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Original Article

Relationship between IL-27 gene polymorphism and susceptibility of rheumatoid arthritis in Chinese Han population

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Abstract: Objective: To investigate the relationship between IL-27 gene polymorphism and the susceptibility of rheumatoid arthritis (RA) in a Chinese Hans population. Methods: 310 RA patients and 310 healthy controls were examined in this study. Polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) technique was used in the detection of the genotype in three loci of IL-27 gene (-964A/G, 2905T/G, and 4730T/C). We compared genotype and allele frequency and distribution of these two groups. Results: The genotype distribution of the case group and the control group were all in accordance with Hardy-Weinberg equilibrium (P>0.05). The difference of genotype and allele frequencies of three loci between these two groups showed no statistically significant (P>0.05). But the frequencies of G-T-C haplotype was significantly higher in the case group than in the control group, the difference showed statistically significant (OR=2.001, 95% CI: 1.121~3.573; P=0.0170). G-T-T haplotype in case group was significantly lower than that in the control group, the difference showed statistically significant (OR=0.715, 95% CI: 0.527~0.970, P=0.030). Conclusion: In Chinese Hans population, IL-27 gene haplotypes were correlated with the risk of RA. G-T-C haplotype was the risk factors for the incidence of RA, but G-T-T haplotype maybe was the protective factor of RA.

Keywords: IL-27, gene polymorphism, rheumatoid arthritis, Chinese Han

Introduction

Rheumatoid arthritis (RA) is a kind of systemic disease which characterized chronic erosive osteoarthritis [1]. Rheumatoid arthritis development always is gradual, if not treated timely, and can cause irreversible joint deformities and joint ankylosis, which has high disability rate [2].

Though the environmental and genetic factors play a major role in the development of the RA [3], the pathogenesis of RA is still unclear. RA is generally believed as an autoimmune disease, variety auto antibodies can be found in the serum of RA patients, including Rheumatoid factor (RF), Anti-cyclic citrullinated peptide Antibody (anti-CCP antibody), Antiperinuclear factor (APF), and Anti-keratin Antibody (AKA). The genetics factors may account for about 50%~60% of the contribution to RA susceptibility. The candidate genes which related with the RA risk, including HLA-DR, PADI4, and PTPN22 have been reported previously [4-6]. IL-27 was discovered and named in 2002 and it is one of a family mumber of IL-6/IL-12, which plays an important role in many immune responses such as anti-infectious and anti-tumor immune response and autoimmune inflammation [7, 8]. Resent research found IL-27 polymorphism was associated with many inflammatory diseases and autoimmune diseases [9, 10]. -964A>G is located in the promoter region of IL-27 gene. In 2007 South Korean scholars found -964A>G genetic polymorphism was related to the asthma susceptibility in Korean population [11]. Also, it was reported that polymorphisms of IL-27 was related to the inflammatory bowel disease [12] and associated with the severity of nasopharyngeal carcinoma [13]. It was also reported that IL-27 gene -964A/G polymorphism was associated with colorectal cancer

Table 1. Sequences of primer and the length of PCR product

Locus	Primers' sequences	Products
-964A>G	Sense: 5'-CCTCTACAGAGCAGAAACACCA-3'	377 bp
	Antisense: 5'-GGCTCTGTCTCCATCTTTAACC-3'	
2905T>G	Sense: 5'-CTGGTTCAAGCTGGTGTCTG-3'	443 bp
	Antisense: 5'-CAGCCTTATCCAGGTTCTATCC-3'	
4730T>C	Sense: 5'-GACCTTTCCTGACCATCTCC-3'	489 bp
	Antisense: 5'-ACTCATACAGACCCACATGCAC-3'	

Table 2. Characteristics of participants

Groups	Age (Years)	Sex (M/F)	ESR (mm/h)	CRP
RA	58 (40~76)	85/225	35.4±12.9	123.4±10.3
Control	57 (35~71)	90/220	10.3±5.8	25.5±7.4
P value	0.781	0.881	<0.001	<0.001

[14]. On the other hand, IL-27 also was associated with chronic hepatitis B virus infection [15]. However, the relation between IL-27 gene polymorphism and RA is remains unclear. In the present study, we aimed to analyze the association of IL-27 genetic polymorphism with RA in a case-control study including 310 RA patients and 310 normal controls.

Material and methods

Subjects

310 RA patients were selected as case group (male: 85; female 225). The average age of patient is 58 (40~76) years old. The course of disease was from 6 months to 15 years. All the selected RA patients were all Han people who were from the same area. The diagnosis was carried out according to the American College of Rheumatology Criteria for Diagnosis of Rheumatic Diseases [16]. Clinical data including onset ages, the number of swelled joint and painful joint, the number of deformed joint, the time of morning stiffness and inspection indexes of X-RAY and biochemistry examination (erythrocyte sedimentation rate, ESR, rheumatoid factor, RF, Anti CCP antibody) were collected. 310 cases of health people were selected as control group who did health examination in the First Affiliated Hospital, Chongqing Medical University including 90 cases of male and 220 cases of female. The average age of control is 57 (age 35~71) years old. All the controls had no symptoms of RA, had no family history of autoimmunity autoimmunization, infection, tumor and other diseases. There were no significant differences between these two groups in gender and age (P>0.05).

Methods

5 ml of peripheral venous blood with EDTA anticoagulation of each participants were collected. Genomic DNA extraction kit (Beijing TianGen Biochemical Company, Catalog number DP319-01) were utilized to extract genomic DNA. The primers were designed according to the gene sequence of IL-27(NC-000016.9) by using the primer 5.0 software. Sequences of primer and the length of PCR product were shown in **Table 1**.

PCR reaction system: DNA template was 1 μl, the primers of upstream and downstream were all 1 μl, 10 μl of Mix, sterile demonized water was added, the final volume is 50 μl. The reaction conditions: 94°C pre-degeneration in 5 min; 94°C pre-degeneration 30 s; 60°C annealed 1 min, 72°C extended 45 s, 0.5°C was decreased every circle; 94°C denatured 30 s, 65°C annealed 1 min,72°C extended 45 s, there were 30 cycle; 72°C extended 3 min, the reaction was terminated till 4°C. PCR reaction kit was purchased from Beijing Ding Guo Lu Sheng Biotech Corp (universal PCR kit). Genotyping were performed according to previous protocol.

Statistical analysis

SPSS17. 0 was used for statistical analysis. Genotype and allele frequency were calculated with direct notation. Hardy-Weinberg Equilibrium (HWE) was tested using χ^2 test, We also used χ^2 test to compare the difference, between these two groups in distribution of genotype and allele three loci (-964A/G, 2905T/G, 4730T/C) in IL-27 gene, a P-value of less than 0.05 was considered to be statistically significant.

Results

General information comparison of two groups

As it was shown in **Table 2**, there were no significant differences between these two groups in gender and age (P>0.05).

Table 3. Distribution of genotype and allele of IL-27 gene in two groups

SNPs	Genotype/allele	RA (n, frequency)	Control (n, frequency)	P	OR	95% CI
-964A/G	AA	153 (0.494)	152 (0.490)	0.069		
	AG	146 (0.471)	134 (0.432)			
	GG	11 (0.035)	24 (0.077)			
	A	452 (0.729)	438 (0.706)	0.377	1.117	0.872~1.431
	G	168 (0.271)	182 (0.294)			
2905T/G	TT	265 (0.855)	262 (0.845)	0.942		
	TG	43 (0.139)	46 (0.148)			
	GG	2 (0.006)	2 (0.006)			
	T	573 (0.924)	570 (0.919)	0.751	0.935	0.617~1.415
	G	47 (0.076)	50 (0.081)			
4730T/C	TT	231 (0.745)	245 (0.790)	0.400		
	TC	76 (0.245)	62 (0.200)			
	CC	3 (0.010)	3 (0.010)			
	T	538 (0.868)	552 (0.890)	0.222	1.237	0.878~1.742
	C	82 (0.132)	68 (0.110)			

Table 4. Haplotype analysis between case and control

Haplotypes	Case (freq)	Control (freq)	Chi^2	*P* value	OR	95% CI
A-T-T	452.00 (0.729)	438.00 (0.706)	0.780	0.377107	1.118	0.873~1.432
G-G-C	47.00 (0.076)	50.00 (0.081)	0.101	0.751040	0.935	0.618~1.416
G-T-C	35.00 (0.056)	18.00 (0.029)	5.697	0.017030	2.001	1.121~3.573
G-T-T	86.00 (0.139)	114.00 (0.184)	4.674	0.030671	0.715	0.527~0.970

Association between IL-27 SNPs and rheumatoid arthritis

Genotyping was performed on 310 patients with RA and 310 controls. Three loci were all in line with Hardy-Weinberg Equilibrium (All *P*>0.05), which indicated that the selected samples can represent the total population. As shown in **Table 3**, the distributions of these three loci between control group and RA group were no significant differences (all *P*>0.05).

Haplotype analysis

We constructed 6 haplotype using these three loci. The results showed that the frequencies of G-T-C haplotype was significantly higher in the case group than in the control group, the difference showed statistically significant (OR=2.001, 95% CI: 1.121~3.573; *P*=0.0170). G-T-T haplotype in case group was significantly lower than that in the control group, the difference showed statistically significant (OR=0.715, 95% CI: 0.527~0.970, *P*=0.030, **Table 4**).

Discussion

IL-27 was found and named as a kind of new cytokines of IL-12 family in 2003 [17]. IL-27 plays an important role in the regulation of different types of immune cells, especially in th-e regulation of CD^{4+} T cell differentiation and proliferation [18]. IL-27 and IL-12 can promote initial CD^{4+} T cells producing IFN-γ, andpromotethedifferentiation of Th0 cell to Th1 cell [19, 20]; at the same time, IL-27 also is of inhibitory effect of Th2 cell [21]. CD4+ T cells (Th1/Th0 cells) play an important role during the pathogenesis of RA, and Th1 presents persistent high reactive activation in RA patients. In addition, it was found that the addition of IL-27 can help to reduce and relieve arthritis [22]. Therefore, IL-27 perhaps can be the better cytokines in the treatment of RA. Many susceptibility genes of autoimmune disease were reported, and IL-27 gene polymorphism has been found to be closely associated with many autoimmune diseases, but the association with RA is unclear.

In this research, 3 SNPs (-964A>G, 2905T>G, and 4730T>C) in IL-27gene were selected. The results showed that, although there was no significantly difference in these three loci genotype and allele frequency between these two groups, it was found that G-T-C haplotype frequency in case group was obviously higher than that in the control group through haplotype analysis, the difference was statistical significance (P<0.0), but G-T-T haplotype frequency in

case group was obviously lower than in the control group.

In conclusion, our study indicated that IL-27 gene polymorphism was significantly related to the risk of RA in the Chinese Han population. G-G-C haplotypes may be a risk factor, but the A-T-T haplotype may be protective factor of RA.

Disclosure of conflict of interest

None.

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