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Common variation of the *CYP17* gene in Iraqi women with endometriosis disease

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ABSTRACT

Common variants among genes coding for enzymes in sex steroid biosynthetic pathways may influence the risk of endometriosis in Iraqi women patients in the last years. Cytochrome P450c17a1 (*CYP17*), a gene that codes for a key enzyme (cytochrome P450c17a1) in a rate-limiting step of estrogen biosynthesis has attracted considerable attention as an important gene for endometriosis. To evaluate the relationship between common genetic variations in *CYP17* and endometriosis risk and determine the main effects of those variations on the gene expression. A women-based case control study of Iraqi women aged range (23–46), the associations between selected single-nucleotide polymorphisms (SNPs) in the *CYP17* gene and endometriosis diagnosis in fifty women and thirty disease-free controls were evaluated. The study found a significant association ($P \leq 0.01$)between endometriosis and selected SNPs of *CYP17* gene, with the homozygous genotype conferring decreased risk. A highly significant difference ($P \leq 0.01$) in *CYP17* gene expression from women with versus without endometriosis and increased by 1.56-fold in women with endometriosis. These findings suggest that variation in or around *CYP17* may be associated with endometriosis development in the Iraqi women.

1. Introduction

A common and painful condition of the female reproductive system and influences female health negatively by causing abdominopelvic pain and infertility is endometriosis [1]. It affects about 10% of women of reproductive age. Nearly two-thirds of adolescents with dysmenorrhea or chronic pelvic pain have laparoscopic evidence of endometriosis. The most widely accepted theory is the retrograde reflux hypothesis, which suggests that endometrial tissues can regurgitate into pelvic cavity during menstruation and develop into endometriosis [2]. Endometriosis is a complex disease arising from the interplay between multiple genetic and environmental factors. The genetic variants potentially underlying the hereditary component of endometriosis were widely investigated through hypothesis-driven candidate gene studies, an approach that generally was proven to be inherently difficult and problematic for a number of reasons [3]. *CYP17A1* gene encodes a member of the cytochrome P450 superfamily of enzymes. It has eight coding exons, which is located on chromosome 10q24.3 and spanning 6569 base pair (bp) and encodes cytochrome p450c17α (CYP17A1), an enzyme which catalyzes the activity of both the 17α-hydroxylase and the 17,20-lyase. These two enzymes play critical roles in two sequential rate-limiting steps in the biosynthesis of testosterone [4]. Genetic variation in *CYP17A1* was associated with steroid hormone levels, menstrual factors and risk of endometrial, breast and prostate cancers [5]. The allele variant T/C at the −34 bp position relative to the start codon in the 5′-UTR promoter region of *CYP17A1* rs743572 was associated with an increased risk of endometriosis [6]. Genotyped G/T and C/T of exon 1 (rs6162) and (rs6163) were a highly correlated with (rs743572), the minor allele of (rs6163) was associated with a modest increase in levels of plasma androstenedione in premenopausal women [7–8]. Genetic studies of *CYP17A1* variants to date have followed a defined biological hypothesis suggesting the 5′UTR promoter region (rs743572) is associated with gene expression [9]. To study further a possible role of *CYP17A1* in endometriosis, we evaluated the risk of endometriosis in relation to common genetic variation of *CYP17A1* and determine the main effects of those variations on the gene expression in a population based case control study conducted in Iraqi women with endometriosis.

2. Materials and methods

2.1. Study population

The present investigation was carried out at Kamal AL-Samarai Hospital between (January–July 2015), in Baghdad, Iraq. The data were collected from fifty women patients as referred by a Gynecologist for endometriosis investigation. A total of thirty healthy female volunteers

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served as controls, aged (23–46) years. Blood samples (3 mL) were placed into containing EDTA tubes, and stored at −20 °C until assayed for genotyping analysis (DNA & RNA extraction) and using conventional-PCR and qRT-PCR technique.

2.2. Genetic analysis (gene polymorphisms)

The Genomic DNA Mini Kit supplied by Geneaid-Taiwan provides an efficient method for purifying total DNA from whole fresh blood. Also Magnesia 16 Genomic DNA whole blood Kit was designed for automated extraction by using automated instrument of Magnesia 16 of Anatolia (Turkey). DNA concentration was measured by nano-spectrophotomoter (Quawell-USA). DNA amplification was carried with a KAPA2G Robust HotStart PCR Kit which contains an engineered DNA polymerase and uniquely-formulated buffers, and requires specialized reaction conditions. Each reaction contained 12.5 μL (1×) master mix, 1.5 μL (10 μM) each primer (Table 1), primers were designed for *CYP17* gene from GenBank (NCBI reference sequence NM-000102.3), Template DNA 3 μL (50 ng/μL), 6.5 μL deionized distilled-water (dd-water). An initial denaturation step was run at 94 °C for 3 min, followed by 30 cycles of amplification with denaturation at 94 °C for 20 s, annealing at 59 °C for 30 s, extension at 72 °C for 30 s and final extension at 72 °C for 10 min. Amplified PCR products were detected by agarose gel electrophoresis. A DNA marker 100 bp (Promega/USA) was run with each gel, and the genotype was determined by direct sequencing as well as for detecting SNPs within these sequences. Our sequences were compared with reference sequence of *CYP17* in NCBI GenBank.

2.3. DNA sequence analysis

The DNA fragments for sequencing were obtained by PCR amplification, the fragments of each PCR products were sequenced with the set of primers by Macrogen Company, Korea. The program (BioEdit) was used for bioinformatic analysis of nucleotide sequences.

2.4. Gene expression (RNA extraction and one-step real time–PCR protocol)

Total RNA purification from white blood cells of 0.4 mL human whole blood was used with automated instrument Magnesia 16 of Anatolia (Turkey).RNA concentration was measured by nano-spectrophotomoter (Quawell-USA). Quantitative PCR was performed using an Agilent Technologies PCR 8800 supercycle-USA. The KAPA SYBR*FAST One-Step qRT-PCR kit was used for real-time PCR using RNA as template. A suitable amount of RNA tamplet 6 μL (100 ng/μL) mixed with 0.5 μL (0.2 μM) of each primer, (10 μL) of KAPA SYBR* FASTqPCR Master mix, dNTP 0.5 μL (200 nM), KAPA RT Mix 1 μL and 1.5 μL of double distilled-water. The reaction after brief spin was submitted to the following PCR condition: cDNA synthesis at 42 °C for 5 min, inactivation reverse transcriptase at 95 °C for 3 min, denaturation at 95 °C for 3 s, annealing 59 °C for 20 s, and polymerization at 72 °C for 20 s for 40 cycles. At the end of PCR run, a melt curve analysis was performed to ensure that only a single product was amplified. The experiment was repeated in triplicate.

Table 1
Primers used for gene analysis.

Gene	Primer sequences(5′-3′)	Size (base pair)
CYP17	F-GGAGAATCTTTCCACAAGGCAAG R-TGGTGGCCGACAATCACTGTAG	366
GAPDH	F-ATCACTGCCACCCAGAAGACTG R-AGGTTTTTCTAGACGGCAGGTCAG	216

F: Forward primer, R: Reverse primer.

2.5. Statistical analysis

All data were expressed as mean ± standard deviation (mean ± SD). Statistical analysis was performed using LSD, considering $P < 0.05$ as the lowest limit of significance. Statistical analysis was performed using a software program (SAS. 2012. Statistical Analysis System, User's Guide. Statistical Version 9. 1st ed. SAS.Inst.Inc. Cary, NC, USA).

3. Results

Three SNPs in the *CYP17* gene (T/C rs 743572) in the 5′UTR, G/T (rs 6162) and C/T (rs 6163 in exon 1) were typed in this study based on their role in hormones action, biosynthesis and metabolism. This is the first study to our knowledge to give frequency distribution genotypes of *CYP17* gene in Iraqi population. The 5′-UTR of *CYP17* contains a single-bp polymorphism T/Cat 34 bp upstream from the initiation of translation. After analysis of T/C genotypes for fifty individuals in this study, it reached to (31) 62.00% of all individuals had TT, (18) 36.00% had CC and only one of them 2.00% had TC alleles as shown in Table 2. The second SNP in exon 1 of *CYP17* gene rs 6162 contains a single-bp polymorphism G to T upstream from the initiation of translation, Table 2 showed analysis of G/T genotypes for fifty individuals in this study, it reached to (34) 68.00% of all individuals had GG, (15) 30.00% had TT and only one of them (2.00%) had GT alleles. The third SNP in exon 1 of *CYP17* gene rs 6163 contains a single-bp polymorphism C to T upstream from the initiation of translation. Analysis of C/T genotypes for fifty individuals in this study has shown that (29) 58.00% of all individuals had CC, (20) 40.00% had TT and only one of them (2.00%) had CT alleles as showed in Table 2. The frequency distribution of *CYP17*T/C, G/T and C/T genotypes according to demographic characteristics is shown in Tables 3 to 5. Fifty-five samples of amplified products (forward and reverse strand) for *CYP17* gene from healthy and endometriotic subjects were further analyzed by direct sequencing for detecting SNPs within these sequences. After alignment of product amplification of *CYP17* gene for five samples having homozygous genotypes T/T, G/G and C/C from healthy subjects with the *CYP17* of *Homo sapiens* from the GenBank using the BioEdit software, we found that part of *CYP17* gene from healthy subjects having 100% compatibility with standard *CYP17* in GenBank. The sequence was submitted to gene bank National Center Biotechnology Information (NCBI), DNA data bank of Japan (DDBJ) and European Bioinformatics institutes (EMBI) under the accession number (LC145028). Sequence alignment for eighteen, fifteen and twenty endometriotic patients have homozygous mutant genotype C/C, T/T and T/T respectively, this sequence were submitted under the accession numbers LC144988.1, LC145026.1 and LC145024.1 respectively. Alignment of the sequences of nucleotides for only sample has heterozygous genotype T/C, G/T and C/T from endometriotic sample with standard *CYP17* gene that was submitted to gene bank under the accession numbers LC145027.1, LC145025.1 and LC145028.1 respectively. The data obtained from real time experiments were detected according to the Ct values which calculated from cycles and was proportional to the starting target copy number (logarithmic scale) used for

Table 2
Distribution of samples study of *CYP17* according to T/C, G/T and C/T genotypes.

Polymorphism	CYP17 according to T/C		CYP17 according to G/T		CYP17 according to C/T	
	No.	(%)	No.	(%)	No.	(%)
CC	31	62.00	34	68.00	29	58.00
TT	18	36.00	15	30.00	20	40.00
CT	1	2.00	1	2.00	1	2.00
Total	50	100	50	100	50	100
Chi-square (χ^2)	11.438*		12.692*		10.957*	
P-value	0.00219		0.0001		0.00388	

* Significant at $P \leq 0.01$.