# Study of the role of CYP17 gene polymorphism in polycystic ovary syndrome

**Thesis**

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by

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# ABSTRACT

**Introduction**: Polycystic ovary syndrome (PCOS) is a prevalent endocrine disorder, which is the most common cause of anovulatory infertility and hirsutism. It is caused by an overproduction of androgen in theca interna cells. In the ovary, androgen synthesis is regulated by CYP17 gene, which encodes a single P450c17with both 17α-hydroxylase/17,20-lyase activities. In PCO patients, the promoter region of CYP17 gene contains a T→ C substitution that leads to overexpression of that gene and creates a Sp1 site at position-34. This polymorphism generates a recognition site for the MspA1 restriction enzyme.

**Subjects and methods:** The present study was conducted on 28 PCOS patients compared to a control group of 26 healthy females of matching age. Serum levels of the hormones LH, FSH, free T. and DHEA-S were measured and the frequency of T→ C substitution CYP17 gene promoter was investigated in all patients and controls.

**Results:** The wild A1A1 alleles were found in 7 patients with PCOS (25%) and in 6 healthy women (23.1%). In 18 patients with PCOS (64.3%) and in 15 healthy women (57.7%), heterozygous A1A2 alleles were found. Homozygous A2A2 alleles were found in 3 patients with PCOS (10.7%) and in 5 healthy women (19.2%) There was no significant difference in the frequencies of CYP17 genotypes (A1A1), (A1A2),(A2A2) in PCOS patients compared with those in healthy women. Moreover, hormonal profiles were not significantly different in the patients with PCOS from both the homozygous and the heterozygous groups.

**Conclusions:** T→ C polymorphism CYP17 gene is not the primary genetic defect in PCOS and is not associated with steroid hormone synthesis in this disorder.



**Key words**: **PCOS**: Polycystic ovary syndrome, **CYP17**: cytochrome P450 17α-hydroxylase gene, **P450c17**:17α-hydroxylase/17,20-lyase, **LH**: luteinizing hormone, **FSH**: follicle stimulating hormone, **DHEA-S**: dehydroepiandrosterone sulfate, **free T**: free testosterone, **T**: thymine, **C**: cytosine

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| **A ……** | **Adenine** |
| **AC…….** | **Adenylate cyclase** |
| **Ah…….** | **Aryl hydroarbon** |
| **ASO……** | **Allele specific oligonucleotide** |
| **ACTH ……..** | **Adreno corticotrophic hormone** |
| **B-HCG ……..** | **Beta-human chorionic gonadotropin** |
| **BMI……** | **Body mass index** |
| **C …….** | **Cytosine** |
| **cAMP …….** | **3',5'cyclic adenosine monophosphate** |
| **COMT ……** | **Catechol-O- methyl transferase** |
| **CYP ……** | **Cytochrome P 450** |
| **DGK …….** | **Diacylglycerol kinase** |
| **DHEA-S ……** | **Dehydroepiandrosterone sulfate** |
| **DNA …….** | **Deoxyribonucleic acid** |
| **dNTPs…….** | **Deoxnucleotide triphosphates** |
| **EDTA…….** | **Ethylene diamine tetraacetate** |
| **ERK……** | **Extracellular –signal regulated kinase** |
| **FAD …….** | **Flavin adenine dinucleotide** |
| **FMN …….** | **Flavin mononucleotide** |
| **Free T. …….** | **Free testosterone** |

| | |
|---|---|
| **FSH ……** | **Follicle stimulating hormone** |
| **G ……** | **Guanine** |
| **GnRH ……** | **Gonadotropin releasing hormone** |
| **H ……** | **Hydrogen** |
| **HAIR-AN ……** | **Hyperandrogenic insulin resistant-acanthosis nigricans** |
| **HDL ……..** | **High- density lipoprotein** |
| **IRMA…….** | **Immunoradiometric assay** |
| **LDL ……..** | **Low- density lipoprotein** |
| **LH ……** | **Luteinizing hormone** |
| **MAPK**..... | **Mitogen-activated protein kinase** |
| **m RNA ……** | **Messenger ribonucleic acid** |
| **NaCl…….** | **Sodium chloride** |
| **NADH ……** | **Reduced nicotinamide adenine dinucleotide** |
| **NADPH …….** | **Reduced nicotinamide adenine dinucleotide phosphate** |
| **NF_1 ……..** | **Nuclear factor _1** |
| **NIH…….** | **National Institute of Health** |
| **NICHHD……** | **National Institute of Child health and Human Development** |
| **P value ……..** | **Propability** |
| **PA ……..** | **Phosphatidic acid** |
| **PCOS ……** | **Polycystic ovary syndrome** |

| | |
|---|---|
| **PCR ……..** | **Polymerase chain reaction** |
| **PKA……..** | **Protein kinase A** |
| **PPAR ……** | **Peroxisome proliferators activated receptor** |
| **r……** | **Regression coefficient** |
| **RBC's……** | **Red blood cells** |
| **RFLP …….** | **Restriction Fragment Length Polymorphism** |
| **RIA……** | **Radioimmunoassay** |
| **RXR ……** | **Retinoid X receptor** |
| **SDS …….** | **Sodium Dodecyl Sulfate** |
| **SHBG …….** | **Sex hormone- binding globulin** |
| **SP_1 ……....** | **Specificity protein_1** |
| **SF_1 ……** | **Steroidogenic factor_1** |
| **T …….** | **Thymine** |
| **TBE…….** | **Tris-borate EDTA.** |
| **TGF- β ……** | **Transforming growth factor _β** |
| **TSH ……** | **Thyroid stimulating hormone** |
| **5'UTR ……** | **5'-Untranslated region** |
| **VNTR ……** | **Variation in number of tandem repeats** |

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# INTRODUCTION

Polycystic ovary syndrome(PCOS) is one of the most common endocrine dysfunctions affecting >10% of women of reproductive age (***Kahsar-Miller et al.,2004).*** This disorder is associated with oligomenorrhea, infertility, hirsutism, obesity, persistent anovulation, fibrotic cystic ovaries and, rarely, virilism ***(Ben-Rafael and Orvieto. 2000).***

Ovarian androgen overproduction has been implicated in the pathogenesis of hyperandrogenism which is characteristic of PCOS (***Maitra et al.,2005).***

The rate-limiting reaction of androgen synthesis in the ovary is the hydroxylation of progesterone at carbon 17 accompanied by cleavage of C17-20 bond to produce androstenedione. The reaction is catalysed by 17α-hydroxylase/17-20 lyase enzyme complex involving cytochrome P450c17 as a terminal electron acceptor ***( Miller.,2002).***

The humam gene encoding cytochrome P450c 17 (CYP17) is expressed in the adrenal gland and the ovarian theca cells, its promoter region has several Sp1 sites (CCACC). It has been demonstrated that in some women with PCOS, the region encoding 5'-UTR of CYP17 gene contains a T→C substitution at position -34bp, thus generating an additional Sp1 site ***(Kahsar-Miller et al, 2004).:***

It has been postulated that the additional Sp1 site might be responsible for the excessive expression of CYP17 gene and the overproduction of androgens, which ultimately could lead to PCOS ***(Wickenheisser JK et al., 2004).***

## AIM OF THE WORK:

This work aimed at finding the possible correlation between CYP17 gene polymorphism and PCOS , by comparing the frequencies of the polymorphic allele in a group of women with PCOS with a group of healthy women and detection of the similarities and differences of the hormonal profiles.

# Subjects and Methods

## Subjects

Twenty eight female patients, of age ranged (18-38) years, recruited from the gynaecology outpatient clinic at Kasr Al-Aini hospital, Cairo University, were studied. Twenty six age matched females with regular ovulatory cycles were taken as control group.

The patients' chief complaints were failure of conception, menstrual disturbance and or undesired body hair. They were diagnosed as polycystic ovary syndrome, by means of history taking, clinical examination and ultrasonography.

Selection criteria

The patient was selected only if she applies to the diagnostic criteria for polycystic ovary syndrome based on the consensus definition specified in Rotterdam conference (2003).

A pelvic ultrasound proving the presence of at least more than 8 subcapsular follicles of 3-8 mm diameter in one plane, in one ovary with an increased stroma. Together with at least one of the following symptoms: oligomenorrhea, or amenorrhea, hirsutism, or acne.

All selected cases were subjected to the following:

**A) Thorough history taking**:

Each patient was questioned for her age, duration of complaint, symptoms suggestive androgen excess in the form of hirsutism and, menstrual disturbance.