Iraqi Journal of Biotechnology, 2015, Vol. 14, No. 2 , 99-110

Association Between Polycystic Ovary Syndrome and Genetic Polymorphisms of CYP 17 Gene in Iraqi Women

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Received: June 14, 2015 / **Accepted**: October 11, 2015

Abstract: Polycystic ovary syndrome (PCOS), whose genetic basis is not completely well understood, it is the most common endocrine disorder in women in their reproductive years. Blood samples were collected from two groups. The first group included 61 females with PCOS and the second group included 30 normal females to detect the presence of mutation in the CYP17 gene. The two groups were genotyped and a comparison was done between them. The results showed a significant difference ($p<0.05$) in FSH levels in patients and normal females and there is no significant difference in the levels of Testosterone, prolactin and LH. Moreover, it showed a significant difference ($p<0.05$) in the levels of HDL in both patients and normal females and no significant difference in the levels of (LDL, VLDL, Triglyceride and cholesterol). Two comparisons for genotype were done: one between age and genotype and the second between BMI and genotype for each group. The results showed two types of genotype, which were a TT wild type and a heterozygote TC mutant type. Furthermore, the results showed a significant difference ($p<0.05$) in genotype TT and TC in the group of age less than 25 years old and no significant difference in these genotypes in age groups (25-35) and those with more than 35 years old. It is concluded from this study that this single nucleotide polymorphism in the CYP 17 gene was not associated with PCOS in Iraqi women.

Key words: PCOS, BMI, CYP17, SNP, RFLP, genotype.

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Introduction

Polycystic ovary syndrome is a highly prevalent heterogeneous endocrine disorder of uncertain etiology, not a specific disease affecting approximately up to 18% of reproductive aged women (1). The syndrome is often associated with obesity, insulin resistance and metabolic syndrome (2).

It is typically characterized by chronic anovulation, excess androgen production, and the presence of polycystic ovaries on ultrasound. Clinically, these women present with irregular menses, hirsutism, acne and alopecia, and elevated LH: FSH ratio along with insulin and androgen excess. This syndrome also confers a greater risk of development of impaired glucose tolerance and

subsequent type 2 diabetes mellitus (T2DM), as well as metabolic syndrome and cardiovascular diseases (CVD) in later life (3).

Women with PCOS also exhibit lower high-density lipoprotein (HDL) levels, higher triglyceride and higher low-density lipoprotein (LDL) levels than age- and weight-matched control women, which is responsible for the increased incidence of hypertension, coronary heart disease and thrombosis (4).

The aetiopathogenesis of PCOS is likely to be multifactorial consisting of both environmental and genetic factors. Polymorphism of several genes like FSHR, CYP17, CYP1A1, CAPN10 and INSR have been found to be associated with hyperandrognemia and infertility found in PCOS (5, 6, 7). It has been hypothesized that the interaction of the environmental factors with some important genes involved with production of androgen and synthesis/ secretion of insulin can most suitably explain PCOS (8).

The adrenal and gonadal steroid hormones are produced in a multistep process that involves the participation of six P450 cytochromes: CYP11A1, CYP17, CYP21, CYP11B1, CYP11B2, and CYP19. Of these enzymes, CYP17 and CYP19 catalyze the rate-limiting steps of estrogen biosynthesis (9, 10).

CYP17, whose gene is located on 10q 24-q25, spanning a 1870-bp region, is also important in estrogen formation. CYP17, encoded by the CYP17 gene, catalyzes the conversion of progesterone and Pregnenolone into precursors of potent androgens (11). CYP17 is predominantly expressed in the adrenal gland, testicular Leydig cells, and ovarian theca cells. At -34 point in the 5' promoter region of the CYP17 gene, a T to C substitution has been hypothesized to alter CYP17 gene expression (12).

The aim of this study is to explain the possible correlation between genetic polymorphism of *CYP* 17 gene and polycystic ovary syndrome in Iraqi women.

Materials and Methods

A case-control study conducted in the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies and Al-Nahrain Forensic DNA Unit, Baghdad, Iraq during the period from February 2014 to July 2014. This study has included sixty one infertile Iraqi women with PCOS and 30 apparently healthy women served as control group. Patients were selected from the Higher Institute of Infertility Diagnosis and Assisted Reproductive Technologies fulfilling at least two of the following three criteria based on the Rotterdam ESHRE/ASRMS sponsored PCOS consensus workshop group (13):

1) Polycystic ovaries on ultrasound.
2) Chronic anovulation or oligovulation.
3) Clinical or biologic Hyperandrogenism.

According to BMI, each group was subdivided into three subgroups (less than 30 $kg/m^{2,}$ 30-40 kg/m^2 and more than 40 kg/m^2). Basal blood samples were obtained from the studied subjects to measure plasma FSH, LH, testosterone and prolactin hormones. The cholesterol, triglyceride, low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) and high-density lipoprotein (HDL) were measured.

Genetic Analysis

The genomic DNA isolated from the whole fresh blood with PCOS and the control women by using gene extraction kit supplied by Gene aid Company (Thailand). The restriction fragment

length polymorphism analysis was performed to determine genotypes for the (-34 T/C, rs743572) in the 5′ promoter region of CYP 17 gene .

This region of CYP17 was amplified by polymerase chain reaction (PCR), using forward primer: 5′-CATTCGCACTCTGGAGTC-3′, reverse primer: 5′-AGGCTCTTGGGGTACTTG-3′. A total volume of 25 µl containing genomic DNA 50 ng which used as template in the reaction mixture, 0.3 µl of each primer and 12.5 µl of Green Master Mix (Promega, USA). Cycling parameters were denatured at 94oC.for 3 minutes, 35 cycles with 94oC for 30 seconds, 55oC for 30 seconds, 72oC for 60 seconds, and 72oC for 10 min.

PCR products (414-bp) digested with MspA1I (Promega, USA) for 4 hours at 37oC. The reaction mixture was prepared to 20 µl final volumes which contains 10 µl of PCR product, 2µl of buffer C, 0.5 µl of Acetylated BSA and 0.2 µl of *MspA*1I enzyme. Digested DNA fragments were electrophoresed on a 2.5% agarose gel containing ethidium bromide and visualized by UV transilluminator (Cleaver Scientific, UK). Hence, a single 414-bp band indicates homozygosity for the TT genotype. The presence of two fragments, 290-bp and 124-bp bands, indicates homozygosity for the CC genotype. The presence of three fragments, 414-, 290-, and 129-bp bands, indicates heterozygosity for the TC genotype.

Statistical Analysis

The Statistical Analysis System-SAS (14) was used to effect of different factors in study parameters. Chi-square test was used to significant compare between percentage and Least significant difference LSD test was used to significant compare between means in this study.

Results and Discussion

Hormonal Assay

The comparison of hormonal assay between patient and control was illustrated in table (1), that shows non-significant difference of LH, Prolactin and Testosterone while the only statistically significant difference was FSH level with (P value = 0.047).

The lack of difference in these hormones could be attributed to prior medical treatment taken by patients participated in the study. This result is agreed with hilt *et al*. (15) who suggested that FSH levels are lower in young women with PCOS than in the early follicular phase of women with normal ovaries. The mechanism behind these low levels, which may partly explain the lack of follicular growth, is probably increased production of inhibit B from the increased number of antral follicles in polycystic ovaries (16). The current result disagreed with Nawras (17), who showed that no significant differences (p>o.05) in level of FSH. Also, the result is disagreed with Mezaal (18), who reported the same findings in the Iraqi population.

Table 1: Comparison between patients and control in hormone profile

Group	No.	Mean ± SD			
		FSH(IU/ml)	LH(IU/ml)	Prolactin(ng/ml)	Testosterone(ng/ml)
Patients	61	5.73 ± 0.23	4.42 ± 0.33	16.04 ± 1.21	0.595 ± 0.05
Control	30	7.01 ± 0.66	4.28 ± 0.59	17.59 ± 2.35	0.525 ± 0.07
LSD value	--	1.268 *	1.592 NS	6.143 NS	0.215 NS
P-value	--	0.047	0.863	0.614	0.515
* (P<0.05), NS: Non-significant.					

FSH = follicular stimulating hormone, LH = luteinizing hormone and LSD: Least Significant Differences.

Lipid Profile

The Comparison between patients and control in lipid profile illustrated in table (2). HDL level was significantly higher in control compared with patients. No significant differences between patients and control in triglyceride, LDL, VLDL and cholesterol levels. Moreover, the same explanation also applies to the lipid profile comparison (as hormonal assay), cases receiving lipid Lowery medication could have cholesterol, LDL, VLDL and Triglyceride with the normal limits. HDL could also be affected by type of nutritional habits, type and duration of medication. Dyslipidemia is defined as a clinically significant change in serum lipids and lipoproteins. In a previous review, Diamanti-Kandarakis *et al.* (19) reported that between 36-70% of women with PCOS has an abnormal lipid profile. Also, those researchers found an overproduction of very low density lipoprotein (VLDL) and increased channelling of fatty acids through lipolysis. Insulin resistance and hyperinsulinemia are thought to contribute to this, with defective insulin signalling within the visceral adipose tissue leading to increased lipolysis. Hyperandrogenemia further adversely impacts lipoprotein metabolism, by both reducing HDL and increasing LDL (19). Wild *et al.* (20), found women with PCOS to have higher TG, higher VLDL cholesterol and lower HDL cholesterol than healthy age-matched controls. Our results agree with this study only with HDL but not with the other parameters.

Table (2): Comparison between patients and control in Lipoprotein (Cholesterol, HDL, Triglycer ide, LDL and VLDL)

Group	No.	Mean ± SD				
		Cholesterol (mg/dl)	**HDL** (mg/dl)	**Triglyceride** (mg/dl)	**LDL** (mg/dl)	**VLDL** (mg/dl)
Patients	**61**	**149.12 ± 6.13**	**30.6**	**118.21 ± 10.81**	**94.80 ± 5.47**	**23.64 ±2.16**
Control	**30**	**159.33 ± 7.93**	**37.03 ± 3.04**	**151.28 ± 28.02**	**92.04 ± 7.97**	**30.25 ± 5.60**
LSD value	--	**19.939 NS**	**6.171 ***	**55.015 NS**	**18.89 NS**	**1.003 NS**
P-value	--	**0.307**	**0.041**	**0.232**	**0.769**	**0.232**

* (P<0.05), NS: nonsignificant.

VLDL = very low density lipoprotein, LDL = low density lipoprotein, HDL = high density lipoprotein and LSD: Least Significant Differences.

Genetic Studies

Genotype Distribution of CYP 17 Gene

Number and percentage of genotype in patients and control women data are shown in table (3), there was a significant difference ($p<0.05$) between percentage of wild type TT and heterozygote TC. The percentage of TT was significant higher than TC between patients 57.38, 42.62 %, respectively. Furthermore, there was significantly higher difference ($p<0.01$) between TT and TC in the control group (60.00, 40.00) % respectively. Also, in this table there was a comparison between patients and control in TT and TC percentage. There was no significant difference between the two groups (control and patient) in TT and TC percentage. CC genotype was not present in this current study.

Genotype assay was not significantly different between cases and controls, but only within each group in the present study. This result strengthens the multifactorial theory of the etiology of this syndrome.

Park *et al*.(21) found seven SNPs of the gene CYP17, which is active in estrogen biosynthesis and located at 10q24.3, and found no significant association between these SNPs and PCOS.

Table (3) Number and percentage of genotype in patients and control

Group	TT		TC		Ch-Square	P-value
	No.	%	No.	%		
Patients (61)	35	57.38	26	42.62	5.193 *	0.0437
Control (30)	18	60.00	12	40.00	7.25 **	0.0149
Chi-square- χ^2	--	0.922 NS	--	0.714 NS	--	--
P-value	--	0.617	--	0.883	--	--
* (P<0.05), ** (P<0.01), NS: Non-significant.						

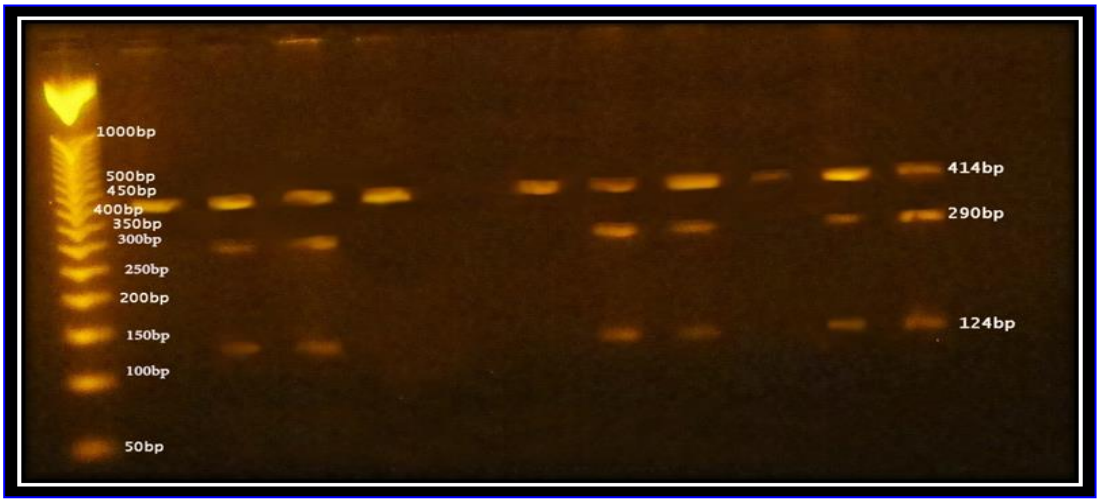

Figure (1): Restriction fragment length polymorphism analysis of the C/T polymorphism of -34 in promoter region of the CYP 17 gene. Agarose gel (2.5%) electrophoresis after *Msp*A1I digestion of the PCR.
Lane 1: TT wild type (414 bp) .
Lane 2: TC heterozygote (414 bp, 290bp, 124bp) .

Genotype and Age Group

There was a significant difference ($p<0.05$) between TT, TC in age group of patients (less than 25 years), TT was a significant higher than TC, (TT=51. 43) and (TC =42. 31). There was no significant difference between TT and TC in age group of patients (25-30, more than 30 years old). In compared between different age groups of patients (less than 25 ,25-30, more than 30 years

old) in TT, TC percentage, there were a high significant difference ($p<0.01$) among different age groups of patients, TT was a significantly higher in the age group (less than 25 years) (51.43%) and follow it TT percentage in group 25-30 years old (40%) and at last in the age group (more than 30 years old) was (8.57%). In TC percentage of patients less than 25 years old was a higher (42.31%) compared to the percentage of patients with 25-30 and more than 30 years old (46.15%, 11.54%) respectively.

Table (4) Number and percentage of genotype in Age group of patients

Age Group (year)	TT (No = 35)		TC (No. = 26)		Ch-Square	P-value
	No.	%	No.	%		
Less than 25	18	51.43	11	42.31	4.913 *	0.054
25-35	14	40.00	12	46.15	1.866 NS	0.361
More than 35	3	8.57	3	11.54	0.752 NS	0.694
Chi-square- χ^2	---	10.826 **	---	8.463 **	---	---
P-value	---	0.0116	---	0.0147	---	---
* (P<0.05), ** (P<0.01), NS: non-significant.						

Genotype and BMI

There was a significant difference showed in table (5) between TT and TC genotype percentage in BMI of patients with the group (less than 30), TT genotype percentage of patients women were higher (65.71%) in BMI than the TC genotype percentage (53.85%) for the same group (less than 30). While there were no significant differences showed in this table between TT, TC genotype percentage in BMI of patients women with (30-40 and more than 40). In comparison among different groups of patients (less than 30, 30-40 and more than 40) in BMI, there were high significant difference ($p<0.01$) among different groups in TT genotype percentage for BMI of cases. The group with less than 30 was a higher percentage (65.71) than other groups 28.57%, 5.71% with age 30-40 and more than 40 in TT genotype for BMI respectively. Also, there were high significant difference ($P<0.01$) among different groups in TC genotype percentage for BMI of patients, percentage of TC in BMI of patients significantly decreased with more than 40.

The present study showed that no relationship between SNP and obesity.

Table (5) Number and percentage of genotype in BMI of patients

BMI	TT (No = 35)		TC (No. = 26)		Ch-Square	P-value
	No.	%	No.	%		
Less than 30	23	65.71	14	53.85	4.924 *	0.052
30-40	10	28.57	9	34.62	1.783 NS	0.394
More than 40	2	5.71	3	11.54	1.805 NS	0.372
Chi-square- χ^2	---	11.972 **	---	9.326 **	---	---
P-value	---	0.0029	---	0.0138	---	---
* (P<0.05) , ** (P<0.01) , NS: Non-significant.						

BMI= body mass index.

Genotype of Control Age Group

The number and percentage of genotype in the age group of control showed in Table (6). There were significant difference between TT, TC genotype percentage in age group (25-35 years) and there were no significant difference between TT, TC genotype percentage and age groups (less than 25 and more than 35 years old) for control. Also there were significant difference ($p<0.01$) between age groups and the TT genotype percentage of control. In TC genotype there were significant difference ($p<0.01$) between age groups and TC genotype percentage. In the age group (25-35) the TT and TC genotype percentage was higher significant (55.56 and 66.67%) respectively, than other age groups (less than 25 and more than 35).

Table (6): Number and percentage of genotype in Age group of control

Age Group (year)	TT (No = 18)		TC (No. = 12)		Ch-Square	P-value
	No.	%	No.	%		
Less than 25	2	11.11	1	8.33	0.834 NS	0.492
25-35	10	55.56	8	66.67	4.509 *	0.037
More than 35	6	33.33	3	25.00	2.39 NS	0.182
Chi-square- χ^2	---	9.773 **	---	11.429 **	---	---
P-value	---	0.0117	---	0.0029	---	---
* (P<0.05), ** (P<0.01), NS: nonsignificant.						

Genotype and BMI of Control Group

The number and percentage of genotype in BMI of control were Illustrated in Table (7). There were significant difference in BMI between TT, TC genotype percentage and the group (30-40) of control. No significant difference between TT, TC genotype percentage and other groups (less than 30 and more than 40) in BMI of control. In addition, a highly significant difference showed in table (7). Between TT percentage and TC percentage with different groups in BMI of control. High percentage for TT, TC (68.75%, 50%) were noted in group (30-40).

Table (7): Number and percentage of genotype in BMI of control

BMI	TT (No = 18)		TC (No. = 12)		Ch-Square	P-value
	No.	%	No.	%		
Less than 30	2	11.11	2	16.67	1.943 NS	0.217
30-40	11	68.75	6	50.00	7.159 **	0.0155
More than 40	5	27.78	4	33.33	2.054 NS	0.209
Chi-square- χ^2	---	11.627 **	---	9.027 **	---	---
P-value	---	0.0029	---	0.0138	---	---
* (P<0.05) , ** (P<0.01) , NS: Non-significant.						

BMI= body mass index

Although hyperandrogenism is a distinct feature of PCOS, studies of CYP17, which encodes the rate-limiting step in androgen synthesis, have yielded mixed results, whichcould have resulted from smaller sample sizes in those studies and the fact that most prior studies looked at only one or two variants per gene. Most studies focused almost exclusively on a variant in the 5′ promoter region(-34 T/C, rs74357).

Carey *et al.* (22) described a change of a single base T into C at -34 point in the promoter region of the gene that created a restriction site for the restriction enzyme Msp-A1,while a few studies reported an association of this variant with PCOS (23 ; 24).

Several others agreed with our findings. They found no such association (25; 26; 27; 21; 6). In another study Marszalek *et al.*(28) illustrate a similar result in Poland population, while they genotyped 56 PCOS women and concluded the T>C polymorphism of cyp17 gene is not associated with steroid hormone synthesis in PCOS and it is not a primary genetic defect in this disease. Chua *et al.* (29) reported that genetic variation in the CYP17 gene was not a major risk factor for PCOS. These studies also confirm our results and agreed with them.

As mentioned above, many studies have aimed at studying the pattern of inheritance of PCOS, though case control studies, family based studies, mostly with small sample size. Few of the studies have concluded that PCOS is inherited in an autosomal dominant pattern. However, they have done ultrasound or physically examined the first degree relatives.

To conclude, PCOS is a complex genetic disorder, so the effect of any one gene may be small. Besides, our study showed that this SNP is similar between patients and control. Which means, it does not play a major or a direct role in the pathogenicity of this syndrome.

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