Association of CD4 Enhancer Gene Polymorphisms with Rheumatoid Arthritis and Systemic Lupus Erythematosus in Taiwan

SUI-FOON LO, LEI WAN, HSIU-CHEN LIN, CHUNG-MING HUANG, and FUU-JEN TSAI

ABSTRACT. ***Objective.*** It has been found that changes in CD4 expression and CD4+ T cell activity may influence tolerance or tissue destruction in autoimmune diseases and contribute to their risk. We examined whether an association of CD4 enhancer gene polymorphisms with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) exists.

Methods. For study of the CD4 –11743A/C polymorphism, 192 patients with RA, 141 patients with SLE, and 96 normal controls participated. For the CD4 –10845A/G polymorphism, 191 patients with RA, 127 patients with SLE, and 92 controls participated. The polymorphism of the CD4 enhancer was examined with the polymerase chain reaction-restriction fragment length polymorphism method. Genotypic and allelic frequencies of the 3 groups of participants were compared. Genotype groups were also compared according to different clinical variables among the patients with RA and SLE.

Results. For the CD4 –11743A/C polymorphism, patients with RA demonstrated significantly higher frequency of the C allele ($p = 0.048$); patients with SLE had significantly higher frequency of the CC genotype ($p = 0.026$), and lower frequency of the AC genotype ($p = 0.013$) compared with controls. For the CD4 –10845A/G polymorphism, patients with RA had significantly higher frequencies of the AA genotype ($p = 0.047$) and the A allele ($p = 0.026$); patients with SLE had significantly higher frequency of the AA genotype ($p = 0.011$) and A allele ($p = 0.001$), and lower frequency of the GG genotype ($p = 0.003$) compared with controls. A comparison of genotype groups according to different clinical variables revealed the association of the respective polymorphisms with mucosal ulcer lesions among patients with SLE.

Conclusion. Our results suggest that the genetic polymorphisms at the CD4 enhancer gene are associated with the risk of development of RA and SLE. They are also associated with mucosal ulcer lesions in patients with SLE. (First Release Nov 1 2008; J Rheumatol 2008;35:2113–8; doi:10.3899/jrheum.070993)

Key Indexing Terms:
CD4 ENHANCER
RHEUMATOID ARTHRITIS
POLYMORPHISM
SYSTEMIC LUPUS ERYTHEMATOSUS

Rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) are autoimmune diseases with a broad spectrum of clinical manifestations and systemic involvement[1-3]. Previous studies have shown that T cells play an important role in the development of such autoimmune diseases[3-6]. The balance between regulatory T cells and proinflammatory effector T cells has been shown to be of pivotal importance in the development and persistence of autoimmune diseases[7]. Kamradt and Mitchison suggested the main role that CD4+ T cells play in the development of most human autoimmune diseases[8]. The CD4 glycoprotein expressed on the surface of helper T cells colocalizes with the T cell receptor (TCR) and major histocompatibility complex (MHC) class II molecules[9]. It interacts with the non-antigen-binding regions in the MHC class II molecule in V-shaped binding mode during antigen recognition[10]. The binding of CD4 to the MHC-TCR-CD3 complex during antigen recognition brings the active Lck close to the immunoreceptor tyrosine-based activation motifs (ITAM) located within the TCR-CD3 complex, thereby phosphory-

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Accepted for publication July 8, 2008.

lating them[3,11]. The Zeta-associated protein of 70 kD (ZAP-70) is then recruited to the complex and activated, allowing further phosphorylation of downstream molecules, eventually leading to gene activation and subsequent change of cellular function[3,11]. The inhibition of CD4-MHC class II interaction severely impairs the response of T cells to antigen exposure[12]. The CD4 gene is located in chromosome 12p-12pter[13], and its expression is regulated primarily at the transcriptional level[14,15]. The CD4 enhancer and promoter seem to be the major regulatory regions for CD4 transcription in T cells[16,17]. Therefore, an analysis of the CD4 enhancer polymorphism can provide insights into regulation of its expression. The MHC class II region has been studied extensively and recognized as an important susceptibility factor for RA and SLE[18-21]. However, the potential role of polymorphisms in other components of the MHC-T cell interaction complex has been neglected in most association studies.

In view of the importance of CD4 in the functional performance of T cells, as well as its consequent crucial effect in the immune response of an individual, we hypothesized that genetic variations in the CD4 enhancer may be associated with the risk of development of autoimmune diseases such as RA and SLE. To verify our hypothesis, we compared the allelic and genotypic frequencies of the –11743A/C polymorphism in the CD4 enhancer of 192 patients with RA, 141 patients with SLE, and 96 normal controls. We also compared the allelic and genotypic frequencies of the –10845A/G polymorphism in the CD4 enhancer of 191 patients with RA, 127 patients with SLE, and 92 controls living in Taiwan. Further, we compared the genotypes among patients with RA and SLE with various clinical variables to investigate whether a relationship exists between CD4 enhancer polymorphisms and the clinical manifestations of RA and SLE.

MATERIALS AND METHODS

Patient selection. Patients with RA and SLE according to the revised American College of Rheumatology criteria[22-24] were enrolled. Nephelometry was used to detect rheumatoid factor (RF). Values ≥ 30 IU/ml were defined as positive. The presence or history of extraarticular manifestations in patients with RA was recorded[25]. Radiographs of wrists, hands, and feet of patients were obtained, and the presence or absence of joint erosion was evaluated by a rheumatologist and a radiologist. Various clinical features were also evaluated and recorded for the patients with SLE. For the CD4 –11743A/C polymorphism,192 patients with RA, 141 with SLE, and 96 controls were included. For the CD4 –10845A/G polymorphism, 191 patients with RA, 127 with SLE, and 92 controls were included. Informed consent was obtained from all participants.

Genomic DNA was prepared from peripheral blood using the DNA extractor kit (Genomaker DNA extraction kit; Blossom, Taipei, Taiwan).

Polymerase chain reaction (PCR). A total of 50 ng of genomic DNA was mixed with 20 pmol of each PCR primer in a total volume of 25 µl containing 10 mM Tris-hydrochloride, pH 8.3; 50 mM potassium chloride; 2.0 mM magnesium chloride; 0.2 mM each deoxyribonucleotide triphosphate; and 1 U of DNA polymerase (Amplitaq; Perkin Elmer, Foster City, CA, USA). PCR amplification was performed in a programmable PCR thermal cycler (GeneAmp PCR System 2400, Perkin Elmer). For the CD4 –11743 polymorphism, a 302-base-pair (bp) fragment of CD4 –11743 was amplified by PCR. The primers used were forward 5'-TCA GAT ATT CTC TGC TCA GC CCA-3' and reverse, 5'-TTC CAG TCT GAA AAA AGT GG-3'. The choice of primers selected was according to the genetic sequence of human CD4 gene (chromosome 12p13, rs11064391). The PCR conditions for CD4 –11743 polymorphism examination were as follows: 1 cycle at 95°C for 5 min, 18 cycles at 95°C for 30 s, touchdown60-51°C for 30 s, and 72°C for 30 s, 17 cycles at 95°C for 30 s, 51°C for 30 s, and 72°C for 30 s, and 1 final cycle of extension at 72°C for 7 min, then holding at 25°C. The CD4 –11743 A/C polymorphism was analyzed by PCR amplification followed by restriction enzyme analysis with MwoI. Two fragments measuring 205 bp and 97 bp would be present if the product is able to be excised. The uncut band showed up as a 302-bp length on the gel. The reaction was then incubated overnight at 37°C, and then 10 µl of the digested products were loaded into a 3% agarose gel with ethidium bromide staining and separated by electrophoresis. The polymorphism of CD4 –11743 was categorized as non-excisable homozygote (AA), excisable homozygote (CC), and heterozygote (AC). For the CD4 –10845 polymorphism, a 300-bp fragment of CD4 –10845 was amplified by PCR. The primers used were forward 5'-GAA ATG AGA AGT AGC ACA CAG T-3' and reverse, 5'-AAA AGT TAA GCA GAA TCA GGC-3'. The choice of primers was according to the genetic sequence of human CD4 gene (chromosome 12p13, rs7956804). The PCR conditions for CD4 –10845 polymorphism examination were as follows: 1 cycle at 95°C for 5 min, 18 cycles at 95°C for 30 s, touchdown60-51°C for 30 s, and 72°C for 30 s, 17 cycles at 95°C for 30 s, 51°C for 30 s, and 72°C for 30 s, and 1 final cycle of extension at 72°C for 7 min, then holding at 25°C. The CD4 –10845A/G polymorphism was analyzed by PCR amplification followed by restriction enzyme analysis with HaeII. Two fragments measuring 202 bp and 98 bp would be present if the product is able to be excised. The uncut band showed up as a 300-bp length on the gel. The reaction was then incubated overnight at 37°C, and then 10 µl of the digested products were loaded into a 3% agarose gel with ethidium bromide staining and separated by electrophoresis. The polymorphism of CD4 (–10845) was categorized as non-excisable homozygote (AA), excisable homozygote (GG), and heterozygote (AG).

Statistical analysis. The genotype distributions and allelic frequencies for the CD4 –11743 and CD4 –10845 polymorphisms of the patients with RA and SLE and controls were compared using the chi-squared test. Among the patients with RA or SLE, genotype groups with different clinical variables were also compared using the chi-squared test. When 1 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 were < 0.05. The odds ratios (OR) were also calculated from the genotypic frequency and allelic frequency, with a 95% confidence interval (95% CI) for the polymorphism of CD4 –11743 and CD4 –10845, respectively. The statistical analysis was performed by using the Statistical Package for the Social Sciences, version 8.01.

RESULTS

The genotypic and allelic frequencies of the CD4 –11743A/C polymorphism of patients with RA and controls are shown in Table 1. Here, patients with RA demonstrated no significant difference in genotypic frequency compared with controls. However, a comparison of the allelic frequencies between patients with RA and controls revealed that the former had a higher frequency of the C allele (71.6% vs 63.5%; p = 0.048, OR 1.448, 95% CI 1.002–2.092) and a lower frequency of the A allele (28.4% vs 36.5%; p = 0.048, OR 0.691, 95% CI 0.478–0.998). The genotypic and allelic frequencies of the CD4 –11743A/C polymorphism of patients with SLE and controls are shown in Table 2. We found that patients with SLE had a significantly higher frequency of the CC genotype (57.4.0% vs 42.7%; p = 0.026,

Table 1. Comparison of CD4 –11743 A/C genotype distributions and allelic frequencies between patients with RA and controls.

	RA Patients, Total = 192, n (%)	Controls, Total = 96, n (%)	p*	Relative Risk (OR)	95% CI
Genotype			0.170†		
A/A	19 (9.9)	15 (15.6)	0.155	0.593	0.287–1.226
A/C	71 (37.0)	40 (41.7)	0.441	0.821	0.498–1.355
C/C	102 (53.1)	41 (42.7)	0.096	1.520	0.928–2.491
Allelic frequency					
Allele A	109 (28.4)	70 (36.5)	0.048	0.691	0.478–0.998
Allele C	275 (71.6)	122 (63.5)	0.048	1.448	1.002–2.092

* Chi-square test. † Comparing the distribution of 3 genotypes between patients with RA and controls.

Table 2. Comparison of CD4 –11743 A/C genotype distributions and allelic frequencies between patients with SLE and controls.

	RA Patients, Total = 141, n (%)	Controls, Total = 96, n (%)	p*	Relative Risk (OR)	95% CI
Genotype			0.037†		
A/A	23 (16.3)	15 (15.6)	0.887	1.053	0.518–2.139
A/C	37 (26.2)	40 (41.7)	0.013	0.498	0.287–0.866
C/C	81 (57.4)	41 (42.7)	0.026	1.811	1.072–3.060
Allelic frequency					
Allele A	83 (29.4)	70 (36.5)	0.108	0.727	0.492–1.073
Allele C	199 (70.6)	122 (63.5)	0.108	1.376	0.932–2.031

* Chi-square test. † Comparing the distribution of 3 genotypes between patients with SLE and controls.

OR 1.811, 95% CI 1.072–3.060) and lower frequency of the AC genotype (26.2% vs 41.7%; p = 0.013, OR 0.498, 95% CI 0.287–0.866) compared with controls. The genotypic and allelic frequencies of the CD4 –10845A/G polymorphism of patients with RA and controls are shown in Table 3. We found that patients with RA had a significantly higher frequency of the AA genotype compared with the controls (55.0% vs 42.4%; p = 0.047, OR 1.659, 95% CI 1.004–2.742). Similarly, further comparison of the allelic frequencies between patients with RA and controls revealed that the former had a higher frequency of the A allele (72.8% vs 63.6%; p = 0.026, OR 1.531, 95% CI 1.052–2.228), and a lower frequency of the G allele (27.2% vs 36.4%; p = 0.026, OR 0.653, 95% CI 0.449–0.951). The genotypic and allelic frequencies of the CD4 –10845A/G polymorphism of patients with SLE and the controls are shown in Table 4. We found that patients with SLE had a significantly higher frequency of the AA genotype (59.8% vs 42.4%; p = 0.011, OR 2.025, 95% CI 1.174–3.492), and lower frequency of GG genotype (3.9% vs 15.2%; p = 0.003, OR 0.228, 95% CI 0.079–0.659) compared with controls. Further comparison of the allelic frequencies between patients with SLE and controls revealed that the former had a significantly higher frequency of the A allele (78.0% vs 63.6%; p = 0.001, OR 2.025, 95% CI 1.328–3.028) and lower frequency of the G allele (22.0% vs 36.4%; p = 0.001, OR 0.494, 95% CI 0.324–0.753). On the other hand, the comparison of the genotypic and allelic frequencies of the CD4 –11743A/C

Table 3. Comparison of CD4 –10845 A/G genotype distributions and allelic frequencies between patients with RA and controls.

	RA Patients, Total = 191, n (%)	Controls, Total = 92, n (%)	p*	Relative Risk (OR)	95% CI
Genotype			0.104†		
A/A	105 (55.0)	39 (42.4)	0.047	1.659	1.004–2.742
A/C	68 (35.6)	39 (42.4)	0.270	0.751	0.452–1.249
C/C	18 (9.4)	14 (15.2)	0.149	0.580	0.274–1.225
Allelic frequency					
Allele A	278 (72.8)	117 (63.6)	0.026	1.531	1.052–2.228
Allele C	104 (27.2)	67 (36.4)	0.026	0.653	0.449–0.951

* Chi-square test. † Comparing the distribution of 3 genotypes between patients with RA and controls.

Table 4. Comparison of CD4 –10845 A/G genotype distributions and allelic frequencies between patients with SLE and controls.

	SLE Patients, Total = 127, n (%)	Controls, Total = 92, n (%)	p*	Relative Risk (OR)	95% CI
Genotype			0.003†		
A/A	76 (59.8)	39 (42.4)	0.011	2.025	1.174–3.492
A/C	46 (36.2)	39 (42.4)	0.355	0.772	0.446–1.337
C/C	5 (3.9)	14 (15.2)	0.003	0.228	0.079–0.659
Allelic frequency					
Allele A	198 (78.0)	117 (63.6)	0.001	2.025	1.328–3.028
Allele C	56 (22.0)	67 (36.4)	0.001	0.494	0.324–0.753

* Chi-square test. † Comparing the distribution of 3 genotypes between patients with SLE and controls.

polymorphism between patients with RA and SLE revealed no significant difference (p = 0.054 and p = 0.768, respectively). Similarly, a comparison of the genotypic and allelic frequencies of the CD4 –10845A/G polymorphism between patients with RA and SLE also revealed no significant difference (p = 0.174 and p = 0.141, respectively). Among patients with RA, the comparison of genotype groups according to different clinical variables revealed no significant findings. Meanwhile, among patients with SLE, lower frequency of the CD4 –11743 CC genotype (16.7% vs 35.1%; p = 0.014, OR 0.370, 95% CI 0.165–0.829) and the CD4 –10845AA genotype (16.2% vs 34.7%; p = 0.018, OR 0.364, 95% CI 0.155–0.855), as well as higher frequency of the CD4 –11743AC genotype (37.1% vs 20.0%; p = 0.042, OR 2.364, 95% CI 1.018–5.49) and the CD4 –10845AG genotype (34.1% vs 17.7%; p = 0.040, OR 2.401, 95% CI 1.027–5.617) were found in patients with mucosal ulcer (Table 5, Table 6).

DISCUSSION

Many studies suggest that T cells play an important role in the development of autoimmune diseases, such as RA and SLE[3-7]. Accumulated evidence has shown that the repertoire of CD4+ T cells in patients with RA is distinct. This includes a high frequency of disease-relevant T cells with a distinct phenotype (CD4+CD28–) and a functional profile (overproduction of interferon-γ and cytotoxicity) that give them the ability to function as proinflammatory cells[26]. The importance of CD4+ CD25+ T cells in the pathogenesis of RA and SLE has also been thoroughly studied[27-31]. However, a recent study has suggested that the defects in Treg cell function previously reported in the autoimmune and inflammatory diseases should be interpreted cautiously[32]. The study's proponents found no quantitative or qualitative alteration in Treg cells from patients with SLE. They did find that SLE-derived CD4+CD25– effector T cells resisted suppression by autologous and allogeneic regulatory cells, which led them to suggest that the defect in T cell suppression observed in SLE was due to effector cell resistance, and not to an abnormal regulatory function[32]. In view of different findings concerning the role CD4+ CD25+ T cells play in the pathogenesis of autoimmune and inflammatory diseases, we studied the association of the coreceptor CD4 enhancer gene polymorphisms with RA and SLE.

Table 5. Relationship between CD4 –11743 genotype and clinical variables in patients with SLE.

CD4 –11743 Genotype	Malar Rash, n (%)	Discoid Lupus, n (%)	Photosensitivity, n (%)	Mucosal Ulcer, n (%)	Arthritis, n (%)	Serositis, n (%)	Renal, n (%)	CNS, n (%)	Hematologic, n (%)	Immunologic, n (%)	ANA, n (%)
C/–	56 (49.6)	15 (13.3)	45 (39.8)	26 (23.0)	54 (47.38)	20 (17.7)	38 (33.6)	13 (11.5)	56 (49.6)	90 (79.6)	109 (97.3)
A/A	10 (45.5)	3 (13.6)	8 (36.4)	7 (31.8)	13 (59.1)	3 (13.6)	11 (50.0)	3 (13.6)	10 (45.5)	19 (86.4)	20 (90.9)
p	0.725	0.964	0.761	0.379	0.332	0.766*	0.144	0.725*	0.725	0.567*	0.189*
OR	0.848	1.032	0.863	1.562	1.578	0.734	1.974	1.215	0.848	1.619	0.275
95% CI	0.339–2.121	0.272–3.914	0.335–2.226	0.575–4.238	0.625–3.986	0.198–2.721	0.785–4.964	0.316–4.675	0.339–2.121	0.441–5.944	0.043–1.753
A/A & C/C	47 (47)	13 (13.0)	37 (37.0)	20 (20.0)	48 (48.0)	18 (18.0)	38 (38.0)	10 (10.0)	50 (50.0)	80 (80.0)	95 (96.0)
A/C	19 (54.3)	5 (14.3)	16 (45.7)	13 (37.1)	19 (54.3)	5 (14.3)	11 (31.4)	6 (17.1)	16 (45.7)	29 (82.9)	34 (97.1)
p	0.458	0.781*	0.364	0.042	0.522	0.615	0.487	0.360*	0.662	0.712	1.000*
OR	1.339	1.115	1.434	2.364	1.286	0.759	0.748	1.862	0.842	1.208	1.432
95% CI	0.619–2.866	0.367–3.390	0.658–3.125	1.018–5.49	0.594–2.784	0.259–2.226	0.329–1.698	0.623–5.567	0.389–1.822	0.442–3.306	0.155–13.26
A/–	29 (50.9)	8 (14.0)	24 (42.1)	20 (35.1)	32 (56.1)	8 (14.0)	22 (38.6)	9 (15.8)	26 (45.6)	48 (84.2)	54 (94.7)
C/C	37 (7.4)	10 (12.8)	29 (37.2)	13 (16.7)	35 (44.9)	15 (19.2)	27 (34.6)	7 (9.0)	40 (51.3)	61 (78.2)	75 (97.4)
p	0.693	0.838	0.563	0.014	0.196	0.428	0.635	0.226	0.515	0.382	0.650*
OR	0.871	0.901	0.814	0.70	0.636	1.458	0.842	0.526	1.255	0.673	2.083
95% CI	0.440–1.726	0.331–2.447	0.405–1.636	0.165–0.829	0.320–1.265	0.572–3.718	0.415–1.711	0.183–1.508	0.633–2.489	0.276–1.642	0.337–12.90

* Fisher's exact test. CNS: central nervous system.

Table 6. Relationship between CD4 –10845 genotype and clinical variables in patients with SLE.

CD4 –10845 Genotype	Malar Rash, n (%)	Discoid Lupus, n (%)	Photosensitivity, n (%)	Mucosal Ulcer, n (%)	Arthritis, n (%)	Serositis, n (%)	Renal, n (%)	CNS, n (%)	Hematologic, n (%)	Immunologic, n (%)	ANA, n (%)
G/–	24 (49.0)	8 (16.3)	20 (40.8)	17 (34.7)	27 (55.1)	7 (14.3)	18 (36.7)	7 (14.3)	18 (36.7)	40 (81.6)	46 (93.9)
A/A	35 (47.3)	9 (12.2)	27 (36.5)	12 (16.2)	32 (43.2)	13 (17.6)	26 (35.1)	7 (9.5)	37 (50.0)	58 (78.4)	72 (98.6)
p	0.855	0.512	0.629	0.018	1.197	0.629	0.856	0.409	0.147	0.661	0.301*
OR	0.935	0.710	0.833	0.364	0.621	1.279	0.933	0.627	1.722	0.816	4.696
95% CI	0.454–1.925	0.253–1.987	0.397–1.747	0.155–0.855	0.300–1.284	0.471–3.474	0.440–1.978	0.205–1.914	0.823–3.603	0.328–2.027	0.474–46.52
A/A & G/G	39 (49.4)	10 (12.7)	29 (36.7)	14 (17.7)	35 (44.3)	13 (16.5)	30 (38.0)	8 (10.1)	40 (50.6)	62 (78.5)	77 (98.7)
A/G	20 (45.5)	7 (15.9)	18 (40.9)	15 (34.1)	24 (54.5)	7 (15.9)	14 (31.8)	6 (13.6)	15 (34.1)	36 (81.8)	41 (93.2)
p	0.677	0.617	0.646	0.040	0.276	0.937	0.495	0.557	0.077	0.659	0.133
OR	0.855	1.305	1.194	2.401	1.509	0.960	0.762	1.401	0.504	1.234	0.177
95% CI	0.408–1.790	0.459–3.712	0.561–2.540	1.027–5.617	0.719–3.165	0.352–2.619	0.349–1.663	0.453–4.336	0.235–1.082	0.484–3.144	0.018–1.761
A/–	55 (46.6)	16 (13.6)	45 (38.1)	27 (22.9)	56 (47.5)	20 (16.9)	40 (33.9)	13 (11.0)	52 (44.1)	94 (79.7)	113 (96.6)
G/G	4 (80.0)	1 (20.0)	2 (40.0)	2 (40.0)	3 (60.0)	0 (0)	4 (80.0)	1 (20.0)	3 (60.0)	4 (80.0)	5 (100.0)
p	0.193*	0.531*	1.000*	0.337*	0.670*	0.591*	0.055*	0.459*	0.656*	1.000*	1.000*
OR	4.582	1.594	1.081	2.247	1.661	—	7.800	2.019	1.904	1.021	—
95% CI	0.497–42.23	0.167–15.18	0.174–6.724	0.357–14.15	0.268–10.30	—	0.844–72.12	0.209–19.46	0.307–11.82	0.109–9.561	—

* Fisher's exact test. CNS: central nervous system.

A previous study found that the expression of CD4 lowers the activation threshold of the cells. This allows the detection of low-affinity TCR reactivities, such as those directed at self-MHC. Changes in CD4 expression and CD4+ T cell activity may influence tolerance or tissue destruction in autoimmune disease[33]. Previous reports also found a possible association of the CD4 gene polymorphism with certain autoimmune-related diseases, such as insulin-dependent diabetes mellitus[34] and schizophrenia[35].

In our study, we compared the frequencies of the CD4 enhancer single-nucleotide polymorphisms (SNP) between the controls and the patients with RA and SLE to determine the association of the CD4 enhancer gene polymorphisms with RA and SLE in Taiwan. In the study of the CD4 –11743AC polymorphism, the patients with RA were found to have a higher frequency of the CD4 –11743C allele, and a lower frequency of the CD4 –11743A allele, compared with the controls. This suggested that while the CD4 –11743C allele increased the risk of development of RA, CD4 –11743A allele achieved the opposite. The patients with SLE were found to have a higher frequency of the CD4 –11743CC genotype, and a lower frequency of the CD4 –11743AC genotype compared with the controls. This result suggested that while the CD4 –11743CC genotype increased the risk of SLE development, the CD4 –11743AC genotype did the opposite. In the study of the CD4 –10845AG polymorphism, the patients with RA were found to have a higher frequency of the CD4 –10845AA genotype, CD4 –10845A allele, and a lower frequency of the CD4 –10845G allele compared with the controls. This suggests that while the CD4 –10845AA genotype and CD4 –10845A allele increased the risk of RA development, CD4 –10845G performed the opposite. The patients with SLE were found to have a higher frequency of the CD4 –10845AA genotype and CD4 –10845A allele. In contrast, it was observed that there was a lower frequency of the CD4 –10845GG genotype and CD4 –10845G allele compared with the controls. This result suggested that while the CD4 –10845AA genotype and CD4 –10845A allele increased the risk of development of SLE, the CD4 –10845GG genotype and CD4 –10845G allele performed otherwise. Further, we also found that CD4 –11743 and CD4 –10845 polymorphisms were associated with mucosal ulcer lesion in patients with SLE.

The enhancer elements, which were often targets for tissue-specific or temporal regulation, played an important role in the initiation of transcription[36]. The polymorphism at these 2 sites of the CD4 enhancer may have affected CD4 gene expression and conferred risk of development of RA and SLE. A previous study found that T cells expressing the CD4 coreceptor responded to just a single agonist peptide-MHC ligand. This study also found that by blocking CD4 with antibodies, T cells failed to detect ligands fewer than 25 in number[12]. Low CD4 expression and the inhibition of CD4 interaction with the TCR/MHC class II complex were also found to impair TCR response to antigen exposure, and decreased immune response[12,33]. In our study, we found association of the CD4 –11743 and CD4 –10845 enhancer gene polymorphism with RA and SLE. The process in which the enhancer initiates gene transcription via interaction with the promotor is complex[36-40]. We tried further delineating the mechanism of such association with a reporter gene assay in order to evaluate the enhancer activity of these CD4 polymorphisms. However, our data revealed that the CD4 enhancer gene polymorphisms had no influence on the transactivation activity of SV40 promoter. It is possible that these SNP may have acted through some unknown mechanisms, affecting gene transcription in T cells and altering selection. This leads to tolerance or susceptibility to autoimmunity, and the

risk of autoimmune diseases such as RA and SLE. However, such a hypothesis cannot be proven by our results.

Our study suggests that the genetic polymorphism at the CD4 enhancer can serve as a genetic marker for the risk of development of RA and SLE. However, the exact mechanism of such association needs to be further investigated.

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