Influence of HLA-DRB1 Genes and the Shared Epitope on Genetic Susceptibility to Rheumatoid Arthritis in Taiwanese

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ABSTRACT. ***Objective.*** To investigate the association of predisposing and protective HLA-DRB1 alleles with rheumatoid arthritis (RA) and its clinical markers in a Taiwanese population.

Methods. A total of 273 patients with RA and 480 healthy controls, all of Taiwanese origin, were genotyped for HLA-DRB1 alleles by polymerase chain reaction and sequence-based typing assays. The associations between RA and HLA-DRB1 alleles and genotypes were investigated by chi-squared test.

Results. The DRB1*0405 and *1001 phenotypes showed the most significant associations with RA (OR 4.04, 95% CI 2.84–5.77, $p_c = 3.2 \times 10^{-14}$; OR 5.25, 95% CI 2.10–13.06, $p_c = 3.0 \times 10^{-3}$, respectively). Individuals carrying single or double doses of the shared epitope (SE/non-SE or SE/SE) had higher risks of RA. The compound heterozygote of DRB1*0405/*1001 showed the largest increase in RA risk (OR 15.8, 95% CI 2.48–100.7, $p_c = 0.004$). Single or double doses of SE alleles were significantly associated with a higher bone erosion rate. Rheumatoid factor positivity and bone erosion were more frequent in patients with at least one copy of DRB1*0405.

Conclusion. Our results show that SE-encoding HLA-DRB1*0405 and *1001 are associated with RA in a Taiwanese population; this is the first time DRB1*1001 has been described in persons of Asian ethnicity. Heterozygotes of DRB1*0405 and *1001 predicted the strongest susceptibility to RA, suggesting that this genotype enhances susceptibility to RA in Taiwanese. (First Release Feb 15 2007; J Rheumatol 2007;34:674–80)

Key Indexing Terms:
HLA-DRB1 RHEUMATOID ARTHRITIS SHARED EPITOPE TAIWANESE

Rheumatoid arthritis (RA) is a common human autoimmune disease of unknown etiology; both genetic and environmental factors are important in its development[1]. Regarding the genetic component, the best characterized genes are those located in the human leukocyte antigen (HLA) class II region, in particular, the HLA-DR4. The associations between HLA-DR4 and RA vary in different populations under study, suggesting that susceptibility alleles may differ according to ethnic origin. For example, HLA-DRB1*0401 and *0404 are the most common alleles found with RA in Caucasians from North America and Northern Europe[2,3]. Interestingly, in keeping with populations in the UK and probably due to the Celtic background of the population[4], RA susceptibility is predominantly associated with DRB1*0401 in the Lugo region of Northwestern Spain. In contrast, as observed in patients with RA from Southern Spain[5], HLA-DRB1*0405 has been found to be the associated allele in Japanese[6], Chinese[7,8], Taiwanese[9], Koreans[10], Asian Indians[11], and Caucasians from Israel[12]. Other allele associations are HLA-DRB1*1001 in Spanish RA patients from the Basque region[5,13], a population also associated with HLA-DRB1*10 but not with HLA-DRB1*04, Greeks[14,15], Israelis[12], Zimbabweans[16], and Indians[11], and DRB1*1402 in Yakima Native Americans[17] and Mexican Americans[18].

The alleles associated with susceptibility to and progression of RA all share a highly conserved amino acid sequence ($Q^{70}KRAA^{74}$, $Q^{70}RRAA^{74}$, or $R^{70}RRAA^{74}$) at positions 70–74 in the third hypervariable region (HVR3) of the DRß1 chain. These sequences are called the rheumatoid epitope or shared epitope (SE)[19]. In contrast, studies on mouse collagen

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induced arthritis, an experimental model for RA, disclosed the importance of aspartic acid (D) at position 70 in protection against the development of arthritis[20-22]. As well, protection against RA in humans was also found to be associated to alleles carrying D at position 70 of the DRß1 chain[23]. The DRB1*0403 allele was shown to have a protective effect against RA in a Taiwanese population[9]. Lee, *et al* reported that DRB1*0701, *0802, *1301, *1302, *1403, and *1405 alleles showed significant protective effects for RA in a Korean population[24]. A study in a Caucasian population also identified a significant underrepresentation of the DRB1*0103 allele among patients with RA[25]. de Vries, *et al* demonstrated that DRB1*07, *1201, *1301, and *1501 were significantly protective against RA in a Dutch population[26].

The SE sequences may also influence clinical manifestations of RA. The $Q^{70}KRAA^{74}$ sequence was found to be associated with more severe disease and a greater frequency of extraarticular involvement[27,28]. This sequence has also been reported to be dominant in rheumatoid factor (RF)-positive patients with RA[29]. However, associations between SE sequences and disease expression are not concordant and remain controversial in other studies[30-32].

To investigate the associations between SE- or DERAA-encoding DRB1 alleles and RA in Taiwanese, we carried out high-resolution sequence-based typing to examine the distribution of all DRB1 alleles. We also tested the associations between these DRB1 alleles and clinical markers such as sex, age at disease onset, RF positivity, and bone erosion rate.

MATERIALS AND METHODS

Two hundred seventy-three Taiwanese patients with RA (221 women, 52 men), all satisfying the 1987 diagnostic criteria[33] of the American College of Rheumatology (ACR) for inflammatory active RA, were recruited consecutively from the outpatient clinic of the Mackay Memorial Hospital, Taipei. The control group consisted of 480 healthy blood donors who were ethnically identical, resided in the same area, and had no history of autoimmune disease. Written informed consent was obtained from all subjects and the study was approved by the Institutional Review Board at Mackay Memorial Hospital.

Clinical evaluation. A structured questionnaire was used to identify demographic characteristics and clinical features of patients, including current age, sex, age at disease onset, and disease duration. Serum concentrations of RF were determined by nephelometric technique, and a titer ≥ 20 IU/ml was considered positive. We examined radiographs of the hands or feet, noting the presence or absence of radiographic changes typical of RA, such as erosions adjacent to the joints, as required for the 1987 ACR criteria[33]. No attempt was made to calculate radiological score. These data are summarized in Table 1.

HLA-DRB1 genotyping. Genomic DNA for HLA-DRB1 typing of blood samples from patients and controls was extracted as described[34]. The protocols and primers used for sequence-based typing of HLA-DRB1 alleles were based on our previous study[35]. This is a high-resolution HLA typing method using polymerase chain reaction (PCR) amplification of genomic DNA, followed by direct DNA sequencing. In brief, the PCR mixture consisted of 50 to 100 ng genomic DNA, PCR buffer (15 mM Tris, pH 8.0, 50 mM KCl, 1.5 mM $MgCl_2$), 0.2 mM each of dNTP, 5% glycerol, 0.1 mg/ml cresol red, 1 ng of each primer that had been modified to amplify the second exon of DRB1 alleles[36], and 0.25 units of AmpliTaq Gold polymerase (Applied Biosystems, Foster City, CA, USA). A reaction mixture volume of 10 μl was then submitted to 32 cycles of amplification using a GeneAmp PCR system (Applied Biosystems). The amplified PCR product was then analyzed on 2% agarose gel electrophoresis and typed for DRB1 alleles by DNA sequencing methods, using fluorescence-labeled dideoxynucleotide terminator chemistry. The analysis was carried out on an ABI 377 DNA sequencer using ABI Sequence Navigator and HLA MatchTool software (Applied Biosystems).

Table 1. Demographic and clinical characteristics in patients with RA and controls.

	Patients, n = 273	Controls, n = 480
Age, mean ± SD yrs	54.1 ± 14.3	41.3 ± 4.7
Sex, no. (%) female/male	221/52 (81/19)	289/192 (60/40)
Age at disease onset, mean ± SD yrs	46.7 ± 14.6	—
Disease duration, mean ± SD yrs	7.3 ± 6.5	—
Rheumatoid factor-positive, no. (%)	214 (78.4)	—
Bone erosion, no. (%)	178 (65.2)	—

Statistical analysis. Allele and phenotype frequencies were determined by direct counting. Agreement with Hardy-Weinberg equilibrium was tested for genotype frequencies of the controls using PyPop statistical software[37] from the International Histocompatibility Working Group based on the method described by Guo and Thompson[38]. The odds ratios (OR) with 95% confidence intervals (95% CI) of the phenotypes, alleles, and genotypes of DRB1 were compared between patients and controls using a freely available program[39] [available on request to yannlee@ms2.mmh.org.tw]. Sex distribution, RF positivity, and bone erosions were also compared using this program. The mean age at disease onset was analyzed by Student's t test, and p values were corrected by multiplying by the number of comparisons. The Bonferroni inequality method was used to correct for multiple comparisons where appropriate; p_c values less than 0.05 (2-tailed) were considered significant. SPSS 12.0 for Windows (SPSS, Chicago, IL, USA) was used for statistical analyses.

RESULTS

HLA-DRB1 phenotype-specific associations. OR and 95% CI values of specific phenotypes in RA patients and controls are shown in Table 2. All the DRB1 genotypes of controls were in Hardy-Weinberg equilibrium (p = 0.36). Phenotypes with the greatest risk for RA were DRB1*0405 and *1001 (OR 4.04, 95% CI 2.84–5.77, $p_c = 3.2 \times 10^{-14}$, and OR 5.25, 95% CI 2.10–13.06, $p_c = 3.0 \times 10^{-3}$, respectively). Although DRB1*0404 tended to be positively associated with RA, and DRB1*0803, *1202, and *1302 were negatively associated, these associations were not significant after correction for multiple comparisons. Further, we performed stratification analysis in terms of RF and bone erosion with HLA-DRB1 phenotypes. HLA-DRB1*0405 and *1001 phenotypes were found to be significantly associated with RF and bone erosion in RA patients (data not shown).

Influence of SE and DERAA alleles on development of RA. To investigate the effect of SE- and DERAA-encoding alleles on RA and whether gene dose affects susceptibility, protection, or the presence of clinical characteristics, patients and controls were divided into 6 genotypes according to their HLA-DRB1 status (Table 3). Here, HLA-DRB1 alleles encoding the RA-susceptible amino acid sequences QKRAA, QRRAA, and RRRAA were considered SE alleles, whereas alleles encoding

Table 2. HLA-DRB1 phenotype frequencies in patients with RA and controls.

DRB1	No. (%) RA Patients	No. (%) Controls	OR	95% CI	p_c
0101	2 (0.7)	4 (0.8)	—	—	—
0301	36 (13.2)	74 (15.4)	0.83	0.54–1.28	NS
0401	5 (1.8)	3 (0.6)	2.97	0.78–11.32	NS
0403	13 (4.8)	35 (7.3)	0.64	0.33–1.21	NS
0404	16 (5.9)	13 (2.7)	2.24	1.08–4.65	NS
0405	107 (39.2)	66 (13.8)	4.04	2.84–5.77	3.2×10^{-14}
0406	17 (6.2)	17 (3.5)	1.81	0.92–3.36	NS
0409	1 (0.4)	0 (0.0)	—	—	—
0410	3 (1.1)	2 (0.4)	2.66	0.53–13.36	NS
0701	8 (2.9)	12 (2.5)	1.18	0.49–2.84	NS
0802	0 (0.0)	6 (1.3)	—	—	—
0803	25 (9.2)	74 (15.4)	0.55	0.34–0.89	NS
0809	4 (1.5)	3 (0.6)	2.36	0.59–9.51	NS
0901	73 (26.7)	135 (28.1)	0.93	0.67–1.30	NS
1001	17 (6.2)	6 (1.3)	5.25	2.10–13.06	3.0×10^{-3}
1101	33 (12.1)	72 (15.0)	0.78	0.50–1.21	NS
1104	1 (0.4)	2 (0.4)	—	—	—
1106	1 (0.4)	0 (0.0)	—	—	—
1201	17 (6.2)	40 (8.3)	0.73	0.41–1.31	NS
1202	40 (14.7)	103 (21.5)	0.63	0.42–0.94	NS
1301	1 (0.4)	2 (0.4)	—	—	—
1302	7 (2.6)	30 (6.3)	0.40	0.18–0.89	NS
1312	3 (1.1)	5 (1.0)	1.06	0.28–4.03	NS
1401	13 (4.8)	36 (7.5)	0.62	0.32–1.17	NS
1402	0 (0.0)	2 (0.4)	—	—	—
1403	0 (0.0)	4 (0.8)	—	—	—
1404	2 (0.7)	3 (0.6)	—	—	—
1405	8 (2.9)	22 (4.6)	0.63	0.28–1.40	NS
1407	0 (0.0)	1 (0.2)	—	—	—
1418	1 (0.4)	1 (0.2)	—	—	—
1443	0 (0.0)	1 (0.2)	—	—	—
1501	35 (12.8)	69 (14.4)	0.88	0.57–1.35	NS
1502	6 (2.2)	19 (4.0)	0.55	0.22–1.34	NS
1504	1 (0.4)	0 (0.0)	—	—	—
1601	0 (0.0)	1 (0.2)	—	—	—
1602	30 (11.0)	50 (10.4)	1.06	0.66–1.71	NS
Total	273 (192.7)	480 (190.2)	—	—	—

P_c were obtained by multiplying the uncorrected p values by 22 using the Bonferroni inequality method. NS: not significant.

the RA-protective amino acid sequence DERAA were considered as DERAA alleles. Non-SE represented all HLA-DRB1 alleles other than SE and DERAA alleles.

These data confirmed, at the genotype level, that patients carrying 2 copies of the SE alleles (SE/SE) or one copy of the SE allele (SE/non-SE) had a higher risk of developing RA compared with the reference group (non-SE/non-SE genotype) or the sum of other groups. Patients carrying DERAA alleles in the absence of SE (DERAA/non-SE) had a lower risk of developing RA, although the observed effect was not statistically significant. Compared to the sum of other groups, the non-SE/non-SE genotype had a lower risk of developing RA. When we compared patients with at least one copy of the SE allele (n = 138) to the sum of other groups, a significantly higher risk was found (OR 4.31, 95% CI 3.11–5.99, $p_c = 5.02 \times 10^{-19}$). However, patients carrying at least one copy of DERAA (n = 8) did not show significantly lower risk ($p_c = 0.06$).

The effects of various genotypes on clinical features were then evaluated. Age at disease onset in subjects with the SE/SE and SE/non-SE genotypes was roughly 4 years younger than in those with the non-SE/non-SE genotype, but the differences were not statistically significant. The result also revealed that patients with genotypes of SE/SE and SE/non-SE had a significantly higher rate of bone erosion. Analyses of RF positivity indicated no significant differences.

*Effect of DRB1*0405 and *1001 alleles on RA.* To determine whether susceptibility to RA was influenced by the combination of the 2 most strongly associated alleles, DRB1*0405 and *1001, we compared frequencies of genotypes grouped according to the presence of DRB1*0405 and *1001 (Table 4). Compared with the reference group (X/X genotype), individuals with either homozygote or heterozygote of these 2 alleles were significantly associated with a risk of RA, except the DRB1*1001/*1001 genotype, which was not present in both patients and controls. However, all the risk genotypes for RA except DRB1*0405 homozygote remained significant when compared to the sum of other groups. Among them, the heterozygote of DRB1*0405/*1001 demonstrated the greatest risk for RA compared with the reference group (OR 15.8, 95% CI 2.48–100.7, $p_c = 0.004$) or with the sum of other groups (OR 10.8, 95% CI 1.69–68.3, $p_c = 0.03$). Further, the X/X genotype indicated a lower risk of developing RA as compared to the sum of other groups.

The mean age at disease onset in subjects with DRB1*0405/*0405, *0405/*1001, *0405/X, and *1001/X was not statistically significant compared with the X/X genotype. Comparisons of RF positivity and bone erosion in RA patients with different risk genotypes revealed no significant differences, except that a significantly higher frequency of bone erosion was found in the DRB1*0405/X genotype (Table 4).

To further differentiate the influence of DRB1*0405 or DRB1*1001 allele on clinical characteristics of RA patients, we did a separate reanalysis (Table 5). The percentages of female patients were significantly lower in subjects with at least one copy of DRB1*0405 and DRB1*1001 than in those with the X/X genotype. The mean age at disease onset in patients with at least one copy of DRB1*0405 and DRB1*1001 was not statistically significant as compared to the X/X genotype. We also observed significant increases of frequency in both RF positivity and bone erosion in patients with at least one copy of DRB1*0405, but not in those with at least one copy of DRB1*1001.

DISCUSSION

Associations between HLA and RA have been studied extensively since the first report in 1976[40]. Based on the studies of Caucasian and other ethnic group patients with RA, the SE

Table 3. Influence of HLA-DRB1 genotypes on RA susceptibility, age at disease onset, rheumatoid factor positivity, and bone erosion.

Genotype[†]	RA (N = 273) no. (%)	Controls (N = 480) no. (%)	OR (95% CI)[a]	OR (95% CI)[b]	Age at Disease Onset, mean ± SD yrs	RF-Positive, %	Bone Erosion, %
SE/SE	20 (7.3)	7 (1.5)	7.93 (3.35–18.7)[c]	5.34 (2.28–12.5)[d]	41.5 ± 11.5	85.0	85.0[††]
SE/non-SE	116 (42.5)	83 (17.3)	3.88 (2.75–5.48)[e]	3.53 (2.53–4.95)[f]	42.2 ± 12.8	83.6	72.4[‡]
SE/DERAA	2 (0.7)	2 (0.4)	2.78 (0.49–15.9)	1.76 (0.31–10.1)	46.1 ± 2.3	50.0	50.0
Non-SE/non-SE	129 (47.3)	358 (74.6)	—	0.31 (0.22–0.42)[g]	46.2 ± 14.2	72.9	56.6
DERAA/non-SE	6 (2.2)	29 (6.0)	0.57 (0.24–1.38)	0.35 (0.15–0.83)	48.5 ± 15.6	83.3	50.0
DERAA/DERAA	0 (0.0)	1 (0.2)	—	—	—	—	—

All odds ratios and 95% confidence intervals were calculated by comparing each group with the reference group[a] (non-SE/non-SE genotype) or with the sum of other groups[b]. Corrected p (p_c) values were obtained by multiplying the uncorrected p value by 4 (compared with the reference group) or 5 (compared with the sum of other groups) using the Bonferroni inequality method. [†] Shared epitope (SE) alleles include DRB1*0101, *0102, *0104, *0404, *0405, *0408, *0409, *0410, *0413, *0416, *0419, *0421, *1001, *1402, and *1406. DERAA alleles include DRB1*0103, *0402, *1102, *1103, *1301, *1302, and *1323. Non-SE represents all DRB1 alleles other than SE and DERAA alleles. [a] OR (95% CI) values for comparison with reference group. [c] $p_c = 4.52 \times 10^{-7}$; [e] $p_c = 1.22 \times 10^{-14}$. [b] OR (95% CI) values for comparison with the sum of other groups. [d] $p_c = 1.57 \times 10^{-4}$; [f] $p_c = 2.37 \times 10^{-13}$; [g] $p_c = 2.30 \times 10^{-13}$. [††] $p_c = 0.032$. [‡] $p_c = 0.02$. RF: rheumatoid factor.

Table 4. Influence of 2 confirmed RA susceptibility alleles on RA susceptibility, age at disease onset, rheumatoid factor positivity, and bone erosion.

DRB1 Genotype	RA (N = 273) no. (%)	Controls (N = 480) no. (%)	OR (95% CI)[a]	OR (95% CI)[b]	Age at Disease Onset, mean ± SD yrs	RF-Positive, %	Bone Erosion, %
*0405/*0405	6 (2.2)	3 (0.6)	5.28 (1.43–19.5)[c]	3.57 (0.97–13.1)	39.9 ± 12.0	66.7	100
*1001/*1001	0 (0.0)	0 (0.0)	—	—	—	—	—
*0405/*1001	6 (2.2)	1 (0.2)	15.8 (2.48–100.7)[d]	10.8 (1.69–68.3)[e]	42.3 ± 19.1	83.3	83.3
*0405/X	95 (34.8)	62 (12.9)	4.04 (2.80–5.85)[f]	3.60 (2.50–5.18)[g]	48.9 ± 15.6	86.3	74.7[†]
*1001/X	11 (4.0)	5 (1.0)	5.81 (2.07–16.3)[h]	3.99 (1.43–11.1)[i]	47.8 ± 14.8	81.8	63.6
X/X	155 (56.8)	409 (85.2)	—	0.23 (0.16–0.32)[j]	45.8 ± 13.9	73.5	57.4

All odds ratios and 95% confidence intervals were calculated by comparing each group with the reference group (X/X genotype)[a] or with the sum of other groups[b]. Corrected p (p_c) values were obtained by multiplying the uncorrected p value by 4 (compared with the reference group) or 5 (compared with the sum of other groups) using the Bonferroni inequality method. X represents all alleles except DRB1*0405 and *1001. [a] OR (95% CI) values for comparison with reference group. [c] $p_c = 0.036$; [d] $p_c = 0.004$; [f] $p_c = 5.88 \times 10^{-14}$; [h] $p_c = 1.27 \times 10^{-3}$. [b] OR (95% CI) values for comparison with the sum of other groups. [e] $p_c = 0.03$; [g] $p_c = 5.98 \times 10^{-12}$; [i] $p_c = 0.03$; [j] $p_c = 2.57 \times 10^{-17}$. [†] $p_c = 0.024$. RF: rheumatoid factor.

Table 5. Comparison of clinical profiles in RA patients, stratified by the presence of the HLA-DRB1*0405 and *1001 alleles.

	DRB1*0405[†]				DRB1*1001[‡]			
	Positive (N = 107)	X/X (N = 155)	OR (95% CI)	p	Positive (N = 17)	X/X (N = 155)	OR (95% CI)	p
Female/male, no. (%)	81/26 (75.7/24.3)	134/21 (86.5/13.5)	0.49 (0.26–0.92) 2.05 (1.09–3.85)	0.04	11/6 (64.7/35.3)	134/21 (86.5/13.5)	0.29 (0.09–0.83) 3.48 (1.21–10.13)	0.03
Age at disease onset, mean ± SD yrs	48.0 15.7	45.8 ± 13.9	—	NS	45.9 ± 16.0	45.8 ± 13.9	—	NS
RF-positive, no. (%)	92 (86.0)	114 (73.5)	2.21 (1.16–4.20)	0.016	14 (82.4)	114 (73.5)	1.68 (0.49–5.71)	NS
Bone erosion, no. (%)	82 (76.6)	89 (57.4)	2.43 (1.41–4.20)	0.001	12 (70.6)	89 (57.4)	1.78 (0.62–5.08)	NS

X represents all alleles except DRB1*0405 and *1001. [†] RA patients homozygous or heterozygous for DRB1*0405. [‡] RA patients homozygous or heterozygous for DRB1*1001. RF: rheumatoid factor; NS: nonsignificant.

hypothesis has been proposed to account for the observed HLA-DRB1 associations. Studies have also shown that DRB1 alleles carrying the DERAA sequence prevent the onset of RA[23,41-44]. We studied SE- and DERAA-encoding HLA-DRB1 allele associations with RA in Taiwanese patients, on whom only sparse information had been reported.

We found that DRB1*0405 ($Q^{70}RRAA^{74}$) and DRB1*1001 ($R^{70}RRAA^{74}$) were the most significant alleles conferring susceptibility to RA. Further stratification analyses also revealed these 2 alleles were significantly correlated with patients who were RF-positive and positive for bone erosions. Previously, only DRB1*0405 had been reported to be associated with RA in Taiwanese[9] and other Asian ethnic populations[6-8,10,11]. In addition, we observed that one DRB1 allele

(DRB1*0404) and 3 DRB1 alleles (DRB1*0803, *1202, and *1302) tended to be positively and negatively associated with RA, respectively. DRB1*0803 and *1202 alleles encoding neither the SE nor the DERAA sequence have also been described by a few studies[11,24,45,46]. These studies also reported there were no significant differences in the frequencies of DRB1*0803 or *1202 alleles between RA patients and controls. Although these associations were not significant after correction for p value, a further study with data sets of larger sample size should be conducted to confirm these possible associations.

Association of RA with HLA-DRB1*10 was observed in some Spanish populations[13], but not in Galician people from the Lugo region of Northwestern Spain[4], and was subsequently reported in different ethnic groups[5,11,12,14-16] other than Asian populations. In Singaporean Chinese subjects, Chan, *et al*[7] observed that frequency of DRB1*1001 was higher in patients with RA, but this did not reach a significant level after correction for multiple comparisons. Our results revealed that the DRB1*1001 phenotype, rare in a Taiwanese population, was strongly associated with RA (OR 5.25, 95% CI 2.10–13.06, $p_c = 3.0 \times 10^{-3}$), which has not been documented before. This novel finding further validates that RA in Taiwanese is associated with the same amino acid motif of the HLA-DRß1 chain, $R^{70}RRAA^{74}$, that confers RA susceptibility in various ethnic populations.

Although the shared epitope and RA protection model hypotheses have been adopted successfully to explain why the Class II regions of the major histocompatibility complex confer susceptibility to or protection against RA, some ethnic exceptions have been observed[47,48]. Thus, we examined whether these hypotheses can be applied to the Taiwanese population. When data were analyzed based on the SE and DERAA classifications, it was found that both the single-dose SE allele (SE/non-SE) and the double SE allele (SE/SE) conferred a higher risk for RA susceptibility. But the dose effect of SE allele was not determined in our study. On the other hand, the frequency of the genotype of the single DERAA allele (DERAA/non-SE) in RA patients was not significantly lower than that in controls. Our analyses clearly showed that the SE hypothesis is applicable to Taiwanese patients with RA. In contrast, the protective effect of DERAA-bearing alleles on development of RA could not be affirmed.

To further illuminate the effect of the susceptibility alleles, we investigated the effect of combinations of DRB1*0405 and *1001. The results revealed that either the single dose of DRB1*0405 allele (*0405/X) or double doses of DRB1*0405 alleles was significantly associated with RA compared to the reference group, but a dose effect of this risk allele was not observed. Because of the findings that homozygosity for DRB1*1001 in both RA patients and controls was null, our study was unable to address the question of a dose effect of DRB1*1001. Intriguingly, the risk of RA conferred by the compound heterozygote DRB1*0405/*1001 was found to be the highest among all combinations. Similar findings have also been reported that the compound heterozygotes DRB1*0401/*0404 and DRB1*0405/*0901 present much higher risks for RA than their respective homozygotes[24,49,50]. The underlying mechanisms that explain the combined effect of HLA-DRB1 heterozygotes remain elusive. One possible explanation might be that heterozygosity of HLA has a greater repertoire of molecules with which to present foreign antigens[51], providing the predisposition to the development of an autoimmune disease such as RA.

There is still controversy whether the influence of HLA-DRB1 alleles on RA is due to effects on disease susceptibility or on disease severity and progression. Gonzalez-Gay, *et al* reported the choice of treatment with cyclosporine A in patients with RA who were refractory to methotrexate therapy was primarily associated with patients carrying DRB1*0401, although interestingly there was no association with seropositivity[52]. In our study, RA patients with single or double doses of SE alleles were significantly associated with bone erosion. Further, analyses of the relationships between the presence of the most susceptible alleles, DRB1*0405 and *1001, and clinical measures revealed that only patients homozygous or heterozygous for DRB1*0405 were significantly associated with RF positivity and bone erosion, as compared to those without this allele (Table 5). Similar correlations between DRB1*0405 and bone erosion of Asian patients with RA have been reported in many studies[9,53,54]. We also found that among RA patients with at least one copy of DRB1*0405 and *1001 there were significantly fewer women compared to those without these alleles. Although our study provides further evidence for the association of the HLA-DRB1*0405 allele with clinical features, larger studies are needed before it can be used as a marker for routine clinical use.

Our results suggest that susceptibility to RA in Taiwanese is associated with the presence of SE-encoding HLA-DRB1 phenotypes. Compound heterozygotes of DRB1*0405 and *1001 confer the highest susceptibility to RA, suggesting that these alleles enhance susceptibility to RA in the Taiwanese population.

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