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Correlation between endometriosis combined with infertility and STAT3 gene polymorphisms

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ABSTRACT

Objective: To investigate the correlation between STAT3 gene polymorphisms and endometriosis complicated with infertility. **Methods:** A total of 35 patients with endometriosis complicated with infertility and 35 cases of healthy volunteer from October 2014 to October 2015 in our hospital were selected as research objects. STAT3 gene polymorphisms of all objects were detected by PCR-RFLP method. **Results:** Polymorphic sites of STAT3 gene rs2293152 were expressed as three genotypes, namely, CC, GC, and GG. There were 18 cases, 10 cases and 7 cases of type CC, GC and GG in the observation group, accounted for 51.43%, 28.57% and 20.00%, respectively. There were 29 cases, 3 cases and 3 cases of type CC, GC and GG in the control group, accounted for 82.86%, 8.57% and 8.57%. There was a statistically difference` between the two groups. The frequency of C and G allele in the observation group and the control group were 65.71%, 34.29% and 87.14%, 12.86%, respectively. There were statistically significant differences between two groups. In addition, compared with the CC genotype, genotype G might increase the risk of the disease. **Conclusions:** The susceptibility of endometriosis complicated with infertility may be associated with STAT3 gene polymorphism and women who carried the G allele may have an increased the risk of the disease.

1. Introduction

Epidemiological studies have shown that endometriosis usually occurs in women of childbearing age, with the morbidity of 6%-10%[2], but in infertile women, the morbidity is as high as 45%-50%[3]. Studies show that endometriosis will cause symptoms such as dysmenorrhea and chronic pelvic pain, and cyclical bleeding of endometrial cells with ectopic growth can increase the risk of infertility in women of childbearing age[4,5]. The pathogenesis of endometriosis is still unclear, but it commonly occurs in neovascularization and cell malignant proliferation, which is similar with the occurrence and metastasis of tumor. Signal Transducer and Activator of Transcript-3 (STAT3) are closely associated with biological behaviors of cells such as proliferation, differentiation, invasion, and apoptosis. It is reported that endometriosis biological behaviors similar to malignant tumor through STAT3 signaling pathway[6,7]. In recent years, it was found that single nucleotide polymorphisms (SNPs) may be the main factor to cause genetic differences among different individuals[8]. The present study aimed to detect the STAT3 gene polymorphisms in patients with endometriosis complicated with infertility by PCR-RFLP so as to investigate the correlation between the disease and STAT3 gene polymorphisms.

2. Materials and methods

2.1. General information

A total of 35 patients with endometriosis complicated with infertility from October 2014 to October 2015 in our hospital were selected as observation group, meanwhile, 35 cases of healthy

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volunteer were selected as control group, aging between 20 and 34 years, with the average age of (25.13 ± 3.34) years. All the objects are Han nationality. The nationality, age, age of menarche, menstrual history and reproductive history of women in observation and control groups had no statistically difference and were comparable (P>0.05). Exclusion criteria: 1. Patients with endometrial polyp, endometrial cancer, myoma of uterus and other gynecological oncologies; 2. Patients with primary or secondary metabolic disorders; 3. Patients recently used hormone or immunosuppressive drugs

2.2. Sampling and DNA extraction

Five mL of whole blood was collected from elbow vein of women in two groups by using a plain tube contained sodium citrate and under aseptic condition. Then the white blood cell was separated from the sample and was conducted to a DNA extraction by using QIAamp DNA extraction kit and afterwards it was stored at 20 ℃ until use.

2.3. The selection of STAT3 polymorphism loci

According to the data of STAT3 gene of Chinese Han population from HapMap database, the selection of STAT3 polymorphism loci and the loci determination were carried out. The screening criteria: (1) there was a sequence which could be used for the PCR primers in SNP loci; (2) the heterozygosity of SNP was more than 8%; (3) the loci had the endonuclease sites and its enzyme product could be segregated by electrophoresis.

2.4. Detection of gene polymorphism

The detection of gene polymorphism was carried out according to the reference[9]. The primer was designed and synthesized by Shanghai genePharma Co.,Ltd. (5'-TCCCCTGTGATTCAGATCCC-3', 3'-CATTCCCACATCTCTGCTCC-5', 233bp). The PCR reaction conditions: 94 ℃/4 min, 94 ℃/30 s, 55 ℃/30 s, 72 ℃/30 s, 30 circulation; 72 ℃/10 min.

2.5. Statistical analysis

The SPSS 19.0 software package was used for the data analysis. The enumeration data were expressed as a percentage and detected by chi-square test. P<0.05 was considered statistically significant.

3. Results

3.1. The comparison of distribution of STAT3 gene polymorphism

There were three genotypes expressed in the STAT3 gene rs2293152 polymorphism sites, namely, CC, GC, and GG. In observation group, 18 cases (51.43%) were CC homozygotic type, 10 cases (28.57%) were GC hybrid subtype and 7 cases (20.00%) were GG homozygotic type. While in control group, there were 29 (82.86%), 3 (8.57%) and 3 cases (8.57%) in CC, GC and GG genotypes, respectively. There were significant differences in the comparison of genotype distribution between two groups (χ^2 = 7.944, P = 0.019). The frequencies of C and G alleles in observation group and control group were 65.71%, 34.29% and 87.14%, 12.86%, respectively, and there were significant differences existed (χ^2 = 8.921, P = 0.003) (Table 1).

Table 1

The comparison of distribution of genotype and allele between two groups [%, (n/N)].

Group	Control group (n = 35)	Observation group (n = 35)	χ^2-value	P-value
Genotype			7.944	0.019
CC	82.86 (29/35)	51.43 (18/35)		
GC	8.57 (3/35)	28.57 (10/35)		
GG	8.57 (3/35)	20.00 (7/35)		
Allele			8.921	0.003
C	87.14 (61/70)	65.71 (46/70)		
G	12.86 (9/70)	34.29 (24/70)		

3.2. The correlative analysis of gene polymorphism and EMs

The Table 2 shows that G allele (CG+GG) may increase the onset risk of EMs complicated with infertility (χ^2 = 7.835, P = 0.005, OR = 4.565, 95% CI: 1.518-13.727) (Table 2).

Table 2

The correlative analysis of STAT3 gene polymorphism and EMs.

Group	Control group (n = 35)	Observation group (n = 35)	χ^2-value	P-value	OR(95% CI)
CC	82.86 (29/35)	51.43 (18/35)	7.835	0.005	1.000 (reference)
CG+GG	17.14 (6/35)	48.57 (17/35)			4.565 (1.518-13.727)

4. Discussions

The patients with EMs have the similar biological behaviors to malignant tumor like a poor prognosis and a high recurrence rate[10]. Up to now, the etiology and pathogenesis related to EMs are still unclear. The correlative reference had shown that the onset of EMs was significantly correlated with the family heredity, therefore it was concluded that EMs might be a polygenic disease[10,11]. The previous study reported that there might be some kind of correlation between the onset of EMs and STAT3 gene polymorphism[12]. The STAT3 exists in various tissues and it can enter into the nucleus by forming a dipolymer by phosphorylation as well as activation, and thereby being involved in various biology processes such as the cell proliferation, cell differentiation, cell invasion and cell apoptosis, etc. through regulating the gene expression (cyclin D1, VEGF, bcl-2, etc.)[13,14]. Besides, the research revealed that STAT3 exerted a significant role in the process of women pregnancy by regulating

the endometrial decidualization[15]. Though investigating 30 cases of patients with endometriosis, the researcher found that the level of STAT3 phosphorylation in endometrial tissues of patients with endometriosis increased significantly. In the study by Wang, et al.[16], it was also reported that there was a high expression of STAT3 protein in patients with EMs complicated with infertility, which showed that the onset of EMs complicated with infertility might closely correlated with the STAT3. However, there is few study on the correlation between the EMs complicated with infertility and STAT3 reported so far.

There is little genetic difference between different individual genome sequence, and the SNPs is a common variation in the sequence. The study showed that SNPs polymorphism was associated with the difference of an individual's susceptibility to disease[17]. By investigating 1021 cases of patients with liver cancer, Xie et al. found that the STAT3 rs2293152 polymorphism might increase the risk of disease[18], and Yan et al. also found a result which was in accordance with that of Xie et al., by a meta-analysis of 15 papers about the STAT3 gene polymorphism and cancer[19,20].

Therefore, the present study attempted to detect the STAT3 gene polymorphism of patients with EMs complicated with infertility and then to discuss the correlation between them. The result revealed that there were significant differences in the genotypes of STAT3 gene rs2293152 polymorphism loci between two groups, and the frequencies between C and G alleles were significantly different. What's more, there might an increased onset risk in patients carried G allele (CG + GG). In the present study, the results showed that the genetic predisposition of EMs complicated with infertility might be associated with the STAT3 gene rs2293152 and the patients carried G allele might have an increased risk of disease.

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