

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

Thesis

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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 P450c17 with both 17 α -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 17 α -hydroxylase gene, **P450c17:** 17 α -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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ARABIC SUMMARY

LIST OF ABBREVIATIONS

A	Adenine
AC.....	Adenylate cyclase
Ah.....	Aryl hydrocarbon
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.....	Deoxynucleotide 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 17 α -hydroxylase/17-20 lyase enzyme complex involving cytochrome P450c17 as a terminal electron acceptor (**Miller.,2002**).

The human 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.