**HLA-DRB1 Shared Epitope-Dependent DR-DQ Haplotypes Are Associated with Both Anti-CCP–Positive and –Negative Rheumatoid Arthritis in Chinese Han**

**Xu Liu1,2, Jianping Guo1,3, Yuan Jia1, Yi Zhao2, Xia Liu3, Feng Cheng4, Xiaoxia Li2, Yi Zheng5, Xuhua Shi5, Haiyun Li5, Cibo Huang6, Yongjing Cheng6, Bei Lai6, Yanhong Huang7, Tian Wang8, Bo Ding9, ZhangGuo Li1,5**

1 Department of Rheumatology and Immunology, Peking University People's Hospital, Beijing, China, 2 Department of Rheumatology, Xuanwu Hospital Capital Medical University, Beijing, China, 3 Department of Rheumatology, China-Japan Friendship Hospital, Chaoyang District, Beijing, China, 4 Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Haidian District, Beijing, China, 5 Department of Rheumatology, Chao-yan Hospital, Chaoyang District, Beijing, China, 6 Department of Rheumatology, Beijing Hospital of the Ministry of Health, Beijing, China, 7 Department of Rheumatology, Beijing Jishuitan Hospital, Beijing, China, 8 Department of Internal Medicine, Beijing Anzen Hospital Capital Medical University, Beijing, China, 9 Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden

**Abstract**

The association between Human Leukocyte Antigen (HLA) class II and rheumatoid arthritis (RA) has been extensively studied, but few reported DR-DQ haplotype. Here we investigated the association of HLA-DRB1, DQA1, DQB1, and DR-DQ haplotypes with RA susceptibility and with anti-CCP antibodies in 281 RA patients and 297 control in Han population. High-resolution genotyping were performed. The HLA-DRB1 shared epitope (SE)-encoding allele *0405 displayed the most significant RA association (P = 1.35 x 10^-6). The grouped DRB1 SE alleles showed great association with RA (P = 3.88 x 10^-13). The DRB1 DRRAA alleles displayed significant protective effects (P = 0.021). The SE-dependent DR-DQ haplotype SE-DQ3/4/5 remained strong association with both anti-CCP-positive (P = 3.71 x 10^-13) and -negative RA (P = 3.89 x 10^-13). Our study revealed that SE alleles and its haplotypes SE-DQ3/4/5 were highly associated with RA susceptibility in Han population. The SE-DQ3/4/5 haplotypes were associated with both anti-CCP positive RA and -negative RA.

**Introduction**

Rheumatoid arthritis (RA) is characterized by chronic inflammation of synovial joints, resulting in progressive destruction of cartilage and bone. Both genetic and environmental factors contribute to development of RA. The most important genetic factors associated with RA are the human leukocyte antigen (HLA) linked genes, accounting for approximately 30% of the total genetic contribution for RA susceptibility [1,2]. Of which, the increase in frequencies of HLA-DRB1*0101, *0102, *0401, *0404, *0405, *0408, *1001 and *1402 were reported in RA patients in different ethnic groups. These HLA-DRB1 alleles encode a conserved amino acid sequence (70QRRKRRAA74), termed the shared epitope (SE) and seemed to be the most recognized and powerful RA genetic risk factors [3]. The DRB1*0901 allele, a corresponding motif 70RRRAE74, was rare in White and African populations but very frequent among Asians[4-6]. Besides the HLA-DRB1 alleles that contribute to RA susceptibility, certain HLA-DRB1 alleles conferred protective effects against RA. The best known DRB1 protective alleles harbored a unique amino acid sequence at position 70 (D70 alleles) [7], among which both DRRAA and DERAA conferred significant protective effects.

In addition to DRB1, the HLA-DQ molecules may also play a role in RA susceptibility. Experimental studies in transgenic mice have suggested that HLA-DQ was predisposes to arthritis and could modulate disease severity by being source of self-peptides presented in the context of DR [8,9]. In human, it has been reported that DQA1*03-DQB1*03 (DQ3) homozygous predisposed more strongly to RA and to a more severe disease while DQA1*01-DQB1*0501 (DQ5) homozygous was weakly associated with RA and often with a mild form of undifferentiated arthritis [10]. The haplotype DQ3 linked to DRBI*0901 or *04 and the haplotype DQ5 linked to DRBI*0101, *0102, *0103, and *1001 were positively associated with RA in Caucasians [11,12]. However, the contribution of DQ genes to RA is undistinguishable from DRBI, due to the strong linkage disequilibrium (LD).
Even though the well known HLA-DRB1 has the strongest genetic effect in RA, the DR-DQ haplotypes, as well as its relationship with RA patients were unknown. It has been shown that the HLA-DRB1 genetic background was more specific for association with anti-cyclic citrullinated peptides (anti-CCP) positive RA [13]. However, the interaction of HLA-DR-DQ haplotypes with anti-CCP in RA susceptibility remains to be undetermined so far. Furthermore, few RA association studies have been so far performed in Chinese Han population. In present study, we aimed to clarify the contribution of HLA-DRB1, DQA1 and DQB1 alleles and DR-DQ haplotypes to RA susceptibility in Han population, and to further determine whether certain DR-DQ haplotypes were specifically associated with RA subsets, e.g. anti-CCP positive/negative RA.

Materials and Methods

Study Subjects

A total of 281 RA patients were recruited from the Department of Rheumatology Peking University People’s Hospital (mean onset age 42.6 ± 8.6 years; 77.7% females). All patients satisfied the American College of Rheumatology 1987 revised criteria for a diagnosis of RA [14]. The data regarding anti-CCP antibodies were available in 204 RA patients (75.0% anti-CCP-positive, n = 153; 25.0% anti-CCP-negative, n = 51), and the data regarding rheumatoid factor (RF) were available in 128 RA patients (71.1% RF-positive, n = 91; 28.9% RF-negative, n = 37). The control group comprised 297 non-related healthy individual (mean age 42.6 ± 8.6 years; 77.7% females) and was recruited from Health Care Center from Peking University People Hospital.

All patients and healthy controls were self-reported Han Chinese originated from the region of northern China. The study was approved by the medical ethics committee of Peking University People’s hospital and the written informed consents were obtained from all participants to publish these case details.

HLa Genotyping

The HLA-DRB1, DQA1 and DQB1 were genotyped by using sequence based typing (SBT). The strategy of both DR and DQ included forward and backward amplification and sequencing of exons 2. The amplification primers for exon 2 were designed on the basis of known intron sequences [15]. Alleles which cannot be separated by exon2 were group together, e.g. HLA-DQA1*03. The sequences of HLA-DRB1, DQA1 and DQB1 were analyzed using Assign software (UTYPE, Invitrogen), which enables assignment of genotypes based on a recent library file of HLA alleles. Ambiguous alleles of HLA-DRB1 were additionally performed by sequence-specific polymerase chain reaction, according to the reference protocol (Invitrogen 45040-4). The time resolved fluorescence hybridization was performed for the ambiguous alleles of DQA1 and DQB1 [16]. All samples were genotyped for DRB1. The DQA1 and DQB1 typing was performed in 269 RA patients and 297 controls.

Table 1. Association of HLA-DRB1 alleles with RA by RPE method in 281 patients and 297 controls.

| HLA-DRB1 motif | RA n (%) | Control n (%) | OR (95%CI) | P value* |
|---------------|----------|---------------|------------|----------|
| SE alleles    |          |               |            |          |
| 0101          | 134 (23.8) | 49 (8.2)      | 3.48 (2.45–4.95) | 3.88 × 10^{-11} |
| 0404          | 8 (1.4)   | 2 (0.3)       | 3.77 (1.68–8.43) | 1.00 × 10^{-3}  |
| 0405          | 53 (9.4)  | 16 (2.7)      | 3.76 (2.12–6.66) | 3.15 × 10^{-6}  |
| 0410          | 11 (1.8)  | 0 (0.0)       | 3.82 × 10^{-4}   |          |
| 0401          | 18 (3.2)  | 11 (1.9)      | 3.01 (0.94–4.30) | 0.067     |
| 1001          | 93 (16.7) | 86 (14.5)     | 0.71 (0.52–0.95) | 0.021     |
| Others        |          |               |            |          |
| 1302          | 5 (0.9)   | 12 (2.0)      | 0.40 (0.14–1.14) | 0.076     |
| 0701          | 49 (8.7)  | 70 (11.8)     | –           |          |
| 0803          | 17 (3.0)  | 20 (3.4)      | –           |          |
| 0301          | 20 (3.6)  | 28 (4.7)      | –           |          |
| 0403          | 7 (1.2)   | 6 (1.0)       | –           |          |
| 0901          | 94 (16.7) | 86 (14.5)     | –           |          |
| 1401          | 10 (1.8)  | 16 (2.7)      | –           |          |
| 1405          | 8 (1.4)   | 15 (2.5)      | –           |          |
| 1501          | 81 (14.4) | 96 (16.2)     | –           |          |
| 1502          | 12 (2.1)  | 12 (2.0)      | –           |          |

Alleles with frequencies less than 1% in both cases and controls were not listed. RPE: relative predispositional effect; *P < 0.05 were listed. 

Table 2. Association of HLA-DQ alleles with RA by RPE method.

| HLA-DQ | RA n (%) | Control n (%) | OR (95%CI) | P value* |
|--------|----------|---------------|------------|----------|
| DQA1   |          |               |            |          |
| 03*    | 189 (35.1)| 152 (25.6)    | 1.56 (1.22–2.03) | 4.76 × 10^{-4} |
| 0102   | 85 (15.8) | 115 (19.4)    | –           | –        |
| 0501/03/05 5 | 74 (13.8) | 107 (18.0)    | –           | –        |
| 0101/04/05 5 | 71 (13.2) | 69 (11.6)    | –           | –        |
| 0201   | 47 (8.7)  | 61 (10.3)     | –           | –        |
| 0103   | 41 (7.6)  | 38 (6.4)      | –           | –        |
| 0601   | 27 (5.0)  | 51 (8.6)      | 0.56 (0.35–0.91) | 0.018     |
| DQB1   |          |               |            |          |
| 0303   | 104 (19.3)| 91 (15.3)     | 1.44 (1.05–1.96) | 0.022     |
| 0501   | 83 (15.4) | 65 (10.9)     | –           | –        |
| 0502   | 6 (1.1)   | 11 (1.9)      | –           | –        |
| 0503   | 5 (0.9)   | 13 (2.2)      | –           | –        |
| 0301   | 99 (18.4)| 150 (25.3)    | 0.67 (0.50–0.89) | 5.00 × 10^{-3} |
| 0601   | 93 (17.3)| 126 (21.2)    | 0.68 (0.50–0.92) | 0.013     |
| 0201   | 65 (12.1)| 78 (13.1)     | –           | –        |
| 0401   | 51 (9.5)  | 21 (3.5)      | 2.86 (1.70–4.82) | 4.27 × 10^{-5} |
| 0302   | 30 (5.6)  | 41 (6.9)      | –           | –        |

Alleles with frequencies less than 1% in both cases and controls were not listed. RPE: relative predispositional effect; *P < 0.05 were listed. 

Alles with frequencies that could not be identified by SBT of exon 2.

RA: rheumatoid arthritis; OR (95% CI): odds ratio (95% confidence interval).

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Anti-CCP Antibody Detection

Anti-CCP antibodies were quantitatively tested using the second generation kit by an enzyme-linked immunosorbent assay (ELISA; Euroimmun, Germany). A cutoff value of 5 relative units (RU/ml) was established as recommended by the manufacturer’s protocol.

Haplotype Computational Estimation

Molecular haplotyping required family-based data to establish phases. For our phase-unknown population-based data, the haplotypes were statistically calculated by using software Arlequin 3.1 (http://cmpg.unibe.ch/software/arlequin3/). The Hardy-Weinberg Equilibrium (HWE) was calculated locus by locus and for whole haplotype. All variants were in HWE in whole cohort (P > 0.05, data not shown). The Markov chain approximation was used with 100000 steps and 1000 dememorization steps definition. The distribution of HLA-DQ-DR haplotypes was analyzed in all patients and controls, using pseudo-Bayesian based ELB approach [17]. The software Arlequin 3.1 was run by using the following settings: ε = 1e–7; 5 significant digits for output 50 starting points for ELB algorithm and a maximum of 1000 iterations.

Statistical Analysis

The differences of allelic/haplotypic distribution between cases and controls were analyzed using chi-square test or Fisher’s exact test (when frequency < 5), with two-tailed P values. Odds ratios (ORs) were calculated with 95% confidence intervals (95% CIs) in 2 x 2 tables. The chi-square values for the individual alleles were determined after stratifying the data using the relative predispositional effect (RPE) method [18]. Statistical analyses were performed with SPSS version 13.0 software. P values < 0.05 were considered statistically significant.

Results

Association of HLA-DRB1, -DQA1 and –DQB1 with RA in Han Population

To clarify the haplotype association of HLA-DR-DQ with RA, first the frequencies of each HLA-DRB1, -DQA1 and -DQB1 allele were measured by sequence based typing for 281 RA patients and 297 ethnically matched healthy controls. A total of 45 HLA–DRB1, 10–DQA1 and 13–DQB1 alleles were identified. The alleles with frequencies more than 1% in cases or controls were listed in Table 1 and Table 2.

Significant RA association were observed with –DRB1 alleles *0101, *0404, *0405 and *0410, compared with healthy controls (Table 1, P = 1.00 x 10^-3, 0.049, 1.35 x 10^-6 and 3.82 x 10^-4, respectively), which was in concordance with the results from other Asian populations [4]. All the susceptible alleles had QRRAA motif at 70–74 amino acid position resided in HLA-DRB1. Unlike in Caucasians [3], DRB1*0401 encoding QKRAA at 70–74 amino acid was not associated with RA in our study cohort (P = 0.067). When the SE alleles were grouped, as shown in Table 1, a strong association with RA susceptibility in Han population (P = 3.86 x 10^-13). In contrast, the allele DRB1*1202 and *1302 which encode DRRAA and DERAA had higher frequencies in healthy controls than that in RA patients (P = 5.00 x 10^-3 and 0.076, respectively). When the DRRAA

Table 3. Association of DQA1-DQB1 haplotype with RA susceptibility.

| DQA1-DQB1 Serotype | RA | Control | OR (95%CI) | P value* |
|--------------------|----|---------|------------|----------|
| Total DQ2          |    |         |            |          |
| 0201-0201 DQ2      | 37 (6.9) | 22 (3.8) | 1.41 (0.80–2.48) | 0.214 |
| 03-0201 DQ2        | 7 (1.3)  | 6 (1.0)  | 1.33 (0.50–3.55) | 0.573 |
| 0501-0201 DQ2      | 17 (3.2) | 14 (2.3) | 1.31 (0.65–2.65) | 0.462 |
| Total DQ3          | 220 (40.9) | 284 (47.5) |            |          |
| 03-0301 DQ3        | 20 (3.7)  | 15 (2.5)  | 1.26 (0.70–2.25) | 0.469 |
| 0501-0301 DQ3      | 52 (9.7)  | 41 (6.8)  | 1.15 (0.74–1.78) | 0.566 |
| 0601-0301 DQ3      | 24 (4.5)  | 23 (3.8)  | 1.06 (0.64–1.74) | 0.807 |
| 03-0302 DQ3        | 24 (4.5)  | 13 (2.1)  | 1.15 (0.70–1.87) | 0.587 |
| 0102-0303 DQ3      | 6 (1.1)   | 4 (0.7)   | 1.46 (0.76–2.80) | 0.276 |
| 0201-0303 DQ3      | 7 (1.3)   | 9 (1.5)   | 0.80 (0.43–1.51) | 0.543 |
| 03-0303 DQ3        | 87 (16.2) | 70 (12.0) | 1.24 (1.00–1.53) | 0.047 |
| Total DQ4          | 46 (8.6)  | 18 (3.0)  | 2.99 (1.71–5.23) | 5.93 x 10^-5 |
| Total DQ5          | 76 (14.1) | 66 (11.1) |            |          |
| 0101-0501 DQ5      | 61 (11.3) | 50 (8.4)  |            |          |
| 0102-0501 DQ5      | 15 (2.8)  | 16 (2.7)  |            |          |
| Total DQ6          | 74 (13.8) | 98 (16.5) |            |          |
| 0103-0601 DQ6      | 35 (6.5)  | 35 (5.9)  |            |          |
| 0102-0601 DQ6      | 39 (7.2)  | 61 (10.6) | 0.66 (0.43–1.00) | 0.049 |

Alleles with frequencies less than 1% in both cases and controls were not listed. *P < 0.05 were listed.

RA: rheumatoid arthritis; OR (95% CI): odds ratio (95% confidence interval).
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alleles were grouped, its RA protective effects still remained \((P = 0.021, \text{ Table 1})\).

HLA-DQ typing was performed in 267 RA patients and 297 controls. As shown in Table 2, the alleles DQA1*03, DQB1*0303 and DQB1*0401 were significantly associated with RA susceptibility \((P = 4.76 \times 10^{-4}, 0.022 \text{ and } 4.27 \times 10^{-5}, \text{ respectively})\). Alleles DQA1*0601, DQB1*0301 and *0601 displayed protective effects against RA \((P = 0.018, 0.005 \text{ and } 0.013, \text{ respectively})\). The DQA1-DQB1 haplotype was also calculated. As shown in Table 3, the haplotype DQA1*03-DQB1*0303(DQ3) and DQA1*03-DQB1*0401(DQ4) displayed increased susceptibility to RA \((P = 0.042 \text{ and } 5.93 \times 10^{-5}, \text{ respectively})\), whereas the haplotype DQA1*0601-DQB1*0301 (DQ3) and DQA1*0102-DQB1*0601(DQ6) displayed protective effects \((P = 0.010 \text{ and } 0.049, \text{ respectively})\). However, as the same serotype were grouped, unlike in Caucasians neither DQ3 nor DQ5 alleles were associated with RA \((P = 0.15 \text{ and } 0.13, \text{ respectively})\).

The SE-grouped but not DQ3/DQ5-grouped HLA-DR-DQ Haplotypes Remained Predominant Association with RA

HLA DR and DQ are in strong linkage disequilibrium [19], therefore the association of DR-DQ haplotypes with RA susceptibility was calculated. The frequencies of the DR-DQ haplotypes were shown in Table 4. All susceptibility haplotypes were SE-related. The highest RA risk was associated with the haplotypes QRRAA-DQ4 \((\text{DRB1*0405/10- DQA1*03-DQB1*0401, } P = 3.05 \times 10^{-6})\).

To test whether there is an independent effect of HLA-DRB1 on RA susceptibility, we stratified the DR-DQ haplotypes by DRB1 and DQ separately. When the haplotypes were stratified by SE status, it remained strong association with RA susceptibility \((Table 4, P = 2.85 \times 10^{-12})\). Unlike SE alleles, when the haplotypes were grouped according to DQ status, there were no association observed between DQ2/D3/D5/D6-haplotypes and RA susceptibility except for the DQ4-related ones.

### Table 4. Association of HLA-DR-DQ haplotypes with RA stratified by DRB1 70–74 motif.

| DRB1 DQ | DR-DQ Haplotype | RA n (%) | Control n (%) | OR (95% CI) | \(P\) value* |
|---------|-----------------|----------|--------------|-------------|-------------|
| Grouped by SE | | | | | |
| QRRAA DQ3 | 0401-0303/0203 | 101 (18.8) | 32 (5.4) | 4.06 (2.68-6.16) | 2.85 \times 10^{-12} |
| QRRAA DQ3 | 0404/05/0103-0301/0203 | 20 (3.7) | 5 (0.8) | 4.55 (1.70-12.2) | 0.010 |
| QRRAA DQ4 | 0405/10030401 | 43 (8.0) | 12 (2.0) | 4.21 (2.20-8.08) | 3.05 \times 10^{-6} |
| QRRAA DQ5 | 0101/02010501 | 24 (4.5) | 5 (0.8) | 5.50 (2.08-14.50) | 1.19 \times 10^{-4} |
| RRRAA DQ5 | 1001010501 | 18 (3.3) | 8 (1.3) | \(-\) | \(-\) |
| Grouped by DRRAA | | | | | |
| DRRAA DQ3 | 110105010301 | 21 (3.9) | 35 (5.9) | \(-\) | \(-\) |
| DRRAA DQ3 | 110101020303 | 0 (0.0) | 6 (1.0) | \(-\) | \(-\) |
| DRRAA DQ3 | 110105010303 | 1 (0.2) | 11 (1.9) | 0.10 (0.01-0.77) | 6.00 \times 10^{-3} |
| DRRAA DQ3 | 120105010301 | 21 (3.9) | 17 (2.9) | \(-\) | \(-\) |
| DRRAA DQ3 | 120206010301 | 19 (3.5) | 45 (7.6) | 0.45 (0.26-0.77) | 0.030 |
| DRRAA DQ5 | 160201020501 | 10 (1.9) | 9 (1.5) | \(-\) | \(-\) |
| Grouped by RRRAE | | | | | |
| RRRAE DQ3 | 0901-0303-0203 | 83 (15.4) | 78 (13.1) | \(-\) | \(-\) |
| RRRAE DQ5 | 1405-01010501 | 6 (1.1) | 7 (1.2) | \(-\) | \(-\) |
| RRRAE DQ5 | 140101010501 | 6 (1.1) | 7 (1.2) | \(-\) | \(-\) |
| Others | | | | | |
| DERAA DQ6 | 130201020601 | 4 (0.7) | 6 (1.0) | \(-\) | \(-\) |
| DRRQG DQ3 | 070102010303 | 7 (1.3) | 11 (1.9) | \(-\) | \(-\) |
| DRRQG DQ2 | 070102010201 | 35 (6.5) | 44 (7.4) | \(-\) | \(-\) |
| DRRAL DQ6 | 080301030601 | 15 (2.8) | 18 (3.0) | \(-\) | \(-\) |
| QRRAE DQ3 | 0403-0303-0202 | 5 (0.9) | 4 (0.7) | \(-\) | \(-\) |
| QRRAE DQ3 | 0406-0303-0202 | 2 (0.4) | 11 (1.9) | \(-\) | \(-\) |
| QARAA DQ3 | 150101020303 | 5 (0.9) | 3 (0.5) | \(-\) | \(-\) |
| QARAA DQ6 | 150101020601 | 44 (8.2) | 69 (11.6) | \(-\) | \(-\) |
| QARAA DQ6 | 150101030601 | 14 (2.6) | 4 (0.7) | \(-\) | \(-\) |
| QARAA DQ6 | 150201030601 | 4 (0.7) | 7 (1.2) | \(-\) | \(-\) |
| QKRG DQ2 | 030105010201 | 18 (3.3) | 24 (4.0) | \(-\) | \(-\) |

Alles with frequencies less than 1% in both cases and controls were not listed.

*\(P < 0.05\) were listed.

RA: rheumatoid arthritis; OR (95% CI): odds ratio (95% confidence interval); SE: Shared Epitope QR(K)RAA or RRRAA.

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Accordingly, the DRRAA-DQ3/DQ5 haplotypes were displayed protective effect against RA. The DRRAA-DQ3 haplotypes were also showed association with RF-positive RA (Table 5), indicating DRB1 may provides more contribution in RA genetics than DQ does. Further, when the haplotypes were grouped according to DRRAA status, a strong RA protective effect was observed (P = 0.001, Table 4).

Table 5. Association of HLA-DR-DQ haplotypes with RA stratified by DQ.

| Haplotypes | RA | Control | OR(95%CI) | P value* |
|------------|----|---------|-----------|----------|
| DR-DQ2+    |    | 61(11.3) | 73(12.3)  |         |
| DR-DQ3     |    | 220(40.9)| 268(45.1) |         |
| DR-DQ4     |    | 46(8.6)  | 18(3.0)   |         |
| DR-DQ5     |    | 76(14.1) | 66(11.1)  |         |
| DR-DQ6     |    | 74(13.8) | 98(16.5)  |         |

*P<0.05 were listed.

RA: rheumatoid arthritis; OR (95% CI): odds ratio (95% confidence interval).

Discussion

In this study, we found that the most significant DRB1 allele in susceptibility to RA in Han population was DRB1*0405 encoding QRRAA, a finding that is consistent with previous studies in other Asian populations [4,3]. When the SE alleles were grouped, it showed a strong association with RA susceptibility. The DRRAA alleles displayed protective effects against RA in Han population.

Among the three HLA loci, DRB1 provided more contribution in RA susceptibility than DQ did. Furthermore, for the first time, we showed that the presence of SE-DQ3/4/5 haplotype was strongly associated with both anti-CCP positive and anti-CCP negative RA.

Table 6. Association of HLA-DR-DQ haplotypes with RA stratified by anti-CCP antibodies and RF status.

| Haplotypes | n (%) | OR(95%CI) | P value* |
|------------|-------|-----------|----------|
| Anti-CCP positive | 65(21.3) | 4.74(3.02–7.42) | 3.71×10⁻¹³ |
| Anti-CCP negative | 17(16.7) | 3.51(1.87–6.60) | 3.89×10⁻⁵ |
| RF positive | 42(22.3) | 5.05(3.08–8.29) | 4.47×10⁻¹² |
| RF negative | 13(17.6) | 3.74(1.86–7.51) | 8.08×10⁻⁵ |
| Control | 32 (5.4) |       |         |

RA: rheumatoid arthritis; anti-CCP antibodies: anti-cyclic citrullinated peptides antibodies; RF: rheumatoid factor.

OR (95% CI): odds ratio (95% confidence interval).}

Previous Lee, et al. has reported that DRB1*1302 was the strongest RA protective allele in Korean population [5]. However, we found that DRB1*1302 encoding DERAA which was crucial in RA protection [RAP] model [20], was rather rare in our study cohort (0.9% in RA and 2.0% in control, P = 0.076). The protective allele DRB1*1202 encoding DRRAA was more frequent than DRB1*1302 and conferred a significant RA protective effect. The grouped DRB1 DRRAA-DQ3/DQ5 haplotypes displayed protective effects against anti-CCP positive RA.

Several studies have reported that SE-encoding HLA-DRB1 alleles were only associated with anti-CCP positive RA but not
with anti-CCP negative RA in Caucasians and Asians [21–23]. Furthermore, there has been so far no any study describing relationship between ACPA and DR-DQ haplotype. In this work, we showed that SE-DQ3/DQ4/DQ5 haplotypes were associated not only with anti-CCP positive RA also with anti-CCP negative RA. As a result, a similar association pattern was observed. Recently, Mackie SL et al. also found that association of SE with both anti-CCP positive and negative RA in a large UK population [24]. Future studies including greater numbers of study subjects are needed to further clarify this effect.

In conclusion, we demonstrated that SE alleles and its haplotypes SE-DQ3/DQ4/DQ5 were highly associated with RA susceptibility in Han population. The DRB1- DRAA alleles and its haplotypes DRAA-DQ3/DQ5 displayed protective effects against RA. The HLA DR-DQ haplotypes containing RA susceptible or protective alleles were mainly associated with anti-CCP positive RA. However, the SE-DQ3/DQ4/DQ5 haplotypes were also associated with anti-CCP negative RA.

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Author Contributions
Conceived and designed the experiments: Xu Liu JG ZL. Performed the experiments: Xu Liu JG YJ Y. Zhao Xia Liu FC X. Li Y. Zheng XS HL CH YC BI. YH TW. Analyzed the data: Xu Liu JG YJ Y. Zhao. Contributed reagents/materials/analysis tools: FC BD. Wrote the paper: Xu Liu JG.

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