Wen-Xin Liu, MD^{1,2}

Yao Jiang*, MD^{1,2}

Qing-Xiang Hu, MD²

Xie-Bo You, MD²

¹Medical College of Soochow University,
Suzhou, China

²Department of Orthopedics, Shanghai Sixth
People's Hospital, Shanghai, 200233, China

HLA-DRB1 shared epitope allele polymorphisms and rheumatoid arthritis: a systemic review and meta-analysis

Abstract

Purpose: A meta-analysis was performed to determine which HLA-DRB1 alleles are associated with increased risk of rheumatoid arthritis (RA) in Asian patients.

Methods: Medline, PubMed, Central, EMBASE, Cochrane, and Google Scholar databases were searched until November 3, 2015 using the following keywords: rheumatoid arthritis; RA, HLA-DRB1; severity; treatment; and, prognosis. Randomized-controlled-trials, prospective, retrospective, cohort and case-controlled studies that examined the HLA-DRB1 allelic association with RA in Asians were included. The frequencies of allelic types and the shared epitope (SE) were compared between patients with or without RA.

Results: A total of 331 articles were identified after duplicates removed, and 40 studies, with 5470 RA patients and 5837 control patients, were included in the analysis. Pooled odds ratios (ORs) revealed the frequencies of HLA-DRB1*04 and *10 were higher in the RA group, and the frequency of *14 was lower in the RA group as compared to controls (*04: OR = 3.35, 95% CI: 2.28-3.99, $p < .001$; *10: OR = 2.08, 95% CI: 1.55-2.78, $p < .001$; *14: OR = 0.70, 95% CI: 0.54-0.90, $p = .006$). No associations were noted for *01 and *09. Pooled ORs revealed associations of *0101 (OR = 1.58), *0401 (OR = 2.17), *0404 (OR = 1.91), *0405 (OR = 3.73), *0410 (OR = 2.24), *1001 (OR = 1.78) and SE positive (OR = 2.38) with RA. HLA-DRB1 *14 subtypes did not show associations with RA.

Conclusions: HLA-DRB1 allelic variations are associated with RA in Asian patients.

Manuscript submitted 14th April, 2016

Manuscript accepted 3rd August, 2016

Clin Invest Med 2016; 39 (6): E182-E203.

Correspondence to:

Yao Jiang

Medical College of Soochow University, Suzhou, China; Department of Orthopedics,
Shanghai Sixth People's Hospital, Shanghai, 200233, China

E-mail: jiangyaodoctor@sina.com

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by synovitis and the localized destruction of cartilage and bone that can eventually lead to profound disability. The disease is more common in females than males, and the worldwide prevalence is estimated to be around 1% [1]. Environmental and genetic factors have both been implicated in the development of RA [1]. In particular, the major histocompatibility complex (MHC) has been shown to influence disease susceptibility and severity.

Human leukocyte antigens (HLAs) are an important part of the immune system – triggering the T cells of the immune system to produce antibodies against non-self (and sometimes self) cells. Variations in HLAs have been estimated to account for 30% to 50% of the overall genetic risk for RA [2,3]. HLA-DRB1 alleles that have been found to be associated with RA (*0401, *0404, *0405, *0408, *0413, *0101, *0102, *1402, and *1001) encode a common sequence at amino acids (70-75) in the third hypervariable region of the β -chain, referred to as the shared epitope (SE) [4]. While various definitions of the SE are found across studies, Mourad *et al.* [5] defined SE+ as the sum of DRB1*01 *04 *10 *14. Associations of RA with HLA-DRB1 alleles have been observed in all racial and ethnic populations, and HLA-DRB1 alleles containing the SE have been observed in approximately 50% to 70% of RA patients who are of European ancestry [1]. Different HLA-DRB1 SE alleles have been reported to be associated with certain ethnicities [6]; for example, HLA-DRB1 *0401 and *0404 are most common in Northern Europeans, whereas *1402 is seen in Native Americans, and *0101 and *1001 in Greek and Spanish populations [7].

Studies in Asian populations of the associations of HLA-DRB1 alleles with RA have provided somewhat inconsistent results. Liu *et al.* [8] reported the frequency of HLA-DRB1 *0404 and *0405 alleles were significantly increased in Chinese patients with RA, while Zhang *et al.* [9] reported no associations. Yang *et al.* [10] reported that HLA-DRB1 *04, *0401, *0405, and *0410 alleles are risk factors for RA in Chinese individuals, whereas Jun *et al.* [11] reported that *0101 *0401 *0405 *0410 and *1010 conferred susceptibility in Asian populations.

The purpose of this study was to perform a meta-analysis of studies that examined HLA-DRB1 alleles in Asian populations with RA to determine which alleles were associated with increased risk of RA, and which might have a protective effect.

Methods

Literature search strategy and study selection criteria

This systematic review and meta-analysis was conducted in accordance with PRISMA guidelines [12]. Medline, PubMed, Central, EMBASE, Cochrane and Google Scholar databases were searched from inception until June 15, 2016 using the following keywords: rheumatoid arthritis; RA; HLA-DRB1; severity; treatment; and, prognosis.

Inclusion criteria for the meta-analysis were as follows: 1) studies included randomized controlled trials (RCTs), prospective and retrospective studies and cohort and case-controlled studies; 2) patients had a proven diagnosis of RA by American College of Rheumatology (ACR) criteria [13]; and, 3) studies examined the association of HLA-DRB1 alleles with RA in Asian patients. Letters, comments, editorials, case reports, proceedings, and personal communications were excluded, as were studies published prior to 1990 and those that reported no quantitative primary outcome. Reference lists of relevant studies were hand-searched. Searches were performed by two independent reviewers, and a third was consulted for resolution of any disagreements.

Data extraction

The following information/data were extracted from studies that met the inclusion criteria: the name of the first author, year of publication, study design, number of participants in each group, participants' age and gender, and associations of HLA-DRB1 alleles with RA.

Quality assessment

The Newcastle-Ottawa scale to assess the included studies [14]. Briefly, the Newcastle-Ottawa scale is a validated tool to evaluate the quality of nonrandomized studies in three broad areas: enrollment criteria; comparability of study groups; and, assessment of outcomes. The quality of each study is scored on the basis the areas mentioned above, with 4 of the 9 total points indicating the quality of the patient selection; 2 points, the quality of between-group comparability; and 3 points, the quality of the outcome assessment. A score of 9 indicates the highest quality, a score of 0, the lowest. Two independent reviewers utilized the NOS list to assess the quality of the included studies.

TABLE 1. Basic characteristics of studies included in the systematic review

1st author (publication year)	Study design	Country or ethnicity	Control group ^a			RA group					
			Number of controls	Age (y)	Males (%)	Number of patients	Age (y)	Males (%)	Duration of disease (y)	RF seropositivity, n (%)	Quality score (NOS)
Wang (2016)	Case-controlled	Chinese (Shaanxi)	111	49.49 (5.38)	0	109	50.94 (6.54)	0		95 (85.3%)	7
Louthrenoo (2015)	Case-controlled	Thai	99	46.5 (15.1)	48%	100	51.69 (11.86)	7%	9.24 (7.55)	91 (91%)	6
Saghafi (2014)	Prospective	Northern Iran	72			177	44.83	18			9
Mourad (2013)	Case-controlled	Syria	200	40.21 (10.11)	20	86	41.41 (10.57)	20	11.26 (6.25)	55 (63.95%)	8
Sariyildiz (2013)	Case-controlled	Turkish	84	45.07 (9.15)	15.6	96	46.97 (13.44)	17.4	7.52 (6.86)	64 (66.7%)	8
Prasannavar (2013)	Case-controlled	Western India	90			100	Early: 26.3 (3.8); Late: 46.5 (12.4)	10	Early: 23; Late: 77	72 (72%)	8
Chang (2012)	Case-controlled	Chinese (Neimenggu)	100	52 (15, 79) [^]	24	140	51 (12, 81) [^]	24			8
Shi (2012)		Chinese (Xinjiangweiwuerzu)	118	47.08 (13.17)	26	157	47.66 (13.24)	20			8
Chen (2010)	Case-controlled	Chinese (Shanxi)	78	48(11)	15	90	52 (10)	23	53 (3) mo		8
Duan (2008)	Case-controlled	Chinese (North Anhui)	40		25	102		21			8
Lin (2007)	Case-controlled	Chinese (Shantou)	100			117	48.8 (18, 71) [^]	31	5.2	90 (77%)	7
Ali (2006)	Case-controlled	Pakistan	91			91	43.4 (11)	18	7.03 (4.48)	79 (87%)	7
Alsaied (2005)	Case-controlled	Kuwaiti	70			47	39 (14.3)	19	9 (7)	35 (74%)	7
Su (2004)	Case-controlled	Chinese (Beijing)	79 ^a	43 (16)	21	136	53 (14)	14	7 (4 mo, 40)*		7
Lee (2004)	Case-controlled	Korean	392	41.9 (14.1)	12	574	50.6 (11.5)	8	12.5 (7.7)	459 (80%)	7
Kinikli (2003)	Case-controlled	Turkey	110	49.8(12.1)	25	104	51.6 (13.8)	23	48 (12, 144)* mo	82 (78.8%)	7
Li (2003)	Case-controlled	Chinese (Shandong)	130	41.5	23	132	46 (19, 76) [^]	21	5.4 (2 mo to 30 y) [^]	101 (76.5%)	8
Zhang (2003)	Case-controlled	Chinese (Fujian)	100			98	51.7 (20, 69)*	24	>2 y: 46 (46.9%)		8
Liu (2002)	Case-controlled	Chinese (Guizhou)	51	32 (19, 53)*	41	35	46 (9, 75)*	20			7
Wu (2001)	Case-controlled	Chinese (Changsha)	76			105	43.5	20			7
Lin (2001)	Case-controlled	Chinese (Shantou)	100			117	40.8 (16, 70.5)*	31			7
Griffiths (2000)	Cross sectional	Asian				107	52 (42–57) [§]	17	7 (3–14) [§]	69 (65%)	9
Liu (2000)	Case-controlled	Chinese (Wuhan)	126			78	39 (10)	15	10 (8)		8
Zhang (1999)		Chinese (Hebei)	100	44.4 (8.4)	30	180	39.9 (14.3)	22	4.5 (3.9) range: 0-20 y	107 (59.4%)	7
Kim (1999)	Case-controlled	Korean	106	28	71	90	38.4				7
Tokunaga (1999)		Japanese (Fukuoka)	493			75					7
Yuan (1998)	Case-controlled	Chinese (Han)	100			35					8
Gu (1998)	Case-controlled	Chinese (Guangdong)	102			47	41.63 (11.02)	43	9.46 (11.19)		8
Yukioka (1998)	Case-controlled	Japanese (Kansai)	652			852	44.4 (13.4)	19	14.6 (9.8)		7
Wakitani (1998)		Japanese (Kansai)	652			379	65.5	23	14		7
Xiao (1997)	Case-controlled	Chinese (Neimenggu)	50	37.1 (19, 58) [^]	24	28	47.6 (19, 72) [^]	21	7.6 (1 mo, 37) [^]		7
Zhao (1997)	Case-controlled	Chinese (North)	106			86					7
Higami (1997)	Case-controlled	Japanese (Tokyo)	150			82	51.5 (15.6-81.5)*	28	5.5 (2.0-12.0) mo*	77 (94%)	7
Toda (1997)	Retrospective	Japanese	493			322	57	9	7		7
Zhao (1996)	Case-controlled	Chinese (Beijing)	106			86					7
Wang (1996)	Case-controlled	Chinese (Anhui)	30	33.5	63	30	37.5	27		20 (66.7%)	7
Takeuchi (1996)	Case-controlled	Japanese	96			96	55.4 (11.7)	11		86 (89.6%)	7
Kim (1995)	Case-controlled	Korean	118			95	(20, 62)	7	8.3 (1.8-14.7) [^]	75 (78.9%)	7
Molkentin (1993)	Case-controlled	Chinese (Shanghai)	21			23		35			7
Seglias (1992)	Case-controlled	Chinese (Southern Chinese)	45		N	66		N	N	N	7

RA, rheumatoid arthritis; NOS, Newcastle-Ottawa scale; y, years; mo, months.

Age, duration of disease, and clinical measurements were summarized as mean, mean (SD), mean (range)[^], median (Range)^{*}, and median (IQR)[§].^a All controls were non-RA patients.

TABLE 2. Summary of the results of the pooled effects of the included studies

Number of studies	HLA DRB1 allele type	Odds ratio	Lower limit	Upper limit	z-value	p-value	Heterogeneity test
19	*01	1.117	0.721	1.729	0.496	0.620	$I^2 = 71.71\%$, $Q = 63.62$, $df = 18$, $p < .001$
32	*04	3.354	2.823	3.985	13.766	0.000	$I^2 = 52.88\%$, $Q = 65.79$, $df = 31$, $p < .001$
16	*09	0.869	0.667	1.133	-1.035	0.301	$I^2 = 44.21\%$, $Q = 26.89$, $df = 15$, $p = .030$
19	*10	2.078	1.552	2.783	4.909	0.000	$I^2 = 25.99\%$, $Q = 24.32$, $df = 18$, $p = .145$
11	*14	0.698	0.540	0.904	-2.729	0.006	$I^2 = 0\%$, $Q = 8.75$, $df = 10$, $p = .556$
10	*0101	1.584	1.317	1.905	4.887	0.000	$I^2 = 16.69\%$, $Q = 10.80$, $df = 9$, $p = .289$
4	*0102	1.312	0.594	2.895	0.672	0.502	$I^2 = 0\%$, $Q = 0.314$, $df = 3$, $p = .957$
20	*0401	2.167	1.618	2.902	5.191	0.000	$I^2 = 0\%$, $Q = 9.04$, $df = 19$, $p = .973$
20	*0404	1.912	1.319	2.772	3.419	0.001	$I^2 = 0\%$, $Q = 14.84$, $df = 19$, $p = .733$
25	*0405	3.731	3.002	4.637	11.875	0.000	$I^2 = 59.51\%$, $Q = 59.28$, $df = 24$, $p < .001$
12	*0408	1.867	0.920	3.790	1.728	.084	$I^2 = 0\%$, $Q = 9.79$, $df = 11$, $p = .550$
12	*0410	2.236	1.231	4.064	2.641	.008	$I^2 = 0\%$, $Q = 4.74$, $df = 11$, $p = .943$
6	*0901	1.101	0.692	1.753	0.407	0.684	$I^2 = 74.91\%$, $Q = 19.93$, $df = 5$, $p = .001$
5	*1001	1.784	1.117	2.850	2.424	.015	$I^2 = 0\%$, $Q = 3.44$, $df = 4$, $p = .488$
4	*1401	0.866	0.544	1.377	-0.609	0.542	$I^2 = 0\%$, $Q = 2.86$, $df = 3$, $p = .414$
3	*1402	1.560	0.333	7.316	0.564	0.573	$I^2 = 0\%$, $Q = 0.378$, $df = 2$, $p = .828$
2	*1403	0.904	0.242	3.375	-0.151	0.880	$I^2 = 0\%$, $Q = 0.009$, $df = 1$, $p = .924$
3	*1405	0.468	0.149	1.476	-1.296	0.195	$I^2 = 56.59\%$, $Q = 4.61$, $df = 2$, $p = .100$
9	SE positive	2.384	1.385	4.104	3.136	0.002	$I^2 = 86.61\%$, $Q = 59.74$, $df = 8$, $p < .001$

Outcome measures and statistical analysis

The primary outcome of the analysis was the association of RA and HLA-DRB1 shared epitope allele frequencies. The frequencies of allelic types *01, *04, *09, *10, *14, *0101, *0102, *0401, *0404, *0405, *0408, *0410, *0901, *1001, *1401, *1402, *1403, *1405, and SE positive were compared between RA patients and patients without RA (control). Data were summarized as number of alleles by group for each study, and odds ratios (ORs) with a 95% confidence interval (CIs) were calculated for binary outcomes for each individual study, as well as for all of the studies combined for each of the alleles. $OR > 1$ indicated the RA group had higher allelic frequency than the control group; $OR < 1$ indicated the RA group had lower allelic frequency than control group; and $OR = 1$ indicated the allelic frequency was similar between the RA and control groups.

Heterogeneity among the studies was assessed by calculating the Cochran Q and the I^2 statistic. A Q statistic with a value of $p < .1$ or an I^2 statistic $> 50\%$ was considered to indicate significant heterogeneity between studies. When significant heterogeneity was present, a random-effects model (DerSimonian-Laird method) of analysis was used; otherwise a fixed-effects model (Mantel-Haenszel method) of analysis was used. Sensitivity analysis was carried out based on the leave-one-out approach. Publication bias was assessed by constructing a funnel plot and by Egger's test. The absence of publication bias is indicated by the data points forming a symmetric, funnel-shaped distribution and by a 1-tailed significance level of $p > .05$ in Egger's test. All data were compiled using Microsoft® Office Excel® 2007, and all analyses were performed using Comprehensive Meta-Analysis statistical software, version 2.0 (Biostat, Englewood, NJ, USA).

FIGURE 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram of study selection.

Results

Literature search

A flow diagram of study selection is shown in Figure 1. A total of 720 articles were identified in the database searches and 44 through other sources; and 327 remained after duplicates were removed. After screening by title and abstract, 215 were excluded based on the inclusion and exclusion criteria. The full texts of the remaining 116 articles were examined and 76 were excluded, for the reasons shown in Figure 1; thus, 40 articles were included in the meta-analysis [5,8,15-52].

Study characteristics

A summary of the study characteristics is shown in Table 1. The total number of RA patients was 5,470 (range, 23 to 852 patients/study), and the total number of control patients was 5,837 (range, 21 to 652 patients/study). The majority of the studies were case-controlled, and the percentage of males ranged from 0% to 71%. The mean/median age ranged from 26 years to 65 years, and disease duration from 7 years to 14 years.

The frequencies of HLA-DRB1 alleles in RA patients and controls, and the association results from the individual studies are available upon request.

FIGURE 2. Forest plot of the combined effect of the association between (A) *01, (B) *04, (C) *09, (D) *10, (E) *14 and (F) SE positive with rheumatoid arthritis. Abbreviations: 1st AU, first author; 95%CI, lower limit and upper limit.

(C)

Heterogeneity test : $I^2=44.21\%$, $Q\text{-value}=26.89$, $df=15$, $p\text{-value}=0.030$

(D)

Heterogeneity test : $I^2=25.99\%$, $Q\text{-value}=24.32$, $df=18$, $p\text{-value}=0.145$

FIGURE 2 continued. Forest plot of the combined effect of the association between (A) *01, (B) *04, (C) *09, (D) *10, (E) *14 and (F) SE positive with rheumatoid arthritis. Abbreviations: 1st AU, first author; 95%CI, lower limit and upper limit.

(E)

Heterogeneity test : $I^2=0\%$, $Q\text{-value}=8.75$, $df=10$, $p\text{-value}=0.556$

(F)

Heterogeneity test : $I^2=86.61\%$, $Q\text{-value}=59.74$, $df=8$, $p\text{-value}<0.001$

FIGURE 2 continued. Forest plot of the combined effect of the association between (A) *01, (B) *04, (C) *09, (D) *10, (E) *14 and (F) SE positive with rheumatoid arthritis. Abbreviations: 1st AU, first author; 95%CI, lower limit and upper limit.

FIGURE 3. Forest plot of the combined effect of the association between *04 subtypes: (A) *0401, (B) *0404, (C) *0405, (D) *0408, and (E) *0410 with rheumatoid arthritis. Abbreviations: 1st AU, first author; 95%CI, lower limit and upper limit.

(D)

FIGURE 3 continued. Forest plot of the combined effect of the association between *04 subtypes: (A) *0401, (B) *0404, (C) *0405, (D) *0408, and (E) *0410 with rheumatoid arthritis. Abbreviations: 1st AU, first author; 95%CI, lower limit and upper limit.

(E)

FIGURE 3 continued. Forest plot of the combined effect of the association between *04 subtypes: (A) *0401, (B) *0404, (C) *0405, (D) *0408, and (E) *0410 with rheumatoid arthritis. Abbreviations: 1st AU, first author; 95%CI, lower limit and upper limit.

Associations between major allelic types *01 *04 *09, *10, and *14 with RA

A total of 19, 32, 16, 19, and 11 studies with completed data of alleles *01, *04, *09, *10, and *14, respectively, were included in this analysis. A random-effects model was used for *01, *04, and *09, and a fixed-effects model for *10, and *14 (*01: $I^2 = 71.71\%$, $Q = 63.2$, $p < .001$; *04: $I^2 = 52.88\%$, $Q = 65.79$, $p < .001$; *09: $I^2 = 44.21\%$, $Q = 26.89$, $p = .030$; *10: $I^2 = 25.99$, $Q = 24.32$, $p = .145$; *14: $I^2 = 0\%$, $Q = 8.75$, $p = .556$). The pooled ORs revealed the frequencies of *04 and *10 were higher in the RA group, and the frequency of *14 was lower in the RA group as compared to controls (*04: OR = 3.35, 95% CI: 2.28-3.99, $p < .001$; *10: OR = 2.08, 95% CI: 1.55-2.78, $p < .001$; *14: OR = 0.70, 95% C : 0.54-0.90, $p = .006$) (Table 2; Figures 2B, D, E). No associations were noted for *01 and *09.

Associations between the subtypes of *01 *04 *09, *10, and *14 with RA

For *01, a total of 10 and 4 studies provided data of the allelic subtypes *0101 and *0102, respectively. A fixed effects model of analysis was used for both subtypes (*0101: $I^2 = 16.69\%$, $Q = 10.8$, $p = .289$; *0102: $I^2 = 0\%$, $Q = 0.314$, $p = .957$). The pooled OR revealed the frequency of *0101 was higher in RA patients than controls, but no association with *0102 was noted (*0101: OR = 1.58, 95% CI: 1.32-1.91, $p < 0.001$; *0102: OR = 1.31, 95% CI: 0.59-2.90, $p = .502$) (Table 2).

For *04, a total of 20, 20, 25, 12, and 12 studies provided data of the allelic subtypes *0401, *0404, *0405, *0408, and *0410, respectively. A fixed-effects model of analysis was used for *0401, *0404, *0408, and *0410, and a random-effect model for *0405. (*0401: $I^2 = 0\%$, $Q = 9.04$, $p = .973$; *0404: $I^2 = 0\%$, $Q = 14.84$, $p = .733$; *0405: $I^2 = 59.5\%$, $Q = 59.3$, $p < .001$; *0408: $I^2 = 0\%$, $Q = 9.79$, $p = .550$; *0410: $I^2 = 0\%$, $Q = 4.74$, $p = .943$). The pooled ORs revealed that all subtypes, except for *0408, were associated with RA (*0401: OR = 2.17, 95% CI: 1.62-2.90, $p < .001$; *0404: OR = 1.91, 95% CI:

1.32-2.77, $p = .001$; *0405: OR = 3.73, 95% CI: 3.00-4.64, $p < .001$; *0408: OR = 1.87, 95% CI: 0.92-3.80, $p = .084$; *0410: OR = 2.24, 95% CI: 1.23-4.06, $p = .008$) (Table 2; Figure 3A-E).

For *09, a total of six studies provided data of the allelic subtype *0901. A random-effects model of analysis was used ($I^2 = 74.91\%$, $Q = 19.93$, $p = .001$), and the pooled OR did not reveal an association between RA and *0901 (OR = 1.10, 95% CI: 0.69-1.75, $p = .684$) (Table 2).

For *10, a total of five studies provided data of the allelic subtype *1001. A fixed effects model of analysis was used ($I^2 = 0\%$, $Q = 3.44$, $p = .488$), and the pooled OR revealed *1001 was associate with RA (OR = 1.78, 95% CI: 1.12-2.01, $p = .015$) (Table 2).

For allelic subtypes of *14, a total of four, three, two and three studies provided data with respect to *1401, *1402, *1403, and *1405. A fixed effects model of analysis was used for subtypes *1401, *1402, and *1403 and random effect model for *1405 (*1401: $I^2 = 0\%$, $Q = 2.86$, $p = .414$; *1402: $I^2 = 0\%$, $Q = 0.378$, $p = .828$; *1403: $I^2 = 0\%$, $Q = 0.009$, $p = .924$; and *1405: $I^2 = 56.59\%$, $Q = 4.61$, $p = .100$). The pooled ORs showed the *14 subtypes were not associate with RA (*1401: OR = 0.87, 95% CI: 0.54-1.38, $p = .542$; *1402: OR = 1.56, 95% CI: 0.33-7.32, $p = .573$; *1403: OR = 0.90, 95% CI: 0.24-3.38, $p = .880$; *1405: OR = 0.47, 95% CI: 0.15-1.48, $p = 0.195$) (Table 2).

Association between SE positive with RA

The SE was defined as the sum of DRB1 *01, *04, *10, and *14 allele s[5], and a total of nine studies with completed SE were included in the analysis. A random-effects model of analysis was used ($I^2 = 86.61\%$, $Q = 59.74$, $p < .001$). The pooled OR revealed SE positive status was associate with RA (OR = 2.38, 95% CI: 1.38-4.10, $p = .002$) (Table 2; Figure 2F).

Sensitivity analysis

Results of the sensitivity analysis using the leave-one-out approach for the associations between the major allelic types and RA are shown in Figure S1. For all allelic types, the pooled estimates remained significant with each of the studies removed in turn, indicating that no obvious influence of individual studies on the pooled estimates.

Publication bias

Funnel plots of the associations between the major allelic types *01, *04, *09, *10, *14, and SE positive are shown in Figure S2, and Egger's test was also performed. The results indicated no publication bias in *04, *09, *14, and SE positive, but possible

publication bias with respect to *01 and *10 (1-tailed p-values Eggers' test = .011, .056, .374, .026, .253, and .371 for *01, *04, *09, *10, *14, and SE positive, respectively).

Discussion

This study aimed to examine the associations of HLA-DRB1 alleles with RA in Asian patients. The results indicated that the frequencies of HLA-DRB1*04, *10, *0101, *0401, *0404, *0405, *0410, *1001, *1401, and SE positive were higher in patients with RA than control patients without RA. Interestingly, the frequency of *14 and *1405 was lower in RA patients than controls.

Accurate identification of genetic markers can aid in the screening and diagnosis of RA, and may allow for early intervention or targeted therapy; for example, an early study found that the absolute risk of developing RA in Caucasians carrying alleles *0401, *0404, or *0101 was 1/46 [53]. HLA class II alleles have also been found to contribute to susceptibility to other autoimmune diseases, including autoimmune hepatitis, systemic lupus erythematosus, Sjögren syndrome and type 1 diabetes mellitus [55].

Although it has been estimated that up to 20% of patients with RA are SE-negative, it is clear that HLA-DRB1 haplotypes are associated with RA, and the associations vary with ethnicity [4]. For example, HLA-DRB1*0402 is associated with Caucasians and *0405 with North Indian and Asian populations [11,55]. Meta-analysis Western European ancestry populations found that HLA-DRB1*11 and the HLA-DRB1*11-HLA-DQA1*05-HLA-DQB1*03 haplotype were strongly associated with systemic juvenile idiopathic arthritis [56]. In contrast to the study by Gorman *et al.* [57] that found no association of the SE and only a weak association of *0401 with rheumatoid nodules, our results indicated strong associations of *0401 and *0405 with RA in Asians. Other study has shown that SE positivity is strongly associated with RA in African-Americans [7].

While many studies have found that certain alleles are more common in different ethnic groups, only a small number of prior meta-analysis have examined HLA-DRB1 allele frequencies in various populations with RA. In a meta-analysis of studies of Latin American patients with RA, Delgado-Vega and Anaya [58] reported that HLA-DRB1*0404 and HLA-DR4 were associated with RA. A meta-analysis has also revealed an association between HLA-DRB1*1402 DQA1*0505 DQB1*0301 DRB3*0101 with RA in full-heritage Native Americans [59]. Interestingly, a meta-analysis by Gorman *et al.* [57], which included 3,272 Caucasian patients with RA, found a nonsignificant

association of the presence of the SE (1 or 2 alleles *vs.* no alleles) and rheumatoid nodules and only a weak relationship with HLA-DRB1*0401 and nodules in this population. Another meta-analysis by Gorman *et al.* [60] examining erosive RA found that when the 3,240 patients were examined as a group, there was a significant association of the presence of the SE (2 or 1 *vs.* 0 SE alleles) with erosive disease (OR = 2.0). Subgroup analyses, however, indicated that ethnic background was important with no association of the SE with erosions Greeks (OR = 0.8), but a significant association in southern European Caucasians and Asians with OR = 6.2 and 5.4, respectively, in patients with 2 SE alleles. Another recent meta-analysis found that BB alleles were significantly associated with anticitrullinated peptide antibodies (ACPA) and structural progression, and protected against the presence of ACPA and structural progression [61].

Three meta-analyses have examined HLA-DRB1 alleles in Asian patients with RA. A 2007 meta-analysis by Jun *et al.* [11] included 12 studies that examined Korean, Japanese, Chinese and Thai patients with RA. Patients with RA had significantly higher frequencies of HLA-DRB1*0101, *0401, *0410, and *1001 than controls (OR = 1.5-2.1). When the analysis was restricted to Koreans, HLA-DRB1*0405 showed a significant susceptibility to RA (OR = 5.65), whereas the HLA-DRB1*0301, *0403, *0406, *0701, *1301, and *1405 alleles showed protective association with RA (OR = 0.32-0.70). A recent meta-analysis by Yang *et al.* [10] examining Chinese RA patients included 22 studies with 1,690 patients and 1,793 controls. Patients with RA had significantly higher frequencies of HLA-DRB1*04, *0401, *0404, *0405 and *0410 than controls (OR = 2.28-4.19). A study of Japanese patients with RA showed that the number of SE alleles was significantly associated with progressive joint destruction as well as anticitrullinated peptide antibody positivity and younger age of disease onset [62].

While most research has been devoted to studying the susceptibility to RA conferred by different alleles, some evidence has suggested a protective effect of certain alleles. The current results found a negative association between *14, especially *1405, with RA. In a study of RA patients in Western India, Prasannavar *et al.* [19] reported that while HLA-DRB1*04 was strongly associated with RA, *03 and *14 appeared to confer a protective effect. Other study has also suggested that some HLA-DRB1 haplotypes may confer a protective effect [62].

While the current study is the largest-scale analysis examining HLA-DRB1 alleles in Asian patients with RA, there are some limitations that need to be considered. Large

heterogeneity was present among some alleles in the different studies, which may have influenced the results. Publication bias was found for alleles *01 and *10, and may possibly be due to the large range of odds ratio for some studies; for example, Saghaei *et al.* [15] reported an OR = 0.003 and Seglias *et al.* [50] reported an OR = 5.016 (Figure 2A). Although our sensitivity analysis found that the results were robust, the numbers are considered at two ends of extreme. The presence of possible reporting bias and publication bias may overestimate the seemed significant analytic results. A large portion of the studies were conducted in China; thus, the results may not be applicable to other Asian populations. Analysis of different ethnic groups may clarify this point, but there were not enough studies for analysis based on different countries/ethnicities. There is inconsistent nomenclature for the description of HLA-DRB1 alleles [64], and the definition of the SE also differed between studies. Analysis of allele frequency in rheumatoid factor (RF) positive and negative patients was not conducted, nor were we able to examine an association of allele frequency with disease severity. We were not able to examine the effect of one *vs.* two copies of the alleles, and prior study has suggested that homozygosity for certain alleles may be associated with more severe RA, earlier mortality, and higher risk of cardiovascular death in RA patients. While the overall quality of the studies was high, confounding factors are difficult to adjust for due to the nature of the studies.

Conclusions

This analysis showed that HLA-DRB1*04, *10, *0101, *0401, *0404, *0405, *0410, *1001, *1401, and SE positive were positively associated with RA in Asian patients. Although evidence was relatively weak, alleles *14 and *1405 were found to be negatively associated with RA. More ethnic specific studies on the associations based on suballeles should be conducted for a better clarification of the genetic susceptibility of RA. Early diagnosis and intervention prior to symptom development may be beneficial in preventing or delaying the onset of this chronic disease.

References

1. Gabriel SE. The epidemiology of rheumatoid arthritis. *Rheum Dis Clin North Am.* 2001;27:269-81.
2. de Vries R. Genetics of rheumatoid arthritis: time for a change! *Curr Opin Rheumatol.* 2011;23:227-32.
3. Bax M, van Heemst J, Huizinga TW, Toes RE. Genetics of rheumatoid arthritis: what have we learned? *Immunogenetics.* 2011;63:459-66.

4. Holoshitz J. The rheumatoid arthritis HLA-DRB1 shared epitope. *Curr Opin Rheumatol.* 2010;22:293-8.
5. Mourad J, Monem F. HLA-DRB1 allele association with rheumatoid arthritis susceptibility and severity in Syria. *Rev Bras Reumatol.* 2013;53:47-56.
6. Furukawa H, Oka S, Shimada K, Hashimoto A, Tohma S. Human leukocyte antigen polymorphisms and personalized medicine for rheumatoid arthritis. *J Hum Genet.* 2015;60:691-6.
7. Hughes LB, Morrison D, Kelley JM, Padilla MA, Vaughan LK, Westfall AO, et al. The HLA-DRB1 shared epitope is associated with susceptibility to rheumatoid arthritis in African Americans through European genetic admixture. *Arthritis Rheum.* 2008;58:349-58.
8. Liu QH, Teng Y. [Relation between shared epitope of HLA-DR antigen B chain and rheumatoid arthritis]. *Chinese J Rheumatol.* 2000;4:153-5.
9. Zhang HW, Chen GQ, Yan MX. HLA DR4 genes and clinical features of rheumatoid arthritis. *Guangdong Med J.* 2005;26:1518-9.
10. Yang M, Kuang X, Li J, Pan Y, Tan M, Lu B, et al. Meta-analysis of the association of HLA-DRB1 with rheumatoid arthritis in Chinese populations. *BMC Musculoskelet Disord.* 2013;14:307.
11. Jun KR, Choi SE, Cha CH, Oh HB, Heo YS, Ahn HY, et al. Meta-analysis of the association between HLA-DRB1 allele and rheumatoid arthritis susceptibility in Asian populations. *J Korean Med Sci.* 2007;22:973-80.
12. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *Ann Intern Med.* 2009;151:W65-W94.
13. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO 3rd, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann Rheum Dis.* 2010;69:1580-8.
14. Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Available at: http://www.ohri.ca/programs/clinical_epidemiology/oxford.htm. Accessed February 2, 2016.
15. Wang J, Zhang H, Wang GQ, Quan Y. HLA-DRB1 gene polymorphisms and its associations with rheumatoid arthritis in Chinese Han women of Shaanxi province, northwest of China. *Int J Immunogenet.* 2016;43:25-31.
16. Louthrenoo W, Kasitanon N, Wangkaew S, Kuwata S, Takeuchi F. Distribution of HLA-DR alleles among Thai patients with rheumatoid arthritis. *Hum Immunol.* 2015;76:113-7.
17. Saghafi M, Nohesara N, Rafatpanah H, Shariati J, Shakeri MT. HLA-DRB1 frequency in patients with familial and sporadic rheumatoid arthritis in north east of Iran. *Clin Rheumatol.* 2014;33:1397-402.
18. Sariyildiz MA, Batmaz I, Guli Çetinçakmak M, Yıldız I, Nas K, Çevik R. Relationship of the HLA-DRB1 alleles and seropositivity, anti-MCV, functional status and radiological damage in Turkish patients with rheumatoid arthritis. *J Back Musculoskelet Rehabil.* 2013;26:63-70.
19. Prasannavar DJ, Yeola A, Pradhan V, Patwardhan M, Rajadhyaksha A, Ghosh K. Distribution of HLA-DRβ1 alleles among well-characterized rheumatoid arthritis patients from Western India. *Rheumatol Int.* 2014;34:705-8.
20. Chang ZF, Wang YF, Shi XX. [The relationship between antigen epitope of HLA-DRB1 and rheumatoid arthritis]. *J Baotou Medical College.* 2012;28:12-5. [Article in Chinese]
21. Shi YM, Luo CN, Wang AX. Association of HLA-DR4 gene with rheumatoid arthritis in the Uygur nationality of Xinjiang region. *J Clin Intern Med.* 2012;29:490-2.
22. Chen CP, He LF, Cheng T. HLA-DR4 gene and its subtypes associated with rheumatoid arthritis disease. *Chinese Remedies and Clinics.* 2010;002:157-8. [Article in Chinese]
23. Duan JM, Li ZJ, Wang RM. [Relationship between HLA-DR4 antigen gene and rheumatoid arthritis in North of Anhui Province]. *Appl J Gen Pract.* 2008;6:469-70. [Article in Chinese]
24. Lin L, Chen Y, Xiao Z, Huang S, Yang Z. The association of HLA-DRB1 alleles with rheumatoid arthritis in the Chinese Shantou population: a follow-up study. *Biochem Cell Biol.* 2007;85:227-38.
25. Ali AA, Moatter T, Baig JA, Iqbal A, Hussain A, Iqbal MP. Polymorphism of HLA-DR and HLA-DQ in rheumatoid arthritis patients and clinical response to methotrexate--a hospital-based study. *J Pak Med Assoc.* 2006;56:452-6.
26. Alsaied K, Alawadhi A, Al-Saeed O, Haider MZ. Human leukocyte antigen DRB1*04 is associated with rheumatoid arthritis in Kuwaiti patients. *Joint Bone Spine.* 2006;73:62-5.
27. Su Y, Li ZG, Jia RL. The relationship between amino-acid motif of HLA-DRB1*04 (B chain) and the susceptibility to rheumatoid arthritis. *Chinese J Rheumatol.* 2004;8:215-9.
28. Lee HS, Lee KW, Song GG, Kim HA, Kim SY, Bae SC. Increased susceptibility to rheumatoid arthritis in Koreans heterozygous for HLA-DRB1*0405 and *0901. *Arthritis Rheum.* 2004;50:3468-75.
29. Kinikli G, Ateş A, Turgay M, Akay G, Kinikli S, Tokgöz G. HLA-DRB1 genes and disease severity in rheumatoid arthritis in Turkey. *Scand J Rheumatol.* 2003;32:277-80.
30. Li XF, Zhang F, Pan ZL. Association between rheumatoid arthritis and shared epitope of HLA2DRB1 gene in Shandong population. *J Rheumatol.* 2003;7:531-6.

31. Zhang SL, Ye DF, Zheng L. [The relationship between HLA-DR4 and rheumatoid arthritis in the Han population of Fujian]. *Fujian Med J*. 2003;25:75-6. [Article in Chinese]
32. Liu YJ, Liu RY, Zhang AH. [HLA-DR4 subtype polymorphism of Han rheumatoid arthritis patients in Guizhou]. *Chinese J Rheumatol*. 2002;6:447-8. [Article in Chinese]
33. Wu H, Gao JS, Li M. [HLA DR4 alleles and rheumatoid arthritis curative effect]. *Chinese J Rheumatol*. 2001;5:213-5. [Article in Chinese]
34. Lin L, Zeng Q, Liu SY. [HLA-DRB1 alleles genotyping in patients of rheumatoid arthritis in Shantou]. *J Shantou Med College*. 2001;14:22-4. [article in Chinese]
35. Griffiths B, Situnayake RD, Clark B, Tennant A, Salmon M, Emery P. Racial origin and its effect on disease expression and HLA-DRB1 types in patients with rheumatoid arthritis: a matched cross-sectional study. *Rheumatology (Oxford)*. 2000;39:857-64.
36. Zhang JQ, Wang JK. HLA-DR4 genetic testing in rheumatoid arthritis disease Chinese. *J Rheumatol*. 1999;3:183-5.
37. Kim TG, Choi HB, Park SH, Kim HY, Han H. DQCAR 113 and DQCAR 115 in combination with HLA-DRB1 alleles are significant markers of susceptibility to rheumatoid arthritis in the Korean population. *Tissue Antigens*. 1999;54:552-9.
38. Tokunaga NK, Noda R, Kaneoka H, Ogahara S, Murata T, Hiratsuka T, et al. Association between HLA-DRB1*15 and Japanese patients with rheumatoid arthritis complicated by renal involvement. *Nephron*. 1999;81:165-71.
39. Yuan GH, Shi GY. Molecular analysis of HLA-DRB polymorphism in Han nationality patients with rheumatoid arthritis. *Nat Med J China*. 1998;78:172-4.
40. Gu JR, Sun CG. [The relationship between HLA-DRB1 and rheumatoid arthritis in the Han population of Guangdong]. *Chinese J Microbiol Immunol*. 1998;18:227-9. [Article in Chinese]
41. Yukioka M, Wakitani S, Murata N, Toda Y, Ogawa R, Kaneshige T, et al. Elderly-onset rheumatoid arthritis and its association with HLA-DRB1 alleles in Japanese. *Br J Rheumatol*. 1998;37:98-101.
42. Wakitani S, Kuwata K, Imoto K, Murata N, Oonishi H, Ochi T. Knee and/or hip joint destruction in rheumatoid arthritis is associated with HLA-DRB1*0405 in Japanese patients. *Clin Rheumatol*. 1998;17:485-8.
43. Xiao Z, Gong L. [The relationship between HLA-DR4 and rheumatoid arthritis in the Han population of Inner Mongolia]. *Acta Academiae Med Neimongo*. 1997;19:57. [Article in Chinese]
44. Zhao Y, Dong Y, Tang FL. [HLA-DRB1 allele and relationship of patients with rheumatoid arthritis in north of China]. *Chinese J Intern Med*. 1997;36:112-5.
45. Higami K, Hakoda M, Matsuda Y, Ueda H, Kashiwazaki S. Lack of association of HLA-DRB1 genotype with radiologic progression in Japanese patients with early rheumatoid arthritis. *Arthritis Rheum*. 1997;40:2241-7.
46. Toda Y, Takemura S, Komuro H, Ono H, Tanabe T, Nakagawa T, Morimoto T, et al. Relationship between duration of rheumatoid arthritis before knee joint surgery and HLA-DRB1 alleles: a retrospective study. *J Rheumatol*. 1997;24:2304-7.
47. Zhao Y, Dong Y, Zhu X, Qiu C. HLA-DRB1 alleles genotyping in patients with rheumatoid arthritis in Chinese. *Chin Med Sci J*. 1996;11:232-5.
48. Wang BL, Gao P, Li XP. [Patients with rheumatoid arthritis HLA-DR4 allele detection]. *Chinese J Clin Lab Sci*. 1996;14:196. [Article in Chinese]
49. Takeuchi F, Nakano K, Matsuta K, Nabeta H, Bannai M, Tanimoto K, et al. Positive and negative association of HLA-DR genotypes with Japanese rheumatoid arthritis. *Clin Exp Rheumatol*. 1996;14:17-22.
50. Kim HY, Kim TG, Park SH, Lee SH, Cho CS, Han H. Predominance of HLA-DRB1*0405 in Korean patients with rheumatoid arthritis. *Ann Rheum Dis*. 1995;54:988-90.
51. Molkentin J, Gregersen PK, Lin X, Zhu N, Wang Y, Wang Y, Chen S, et al. Molecular analysis of HLA-DR beta and DQ beta polymorphism in Chinese with rheumatoid arthritis. *Ann Rheum Dis*. 1993;52:610-2.
52. Seglias J, Li EK, Cohen MG, Wong RW, Potter PK, So AK. Linkage between rheumatoid arthritis susceptibility and the presence of HLA-DR4 and DR beta allelic third hypervariable region sequences in southern Chinese persons. *Arthritis Rheum*. 1992;35:163-7.
53. Nepom GT, Nepom BS. Prediction of susceptibility to rheumatoid arthritis by human leukocyte antigen genotyping. *Rheum Dis Clin North Am*. 1992;18:785-92.
54. Cruz-Tapias P, Pérez-Fernández OM, Rojas-Villarraga A, Rodríguez-Rodríguez A, Arango MT, Anaya JM. Shared HLA class II in six autoimmune diseases in Latin America: A meta-analysis. *Autoimmune Dis*. 2012;2012:569728.
55. Jaini R, Kaur G, Mehra NK. Heterogeneity of HLA-DRB1*04 and its associated haplotypes in the North Indian population. *Hum Immunol*. 2002;63:24-9.
56. Ombrello MJ, Remmers EF, Tachmazidou I, Grom A, Foell D, Haas JP, et al. HLA-DRB1*11 and variants of the MHC class II locus are strong risk factors for systemic juvenile idiopathic arthritis. *Proc Natl Acad Sci U S A*. 2015;112:15970-5.
57. Gorman JD, David-Vaudey E, Pai M, Lum RF, Criswell LA. Lack of association of the HLA-DRB1 shared epitope with rheumatoid nodules: an individual patient data meta-analysis of 3,272 Caucasian patients with rheumatoid arthritis. *Arthritis Rheum*. 2004;50:753-62.
58. Delgado-Vega AM, Anaya JM. Meta-analysis of HLA-DRB1 polymorphism in Latin American patients with rheumatoid arthritis. *Autoimmun Rev*. 2007;6:402-8.
59. Williams RC, Jacobsson LT, Knowler WC, del Puente A, Kostyu D, McAuley JE, et al. Meta-analysis reveals association

- between most common class II haplotype in full-heritage Native Americans and rheumatoid arthritis. *Hum Immunol.* 1995;42:90-4.
60. Gorman JD, Lum RF, Chen JJ, Suarez-Almazor ME, Thomson G, Criswell LA. Impact of shared epitope genotype and ethnicity on erosive disease: a meta-analysis of 3,240 rheumatoid arthritis patients. *Arthritis Rheum.* 2004;50:400-12.
 61. Ruysen-Witrand A. A new classification of HLA-DRB1 alleles based on acid-base properties of the amino acids located at positions 13, 70 and 71: impact on ACPA status or structural progression, and meta-analysis on 1235 patients with rheumatoid from two cohorts (ESPOIR and EAC cohort). *RMD Open.* 2015;1(1):e000099. doi: 10.1136/rmdopen-2015-000099.
 62. Suzuki T, Ikari K, Yano K, Inoue E, Toyama Y, Taniguchi A, et al. PADI4 and HLA-DRB1 are genetic risks for radiographic progression in RA patients, independent of ACPA status: results from the IORRA cohort study. *PLoS One.* 2013;8:e61045.
 63. Feitsma AL, Worthington J, van der Helm-van Mil AH, Plant D, Thomson W, Ursum J, et al. Protective effect of noninherited maternal HLA-DR antigens on rheumatoid arthritis development. *Proc Natl Acad Sci U S A.* 2007;104:19966-70.
 64. Marsh SG, Albert ED, Bodmer WF, Bontrop RE, Dupont B, Erlich HA, et al. Nomenclature for factors of the HLA system, 2010. *Tissue Antigens.* 2010;75:291-455.

Supplement Figures

FIGURE S1. Sensitivity analysis showing the influence of each study on the pooled estimate for the combined effect of the association between (A) *01, (B) *04, (C) *09, (D) *10, (E) *14, and (F) SE positive with rheumatoid arthritis. Abbreviations: 1st AU, first author; 95%CI, lower limit and upper limit.

FIGURE S1 continued. Sensitivity analysis showing the influence of each study on the pooled estimate for the combined effect of the association between (A) *01, (B) *04, (C) *09, (D) *10, (E) *14, and (F) SE positive with rheumatoid arthritis. Abbreviations: 1st AU, first author; 95%CI, lower limit and upper limit.

(E)

(F)

FIGURE S1 continued. Sensitivity analysis showing the influence of each study on the pooled estimate for the combined effect of the association between (A) *01, (B) *04, (C) *09, (D) *10, (E) *14, and (F) SE positive with rheumatoid arthritis. Abbreviations: 1st AU, first author; 95%CI, lower limit and upper limit.

FIGURE S2. Funnel plot of the association between allelic types (A) *01, (B) *04, (C) *09, (D) *10, (E) *14, and (F) SE positive with rheumatoid arthritis. The one-tailed p-values from Egger's test were .011 for *01, .056 for *04, .374 for *09, .026 for *10, .253 for *14 and .371 for SE positive.

FIGURE S2 continued. Funnel plot of the association between allelic types (A) *01, (B) *04, (C) *09, (D) *10, (E) *14, and (F) SE positive with rheumatoid arthritis. The one-tailed p-values from Egger's test were .011 for *01, .056 for *04, .374 for *09, .026 for *10, .253 for *14 and .371 for SE positive.

FIGURE S2 continued. Funnel plot of the association between allelic types (A) *01, (B) *04, (C) *09, (D) *10, (E) *14, and (F) SE positive with rheumatoid arthritis. The one-tailed p-values from Egger's test were .011 for *01, .056 for *04, .374 for *09, .026 for *10, .253 for *14 and .371 for SE positive.