To Screen Inactivation Mutation of Exon 1 of FSHR Gene in Polycystic Ovarian Syndrome: A South Indian Cohort Study

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To Screen Inactivation Mutation of Exon 1 of FSHR Gene in Polycystic Ovarian Syndrome: A South Indian Cohort Study

NishuSekar, Samiksha Yeole, Rashmi Pradeep, Yogamaya D Prabhu, KaviyarasiRenu, Shalaka S Ramgir and V G Abilash
Department of Biomedical Sciences, School of Bioscience and Technology, VIT Univeristy, Vellore-632014, Tamilnadu, India.

E-mail: abilash.vg@vit.ac.in

Abstract. Polycystic ovary syndrome is an endocrine disorder. Irregular menstrual cycle, acne, facial hair and elevated androgen levels are the most common signs for PCOS. PCOS has an estimated prevalence of 4-12% among reproductive age women, thus making it a forerunner in female infertility. FSHR plays an important role in FSH signaling pathway making it an important gene for PCOS. In this study, we aim to focus on any association between the FSHR gene and PCOS. Our study was to evaluate any polymorphism of exon 1 of FSHR gene associated with PCOS.PCR-RFLP technique was performed on the PCOS samples. Hormonal changes were found in the patients. Exon 1 inactivation mutation of FSHR gene was not observed in the patient sample. A study of this association needs to be done using large sample size.

1. Introduction

Polycystic Ovarian Syndrome (PCOS) being an endocrine disorder is characterized by polycystic ovarian morphology, hyperandrogenism, and menstrual irregularities. Correct diagnostic criteria are elevated androgen levels and anovulation and not just ovarian cysts. Studies suggest that PCOS might involve multiple physiological systems, but the exact cause still remains unknown. Females with this syndrome show resistance to insulin which majorly casts its effect on the ovaries. Acne, the presence of unwanted hair and high blood pressure are also seen in many affected women. Also, the term ‘polycystic" can be pretty misleading, as, many affected women, may or may not show cysts in their ovaries. Thus, presence or absence of ovarian cysts should not be the only criterion while discerning possible PCOS affected women. Though all the causes of PCOS have not been identified yet, studies show that there is a possibility of getting affected, if a woman's mother or sister already suffering from the disease. It could also develop as a result of health conditions that make the body produce excess insulin, which may affect the ovulatory capacity of the ovaries.

Follicle stimulating hormone (FSH) is expressed as a pituitary glycoprotein that stimulates the granulosa cell proliferation and differentiation and in the growth and development of follicles, thus playing an important role in folliculogenesis [6]. FSH receptor, a member of the family of G protein-coupled receptors mediates the effect of FSH and is characterized by three domains, extracellular ligand-binding domain, the transmembrane domain and intracellular domain. The FSHR mediates the action of FSH, mediating FSH signal transduction by activation of adenylate cyclase and intracellular cAMP elevation. The FSHR gene has a single large exon and nine smaller ones. It was in 1995, in a Finnish population that the first FSHR gene inactivating mutation was described. The FSHR gene is located on chromosome 2p21 (Figure 1) and comprises of 10 exons and 9 introns [10]. FSHR gene contains two important single nucleotide polymorphisms (SNPs) in exon 10.

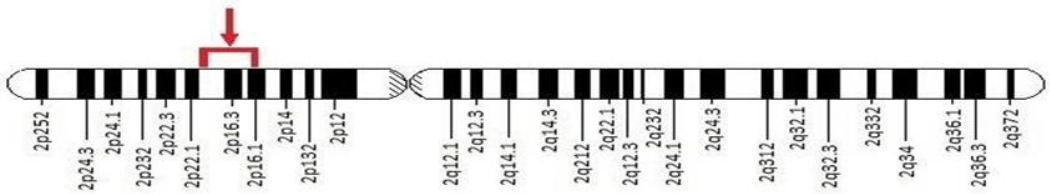

Figure 1. Location of FSHR on chromosome 2 [11]

In our study, we have incorporated the PCR-RFLP technique to identify polymorphism in exon 1 of the FSHR gene so as to determine its relation with PCOS. The diagnostic criteria used for evaluation is the most widely accepted Rotterdam criteria. According to this criteria, at least two of the three clinical manifestations namely oligo/anovulation, hyperandrogenism, polycystic ovaries on ultrasound should be seen for diagnosis of PCOS.

2. Materials and Method

2.1 Study population

The study was done using 10 samples collected from PCOS patients. The sample was collected from Sandhya Hospital, Vellore duly approved by the Institute of Ethical Community, VIT, Vellore, India. The study was confined to South Indian cohort. The controls were selected by excluding the diagnosis of PCOS according to the 2003 Rotterdam Criteria and exhibiting normal menstrual cycles (32 days). The control group consisted of healthy females without hirsutism. The patient's data contains the information like age, height, BMI, marital status, clinical features, hormonal reports and ovarian report. The data of individual patients is summarized below.

2.2 Genetic determination of Exon 1 of FSHR

The blood samples of patients were used for DNA extraction. Amplification of DNA samples was done through PCR. The primer sequences used were 5′ GGAGCTTCTGAGATCTGTGG C 3′ as the forward primer and 5′ AAATGCCAGCCATGCAGTTG 3′ as reverse primer. A 20µl reaction mixture was prepared by PCR. The PCR conditions for 35 cycles were, initial denaturation at 95°C for 5 mins, final denaturation at 95° C for 1 min, annealing temperature at 57°C for 30 seconds, initial extension at 72°C for 50 seconds, final extension at 72°C for 10 seconds. The PCR product was run on 2% agarose gel and visualized under UV Transilluminator (Medox) [9]. Presented in Figure3.

The products were digested at 37°C for 5 minutes using 0.5 units of restriction enzyme *Mbo II*. The digests were electrophoresed on 3% agarose gel and visualized under UV Transilluminator (Medox) and photographed using Gel Dock software.

3. Results

Among the 10 patients from whom the samples were collected, all reported the problem of the irregular menstrual cycle. The patients had normal insulin levels. Out of 10, only 1 was found to have Acanthosis Nigricans as seen in Table 1. Acanthosis nigricans is a brown or black notable patch of skin having a velvety texture. Some patients also displayed acne problems.

Table 1. Clinical reports of patients.

Patients Code	Irregular periods	Insulin problems	Obesity problem	Cardio problem	Acne	Acanthosis Nigricans
PCOS 1	Yes	No	No	No	DURING PERIODS	No
PCOS 2	Yes	No	No	No	DURING PERIODS	No
PCOS 3	Yes	No	No	No	NO	No
PCOS 4	Yes	No	No	No	MEDIUM	No
PCOS 5	Yes	No	No	No	NO	No
PCOS 6	Yes	No	No	No	MEDIUM	No
PCOS 7	Yes	No	No	No	DURING PERIODS	No
PCOS 8	Yes	No	No	No	NO	No
PCOS 9	Yes	No	No	No	NO	No
PCOS 10	Yes	No	No	No	DURING PERIODS	Yes

The LH and FSH levels among the PCOS patients was found to be normal. The level of T3 among all patients except one is normal. Only 30% of the patients had normal total testosterone level. Among all patients, only 20% had high free testosterone level and one had a slightly high level as seen in Figure 2. Also only 10% patient had high prolactin levels.

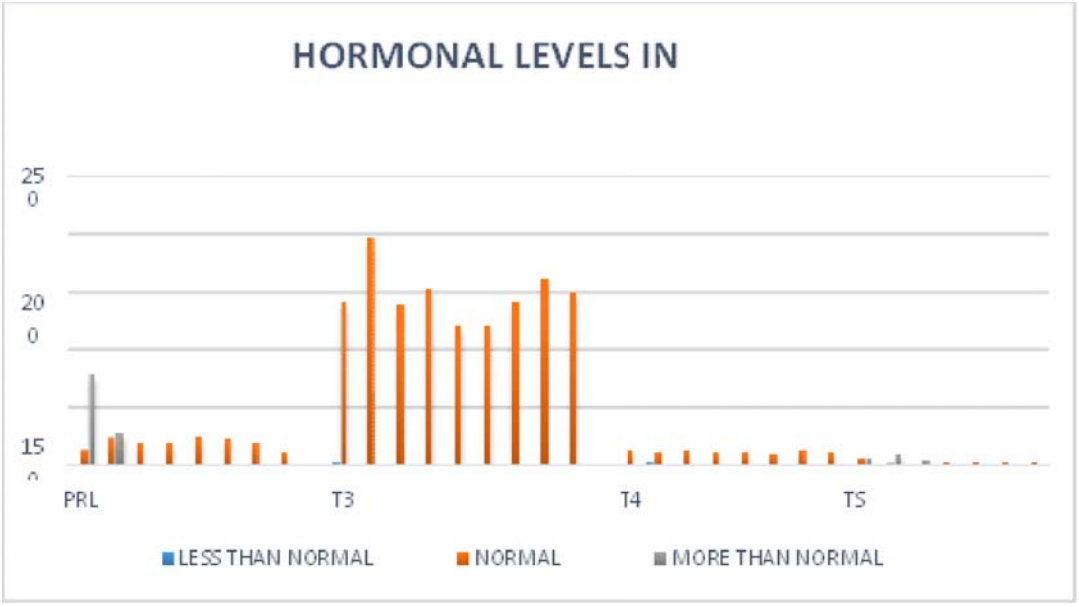

Figure 2. Hormonal levels in the PCOS patients

The table 2 gives the patient details showing the BMI ranges among all the patients.

Table 2. Patient details

Patient Code	Age	Height	Weight	BMI	Age of problem Recognized	Marital Status	Problem Recognized Since
PCOS 1	21	158	86.5	34.64	19	YES	3 year
PCOS 2	21	154	39.5	16.65	19	NO	3 year
PCOS 3	24	153	81.6	34.85	21	YES	3 year
PCOS 4	25	155	37.2	15.48	20	YES	5 year
PCOS 5	29	163	74	27.85	28	YES	1 year
PCOS 6	23	145	51	24.25	15	YES	8 year
PCOS 7	31	153	74	35.79	31	YES	4 months
PCOS 8	14	145	66.4	31.58	14	NO	5 months
PCOS 9	19	162	72.54	27.64	16	NO	3 years
PCOS 10	22	158	60.1	24.07	21	YES	1 years

Ultrasonography was utilized to study the morphology of the right and left ovary, along with the size and number of follicles. All these were satisfying the Rotterdam criteria. Ovarian report of the patients were presented in Table 3

Table 3. Ovarian reports of the patients

	Ovary Measurement (cm)		No of Follicles cyst		Size of Follicles (mm)	
Patients code	Right	Left	Right	Left	Right	Left
PCOS 1	3.9× 2.2 × 1	4.5× 2.7 × 2	8	10	4	3
PCOS 2	29.8× 24.3 × 2	30.3× 18 × 2	11	12	5	4
PCOS 3	3.2× 4.3 × 1	3.5× 3.0 × 1	10	11	4	5
PCOS 4	42× 20 × 31	51× 20 × 36	12	11	5	3.5
PCOS 5	39× 29 × 23	34× 23 × 34	11	8	5	5
PCOS 6	6.7× 6.1 × 2	4.7× 3.9 × 2	8	16	3	4.5
PCOS 7	3.5× 3.8 × 2	6× 4.4 × 2	11	4	4	3
PCOS 8	29.8× 24.3 × 2	3.7× 2.8 × 2	9	10	4.5	4
PCOS 9	4.5× 3.5 × 2.9	3.7× 3.1 × 2.7	11	12	4	5
PCOS 10	5.8× 5.4 × 1	4.8× 4.5 × 1	12	10	3	4

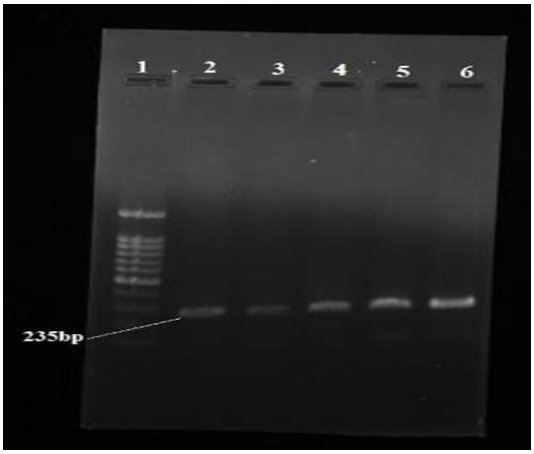

Figure 3. PCR amplification of Exon 1 of FSHR gene: Lane 1:100bp DNA Ladder, Lane 2-6: Amplified PCR Product of Exon 1 of FSHR gene

4. Discussion

Stein-Leventhal syndrome**,** better known as polycystic ovary syndrome (PCOS), is a disorder in women, characterized by an increased level of male hormone - androgen, and the absence or infrequent ovulation (anovulation), due to deficit levels of progesterone hormone. Women with this hormonal condition may have impaired fertility. First described in 1935 by American gynecologists Irving F. Stein, Sr., and Michael L. Leventhal, they associated the symptom with the presence of ovarian cysts with anovulation, affects approximately 5 percent of women, thereby being responsible for a substantial proportion of female infertility cases.

Our study showed no inactivation mutation in Exon 1 of FSHR in the PCOS patients. Abdel. Aziz, et al. demonstrated that the Ala307Thr polymorphism of FSHR is associated with PCOS in the studied Egyptian population. This polymorphism correlates with the increased levels of total testosterone among PCOS women.

The findings that FSHR genotype modifies the ovarian response to FSH ought to have an impact on the delineation of stimulation protocols [6]. No inactivating mutations in exon 10 of the FSH receptor gene were identified in Brazilian patients with premature ovarian failure [12].

Not much study has been done on the polymorphism of Exon 1 of FSHR gene as a risk factor for PCOS. There is not enough literature work found on exon1 of FSHR gene. Furthermore, studies are needed to be undertaken to conclude the mutations in FSHR can cause PCOS.

5. Conclusion

The absence of inactive mutation was found in the PCOS patients. As the sample size is small, Hardy-Weinberg law cannot be applied in our study. More study need to be carried out to identifying the any genetic risk factors for PCOS. The association of FSHR exon 1 and PCOS can be studied further using large sample size, along with SSCP (Single Strand Confirmation Polymorphism) analysis.

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