, AA, and GG, and the allelic frequency of the CYP2C8 gene at the variant site C/1058932 G/A in the study groups, according to the Hardy probability of 0.3272 and 0.6242, are balanced, as there are no statistically significant differences between the observed and expected values. For the three genotypes and alleles in the two groups of patients and healthy controls, respectively, in a study published in 2022, researcher Geo *et al.* examined the relationship between *CYP450* gene polymorphisms and blood levels of the medication hydroxyl chloroquine

(HCQ), its receptors, and adverse drug interactions in 146 patients receiving treatment for rheumatoid arthritis (RA) and lupus erythematous (SLE). Among the 29 polymorphisms, HCQ discovered that there is an rs1058932 variation of the *CYP2C8* gene. Those with the AG genotype are more likely to have defective kidney function than those with the genotype (AA + GG) [P = 0.07]. The study's investigator came to the conclusion that the polymorphism The CYP2C8 gene's two variations (rs1058932 and rs10882526) and the *CYP3A5* gene's variant (rs776746) are involved in the drug interactions of the medication (HCQ).A different study by researchers in Wuhan, China [14] found sex-specific correlations between cerebral hemorrhage (HICH) and the *CYP2C8* gene in the rs105932 and rs2275622 variants and the CY2J2 gene in the rs2271800 and rs1155002 variants. Particularly in females, the A allele carrying the rs1058932 variation and the C allele carrying the rs2275622 variant exhibit a strong correlation with HICH; in men, however, this association is just marginal. This study's researchers anticipated that variations in the *CYP2C8* gene may lessen the pharmacological action of estrogen in females, increasing the likelihood that they may develop HICH, In addition, HICH patients had a greater frequency of the CYP2J2 gene's rs1155002 C/A mutation [P = 0.042] in comparison to the control group. The genotype frequencies of the CYP2J2 gene within the rs2271800 and rs1155002 variations did not, however, differ statistically significantly between the HICH patient group and the control group (P > 0.05).

Table 3's results indicate that there were 23 patients with the homozygous TT genotype and 47 with the G allele. In comparison to the control group, which had percentages of 87.5 and 45, respectively, it showed a considerable rise in the polycystic ovary syndrome patient group, with percentages of 95.83 and 97.92. Fisher's probability P = 0.359 indicates that there are no statistically significant differences between the patients and the control group. Therefore, the T allele and the TT genotype are thought to be causal factors for the disease, as indicated by the odds ratio values of 3.13 and 3.29, respectively. However, the data indicated that there was just one patient with the variant genotype TC. Fisher's probability P = 0.359 showed that there was a drop in the patient group, with the percentages reaching 4.16 and 2.08, respectively, compared to the control group, which reached 3, and the percentages were 12.5, 3., and 6.25. There aren't any notable variations. The odds ratio values of 0.30 and 0.32, respectively, indicate that the TC genotype and the C allele are protective factors against the illness between the female patients and the control group, whereas the CC genotype was absent from both groups. There are no statistically significant differences between the observed and expected values for any of the three genotypes and alleles, as indicated by the results in Table 4, which also showed that the distribution of the TT, TC, and CC genotypes and the allelic frequency of the *CYP2C8* gene at the rs10509681 variant site in the study groups are balanced according to the Hardy-Weinberg law. The Hardy Weinberg probability values reached 0.917 and 0.744 for the patient group and the control group, respectively. [15] conducted a study comprising 360 patients with type 2 diabetes from northern India, ranging in age from 59 to 34 years. The findings demonstrated that the CC genotype frequency distribution for the rs10509681 variant was absent in T2DM patients, with the frequency of this variant being higher in other populations. The trend was five percent. In contrast, the majority of the investigated samples (92.78%) had the TT genotype. The prevalence of the T allele was 96.39%, but the heterozygous CT genotype had a low allelic frequency of 3.61% and a frequency of 7.22%. As a result, this study's conclusion was that T2DM patients had a greater frequency of the T allele of the single nucleotide polymorphism at the variant site rs10509681 than did healthy individuals. According to a study [16], there are two variants of the CYP2C8 gene (rs0509681 and rs11572080) that raise the risk of peripheral neuropathy when taking Txan, a drug used in the first line of chemotherapy for breast cancer. The odds ratios for the two variants were, respectively, (2.03 - 1.08 CI 95%, OR = 1048) and (CI = 1.02 - 1073, OR = 1.37).

Limitations

This study has several limitations that must be acknowledged. First, the small sample size (24 PCOS patients and 24 controls) limits the statistical power of the findings, making it difficult to generalize results to a larger population. Second, the lack of randomization in participant selection may introduce selection bias, potentially compromising the reliability of the results. Third, blinding was not fully implemented; while laboratory staff were blinded during genetic testing, the overall study design did not adopt a double-blind approach, which could lead to detection bias. Additionally, important confounding factors such as body mass index (BMI), insulin resistance, diet, and physical activity were not considered, potentially influencing PCOS risk independently of genetic factors. Furthermore, the study did not include replication of the PCR results or specific quality control measures, which could impact the accuracy of the genetic data. Lastly, the study's focus on a specific geographic region (Diyala

Governorate, Iraq) may limit the applicability of the findings to other populations with different genetic backgrounds. Future research should address these limitations by incorporating larger, randomized, and more diverse sample sizes, implementing rigorous blinding protocols, and controlling for potential confounders to provide more robust insights into the genetic basis of PCOS.

CONCLUSION

Two genetic variations were detected in the 3-UTR and exon sections of the*CYP2C8*gene: rs1058932 G/A/C and rs10509681 T/C. Polycystic ovarian syndrome is more common in women who have the homozygous genotype TT for the variation rs10509681 T/C and the heterozygous genotype GA for the variant rs1934953.

Conflict of Interest: The authors declare that they have no conflict of interest

Funding: No funding sources

Ethical approval: The study was approved by the University of Diyala, Baaquba, Iraq.

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