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Original Article

Correlations between the polymorphism of +869T/C in TGF-β1 and rheumatoid arthritis

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Abstract

Objective: To explore the correlations between the polymorphism of the gene first exon +869T/C in transforming growth factor-β1 (TGF-β1) and rheumatoid arthritis (RA). **Methods:** The patient group included 150 RA patients at the Department of Rheumatology in the First Affiliated Hospital of Chengdu Medical College between March 2014 and May 2017 and 150 healthy cases as the control group. The polymorphism was analyzed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and relationships between RA patients and genotypes were analyzed using logistic regression. **Results:** The genotype frequency distribution and the genotype frequency of +869T/C locus was statistically different between two groups ($P<0.05$). Compared to the control group, the genotype frequency of +869 CC in the inpatient group was significantly lower (17.3% vs 32.7%), while the genotype frequency of +869 TT increased significantly (29.3% vs 20.7%). The T allele frequency in inpatient group was significantly higher than that in control group (57.83% vs 48.82%), while the C allele frequency in control group was significantly higher than that in inpatient group (51.18% vs 42.17%). **Conclusion:** The polymorphism of the gene first exon +869T/C in TGF-β1 significantly correlated with RA and CC genotype might be the susceptible gene of RA.

Keywords: TGF-β1, +869T/C, Polymorphism, Rheumatoid Arthritis

Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease[1]. RA can cause joint pain, deformation, and even disability, having a serious impact on the quality of life of patients. At present, it is still not clear what the etiology of RA is, but most scholars think it is caused by geographical environment and genetic factors[2,3]. A study showed[4] that approximately 20 million people would have been influenced by RA by 2015 worldwide, and the majority of RA patients are in 30~50 years old, the morbidity rate of which in women is three times higher than that in men. The study of Abubakar Il et al. showed[5] that the death rate of RA patients increased by nearly 1.3 times from 28000 in 1990 to 38000 in 2013 in the past 20 years.

The continuous development of molecular biology provides new conditions for studying the mechanism of RA at the gene level. The main manifestation of RA is the abnormal proliferation of synovial tissue contributing to progressive tissue damage, and important biological changes take place in synovial cells in the process of proliferation[6]. In addition to binding to elastase and collagenase, synovial cells are also involved in the recruitment of lymphocytes and macrophages, activating cytokines and adhesion molecules[7]. Any block in these links has an impact on the occurrence and development of RA, and these links are potential therapeutic targets for RA. In the present study, it is shown[8] that the pathological feature of RA was mainly the immune inflammation mediated by the activation of macrophages, plasma cells and T cells caused by synovial infiltration and the release of a large

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number of inflammatory cytokines after the activation of inflammatory cells. Transforming growth factor-β1 (TGF-β1), which is a multifunctional cytokine, is an important member of the transforming growth factor β superfamily regulating the cell response and involved in bone metabolism through joint soft tissue cells or synovial tissues[9]. At present, it is found that there is a gene polymorphism in TGF-β1. Some of the locus gene polymorphisms were associated with the expression level of TGF-β1 and the gene polymorphism of TGF-β1 was associated with the existence of RA.

Therefore, in this study, relationships between the polymorphism of the gene first exon +869T/C and rheumatoid arthritis were examined.

Materials and methods

In this study, one hundred and fifty patients who were diagnosed with RA at the Department of Rheumatology between March 2014 and May 2017 were selected as subjects (inpatient group), including 116 female and 34 male, aged from 33 to 62 years with an average age of 48.58±10.55 years. All subjects in the inpatient group were in accordance with the diagnostic criteria of rheumatoid arthritis established by the American Society of Rheumatology[10]. In addition, the volunteers for physical examination (control group) were selected at the same time in the physical examination center of our hospital to carry out the comparison, including 100 female and 50 male, aged from 30 to 60 years old with an average age of 47.94±11.05 years. This study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Chengdu Medical College. All the patients and their family members were informed and signed the consent.

Inclusion and exclusion criteria

Inclusion criteria: all the patients were more than 18 years old; no major operation had been performed before examination; no blood transfusion treatment had been performed one month before examination; patients had no depression or memory impairment; patients had complete clinical data, cooperated with treatment and were followed up.

Exclusion criteria: patients with less than half a year course of disease; patients with other hereditary diseases; patients with congenital defects; patients with mental illness or disability; patients who have received radiotherapy and chemotherapy within half a year; patients with cancer and immune diseases; pregnant and lactating women.

DNA extraction and RFLP-PCR detection

The peripheral blood (10 mL) was extracted from the venous blood of the subjects and the collected samples were labeled with patients'information. 2 mL was extracted using EDTA-k anticoagulant tube and the other blood samples were packed with different blood vessels and sent to the laboratory of our hospital for the detection of ESR, rheumatoid factor, C reactive protein, acute phase reaction protein and blood routine tests.

The blood samples in EDTA-k anticoagulant tube were stored in -80°C refrigerator and Genomic DNA was extracted from EDTA whole blood using a spin column method according to the protocol (QIAamp Blood Kit; Qiangen, Germany). DNA purity was detected by UV spectrophotometer.

The design and synthesis of +869T/C primers were carried out by Shanghai Bioengineering Co., Ltd and the primer sequence used for +869T/C in upstream was 5'-CGGCACCTCCCCCTGGCTCG-3' and that in downstream was 5'--CCTCCCCACCACACCAG-3'. Reaction system: 2.5 μL for 10XBuffer, 2.5 μL for 25 mmol/L Mg^{2+}, 0.5 μL for upstream primer, 0.5 μL for downstream primer, 2.0 μL for 1 μg/μL DNA template, 1U/μL for Taq enzyme (ABI, United States) and double distilled water added to 25 μL. Reaction conditions: 10 min after pre-denaturation at 94°C, 30 s for denaturation at 94°C, 30 s for annealing at 60°C, 1 min for an extension at 72°C, 40 cycles, and 10 min for an extension at 72°C after the completion of the cycle. Gel Imaging Analysis was obtained from Beijing Maxwell High Technology Co., Ltd.

The PCR products were preserved at 4°C, restriction endonuclease was purchased from Baosheng Biological Co., Ltd and digested with 1U restriction endonuclease Bsu36 I for 2.5 h at 37°C. The genotypic identification of enzyme-digested products identification was performed by 2.5% agarose gel electrophoresis stained by EB, and three genotypes of enzyme-digested products were wild homozygous (CC)(190 bp, 229 bp), mutant heterozygote (CT)(419 bp, 229 bp, 190 bp) and mutant homozygous (TT) (419 bp).

Statistical analysis

The data was examined by Hardy-Weinberg balance using R language genetics package and $P \geq 0.05$ suggested that the gene frequency was in accordance with Hardy-Weinberg's law. SPSS 22.0 (Beijing Situangweida Technology Co., Ltd.) was used for statistical analysis, and all data were expressed by Means ± SD. Comparing the data of the two groups, t-test was used to examine the inter-group measurement data, x^2 was used to examine the inter-group counting data, and relationships between RA patients and genotypes were analyzed using logistic regression. $P<0.05$ suggested that the difference was statistically significant.

Results

The contrast of clinical data between the two groups of patients

Comparison of clinical data and clinical test results between the inpatient group and the control group indicated that there was no statistical difference in the two groups in age, gender, exercise status, residence, smoking history, white blood cell, red blood cell, platelet, hemoglobin and acute phase reaction protein ($P>0.05$), while the rheumatoid factor, ESR and C reactive protein of patients significantly increased, and the difference was significant ($P<0.01$). The details were shown in Table I.

Table I. Contrast of Clinical Data and Clinical Detection Index between the two groups of patients [n(%)].

		Inpatient Group (n=150)	Control Group (n=150)	x^2/t	*P* Value
Gender					0.053
	Male	34 (22.67)	50 (33.33)		
	Female	116 (77.33)	100 (66.67)		
Age (Years)				0.482	0.487
	≥45	83 (55.33)	77 (51.33)		
	<45	67 (44.67)	73 (48.67)		
Exercise Status					0.400
	Yes	50 (33.33)	58 (38.67)		
	No	100 (66.67)	92 (61.33)		
Smoking History					0.257
	Yes	40 (26.67)	50 (33.33)		0.053
	No	110 (73.33)	100 (66.67)		
White Blood Cell ($x10^9$/L)		6.84±2.58	6.65±1.92	0.725	0.470
Red Blood Cell ($x10^9$/L)		4.68±0.35	4.60±0.58	1.446	0.149
Platelet ($x10^9$/L)		294.50±75.35	285.64±62.31	1.110	0.268
Hemoglobin (g/L)		135.54±11.33	133.61±10.83	1.508	0.133
Rheumatoid Factor (IU/mL)		45.88±4.25	13.07±1.58	88.624	0.000
ESR (mm/h)		37.84±3.66	15.32±1.36	70.639	0.000
C Reactive Protein (mg/L)		14.84±9.36	2.54±0.33	16.084	0.001
Acute Phase Reaction Protein (IU/mL)		46.38±4.55	45.76±8.32	0.878	0.380

Table II. Hardy-weinberg balance test.

Gene Locus	Inpatient Group		Control Group	
	X^2	*P*	X^2	*P*
+869T/C	0.747	0.382	0.443	0.506

Table III. Distribution of gene and genotype frequency.

SNP		Inpatient Group	Control Group	X^2	*P*
+869T/C	Genotype				
	CC	26 (17.3%)	49 (32.7%)		0.003
	CT	80 (53.3%)	70 (46.7%)	1.333	0.248
	TT	44 (29.3%)	31 (20.7%)		0.109
	Allele			8.640	0.003
	C	132 (44.00)	168 (56.00)		
	T	168 (56.00)	132 (44.00)		

Table IV. Logistic regression analysis of the relationships between rheumatoid arthritis and genotypes.

Genotype	β	Wald X^2	*P*	OR	95%CI
Sex	0.884	0.384	0.825	1.084	0.358~1.786
CC	0.984	5.758	0.015	2.834	1.384~6.547
CT	0.745	0.358	0.873	1.025	0.325~1.635
TT	0.835	0.425	0.892	1.063	0.348~1.684

Notes: β means regression coefficient and intercept; OR means hazard ratio; Wald X^2 means Chi-square value; 95%CI means confidence interval.

Analysis of genotype and gene frequency of +869T/C locus

The two groups were examined by Hardy-Weinberg balance using R language genetics package, and it is found that all groups were in accordance with Hardy-Weinberg's law suggesting that the study group was representative. The details were shown in Table II. By comparing the genotype frequency of the two groups, we found that the composition ratio was significantly different (x^2=9.972, *P*=0.007) in Table III, and compared to control group, the genotype frequency of CC inpatient group significantly decreased, while that of CT and TT increased. The C allele frequency the inpatient group showed a downward trend, but the T allele frequency increased, and the difference was statistical (*P*<0.05).

Relationships between RA patients and genotypes

Analysis of logistic regression showed that there was a statistical difference suggesting that patients with CC genotype were more likely to develop RA than those with non-CC genotype, and the risk of patients with CC genotype was about 2.8 times higher than that of those with non-CC genotype. The details were shown in Table IV.

Discussion

Rheumatoid arthritis is a chronic systemic inflammatory disease of unclear etiology at present. The main clinical manifestations of RA are symmetrical multiple joint recurrent attacks and cartilage and bone injury caused by the progressive development of synovium and hyperplastic tissue, thus contributing to dysfunction of joint function inpatients[11]. Studies have shown[12] that RA patients without effective treatment have a disability rate of more than 60% within 2~3 years. However, at present, there is no satisfactory specific medicine for the treatment of RA, and normal routine treatment can only alleviate the inflammation of the patients and relieve the pain, so as to improve the mobility and quality of life of patients, but it cannot cure the disease fundamentally. In recent years, the development of many types of drugs has alleviated the course of RA, but it is only a kind of exploratory control therapy and still unable to cure the disease fundamentally[13-15]. Therefore, it is imperative to seek new treatment methods and corresponding nursing methods to delay the conditions of patients and improve their quality of life.

TGF-β1, which is a polypeptide isolated from the cultivation process of mouse T cells transformed by Moloney tumor virus, can induce non-tumor cells to lose their inhibitory proliferation characteristics and further obtain growth function[16]. Studies have shown that TGF-β is expressed by three subtypes in mammalian tissues. TGF-β1 is expressed in connective tissues, and TGF-β2 and TGF-β3 are expressed in epithelial neuron cells and in interstitial cells respectively[17]. A study has shown[18] that a high concentration of TGF-β1 has a function in the promotion of multiple growth factors synthesis and in the protection of newly formed fibroblasts. However, it can also destroy macrophages to produce H_2O_2 destructive to the newly formed fibroblasts. Study of Cheon H reported that the high expression of TGF-β1 in fibroblast-like synovial cells could promote the expression of various inflammatory cytokines (IL-1β, TNF-α, IL-8) in RA patients, thus promoting the occurrence of inflammatory reaction in RA patients. It also showed that the high expression of TGF-β1 in fibroblast-like synovial cells of RA patients was specific indicating that TGF-β1 was involved in the occurrence and development of RA patients' conditions[19].

At present, the gene polymorphism of TGF-β1 has been studied in various diseases. Diseases, related to the polymorphism of TGF-β1 such as myocardial infarction, cancer, hypertension, diabetes, organ fibrosis, and autoimmune diseases, have been reported, and different expression levels of TGF-β1 in blood plasma have been found among individuals[20]. In this study, by detecting the gene polymorphism of RA patients and normal patients, we found that the genotype frequency of CC in +869T/C locus of RA patients in inpatient group significantly decreased when compared to control group, while that of CT genotype and TT genotype increased. The C allele frequency the inpatient group showed a downward trend, but the T allele frequency increased, and there was a difference. We speculated that patients with CC genotype were more likely to develop RA than those with non-CC genotype, and we confirmed our point of view using the following logistic regression analysis. We also cannot explain the reason why these patients lack or carry CC genotype, but we speculate that this might be associated with the living environment of patients.

However, there are a few limitations in this study. First of all, the data of only two groups of patients were contrasted and analyzed, three genotypes of patients were not compared, and it was still not clear whether there was any difference among them. Second, the small number of subject may have an impact on our research and we cannot be certain whether the accuracy of the outcomes was biased. Therefore, we hope that the sample size and content of analysis will be increased to better verify the accuracy of our results in future studies.

In conclusion, the polymorphism of the gene first exon +869T/C in TGF-β1 significantly correlates with rheumatoid arthritis (RA) and CC genotype may be a susceptible gene of RA.

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Authors' contributions

WS and MY collected and analyzed the clinical data of patients. YB, LW, JC, and YR analyzed Genotype and Gene Frequency of +869T/C Locus. XL, HW, and YM were responsible for DNA Extraction. QZ performed PCR. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the First Affiliated Hospital of Chengdu Medical College. Patients who participated in this research signed the informed consent and had complete clinical data. Signed written informed consents were obtained from the patients and/or guardians.

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