

Original Article

Association study of polymorphism of FXIIIVal34Leu gene and polycystic ovary syndrome

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Abstract: Objective: To study the distribution of polymorphism of FXIIIVal34Leu gene in patients with polycystic ovary syndrome and to explore its relationship with polycystic ovary syndrome. Methods: FXIIIVal34Leu genotype of 120 patients with polycystic ovary syndrome (PCOS) and 90 controls was detected, and the endocrine and metabolic conditions of PCOS patients and the control group were analyzed. Results: Body mass index (BMI) of PCOS patients (26.45 ± 5.81) kg/m² was higher than the control group (22.33 ± 5.58) kg/m², ($p=0.00$); the androgen level of PCOS group was (0.67 ± 0.85) ng/mL higher than the control group (0.42 ± 0.22) ng/mL, ($p=0.02$), and the luteinizing hormone (LH) (16.8 ± 3.61 IU/L) level is higher than the control group (9.23 ± 4.67) IU/L, ($p=0.01$). Frequency of Val34Leu allele in PCOS group was 1.25%, which was not statistically significant compared to the control group ($P=0.56$). BMI of patients with the genotype of FXIIIVal34Leu was relatively higher (with the average 34 ± 1 kg/m²), and serum HDL level was significantly lower (0.23 ± 0.11 mmol/L). Polymorphism of FXIIIVal34Leu gene had no correlation with susceptibility of PCOS. Conclusion: Polymorphism of FXIIIVal34Leu gene may be related to metabolism of PCOS patients; the mutation of FXIIIVal34Leu is not at the common site for PCOS; polymorphism of FXIIIVal34Leu gene may not have correlation with PCOS.

Keywords: Coagulation factor XIII, polycystic ovary syndrome, polymorphism of Val34Leu gene

Introduction

Polycystic ovary syndrome (PCOS), a common heterogeneous endocrine disease among females, is characterized by oligomenorrhea or amenorrhea, anovulation, hyperandrogenism, hirsutism, acne, obesity and insulin resistance (IR). The incidence of PCOS is about 5% to 10% among women of childbearing age, and is the main cause leading to female infertility [1]. As a polygenic disease, the pathophysiological basis of occurrence and development of PCOS are insulin resistance and hyperinsulinemia. The significant familial aggregation tendency of PCOS also suggests genetic factors play an important role in the pathophysiological process of PCOS. Molecular genetic studies show that mechanism of PCOS is closely related to the genes that affect synthesis and metabolism of steroid hormones, gonadotropins and insulin as well as the genes that regulate material and energy. PCOS is a polygenic disease, in which polymor-

phism of some key genes especially that of single nucleotide may play an important role [2, 3]. The polymorphism of FXIIIVal34Leu gene is not only associated with the concentration of fiber protein, but also associated with insulin resistance, which suggests that polymorphism of FXIIIVal34Leu gene may affect the metabolism of PCOS by changing insulin resistance [4, 5]. Through the preliminary test on expression of polymorphism of FXIIIVal34Leu gene in PCOS patients (polymorphism of FXIIIVal34Leu gene in coagulation factor subunit IIIA), the study explored the relations between polymorphism of FXIIIVal34Leu gene with susceptibility of PCOS and endocrinology and metabolism.

Materials and methods

Subjects

There are 120 cases included in the PCOS group from January 2009 to December 2012.

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Diagnostic criteria refers to the revised standard 2003 Rotterdam European Reproductive Annual conference (ASRM/ESHRE), namely: 1). Oligo-ovulation or no ovulation; 2). Clinical or biochemical hyperandrogenism; 3). Polycystic ovary-like changes observed by ultrasound: unilateral or bilateral ovarian follicles with the diameter of 2-9 mm ≥ 12 , and/or ovarian volume > 10 ml. Cases that comply with 2 of the above-mentioned three requirements and exclude other diseases, such as congenital adrenal hyperplasia, androgen-secreting tumors, Cushing's syndrome, thyroid dysfunction, hyperprolactinemia, delayed 21-hydroxylase deficiency, etc. can be diagnosed as PCOS. Inclusion criteria of subjects in the study is B-mode ultrasound showing unilateral or bilateral ovarian basal follicles > 12 and elevated serum testosterone or B-mode ultrasound showing unilateral or bilateral ovarian basal follicles > 12 and Oligomenorrhea (cycle > 37 days), while excluding other hyperandrogenism caused by endocrine disorders. Select 90 patients treated in our hospital at the same period due to infertility caused by tubal obstruction or azoospermia of their husbands as the control group, with the inclusion criteria as: normal menstrual cycle, biphasic basal body temperature; ultrasound showing the uterus and accessories are morphologically normal and no polycystic ovary changes; no basic endocrine disorders, no high blood pressure, cardiovascular disease or family history of diabetes. For patients in the two groups measure and record the details of relevant information including age, height, weight, abdominal circumference, luteinizing hormone (LH), follicle stimulation hormone (FSH), testosterone (T), estradiol (E2) and prolactin (PRL), total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein (LDL). And calculate the body mass index (BMI)=weight (kg)/height² (m).

Collection and handling of specimens

Blood samples from cubital vein of patients in 2-4 days of the menstrual cycle or patients with amenorrhea at any period were collected and anticoagulated with ethylenediamine tetra-acetic acid (EDTA) for 30 min, then the samples were centrifuged for 15 min at 3000 rpm, supernatant plasma and blood cells pellet were collected respectively, and the plasma were

stored at -80°C for test. Use the whole blood DNA isolation kit to extract leukocyte genomic DNA from cell pellet.

PCR amplification of the target fragment

The primer of FXIII gene was designed as according to Balogh et al. [6]: upstream primer sequence 5-ACTTCCAGGACCGCCTTTGGAGGC-3; downstream primer sequence 5-GTTGAC-GCCCCGGGGCACC-3, underlined G in the 3' end of downstream primer is a mismatched base and it should be A. A restriction site of Hin6I (GCGC) can be introduced into the normal sequence and there is no restriction site of Hin6I in the sequence of Leu mutant type. PCR primers were synthesized by Beijing Genomics Institute. Hind6I restriction endonuclease was provided by Fermentas Company, DNA Marker was provided by TransGen Biotech, and DNA extraction kit purchased from Tiangen Biotech Co., Ltd. PCR system was 25 μ l, which included 1.5 μ l of DNA template, 2.5 μ l of 10 \times PCR buffer, 2.0 μ l of MgCl₂, 2.0 μ l of dNTP (10 mmol/L), respective 0.5 μ l (5 μ mol/L) of upstream primer and downstream primer, 0.125 μ l of TaqDNA polymerase, and the remaining was filled with sterile deionized tri-distilled water. Cycling parameters are: pre-denaturation at 95°C for 4 min, 37 cycles, denaturation at 94°C for 30 s, annealing at 66°C for 30 s, extension at 72°C for 30 s, final extension at 72°C for 5 min. Use 2.0% agarose gel electrophoresis and detection method of G to test PCR products. Analysis of restriction endonuclease: Total reaction volume was 20 μ l (containing 10 μ l of PCR products and 10 U of restriction endonuclease Hin6I), after mixing, put at 37°C for digestion of 4 h. Use 4% agarose gel electrophoresis and Gold View nucleic acid dye staining to analyze the results. Take DNA Marker as the standard to identify the genotype of the PCR product. Select the genotypes identified as wild-type, heterozygous mutant type and homozygous mutant type for sequencing of DNA fragments.

Statistical processing

Use the SPSS 17.0 software for statistical analysis. Results of measurement data are shown as Mean \pm Standard Deviation (\pm s). Clinical and biochemical indicators between the PCOS group and the control group were compared using independent sample t-test. Genotype and allele frequency between the two groups were com-

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Table 1. Clinical characteristics and biochemical indicators in PCOS group and the control group

Parameters	PCOS group	Control group	T	P
Age (Year old)	28.56±7.42	29±9.13	1.81	0.06
BMI (kg/m ²)	26.45±5.81*	22.33±5.58	7.23	0.00
FSH (IU/L)	5.19±2.99	4.97±3.53	1.53	0.67
LH (IU/L)	16.8±3.61*	9.23±4.67	4.56	0.01
LH/FSH	3.52±0.57	1.9±0.67	4.92	0.03
T (ng/mL)	0.67±0.85*	0.42±0.22	3.45	0.02
TC (mmol/L)	4.15±0.54	4.09±0.54	1.16	0.25
TG (mmol/L)	2.63±0.98	1.24±0.34	5.39	0.00
HDL (mmol/L)	0.44±0.47*	0.93±0.56	3.45	0.02
LDL (mmol/L)	2.58±0.73	2.45±0.73	4.87	0.16

Footnotes: *P < 0.05 vs control group.

Table 2. Distribution of genotypes and alleles of FXIIIVal34Leu (n, %)

Group	Genotype			Allele	
	Val/Val	Val/Leu	Leu/Leu	Val	Leu
PCOS group	118 (98.34%)	1 (0.83%)	1 (0.83%)	237 (98.75%)	3 (1.25%)
Control group	89 (98.9%)	1 (1.11%)	0 (0)	179 (99.46%)	1 (0.56%)
χ ² value	0.009	0.757	2.890	0.316	
P value	0.93	0.09	0.39	0.56	

Footnotes: There is no significant difference of Leu allele frequency between PCOS group and the control group, P > 0.05.

pared using chi-square test χ^2 . P < 0.05 was considered as statistically significant differences between the two groups.

Results

Determination of genotype

The amplification product of PCR was an 114 bp DNA fragment containing a Hin6I restriction site, which can be cut into two fragments of 94 bp and 20 bp. The mutation of codon 34 on FXIII A subunit led to replacement of its encoding valine (Val) by leucine (Leu) and elimination of Hin6I restriction site. When mutation of homozygotes occur, after digested by Hin6I, the agarose gel electrophoresis of PCR product can only get a 114 bp band; while that of Val34/Leu34 heterozygous can see 3 bands of 114 bp, 94 bp and 20 bp; that of Val34 wild-type can see 2 bands of 94 bp and 20 bp. wild-type and Leu34 genotype was selected for sequencing after PCR amplification. Sequencing results of Val34/Val34 wild-type showed the restriction site of Hin6I was GCGC, and restriction site of mutant type was GCTC.

Clinical characteristics and biochemical indicators of PCOS patients and the control group are seen in **Table 1**. Compared to the control group, the level of BMI, T and LH in the PCOS patient group were significantly higher, and the level of HDL was significantly lower (P < 0.05). Also the average BMI of patients with polymorphism of FXIII AVal34Leu gene was (34.56±4.83) kg/m², which was significantly higher than patients without polymorphism of FXIII AVal34Leu gene and patients in the control group, and HDL level in the blood was significantly lower (0.23±0.11) mmol/L.

After the test, there were 118 cases of Val/Val genotype, 1 case of Val/Leu genotype and 1 case of Leu/

Leu genotype of FXIIIVal34Leu in PCOS group; 1 case of Val/Leu genotype was found in the control group. There is no significant difference of Leu allele frequency between PCOS group and the control group, P > 0.05. The distribution of genotypes and alleles of PCOS group and the control group are seen in **Table 2**.

Discussion

PCOS is a common gynecological endocrine disease and is often associated with type 2 diabetes and cardiovascular disease. However, so far the cause of PCOS remains unclear. Various studies have showed that PCOS are a gene-related disease and is likely to be caused by some disease-related genes and environmental factors. In recent years, searching for the disease-related genes and susceptibility genes of PCOS has become a hot spot of research.

PCOS and type 2 diabetes are all polygenic diseases, both of which have many common genetic features. Insulin resistance and hyperinsulinemia are the common pathophysiological basis of occurrence and development of

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PCOS and type 2 diabetes. About 50%~70% of PCOS patients have different degrees of insulin resistance and compensatory hyperinsulinemia. Insulin resistance and hyperinsulinemia are the basic characteristics of abnormal glucose metabolism of PCOS patients, and are closely linked to long-term complications of PCOS such as type 2 diabetes, hypertension and cardiovascular disease [7-9]. Action of excess insulin on insulin receptors can promote release of LH and ovarian and adrenal androgen secretion, inhibit synthesis of sex hormone-binding globulin in the liver, increase the free testosterone and suppress lipolysis, thereby leading to fat accumulation and increase of TG and LDL as well as decrease of HDL in PCOS patients [10, 11], thus abnormal lipid metabolism occur. The BMI (26.45 ± 5.81) kg/m², androgen level (0.67 ± 0.85) ng/mL and LH (16.8 ± 3.61) IU/L of PCOS patients in the study were all significantly higher than the control group. While HDL level of PCOS patients (0.44 ± 0.47) mmol/L was significantly lower than the control group (0.93 ± 0.56) mmol/L, and TG level (2.63 ± 0.98) mmol/L was significantly higher than the control group (1.24 ± 0.34) mmol/L. This was consistent with results of former research, which confirmed the metabolic characteristics of high cholesterol in PCOS patients.

Is there any gene polymorphism that causes insulin resistance of patients, thereby causing metabolic abnormalities in PCOS patients? In 2013, Lin et al. found in their study that Fetuin-A gene rs2248690 polymorphism may be related to insulin sensitivity of PCOS patients of Han population in Chongqing [12]. Thus the study of gene polymorphism associated with insulin sensitivity has become an important aspect in etiologic research of PCOS. Search for gene polymorphism associated with insulin sensitivity has provided a new way of thinking for etiologic research of PCOS.

Factor XIII (FXIII), the last clotting factor in clotting process, was identified in 1991 as a family member of transglutaminase (TG) in plasma. It functions through tetramer composed of two A subunits and two B subunits, but the A subunit and the B subunit are encoded by different genes and expressed in different cells. FXIII A1 was found in its protein sequences a large number of polymorphisms with genetic heterogeneity. On codon 34 on the exon 2 in FXIII A subunit gene, G→T mutation occurs, leading to

the replacement of its encoding Val by Leu, namely Val34Leu polymorphism, which can significantly increase the transglutaminase activity of plasma FXIII. It can affect activity of plasma FXIII and stability of fibrin cross-linking structure stability, which forms more easily a stable fibrin polymer and promotes thrombosis, and is related to the occurrence of many diseases [13, 14]. Studies have shown that polymorphism of FXIII A1 gene is not only related to concentration of fibrin, but also related to insulin resistance [4, 5]. Naukkarinen et al. found in their study that the mutation of FXIII A1 gene is highly correlated with BMI and high-density lipoprotein level [15], and polymorphism of FXIII A1 gene may be related to metabolism of PCOS patients [16]. In the study, the BMI (34.56 ± 4.83) kg/m² of patients with polymorphism of FXIII A1 gene was significantly higher than patients in the control group and other patients and the HDL level in the blood was significantly lower (0.23 ± 0.11) mmol/L, suggesting polymorphism of FXIII A1 gene may be related to BMI and lipid metabolism of patients. However, as the sample size of the study is relatively small, it remains to be confirmed by large sample studies.

For different races, frequency of Val34Leu polymorphism gene differs greatly. Leu34 allele frequency of Australians is 0.27, Finns 0.14 and Germans 0.11 [17, 18]. Leu frequency of the control group in the study was 0, which demonstrated the distribution rate of this allele is very low. In PCOS group there was 1 patient with Val/Leu heterozygotes and 1 patient with Leu/Leu homozygotes, whose Leu34 allele frequency was not significantly different from the control group. It is suggested that FXIII Val34Leu mutation may not be the common site of PCOS gene mutation and polymorphism of FXIII A1 gene may not be related to PCOS. However, as the sample size of the study is relatively small, it remains to be confirmed by large sample studies.

Disclosure of conflict of interest

None.

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