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Human leukocyte antigen (HLA)-Cw0303, HLA-Cw04, and HLA-Cw07 polymorphisms are associated with susceptibility of rheumatoid arthritis in Chinese Han patients from Southern China

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ABSTRACT

Objective(s): This study aimed to investigate the association between human leukocyte antigen Cw (HLA-Cw) polymorphisms and rheumatoid arthritis (RA) in Chinese Han patients in the Jiangsu area (Southern China).

Materials and Methods: Polymerase chain reaction-sequence specific primers were used to detect HLA-Cw01–08 of 201 RA patients and 211 healthy individuals from Zhongda Hospital (China). The allele frequency distribution of HLA-Cw and genotypic differences between the two groups were analyzed.

Results: The frequency of HLA-Cw0303 in patients with RA was significantly higher than that in controls, while the frequency of HLA-Cw04 was lower than that in controls (P<0.05). The gene frequency of HLA-Cw07 in anti-cyclic citrullinated peptide (anti-CCP)-negative patients was higher than that in controls (P=0.044). The frequency of HLA-Cw04 was decreased in the short duration subgroup and increased in the long duration subgroup (P<0.05). Compared to controls, the frequency of HLA-Cw0303 in patients with RA and morning stiffness was increased (P=0.004), while the frequency of HLA-Cw04 was decreased (0.005).

Conclusion: These results suggest that HLA-Cw0303 is a susceptibility gene for RA in Chinese Han patients in the Jiangsu area of southern China. The HLA-Cw04 gene may be a protective factor against RA, while HLA-Cw07 might play a protective role in the production of anti-CCP in the long-term course in patients with RA.

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Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic systemic inflammation that primarily affects the joints. Its clinical manifestations include joint pain, cartilage destruction, joint gap narrowing, joint deformity, and different levels of disability (1). Genetic, hormonal, and environmental factors are considered to be associated with RA, and genetic factors may play an important role in disease development. Approximately, 80% of the patients are aged 20–45 years, and women are affected more frequently (1).

The major histocompatibility complex (MHC) (Chr6: 26-34 Mb) is known as one of the most polymorphic regions in the genome. The MHC can be divided into the class I, II, and III regions. Classical class I and II loci encode human leukocyte antigen (HLA) proteins that are involved in antigen presentation to T cells. The class III region contains the greatest density of genes in the human genome, many of which produce proteins with immune functions such as the complement system and tumor necrosis factor gene cluster. Genome-wide association studies revealed that the HLA region contains the strongest disease risk genes for RA (2). Killer-cell immunoglobulin-like receptors (KIR) specifically recognize MHC class I molecules on target cells and transmit either stimulatory or inhibitory signals, depending on the sequence of their intracellular domain, into KIR-bearing cells (3). HLA-KIR gene interactions were shown to be involved in disease outcomes for a number of viral infections and autoimmune diseases, including RA (4).

HLA-Cw genes are HLA I molecules that are widely distributed on the surface of nucleated cells (5). HLA-Cw is a classical HLA I gene with specific KIR molecules that regulate natural killer (NK) cell activity (6). HLA-Cw01-08 is recognized by KIR2DL (7) and KIR2DL2/2DL3 recognizes HLA-Cw01, Cw03, Cw07, and Cw08; these molecules are known as the first group (HLA-C1). KIR2DL1 can recognize the HLA-Cw02, Cw04, Cw05, and Cw06 molecules, which are known as the second group (HLA-C2). Among Han Chinese, the HLA-C1 group (HLA-Cw01, Cw03, Cw07, and Cw08) is the main genotype group, while HLA-C2 is the minor group (8). Polymorphisms of HLA-Cw are associated with a number of autoimmune diseases including scleroderma (9), ankylosing spondylitis (10), systemic lupus erythematosus (SLE) (11), recurrent aphthous stomatitis (12), and psoriatic arthritis (13). Besides, HLA-C polymorphisms are reported to be associated

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Table 1. Primers used for human leukocyte antigen-cw genotyping

Primers	Sequences (5′→3′)	Fragment size (bp)	Annealing temperature (˚C)
HLA-Cw01	F: CACAGACTGACCGAGTGAG R: CCCCAGGTCGCAGCCAC	341	63
HLA-Cw02	F: CCGAGTGAACCTGCGGAAA R: GAGCCACTCCACGCACTC	521	63
HLA-Cw03	F: CACAGACTGACCGAGTGAG R: AGCGTCTCCTTCCCATTCTT	563	63.5
HLA-Cw04	F: CCGAGTGAACCTGCGGAAA R: GCCCCAGGTCGCAGCCAA	330	63.5
HLA-Cw05	F: CCGAGTGAACCTGCGGAAA R: CGCGCGCTGCAGCGTCTT	563	63
HLA-Cw06	F: TACTACAACCAGAGCGAGGA R: GGTCGCAGCCATACATCCA	296	64
HLA-Cw07	F: CCGCGGGTATGACCAGTC R: CAGCCCCTCGTGCTGCAT	1056	68.9
HLA-Cw08	F: ACGACACGCAGTTCGTGCA R: GCGCAGGTTCCGCAGGC	161	58.5
HLA-Cw0303	F: TACAACCAGAGCGAGGCCA R:AGCGTCTCCTTCCCATTCTT	522	59.5
HLA-Cw0704	F: TACTACAACCAGAGCGAGGA R: CGCGCGCTGCAGCGTCTT	535	66
HLA-DRB1	F: TGCCAAGTGGAGCACCCAA R: GCATCTTGCTCTGTGCAGAT	796	-

HLA: human leukocyte antigen

with RA in previous studies (14, 15). However, the HLA-Cw allele distribution is highly variable among different populations, even within the same country. There are few reports on the association of HLA-Cw polymorphisms in Chinese population.

The aim of the present study was to investigate the association between HLA-Cw polymorphisms and RA in Chinese Han patients in the Jiangsu area (southern China). The results of this study improve the understanding of the development and susceptibility to RA.

Materials and Methods

Patients

This was a prospective study of 201 patients with RA from Zhongda Hospital. Patients were diagnosed according to 2010 American College of Rheumatology/ European League Against Rheumatism (ACR/EULAR) diagnostic criteria (16). During the same period, 211 healthy individuals were recruited from the Nanjing Blood Center, Changzhou Blood Center, and Zhongda Hospital Medical Center. All participants with cancer, transplantation, SLE, or other autoimmune diseases were excluded. The study was approved by ethics committees of all participating centers. Written informed consent was obtained from each participant.

HLA-Cw genotyping

Genomic DNA was isolated from 500 µl of di-potassium ethylenediaminetetraacetate (EDTA-K_2) peripheral blood using a commercial kit (Qiagen, Hilden, Germany). DNA concentration and purity were verified using an UV spectrophotometer (Bio-Rad, Hercules, CA, USA). Ten pairs of genotyping primers were designed according to the literature (Table 1) (17) and commercially synthesized (Invitrogen, Carlsbad, CA, USA). The internal control primer was from intron 3 of the HLA-DRB1 gene and was included in every polymerase chain reaction (PCR) run. HLA-Cw genotyping was performed by PCR using sequence-specific primers (PCR-SSP). The reactions were performed in a total volume of 20 µl containing 1 µl of 100 ng/µl genomic DNA, 1.2 µl of 10 µM specific primers, 1 µl of 1 µM internal reference primer, 0.2 µl of 5 U/µl Taq polymerase, 2 µl 10× PCR buffer, 1.5 µl 5 mM $MgCl_2$ and 20 µl of distilled water. The reaction was carried out using the MJ Mini PCR system (Bio-Rad) and under the following conditions: denaturation for 5 min at 96 ˚C; 35 cycles of 30 sec at 95 ˚C, 45 sec at 63 ˚C, and 2 min at 72 ˚C, and a final elongation step for 5 min at 72 ˚C. Temperatures were adjusted according to the different primer pairs. PCR products were visualized under ultraviolet light after electrophoresis on 1.5% agarose gels containing ethidium bromide.

Subgroup analyses

The RA group was subdivided into the pure RA group and RA and Sjogren's syndrome (RA+SS) group. SS was diagnosed as described by Fox and Saito (18). The RA group was also divided into the short disease duration subgroup (≤7 years) and long disease duration subgroup (>7 years). The cutoff point of 7 years was used because it was the median disease duration in the present study. Finally, the RA group was divided according to with/without morning stiffness, positive/ negative anti-cyclic citrullinated peptide (anti-CCP), and positive rheumatoid factor (RF)/negative RF with morning stiffness.

Statistical analysis

Fisher's exact tests were used to compare the distribution of the allele frequencies between two groups. Analyses were carried out using SPSS 17.0

Table 2. Allelic frequencies of human leukocyte antigen-cw in patients with rheumatoid arthritis and healthy unrelated individuals

	RA (n=201)				Control (n=211)			
HLA-Cw	N	+	f (%)	GF	+	f (%)	GF	*P*
01	163	57	34.96	0.194	64	30.33	0.165	0.342
02	190	10	5.26	0.027	5	2.37	0.012	0.127
03	142	38	26.76	0.144	67	31.75	0.174	0.341
04	172	6	3.49	0.017	25	11.85	0.061	0.006
05	176	0	0.00	0.000	0	0.00	0.000	---
06	163	25	15.34	0.080	42	19.91	0.105	0.253
07	155	54	34.84	0.193	58	27.59	0.149	0.132
08	153	35	22.88	0.122	44	20.85	0.110	0.644
0303	129	22	17.05	0.089	12	5.69	0.029	0.001
0704	186	2	1.08	0.005	0	0.00	0.000	0.131

RA: Rheumatoid arthritis; N: number of cases; +: positive; GF: gene frequency; GF was calculated by 1- (1-f)1/2; f (%) was calculated by (+/N) * 100%

(SPSS, Inc., Chicago, IL, USA). Two-sided *P*-values <0.05 were considered statistically significant.

Results

Characteristics of the subjects

All 201 patients with RA were unrelated Han Chinese, including 39 males and 162 females, aged 18–84 (mean: 59.3±13.6) years. The 211 controls were unrelated Han Chinese, including 42 males and 169 females, aged 18–78 (mean: 55.3±12.7) years.

Allelic frequency of HLA-Cw

As shown in Table 2, compared to the control group, the frequency of HLA-Cw0303 in patients with RA was significantly higher (*P*=0.001, odds ratio [OR]: 3.410, 95% confidence interval [95% CI]: 1.624–7.158), while the frequency of HLA-Cw04 was lower (*P*=0.006, OR: 0.316, 95% CI: 0.133–0.749) in these patients.

Allelic frequency of HLA-Cw in pure RA and RA+SS subgroups

The 201 patients with RA were divided into two subgroups: pure RA subgroup (n=117) and RA+SS subgroup (n=33). As shown in Table 3, compared to the RA+SS subgroup, the frequency of HLA-Cw0303 in the pure RA subgroup was significantly higher (*P*=0.001, OR: 3.935, 95% CI: 1.726–8.970), while the frequency of HLA-Cw04 was significantly lower (*P*=0.012, OR: 0.235, 95% CI: 0.069–0.798) in these patients.

Allelic frequency of HLA-Cw according to disease duration

Patients with RA were divided into two subgroups: the short duration subgroup (≤7 years) (n=103) and

Table 3. Human leukocyte antigen-cw allelic frequencies in the rheumatoid arthritis and rheumatoid arthritis+Sjogren's syndrome subgroups

HLA-Cw	Control (n=211)			Pure RA subgroup (n=117)				RA+SS subgroup (n=33)			
	+	f (%)	GF	N	+	GF	*P*	N	+	GF	*P*
01	64	30.33	0.165	96	33	0.190	0.480	30	11	0.221	0.483
02	5	2.37	0.012	109	4	0.018	0.496	30	2	0.034	0.212
03	67	31.75	0.174	85	25	0.160	0.694	21	3	0.074	0.096
04	25	11.85	0.061	98	3	0.015	0.012	29	1	0.017	0.333
05	0	0.00	0.000	103	0	0.000	----	30	0	0.000	----
06	42	19.91	0.105	97	12	0.064	0.106	24	5	0.110	1.000
07	58	27.59	0.149	86	30	0.193	0.206	29	8	0.149	0.991
08	44	20.85	0.110	88	21	0.127	0.565	26	4	0.080	0.513
0303	12	5.69	0.029	73	14	0.101	0.001	23	4	0.091	0.058
0704	0	0.00	0.000	106	1	0.004	0.334	32	1	0.015	0.132

RA: Rheumatoid arthritis; SS: Sjogren's syndrome; N: number of cases; +: positive; GF: gene frequency; GF was calculated by 1- (1-f)1/2; f (%) was calculated by (+/N) * 100%. P was calculated between each subgroup and control group by using Fisher's exact tests

Table 4. Human leukocyte antigen-cw allele frequencies according to disease duration

HLA-Cw	Control (n=211)			Short course subgroup (n=103)					Long course subgroup (n=98)				
	+	f (%)	GF	N	+	f (%)	GF	*P*	N	+	f (%)	GF	*P*
01	64	30.33	0.165	81	25	30.86	0.169	0.929	82	32	39.02	0.219	0.155
02	5	2.37	0.012	98	5	5.10	0.026	0.298	92	5	5.43	0.027	0.178
03	67	31.75	0.174	78	22	28.21	0.153	0.562	64	16	25.00	0.134	0.303
04	25	11.85	0.061	85	3	3.53	0.018	0.027	87	4	3.45	0.017	0.055
05	0	0.00	0.000	93	0	0.00	0.000	----	83	0	0.00	0.000	----
06	42	19.91	0.105	81	11	13.58	0.070	0.209	82	14	17.07	0.089	0.580
07	58	27.59	0.149	84	25	29.76	0.162	0.695	71	29	40.85	0.231	0.035
08	44	20.85	0.110	77	17	22.08	0.117	0.822	76	18	23.68	0.126	0.607
0303	12	5.69	0.029	71	10	14.08	0.073	0.022	57	12	21.05	0.111	0.001
0704	0	0.00	0.000	94	1	1.06	0.005	0.308	91	1	1.09	0.005	0.301

N: number of cases; +: positive; GF: gene frequency; GF was calculated by 1- (1-f)1/2; f (%) was calculated by (+/N) * 100%. P was calculated between each subgroup and control group by using Fisher's exact tests

long duration subgroup (>7 years) (n=98). Compared to the long duration subgroup, the frequency of HLA-Cw04 was lower in the short duration subgroup (*P*=0.027, OR: 0.272, 95% CI: 0.080–0.927) (Table 4).

Allelic frequency of HLA-Cw according morning stiffness

The patients were subdivided into subgroups of with morning stiffness (n=153) and without morning stiffness (n=6). Compared to the control group, the frequency of HLA-Cw0303 in patients with RA and morning stiffness was higher (*P*=0.004, OR: 3.827, 95% CI: 1.761–8.315), while the frequency of HLA-Cw04 was lower in these patients (*P*=0.005, OR: 0.234, 95% CI: 0.080–0.690) (Table 5).

HLA-Cw allelic frequency according to anti-CCP status

Patients were subdivided into the anti-CCP-positive subgroup (n=131) and anti-CCP-negative subgroup (n=22). Compared to the control group, the frequency of HLA-Cw0303 in the anti-CCP-positive subgroup was higher (*P*=0.013, OR: 2.803, 95% CI: 1.204–6.523), while the frequency of HLA-Cw04 was lower (*P*=0.012, OR: 0.271, 95% CI: 0.092–0.798) in these patients. In addition, the frequency of HLA-Cw07 in the anti-CCP-negative subgroup was higher (*P*=0.044, OR: 2.638, 95% CI: 0.998–6.973) (Table 6).

HLA-Cw allelic frequency in RA according to RF status

Patients were subdivided into the positive RF subgroup (n=147) and negative RF subgroup (n=28). Compared to the control group, the frequency of HLA-Cw0303 in the positive RF subgroup was significantly higher (*P*=0.004, OR: 3.149, 95% CI: 1.411–7.026), while the frequency of HLA-Cw04 was lower (*P*=0.007, OR: 0.248, 95% CI: 0.084–0.730) in these patients (Table 7).

Table 5. Human leukocyte antigen-cw allele frequencies according morning stiffness

HLA-Cw	Control (n=211)			RA with morning stiffness subgroup (n=153)					RA without morning stiffness subgroup (n=6)				
	+	f (%)	GF	N	+	f (%)	GF	*P*	N	+	f (%)	GF	*P*
01	64	30.33	0.165	124	44	35.48	0.197	0.330	6	1	16.67	0.087	0.671
02	5	2.37	0.012	146	9	6.16	0.031	0.069	6	0	0.00	0.000	1.000
03	67	31.75	0.174	108	31	28.70	0.156	0.576	4	0	0.00	0.000	0.312
04	25	11.85	0.061	131	4	3.05	0.015	0.005	6	0	0.00	0.000	1.000
05	0	0.00	0.000	135	0	0.00	0.000	----	6	0	0.00	0.000	----
06	42	19.91	0.105	130	20	15.38	0.080	0.293	5	1	20.00	0.106	1.000
07	58	27.59	0.149	122	43	35.24	0.195	0.138	4	1	25.00	0.134	1.000
08	44	20.85	0.110	117	29	24.79	0.133	0.412	6	1	16.67	0.087	1.000
0303	12	5.69	0.029	96	18	18.75	0.098	0.004	5	0	0.00	0.000	1.000
0704	0	0.00	0.000	138	2	1.45	0.007	0.156	6	0	0.00	0.000	----

N: number of cases; +: positive; GF: gene frequency; GF was calculated by 1- (1-f)1/2; f (%) was calculated by (+/N) * 100%. P was calculated between each subgroup and control group by using Fisher's exact tests

Table 6. Human leukocyte antigen-cw allele frequency according to anti-cyclic citrullinated peptide status

HLA-Cw	Control (n=211)			Positive anti-CCP subgroup (n=131)					Negative anti-CCP subgroup (n=22)				
	+	f (%)	GF	N	+	f (%)	GF	*P*	N	+	f (%)	GF	*P*
01	64	30.33	0.165	109	40	36.70	0.204	0.249	17	8	47.06	0.272	0.153
02	5	2.37	0.012	123	8	6.50	0.033	0.078	22	0	0.00	0.000	1.000
03	67	31.75	0.174	93	29	31.18	0.170	0.921	18	3	16.67	0.087	0.182
04	25	11.85	0.061	114	4	3.51	0.194	0.012	21	1	4.76	0.024	0.482
05	0	0.00	0.000	120	0	0.00	0.000	----	15	0	0.00	0.000	----
06	42	19.91	0.105	104	14	13.46	0.069	0.160	20	2	10.00	0.051	0.381
07	58	27.59	0.149	98	32	32.65	0.179	0.352	18	9	50.00	0.293	0.044
08	44	20.85	0.110	99	26	26.26	0.141	0.288	17	5	29.41	0.160	0.374
0303	12	5.69	0.029	83	12	14.46	0.075	0.013	14	2	14.28	0.074	0.213
0704	0	0.00	0.000	122	2	1.64	0.008	0.134	21	0	0.00	0.000	----

CCP: cyclic citrullinated peptide; N: number of cases; +: positive; GF: gene frequency; GF was calculated by 1- (1-f)1/2; f (%) was calculated by (+/N) * 100%. P was calculated between each subgroup and control group by using Fisher's exact tests

Table 7. Human leukocyte antigen-cw allelic frequencies in rheumatoid arthritis according to rheumatoid factor status

HLA-Cw	Control (n=211)			Positive RF subgroup (n=147)					Negative RF subgroup (n=28)				
	+	f (%)	GF	N	+	f (%)	GF	*P*	N	+	f (%)	GF	*P*
01	64	30.33	0.165	123	43	34.96	0.193	0.382	19	8	42.11	0.239	0.289
02	5	2.37	0.012	139	7	5.04	0.026	0.232	28	2	7.14	0.036	0.192
03	67	31.75	0.174	106	33	31.13	0.170	0.911	18	4	22.22	0.118	0.401
04	25	11.85	0.061	124	4	3.23	0.016	0.007	25	2	8.00	0.041	0.748
05	0	0.00	0.000	127	0	0.00	0.000	----	25	0	0.00	0.000	----
06	42	19.91	0.105	120	16	13.33	0.069	0.131	25	3	12.00	0.062	0.429
07	58	27.59	0.149	113	37	32.74	0.180	0.322	23	9	39.13	0.220	0.241
08	44	20.85	0.110	115	27	23.48	0.125	0.583	17	5	29.41	0.160	0.374
0303	12	5.69	0.029	94	15	15.96	0.083	0.004	14	2	14.28	0.074	0.213
0704	0	0.00	0.000	134	2	1.49	0.007	0.150	27	0	0.00	0.000	----

RA: Rheumatoid arthritis; RF: Rheumatoid factor; N: number of cases; +: positive; GF: gene frequency; GF was calculated by 1- (1-f)1/2; f (%) was calculated by (+/N) * 100%. P was calculated between each subgroup and control group by using Fisher's exact tests

Discussion

RA is a complex systemic autoimmune disease with a genetic background. Studies have shown that KIR is associated with autoimmune disease (19). A previous study showed that the major HLA-Cw alleles in the Jiangsu Han population were HLA-Cw01, Cw03, Cw06, and Cw07 (20). In the present study, the genotyping results were consistent with those of studies detecting HLA-C in Han Chinese (21). Additionally, this previous study suggested that HLA-Cw0303 is related to SLE. Therefore, the aim of the present study was to investigate the association between HLA-Cw polymorphisms and RA in Chinese Han patients in the Jiangsu area. The results showed that the frequency of HLA-Cw0303 in patients with RA was significantly higher than that in controls, while the frequency of HLA-Cw04 was lower than that in controls. The gene for HLA-Cw07 was higher in anti-CCP-negative patients than in controls. The frequency of HLA-Cw04 was lower in the short duration subgroup and higher in the long duration subgroup. Compared to controls, the frequency of HLA-Cw0303 in patients with RA and morning stiffness was higher, while the frequency of HLA-Cw04 was lower. These results suggest that the HLA-Cw0303 gene is a RA-susceptibility gene and that the HLA-Cw04 gene is a protective factor in patients with RA.

HLA-Cw has weak antigenic potential and its expression on the surface of T cells is low. Therefore, conventional serological tests have some limitations (22). With the development of molecular biology techniques for HLA typing such as PCR- restriction fragment length polymorphism (RFLP), PCR- single-stranded conformation polymorphism (SSCP), PCR-sequence-specific oligonucleotide primed (SSO), PCR-SSP, and DNA sequencing, it is now easier to genotype HLA-Cw. The PCR-SSP genotyping method is widely

used (23) because of its high sample throughput, but high-quality DNA and perfect primer design are required to avoid pseudo-gene amplification 23. In the present study, PCR-SSP was used to detect and analyze the HLA-Cw01-08 genotypes in 412 participants and was successful in all samples.

We hypothesized that HLA-Cw0303 increases the expression of KIR-related genes. The HLA-Cw0303 gene belongs to HLA-C1 and corresponds to KIR2DL2-3. Inhibition of NK cell function of HLA-C2/KIR2DL2-3 is weaker than that of HLA-C1/KIR2DL1 (24, 25), which may lead to symptoms associated with RA. However, the frequency of HLA-Cw03 was not significantly different from that of controls, suggesting that overexpression of HLA-Cw0303 is involved in a complex interaction with other factors. HLA-Cw is composed of multiple introns and exons, and its polymorphisms mainly include single-base substitutions within exons and internal rearrangements (26, 27), but the exact mechanisms remain poorly understood.

The present study showed that the frequency of KIR2DS4 (HLC-Cw04) in patients with RA was lower than that in controls, which is consistent with the results of Sun *et al.* (28). HLA-Cw04 belongs to HLA-C2 and is the possible ligand for KIR2DS1/2DS4. Under physiological conditions, the binding force of activating KIR molecules and HLA-Cw is extremely weak, mainly maintaining an inhibitory state and effectively suppressing NK cell from killing normal cells identified as "self". However, when the inhibitory signal is weak, the effect of activated HLA-Cw-KIR overwhelms the suppression, leading to NK cell activation and symptoms of autoimmune diseases (29). Stewart *et al.* (30) suggested that the combined KIR2DS1/HLA-Cw04 peptide is similar to the KIR2DL1/HLA-Cw04 combination. This likely affects the role of activated HLA-Cw-KIR. Therefore, our results suggest that in RA, the gene frequency of HLA-Cw04 was lower and inhibition of KIR2DL1/HLA-C2 was weaker, leading to immune system disorders and triggering a series of RA symptoms, which is supported by a previous study of RA (28).

Subgroup analyses showed that HLA-Cw04 was lower in the pure RA subgroup compared to that in the RA+SS subgroup, while HLA-Cw0303 (P=0.001) was higher. These results strongly suggest that HLA-Cw0303 is a susceptibility gene for RA, while HLA-Cw04 may be protective. Additionally, in each positive subgroup of morning stiffness, RF, and anti-CCP, the frequency of HLA-Cw0303 was higher. This further suggests that the HLA-Cw0303 gene is a disease susceptibility factor and affects the clinical manifestations of RA. Additionally, the frequency of HLA-Cw04 was lower and consistent with the total RA group. Finally, the frequency of HLA-Cw0303 was higher and that of HLA-Cw04 was lower among patients with longer disease duration compared to those with shorter disease duration.

The frequency of the HLA-Cw07 gene was higher in the long duration group and anti-CCP-negative group. Previous studies showed that RA is associated with specific HLA-DRB1 alleles. The alleles encoding the β chain of the third hypervariable region have an amino acid sequence of QK/QR/RRRAA, known as the "shared epitope" (31), which is the antigen-presenting epitope. Studies confirmed that synovial CCP epitope expression and anti-CCP antibodies in patients with RA were closely associated with specific shared epitope alleles (32, 33). Sun *et al.* (11) demonstrated that HLA-Cw07 is a predisposing factor for SLE. However, additional studies are necessary to explore these associations.

There were some limitations to this study. First, the sample size was small and from a single geographic region. Second, since the RA cases were retrospectively selected from the electronic medical records in our department, the disease activity score 28-ESR (DAS28-ESR) and visual analogue scale (VAS) were not recorded in the electronic medical records. Third, only a small panel of genes was examined, and it will be necessary to examine a larger number of polymorphisms and their relation to manifestations of RA. Additional studies are necessary to better characterize RA.

Conclusion

These results suggest that HLA-Cw0303 is a susceptibility gene for RA in Chinese Han patients in the Jiangsu area of southern China. The HLA-Cw04 gene may be a protective factor against RA, while HLA-Cw07 might play a protective role in the production of anti-CCP in the long-term course of patients with RA.

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Conflict of Interest

All authors declared that they have no conflict of interest.

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