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Original Article

Molecular analysis of FSH receptor gene in Iraqi women with PCOS syndrome ☆

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ABSTRACT

Polycystic Ovarian Syndrome (PCOS) is a common endocrine disorder in women during their reproductive age. This study was design to establish the relationship between this syndrome and follicle stimulating hormone receptor defect by determination of lethal single nucleotide polymorphism that may play a vital role in this syndrome. A total number of 500 women attending Kamal Al- Samarrai Hospital diagnosed with PCOS were selected and divided according to their age into group one which includes (20–30) years old women, group two included (31–40) years old women, and group three which included (41–50) years old women. Fertility hormones (FSH, LH, and testosterone) were tested for all groups. Results showed that LH increased significantly in groups three with low FSH, whereas testosterone increased significantly in age group two. Molecular analysis of whole FSHR gene amplified using specific primers showed the presence two SNPs rs6166, and rs6165 which are associated with drug response and 9 lethal missense mutation that caused sever effect on FSHR, that probably render this receptor more sensitive to FSH without the possibility of feed back inhibition.

1. Introduction

Family studies proposed that polycystic ovary syndrome (PCOS) has a genetic basis because of the high number of female relatives of PCOS patients are affected [7]. PCOS affects 4–12% of women at reproductive age [5]. Despite its frequency, the PCOS is still a difficult to diagnose in endocrinology, gynaecology and reproductive medicine [12]. The etiology of this syndrome is still to be speculated while its path physiology appears to be both multifactorial and polygenic [4]. Identification of specific causes, and exclusion of the multiple phenol types that make up PCOS, will assist its diagnosis [14]. A high occurance of similar phenotypes in family members of PCOS patients suggests that genetics may play a role [3,15]. Though the polymorphisms in genes encoding sex hormones and their receptors have been investigated, results are still under debate [16]. Follicle stimulating hormone (FSH) plays an important role in follicular growth and ovarian teroidogenesis. Mutations or lethal polymorphisms in the FSH receptor (FSHR) gene can affect reproductive ability [11]. The FSHR gene contains two important single nucleotide polymorphisms (SNPs) [10]. Few genetic studies have examined the association between FSHR polymorphisms and PCOS [17]. Since PCOS prevalence and clinical manifestations, as well as frequency of the FSHR polymorphisms, can differ between ethnic and racial groups [18].

2. Materials and methods

Sample collection: The study included five hundreds blood samples from women suffering Poly Cystic Ovary Syndrome (PCOS) during the period from November 2016 to April 2017, collected from Kamal Al- Samarrai Hospital (Baghdad/Iraq) and 100 blood samples from healthy women served as control. The ages of patients and control group were 20–50 years.

2.1. Measurment of fertility hormones

Anovulatory subjects went through routine examinations, which included a detailed history, physical and pelvic examination, ultrasound, and other laboratory studies as deemed necessary by the attending gynecologists. In addition, blood samples were obtained for hormonal studies and DNA analysis. Plasma concentrations of follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone (T), were measured with Enzyme-linked Immunosorbent Assay (ELISA, VIDAS Biomerieux, France)

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Subjects in the control group had routine physical and obstetrical examinations, and blood tests as usual for those attending the antenatal clinic for the first time. In addition, three milliliters of blood were obtained in tubes containing ethylenediaminetetraacetic acid for DNA analysis.

2.2. DNA extraction

Total cellular DNA was extracted from blood samples by using the Reliaprep Blood genomic DNA MiniPrep System from Favorgene Taiwan, determination of concatenation and purity of the extracted DNA was done using nanodrop (Techne /UK).

2.3. PCR protocols

Extracted DNA from blood samples and healthy was subjected to PCR amplification using specific primers shown in Table 1 to amplify FSHR gene using the following program Initial denaturation at 94 °C for 5 min., 35 cycle of denaturation at 94 °C for 1 min, Annealing at 55 °C, 59 °C, and 55 °C respectively for each primers for 1 min, extension at 72 °C for 1 min., and final extension at 72 °C for 10 min.

Table 1
Primers [20].

Primer name	Sequence 5′–3′
(RS1)	F: TTTGTGGTCATCTGTGGCTGC
	R: AGGCAAGGACTGAATTATCATT
(RS2)	F: CAAATCTATTTTAAGGCAAGAAGTTGATTATATGCC TCAG
	R: GTAGATTCCAATGCAGAGATCA
(RS3)	F: CATGGTGAAGGAAGTTGTC
	R: AAAGCCAGGGATCTTCTC

2.4. DNA sequencing

The purified PCR products of the amplified FSHR gene region were sequenced by Macrogen Company in Korea. The obtained sequences of these samples were analyzed at National Center for Biotechnology Information (NCBI) web site using the BLAST search tool and examined for the presence of SNPs using available analysis tools.

2.5. Statistical analysis

The Statistical Analysis System- SAS (2012) program was used to determine the association of Poly Cystic Ovary Syndrome (PCOS) to age group and gender using chi square test.

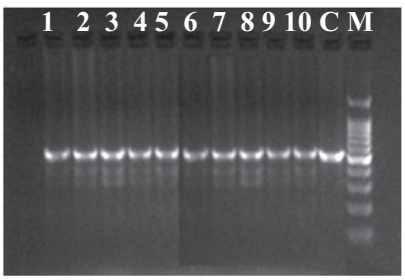
3. Result

In this study, five hundreds samples have been collected from female patients who had Polycystic ovary syndrome. Their ages were 20–50 years. T- test and P- value were used to signify the correlation between fertility hormones and age as shown in Table 2.

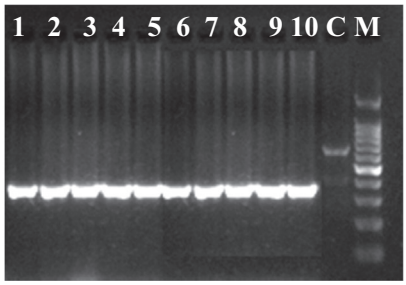
Table 2
Effect of age group in level of LH, FSH and Testosterone.

Age group (year)	Mean ± SE		
	LH (IU/ml)	FSH (IU/ml)	Testosterone (ng/ml)
20–30	5.23 ± 0.83b	4.97 ± 0.64b	0.585 ± 0.09b
31–40	5.51 ± 0.79b	3.13 ± 0.32b	0.696 ± 0.09 a
41–50	7.48 ± 1.40 a	2.30 ± 0.46 a	0.518 ± 0.08b
Control (Healthy)	2.78 ± 0.23	5.42 ± 0.26	0.205 ± 0.03
LSD (T-test)	0.974*	0.627*	0.825*
P-value	0.0392	0.0484	0.0475

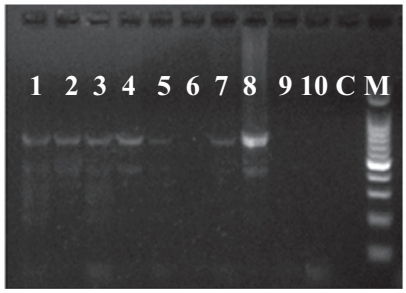
* ($P < 0.01$).



(A)



(B)



(c)

Fig. 1. PCR product of (A) RS1 primer, (B) RS2 Primer, and (C) RS3 Primer. Agarose gel electrophoresis conditions were (2% agarose gel, 10 min at 100 voltage and then lowered to 70 Volts, 80 min). Visualized under U.V light after staining with ethidium bromide, Line M: 100 bp marker. For (A), lane 1–10: DNA isolated from blood samples of patients, and lane C: from control (healthy). For (B), lane 1–10: DNA isolated from blood samples of patients, and lane C: from control (healthy). For (C), lane 1–10: DNA isolated from blood sample of patients, and lane C: from control (healthy).

Table 3
Point mutations detect in patient samples.

Chr.position	mRNA pos	dbSNP rs# cluster id	Clinical significance	Function	dbSNP allele	Protein residue	Codon pos	Amino acid pos
48,962,782	2149	rs6166	drug-response	missense	A	Asn [N]	2	680
				contig reference	G	Ser [S]	2	680
48,963,020	1911	rs386833513	Likely pathogenic	missense	G	Val [V]	1	601
				contig reference	C	Leu [L]	1	601
48,963,061	1870	rs386833512	other	missense	A	His [H]	2	587
				contig reference	C	Pro [P]	2	587
48,963,097	1834	rs386833511	Likely pathogenic	missense	T	Val [V]	2	575
				contig reference	C	Ala [A]	2	575
48,963,104	1827	rs121909660	Pathogenic	missense	T	Cys [C]	1	573
				contig reference	C	Arg [R]	1	573
48,963,122	1809	rs28928871	Pathogenic	missense	A	Asn [N]	1	567
				contig reference	G	Asp [D]	1	567
48,963,187	1744	rs121909664	Pathogenic	missense	C	Thr [T]	2	545
				contig reference	T	Ile [I]	2	545
48,963,225	1706	rs757909841	Uncertain significance	missense	A	Ile [I]	3	532
				contig reference	G	Met [M]	3	532
48,963,245	1686	rs138281715	Uncertain significance	missense	G	Val [V]	1	526
				synonymous	C	Leu [L]	1	526
				contig reference	T	Leu [L]	1	526
48,963,266	1665	rs121909662	Pathogenic	missense	A	Thr [T]	1	519
				contig reference	C	Pro [P]	1	519
48,963,475	1456	rs28928870	Pathogenic	missense	A	Asn [N]	2	449
				missense	T	Ile [I]	2	449
48,963,476	1455	rs121909663	Pathogenic	missense	G	Ala [A]	1	449
				contig reference	A	Thr [T]	1	449
48,963,491	1440	rs202162496	Uncertain significance	missense	A	Thr [T]	1	444
				contig reference	G	Ala [A]	1	444
48,963,566	1365	rs121909661	Pathogenic	missense	A	Thr [T]	1	419
				contig reference	G	Ala [A]	1	419
48,963,778	1153	rs386833510	Likely pathogenic	missense	G	Arg [R]	2	348
				contig reference	C	Pro [P]	2	348
48,963,791	1140	rs772756688	Uncertain significance	missense	A	Met [M]	1	344
				contig reference	G	Val [V]	1	344
48,963,874	1057	rs886056150	Uncertain significance	missense	G	Gly [G]	2	316
				contig reference	A	Glu [E]	2	316
48,963,902	1029	rs6165	drug-response	missense	A	Thr [T]	1	307
				contig reference	G	Ala [A]	1	307
48,968,766	896	rs150863050	Likely benign	synonymous	T	Val [V]	3	262
				contig reference	C	Val [V]	3	262
48,968,881	781	rs386833515	Likely pathogenic	missense	T	Val [V]	2	224
				contig reference	A	Asp [D]	2	224
48,982,918	772	rs386833514	Likely pathogenic	missense	G	Gly [G]	2	221
				contig reference	T	Val [V]	2	221
48,982,977	713	rs75552966	Likely benign	synonymous	T	Ser [S]	3	201
				contig reference	C	Ser [S]	3	201
48,983,125	676	rs121909658	Pathogenic	missense	T	Val [V]	2	189
				contig reference	C	Ala [A]	2	189
48,989,016	595	rs111883853	Likely benign	missense	A	Lys [K]	2	162
				contig reference	G	Arg [R]	2	162
48,989,022	589	rs121909659	Pathogenic	missense	C	Thr [T]	2	160
				contig reference	T	Ile [I]	2	160
48,990,629	493	rs121909665	Pathogenic	missense	A	Tyr [Y]	2	128
				contig reference	C	Ser [S]	2	128
49,017,490	483	rs886056151	Uncertain significance	missense	A	Met [M]	1	125
				contig reference	C	Leu [L]	1	125
49,017,551	422	rs147355964	Uncertain significance	synonymous	A	Lys [K]	3	104
				contig reference	G	Lys [K]	3	104
49,068,224	329	rs377397067	Uncertain significance	synonymous	A	Glu [E]	3	73
				contig reference	G	Glu [E]	3	73
49,154,394	134	rs115030945	Likely benign	missense	T	Phe [F]	3	8
				contig reference	G	Leu [L]	3	8

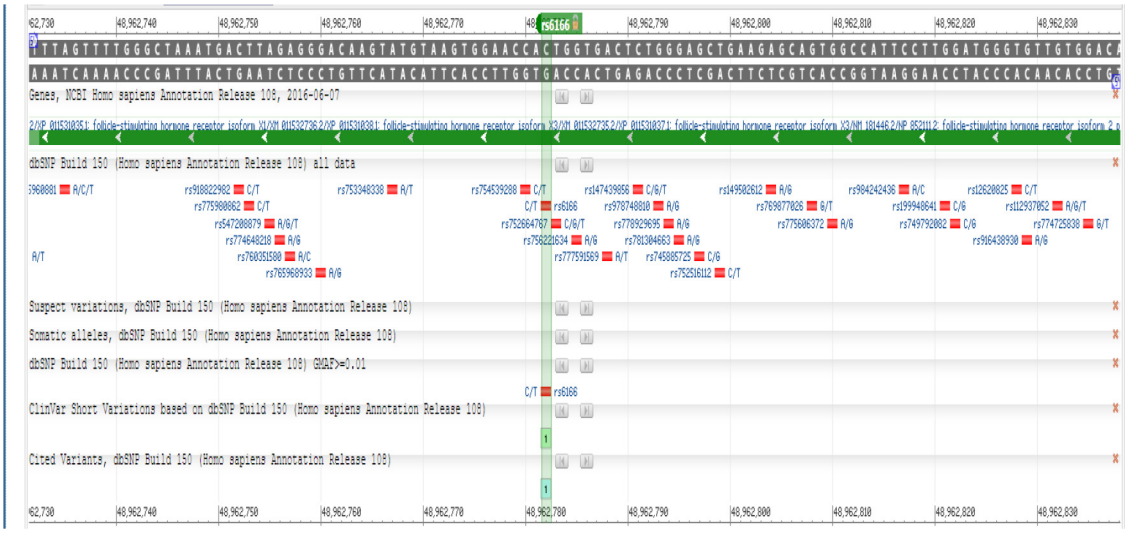


Fig. 2. Graphical distribution of SNPs detected on FSHR red dots represent the change in nucleotide sequence identified by nucleotide shift and accession number.

Test of fertility hormones, (LH, FSH and Testosterone) was done for all patient to establish the correlation between PCOS and their level. A significant elevation in LH hormone was recorded in (41–50) years old women, with a mean ± standard deviation of 7. 48 ± 1.40 a. In contrast, a significant decrease in FSH it was noticed the age groups (41–50) as the difference of level of this hormone was more than the LSD value which was 0.627. Testosterone increased significantly in the age groups (31–40) as the difference of this hormone was more than the LSD value which was 0.825.

3.1. Molecular analysis of FSHR Gene.

The DNA samples from patients were subjected to molecular analysis after PCR amplification using specific primers of the FSHR gene and DNA sequencing. The three primers amplify exon (10) giving amplicon size (520, 364 and 700 bp) with RS1, RS2, and RS3 primers respectively as shown in Fig. 1.

DNA sequences of FSHR gene obtained from patients were subjected to analysis. We detected the presence of genetic change in several main locations at FSHR gene in individuals with PCOS specifically on exon 10. Total genetic variation is listed in Table 3 and represented in Fig. 2.

4. Discussion

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy affecting women of childbearing age causing not only reproductive but also metabolic anomalies. Women with PCOS present ovulatory dysfunction, abnormal hormones, hyperandrogenemia, obesity, and hyperinsulinemia [6]. It is a heterogeneous disorder which results from interaction of multiple genes along with environmental factors [22]. For a number of genes altered patterns of expression have been detected, suggesting that the genetic abnormality in PCOS affects signal transduction pathways controlling the expression of multiple genes rather than abnormal expression of a single gene [13].

Familial clustering of PCOS cases suggests that genetic factors play an important role in PCOS's etiology. Although the studies of familial cases of PCOS have produced results suggesting an autosomal dominant trait, the mode of inheritance has not been firmly established [8]. There are several reasons for this. First, genetic heterogeneity makes the genetic studies of PCOS hard to perform. Second, several pathways are implicated in the etiology of PCOS; therefore, several candidate genes may be responsible for this 'complex' genetic trait, making the identification of each contributing gene very difficult. Until recently, the approach to understand the molecular basis of this complex syndrome was to study the functions of individual genes.

Endocrine and genetic studies of FSHR gene polymorphisms showed the association with different levels of serum FSH within the normal levels, duration of menstrual cycles and expression of FSHR transcripts, [9,1]. Polymorphism rs6166 (G/G) is associated with high levels of FSH and followed by rs6166 (A/G) and rs6166 (A/A) genotype in PCOS [19].

A study of Han on Chinese women with PCOS revealed the rs6165/rs6166 (G/A) haplotype of FSHR gene as a risk for PCOS [2] which was detected in this study resembled with change of A/G for rs6166 and change of A/G for rs6165. FSHR gene polymorphisms influence basal FSH levels. Many studies independently tried to found the relation between serum FSH levels and FSHR gene polymorphisms. Polymorphism rs1394205 showed differing results without affecting basal FSH levels in women from Germany and Indonesian women undergoing In-vitro fertilization [21] and study from India showed the association with higher serum FSH levels in women with primary amenorrhea [1] In Iraqi women other types of mutation designated as pathogenic. These, in our openion, might involve in elevated levels of FSH. The mechanism is throught to involve interrupting feed back inhibition signal that is crucial in FSH balance in the body.

5. Conclusions

Hormonal imbalance was a direct result for PCOS, and was a significant cause in infertility in women suffering from this disease. The FSHR gene was affected dramatically with genetic change especially SNPs rs6165, rs6166, and a pathogenic SNPs at position rs121909660, rs28928871, rs121909664, rs121909662, rs28928870, rs121909663, rs121909661, rs121909659, and rs121909665.

Ethics approval and consent to participate

This study did was performed after the approval of Scientific Committee and ethical committee, and consent of patients without mentioning any personal information.

Consent for publication

This work did not include any personal, written information, pictures and videos to any person.

Availability of data and material

All data and materials used in this study are available at College of Biotechnolyg, Dept. of Clinical Biotechnology.

Competing interests

This work was conducted without conflict of interest among authors or any other research group in others institutes.

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Authors' contributions

This work was conducted by personal efforts without the contribution of any other researchers.

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