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Correlation between IL-1β, IL-1Ra gene polymorphism and occurrence of polycystic ovary syndrome infertility

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ABSTRACT

Objective: To explore the relationship between IL-1β, IL-1Ra gene polymorphism and the occurrence of polycystic ovary syndrome (PCOS) infertility. **Methods:** A total of 59 PCOS infertility cases visiting the reproductive center of our hospital from Mar. 2010 to Mar. 2012 and 56 healthy women were selected. ELISA method was used for the detection of IL-1β, IL-1Ra levels, and the levels of serum supersensitivity C reaction protein (US-CRP), insulin (FINS), follicule-stimulating hormone (FSH) and fasting blood-glucose (FBG) were detected. PCR analysis technology was adopted to detect the gene polymorphism of the 511 site of IL-1β and the second introne of IL-1Ra. **Results:** The levels of IL-1β, IL-1Ra, US-CRP, FINS and FBG in blood serum of patients in PCOS group were significantly higher than those in control group ($P<0.05$ or $P<0.01$). The level of FSH in PCOS group was significantly lower than that in control group ($P<0.05$). The genotypic frequency of T/T, the 511 site of IL-1β in PCOS group was 42.37%, significantly higher than 12.50% in control group ($P<0.01$). The frequency of T allele was also significantly higher than that in control group ($P<0.01$). The genotypic frequency of Ⅰ/Ⅴ, the second introne of IL-1Ra in PCOS group was 20.34%, signiciantly higher than 3.57% in control group ($P<0.05$). The frequency of Ⅴ allele in PCOS group was significantly higher than that in control group ($P<0.05$). **Conclusions:** T allele of the 511 site of IL-1β gene and Ⅴ allele of the second introne of IL-1Ra gene might be the genetic basis of the rising of IL-1β, IL-1Ra and US-CRP levels in blood serum of PCOS patients, and are associated with the infertility occurrence of PCOS patients.

1. Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disturbance disease leading to the reproductive dysfunction in females. The main manifestations are anovulia or oligo-ovulation, hyperandrogenism, hyperinsulinemia, fat and so on. The incidence rate of female in child-bearing period is 5%-10%[1,2]. The pathogenesis of PCOS is not very clear, and because of its strong familial aggregation, many researchers believe that its incidence is related to the hereditary factors and may be the results of the interaction of gene and environment factor. The recent studies[3] show that chronic subclinical inflammation is closely related to the incidence of PCOS. IL-1, as an important cell inflammatory factor, plays an important role in cell growth, cell differentiation and immune response, and the levels of IL-1β and IL-1Ra in blood serum of PCOS patients increase significantly[4]. In order to explore the relationship between IL-1β, IL-1Ra and PCOS incidence, this work detected the levels of IL-1β and IL-1Ra in blood serum of PCOS patients and the gene polymorphism, and made the contrastive analysis with the healthy control group, aiming at providing new clues for genetic studies of PCOS.

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2. Material and methods

2.1. General information

Fifty-nine PCOS infertility patients with age (29.75 ±3.62) years old, vsiting reproductive center of our hospital from Mar. 2010 to Mar. 2012, were selected. The inclusion criteria took the diagnostic standard which was established by European Human Reproduction and Embryology Association and American Reproductive Medical Association in May, 2003 as the reference[5]. That was: 1) oligo-ovulation or anovulia; 2) having the clinical or biochemistry manifestation of hyperandrogenism; 3) ovarian polycystic changes (any one ovary existed twelve or more than twelve follicles with diameter 2-9 mm, or the ovarian volume increased more than 10 mL). The patients should meet two of the three items and patients with other diseases (congenital adrenal hyperplasia, androgen secretory tumor, Cushing's symptom complex) must be excluded. Another fifty-six cases of healthy women who received treatment because of their husband with azoospermatism in the same period were selected as the control group. Their ages were (29.83±3.91) years old; the menstrual cycle was normal; the basal body temperature was biphasic; serum hormone level and ultrasonic inspection were normal; bilateral tubal was patent. Other endocrine diseases should be excluded for all subjects investigated. They had no the history of hormone drugs application within three months, and had no diseases of heart, liver, kidney and others. All of the subjects signed the informed consent.

2.2. Methods

2.2.1. Laboratory parameter detection

Five mL venous blood was drawn in the condition of limosis from all subjects investigated in the 3-5 d of the menstrual cycle (when dominant follicle were not found on PCOS amenorrhea patients in B-ultrasound examination), and the following laboratory parameters were detected. 1) IL-1 β and IL-1Ra levels were detected using ELISA method (ELISA kit, made by American Biosource Company); 2) supersensitivity C reaction protein (US-CRP) level was detected using latex-enhanced immunoturbidimetry (AU5400 automatic biochemical analyzer, Japanese Olympus Company); 3) insulin (FINS) and follicule-stimulating hormone (FSH) levels were detected using enzyme amplification chemoluminescence method; 4) fasting blood-glucose (FBG) level was detected using glucose oxidase method.

2.2.2. DNA extraction

Two mL peripheral venous blood was drawn in the condition of limosis from all subjects investigated, and 0.2% EDTA-Na_2 was used for anticoagulation. The modified salting-out method[6] was used for DNA extraction (Genomic DNA fast extraction kit, Beijing SBS Genetech Co., Ltd.), and the DNA was kept under 70 ℃ for test.

2.2.3. Detection of IL-1 β 511 site gene polymorphism

The primer was designed and synthesized by Invitrogen Company. The upstream primer was 5′-TGGCATTGATCTGGTTCATC-3′ and the downstream primer was 5′- GTTTAGGAATC- TTCCCACTT -3′. PCR reaction system: 2 μL DNA sample, 2.5 μL 10×PCR buffer solution, 0.5 μL dNTP, 0.5 μL *Taq* DNA polymerase, 0.5 μL upstream primer and 0.5 μL downstream primer, sterile deionized water supplemented to 25 μL, blending. PCR cyclic conditions: pre-denaturation at 95 ℃ for 5 min; denaturation at 94 ℃ for 40 s, annealing at 51℃ for 40 s, elongation at 72 ℃ for 30 s, and 33 cycles in total; elongation at 72 ℃ for 5 min. After 2% agarose gel electrophoresis, the allele type of IL-1 β 511 site was obtained according to the electrophoresis results. Gene counting method was used for calculating gene frequency.

2.2.4. Gene polymorphism detection of the second Introne of IL-1Ra

The primer was designed and synthesized by Invitrogen Company. The upstream primer was 5′-CTCAGCAACACACCTAT-3′ and the downstream primer was 5′- TCCTGGTCTGCAGGT- AA-3′. PCR reaction system: 25 μL of the total volume, 2 μL DNA sample, 2.5 μL 10×PCR buffer solution, 0.5 μL dNTP, 0.5 μL *Taq* DNA polymerase, 0.5 μL upstream primer and 0.5 μL downstream primer. PCR cyclic conditions: pre-denaturation at 94 ℃ for 5 min; denaturation at 95 ℃ for 30 s, annealing at 55 ℃ for 30 s, elongation at 72 ℃ for 30 s, and 30 cycles in total; elongation at 72 ℃ for 5 min. After 2% agarose gel electrophoresis, the allele type of the second introne of IL-1Ra was obtained according to the electrophoresis results. Gene counting method was used for calculating gene frequency.

2.3. Statistical analysis

SPSS15.0 software was used for statistical treatment and allele frequency and genotype distribution in each group were calculated. The goodness-of-fit χ^2 was used for inspecting if the genotype distribution frequency was accord with Hardy-Weinberg balance. Analysis of variance, t test or χ^2 test was used for the comparison of clinical and laboratory parameters between different genotypes. $P<0.05$ indicated that there were statistical significance.

3. Results

3.1. Comparison of IL-1β, IL-1Ra, US-CRP, FINS, FSH and FBG levels between two groups

The levels of IL-1β, IL-1Ra, US-CRP, FINS and FBG in PCOS group were significantly higher than those in control group ($P<0.05$ or $P<0.01$); FSH level in PCOS group was significantly lower than that in control group ($P<0.05$) (Table 1).

3.2. Results of IL-1β and IL-1Ra genotype detection in PCOS group

There were three kinds of genes in 511 site of IL-1β: TT type (only appearing one strap, 304 bp), CT type (appearing three straps, 304 bp, 190 bp and 114 bp) and CC type (appearing two straps, 190 bp and 114 bp) (Figure 1). The gene polymorphism of IL-1Ra was expressed as existing 86 bp variable number of tandem repeat (VNTR) in the second introne and four kinds of allelic products, 410, 595, 240 and 325 bp, which were corresponding to four alleles of IL-1Ra, IL-1Ra *1 (Ⅰ), IL-1Ra *5 (Ⅴ), IL-1Ra *2 (Ⅱ) and IL-1Ra *4 (Ⅳ) (Figure 2).

Table 1
Comparison of each indicator of patient serology in two groups.

Items	PCOS group (n=59)	Control group (n=56)	t value	P value
IL-1β (ng/L)	138.76±35.35	107.23±41.89	4.37	<0.01
IL-1Ra (ng/L)	273.73±89.45	219.90±85.51	3.29	<0.01
US-CRP (mg/L)	1.58±0.63	1.07±0.49	4.83	<0.01
FINS (mU/L)	18.65±9.32	14.51±8.99	2.42	<0.05
FSH (IU/L)	6.29±1.27	6.85±1.61	2.08	<0.05
FBG (mmol/L)	5.26±0.61	4.85±0.77	3.17	<0.01

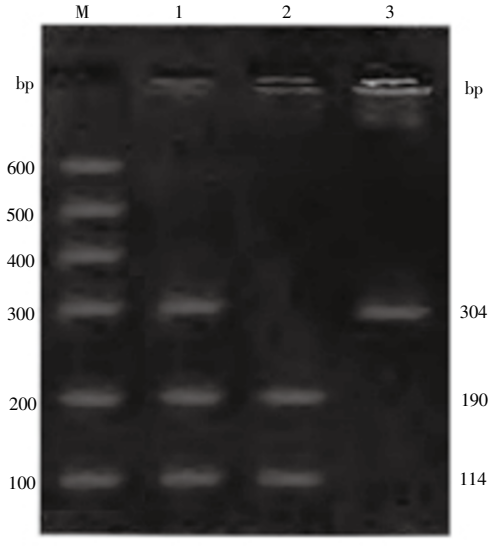

Figure 1. Gene polymorphism electrophoretogram at 511 site of IL-1β.
Note: M indicated DNA marker; 1-3 indicated the three genotypes at 511 site of IL-1β, CT type, CC type and TT type.

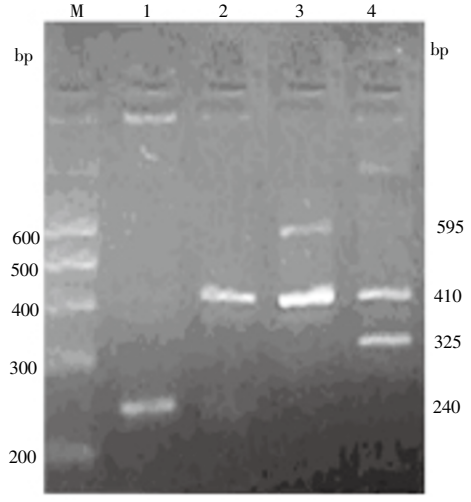

Figure 2. Gene polymorphism electrophoretogram of IL-1Ra.
Note: M indicted DNA marker; 1-4 indicated the four genotypes at the second introne of IL-1Ra, Ⅱ/Ⅱ type, Ⅰ/Ⅰ type, Ⅰ/Ⅴ type and Ⅰ/Ⅳ type.

3.3. Comparison of genotypes and allele frequency distribution at 511 site of IL-1β between the two groups

The genotype frequency of T/T at 511 site of IL-1β in PCOS group was 42.37%, significantly higher than that 12.50% in control group (χ^2=12.78, $P<0.01$). There existed C and T, the two kinds of allele at the 511 site of IL-1β, and T allele frequency in PCOS group was significantly higher than that in control group (χ^2=9.19, $P<0.01$) (Table 2).

Table 2
Comparison of genotypes and allele frequency distribution at 511 site of IL-1β between the two groups [Type (%)].

Groups	n	Genotype frequency			Allele frequency	
		C/C	C/T	T/T	C	T
Control group	56	18(32.14)	31(55.36)	7(12.50)	67(59.82)	45(40.18)
PCOS group	59	13(22.03)	21(35.59)	25(42.37)	47(39.83)	71(60.17)
Total	115	31(26.96)	52(45.22)	32(27.83)	114(49.57)	116(50.43)

3.4. Comparison of genotypes and allele frequency distribution at the second introne of IL-1Ra between two groups

The genotype frequency of T/V at the second introne of IL-1Ra in PCOS group was 20.34%, significantly higher than that 3.57% in control group (χ^2=9.32, $P<0.05$). There existed Ⅰ, Ⅱ, Ⅳ and Ⅴ alleles of IL-1Ra, and Ⅴ allele frequency in PCOS group was significantly higher than that in control group (χ^2=9.99, $P<0.05$) (Table 3).

Table 3

Comparison of genotypes and allele frequency distribution at the second introne of IL-1Ra between the two groups [Type (%)].

Groups	*n*	Genotype frequency					Allele frequency		
		Ⅰ/Ⅰ	Ⅰ/Ⅳ	Ⅰ/Ⅴ	Ⅱ/Ⅱ	Ⅰ	Ⅱ	Ⅳ	Ⅴ
Control	56	52	1	2	1	107	2	1	2
group		(92.86)	(1.79)	(3.57)	(1.79)	(95.54)	(1.79)	(0.89)	(1.79)
PCOS	59	47	0	12	0	106	0	0	12
group		(79.66)	(0.00)	(20.34)	(0.00)	(90.18)	(0.00)	(0.00)	(9.82)
Total	115	99	1	14	1	213	2	1	14
		(86.09)	(0.87)	(12.17)	(0.87)	(92.61)	(0.87)	(0.43)	(6.09)

4. Discussion

PCOS is a kind of female endocrine and metabolic disorder disease and the main manifestations are oligomenorrhea or even amenorrhea, anovulia, hyperandrogenism, hyperinsulinemia, and easily leading to type Ⅱ diabetes, fat and other metabolic diseases. In this work, the levels of FINS and FBG in PCOS group were significantly higher than those in control group ($P<0.05$ or $P<0.01$). CRP is an important inflammatory mediator and regulated by blood serum TNF-Ⅱ, IL-1 and other factors. It participates in various inflammatory reactions and is a sensitive indicator of subclinical system infection[7]. In this work, the level of US-CRP in PCOS patient blood serum increased ($P<0.01$).

Recently, the studies on the related genes of PCOS inheritance are focused on the related genes of sex hormone regulation, steroid hormone biosynthesis, cardiovascular risk and insulin sensitivity[8,9]. The latest studies discover that chronic subclinical inflammation is closely related to PCOS[10], especially for IL-1 family, the important markers of inflammatory reaction, which not only play the role of regulation and induction in the process of cell growth, differentiation and immune response, but also influence the process of ovulation and pregnancy[11]. IL-1 in ovary comes from ovarian granular cells and macrophages, and can promote the proliferation of granular cells and inhibit the generation effect of FSH on trophic hormone in granular cells. Before ovulation, IL-1 which is secreted in follicle can regulate granular cells to synthesize prostaglandin, activator of plasminogen and mucoprotein, and regulate the activation of collagenase, which plays an important role in ovulation. After ovulation, IL-1 can enhance the luteal function of granular cell through paracrine manner, which can make the dissolved activities of protein and collagenous fibre in ovary stop in time after ovulation[12-14].

The domestic and foreign scholars have made numerous studies on the relationship between PCOS and IL-1 gene, and they have discovered that the increase of IL-1β, IL-1Ra and US-CRP levels in patient blood serum may be related to the occurrence of PCOS[15,16]. Through the studies of PCOS patients in Caucasus area, some scholars have found that the gene polymorphism at C-889T site of IL-1β gene is related to the occurrence of PCOS, while the fifth exon of IL-1β gene and the gene polymorphism of IL-1Ra are unrelated to PCOS occurrence and its clinical features[17,18]. Another study has shown that IL-1β gene and IL-6 gene-597 polymorphism are related to PCOS occurrence[19]. The results of this work showed that the levels of IL-1β, IL-1Ra, US-CRP, FINS and FBG in PCOS patient blood serum were significantly higher than those in control group ($P<0.05$ or $P<0.01$); FSH level was lower than that in control group ($P<0.05$). The results were the same with part of Poldan research results and the reasons might me closely related to metabolic syndrome of PCOS patients, with most PCOS patients getting adiposity. It has been recognized that lipocyte is a kind of endocrine cell[21,22] and can secrete a large number of inflammatory factors.

This work also discovered that T/T genotype frequency and T allele frequency at 511 site of IL-1β were higher than those in control group ($P<0.01$); Ⅰ/Ⅴ genotype frequency and Ⅴ allele frequency were higher than those in control group ($P<0.05$). It could be concluded that the 511 site of IL-1β and the genotype frequency of IL-1Ra might be the genetic bases for the increase of IL-1β and IL-1Ra levels in PCOS patient blood serum, and were related to the occurrence of PCOS sterility. Namely, females with T allele at the 511 site of IL-1β and Ⅴ allele carried by IL-1Ra had more tendency of PCOS sterility.

In sum, this work explored the relationship between inflammatory cytokines and PCOS sterility, and the results showed that T allele at the 511 site of IL-1β gene and Ⅴ allele in the second introne of IL-1Ra gene might be the genetic bases for the increase of IL-1β, IL-1Ra and US-CRP levels in PCOS patient blood serum, and were related to the occurrence of PCOS sterility. Meanwhile, females with the previous two alleles had more tendency of PCOS sterility. The patients with these high-risk genes should make early prevention and avoid the occurrence of metabolic disturbance and other long-term complications.

Conflict of interest statement

We declare that we have no conflict of interest.

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