Association of HLA-DRB1 genotype with younger age onset and elder age onset rheumatoid arthritis in Japanese populations

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Abstract

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by joint destructions and human leukocyte antigen (HLA)-DRB1 is an important genetic risk factor for RA and influences the phenotype of RA. The clinical features of elder age onset RA (EORA) were known to be different from those of younger age onset RA (YORA). Previous studies reported the different association pattern of DRB1 alleles with YORA or EORA. The associations of DRB1 genotype with these RA subsets remained almost unknown. We investigated the genotype association of DRB1 with YORA or EORA in Japanese populations.

HLA genotyping was performed in Japanese RA patients and the association of allele or genotype carrier frequencies were analyzed.

The genotype frequency of DRB1*04:05/DRB1*04:06 (P=0.0240, OR 7.69, 95%CI 1.39-42.72), DRB1*04:05/DRB1*12:01 (P=0.0050, OR 5.53, 95%CI 1.71-17.88), and DRB1*04:05/DRB1*15:01 (P=0.0124, OR 3.34, 95%CI 1.39-8.02) in YORA was different from those in EORA.
higher than EORA. However, the frequencies of DRB1∗01:01/DRB1∗04:05 in YORA was tended to be lower than EORA (P = 0.0784, OR 0.14, 95% CI 0.01–2.42). The gene dosage effect of the shared epitope alleles was detected in EORA, but not in YORA. Trans-complementing DQ heterodimer molecules, formed by DQA1 and DQB1 of the haplotypes with and without shared epitope alleles, might explain the higher genotype frequencies of “shared epitope/not shared epitope”. Linear regression analyses showed the primary role of DQB1∗04:01 allele for the age at onset of RA.

This is the first report for the associations of DRB1 genotype with YORA or EORA in the Japanese population and the differential distribution of the genotypes was noted between these RA subsets. The involvement of DQ molecules for the age at onset of RA was suggested.

**Abbreviations:** CI = confidence interval, EORA = elder age onset RA, HLA = human leukocyte antigen, MORA = moderate age onset RA, OR = odds ratio, Pc = corrected P, PRC = partial regression coefficient, RA = rheumatoid arthritis, SE = shared epitope, YORA = younger age onset RA.

**Keywords:** elder age onset rheumatoid arthritis, genotype, HLA-DRB1, younger age onset rheumatoid arthritis

### 1. Introduction

Rheumatoid arthritis (RA) is an inflammatory disease characterized by structural destruction of cartilage and bone. RA patients produced specific auto-antibodies including anti-citrullinated peptide antibodies and rheumatoid factor. The specificity of anti-citrullinated peptide antibodies with RA is higher than that of rheumatoid factor. It was reported that the age at onset of RA had been increased in Japanese populations and the clinical features of elder age onset RA (EORA) were different from those of younger age onset RA (YORA) on the gender distribution, the frequency of acute onset of RA, the involvement of large joints and extra-articular manifestations, the positivity of anti-citrullinated peptide antibody and rheumatoid factor.[2–7]

The etiology of RA is believed to be influenced by genetic and environmental factors. In particular, human leukocyte antigen (HLA)-DRB1 is one of the most important genetic risk factors for RA. Diverse DRB1 alleles are associated with RA in different ethnic populations. In Japanese populations, DRB1∗04:05 is associated with RA[8] and DRB1∗04:01 in European populations.[9] In the RA-associated DRB1 alleles, amino acid sequences at position 70 to 74 in the DRβ chain were conserved. These alleles were defined as the shared epitope (SE) alleles.[9] The homozygosity for the SE alleles conferred higher risk for the susceptibility of RA than the heterozygosity for them. This gene dosage effect was a distinctive feature of the SE alleles. Previous studies reported the different association pattern of DRB1 alleles with YORA or EORA. The gene dosage effect of the SE alleles was not confirmed in stratified analyses with the age at onset of RA.[10,11] DRB1∗04 was strongly associated with YORA,[12,13] but the frequency of DRB1∗04 was lower in EORA compared with YORA.[14–16] Moreover, DRB1∗01 was associated with EORA.[13,16] However, the sample sizes of these studies were modest, the resolution of the genotyping methods used in these studies was lower and DRB1 genotype was not analyzed. Thus, larger scale studies on the association of DRB1 genotype with YORA or EORA should be conducted to validate these results. In the present study, we investigated the association of DRB1 genotype with Japanese YORA and EORA, using the genotyping methods with higher resolution.

### 2. Materials and methods

#### 2.1. Materials

In this study, RA patients were recruited in Hyogo College of Medicine, Miyakonojo Medical Center, Nagasaki Medical center, Nagoya Medical Center, Sagamihara National Hospital, and Tochigi Rheumatology Clinic. The healthy individuals (n = 1026) were recruited in Kanazawa University, Sagamihara Hospital, Teikyo University, University of Tokyo or by Pharma SNP Consortium (Tokyo, Japan).[17,18] The patients and the healthy individuals were native Japanese living in Japan. All the patients fulfilled American College of Rheumatology criteria for RA[19] or Rheumatoid Arthritis Classification Criteria.[20] Anti-citrullinated peptide antibody was detected by Mesacup-2 test CCP (Medical & Biological Laboratories, Nagoya, Japan). Rheumatoid factor was measured by N-latex RF kit (Siemens Healthcare Diagnostics, Munich, Germany). RA patients with age at onset lower than 30 years old and equal or higher than 16 were defined as YORA[21] to eliminate juvenile idiopathic arthritis. RA patients with age at onset lower than 60 years old and equal or higher than 30 years old were defined as moderate age onset RA (MORA) based on the distribution of age at onset in Japanese RA patients.[1] RA patients with age at onset equal or higher than 60 years old were defined as EORA.[16] Steinbrocker stage and class were evaluated to measure the disease progression and the activities of daily living of RA patients.[22]

This study was reviewed and approved by Hyogo College of Medicine Research Ethics Committee (178, 2012), Miyakonojo Medical Center Research Ethics Committee (approval number was not provided, 2009), Nagasaki Medical Center Research Ethics Committee (22081, 2010), Nagoya Medical Center Research Ethics Committee (2012–526, 2012), Sagamihara National Hospital Research Ethics Committee (2009061621, 2009), University of Tsukuba Research Ethics Committee (250, 2015), and Tokyo National Hospital Research Ethics Committee (190010, 2019). Written informed consent was obtained from all the participants. This study was conducted in accordance with the principles expressed in the Declaration of Helsinki.

#### 2.2. Genotyping

HLA genotyping was performed by the polymerase chain reaction with sequence-specific oligonucleotide probes (WAK Flow HLA typing kits, Wakanaga, Akitakata, Japan), using Bio-Plex system (Bio-Rad, Hercules, CA). DRB1∗01:01, DRB1∗04:01, DRB1∗04:04, DRB1∗04:05, DRB1∗04:10, DRB1∗10:01, DRB1∗14:02, and DRB1∗14:06 were included in the SE alleles.[19] Genotyping results for some of the RA patients and the healthy controls were reported in previous studies.[8,23]
3. Results

3.1. Demographic features of YORA and EORA

The characteristics of the RA patients were shown in Table 1. Eighty-nine patients were defined as YORA, 714 as MORA, and 329 as EORA. The ratio of male was higher in EORA than YORA. Steinbrocker stage and class were higher in YORA than EORA. Thus, the association pattern of YORA with EORA was different from that of EORA.

3.2. HLA-DRB1 allele carrier frequency in YORA and EORA

HLA genotyping was performed to compare allele carrier frequency (Table 2, Supplementary Table S1, http://links.lww.com/MD/D419). DRB1∗04:01 was associated with the susceptibility of EORA (P = .0004, OR = 0.41, 95% confidence interval [CI] 1.69–5.73). DRB1∗04:05 was associated with the susceptibility of YORA (P = 8.33 × 10^{-14}, OR = 2.42 × 10^{-12}, OR = 5.47, 95% CI 3.47–8.61), MORA (P = 1.02 × 10^{-13}, OR = 3.15 × 10^{-10}, OR = 3.35, 95% CI 2.73–4.12), and EORA (P = 2.45 × 10^{-12}, OR = 7.11 × 10^{-11}, OR = 2.57, 95% CI 1.98–3.34), compared with the controls. Furthermore, the allele carrier frequency of DRB1∗01:01 in YORA was tended to be lower than EORA and that of DRB1∗04:05 in YORA was tended to be higher than EORA. The association pattern of DRB1 allele carrier frequency in MORA was similar to that of RA per se.8 Thus, the association pattern of YORA with DRB1 alleles was different from that of EORA.

3.3. HLA-DRB1 genotype frequency in YORA and EORA

The DRB1 genotype frequencies in the RA patients were analyzed (Table 3, Supplementary Table S2, http://links.lww.com/MD/D420). The genotype frequencies of DRB1∗04:01/DRB1∗04:05 (YORA: P = .0179, OR = 23.56, 95% CI 2.12–26.47, EORA: P = .0111, OR = 19.04, 95% CI 2.28–158.74) and DRB1∗04:05/DRB1∗01:01 (YORA: P = .0028, OR = 3.38, 95% CI 1.62–7.06, EORA: P = .0014, OR = 2.39, 95% CI 1.43–3.99) were increased in both YORA and EORA, compared with the controls.

The genotype frequency of DRB1∗04:05/DRB1∗12:01 (P = .0004, OR = 7.21, 95% CI 2.76–18.82) was increased in YORA, compared with the controls, but not in EORA. The frequencies of DRB1∗01:01/DRB1∗04:05 (P = .064, OR = 3.20, 95% CI 1.42–7.19) and DRB1∗04:05/DRB1∗12:01 (P = .0005, OR = 5.53, 95% CI 1.71–17.88, and DRB1∗04:05/DRB1∗15:01 (P = .0124, OR = 3.34, 95% CI 1.39–8.02) were increased in EORA, compared with the controls, but not in YORA. Furthermore, the frequency of DRB1∗04:05/DRB1∗04:06 (P = .0204, OR = 7.69, 95% CI 1.39–4.27), DRB1∗04:05/DRB1∗12:01 (P = .0124, OR = 5.53, 95% CI 1.71–17.88, and DRB1∗04:05/DRB1∗15:01 (P = .0124, OR = 3.34, 95% CI 1.39–8.02) was increased in EORA, compared with the controls, but not in YORA. However, the frequencies of DRB1∗01:01/DRB1∗04:05 in YORA was tended to be lower than EORA (P = .0784, OR = 0.14, 95% CI 0.01–2.42). The homozygosity for the SE alleles conferred higher risk for EORA than the heterozygosity (SE/SE: P = 2.65 × 10^{-14}, OR = 2.88, 95% CI 2.18–3.79, SE/SE: P = 2.18 × 10^{-13}, OR = 5.47, 95% CI 3.52–8.50), though this gene dosage effect was not observed in YORA (SE/SE: P = 3.35 × 10^{-10}, OR = 4.70, 95% CI 2.81–7.86, SE/SE: P = .0007, OR = 4.92, 95% CI 2.15–11.29). The association pattern of DRB1 genotype frequency in MORA was similar to those of YORA or EORA. Thus, the association pattern of YORA with DRB1 genotypes was different from that of EORA.

3.4. Associations of amino acid residues in the HLA-DRβ chain with YORA and EORA

The associations of amino acid residues in the HLA-DRβ chain with RA were shown in Figure 1. Tyrosine at position 37 (37Y, P = 8.10 × 10^{-10}, OR = 4.07, P = 2.75 × 10^{-10}, 95% CI 2.31–7.19) in the DRβ chain was associated with YORA (Fig. 1A), but not with EORA (Fig. 1C). On the other hand, leucine at position 67 (67L) and aspartic acid at position 70 (70D) were not associated.

Table 1. Characteristics of RA patients.

|              | YORA | MORA | EORA | P   |
|--------------|------|------|------|-----|
| Number       | 89   | 714  | 329  | NA  |
| Mean age, years (SD) | 50.1 (15.3) | 61.5 (10.1) | 72.4 (5.9) | NA |
| Age at onset (SD) | 23.7 (5.6) | 46.9 (8.1) | 67.3 (2.5) | NA |
| Male, n (%)  | 10 (11.2) | 116 (16.4) | 88 (26.8) | .0018 |
| Steinbrocker stage III and IV, n (%) | 69 (77.5) | 396 (55.5) | 88 (26.8) | 5.03 × 10^{-18} |
| Steinbrocker class 3 and 4, n (%) | 27 (30.3) | 110 (15.4) | 51 (15.5) | .0032 |
| Rheumatoid factor positive, n (%) | 75 (84.3) | 611 (85.6) | 271 (82.4) | .7532 |
| Anti-citrullinated peptide antibody positive, n (%) | 78 (87.6) | 639 (89.5) | 280 (85.1) | .6126 |

Association was tested between YORA and EORA by Fisher exact test using 2×2 contingency tables. EORA = elder age onset RA, MORA = moderate age onset RA, NA = not applicable, RA = rheumatoid arthritis, YORA = younger age onset RA.
### Table 2

**HLA-DRB1 allele carrier frequency in RA patients and the controls.**

|       | YORA vs control | MORA vs control | OR 95%CI                        | YORA vs control | MORA vs control | OR 95%CI                        | Control | YORA vs EDRA | MORA vs EDRA | OR 95%CI | YORA vs MORA | MORA vs EDRA | OR 95%CI |
|-------|----------------|----------------|--------------------------------|----------------|----------------|--------------------------------|---------|--------------|--------------|---------|--------------|--------------|---------|
| DRB1*01:01 | 4.15 | 0.676 | 0.39 | 0.14 (1.0-0.4) | 2.27 | 1.23 | 1.38 | 1.26 (0.9-1.8) | 1.16 | 0.676 | 0.39 | 0.14 (1.0-0.4) | 2.27 | 1.23 | 1.38 | 1.26 (0.9-1.8) |
| DRB1*04:01 | 7.0% | 0.000 | 0.90 | 1.02 (0.9-1.0) | 36 | 1.76 | 1.38 | 1.26 (0.9-1.8) | 1.16 | 0.676 | 0.39 | 0.14 (1.0-0.4) | 2.27 | 1.23 | 1.38 | 1.26 (0.9-1.8) |
| DRB1*07:05 | 5.6 | 0.037 | 4.57 | 2.42 (1.1-6.1) | 29 | 1.38 | 1.38 | 1.26 (0.9-1.8) | 1.16 | 0.676 | 0.39 | 0.14 (1.0-0.4) | 2.27 | 1.23 | 1.38 | 1.26 (0.9-1.8) |
| DRB1*08:01 | 56 (0.9) | 8.34 | 5.14 | 5.14 | 2.42 (1.1-6.1) | 29 | 1.38 | 1.38 | 1.26 (0.9-1.8) | 1.16 | 0.676 | 0.39 | 0.14 (1.0-0.4) | 2.27 | 1.23 | 1.38 | 1.26 (0.9-1.8) |
| DRB1*09:01 | 27 (0.3) | 5.73 | 1.76 | 1.76 | 1.26 (0.9-1.8) | 29 | 1.38 | 1.38 | 1.26 (0.9-1.8) | 1.16 | 0.676 | 0.39 | 0.14 (1.0-0.4) | 2.27 | 1.23 | 1.38 | 1.26 (0.9-1.8) |
| DRB1*12:01 | 9 (0.1) | 3.04 | 1.43 | 1.43 | 1.26 (0.9-1.8) | 29 | 1.38 | 1.38 | 1.26 (0.9-1.8) | 1.16 | 0.676 | 0.39 | 0.14 (1.0-0.4) | 2.27 | 1.23 | 1.38 | 1.26 (0.9-1.8) |
| DRB1*15:01 | 12 (0.5) | 1.000 | 0.99 | 0.99 | 1.26 (0.9-1.8) | 29 | 1.38 | 1.38 | 1.26 (0.9-1.8) | 1.16 | 0.676 | 0.39 | 0.14 (1.0-0.4) | 2.27 | 1.23 | 1.38 | 1.26 (0.9-1.8) |
| SE | 69 (76.4) | 5.80X10^-3 | 4.73 | (2.8-7.4) | 516 (72.3) | 1.40X10^-3 | 3.81 | (1.0-4.6) | 236 (68.7) | 5.00X10^-3 | 3.20 | (0.4-4.1) | 417 (64.8) | 1.90 (1.4-3.0) | 240 (72.4) | 1.19 (0.8-1.8) |

Allele carrier frequencies are shown in parenthesis (%). Association was tested with the control by Fisher exact test using 2X2 contingency tables. RA = rheumatoid arthritis, YORA = younger age onset RA, MORA = moderate age onset RA, OR = odds ratio, CI = confidence interval, P = corrected P value, NS = not significant, SE = Shared epitope.

### Table 3

**HLA-DRB1 genotype frequency in RA patients and the controls.**

|       | YORA vs control | MORA vs control | EDRA vs control | Control | YORA vs EDRA | MORA vs EDRA | OR 95%CI | YORA vs MORA | MORA vs EDRA |
|-------|----------------|----------------|----------------|---------|--------------|--------------|---------|--------------|--------------|
| DRB1*04:01/DRB1*07:05 | 0.15 | 0.004 | 0.037 | 0.014 (0.6-0.3) | 0.014 | 0.004 | 0.037 | 0.014 (0.6-0.3) | 0.014 | 0.004 | 0.037 | 0.014 (0.6-0.3) |
| DRB1*04:01/DRB1*08:01 | 0.36 | 0.017 | 0.236 | 1.236 | 1.236 | 1.236 | 1.236 | 1.236 | 1.236 | 1.236 | 1.236 | 1.236 |
| DRB1*04:01/DRB1*07:05 | 0.22 | 0.012 | 0.232 | 1.232 | 1.232 | 1.232 | 1.232 | 1.232 | 1.232 | 1.232 | 1.232 | 1.232 |
| DRB1*04:01/DRB1*07:05 | 0.22 | 0.012 | 0.232 | 1.232 | 1.232 | 1.232 | 1.232 | 1.232 | 1.232 | 1.232 | 1.232 | 1.232 |
| DRB1*04:01/DRB1*07:05 | 0.22 | 0.012 | 0.232 | 1.232 | 1.232 | 1.232 | 1.232 | 1.232 | 1.232 | 1.232 | 1.232 | 1.232 |
| DRB1*04:01/DRB1*07:05 | 0.22 | 0.012 | 0.232 | 1.232 | 1.232 | 1.232 | 1.232 | 1.232 | 1.232 | 1.232 | 1.232 | 1.232 |
| SE | 9 (10.1) | 0.007 | 0.042 | 0.015 (0.6-0.3) | 0.014 | 0.004 | 0.037 | 0.014 (0.6-0.3) | 0.014 | 0.004 | 0.037 | 0.014 (0.6-0.3) |

Genotype frequencies are shown in parenthesis (%). Association was tested with the control by Fisher’s exact test using 2X2 contingency tables. Association of SE genotypes were compared with “non SE/non SE” genotype.

CI = confidence interval, EDRA = elder age onset RA, MORA = moderate age onset RA, OR = odds ratio, RA = rheumatoid arthritis, SE = Shared epitope, YORA = younger age onset RA.
with YORA (Fig. 1A), though these amino acid residues were associated with EORA (Fig. 1C, 67L: $P = 3.20 \times 10^{-8}$, OR = 2.19, $P_c = 1.10 \times 10^{-6}$, 95% CI 1.65–2.92, 70D: $P = 1.50 \times 10^{-8}$, OR = 0.48, $P_c = 5.12 \times 10^{-7}$, 95% CI 0.37–0.62). The association pattern of YORA (Fig. 1B) was similar to that of RA per se.\[19\] Thus, the association pattern of YORA with amino acid residues in the DRβ chain was different from that of EORA.

### 3.5. Linear regression analysis of HLA alleles for the age at onset of RA

Simple linear regression analyses of HLA alleles were conducted to reveal the effects of alleles for the age at onset of RA (Table 4, Supplementary Table S3, http://links.lww.com/MD/D421). $DQB1^*04:01$ was associated with the age at onset of RA ($P = .0005$, $P_c = .0079$, PRC = 2.44), though some other alleles were also tended to be associated (Table 4). The haplotype including $B^*44:03$, $DRB1^*13:02$, $DQB1^*06:04$, and $DPB1^*04:01$ was known\[24\], $DRB1^*01:01$ and $DQB1^*05:01$ were in strong linkage disequilibrium in Japanese populations.\[25\]$DRB1^*04:05$, $DQB1^*04:01$, and $DPB1^*02:01$ were also included in the other haplotype.\[24\] Multiple linear regression analyses of HLA alleles were performed to identify the independent association of these alleles for the age at onset of RA (Supplementary Table S4, http://links.lww.com/MD/D422).

The significant association of $B^*44:03$, $DRB1^*13:02$, $DQB1^*06:04$, and $DPB1^*04:01$ was disappeared, when conditioned on each allele. These findings supported the strong linkage disequilibrium in the haplotype and the primary allele could not be detected in this analysis. The association of $DQB1^*04:01$ still remained significant ($P = .0081$, PRC = 4.89), when conditioned on $DRB1^*04:05$, suggesting the independent association of $DQB1^*04:01$ from $DRB1^*04:05$. However, the association of $DRB1^*04:05$ was not detected ($P = .1403$, PRC = 2.83), when conditioned on $DQB1^*04:01$, suggesting the dependent effects of $DRB1^*04:05$ on $DQB1^*04:01$. These data suggested the primary role of $DQB1^*04:01$ for the age at onset of RA. The association of $DRB1^*04:05$ ($P = .0044$, PRC = 2.02) and $DQB1^*04:01$ ($P = .0003$, PRC = 2.44) still remained significant, when conditioned on $DPB1^*02:01$. The association of $DPB1^*02:01$ still remained significant, when conditioned on $DRB1^*04:05$ ($P = .0051$, PRC = 1.86) or $DQB1^*04:01$ ($P = .0052$, PRC = 1.85). These data suggested the independent association of $DPB1^*02:01$ form $DQB1^*04:01$. The significant association of $DRB1^*01:01$ and $DQB1^*05:01$ was disappeared, when conditioned on each allele. These also suggested the strong linkage disequilibrium between $DRB1^*01:01$ and $DQB1^*05:01$ and the primary allele could not be detected in the analysis. Thus, some HLA haplotypes or alleles were detected to be responsible for the age at onset of RA.
DQB1 and DQβ1 of the haplotypes with and without SE, might explain the higher genotype frequencies of “SE/not SE”, as proposed in the studies on other diseases.\(^{23,24}\) The primary effect of DQB1*04:01 for the age at onset of RA detected in the multiple linear regression analyses also support the role of DQ molecules. The frequencies of DRB1*04:05/DRB1*04:05, DRB1*04:05/DRB1*04:05, DRB1*04:05/DRB1*04:05/DRB1*04:05/DRB1*15:01 were increased in EORA (Table 3). In these genotypes increased in EORA, all the genotypes except DRB1*04:05/DRB1*09:01 and DRB1*04:05/DRB1*15:01 were “SE/SE”. These results suggested the important roles of the homozygous genotypes of “SE/SE” in the susceptibility of EORA and interpreted the gene dosage effect of the SE alleles in EORA. Thus, the differential distribution of the genotypes including DRB1*04:05 were noted between YORA and EORA, suggesting the different pathogenesis in YORA from EORA.

The amino acid residues of 11V, 13H, 33H, 57S, and 96Y in the DRβ chain were associated with both of YORA and EORA (Fig. 1A and 1C). The predominant roles of DRB1*04:05 on the susceptibility of YORA and EORA could account for the results. However, leucine at position 67 (67L) was associated with EORA, but not with YORA, suggesting the effects of DRB1*01:01 allele on EORA. Similarly, DRB1*12:01 might explain that aspartic acid at position 70 (70D) was not associated with YORA. Additionally, tyrosine at position 37 (37Y) was associated with YORA, but not with EORA, supported by the higher frequency of DRB1*04:05 in YORA. Some HLA alleles were predicted to be selected in the pathogen-driven manner.\(^{24,25}\) Analogously, SE alleles would be selected in the similar manner, increasing the susceptibility of YORA.

Linear regression analyses revealed the role of some HLA haplotypes or alleles for the age at onset of RA. Because of the strong linkage disequilibrium of HLA region, it is difficult to identify the primary role of the responsible allele for the age at onset of RA. DQB1*04:01 was the sole significantly associated allele with the age at onset of RA in simple linear regression analyses. In multiple linear regression analyses, the association of DQB1*04:01 still remained significant, when conditioned on DRB1*04:05. However, the association of DRB1*04:05 was not detected, when conditioned on DQB1*04:01. Thus, the results of the multiple linear regression analyses showed the primary role of DQB1*04:01 for the age at onset of RA, suggesting the involvement of DQ molecules on the age at onset. It is possible that other HLA loci than DRB1 might contribute to the age at onset of RA. However, this primary association of DQB1*04:01 should be confirmed in future replication studies, as DQAI is a risk factor for RA in Chinese populations.\(^{23}\) The association of DRB1 genotypes with YORA and EORA should also be validated in larger scale studies, because of the limited sample size of this study. Since the distribution pattern of DRB1 is different in other ethnic populations, DRB1 genotypes of YORA and EORA should be investigated in other populations.

In the present study, it was revealed that the association pattern of DRB1 genotype was widely different between YORA and EORA. The genotype frequencies of DRB1*04:05/DRB1*12:01 and DRB1*04:05/DRB1*15:01 were increased in YORA. On the other hand, the frequencies of DRB1*01:01/DRB1*04:05 and DRB1*04:05/DRB1*15:01 were increased in EORA. The gene dosage effect of the SE alleles was detected in EORA, but not observed in YORA.

### Table 4

Linear regression analysis of HLA alleles for the age at onset of RA.

| HLA allele | PRC | 95%CI | P  | Pe |
|------------|-----|-------|----|----|
| B*15:07   | 11.41 | (2.16–20.65) | 0.036 | 0.5957 |
| B*44:03   | 3.02 | (0.35–6.59) | 0.265 | NS |
| B*67:01   | 9.69 | (3.61–15.76) | 0.018 | 0.0683 |
| DRB1*01:01 | 2.44 | (0.21–6.47) | 0.031 | 0.9205 |
| DRB1*03:01 | -26.56 | (-46.10–7.02) | 0.078 | 0.2251 |
| DRB1*04:05 | -1.90 | (-3.29–0.51) | 0.074 | 0.2151 |
| DRB1*06:01 | -4.83 | (-8.82–0.83) | 0.019 | 0.5202 |
| DRB1*13:02 | 3.59 | (0.62–6.55) | 0.017 | 0.5138 |
| DQB1*04:01 | -2.26 | (-3.70–1.03) | 0.005 | 0.0076 |
| DQB1*05:01 | 2.48 | (0.45–4.50) | 0.016 | 0.2520 |
| DQB1*06:01 | 3.16 | (0.12–6.19) | 0.041 | 0.6236 |
| DQB1*07:01 | -1.75 | (-3.05–0.45) | 0.005 | 0.1357 |
| DQB1*04:01 | 3.15 | (0.02–6.28) | 0.046 | 0.7781 |

Association for age at onset of RA was tested by linear regression analysis. nCi = confidence interval, NS = not significant, Pcorr = corrected P value, PRC = partial regression coefficient, RA = rheumatoid arthritis.

### 4. Discussion

Many reports on the associations of DRB1 with RA were published, so far. However, a few studies on the role of DRB1 on YORA or EORA were reported. The associations of DRB1 genotype with these RA subsets remained almost unknown. It was reported that the frequencies of DRB1*04 in EORA were lower than those in YORA\(^{14,16}\) and that DRB1*01 was associated with EORA.\(^{13,16}\) Similar tendencies on DRB1 allele carrier frequencies were observed in this study (Table 2), confirming the results of the previous studies. These results suggested the differential roles of DRB1*04:05 and DRB1*01:01 in the pathogenesis of YORA and EORA, respectively. The differential diagnosis is difficult in some patients with EORA from polymyalgia rheumatica and DRB1*01 and DRB1*04 were reported to be associated with polymyalgia rheumatica.\(^{13,26–28}\) The susceptible DRB1 alleles were shared between EORA and polymyalgia rheumatica, suggesting the common etiological bases between them. Thus, this association study on DRB1 alleles with YORA or EORA sheds light on the pathogenesis of the subsets of RA.

DRB1*04:05, one of the SE alleles, is considered to be the most important risk factor for RA in Japanese.\(^{26}\) In the present study, the frequencies of some genotypes including DRB1*04:05 were differentially distributed between YORA and EORA, though the frequencies of some genotypes were reported to be increased in overall RA.\(^{23,24}\) The genotype frequencies of DRB1*04:01/DRB1*04:05, DRB1*04:05/DRB1*09:01, DRB1*04:05/DRB1*12:01, and DRB1*04:05/DRB1*15:01 were increased in YORA (Table 3). In these genotypes increased in YORA, all the genotypes except DRB1*04:01/DRB1*04:05 were “SE/not SE”. These results suggested the important roles of the heterozygous genotypes of “SE/not SE” in the susceptibility of YORA and explained that the gene dosage effect of the SE alleles was not found in YORA. The heterozygous genotypes of “SE/not SE” might increase the variety of the self-antigens presented by DR molecules; more than two types of self-antigens, antigens presented by the SE alleles and those by the non-SE alleles, would increase the susceptibility risk of YORA. Alternatively, trans-complementing DQ heterodimer molecules, formed by DQA1 and DQB1 of the haplotypes with and without SE, might explain the higher genotype frequencies of “SE/not SE”, as proposed in the studies on other diseases.\(^{23,24}\) The primary effect of DQB1*04:01 for the age at onset of RA detected in the multiple linear regression analyses also support the role of DQ molecules. The frequencies of DRB1*01:01/DRB1*04:05, DRB1*04:05/DRB1*04:05, DRB1*04:05/DRB1*04:05/DRB1*15:01 were increased in EORA (Table 3). In these genotypes increased in EORA, all the genotypes except DRB1*04:05/DRB1*09:01 and DRB1*04:05/DRB1*15:01 were “SE/SE”. These results suggested the important roles of the homozygous genotypes of “SE/SE” in the susceptibility of EORA and interpreted the gene dosage effect of the SE alleles in EORA. Thus, the differential distribution of the genotypes including DRB1*04:05 were noted between YORA and EORA, suggesting the different pathogenesis in YORA from EORA.
5. Conclusion

To the best of our knowledge, this is the first report for these associations of DRB1 genotype with these RA subsets in the Japanese population and the differentially associated DRB1 genotypes were detected between these subsets. Although accumulated genetic risk factors were thought to cause autoimmune diseases in younger age, our results suggested that different genetic risk factors contribute to the pathogenesis of YORA from EORA. The involvement of DQ molecules on the age at onset of RA was suggested.

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