Cytokine (IL-6) and chemokine (IL-8) gene polymorphisms among rheumatoid arthritis patients in Taiwan

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Abstract

Objective

The involvement of cytokines and chemokines in the pathogenesis of rheumatoid arthritis (RA) is well studied; however, the genetic bases behind this is not well understood. The aim of this study was to examine whether -572 G/C polymorphism in the IL-6 gene and 2767 A/G polymorphism in the 3'-untranslated region (UTR) of the IL-8 gene are associated with rheumatoid arthritis (RA).

Methods

We enrolled 199 RA patients and 130 normal controls. Polymerase chain reaction was used to identify the IL-6 -572G/C and IL-8 3'-UTR 2767A/G polymorphisms. The relationships between clinical manifestations of RA and the polymorphisms of each gene were investigated by comparing the genotypes among RA patients with different clinical variables.

Results

We found no significant difference in the genotypic and allelic frequencies of the single nucleotide polymorphisms of IL-6 and IL-8 genes between RA patients and controls. Clinical characteristics such as age at onset, rheumatoid factor positivity, joint erosion and extra-articular manifestations were compared among patients with different genotypes of the IL-6 and IL-8 genes. We found that patients with IL-8 3'-UTR 2767AA genotype had a significantly younger age of onset of RA than patients without that genotype.

Conclusion

The IL-6 -572 G/C and IL-8 3'-UTR 2767A/G polymorphisms are not associated with the risk of developing rheumatoid arthritis. However, the finding that patients with IL-8 3'-UTR 2767AA developed RA at a younger age suggests that this genotype may influence the etiopathology of RA in patients in Taiwan. Therefore, further single nucleotide polymorphism studies of this 3'UTR region may give more novel findings and understanding of the genetic basis of rheumatoid arthritis.

Key words

Interleukin-6, interleukin-8, gene polymorphism, rheumatoid arthritis.

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Introduction

Rheumatoid arthritis (RA) is a heterogeneous disease dominated by chronic joint inflammation, and accompanied by several peripheral inflammatory manifestations (1). RA is characterized by chronic synovitis which leads to the destruction of joint tissue and consequently, serious impairment of joint function (2). Fibroblast-like synoviocytes and macrophage-like synoviocytes play critical roles in the destructive process (3, 4). In RA patients,fibroblast-like synoviocytes synthesize and secrete mediators of inflammation such as interleukin(IL)-6 and interleukin(IL)-8 (5-7). Interleukin-6, a multifunctional cytokine, plays an important role in the regulation of acute phase response (8, 9). High levels of IL-6 have been detected in synovial fluid from joints of patients with active RA, suggesting the possible involvement of IL-6 in RA (10). Interleukin-8, which belongs to the CXC chemokine family, is a strong chemoattractant for lymphocytes and neutrophils (11, 12). It is recognized as an important factor that contributes to neutrophil infiltration into synovial fluids in RA patients (13). The involvement of cytokines and chemokines in the pathogenesis of RA is well studied, but the genetic bases behind it is not well understood.

The human IL-6 gene is located on chromosome 7p21,rs1800796. There are several naturally occurring halplotype polymorphisms in the IL-6 promotor (-597G/A, -572 G/C,-373A(n)T(n), -174G/C) (14). A previous study found an association between the -174 G/C polymorphism and juvenile chronic arthritis (15). Another study suggested that the -373A(n)T(n) polymorphism influences RA disease progression (16). A more recent study in postmenopausal women found that the -572G/C polymorphism influences levels of circulating C-reactive protein and bone resorption markers (17). Therefore, the -572G/C IL-6 gene polymorphism may play a role in the development of chronic inflammatory joint disease,such as rheumatoid arthritis. The human IL-8 gene is located on chromosome 4q12-q21,rs10938092. It contains three introns and four exons (12, 18). The 3'-untranslated region (UTR) of the IL-8 gene which contains AU rich elements (AREs) in its transcript, has been suggested to contribute to its post-transcriptional regulation (19). The binding of different RNA-binding proteins to distinct ARE domains exert different functions in mRNA destabilization and stabilization (20), and the stabilization effect of the 3'UTR led to increased protein expression (21). A study found that IL-8 3'-UTR 2767A/G polymorphism influences the susceptibility to nephritis in cutaneous vasculitis (22). Another study found an opposing role of IL-8 3'-UTR 2767A/T polymorphism in juvenile idiopathic arthritis and asthma (23).

Recently, one study found that polymorphisms in the promotor region of RANTES gene are associated with RA susceptibility (24), but another study found no association of interleukin-4 (intron 3 and promotor) gene polymorphisms in Chinese patients with rheumatoid arthritis in Taiwan (25).

In view of the aforementioned studies, we hypothesized that variations in the IL-6 and IL-8 genes may be associated with the risk of developing RA. To verify our hypothesis, we evaluated the -572G/C polymorphism in the IL-6 gene and the 2767A/G polymorphism in the 3'-UTR of the IL-8 gene by comparing the allelic and genotypic frequencies occurring in 199 RA patients with those in 130 healthy individuals living in Taiwan. Furthermore, we compared the genotypes among RA patients with various clinical variables to investigate whether there is a relationship between either the IL-6 or IL-8 genetic polymorphism and the clinical manifestation of RA.

Materials and methods

Patient selection

Patients with rheumatoid arthritis diagnosed according to the 1987 revised American College of Rheumatology criteria (26) were consecutively recruited. Rheumatoid factors (RF) was detected by nephelometry, values ≥30IU/ml were classified as positive. The presence or history of extra-articular manifestation in RA patients was recorded (27). Radiographs of hands,

wrists, and feet of patients were taken, and the presence or absence of joint erosion was evaluated by a rheumatologist and a radiologist. We enrolled 199 patients and 130 unrelated healthy individuals living in Taiwan to serve as controls. Informed consent was obtained from all participants involved. Genomic DNA was prepared from peripheral blood using the Genomic DNA isolation reagent kit (Genomaker Inc., Taipei, Taiwan).

Polymerase chain reaction

Polymerase chain reaction (PCR) was used to identify the polymorphisms in the IL-6 gene promoter region and IL-8 gene 3'-untranslated region (3'-UTR). Polymerase chain reaction of the polymorphisms was achieved to a total volume of 50 μL, containing genomic DNA 50ng, 2–6pmol of each primer, 1X Taq polymerase buffer (1.5mM $MgCl_2$) and 2.5U of AmpliTaq DNA polymerase (Perkin Elmer, Foster City, CA, USA). In the study of the IL-6 gene promoter region, the primers used were upstream 5'-GCAAAGTCCTCACTGGGAGGA-3' and downstream 5'-TCTGACTCCATCGCAGCCC-3'. For the IL-8 gene 3'-untranslated region (3'-UTR) the primers used were upstream 5'-CTTTAGTGTTTTTATGTGCTCTCCA-3' and downstream 5'-GCAAATATGCTTAGGCTTTAACC-3'. The choices of primers being selected were according to the genetic sequence of human IL-6 (chromosome 7p21,rs1800796) and IL-8 (chromosome 4q12-q21,rs10938092). Polymerase chain reaction amplification was performed in a programmable PCR thermal cycler (GeneAmp PCR System 2400, Perkin Elmer). The PCR cycling conditions for IL-6 promotor -572 G/C polymorphism examination were as follows: 35 cycles at 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 45 seconds, then stand at 72°C, and hold at 4°C. The PCR cycling conditions for examination of the 2767A/G polymorphism in the 3'-UTR of IL-8 gene were as follows: 35 cycles at 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 45 seconds, then stand at 72°C, and hold at 4°C.

The polymorphisms of the IL-6 and IL-8 genes of interest were identified by digestion with *Bsr*BI *and Apa*LI respectively. The PCR products were mixed separately using the respective enzymes and reaction buffer according to the manufacturer's instructions, with both reactions incubated for three hours at 37°C. After this, 10μl of each product was loaded into a 3% agarose gel containing ethidium bromide and undergone electrophoresis. The resultant genotypes were classified into the categories of non-excisable homozygote allele (CC), excisable homozygote allele (GG) and heterozygote allele (CG) for the IL-6 gene; non-excisable homozygote allele (GG), excisable homozygote allele (AA) and heterozygote allele (AG) for the IL-8 gene.

Statistical analysis

The genotypic and allelic frequencies of the IL-6 gene and IL-8 gene polymorphisms for the RA patients and controls were compared using the chi-square test. When one cell had an expected count of <1 or >20% of the cells had an expected count of <5, Fisher's exact test was used. Results were considered statistically significant when p values less than 0.05.The odds ratios (OR) were calculated from the genotypic frequency and allelic frequency with a 95% confidence interval (95% CI) for the polymorphism of the IL-6 gene and IL-8 gene respectively. The statistical analysis was performed by using the Statistical Analysis System (SAS version 8.02).

Results

The clinical and demographic features of the participants are shown in Table I. The genotype distributions of the IL-6 -572G/C and the IL-8 3'-UTR 2767A/G polymorphisms in the patient and control groups complied with Hardy-Weinberg quilibrium. The genotypic frequency distribution of the IL-6 -572G/C polymorphism revealed no significant difference between patients and controls. Among the 199 RA patients, 121 patients (60.8%) had genotype CC, 69 patients (34.7%) had genotype CG, and 9 patients (4.5%) had genotype GG which were similar to the genotypic distribution of the 130 control participants. Further comparison of the allelic frequencies of the IL-6 -572 G/C polymorphism between RA patients and controls revealed no significant difference. The genotypic frequency distribution of the IL-8 3'-UTR 2767A/G polymorphism also revealed no significant difference between patients and controls. Among the 199 RA patients, 22 patients (11.1%) had genotype AA, 83 patients (41.7%) had genotype AG, and 94 patients (47.2%) had

Table I. Clinical and demographic features of the participants.

	Sample size	Age (yrs.)		Onset age (yrs.)	RF+	EA+	JE+
	n (%)	Mean ± SD	Range	Mean ± SD	n (%)	n (%)	n (%)
RA patients (n=199)							
Female	152 (76.4%)	49.8 ± 13.4	18 - 75	42.8 ± 13.6	102 (72%)	73 (48.0%)	77 (50.7%)
Male	47 (23.6%)	57.0 ± 11.2	29 - 82	53.2 ± 11.7	35 (74.5%)	19 (40.4%)	22 (46.8%)
Control subjects (N=130)							
Female	68 (52.3%)	41.3 ± 14.2	22 - 70				
Male	62 (47.7%)	43.7 ± 16.5	16 - 79				

RF: rheumatoid factor; EA: extra-articular manifestation; JE: joint erosion.

Table II. Relationship between IL-6 -572 polymorphism and clinical variables in RA patients.

	Onset age		RF+		EA+		JE+	
IL-6 genotype	no.	(yrs.)	n	%	n	%	n	%
C/-	77	45.7 ± 14.7	58	75.3%	42	54.5%	41	53.2%
A/A	120	44.9 ± 13.4	79	65.3%	50	41.3%	58	47.9%
p-value		0.681	0.136		0.069		0.466	
Odd ratio (95% CI)			0.616 (0.325-1.168)		0.587 (0.330-1.044)		0.808 (0.456-1.433)	
A/A & C/C	128	44.8 ± 13.3	87	67.4%	56	43.4%	61	47.3%
A/C	69	46.1 ± 15.1	50	72.5%	36	52.2%	38	55.1%
p-value		0.517	0.466		0.239		0.296	
Odd ratio (95% CI)			1.270 (0.667-2.419)		1.422 (0.791-2.557)		1.366 (0.760-2.458)	
A/-	189	45.3 ± 14.0	129	67.9%	86	45.3%	96	50.5%
C/C	8	42.6 ± 11.0	8	100%	6	75.0%	3	37.5%
p-value		0.583	0.110*		0.148*		0.721*	
Odd ratio (95% CI)			--		3.628 (0.714-18.435		0.588 (0.137-2.528)	

RF: rheumatoid factor; EA: extra-articular manifestation; JE: joint erosion.
*Fisher's exact test.

genotype GG which were similar to the genotypic distribution of the 130 control participants. Further comparison of the allelic frequencies of the IL-8 3'-UTR 2767A/G polymorphism between RA patients and controls also revealed no significant difference.

The relationships between the single nucleotide polymorphisms and clinical manifestations in RA patients are shown in Table II and Table III. The clinical variables of RA did not differ significantly in relation to IL-6 -572G/C polymorphism (Table II). However, we did find significant differences in the disease onset age between patients homozygous at 3'-UTR 2767AA (39.1±15.3yrs) and those homozygous at 3'-UTR 2767GG (46.8±12.3yrs) and heterozygous at 3'-UTR 2767AG (45.1±14.9yrs). Patients with the AA genotype developed RA at a much earlier age than patients with either the GA or GG genotype (p=0.029) (Table III).

Discussion

Rheumatoid arthritis (RA) is a heterogeneous chronic joint disease involving autoimmune response and chronic proliferative inflammation. Synovial cells play an important role in the pathogenesis of RA by synthesizing various cytokines and chemokines. Studies have revealed that cytokines play a pivotal role in immune response and inflammation (28, 29).

High levels of IL-6 detected in synovial fluid from patients with RA have suggested the involvement of IL-6 in RA (8-10). Previous studies have also found a significant correlation between serum IL-6 activity and the serum levels of various acute phase reactants (8, 9). Brozik *et al*. and Swada *et al*. found a correlation between synovial fluid IL-6 activity and rheumatoid factor in RA patients (9, 30). A recent study also found decreases in circulating IL-6 concentrations were consistently and independently associated with reductions in endothelial activation, suggest that suppression of IL-6 production attenuates atherogenesis in RA (31). These studies all suggest the importance of IL-6 in the pathogenesis of RA. Polymorphisms of this cytokine gene may alter gene expression, and lead to the development of RA or change its clinical manifestation; however our investigation revealed no association between the IL6 -572 G/C polymorphism and RA. Our result is in agreement with previous studies which found no association of IL-6 gene polymorphism with the risk of RA development (32-34).

Chemokines are upregulated during inflammation and act mainly on leukocytes by inducing migration and release responses through a series of coordinated biochemical and cellular events (35). In RA patients, monocyte-derived fibroblasts and macrophages that comprise the synovial lining are the major sources of chemokines. Circulating monocytes have been shown to express higher IL-8mRNA levels in RA patients than in healthy people (36). The induction of IL-8 by macrophage migration inhibitory factor in RA synovial fibroblasts has also been observed (37).One study hypothesized that the innate property of activated RA macrophages to produce increased levels of IL-8mRNA is a major determinant of the chemotactic gradient for neutrophils in rheumatoid joints (38). These studies all suggest the importance of IL-8 in the pathogenesis of RA. Polymorphisms of this chemokine gene might affect the gene expression and production of IL-8, and therefore might be associated with

Table III. Relationship between IL-8 3'-UTR2767 polymorphism and clinical variables in RA patients.

	Onset age		RF+		EA+		JE+	
IL-8 genotype	n	(yrs.)	n	%	n	%	n	%
G/-	175	46.0 ± 13.6	121	68.8%	81	46.0%	90	51.1%
A/A	22	39.1 ± 15.3	16	72.7%	11	50.0%	9	40.9%
p-value		0.029	0.730		0.724		0.366	
Odd ratio (95% CI)			1.212 (0.450-3.265)		1.173 (0.483-2.847)		0.662 (0.269-1.627)	
A/A & G/G	115	45.3 ± 13.2	84	72.4%	57	49.1%	58	50.0%
A/G	82	45.1 ± 14.9	53	64.6%	35	42.7%	41	50.0%
p-value		0.930	0.243		0.370		1.000	
Odd ratio (95% CI)			0.696 (0.379-1.280)		0.771 (0.436-1.362)		1.000 (0.568-1.760)	
A/-	104	43.9 ± 15.1	69	66.3%	46	44.2%	50	48.1%
G/G	93	46.8 ± 12.3	68	72.3%	46	48.9%	49	52.1%
p-value		0.140	0.362		0.507		0.569	
Odd ratio (95% CI)			1.327 (0.722-2.437)		1.208 (0.690-2.115)		1.176 (0.673-2.055)	

RF: rheumatoid factor; EA: extra-articular manifestation; JE: joint erosion.

the development of RA. Nevertheless, to the best of our knowledge, no previous literature has reported the association of IL-8 gene polymorphism with RA. Our present study may be the first study reported no direct association between the IL-8 3'-UTR 2767A/G polymorphism and the development of RA. However, we did find that RA patients with the AA genotype developed RA at a much earlier age than patients with either the GA or GG genotype. Therefore, it is possible that the IL-8 3'-UTR 2767A/G polymorphism contributes to the etiopathology of rheumatoid arthritis by influencing the onset of disease. Whether the association between this IL-8 gene polymorphism and age of onset is related to its post-transcriptional regulation or to other unknown mechanism remains to be explored.

The lack of direct association among the IL-6 -572 G/C and IL-8 3'-UTR 2767A/G polymorphisms and RA found in the present study could have been due to the fact that the number of controls was insufficient. The other reason could be that single nucleotide polymorphisms in different locations may act in concert and contribute to the development of disease. The IL-6 -572 G/C and IL-8 3'-UTR 2767A/G polymorphisms may be in linkage disequilibrium with other single nucleotide polymorphisms which dominate their functional expression. This argument is supported by a study which found that the base differences at distinct polymorphic sites influence each other and do not act independently (39).

In summary, although we did not find a direct association among IL-6 -572 G/C and IL-8 3'-UTR 2767A/G polymorphisms and RA, our study did reveal an association between IL-8 3'-UTR 2767A/G polymorphism and disease onset in rheumatoid arthritis patients in Taiwan. Although the exact mechanism remains unknown, such disease onset association could have important implications. Therefore, further single nucleotide polymorphism studies of this 3'UTR region involving more patients and controls of different ethnic groups may give more novel findings and understanding of the genetic basis of rheumatoid arthritis.

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