HLA-DRB1 Alleles and Rheumatoid Arthritis-related Pulmonary Fibrosis

To the Editor:

Pulmonary fibrosis is a well recognized extraarticular manifestation of rheumatoid arthritis (RA)[1]. Several studies have shown an association between the HLA-DRB1 gene and extraarticular manifestations of RA[2,3]. We analyzed the effect of the HLA-DRB1 gene and cytokine gene polymorphisms on the association of pulmonary fibrosis in Japanese patients with RA.

We examined 131 adult patients with RA, who fulfilled the American Rheumatism Association 1987 revised criteria for RA[4]. Clinical characteristics were (mean ± SD) age at study 62.4 ± 13.0 years and disease duration 11.2 ± 4.7 years. Pulmonary fibrosis was diagnosed by chest radiograph and chest high-resolution computed tomography (HRCT). Patients with dry cough or exertional dyspnea or suspicious findings for interstitial changes in chest radiography were further investigated with HRCT at suspended end-inspiratory effort without intravenous contrast material. The abnormalities used to screen for pulmonary fibrosis were ground-glass attenuation and honeycombing defined as areas of cystic spaces with thickened walls. Patients with RA with other autoimmune diseases or those with other pulmonary complications, such as drug (methotrexate)-induced interstitial pneumonia diagnosed according to the criteria of Kremer, *et al*[5] or airway diseases, were excluded. Table 1 presents the characteristics of the patients with RA. Pulmonary fibrosis was diagnosed in 44 patients among these 131 patients with RA. The remaining 87 patients showed no radiographic evidence of pulmonary fibrosis.

Table 1. Clinical characteristics of patients with RA. Values are mean ± SD or n.

	Without Pulmonary Fibrosis, n = 87	With Pulmonary Fibrosis, n = 44	p
Age, yrs	60.8 ± 14.3	65.3 ± 9.4	0.062
Female:male	73:14	34:10	0.354
Duration of RA, yrs	13.2 ± 10.7	10.6 ± 9.2	0.163
RF positive:negative	73:14	42:2	0.088
CRP, mg/ml	2.00 ± 1.73	2.35 ± 1.82	0.081

RF: rheumatoid factor; CRP: C-reactive protein.

Biallelic polymorphism within the transforming growth factor-ß (TGF-ß) gene in codon 10 was determined by a polymerase chain reaction-scanning spectral polarimeter (PCR-SSP) technique employing commercial primers (One Lambda Inc., Canoga Park, CA, USA). Interleukin 4 (IL-4) promoter (–590) and intron 3 polymorphisms were studied by PCR-restriction fragment-length polymorphism (PCR-RFLP) or PCR methods as described[6]. Typing for HLA-DRB1 was performed by PCR and sequence-specific oligonucleotide probe hybridization method using the Genoscience HLA-DRB1 kit (G&G Science, Fukushima, Japan). Genotype frequencies were compared in cases and controls by 2×2 contingency tables, and 2-tailed probabilities were calculated using the Fisher exact test. Adjustment for multiple comparisons was made after second-stage typing using the Bonferroni method. Corrected p value was calculated by multiplying the p value by the number of alleles found in Japanese patients (n = 26).

The genotypes at TGF-ß codon 10, IL-4 promoter (–590), and intron 3 polymorphisms were in Hardy-Weinberg equilibrium in both healthy subjects and patients with RA (data not shown). The genotype distributions of TGF-ß polymorphisms at codon 10, IL-4 promoter (–590), and intron 3 polymorphisms were not significantly different among patients with RA with pulmonary fibrosis and those without pulmonary fibrosis (Table 2). As shown in Table 3A, the allele frequencies of HLA-DRB1 shared epitope (SE) were significantly increased in patients with RA compared to healthy controls (48.1% vs 26.2%, respectively; $p < 0.0001$). We also compared the HLA-DRB1 genotypes between patients with RA with and without pulmonary fibrosis (Table 3B). There were no significant differences in the frequencies of SE between RA patients with and without pulmonary fibrosis (45.5% vs 49.4%). Further, the HLA-DR4 (*04) status had no significant effect on the association of pulmonary fibrosis in patients with RA (patients with pulmonary fibrosis 42.0% vs patients without pulmonary fibrosis 47.1%). On the other hand, the allele frequencies of HLA-DRB1*1502 were significantly associated with pulmonary fibrosis in patients with RA.

Despite advances in molecular genetic techniques, there have been few genetic studies of RA-related pulmonary fibrosis to date. Several studies have demonstrated an association between HLA-DRB1 SE and extraarticular manifestations or disease severity of RA[2,7,8]. We analyzed the genetic background of RA patients with pulmonary fibrosis compared to those without pulmonary fibrosis and healthy controls. However, the frequencies of the HLA-DR1 SE in patients with RA with pulmonary fibrosis were not significantly different compared to those without pulmonary fibrosis. Thus, the SE was not implicated in the association with pulmonary fibrosis in Japanese patients with RA. These findings suggest that the importance of

Table 2. Genotype frequencies of TGF-ß and IL-4 genes in patients with RA.

Genotype	Without Pulmonary Fibrosis, n = 87 n (%)	With Pulmonary Fibrosis, n = 44 n (%)	OR (95% CI)	p
TGF-ß codon 10				
T/T	23 (26.4)	12 (27.3)	1.04 (0.46–2.36)	0.919
T/C	43 (49.4)	16 (36.4)	0.58 (0.28–1.23)	0.156
C/C	21 (24.1)	16 (36.4)	1.80 (0.82–3.94)	0.142
IL-4 intron 3				
RP1/RP1	46 (52.9)	18 (40.9)	0.62 (0.30–1.29)	0.196
RP1/RP2	32 (36.8)	21 (47.7)	1.57 (0.75–3.27)	0.228
RP2/RP2	9 (10.3)	5 (11.4)	1.11 (0.35–3.54)	> 0.999
IL-4 –590				
T/T	43 (49.4)	18 (40.9)	0.71 (0.34–1.47)	0.356
C/T	37 (42.5)	21 (47.7)	1.23 (0.60–2.56)	0.572
C/C	7 (8.0)	5 (11.4)	1.47 (0.44–4.91)	0.536

TGF: transforming growth factor; IL-4: interleukin 4.

Table 3A. Frequencies of HLA-DRB1 alleles in RA patients and controls.

HLA-DRB1	Alleles	RA, n = 131 (%)	Control, n = 63 (%)	p	OR (95% CI)	p_c
SE+	*0101	14 (5.3)	7 (5.6)	0.9311	0.96 (0.38–2.44)	22.3464
	*0401	14 (5.3)	2 (1.6)	0.0814	3.50 (0.79–15.6)	1.9536
	*0404	1 (0.4)	0 (0)	> 0.9999		
	*0405	86 (32.8)	18 (14.3)	0.0001	2.93 (1.67–5.14)	0.0024
	*0410	6 (2.3)	1 (0.8)	0.4355	2.93 (0.35–24.6)	10.4520
	*1001	1 (0.4)	1 (0.8)	0.5446	0.48 (0.03–7.72)	13.0704
	*1406	4 (1.5)	4 (3.2)	0.2811	0.47 (0.12–1.92)	6.7464
SE–	*0403	2 (0.8)	1 (0.8)	> 0.9999	0.96 (0.09–10.7)	
	*0406	10 (3.8)	7 (5.6)	0.4333	0.67 (0.25–1.82)	10.3992
	*0802	3 (1.1)	6 (4.8)	0.0635	0.23 (0.06–0.94)	1.5240
	*0803	9 (3.4)	13 (10.3)	0.0061	0.31 (0.13–0.74)	0.1464
	*0809	0 (0)	1 (0.8)	0.3247		7.7928
	*0901	43 (16.4)	16 (12.7)	0.3401	1.35 (0.73–2.50)	8.1624
	*1101	6 (2.3)	3 (2.4)	> 0.9999	0.96 (0.24–3.91)	
	*1201	10 (3.8)	0 (0)	0.0030		0.0720
	*1202	4 (1.5)	2 (1.6)	> 0.9999	0.96 (0.17–5.32)	
	*1301	0 (0)	2 (1.6)	0.1049		2.5176
	*1302	7 (2.7)	9 (7.1)	0.0381	0.36 (0.13–0.98)	0.9144
	*1401	2 (0.8)	6 (4.8)	0.0162	0.15 (0.03–0.77)	0.3888
	*1403	1 (0.4)	3 (2.4)	0.1025	0.16 (0.02–1.53)	2.4600
	*1405	0 (0)	4 (3.2)	0.0108		0.2592
	*1501	7 (2.7)	12 (9.5)	0.0034	0.26 (0.10–0.68)	0.0816
	*1502	29 (11.1)	8 (6.3)	0.1383	1.84 (0.81–4.14)	3.3192
	*1602	3 (1.1)	0 (0)	0.5540		
SE+ total		126 (48.1)	33 (26.2)	< 0.0001	2.61 (1.64–4.16)	
SE– total		136 (51.9)	93 (73.8)			

SE: shared epitope.

Table 3B. Frequencies of HLA-DRB1 alleles in RA patients with or without pulmonary fibrosis.

HLA-DRB1	Alleles	With Pulmonary Fibrosis, n = 44 (%)	Without Pulmonary Fibrosis, n = 87 (%)	p	OR (95% CI)	p_c
SE+	*0101	4 (4.5)	10 (5.7)	0.7789	0.78 (0.24–2.56)	16.3569
	*0401	5 (5.7)	9 (5.2)	> 0.9999	1.10 (0.36–3.40)	
	*0404	1 (1.1)	0 (0)	0.3359		7.0539
	*0405	27 (30.7)	59 (33.9)	0.5994	0.86 (0.50–1.50)	12.5874
	*0410	2 (2.3)	4 (2.3)	> 0.9999	0.99 (0.18–5.50)	
	*1001	1 (1.1)	0 (0)	0.3359		7.0539
	*1406	0 (0)	4 (2.3)	0.3039		6.3819
SE–	*0403	0 (0)	2 (1.1)	0.5522		11.5962
	*0406	2 (2.3)	8 (4.6)	0.5030	0.48 (0.10–2.32)	10.5630
	*0802	2 (2.3)	1 (0.6)	0.2618	4.02 (0.36–44.9)	5.4978
	*0803	0 (0)	9 (5.2)	0.0311		0.6531
	*0809	0 (0)	0 (0)	> 0.9999		
	*0901	15 (17.0)	28 (16.1)	0.8440	1.07 (0.54–2.13)	17.7240
	*1101	1 (1.1)	5 (2.9)	0.6670	0.39 (0.04–3.38)	14.0070
	*1201	3 (3.4)	7 (4.0)	> 0.9999	0.84 (0.21–3.34)	
	*1202	2 (2.3)	2 (1.1)	0.6042	2.00 (0.28–14.4)	12.6882
	*1301	0 (0)	0 (0)	> 0.9999		
	*1302	1 (1.1)	6 (3.4)	0.4294	0.32 (0.04–2.72)	9.0174
	*1401	1 (1.1)	1 (0.6)	> 0.9999	1.99 (0.12–32.2)	
	*1403	0 (0)	1 (0.6)	> 0.9999		
	*1405	0 (0)	0 (0)	> 0.9999		
	*1501	2 (2.3)	5 (2.9)	> 0.9999	0.78 (0.15–4.14)	
	*1502	18 (20.5)	11 (6.3)	0.0006	3.81 (1.71–8.49)	0.0126
	*1602	1 (1.1)	2 (1.1)	> 0.9999	0.99 (0.09–11.1)	
SE+ total		40 (45.5)	86 (49.4)	0.5435	0.85 (0.51–1.43)	
SE– total		48 (54.5)	88 (50.6)			

SE: shared epitope.

the SE may be variable for different manifestations and other genetic factors, or environmental factors may have more influence on RA-related pulmonary fibrosis. By contrast, significantly increased frequencies of DRB1*1502 were observed in patients with RA with pulmonary fibrosis compared with those without pulmonary fibrosis, and healthy subjects. Genetic associations between pulmonary fibrosis and the genes encoding TGF-ß and IL-4 have been reported[9,10]. However, we did not find any significant association between these genetic polymorphisms and RA-related pulmonary fibrosis.

Our data indicate that HLA-DRB1 SE is not associated with an increased risk for RA-related pulmonary fibrosis. However, we found that the presence of HLA-DRB1*1502 is associated with pulmonary fibrosis in Japanese patients with RA. To firmly establish the relationship between the HLA-DRB1 genotype and pulmonary fibrosis in RA patients, further large-scale studies are required including individuals of other ethnicities.

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