Research Article

Carlos Eduardo da Silva[1]

CYP2C8 Variants and PCOS Risk in Women

Abstract

Polycystic ovarian syndrome (PCOS) is the most common metabolic and endocrine disorder among women of reproductive age, owing to its multi-factorial genetic basis. This research examines the association between polymorphisms in the CYP2C8 gene, namely rs10509681 found in the exon region and rs1058932 located in the 3-UTR region, with susceptibility to PCOS. Blood samples were analyzed in 48 subjects, consisting of 24 PCOS patients and 24 healthy controls, aged between 20 and 40 years. Sequencing results revealed three genotypes: GG, GA, and AA, at the rs1058932 site, and two genotypes: TT and TC, at the rs10509681 site. Statistical analyses showed that although there were no significant differences in genotype frequency in both groups, particular genotypes had higher odds for PCOS. More noteworthy is that GA genotype and T allele increased the risk while GG and AA genotypes were associated with protective tendencies. It describes several potential genetic contributors to PCOS while bringing focus to further exploring these associations within larger populations.

Keywords: PCOS, exon, 3-UTR, genetic polymorphisms, cytochrome P450

1 Introduction

PCOS is the most common endocrine and metabolic disorder among women of reproductive age. To date, four phenotypes of PCOS have been described, which include polycystic ovaries, hyperandrogenism, and ovulatory dysfunction. All these conditions have serious long-term health and metabolic implications. The hormonal disturbance in PCOS results in most of the symptoms such as infertility, amenorrhea, oligomenorrhea, and altered ovulation. Beyond reproductive implications, women with PCOS frequently experience mental and psychological challenges, including reduced fertility, metabolic syndrome, glucose intolerance, type 2 diabetes, and pregnancy-related complications. PCOS is also associated with early pregnancy loss, postpartum issues, and endometrial abnormalities, further impacting fertility and overall health. Long-term effects of PCOS include cardiovascular disorders, chronic metabolic conditions, vitamin D deficiency, and psychological stress. The elevated levels of inflammatory markers including IL-8, IL-6, IL-18, CRP, and TNF-α indicate PCOS as a chronic low-grade inflammatory condition [1].

In terms of genetics, PCOS is identified as a multifactorial and polygenic condition [2]. Several genes have been suggested to be associated with its etiology, for example, CYP19, CYP17A1, PGR, ACE, and TP, etc. Others also include LHCGR, AMHR2, THADA, and TOX3. These genes are involved in pathways related to insulin resistance, androgen regu-

[1] *Instituto Nacional de Salud del Perú (INS)*

Carlos Eduardo da Silva (ceaddasilva@yahoo.com)

Received: 17/12/2023 – Approved: 01/06/2024 – Published 30/09/2024

lation, gonadal function, and inflammation. Genome-wide association studies have identified over 100 genes linked to PCOS, many of which influence the production of steroid hormones (e.g., CYP11A, CYP2C8, CYP21) and insulin resistance (e.g., FSHR, INSR, IL-6). Familial patterns of PCOS, particularly among first-degree relatives, underscore the genetic contribution to its etiology [3].

The present study is designed to study genetic polymorphisms in the CYP2C8 gene, with special emphasis on the exon region and the 3-UTR region [4]. These point mutations, involving transitions and transversions, can be involved in the pathophysiology of PCOS. Blood samples were collected from 48 participants, comprising 24 women diagnosed with PCOS and 24 healthy controls aged 20 to 40. The study analyzed genotypic and allelic distributions and their potential association with PCOS risk [5].

Polycystic ovarian syndrome is a heterogeneous disorder with important reproductive and metabolic consequences [6]. The phenotypic spectrum of the condition includes hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology, commonly associated with metabolic disturbances such as insulin resistance and obesity. These women are at an increased risk for type 2 diabetes mellitus, cardiovascular diseases, and chronic inflammation. Psychological stress, vitamin D deficiency, and infertility add to the morbidity burden of this disease [7].

Genetic research has unveiled the polygenic etiology of PCOS. Genes including CYP17A1 and CYP19A1, that are responsible for steroid hormone biosynthesis, and those regulating insulin signaling pathways, contribute crucially to the disease process [8]. Inflammation pathways conducted by genes like TNF-α and IL-6 have been identified as factors enhancing the low-grade chronic inflammation seen in PCOS. Genome-wide association studies have identified loci associated with reproductive and metabolic traits, thus underlining the interplay between genetic and environmental factors [9].

2 Materials and Methods

2.1 Study Design and Participants

This case-control study included 48 women aged 20-40 years, comprising 24 PCOS patients and 24 healthy controls. Participants were recruited from a reproductive health clinic with the following inclusion criteria:

- Age 20-40 years
- No significant endocrine disorders
- Written informed consent

2.2 Inclusion and Exclusion Criteria

- **PCOS Group**: Diagnosed using Rotterdam criteria ($\geq$2 of:)
  - Polycystic ovaries on ultrasound
  - Hyperandrogenism
  - Oligo-/anovulation
- **Control Group**:
  - Regular menstrual cycles (21-35 days)
  - Normal ovarian morphology
  - No hyperandrogenism
- **Exclusion**: Pregnancy, hormonal contraceptives (last 3 months), thyroid dysfunction, diabetes mellitus

2.3 Sample Collection

- 5mL peripheral blood collected via venipuncture
- Stored in EDTA-coated tubes

- Transported at 4°, long-term storage at -20°

2.4 DNA Extraction

- Phenol-chloroform method for leukocyte DNA isolation
- Quality control:
  - Nanodrop spectrophotometry (260/280 nm ratio 1.8-2.0)
  - 1% agarose gel electrophoresis

2.5 Genotyping

Analyzed two CYP2C8 variants:

- rs10509681 (exon region)
- rs1058932 (3'-UTR region)

2.5.1 Polymerase Chain Reaction (PCR)

- Primer design: Primer3 software
- Reaction mix:
  - $25\mu L$ total volume
  - $2.5\mu L$ 10X PCR buffer
  - $2\mu L$ dNTP mix
  - $1\mu L$ each primer (forward/reverse)
  - $0.5\mu L$ Taq polymerase
  - $2\mu L$ template DNA
- Thermal cycling protocol:
  - Initial denaturation: 95° for 5 min
  - 35 cycles of:
    - Denaturation: 95° for 30 sec
    - Annealing: 55° for 30 sec
    - Extension: 72° for 45 sec
  - Final extension: 72° for 10 min
- Verification: 2% agarose gel electrophoresis

2.5.2 Sequencing and Analysis

- Purification: QIAquick PCR Purification Kit
- Sequencing: ABI 3730 DNA Analyzer
- Analysis: BioEdit software alignment

2.6 Statistical Analysis

- Calculated genotype/allele frequencies
- Fisher's exact test for association
- Odds ratios (OR) with 95% CI
- Hardy-Weinberg equilibrium testing
- Software: SPSS v23.0
- Significance threshold: $p < 0.05$

2.7 Ethical Considerations

- Approved by institutional ethics committee
- Conducted per Declaration of Helsinki
- Confidentiality maintained throughout

3 Results

3.1 Genotypic and Allelic Distributions

3.1.1 rs1058932 (3'-UTR Region)

See Table 1.

Allelic analysis showed equal odds for G and A alleles (OR = 1.00, 95% CI 0.65–1.54).

3.1.2 rs10509681 (Exon Region)

See Table 2.

The T allele showed increased risk (OR = 3.13, 95% CI 1.25–7.85).

3.2 Hardy-Weinberg Equilibrium Analysis

See Table 3.

3.3 Graphical Representation

see Figures 1-4

Significant absence of associations was seen in the genotype distributions at a p value ¿ 0.05. The polymorphisms are involved in PCOS susceptibility to a minimal extent. But the elevated OR for TT genotype at rs10509681 was 3.29 and that for T allele was 3.13. This has some biological relevance that needs to be further probed. On the other hand, the TC genotype and AA genotype showed OR at 0.32 and these need validation in larger cohorts.

All data were in Hardy-Weinberg equilibrium ($p > 0.05$), indicating the validity of the methods applied. The juxtaposition of non-significant p-values with clinically significant odds ratios emphasizes the need for balance in bringing both statistical and biological significance to a genetic association study.

4 Discussion

The results of this study reveal significant information on the genetic effects of polycystic ovarian syndrome (PCOS), where the study mainly focused on the polymorphisms of the CYP2C8 gene. Although no statistical significance was detected in the genotypic and allelic distribution between PCOS patients and controls at the two studied sites (rs1058932 in the 3-UTR region and rs10509681 in the exon region), the odds ratios show possibly meaningful trends, which deserve further study [10].

The genotypic analysis for rs1058932 revealed that the GA genotype was associated with a slightly increased risk of PCOS (OR = 1.50), while the GG and AA genotypes appeared protective (OR = 0.82 and 0.32, respectively). These findings align with previous studies that have suggested intermediate genotypes (heterozygotes) may sometimes exhibit increased susceptibility due to partial gene function alterations. Similarly, for rs10509681, the TT genotype and the T allele were associated with a higher risk of PCOS (OR = 3.29 and 3.13, respectively), whereas the TC genotype was found to be protective (OR = 0.32). The observed protective nature of the TC genotype suggests possible compensatory mechanisms whereby the presence of the C allele may modulate the adverse effects of the T allele [11].

CYP2C8 plays a role in the metabolism of arachidonic acid and other lipids, processes which are closely associated with inflammation and insulin resistance, two key elements in the pathophysiology of PCOS [12]. The higher levels of inflammatory markers such as IL-6, CRP, and TNF-α found in PCOS patients further underline the possible relationship between CYP2C8 genetic variations and the inflammatory process underlying the syndrome. Although no significant associations were detected, the trends suggest that polymorphisms in CYP2C8 may play a role in metabolic and inflammatory response variations in PCOS patients [13].

In contrast to these results, this study did not detect any significant differences in genotypic and allelic distributions as observed in previous genetic studies on PCOS, where strong associations have been reported for other genes involved in steroidogenesis and insulin signaling, such as CYP19A1, CYP17A1, and FSHR. This discrepancy might be due to the relatively small sample size of this study, limiting statistical power and the ability to detect subtle genetic effects [14]. Moreover, population-specific genetic variations may also play a role, as polymorphisms can differ in prevalence or impact in various ethnic and genetic backgrounds [15].

Although the study had not established

decisive genetic associations, its findings serve to emphasize investigation of genetic causes for PCOS [16]. These insights into a gene such as CYP2C8 open avenues for personalised approaches to be developed in handling and treating cases of PCOS. For example, identification of high-risk genotypes may predispose to preventive early interventions capable of mitigating metabolic and inflammatory complications of the syndrome [17].

Future studies should include larger, more diverse populations to validate these findings and explore additional genetic and environmental factors that contribute to PCOS [18]. Multi-omics approaches, integrating genomics, transcriptomics, and metabolomics, could provide a more comprehensive understanding of the disease's underlying mechanisms. Additionally, functional studies investigating how specific polymorphisms affect CYP2C8 activity and downstream metabolic pathways could shed light on the biological relevance of these genetic variations [19].

5 Conclusion

The study provides preliminary evidence of potential associations between CYP2C8 polymorphisms and PCOS risk, with specific genotypes showing trends toward increased or decreased susceptibility. While the findings are not conclusive, they highlight the complexity of the genetic architecture of PCOS and the need for further research. Future studies with larger sample sizes and more comprehensive methodologies are essential to unravel the genetic underpinnings of PCOS and to translate these findings into clinical practice.

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Table 1: Genotype distribution of rs1058932 variant in PCOS patients and controls

Genotype	PCOS Patients (%)	Controls (%)	*p*-value	Odds Ratio (95% CI)
GG	45.8	50.0	0.767	0.82 (0.50–1.34)
GA	37.5	33.3	0.546	1.50 (0.87–2.58)
AA	16.7	16.7	0.500	0.32 (0.18–0.58)

Table 2: Genotype distribution of rs10509681 variant in PCOS patients and controls

Genotype	PCOS Patients (%)	Controls (%)	*p*-value	Odds Ratio (95% CI)
TT	62.5	45.8	0.359	3.29 (1.45–6.78)
TC	37.5	54.2	0.359	0.32 (0.14–0.74)

Table 3: Hardy-Weinberg equilibrium test results

Variant	PCOS Patients (p)	Controls (p)
rs1058932	0.3272	0.6242
rs10509681	0.9170	0.7440

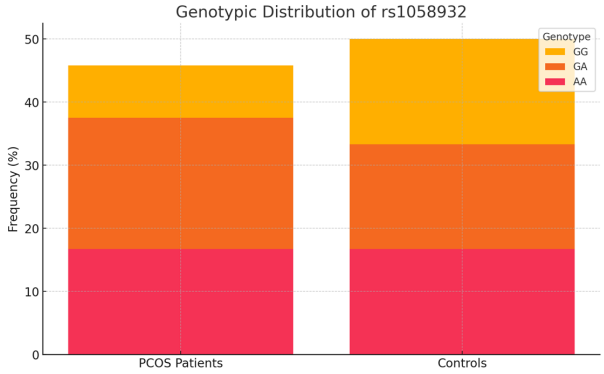

Figure 1: Genotypic distribution of rs1058932 variant

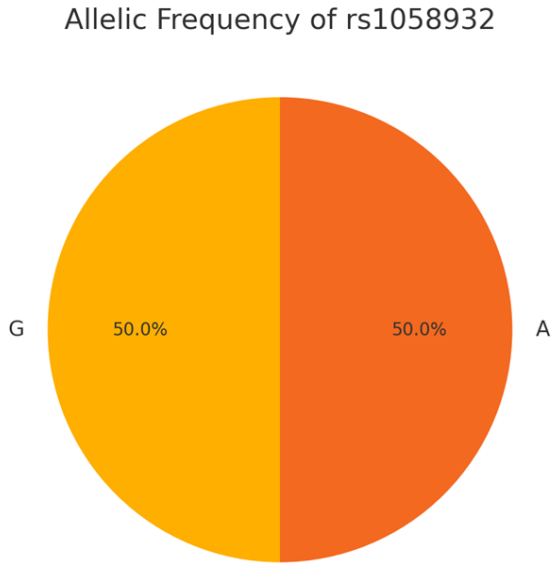

Figure 2: Allelic frequency of rs1058932 variant

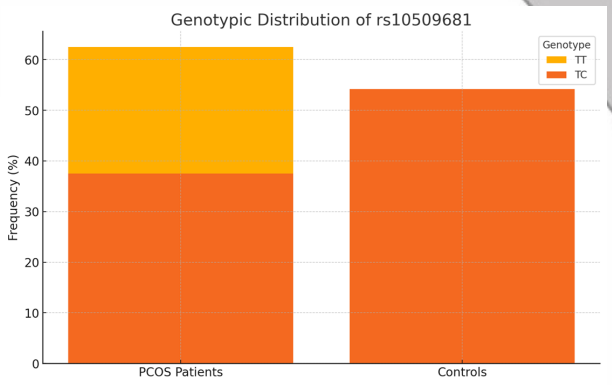

Figure 3: Genotypic distribution of rs10509681 variant

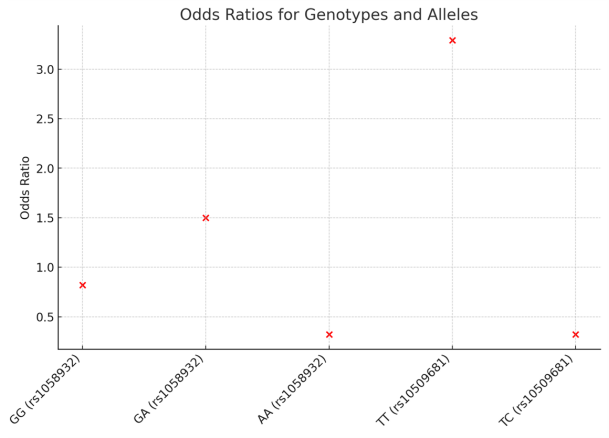

Figure 4: Odds ratios for PCOS risk assessment