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Effect of CYP19 Gene on Polycystic Ovary Syndrome Phenotype in Iraqi Women

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Abstract

Background Ovarian androgen overproduction is the key physiopathologic feature of polycystic ovary syndrome and the bulk of evidence points to the ovary being the source of excess androgens, which appears to result from an abnormal regulation (dysregulation) of steroidogenesis. Aromatase is an enzyme complex responsible for a key step in the biosynthesis of estrogen. It is encoded by CYP19.

Objective To examine whether the rs2414096 of *CYP19* gene contributes to genetic susceptibility to the polycystic ovary syndrome hyperandrogenism in Iraqi women.

Methods A Case control study was conducted in the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University and Al-Nahrain Forensic DNA Unit, Baghdad, Iraq for the period from February 2012 to February 2013. Sixty-five healthy women serves as the control group and eighty-four infertile women with polycystic ovary syndrome, divided into two subgroups depending on the body mass index (< and ≥30 kg/m2) were studied. Restriction fragment length polymorphism analysis was performed to determine the genotypes of rs2414096 of *CYP19*. Clinical, anthropometric, hormonal and biochemical parameters were also estimated.

Results Genotypic distribution of rs2414096 of *CYP19* was significantly different in polycystic ovary syndrome patients from that of control women. The frequency of GG genotype was higher in the patients, while AA genotype was higher in control women. Those with GG genotype have lower estradiol, estradiol/testosterone and higher testosterone, luteinizing hormone, follicular stimulating hormone than those with AA genotype.

Conclusion The present data suggests that single-nucleotide polymorphisms rs2414096 in the *CYP19* gene is associated with susceptibility to polycystic ovary syndrome hyperandrogenism in Iraqi women.

Keywords Polycystic ovary syndrome, *CYP19* gene, reproductive hormones, Iraqi women.

List of Abbreviation: PCOS = polycystic ovary syndrome, FSH = follicle stimulating hormone, Kb = kilobase, SNP = single nucleotide polymorphism, BMI = body mass index, LH = lutinizing hormone, E_2 = estradiol, FBS = fasting blood sugar, LDL = low density lipoprotein, HDL = high density lipoprotein, VLDL = very low density lipoprotein, GnRH = Gonadotropin releasing hormone.

Introduction

The etiology of polycystic ovary syndrome (PCOS) is still unknown; there is increasing evidence to support a major genetic basis, since the syndrome is strongly familial [(1)]. It is clear, however, that more than one gene (and probably several) contributes to the heterogeneous phenotype [(2,3)].

Ovarian androgen overproduction is the key physiopathologic feature of PCOS and the bulk of evidence points to the ovary being the source of excess androgens, which appears to result from dysregulation of steroidogenesis [(4)]. Genetic variation at androgen receptor [(5)], suggesting that hyperandrogenism in PCO may be partly genetically determined.

Aromatase is an enzyme complex responsible for a key step in the biosynthesis of estrogens. This enzyme complex is composed of the cytochrome P450 aromatase and the nicotinamide adenine dinucleotide phosphate (NADPH) cytochrome P450 reductase. It is a member of the cytochrome P450 superfamily, which are monooxygenases that catalyze many reactions involved in steroidogenesis [6] and it catalyzes the conversion of C19 androgens to aromatic C18 estrogens. It is induced by follicular stimulating hormone (FSH) and is present in a number of different tissues including adrenals, muscle, placenta, skin, adipose and nervous tissue. Reduced aromatase activity may lead to the development of PCOS [7].

CYP19 gene encodes P450arom which is located on the long arm of chromosome 15 at position 15p21.1 and 130 kilobase (kb) long. Its 10 exons (the final nine of which are coding) are located within 30 kb of each other, and the 93 kb 50-flanking region is thought to have a regulatory role [8].

Several studies have reported the association of the single nucleotide polymorphism (SNP) rs2414096 in the CYP19 gene with hyperandrogenism [9,10].

The aim of this study is to examine whether the rs2414096 of *CYP19* gene contributes to genetic susceptibility to the PCOS hyperandrogenism in Iraqi women.

Methods

A Case control study conducted in the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University and Al-Nahrain Forensic DNA Unit, Baghdad, Iraq from February 2012 to February 2013. The study was approved by the Institute review Board of the College of Medicine, Al-Nahrain University, and written ethical consent was obtained from patients to participate in the study.

Sixty five healthy control group and 84 women who were diagnosed as PCOS patients according to the 2006 Rotterdam criteria were studied. All patients had a history of oligomenorrhea and evidence of hyperandrogenism (on clinical examination or documented by elevated testosterone levels). Women with any other cause of oligomenorrhea and hyperandrogenism were excluded. Only women who had PCO on ultrasonography were enrolled to ensure that the phenotype was definitely PCOS. Clinical and biochemical characteristics of women with PCOS patients and control women are given in Table 1. According to body mass index (BMI), each groups were subdivided in two subgroups (obese ≥30 kg/m^2 and non-obese <30 kg/m^2)

Whole blood samples were obtained from PCOS patients and control women to measure plasma FSH, luteinizing hormone (LH), estradiol (E_2), and testosterone hormones. Fasting blood sugar (FBS), blood glucose level after half, one and two hours, and lipid profile levels: cholesterol, triglyceride, low density lipoprotein (LDL), high density lipoprotein (HDL) and very low density lipoprotein (VLDL) were measured.

Genetic Analysis

Blood samples for molecular genetic studies were collected in tubes containing ethylenediaminetetraacetate as an anticoagulant. The genomic DNA was extracted from the blood of patients with PCOS and control group by using gene extraction kit supplied by Geneaid Company (Thailand). The restriction fragment length polymorphism analysis was performed to determine genotypes of rs2414096 of CYP19. Using Forward primer: 5'-TCT GGA AAC TTT TGG TTT GAG TG-3' Reverse primer: 5'-GAT TTA GCT TAA GAG CCT TTT CTT ACA-3'. A total volume of 25 µl containing genomic DNA 50 ng was used as template in the reaction mixture In addition to 6.25 pmol of each primer and 12.5 µl of Green Master Mix (Promega, USA). Cycling parameters were denaturation at 94^{o}C for 5 minutes, 30 cycles with 94^{o}C for 1 minute, 60^{o}C for 1 minute, 72^{o}C for 1 minute, and 72^{o}C for 10 [13]. Polymerase chain reaction (PCR)

products (189-bp) were digested with HSP92 II (promega, USA) for 4 hours at 37°C. Digested deoxyribonucleic acid (DNA) fragments were electrophoresed on a 2% agarose gel containing ethidium bromide and visualized by UV trans-illuminator spectroline (USA). Hence, a single 189-bp band indicates homozygosity for the GG genotype. The presence of two fragments, 161-bp and 28-bp bands, indicates homozygosity for the AA genotype. The presence of three fragments, 189-, 161-, and 28-bp bands, indicates heterozygosity for the AG genotype (Fig. 1).

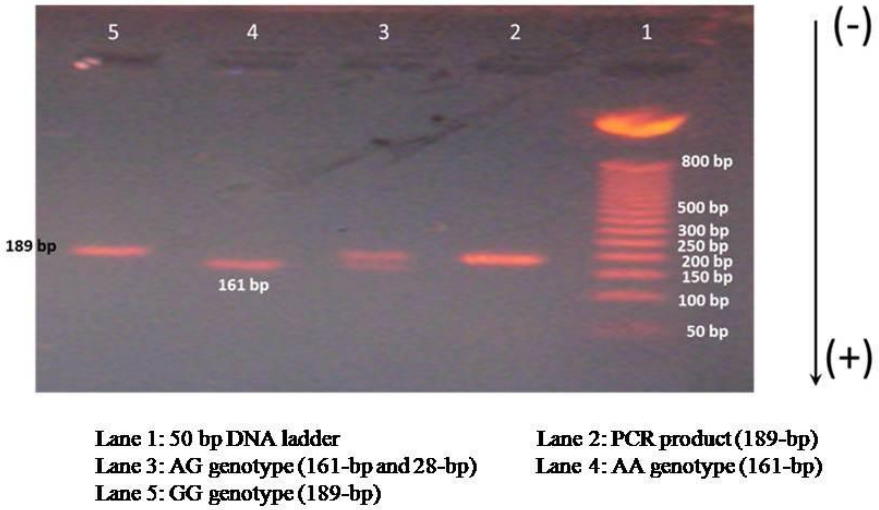

Fig. 1. Restriction fragment length polymorphism analysis of the A/G polymorphism of rs2414096 CYP19 genes. Agarose gel (2%) electrophoresis after HSP92 II digestion of the PCR.

Statistical analysis was performed by using statistical package of Science (SPPS); Version 17.0 and, Microsoft Excel Worksheet 2010. Numerical data analysis was done by using paired sample t-test for tables with mean and standards deviation of mean to compare PCOS and control subjects. Chi-square was used to examine the significance of gene and genotypes distribution in the two major groups and the subgroups. Analysis of variance (ANOVA) test was used to test the anthropometric parameters and its relation to genes. The differences between values were considered statistically significant at the level of ($p < 0.05$).

Results

The demographic data of the PCOS patients and control women are illustrated in table 1. Genotyping distributions of rs2414096 of *CYP19* gene (AA, AG and GG) in PCOS women as a whole or those who were obese were significantly different from that of the control group as a whole or those who were obese, respectively ($p < 0.05$).

On the reverse, the genotypic distribution of the non-obese PCOS patients was not significantly different from non-obese control women (Table 2). Furthermore, the genotypic distribution shows no significant difference between obese and non-obese PCOS patients and between obese and non-obese control women.

The frequency of rs2414096 *CYP19* genotype demonstrated that PCOS patients present low frequency of AA genotype ($p < 0.01$) but not with AG and GG genotypes as compared to the control women (Table 3).

Furthermore, lower A allele ($p < 0.05$), higher G allele ($p < 0.05$) but no difference in the frequency of A/G alleles was noticed in the obese PCOS patients versus obese control women. The alleles were not different in their frequencies in the non-obese PCOS patients

versus non-obese control women, obese PCOS versus non-obese PCOS patients and obese versus non-obese control women (Table 4).

Table 1. Comparison of demographic parameters between polycystic ovary syndrome patients and control women (using unpaired T-test)

Parameters	PCOS patients N = 84 (mean ± SD)	Control women N = 65 (mean ± SD)	p value
Age (yrs)	29.02 ± 4.50	30.31 ± 3.71	0.0584
BMI (kg/m^2)	29.9 ± 5.13	28.10 ± 4.51	0.0270
Waist/ Hip Ratio	0.82 ± 0.07	0.80 ± 0.06	0.0382
Waist/thigh ratio	1.42 ± 0.18	1.33 ± 0.11	0.0002
FSH (IU/ml)	5.35 ± 1.55	7.22 ± 2.32	0.0000
LH (IU/ml)	6.24 ± 3.00	3.97 ± 1.55	0.0000
LH/FSH	1.20 ± 0.52	0.56 ± 0.15	0.0000
E_2 (pg/ml)	60.27 ± 19.68	50.28 ± 18.90	0.0181
Testosterone (ng/ml)	0.79 ± 0.44	0.26 ± 0.15	0.0000
E_2/Testosterone	103.22 ± 58.34	230.62 ± 132.57	0.0000
FBS (mg/dl)	94.81 ± 15.30	87.07 ± 12.78	0.0132
BS (after 1/2 hr.) (mg/dl)	148.53 ± 36.84	133.08 ± 18.77	0.0092
BS (after 1 hr) (mg/dl)	142.79 ± 33.16	124.63 ± 17.39	0.0007
BS (after 2 hr) (mg/dl)	112.71 ± 24.32	99.12 ± 11.97	0.0005
Cholesterol (mg/dl)	163.70 ± 30.72	140.92 ± 17.87	0.0000
Triglyceride (mg/dl)	124.68 ± 33.51	111.25 ± 23.09	0.0109
VLDL (mg/dl)	24.85 ± 6.76	22.25 ± 4.62	0.0140
LDL (mg/dl)	98.90 ± 29.36	77.98 ± 17.36	0.0000
HDL (mg/dl)	39.11 ± 2.49	40.73 ± 2.30	0.0004

* = $P < 0.05$, ** = $P < 0.01$, PCOS = polycystic ovary syndrome, FSH = follicular stimulating hormone, LH = luteinizing hormone, E_2 = estradiol, FBS = fasting blood sugar, BS = blood sugar, VLDL = very low density lipoprotein, LDL = low density lipoprotein, HDL = high density lipoprotein

Table 2. Distribution of A/G Alleles of CYP19 gene in classified polycystic ovary syndrome patients and control women (using Chi square test)

Group	CYP 19 genotype			p value
	AA	AG	GG	
PCOS patients Control women	17 (20.24%) 26 (40%)	34 (40.48%) 23 (35.38%)	33 (39. 28%) 16 (24.62%)	< 0.022
Obese PCOS patients Obese Control women	5 (14.71%) 9 (40.91%)	12 (35.29%) 8 (36.36%)	17 (50%) 5 (22.73%)	< 0.045
Non-obese PCOS patients Non-obese Control women	12 (24%) 17 (39.54%)	22 (44%) 15 (34.88%)	16 (32%) 11 (25.58%)	0.273
obese PCOS patients Non-obese PCOS patients	5 (14.71%) 12 (24%)	12 (35.29%) 22 (44%)	17 (50%) 16 (32%)	0.233
Obese Control women Non-obese Control women	9 (40.91%) 17 (39.54%)	8 (36.36%) 15 (34.88%)	5 (22.73%) 11 (25.58%)	0.968

PCOS = polycystic ovary syndrome

Table 3. Allele frequencies of C/T polymorphism of rs2414096 CYP19 genes in polycystic ovary syndrome patients and control women (using Chi square test)

Genotype		PCOS N = 84	Control Group N = 65	p value
CYP19	AA genotype	17 (20.24%)	26 (40%)*	0.008
	AG genotype	34 (40.48%)	23 (35.38%)	0.526
	GG genotype	33 (39. 28%)	16 (24.62%)	0.059

* = p < 0.01, PCOS = polycystic ovary syndrome.

Table 4. Allele frequencies of A/G polymorphism of rs2414096 CYP19 genes in polycystic ovary syndrome patients versus control women (obese and non-obese) by BMI (using Chi square test)

Group	CYP19 gene					
	A allele	p value	AG allele	p value	G allele	p value
Obese PCOS patients Obese control women	5 (14.71%)* 9 (40.91%)	0.027	12 (35.29%) 8 (36.36%)	0.935	17 (50%)* 5 (22.73%)	0.041
Non-obese PCOS patients Non-obese control women	12 (24%) 17 (39.54%)	0.107	22 (44%) 15 (34.88%)	0.371	16 (32%) 11 (25.58%)	0.497
Obese PCOS patients Non-obese PCOS patients	5 (14.71%) 12 (24%)	0.298	12 (35.29%) 22 (44%)	0.425	17 (50%) 16 (32%)	0.097
Obese control women Non-obese control women	9 (40.91%) 17 (39.54)	0.915	8 (36.36%) 15 (34.88%)	0.906	5 (22.73%) 11 (25.58%)	0.800

* = p < 0.05, PCOS = polycystic ovary syndrome.

With respect to different CYP19 genotypic (AA, AG and GG) of PCOS patients, FSH (4.61 ± 1.01, 5.03 ± 1.15 and 6.07 ± 1.85; respectively) and LH (4.50 ± 2.21, 6.2 ± 2.75 and 7.22 ± 3.24 respectively) levels were significantly different (p = 0.002, p = 0.009; respectively) while no difference (p = 0.088) was observed in LH/FSH ratio (0.95 ± 0.42, 1.25 ± 0.51 and 1.29 ± 0.55; respectively). Likewise, in the control women, FSH (6.15 ± 1.66, 7.22 ± 2.12 and 8.71 ± 2.79; respectively) and LH (3.09 ± 0.95, 4.04 ± 1.41 and 5.07 ± 1.80; respectively) levels were significantly different (p = 0.024; p = 0.005; respectively) but not significant (p = 0.674) in the LH/FSH ratio (0.53 ± 0.16, 0.56 ± 0.17 and 0.59 ± 0.11; respectively).

With regard to the different genotype (AA, AG and GG), E_2 (72.78 ± 16.54, 61.66 ± 14.24 and 54.93 ± 22.61; respectively) and testosterone (0.54 ± 0.24, 0.63 ± 0.35 and 1.07 ± 0.45; respectively) levels and E_2/testosterone ratio (147.22 ± 44.90, 138.32 ± 49.27 and 61.17 ± 34.69; respectively) in PCOS patients were significantly different (p = 0.045; p = 0.000; p = 0.000; respectively). Similarly, in the control women, E_2 (60.62 ± 20.05, 48.87 ± 19.40 and 38.34 ± 10.42; respectively) and testosterone (0.18 ± 0.09, 0.21 ± 0.08 and 0.39 ± 0.16; respectively) levels and E_2/testosterone ratio (337.52 ± 148.13, 246.37 ± 81.07 and 116.88 ± 44.62; respectively) were significantly differ among the three genotypes (p = 0.011; p = 0.000; p = 0.000 respectively).

Discussion

Hyperandrogenism is a key diagnostic physiopathologic feature of PCOS [4]. Sisters of women with PCOS have increased androgen levels suggesting that hyperandrogenism may be partly genetically determined [11]. The *CYP19*

gene encodes aromatase enzyme (P450arom), is a key steroidogenic enzyme that catalyzes the final step of estrogen biosynthesis by converting testosterone and androstenedione to estradiol and estrone separately [12]. It is reported that several SNPs of the *CYP19* gene were associated with variation in serum androgen concentrations among women, both within and between racial/ethnic groups. Several studies have reported the association of the SNP rs2414096 in the *CYP19* gene with hyperandrogenism [9,10,13].

In the present study, there were significant differences in genotypic frequencies for the SNP rs2414096 in *CYP19* gene between PCOS patients and control women. The frequency of the AA genotype in the PCOS patients was significantly lower than that in the control group while the GG genotype was higher in PCOS patients (Table 3) which was comparable to that found by Jin *et al* [14].

According to BMI, GG genotype was presented more frequently in PCOS patients than control women (significantly in the obese and insignificantly in the non-obese). Likewise, the frequency of AA genotype was higher in the control women than PCOS patients also in both subgroups. On the other hand, the frequency of these genotypes did not differ significantly between both obese and non-obese PCOS and control women. The significant difference in genotype distribution probably indicates that the SNP of rs2414096 in *CYP19* gene is associated with the aromatase activity variation in PCOS women [14].

The estradiol/testosterone ratio provides important information about aromatase activity because conversion of androgens to estrogens is mediated by *CYP19*, which suggests that E_2/ testosterone ratio may be a direct marker of aromatase activity. The present study demonstrates that the rs2414096 AA genotype may be associated with activity of the aromatase and further affect the conversion of androgens to estrogens.

The E_2/ testosterone ratio of the AA genotype in PCOS was significantly higher than that of the other two genotypes and this agreed by Jin *et al* [14], who suggested that aromatase activity was augmented in the AA genotype. Reduced aromatase activity may lead to ovarian hyperandrogenism and the development of PCOS [9]. This is what was found in the present data where the testosterone level was higher in group of GG genotype both in PCOS patients and control women.

Also there was significant higher estradiol level in group of AA genotype which can be deduced from the facts that a higher frequency of PCOS is observed in people with augmented aromatase activity caused by mutant functional loss [7,15] and antral follicles taken from PCOS women exhibits no aromatase activity [16]. The augmented activity of the aromatase in the AA genotype may protect the ovary from the development of hyperandrogenism in PCOS patients.

FSH can induce aromatase activity, and this activity is positively correlated to the E_2 level. Thus, a reduced E_2 level can stimulate the production of FSH by negative feedback. This may account for the present observation that the concentration of FSH in the GG genotype, which demonstrated lower aromatase activity, was higher compared with the other two genotypes.

Furthermore, the data of this study shed the light on the effect of testosterone on the regulation of LH secretion, in which the LH level was higher in group of GG genotype, that have higher testosterone level, than other two genotypes. Testosterone augment pituitary sensitivity to gonadotrophin releasing hormone (GnRH) both by direct action on gonadotrophin synthesis and by enhancing GnRH-induced GnRH receptors [17], this is associated with an estradiol-related sensitization of pituitary LH release and hence an increase in LH secretion [18].

In conclusion, this study suggests that the SNP of rs2414096 in *CYP19* gene is positively

associated with PCOS hyperandrogenism in Iraqi PCOS women.

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Author contribution

Dr Muteb collects the data and analyzes it; Dr Hamdan and Al-Salihi interpret the data and revise the manuscript.

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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