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Relationship of follicle stimulating hormone receptor Gene Thr307Ala polymorphism with polycystic ovary syndrome in Iraqi women

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Abstract

Polycystic ovary syndrome (PCOS) is a polygenic multifactorial status affecting millions of females worldwide. It is a common cause of anovulatory subfertility. Because of follicle stimulating hormone (FSH), is an important agent in human reproduction. Therefore, the correlation between follicle stimulating hormone receptor (FSHR) gene polymorphisms and polycystic ovary syndrome attracts broad attention.

The objective of this study is to investigate the potential association between the follicle stimulating hormone receptor gene Thr307Ala polymorphism with polycystic ovary syndrome in the Iraqi women. A case-control study including 135 Iraqi women of Arab ethnicity (75 PCOS patients and 60 age-matched control women). The age of subjects ranged from 18 to 38 years. PCOS diagnosis was established by Rotterdam consensus criteria. The FSHR (Thr307Ala) variant was tested by conventional polymerase chain reaction (PCR)–restriction fragment length polymorphism (RFLP) followed by deoxyribonucleic acid (DNA) sequencing.

The heterozygote Thr/Ala (AG) genotype of Thr307Ala (rs6165) polymorphism of follicle stimulating hormone receptor gene was giving a significant risk (odds ratio=19.4, 95%CI=1.14-30.40, P value=0.002) of developing PCOS in Iraqi women compared with control group. Sequencing analysis of DNA confirms RFLP analysis.

In conclusion; the variant Thr307Ala (rs6165) of the follicle stimulating hormone receptor gene is associated with polycystic ovary syndrome and may consider as the causal factor of polycystic ovary syndrome in Iraqi women.

Keywords: PCOS; FSHR geneThr307Ala polymorphism; Women

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Introduction

Polycystic ovary syndrome (PCOS) is a polygenic multifactorial condition affecting millions of women worldwide. It is a common cause of WHO group 2 anovulatory disorders that accounts for 80% to 90% of anovulatory subfertility [1]. PCOS prevalence is specified, by the diagnostic criteria, ethnicity, and population of women studied [1, 2]. Diagnosis of PCOS is based on Rotterdam consensus criteria [3, 4]. The pathogenesis of PCOS is multifactorial and far from being fully understood. It has been deduced that PCOS can be a familial in origin resulted by a mixture of genetic with environmental agents [5-7]. In addition, a previous study showed a direct association among the genotypes and PCOS phenotype and suggested that each characteristic of PCOS has a particular genetic correlation joined to the etiological pathway [8]. A major collection of studies has been dedicated to fixing the genetic causes of PCOS looking, especially, the release and action of gonadotrophin, secretion and action of insulin and other genes that had interest with regard to PCOS [9-12].

Since follicle-stimulating hormone receptor (FSHR) has been identified as an important vulnerability locus in many genome-wide association studies, there have been multiple researches on FSHR polymorphisms [12-14]. Follicle-stimulating hormone (FSH) is an important agent in human reproduction, boost proliferation and discrimination of the granulosa cell in addition to the estrogen production [15]. It acts by joining to the FSHR that found on the ovarian granulosa cell surface. The FSHR is encoded by the FSHR gene that present on the short arm (p) of chromosome two (2p21-16) and involves ten exons [16, 17].

Various single nucleotide polymorphisms of the FSHR gene have been found in different populations, of these, two polymorphisms present in exon ten at nucleotide position 919 (Thr307Ala) and position 2039 (Asn680Ser) are widely studied to determine the FSHR response to FSH stimulation and to portend the risk of PCOS development [18]. The Thr307Ala polymorphism is situated in the extracellular domain of FSHR at codon position 307, which can be taken either by alanine (Ala=GCT)) or by threonine (Thr=ACT) [16]. It can influence on the binding of hormone to their receptor and is critical for FSH- interceded signal transduction proceedings [19].

Because of the prevalence of PCOS and clinical features and the frequency of FSHR polymorphisms vary among different races of population [2, 20]. Moreover, activation of the receptor that coded by the FSHR gene is important for the functioning of FSH [21]. So, the aim of this study was to investigate the potential association between the FSHR Thr307Ala (rs6165) polymorphism with PCOS in the Iraqi women.

Patients and Method

A case-control study was happened in the High Institute of Infertility Diagnosis and ART/ Al-Nahrain University (Baghdad/Iraq) from February 2017 to February 2018. This work was approved by the Local Medical Ethical Committee of the High Institute of Infertility Diagnosis and ART/Al-Nahrain University. Each woman that participates in the present study signed a consent form. A total of 135 Iraqi women were studied. All women were of Arab ethnicity and were matched according to age. The age of subjects ranged from 18 to 38 years. Two groups were enrolled in this study. The first was seventy-five PCOS patients group were chosen from those women who attending the Higher Institute of Infertility Diagnosis and ART. The PCOS women were chosen based on the Rotterdam criteria [3]. The enrolled women were only those who had polycystic ovaries on transvaginal sonography to ensure that the phenotype was definitely PCOS.

The second group was the control group, which included 60 healthy women recruited randomly from the Higher Institute of Infertility Diagnosis and ART and Al-Imamian Al-Kadhimiyain Medical City (Baghdad/Iraq), with regular menstrual cycle, no clinical appearance of hirsutism, acne and alopecia, normal basic endocrine detection results, and transvaginal sonography revealed no organic lesions at ovary (and the ovary showed no polycystic manifestations) and no family history of PCOS.

Genotype Analysis

For deoxyribonucleic acid (DNA) isolation from all the subjects, two and half milliliters of venous blood were collected in K2-EDTA collecting tube containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant. According to the protocol of G-spin™ Kit (Intron - Korea), genomic DNA was isolated from venous whole blood. After genomic DNA extraction, agarose gel electrophoresis was selected to confirm the presence and integrity of the extracted DNA [22] (Figure 1.A). In addition, all the extracted DNA samples were tested to estimate the genomic DNA concentration and purity by BioSpec-nano spectrophotometer (Shimadzu /Japan). The Thr307Ala polymorphism of FSHR was detected by restriction fragment length polymorphism (RFLP) technique [23, 24]. A 577 base pair (bp) fragment of FSHR was amplified by conventional polymerase chain reaction (PCR) using the following primers (Integrated DNA technologies /USA):

Forward primer: F: 5'-CCT GCA CAA AGA CAG TGA TG - 3'

Reversed primer: R: 5'- TGG CAA AGA CAG TGA AAA G- 3'

Conventional PCR amplification was carried out by using a MultiGene™ OptiMax Thermal Cycler (USA- Labnet), in overall volume of 25 µl mixture containing: 1.5 µl of genomic DNA; 1 µl of each primer,5 µl Maxime PCR Premix(i-Taq) manufactured by (Intron / Korea) and 16.5 µl double distilled water. The Thermal Cycler was adjusted for PCR status as shown in Table 1. All

PCR products (Figure 1.B) were subjected to restriction enzyme digestion. The 577 bp amplified PCR product was digested with Ahd I (Biolab/New England) as shown in Table 2.

Table 1.

The optimum PCR conditions of detection Thr307Ala polymorphism

No.	Phase	Tm (°C)	Time	No. of cycle
1	Initial Denaturation	95°C	3 min.	40 cycle
2	Denaturation -2	95°C	45sec	
3	Annealing	60 °C	45sec	
4	Extension-1	72°C	45sec	
5	Extension -2	72°C	7min.	

Table 2.

Reaction condition of restriction enzyme Ahd I (Biolab/New England)

Protocol	Volume
Product PCR	5 µl
Restriction Enzyme	0.5 µl
Buffer	4.5 µl
Final volume	10µl
Temperature/Time	37°C /30 min

The evaluation of PCR and enzyme digested products were done by gel electrophoresis (2%) that visualized by UV trans-illuminator (Vilberlourmat-France). Three bands: 403/143/ 31 bp in case of AA (Thr/Thr) genotype; four bands: 403/174/143/31 bp in AG (Thr/Ala) genotype; and two bands 403/174 bp in GG (Ala/Ala) genotype were observed (Figure 1.C). To verify genotyping results in RFLP, 20 samples (randomly selected from all samples processed) of PCR products and forward primers were sent to South Korea (Macrogen Company) for DNA sequencing.

Figure 1.

(A) Gel electrophoresis of genomic DNA extraction from blood .1% agarose gel at 1 hour, visualized under UV light after staining with red safe staining. (B) Agarose gel electrophoresis of PCR products for FSHR Gene (Thr307Ala). The amplified fragment of molecular size of 577 bp. (C) The PCR products after digestion with AhdI: (AA genotype= 403 bp, 143 bp, and 31 bp); (GG genotype = 403 bp and 174 bp); (AG genotype = 403 bp, 174 bp, 143 bp, and 31 bp); (Note:

31 bp bands were run off the gel). The PCR and enzyme digested products was electrophoresis on 2% agarose for 1:30 hours. M=DNA ladder 100bp.

Data and Statistical Analysis

Statistical analysis was performed utilizing SPSS Statistics Version 23.0. The continuous variable with normal distribution (age) are presented as mean and standard error (SE). While categorical variables (alleles and genotypes) were expressed as percentages of total. The student t test was utilized to test the differences of continuous variable with normal distribution among the two groups. Chi square (χ^2) test was utilized to show the significance of difference among the frequencies of study groups. Odds ratio (OR), and 95% confidence interval (CI) were achieved to estimate the association among the groups. Significance of tests was specified at P value $\leqslant$ 0.05.

Results

Table 3, shows that there was no significant (P value=0.335) difference in the mean age between PCOS group and controls.

Table 3.

Comparison of age among control and PCOS groups

Parameter	Controls n=60 Mean±SE	PCOS n=75 Mean±SE	P value*
Age (yr)	26.7+0.51	26.0+0.51	0.335 NS

*Student's t-test; Data are shown as mean ± SE (standard error); PCOS=Polycystic Ovary Syndrome; NS: Non- Significant; *$\leqslant$ 0.05.: Significant

The allele and genotype frequencies of FSHR Thr307Ala polymorphism in PCOS patients and control women were assessed (Table 4). In the present study, no significant (P value=0.873) difference was observed in the allele frequencies (Thr=A and Ala=G) between PCOS patients and control groups (82.7 % in PCOS vs. 81.7% in control, OR=1.07, 95% CI =0.57-2.00). According to the genotypes distributions of Thr307Ala polymorphism in PCOS patients and control groups, in the present study, the heterozygote AG genotype showed a significant (P value=0.002) higher frequency in PCOS patients than in control (13.3% and 0.0%, respectively), the OR for such a positive relation was 19.4 with etiological

fraction (EF) =0.13. No significant differences were observed in the distribution of the homozygote AA genotype between the PCOS and control groups (76.0% vs. 81.7%, OR=0.71, 95% CI = 0.30-1.64, P value = 0.528). In addition, no significant differences were observed in the distribution of the homozygote genotype GG between the PCOS and control groups (10.7% vs. 18.3%, OR=0.53, 95%CI=0.20- 1.41, P value=0.222). Sequencing analysis of DNA confirms RFLP analysis as shown in Figure 2. The exon ten sequence of the FSHR gene was submitted to the Gene Bank National Center Biotechnology Information (NCBI) under the accession number (MH910501.1).

Table 4.
Distribution of Thr307Ala Polymorphism in PCOS and controls

Thr307Ala Polymorphism	PCOS (n=75)	Control (n=60)	EF and PF	OR (95% C.I.)	Fisher's P
Allele					
Thr(A)	124 (82.7%)	98 (81.7%)	0.05	1.07 0.57 to 2.00	0.873 NS
Ala(G)	26 (17.3%)	22 (18.3%)	0.012		
Genotypes					
AA(Thr/Thr) versus AG+GG	57 (76.0%)	49 (81.7%)	0.23	0.71 (0.30 - 1.64)	0.528 NS
AG(Thr/Ala) versus GG+AA	10 (13.3%)	0 (0.0%)	0.13	19.4 (1.14 - 30.40)	0.002*
GG(Ala/Ala) versus AG+AA	8 (10.7%)	11 (18.3%)	0.08	0.53 (0.20 - 1.41)	0.222 NS

* Fisher exact test, OR: Odds Ratio, CI: Confidence Interval, EF: Etiological Fraction when odds ratio > 1, PF: Preventive Fraction when odds ratio<1, NS: Non-Significant, * p⩽ 0.05: Significant.

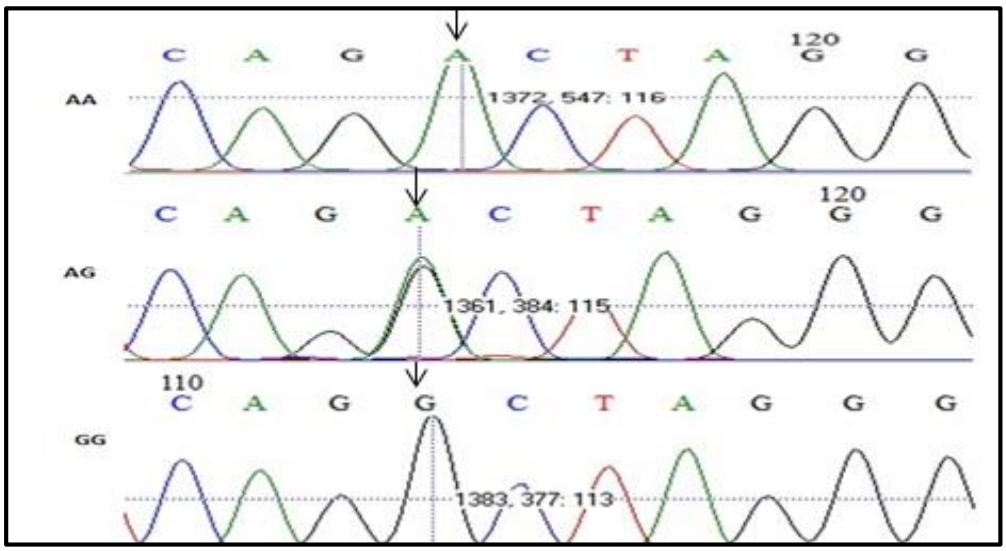

Figure 2.
Sequencing analysis for exon ten of FSHR gene (AA genotype=one peak), (AG genotype=two peaks) and (GG genotype=one peak) as indicated by arrows.

Discussion

Since the cause of the anovulation in PCOS can be due to defect in the development of ovarian follicle with too much early follicular growth and abnormal later stages of arrested growth of follicle before expected maturation [25].

Therefore, the correlation among FSHR gene polymorphisms and this syndrome has been widely investigated across different races with inconsistent results. In the current study, the genotype analysis of FSHR Thr307Ala polymorphism showed that the heterozygote AG genotype gave a significant risk of PCOS development among women in Iraq (Table 4). This is in agreement with previous studies conducted between different races and also confirmed a significant increase in frequency in the Thr307Ala variant.

Abdel-Aziz *et al*. have stated that the heterozygote AG genotype gave a significant risk of PCOS development among Egyptian women [26]. In addition, Khafagi *et al.* and Dolfin *et al*. they concluded that the heterozygote AG genotype gave a significant risk of PCOS development among Iranian women and among Italian women respectively [27,28]. Finally, Sudo *et al*. and Kim *et al.* they suggested a significant association among FSHR gene Thr307Ala polymorphism and PCOS among Japanese women and among Korean women [29,30].

On the contrary, the presence of significant risk of PCOS development was not proven between Turkish adolescent girls [31], Northern Chinese Han women [24, 32], among Thai women [33]

and among Indian women [34]. Finally, in Erbil province, north of Iraq, the variant of Thr307Ala was not associated with PCOS in Kurdistan women [35].

The differences of results that observed in multiple studies may be due to a different PCOS definition and sample size. Moreover, PCOS is a complex and diverse disorder which results from interaction of genetic and environmental agents [36-38]. Therefore, the prevalence and the differences in phenotypes in PCOS women in various populations may be affected by regional and ethnic setting [36, 39, 40]. Finally, PCOS is a multifaceted disorder whose consequences extend beyond the reproductive axis and which has an important effect on PCOS women's health [41]. Thus, distinguishing and treatment of girls that at risk of PCOS development may be an efficient way of prohibit some of the long-term comorbidities that associated with PCOS.

Conclusions

The variant Thr307Ala (rs6165) of the FSHR gene is associated with PCOS and may consider as the causal factor of PCOS in Iraqi women.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

ZK is participated in the conception and design of the study. ZK, EA, NK and BA collected and analyzed the data, EA, NK is interpreted the data, drafted the initial manuscript. All authors read and reviewed the final manuscript.

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