



A population-based case-control study to evaluate the role of codon 164 of beta 2-adrenergic receptor gene polymorphism in Iranian PCOS patients

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ABSTRACT

Polycystic ovary syndrome (PCOS) is a common endocrine disorder. This syndrome is a genetic disorder that involves about 5 to 10 percent of women in reproductive age. The purpose of this study was to investigate the effect of codon 164 of beta-2 adrenergic receptor (NM_000024.5:c.491C>T) polymorphism in patients with PCOS. The normal form of this codon is ACC and when polymorphism occurs, it changes to the ADRB2rs1800888 (Thr164Ile) with codon ATC. This case-control study consisted of 14 Iranian patients (mean age 26.86±1.03), along with 13 healthy controls, from Infertility Center of Valie Asre, Imam Khomani Hospital Complex in May 2013 until Feb 2014. Data analysis was done by statistical software SPSS, version 19.0. The result shows that, two persons of control group and one individual of patient group had polymorphism codon 164. The P-value greater than 0.05 (P>0.05) demonstrated that there is no correlation between Thr164Ile polymorphism and PCOS.

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KEYWORDS

Polycystic ovary syndrome;
PCOS;
Polymorphism;
Beta-2 adrenergic receptor.

INTRODUCTION

The polycystic ovary syndrome (PCOS) is the most common endocrine and metabolic disorder that involves about 5 to 10 percent of women in reproductive life^[1]. The endocrine manifestations of PCOS consist of extra androgen production of ovarian or adrenal origin and prevented follicular development leading to chronic anovulation or infrequent ovula-

tion^[1]. The syndrome is characterized with Amenorrhea, oligomenorrhea, infertility, Hirsutism, obesity and bilaterally enlarged ovaries with cysts^[2]. The Rotterdam criteria were founded in 2003 by the European Society for Human Reproduction and Embryology (ESHRE) in cooperation with the American Society for Reproductive Medicine (ASRM). This definition required at least two of the three following criteria: hyperandrogenism, ovulatory dys-

function and PCO morphology on ultrasound^[2-3]. Many factors have been demonstrated to be the most important contributory factors in the development of PCOS such as endocrine disturbances, Hypothalamus/pituitary ovarian axis dysfunction, Obesity, Insulin resistance, prenatal exposure to androgens^[2]. In spite of extensive research, the accurate etiology and mechanisms of PCOS remain unclear. Significant issue in the genetics of PCOS has increased in recent years following increasing number of genetically studies^[4]. Studies have shown that, several factors, including genetics, could play a role. because Women with PCOS are more likely to have a mother or sister with PCOS compared with that of the general population^[4]. One of genetic factors is Beta-2 adrenergic receptor (ADRB2).

Beta-2 adrenergic receptor is a G protein-coupled receptor that mediate the actions of catecholamine's in multiple tissues, and coded by an 1242 bp intron less gene located on the long arm of chromosome5 (5q31-32)^[5-6]. ADRB2 mediate the regulation of many physiological functions Such as, blood vessels, the heart, bronchial and vascular smooth muscle relaxation, and the uterus^[6-7]. In multiple populations, the ADRB2 gene has been sequenced and more than 80 polymorphisms have been identified in the coding and promoter region^[8]. Most reported studies relate to the no synonymous polymorphisms substitution of glycine for arginine at amino acid position 16 (Arg16Gly), glutamate for glutamine at amino acid position 27 (Gln27Glu) and, more rarely, threonine at position 164 replaced by isoleucine (Thr164Ile)^[7].

The ADRB2rs1800888 (Thr164Ile) polymorphism is uncommon in the general population of various ethnicities, typically being existent in <4% in its heterozygous form, and it may not exist homozygous form for the isoleucine at position 164 (Ile164) genotype^[7]. The Thr164Ile SNP of the ADRB2 gene is associated with an increased risk of obesity, coronary and peripheral artery disease, lipolysis in isolated adipocytes, and airway dilation and lymphocyte cyclic adenosine monophosphate formation in cystic fibrosis patients^[7-9]. Unfortunately, So far, the report has not been published in relation to PCOS and Thr164Ile, but Studies have demonstrated that

ADRB2 gene mutations may change the structure or function of the ADRB2, either the polymorphism in codon 27 (Gln27Glu) of ADRB2 is linked to the expression of PCOS^[10].

MATERIAL AND METHODS

Subjects

This case-control study consisted of 14 Iranian patients with polycystic ovary syndrome along with 13 healthy controls. The enrolment was performed from "May 2013 until Feb 2014". The trial was approved by the Ethics Committee of health reproductive Centers. All of patients were selected from Infertility Center of Valie Asre Hospital, Imam Khomani Hospital, Tehran, Iran and control group were randomly selected Among healthy women and from out of the above-mentioned Hospital.

Determine the health of control women was performed through questionnaire, physical checkup, hormonal tests and ultrasound examination. all patients with PCOS was diagnosed according to the 2003 Rotterdam criteria^[2]: Two out of three of the following criteria were met for the diagnosis: oligo-ovulation and/or anovulation (irregular menstrual cycle), clinical and/or biochemical signs of hyperandrogenism, polycystic ovaries (PCO) by ultrasound (e"12 or more follicles in each ovary measuring 2–8 mm in diameter)^[11].

DNA extraction

Five macrolitr (5µl) from peripheral blood leukocytes of case and control blood samples were collected in EDTA treated tubes and maintained at -20°C. Total Genomic DNA was isolated from these samples using CinnaGen Kit (Iran) according to manufacturer's instructions. Purity and concentration of genomic DNA was evaluated using Nanodrop and prepared a concentration 50 µg/ml as working tubes.

Genotyping of ADRB2gene

ADRB2 Information (NC_000005.10:148826593...148828634) were extracted from NCBI Site. One ADRB2 functional SNPs was selected for genotyping in this study: rs180088 (Thr164Ile). The SNP genotypes were

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determined by polymerase chain reaction (PCR). PCR reactions were performed using specific primers was designed by Primer3 online software. The reactions prepared in tube labeled for final volume 25 μ l containing 2 μ l total DNA from the patient or control, 1 μ l of each primers, 14 μ l distilled water and 7 μ l Taq DNA Polymerase 2x Master Mix Red (Ampliqon, Danmark). The PCR cycling conditions were carried out with an initial denaturation step for 5 min at 95 °C, followed by 34 cycles of 30 s at 95 °C, 30 s at 61°C and 30 s at 72°C and final extension step at 72°C for 5 min.

Sequence of Primers was 5'-AAGCGGCTTCTTCAGAGCA-3' as forward primer, and 5'-GATGGCTTCCTGGTGGGTG-3' as reverse primer. Then the PCR product was run on a 1.5% Arose gel in 1 \times TBE buffer and visualized on a Gel Documentation System using Gel Red dye TABLE 1.

Statistical analysis

Analyses of phenotypic values, included descriptive statistics (s.e.mean by genotype) were per-

formed by analysis of covariance, adjusting for sex, obesity status and age. Chi-square and Student's unpaired t-test were performed for analyzing the associations between categorical variables. Fisher's exact test was used to determine accuracy. All analyses were performed by SPSS software version 19.0. Tests of statistical significance were two sided and not taken as significant when $p < 0.05$.

RESULTS AND DISCUSSION

Clinical parameters

This analysis included 14 patients with Polycystic ovary syndrome (average about 26.9 years), and 13 healthy controls (range 19 to 39), for the Thr164Ile polymorphism. The basal demographical, hormonal and biochemical parameters of controls and PCOS women are summarized in TABLE 2. The age between two groups was similar. LH, FSH levels and other paraclinical parameters were lower in PCOS patients compared with that of controls, except prolactin level. BMI significantly higher in PCOS group TABLE 2.

TABLE 1 : PCR primer pairs

Exon No	Segment Size	Length	T _m	Primer Type	Primer Sequence (5' ->3')
E ₁	759 bp	19	61°C	Forward	AAGCGGCTTCTTCAGAGCA
				Reverse	CACCCACCAGGAAGCCATC

TABLE 2 : Clinical and endocrine-metabolic parameters in PCOS and control women

Parameters	PCOS(N=14)	Controls(N=13)
Age(y)	26.86 \pm 1.03	27.31 \pm 1.39
BMI(kg/m ²)	27.36 \pm 0.64	20.88 \pm 0.34
Oligo- or anovulation (%)	71.43 \pm 0.11	0
PCO (%)	100 \pm 0.0	0
Family history of PCOS (%)	78.57 \pm 0.09	0
Infertility (%)	100 \pm 0.0	0
Abortion (%)	21.43 \pm 0.09	0
Hirsutism (%)	71.43 \pm 0.11	0
LH (mIU/ml)	2.19 \pm 0.22	12.50 \pm 0.49
FSH (mIU/ml)	5.02 \pm 0.44	8.81 \pm 0.71
Prolactin (ng/ml)	30.40 \pm 0.73	12.96 \pm 1.67
T3 (nmol/L)	6.81 \pm 1.72	1.68 \pm 0.13
T4 (nmol/l)	7.36 \pm 0.84	9.03 \pm 0.66

Data are described in mean \pm SD; BMI: body mass index; PCO: polycystic ovary syndrome; LH: luteinizing hormone; FSH: Follicle-stimulating hormone; T3 and T4: Thyroid Hormones

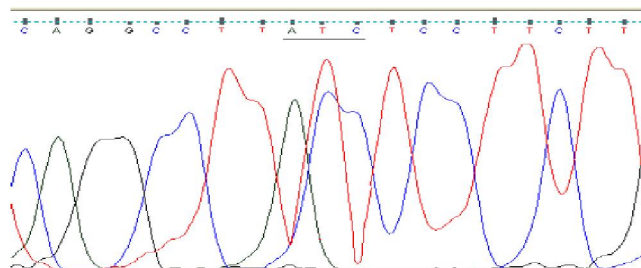


Figure 1 : 1 Case of patient group had Thr164Ile polymorphism

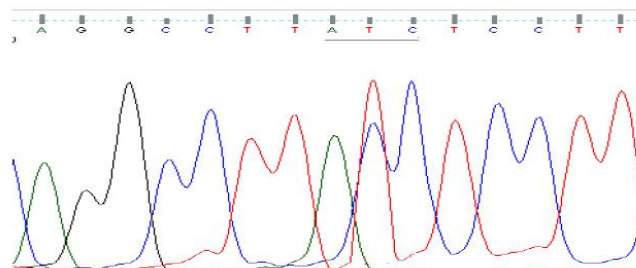


Figure 2 : 1 Case of control group had Thr164Ile polymorphism

TABLE 3 : Genotype distribution in PCOS and control women

Thr164Ile	Genotype (frequency %)			Total (%)
	CC	CT	TT	
Case	13 (92.86)	1 (7.14)	0 (0.00)	14 (100%)
Control	11 (84.62)	2 (15.38)	0 (0.00)	13 (100%)
Total	24	3	0	27

TABLE 4 : Allele distribution in PCOS and control women

Thr164Ile	Allele (frequency %)		Total (%)
	T allele	C allele	
Case	1 (3.57)	27 (96.43)	28 (100%)
Control	2 (7.69)	24 (92.31)	26 (100%)
Total	3	51	54

Sequencing results

Sequencing results show that, 2 person of control group and 1 case of patient group had Thr164Ile polymorphism (Figure 1, 2). In PCOS group, there are 1 Thr164Ile heterozygous patients, the gene variants frequencies were 7.14% and there is no homozygous case. In controls, there were 2 Thr164Ile heterozygous subjects, the gene variants frequencies were 15.38% (TABLE 3).

Both groups of, healthy controls and patients had similar gene frequencies, suggesting that this polymorphism isn't related to Polycystic ovary syndrome in Iranian population so, there was no significant difference in the between PCOS and codon164 of ADRB2, ($P > 0.05$).

The following genotypes were identified for ADRB2 gene Thr164Ile polymorphism: In control samples and PCOS patients. The results of genotyping and allele frequency are depicted in TABLE 3,4.

It has been shown ADRB2 Polymorphisms play role in some disease related to PCOS. Johan Hoffstedt and etal in 2001 have demonstrated that the 164Ile variant of the b2-adrenoceptor is associ-

ated with a decreased native adipocyte receptor function in Sweden population. This suggests that genetic variance in the b2-adrenoceptor gene might be important for catecholamine function in humans; at least as far as adipocyte lipolysis is concerned^[12]. In 2012, in Danish Population, Thomsen M and et,al, suggest that ADRB2rs1800888 (Thr164Ile) rare vs. common homozygotes are not significantly associated with an increase in BMI measured continuously but may be associated with an increased risk of obesity^[9].

In an attempt to discover a genetic marker to screen patients who are more susceptible to PCOS ADRB2 gene Thr164Ile polymorphism were investigated in this study. Our results demonstrated that above-mentioned polymorphism may not be a genetic predisposing factor for PCOS in Iranian population. Although in contrast to our findings, other study reported that the ADRB2 Gln27Glu polymorphism had a significant association with polycystic ovary syndrome in Japanese PCOS patients with P-value = 0.02^[10] but in this study, the P-value greater than 0.05, ($P > 0.05$) demonstrated that there was no significant difference between PCOS and codon164

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of ADR β 2. This study is the first report of ADRB2 gene Thr164Ile polymorphism with PCOS from Iran and our study has some limitations. The number of patients and controls were low, so future studies will be important to understand the role of the Thr164Ile polymorphism and other polymorphisms of ADRB2 gene in Iranian women affected by PCOS in the multitude population.

CONCLUSIONS

In summary, this population-based case-control study failed to find evidence to support our hypothesis that the rare variant of ADRB2 gene Thr164Ile polymorphism, associated with polycystic ovary syndrome. Then we couldn't find an association between the 164Ile allele and PCOS in the small population of the Iranian women. However, the difference between patients and controls wasn't significant in this study, but suggests that the Thr164Ile polymorphism investigated in large scale population and probe other SNP in the ADRB2 Gene.

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