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Effects of gene polymorphism and serum levels of IL-2 and IL-6 on endometriosis

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Abstract. – OBJECTIVE: EMT is closely related to gene polymorphism and the expression level of immune-related substances in patients. Therefore, the aim of this study was to investigate the relationship between single nucleotide polymorphism (SNP), as well as serum levels of interleukin 2 (IL-2) and interleukin 6 (IL-6) in patients with endometriosis (EMT) and disease susceptibility.

PATIENTS AND METHODS: Peripheral blood of EMT patients and healthy people were collected, respectively. Genomic deoxyribonucleic acid (DNA) was extracted and sequenced to obtain gene polymorphisms of IL-2 rs11575812 (T>C), rs2069772 (A>G), rs2069762 (T>G), and IL-6 rs1800795 (C>G). Meanwhile, the serum levels of IL-2 and IL-6 were determined by the relative kits.

RESULTS: For IL-2 rs11575812 allele (C>G), the odds ratio (OR) was 0.49, the 95% confidence interval (CI) was 0.37-0.66, and the p-value was 0. For IL-2 rs2069772 allele (C>G), the OR was 0.97, the 95% CI was 0.73-1.27, and the p-value was 0.83. For IL-2 rs2069762 allele (T>G), the OR was 1.73, the 95% CI was 1.31-2.29, and the p-value was 0. For IL-6 rs1800795 allele (C>G), the OR was 1.26, the 95% CI was 0.96-1.66, and the p-value was 0.09. CC genotype (p=0.000) and TT genotype (p=0.040) of IL-2 rs11575812 (T>C), AG genotype (p=0.000) of IL-2 rs2069772 (A>G), and GT genotype (p=0.000) of rs2069762 (T>G) were remarkably associated with the serum level of IL-2 in patients with EMT. Similarly, the CG genotype (p=0.000) of IL-6 rs1800795 (C>G) was significantly correlated with the serum level of IL-6 in patients with EMT. IL-2 haplotype CAG (p=0.005), CAT (p=0.001), CGG (p=0.047), TAG (p=0.000), and TGG (p=0.000) were significantly different from other haplotypes. Furthermore, there was a significant correlation between the serum levels of IL-2 and IL-6 (r=0.63, p<0.001).

CONCLUSIONS: IL-2 rs11575812 (T>C) TT genotype, rs2069772 (A>G) AG genotype and rs2069762 (T>G) GG genotype increases the risk of EMT, which are related to the serum levels of IL-2 and IL-6.

Key Words:
IL-2, IL-6, Single nucleotide polymorphism (SNP), Endometriosis (EMT).

Introduction

Endometriosis (EMT) is an increasingly common gynecological disease, which affects 6-10% of young and middle-aged women worldwide[1]. Eventually, EMT can lead to long-term chronic pelvic pain, dysmenorrhea, severe dyspareunia, infertility, and pelvic organ dysfunction. Currently, the difficulty in the detection and diagnosis of EMT may result in a considerable probability of delayed diagnosis[2]. Meanwhile, no reliable laboratory biomarker has been identified to help diagnose this disease. As a result, a special observation on highly susceptible population is the best prevention and treatment method for EMT.

The best-accepted pathogenesis of EMT is the retrograde menstruation theory proposed by Sampson JA[3]. As up to 90% of healthy women undergo retrograde menstruation, there may be other pathogenic mechanisms, such as immune factors. EMT is an inflammatory disease that activates macrophages and cytokines, including interleukin-8 (IL-8), IL-1β, tumor necrosis factor-α (TNF-α), as well as the migratory inhibitory factor (MIF) in the peritoneal fluid of patients. Moreover, some inflammatory biomarkers have also been found significantly elevated in serum, such as IL-2, IL-4, IL-6, IL-8, MCP-1, CCL5, and YKL-40[4]. Current studies have demonstrated that the activation of various cytokines and biological markers in the immune system is one of the most important characteristics of EMT.

Single nucleotide polymorphism (SNP) of cytokine genes has been confirmed to be associated

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with multiple diseases. Such correlation can be found between the gene polymorphism of interferon-γ (IFN-γ) (+874A/T) and schizophrenia[5], the gene polymorphism of IL-6 (-174 G/C) and stomatitis[6], gene polymorphisms of IL-17A and IL-17F and psoriasis[7], as well as the gene polymorphism of SOCS3 and liver cancer[8]. Furthermore, cytokine gene polymorphism may affect the level of immune-related substances in patients with EMT, exerting an influence on its occurrence.

IL-2 and IL-6 may play a key role in the immune system of patients with EMT. Starting from the SNPs of IL-2 and IL-6 genes, we first explored the gene polymorphisms of IL-2 rs11575812 (T>C), rs2069772 (A>G), rs2069762 (T>G), and IL-6 rs1800795 (C>G). Moreover, their roles in EMT were investigated *via* analyzing the content of IL-2 and IL-6 in patients' peripheral blood (PB).

Patients and Methods

Basic Information

A total of 212 EMT patients who received treatment in our hospital from 2013 to date were enrolled in the EMT group. Meanwhile, 201 healthy people who underwent physical examinations during the same period were enrolled in the control group. PB samples of patients in the EMT group and control group were collected, respectively. Inclusion criteria of the EMT group were as follows: patients with progressive aggravating dysmenorrhea, abnormal menstruation or unexplained infertility and other symptoms; patients had small hard nodules with a volume of about 2-10 mm^3; patients with pain in rectouterine fossa, uterosacral ligament, and cervical posterior wall found by gynecological examination; and patients with cyst or mass revealed by ultrasonography and confirmed by laparoscopy combined with biopsy. In this study, no significant difference was observed in the basic information between the EMT group and control group ($p>0.05$). This investigation was approved by the Ethics Committee of Ningbo Women & Children's Hospital. Signed written informed consents were obtained from all participants before the study.

Sample Processing

A total of 5 mL of PB was collected from patients in the EMT group and control group, respectively. The collected samples were centrifuged at 3000 rpm for 10 min within 2 h, followed by separation of the serum of the upper layer and the nucleated cells of the middle layer into new centrifuge tubes. Subsequently, the serum was stored in liquid nitrogen for detection. The nucleated cells were used to extract genomic deoxyribonucleic acid (DNA).

DNA Extraction and Gene Polymorphism Analysis of IL-2 and IL-6

Genomic DNA in the nucleated cells was extracted according to the instructions of QIAamp DNA Blood Kit (Qiagen, Hilden, Germany). The primers for IL-2 and IL-6 genes were designed. Genomic DNA was amplified *via* PCR. 2 μL of the product was taken for agarose gel electrophoresis until the UV imaging was qualified. Next, amplified product was sent to IGE Biotechnology Co., Ltd. (Madhyamgram, Kolkata, India) for sequencing. After obtaining sequencing data, the gene polymorphisms of IL-2 rs11575812 (T>C), rs2069772 (A>G), rs2069762 (T>G), and IL-6 rs1800795 (C>G) were analyzed.

Detection of Serum Levels of IL-2 and IL-6

Serum samples stored in liquid nitrogen were taken out, and the levels of IL-2 and IL-6 were measured in accordance with multiple cytokine kit (Invitrogen Human Cytokine 30-Plex Panel, Carlsbad, CA, USA). Based on the specifications of Thermo Fisher Scientific Corporation (Waltham, MA, USA), the detection was carried out with Luminex 200 system (Luminex Corporation, Austin, TX, USA). The results showed that the average sensitivity was less than 1.0 pg/mL, and the coefficient of variation between batches was 6.7%.

Statistical Analysis

IBM Statistical Product and Service Solutions (SPSS) software (version 22.0, IBM Corp., Armonk, NY, USA) was adopted for all statistical analyses. IL-6 level showed a mixture of normal and abnormal distributions through Shapiro-Wilk test, therefore, the nonparametric test was used to compare the differences between the groups. Enumeration data were expressed by frequency, and the χ^2-test was used for comparison. HaploView software was chosen for IL-2 haplotype analysis. Pearson method was used for the correlation analysis. $p<0.05$ was considered statistically significant.

Table I. Alleles and genotypes [number (frequency)] of IL-2 rs11575812 (T>C), rs2069772 (A>G), rs2069762 (T>G), and IL-6 rs1800795 (C>G) in EMT group and control group.

Locus		Control group	EMT group	OR	95% CI	χ^2	*p*
rs11575812	C	187 (0.465)	128 (0.302)	0.49	0.37-0.66	23.32	0
	T	215 (0.535)	296 (0.698)				
	CC	48 (0.239)	32 (0.151)	0.98	0.72-1.22	24.01	0
	CT	91 (0.453)	64 (0.302)				
	TT	62 (0.308)	116 (0.547)				
rs2069772	A	183 (0.455)	190 (0.448)	0.97	0.73-1.27	0.04	0.83
	G	219 (0.545)	234 (0.552)				
	AA	41 (0.204)	17 (0.080)	1.22	0.98-1.43	25.51	0
	AG	101 (0.502)	156 (0.736)				
	GG	59 (0.294)	39 (0.184)				
rs2069762	G	205 (0.510)	273 (0.644)	1.73	1.31-2.29	15.18	0
	T	197 (0.490)	151 (0.356)				
	GG	51 (0.254)	97 (0.458)	1.32	0.89-1.44	18.64	0
	GT	103 (0.512)	79 (0.373)				
	TT	47 (0.234)	36 (0.170)				
rs1800795	C	187 (0.465)	222 (0.524)	1.26	0.96-1.66	2.81	0.09
	G	215 (0.535)	202 (0.476)				
	CC	43 (0.214)	56 (0.264)	1.01	0.78-1.23	2.97	0.22
	CG	101 (0.502)	110 (0.519)				
	GG	57 (0.284)	46 (0.217)				

Results

Alleles and Genotypes of IL-2 rs11575812 (T>C), rs2069772 (A>G), rs2069762 (T>G), and IL-6rs1800795 (C>G) in the EMT Group and the Control Group

The number and frequency of alleles and genotypes of IL-2 rs11575812 (T>C), rs2069772 (A>G), rs2069762 (T>G), and IL-6 rs1800795 (C>G) among 413 research subjects were shown in Table I. For IL-2 rs11575812 allele (C>G), the odds ratio (OR) was 0.49, the 95% confidence interval (CI) was 0.37-0.66, and the *p*-value was 0. For IL-2 rs2069772 allele (C>G), the OR was 0.97, the 95% CI was 0.73-1.27, and the *p*-value was 0.83. For IL-2 rs2069762 allele (T>G), the OR was 1.73, the 95% CI was 1.31-2.29, and the *p*-value was 0. For IL-6 rs1800795 allele (C>G), the OR was 1.26, the 95% CI was 0.96-1.66, and the *p*-value was 0.09.

Relationship Between Serum Level of IL-2 and IL-2 Gene Polymorphism in the EMT Group and the Control Group

The serum level of IL-2 in the EMT group and control group was shown in Table II, Figure 1, Figure 2, and Figure 3. For IL-2 rs11575812

Table II. Serum level of IL-2 (ng/L) in EMT group and control group.

	Genotype	Control group	EMT group	*p*
rs11575812	CC	0.45	1.53	0.000
	CT	0.99	1.12	0.070
	TT	0.88	1.23	0.040
rs2069772	AA	1.23	1.27	0.564
	AG	0.46	1.55	0.000
	GG	0.99	1.05	0.783
rs2069762	GG	0.91	1.23	0.080
	GT	0.73	2.01	0.000
	TT	0.98	1.03	0.329

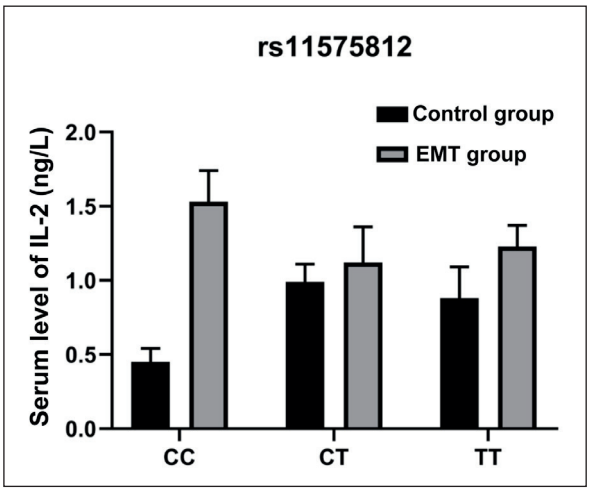

Figure 1. Relationship between serum level of IL-2 and genotype of rs11575812 (T>C).

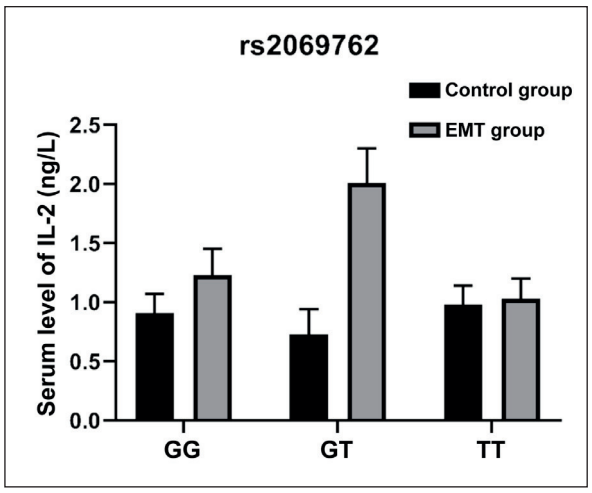

Figure 3. Relationship between serum level of IL-2 and genotype of rs2069762 (T>G).

(T>C), the *p*-value was 0.000 for CC genotype, 0.070 for CT genotype, and 0.040 for TT genotype. For IL-2 rs2069772 (A>G), AA genotype (p=0.564), AG genotype (p=0.000), and GG genotype (p=0.783). For rs2069762 (T>G), GG genotype (p=0.080), GT genotype (p=0.000), and TT genotype (p=0.329).

Relationship Between Serum Level and Polymorphism of IL-6 in the Two Groups

The serum level of IL-6 in the EMT group and the control group was shown in Table III. The results demonstrated that IL-6 rs1800795 (C>G) CC genotype (p=0.132), CG genotype (p=0.000), GG genotype (p=0.070) (Figure 4).

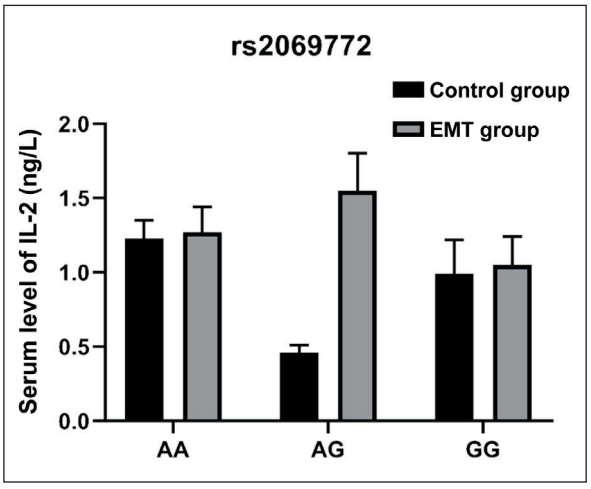

Figure 2. Relationship between serum level of IL-2 and genotype of rs2069772 (A>G).

Haplotype Analysis of IL-2 rs11575812 (T>C), rs2069772 (A>G), and rs2069762 (T>G)

Haplotype analysis of IL-2 rs11575812 (T>C), rs2069772 (A>G), and rs2069762 (T>G) in the EMT group and control group was shown in Table IV. Meanwhile, the results of haplotype analysis *via* the HaploView software were shown in Figure 5. IL-2 haplotype CAG (p=0.005), CAT (p=0.001), CGG (p=0.047), TAG (p=0.000), and TGG (p=0.000) were significantly different from other haplotypes.

Correlation Analysis of Serum Levels of IL-2 and IL-6

There was a remarkable correlation between the serum levels of IL-2 and IL-6 (Figure 6). Pearson correlation coefficient: r=0.63, p<0.001.

Discussion

Currently, the etiology of EMT remains unclear, which may involve a number of environmental and genetic factors[9]. Social relations and psychology of EMT patients have been markedly disturbed[10]. A variety of substances in the immune system, including cytokines, play an important role in the progression of EMT. Previous studies have demonstrated that SNPs in the promoter region of cytokine-related genes may be closely associated with EMT development. Numerous researches[11,12] have pointed out that SNPs of several members in the IL family show

Table III. Serum IL-6 level (ng/L) in EMT group and control group.

	Genotype	Control group	EMT group	p
rs1800795	CC	1.35	1.51	0.132
	CG	0.59	1.74	0.000
	GG	1.21	1.34	0.070

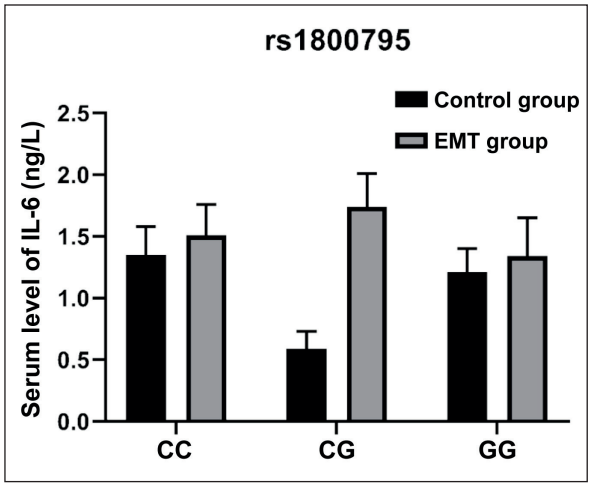

Figure 4. Relationship between serum level of IL-6 and genotype of rs1800795 (C>G).

regulatory effects on EMT, including IFN-γ, IL-1b, IL-4, IL-10, IL-1R1, IL-1 receptor antagonist, and IL-2R. In addition, a research[13] has indicated that IL-37, IL-17A, IL-10, and IL-2 may be important in the immune response of EMT. Meanwhile, IL-37 level can serve as a diagnostic marker for EMT. These findings suggest that cytokines have a notable influence on EMT. Cytokine gene polymorphism may affect its serum level, thereby playing an important role in EMT. This study mainly investigated the correlation between gene polymorphism, as well as the serum levels of IL-2 and IL-6 and EMT.

IL-2 and IL-6 systems have been considered to play a crucial role in the pathophysiology of EMT. IL-2 is able to activate leukocytes in patients with EMT[14], or increase T helper type 1 (Th1) and Th2 immune cells[15,16]. This may eventually aggravate the activation and response of the immune system. IL-6 and IL-6R are related to the etiology of EMT. Increasing sIL-6R in the peritoneal fluid by enhancing the biological activity of IL-6 further promotes the development of EMT[17]. Meanwhile, IL-6 also exerts an influence on patients with EMT by inhibiting NK cells[18]. The correlations of EMT with IL-2 and IL-6 gene polymorphisms were explored in this study. The results found that IL-2 rs11575812 (T>C) allele T and genotype TT, rs2069772 (A>G) genotype AG and rs2069762 (T>G) allele G and genotype GG were remarkably linked with the onset risk of EMT. However, no specific allele or genotype of the IL-6 rs1800795 (C>G) was associated with the onset of EMT (Table I).

IL-2 and IL-6 have been found to be correlated with the susceptibility of different diseases, such as chronic lymphocytic leukemia[19] and Behcet's disease[20]. Gene polymorphism of IL-2 and IL-6 may be associated with the serum levels of IL-2 and IL-6 in patients with EMT, thus affecting immune function and regulating the microenvironment. Hence, the serum levels of L-2 and IL-6 in EMT patients were examined in this study. IL-2 rs11575812 (T>C) CC genotype (p=0.000) and TT genotype (p=0.040), IL-2 rs2069772 (A>G) AG genotype (p=0.000), and rs2069762 (T>G) GT genotype (p=0.000) were markedly related to the serum level of IL-2 in patients with EMT (Table II). Similarly, the CG genotype (p=0.000) of IL-6 rs1800795 (C>G) was significantly cor-

Table IV. The haplotype analysis of IL-2 rs11575812 (T>C), rs2069772 (A>G), and rs2069762 (T>G).

Haplotype	Control group	EMT group	χ^2	OR	95% CI	p
CAG	0.799	1.430	7.836	0.498	0.304-0.818	0.005
CAT	0.517	1.163	10.175	0.397	0.221-0.712	0.001
CGG	0.945	1.383	3.959	0.619	0.385-0.996	0.047
CGT	1.633	1.579	0.233	0.905	0.603-1.358	0.629
TAG	1.470	3.062	19.492	2.284	1.573-3.315	0.000
TAT	1.426	1.322	0.431	0.865	0.560-1.335	0.512
TGG	1.866	3.384	15.301	1.980	1.401-2.797	0.000
TGT	1.687	1.112	5.666	0.589	0.380-0.914	0.017

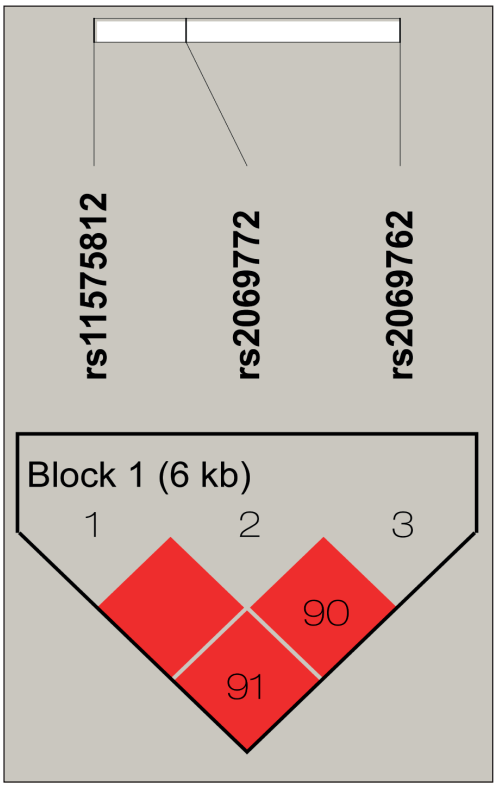

Figure 5. Haplotype analysis of three loci via the HaploView software.

related with the serum level of IL-6 in patients with EMT (Table III). In addition, the serum level of ILs exerted the most direct impact on patients' conditions. All these findings indicated that the above genotypes might further deteriorate the conditions of EMT patients by affecting the serum expression of IL-2 and IL-6.

Haplotype analysis of IL-2 was performed by the HaploView software. The results showed that haplotypes CAG (p=0.005), CAT (p=0.001), CGG (p=0.047), TAG (p=0.000), and TGG (p=0.000) were significantly different from other haplotypes. Meanwhile, these haplotypes were highly correlated with the onset risk of EMT, as well as the serum level of IL-2 (Table IV). By analyzing clinical patients' IL-2 gene haplotypes, the level of IL-2 *in vivo* could be predicted, and the condition of the immune system and the incidence risk of EMT could be indicated. Patients with such IL-2 haplotypes as CAG, CAT, CGG, TAG, and TGG were regarded as high-risk population. As a result, diagnosis and intervention should be made as early as possible to help the treatment of EMT.

The link between the serum levels of IL-2 and IL-6 in EMT patients was discussed. It was

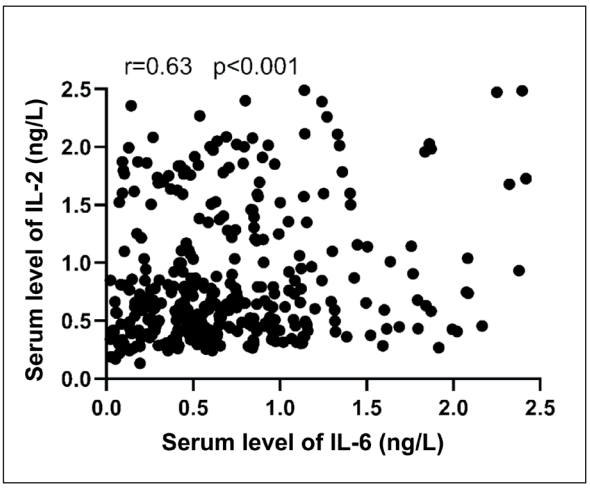

Figure 6. Pearson correlation analysis of serum levels of IL-2 and IL-6.

found that the two molecules had a significant correlation (r=0.63, p<0.001). A high correlation between serum levels of IL-2 and IL-6 in patients with EMT indicated that the immune system was activated. Moreover, a strong inflammatory reaction occurred, deteriorating the disease. Our findings proved that the synergistic effect of IL-2 and IL-6 in EMT might be exerted by combining with other cytokines like IFN-γ and IL-1b to generate a positive feedback effect on the immune system and continuously activate it, thus producing great adverse effects on EMT patients.

Conclusions

We first showed that IL-2 rs11575812 (T>C) TT genotype, rs2069772 (A>G) AG genotype, and rs2069762 (T>G) GG genotype increase the risk of EMT, which are correlated with the serum levels of IL-2 and IL-6.

Conflict of Interests

The authors declared that they have no conflict of interests

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