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ORIGINAL RESEARCH

# Human leucocyte antigens and Japanese patients with polymyalgia rheumatica: the protective effect of *DRB1*09:01*

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## ABSTRACT

**Objective** The hallmarks of the chronic inflammatory disease polymyalgia rheumatica (PMR) include pain, and morning stiffness in areas of the neck, shoulder and pelvic girdle. The human leucocyte antigen (*HLA*) gene was reported to be an important risk factor for PMR, but it has not been analysed precisely, especially in populations other than Europeans.

**Methods** Genotyping of *DRB1* and *DQB1* was performed in Japanese PMR patients (n=270) and controls (n=413). Associations between allele carrier and genotype frequencies were determined for PMR.

**Results** *DRB1*04:05* was associated with a predisposition to PMR (p=0.0006, *Pc*=0.0193, OR 1.85, 95% CI 1.31 to 2.62). *DRB1*09:01* was associated with protection against PMR ($p=1.46\times10^{-5}$, *Pc*=0.0004, OR 0.40, 95% CI 0.26 to 0.61). A shared epitope (SE) associated with PMR ($p=3.07\times10^{-6}$, OR 2.11, 95% CI 1.54 to 2.88). *DQB1*03:03* (p=0.0010, *Pc*=0.0140, OR 0.52, 95% CI 0.35 to 0.77) was associated with protection against PMR and *DQB1*04:01* (p=0.0009, *Pc*=0.0140, OR 1.82, 95% CI 1.28 to 2.58) was associated with predisposition to PMR. A gene dosage effect was observed for *DRB1*09:01* and *DQB1*03:03*, but not for *DRB1*04:05,* SE or *DQB1*04:01*. Haplotype and logistic regression analyses suggested a protective effect for *DRB1*09:01*.

**Conclusion** This study is the first to demonstrate predisposing associations of *DRB1*04:05,* SE, and *DQB1*04:01*, and protective associations of *DRB1*09:01* and *DQB1*03:03* with PMR in Japanese patients. Our data indicate *HLA* has predisposing and protective effects on the pathogenesis of PMR.

### WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Associations of human leucocyte antigen (*HLA*) and polymyalgia rheumatica (PMR) were reported, but it has not been analysed precisely, especially in populations other than Europeans.

### WHAT THIS STUDY ADDS

⇒ This study reported that *DRB1*04:05* was associated with a predisposition to PMR and that *DRB1*09:01* was associated with protection against PMR.

### HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The distribution pattern of *DRB1* in PMR was similar to that of rheumatoid arthritis, but it was different from that of rheumatoid arthritis.

## INTRODUCTION

The hallmarks of the chronic inflammatory rheumatic disease, polymyalgia rheumatica (PMR), are musculoskeletal pain and morning stiffness in the regions of the neck, shoulder and pelvic girdle, and it was reported to affect individuals aged ≥50 years.[1 2] Initially considered senile rheumatic gout by Bruce,[3] it was labelled PMR by Barber.[4] The incidence rate of PMR was 13–113 per 100 000 individuals ≥50 years of age in European populations,[1 5] and 13 per 100 000 individuals ≥50 years of age in Japanese populations.[6] The prevalence of PMR was 600–800 per 100 000 individuals ≥50 years of age in European populations,[7 8] and 300 per 100 000 individuals ≥50 years of age in Japanese populations.[9] PMR is thought to be complicated with giant cell arteritis at a high frequency in European populations (16%–21%),[10] but not in Japanese populations (0%–1%).[11 12] This information contradicts the hypothesis that PMR and giant cell arteritis are diverse incarnations of the same disorder.[13] Furthermore, the epidemiological features of PMR are different between European and Japanese populations.

The aetiology of PMR is not fully understood but might be acted on by genetic and/or environmental factors. The human leucocyte antigen (*HLA*) genes on chromosome 6 are significant risk factors for PMR. The

associations of *DRB1* alleles with PMR were reported in several studies. However, the pathological analysis of giant cell arteritis has been studied more often than PMR. Genome-wide association studies performed for giant cell arteritis,[14 15] indicated a strong association with the *DRB1* locus, but this has not been determined for PMR. An increase in the frequency of *DRB1*01* and *DRB1*04* were observed in PMR[16–23] and the pattern of *DRB1* distribution in PMR was comparable with *DRB1* distribution patterns in patients with giant cell arteritis.[10 24] *DRB1*01:01*, *DRB1*04:01* and *DRB1*04:04* are associated with PMR in European populations[25–27] and the distribution pattern of *DRB1* in PMR was similar to that in rheumatoid arthritis (RA),[21] especially older age-onset RA.[28] Some sequences of amino acids (positions 70–74 in the DRβ chain) are conserved in *DRB1* alleles that were reported to be associated with RA, and were termed 'shared epitope (SE) alleles'.[29 30] Furthermore, SE alleles were shown to have a gene dosage effect, and SE allele homozygosity is a higher risk factor for RA compared with SE allele heterozygosity. Some previous reports indicated a different distribution of *DRB1* alleles in older age-onset RA compared with younger age-onset RA.[31–33] Nevertheless, apart from Europeans, there has been little analysis of *HLA* in other populations of PMR patients. The roles of amino acid residues in DRβ or DQβ chains have not been investigated for their role in susceptibility to PMR. Furthermore, the gene dosage effects of predisposing or protecting alleles have not been assessed. The resolution of previous genotyping methods suffered from low resolution. Moreover, high numbers of European PMR patients complicated with giant cell arteritis have made it difficult to perform precise analyses of the genetic associations of *HLA* with PMR, per se; PMR is not frequently complicated with giant cell arteritis in Japanese patients. Thus, the roles of *HLA* in PMR are poorly understood. Therefore, we analysed the potential associations of *HLA* with PMR in Japanese patients, using genotyping methods with high resolution.

## MATERIALS AND METHODS

### Patients and controls

PMR patients (n=269, mean age±SD; 75.6±8.1 years, 104 male; 38.7%) were recruited at Tama Medical Center, Nagoya Medical Center, Sagamihara National Hospital, Azuma Rheumatology Clinic, Tokyo National Hospital, Shimoshizu National Hospital, Asahikawa Medical Center, Himeji Medical Center, Yokohama Medical Center, Nagasaki Medical Center and Fukushima Medical University. Giant cell arteritis was complicated in six PMR patients and anticitrullinated peptide antibodies were detected in seven PMR patients. Patients with RA were recruited at Miyakonojo Medical Center, Hyogo College of Medicine, Nagoya Medical Center, Sagamihara National Hospital, Nagasaki Medical Center and Tochigi Rheumatology Clinic. Controls (n=413) were recruited by the Pharma SNP Consortium (Tokyo, Japan)[34 35] or at Kanazawa University, Teikyo University, Sagamihara National Hospital. Patients with PMR had to fulfil Bird's criteria for PMR[36] or the 2012 Provisional Classification Criteria for PMR.[37] Patients with RA fulfilled Rheumatoid Arthritis Classification Criteria[38] or the American College of Rheumatology criteria for RA.[39] Patients enrolled in this study were native Japanese and were not related to each other.

### Genotyping

PCR was used for the *HLA* genotyping of *DRB1* and *DQB1* loci in PMR patients and controls. Reverse sequence-specific oligonucleotide probes (WAKFlow HLA typing kits, Wakunaga, Akitakata, Japan) and a Bio-Plex system (Bio-Rad, Hercules, CA) were used, as previously described.[40 41] SE alleles were defined as *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*.[30] Some of the genotyping results from patients with RA and healthy controls were published previously.[33]

### Statistical analysis

Deviation from Hardy-Weinberg equilibrium was tested by Genepop (http://genepop.curtin.edu.au/),[42] as previously described.[40 41] Associations between *HLA* genotype frequencies, amino acid residue carrier frequencies, allele carrier frequencies and haplotype carrier frequencies were analysed by Fisher's exact test. Multiple logistic regression analysis was performed to clarify which allele was a primary contributor to the predisposition to, or protection against, PMR. Principal component analysis (PCA) was conducted to differentiate between PMR, older age-onset RA (age at onset ≥60 years), moderate age-onset RA (age at onset <60 years and ≥30 years), younger age-onset RA (age at onset <30 years and ≥16 years) and healthy controls based on the allele frequencies of *DRB1*. The correction of multiple comparisons was determined by the Bonferroni method and multiplying p values by the number of alleles or amino acid residues tested was used to calculate corrected p (*Pc*) values.

## RESULTS

### Carrier frequencies of DRB1 and DQB1 alleles in PMR

*DRB1* and *DQB1* genotyping was used to determine potential associations of *HLA* alleles with PMR (table 1, online supplemental tables S1 and S2). Deviations from Hardy-Weinberg equilibrium were not detected in PMR patients (*DRB1: p*=0.3490, *DQB1: p*=0.9734) or controls (*DRB1: p*=0.4239, *DQB1: p*=0.2144). *DRB1*04:05* (p=0.0006, *Pc*=0.0193, OR 1.85, 95% CI 1.31 to 2.62) and *DQB1*04:01* (p=0.0009, *Pc*=0.0120, OR 1.82, 95% CI 1.28 to 2.58) were associated with predisposition to PMR. *DRB1*09:01* ($p=1.46\times10^{-5}$, *Pc*=0.0004, OR 0.40, 95% CI 0.26 to 0.61) and *DQB1*03:03* (p=0.0010, *Pc*=0.0140, OR 0.52, 95% CI 0.35 to 0.77) were associated with protection against PMR. SE ($p=3.07\times10^{-6}$, OR 2.11, 95% CI 1.54 to 2.88) and *DRB1*04* (p=0.0052, OR 1.57, 95% CI 1.15 to 2.14) were associated with PMR. Thus, higher carrier

Table 1 *DRB1* and *DQB1* allele carrier frequency in PMR patients and controls

| | PMR (n=269) | Control (n=413) | P value | OR | *Pc* | 95% CI |
|---|---|---|---|---|---|---|
| *DRB1*04:05* | 89 (33.1) | 87 (21.1) | 0.0006 | 1.85 | 0.0193 | (1.31 to 2.62) |
| *DRB1*04:10* | 13 (4.8) | 14 (3.4) | 0.4221 | 1.45 | NS | (0.67 to 3.13) |
| *DRB1*09:01* | 32 (11.9) | 105 (25.4) | $1.46\times10^{-5}$ | 0.40 | 0.0004 | (0.26 to 0.61) |
| *DRB1*12:01* | 25 (9.3) | 29 (7.0) | 0.3109 | 1.36 | NS | (0.78 to 2.37) |
| *DRB1*15:01* | 57 (21.2) | 68 (16.5) | 0.1292 | 1.36 | NS | (0.92 to 2.02) |
| SE | 149 (55.4) | 153 (37.0) | $3.07\times10^{-6}$ | 2.11 | NA | (1.54 to 2.88) |
| *DRB1*04* | 127 (47.2) | 150 (36.3) | 0.0052 | 1.57 | NA | (1.15 to 2.14) |
| *DQB1*03:01* | 76 (28.3) | 96 (23.2) | 0.1495 | 1.30 | NS | (0.92 to 1.85) |
| *DQB1*03:03* | 43 (16.0) | 111 (26.9) | 0.0010 | 0.52 | 0.0140 | (0.35 to 0.77) |
| *DQB1*04:01* | 87 (32.3) | 86 (20.8) | 0.0009 | 1.82 | 0.0120 | (1.28 to 2.58) |
| *DQB1*04:02* | 17 (6.3) | 29 (7.0) | 0.7573 | 0.89 | NS | (0.48 to 1.66) |
| *DQB1*06:02* | 57 (21.2) | 65 (15.7) | 0.0820 | 1.44 | NS | (0.97 to 2.14) |

Allele carrier frequencies are shown in parenthesis (%). Associations were tested by Fisher's exact test using 2×2 contingency tables.
NA, not applicable; NS, not significant; PMR, polymyalgia rheumatica; SE, shared epitope.

frequencies of *DRB1*04:05*, SE and *DQB1*04:01*, and lower frequencies of *DRB1*09:01* and *DQB1*03:03* were detected in PMR.

### Associations of DRB1*04:05 and DRB1*09:01 with the clinical characteristics of PMR

Associations between *DRB1*04:05* and *DRB1*09:01* and the clinical characteristics of PMR were analysed (table 2). *DRB1*04:05* was associated with female PMR (p=0.0006, *Pc*=0.0192, OR 2.03, 95% CI 1.36 to 3.02) and PMR with age at onset higher than 75 years (p=0.0004, *Pc*=0.0123, OR 2.28, 95% CI 1.47 to 3.54). *DRB1*09:01* was protectively associated with female PMR (p=0.0022, *Pc*=0.0192, OR 0.36, 95% CI 0.21 to 0.61). Thus, the frequencies *DRB1*04:05* and *DRB1*09:01* were skewed in some PMR subsets.

### Genotype frequencies of DRB1 and DQB1 in PMR

Associations between the *DRB1* genotype and PMR were analysed (table 3). Homozygosity for *DRB1*04:05* did not provide a higher risk for PMR compared with heterozygosity (**04:05/*not **04:05:* *p*=0.0002, OR 1.96, 95% CI 1.37 to 2.79, **04:05/ *04:05:* *p*=0.7475, OR 0.65, 95% CI 0.17 to 2.55) and SE homozygosity did not provide a higher risk for PMR compared with heterozygosity (SE/not SE: $p=9.84\times10^{-5}$, OR 1.88, 95% CI 1.37 to 2.59, SE/SE: p=0.0957, OR 1.66, 95% CI 0.93 to 2.97). However, homozygosity for *DRB1*09:01* conferred stronger protection against PMR compared with heterozygosity (**09:01/*not **09:01:* *p*=0.0004, OR 0.46, 95% CI 0.30 to 0.72, **09:01/ *09:01:* *p*=0.0046, OR 0.06, 95% CI 0.00 to 1.01). This indicated a gene dosage effect for *DRB1*09:01* but not *DRB1*04:05* or SE.

Increased genotype frequencies of *DRB1*04:05/DRB1*04:10* (p=0.0378, OR 7.80, 95% CI 0.91 to 67.16), *DRB1*04:05/DRB1*12:01* (p=0.0040, OR 5.83, 95% CI 1.61 to 21.09) and *DRB1*04:05/DRB1*15:01* (p=0.0059, OR 4.14, 95% CI 1.46 to 11.76) were observed in PMR. However, *DRB1*04:05/DRB1*09:01* (p=0.3407, OR 0.58, 95% CI 0.21 to 1.65) frequencies tended to be decreased. Genotype frequencies of SE/*DRB1*12:01* (p=0.0108, OR 3.81, 95% CI 1.33 to 10.94) and SE/*DRB1*15:01* (p=0.0003, OR 4.29, 95% CI 1.87 to 9.83) were increased in PMR and SE/*DRB1*09:01* (p=0.7289, OR 0.86, 95% CI 0.43 to 1.73) frequencies tended to be decreased. Thus, *DRB1*04:05/DRB1*04:10*, *DRB1*04:05/DRB1*12:01*, *DRB1*04:05/DRB1*15:01*, SE/*DRB1*12:01* and SE/*DRB1*15:01* heterozygous genotypes predisposed individuals to PMR.

The associations of the *DQB1* genotype with PMR were analysed (online supplemental table S3). Homozygosity for *DQB1*04:01* did not provide a higher risk for PMR compared with heterozygosity (**04:01/*not **04:01:* *p*=0.0010, OR 1.83, 95% CI 1.28 to 2.61, **04:01/ *04:01:* *p*=0.7444, OR 1.23, 95% CI 0.33 to 4.63, online supplemental table S3). However, homozygosity for *DQB1*03:03* conferred stronger protection against PMR than heterozygosity (**03:03/*not **03:03:* *p*=0.0069, OR 0.58, 95% CI 0.39 to 0.86, **03:03/ *03:03:* *p*=0.0337, OR 0.14, 95% CI 0.02 to 1.06). There was an increase in the genotype frequency of *DQB1*04:01/DQB1*06:02* (p=0.0059, OR 4.14, 95% CI 1.46 to 11.76) in patients with PMR. These results indicated a gene dosage effect related to *DQB1*03:03* but not *DQB1*04:01*.

### Haplotype frequencies of DRB1-DQB1 in PMR

Associations of *DRB1-DQB1* haplotypes with PMR were analysed (table 4); the *DRB1*04:05-DQB1*04:01* (p=0.0004, OR 1.90, 95% CI 1.34 to 2.70) haplotype frequency was increased in patients with PMR, but no difference was detected for *DRB1*04:05-DQB1*03:02*. *DRB1*09:01-DQB1*03:03* ($p=6.60\times10^{-5}$, OR 0.42, 95% CI 0.27 to 0.65) haplotype frequencies were decreased,

**Table 2** Association of *DRB1*04:05* and *DRB1*09:01* carrier frequencies with the clinical characteristics of PMR

| Phenotype | No | *DRB1*04:05*(+) | P value | OR | *Pc* | 95% CI | *DRB1*09:01*(+) | P value | OR | *Pc* | 95% CI |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Female | 165 | 58 (35.2) | 0.0006 | 2.03 | 0.0192 | (1.36 to 3.02) | 18 (10.9) | $7.41\times10^{-5}$ | 0.36 | 0.0022 | (0.21 to 0.61) |
| Age at onset >75 years | 119 | 45 (37.8) | 0.0004 | 2.28 | 0.0123 | (1.47 to 3.54) | 16 (13.4) | 0.0061 | 0.46 | 0.1769 | (0.26 to 0.81) |
| Overlap of rheumatoid arthritis | 20 | 5 (25.0) | 0.7790 | 1.25 | NS | (0.44 to 3.53) | 2 (10.0) | 0.1819 | 0.33 | NS | (0.07 to 1.43) |
| Diabetes mellitus | 51 | 19 (37.3) | 0.0130 | 2.22 | 0.3650 | (1.20 to 4.12) | 5 (9.8) | 0.0137 | 0.32 | 0.3822 | (0.12 to 0.82) |
| Malignancy | 43 | 13 (30.2) | 0.1768 | 1.62 | NS | (0.81 to 3.25) | 4 (9.3) | 0.0225 | 0.30 | 0.6304 | (0.11 to 0.86) |
| Rheumatoid factor positive | 21 | 7 (33.3) | 0.1820 | 1.87 | NS | (0.73 to 4.78) | 0 (0.0) | 0.0035 | 0.07 | 0.0968 | (0.00 to 1.13) |
| Controls | 413 | 87 (21.1) | | | | | 105 (25.4) | | | | |

Phenotype and *DRB1*04:05* allele carrier frequencies are shown in parenthesis (%). Associations were tested by Fisher's exact test using 2×2 contingency tables. Each PMR subset with clinical characteristics was compared with controls.
NS, not significant; PMR, polymyalgia rheumatica.

and that of *DRB1*09:01-DQB1*03:01* (p=0.1576, OR 0.17, 95% CI 0.01 to 3.15) tended to be decreased, indicating a primary protective association of *DRB1*09:01* against PMR.

## Associations of DRβ and DQβ chain amino acid residues with PMR

Associations of DRβ chain amino acid residues with PMR were analysed (figure 1A) and glutamine at position 4 (4Q, $p=1.70\times10^{-5}$, OR 0.40, *Pc*=0.0006, 95% CI 0.26 to 0.62), lysine at 9 (9K, $p=2.15\times10^{-5}$, OR 0.40, *Pc*=0.0007, 95% CI 0.26 to 0.62), aspartic acid at 11 (11D, $p=2.15\times10^{-5}$, OR 0.40, *Pc*=0.0007, 95% CI 0.26 to 0.62), tyrosine at 26 (26Y, $p=1.70\times10^{-5}$, OR 0.40, *Pc*=0.0006, 95% CI 0.26 to 0.62), histidine at 28 (28H, $p=2.15\times10^{-5}$, OR 0.40, *Pc*=0.0007, 95% CI 0.26 to 0.62), glycine at 30 (30G, $p=2.15\times10^{-5}$, OR 0.40, *Pc*=0.0007, 95% CI 0.26 to 0.62), phenylalanine at 67 (67F, $p=1.78\times10^{-5}$, OR 0.47, *Pc*=0.0006, 95% CI 0.33 to 0.66) and valine at 78 (78V, $p=1.70\times10^{-5}$, OR 0.40, *Pc*=0.0006, 95% CI 0.26 to 0.62) in the DRβ chain were associated with protection against PMR. Glutamine at position 70 (70Q, $p=1.38\times10^{-5}$, OR 2.11, *Pc*=0.0047, 95% CI 1.42 to 3.13) and alanine at 74 (74A, $p=3.51\times10^{-5}$, OR 2.87, *Pc*=0.0012, 95% CI 1.69 to 4.88) were associated with PMR.

We also analysed potential associations of DQβ chain amino acid residues with PMR (figure 1B). Serine at position 3 (3S, $p=7.97\times10^{-7}$, OR 5.63, $Pc=2.47\times10^{-5}$, 95% CI 2.52 to 12.57) and leucine at 23 (23L, p=0.0008, OR 1.82, *Pc*=0.0265, 95% CI 1.28 to 2.58) were associated with PMR, indicating some amino acid residues in the DRβ and DQβ chains were associated with predisposition to, or protection against, PMR.

## Logistic regression analysis of HLA alleles in PMR

*DRB1* and *DQB1* were in strong linkage disequilibrium; therefore, we performed conditional logistic regression analyses between *DRB1* and *DQB1* alleles to identify alleles associated with the observations described above (online supplemental table S4). *DRB1*04:05* did not appear to be associated ($P_{adjusted}$=0.9701, $OR_{adjusted}$ 1.02, 95% CI 0.35 to 3.01) when conditioned on *DQB1*04:01*. In addition, *DQB1*04:01* did not appear to be associated ($P_{adjusted}$=0.3684, $OR_{adjusted}$ 1.65, 95% CI 0.55 to 4.93) when conditioned on *DRB1*04:05*. The protective effect of *DQB1*03:03* was not detected ($P_{adjusted}$=0.2231, $OR_{adjusted}$ 1.61, 95% CI 0.75 to 3.45) when conditioned on *DRB1*09:01*. The protective effect of *DRB1*09:01* remained ($P_{adjusted}$=0.0011, $OR_{adjusted}$ 0.25, 95% CI 0.11 to 0.58) when conditioned on *DQB1*03:03*, suggesting a primary protective effect of *DRB1*09:01*. Thus, these findings suggested that *DRB1*09:01* was primarily associated with protection against PMR.

## PCA of PMR, RA subsets and healthy controls

PCA was conducted to differentiate between PMR, RA subsets and healthy controls based on *DRB1* allele frequencies (online supplemental figure S1). PCA

Table 3 *DRB1* genotype frequency in PMR patients and controls

| | PMR (n=269) | Control (n=413) | P value | OR | 95% CI |
|---|---|---|---|---|---|
| **04:05*/not **04:05* | 86 (32.0) | 80 (19.4) | 0.0002 | 1.96 | (1.37 to 2.79) |
| **04:05/*04:05* | 3 (1.1) | 7 (1.7) | 0.7475 | 0.65 | (0.17 to 2.55) |
| **09:01*/not **09:01* | 32 (11.9) | 93 (22.5) | 0.0004 | 0.46 | (0.30 to 0.72) |
| **09:01/*09:01* | 0 (0.0) | 12 (2.9) | 0.0046 | 0.06 | (0.00 to 1.01) |
| **04:05/*04:10* | 5 (1.9) | 1 (0.2) | 0.0378 | 7.80 | (0.91 to 67.16) |
| **04:05/*09:01* | 5 (1.9) | 13 (3.1) | 0.3407 | 0.58 | (0.21 to 1.65) |
| **04:05/*12:01* | 11 (4.1) | 3 (0.7) | 0.0040 | 5.83 | (1.61 to 21.09) |
| **04:05/*15:01* | 13 (4.8) | 5 (1.2) | 0.0059 | 4.14 | (1.46 to 11.76) |
| SE/not SE | 124 (46.1) | 129 (31.2) | $9.84\times10^{-5}$ | 1.88 | (1.37 to 2.59) |
| SE/SE | 25 (9.3) | 24 (5.8) | 0.0957 | 1.66 | (0.93 to 2.97) |
| SE/**09:01* | 13 (4.8) | 23 (5.6) | 0.7289 | 0.86 | (0.43 to 1.73) |
| SE/**12:01* | 12 (4.5) | 5 (1.2) | 0.0108 | 3.81 | (1.33 to 10.94) |
| SE/**15:01* | 21 (7.8) | 8 (1.9) | 0.0003 | 4.29 | (1.87 to 9.83) |

Genotype frequencies are shown in parenthesis (%). Associations were tested by Fisher's exact test using 2×2 contingency tables.
PMR, polymyalgia rheumatica; SE, shared epitope.

showed the *DRB1* distribution pattern in PMR was close to that of the older age-onset RA group.

## DISCUSSION

Here, we examined predisposing associations of *DRB1*04:05* and *DQB1*04:01*, and protective associations of *DRB1*09:01* and *DQB1*03:03*, with Japanese PMR. Associations between SE alleles and PMR were confirmed in Japanese PMR patients. Predisposing alleles for PMR were comparable with RA,[43] especially for older age-onset RA, except that *DRB1*09:01*, an RA risk allele, was protective against PMR. Haplotype and logistic regression analyses suggested primary roles for *DRB1*09:01* in protection against PMR. Additionally, some amino acid residues in DRβ and DQβ chains were associated with predisposition to, or protection against, PMR.

Susceptibility to PMR was associated with harbouring *DRB1*01:01*, *DRB1*04:01* and *DRB1*04:04* in populations of Europeans,[25–27] although these associations were not observed in this study. This might be because of the diverse distribution pattern of *DRB1* alleles in diverse ethnic populations; however, these associations might be observed in larger-scale studies. Association studies of PMR in other ethnic populations might help to understand this phenomenon further. *DRB1* distribution patterns in PMR were similar to those of RA in populations of Europeans.[21 28] We verified this similarity. Although *DRB1*09:01* is not an SE allele, it is a risk allele for Japanese RA.[43] However, *DRB1*09:01* was revealed to be protective against PMR, similar to other studies showing *DRB1*09:01* was protective against autoimmune diseases including polymyositis or multiple sclerosis.[44 45]

Table 4 *DRB1-DQB1* haplotype carrier frequency in PMR patients and controls

| *DRB1-DQB1* haplotype | PMR (n=269) | Control (n=413) | P value | OR | 95% CI |
|---|---|---|---|---|---|
| **04:05-*03:02* | 0 (0.0) | 4 (1.0) | 0.1576 | 0.17 | (0.01 to 3.15) |
| **04:05-*04:01* | 88 (32.7) | 84 (20.3) | 0.0004 | 1.90 | (1.34 to 2.70) |
| **04:10-*04:02* | 10 (3.7) | 11 (2.7) | 0.4987 | 1.41 | (0.59 to 3.37) |
| **09:01-*03:01* | 0 (0.0) | 4 (1.0) | 0.1576 | 0.17 | (0.01 to 3.15) |
| **09:01-*03:03* | 32 (11.9) | 100 (24.2) | $6.60\times10^{-5}$ | 0.42 | (0.27 to 0.65) |
| **12:01-*03:01* | 18 (6.7) | 17 (4.1) | 0.1563 | 1.67 | (0.85 to 3.30) |
| **12:01-*03:03* | 7 (2.6) | 8 (1.9) | 0.5997 | 1.35 | (0.48 to 3.77) |
| **15:01-*03:01* | 0 (0.0) | 3 (0.7) | 0.2824 | 0.22 | (0.01 to 4.23) |
| **15:01-*06:02* | 57 (21.2) | 65 (15.7) | 0.0820 | 1.44 | (0.97 to 2.14) |

Haplotype carrier frequencies are shown in parenthesis (%). Associations were tested by Fisher's exact test using 2×2 contingency tables.
PMR, polymyalgia rheumatica.

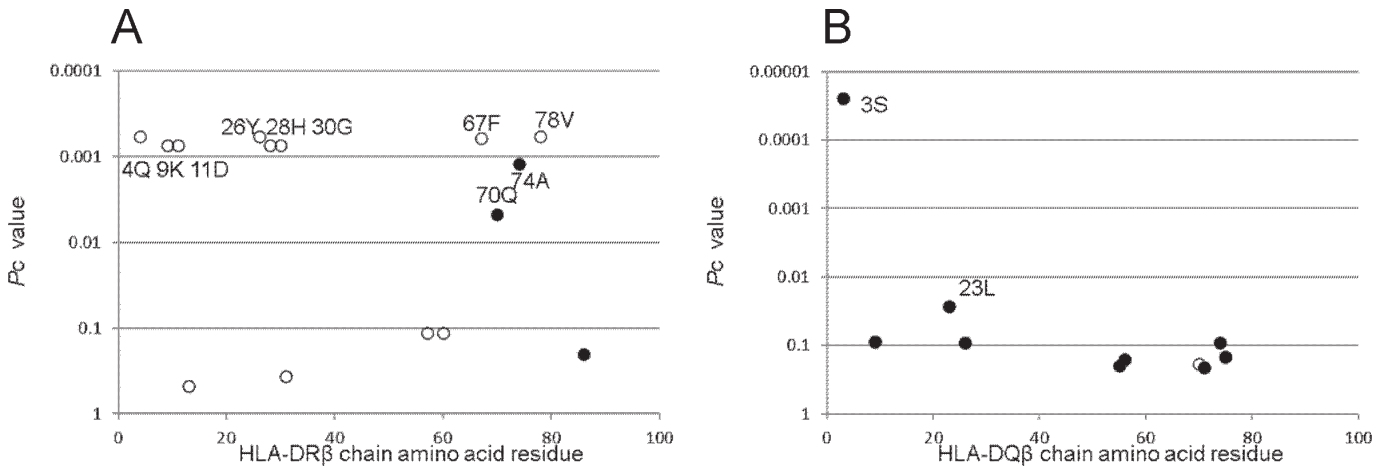


**Figure 1** Associations of amino acid residues in the DRβ (A) and DQβ (B) chains with PMR. Associations were analysed by Fisher's exact test using 2×2 contingency tables. Corrected p (Pc) values were generated by multiplying p values by the number of amino acid residues analysed. Predisposing associations are indicated by filled circles and protective associations by open circles. PMR, polymyalgia rheumatica.

This indicates a difference between susceptibility alleles between RA and PMR. Associations of *DRB1* risk alleles with RA were subject to a gene dosage effect.[43] Here, a gene dosage effect of *DRB1*04:05* or SE was not detected, but it was found for protection against PMR by *DRB1*09:01*. Thus, distinct roles of associated alleles were identified to be important in the pathophysiology of PMR and RA.

Because *HLA* in PMR has rarely been reported in populations other than European populations, our findings might aid our understanding of PMR aetiology. Many studies of the relationship between *HLA* alleles with PMR and giant cell arteritis have been reported in European patients,[10 24–27] but few studies of small sample sizes have been reported in Japanese populations.[46 47] Because PMR is complicated with giant cell arteritis at a high frequency in European populations, the effect of giant cell arteritis could not be eliminated. It is not frequently associated with giant cell arteritis in Japanese populations; thus, it was not necessary to consider the influence of giant cell arteritis in this study.

Type 1 diabetes, an autoimmune disease that affects pancreatic β cells, is associated with *DRB1*03/DRB1*04* and *DRB1*04/DRB1*08* heterozygous genotypes.[48] Autoimmune hepatitis, an autoimmune disease of the liver, is also associated with *DRB1*04/DRB1*08* heterozygous genotypes.[49] PMR was revealed to be associated with *DRB1*04:05/*04:10*, *DRB1*04:05/*12:01* and *DRB1*04:05/*15:01* heterozygous genotypes in Japanese populations. These heterozygous genotypes could be explained by transcomplementing DQα-β heterodimer molecules encoded by the *DQA1* allele for one haplotype and the *DQB1* allele for the second haplotype.[48] Thus, the pathogenesis of autoimmune diseases without gene dosage effects in the association of *DRB1* alleles might be explained by transcomplementing DQα-β heterodimer molecules, which might also explain the predisposing association of *DRB1*04:05* to PMR.

Amino acid residues 4Q, 9K, 11D, 26Y, 28H, 30G, 67F and 78V (encoded by *DRB1*09:01*) in the DRβ chain were associated with protection against PMR. Amino acid residues 70Q and 74A (encoded by *DRB1*04:05*) in the DRβ chain were associated with predisposition to PMR. Amino acid residues 3S, 9F and 23L (encoded by *DQB1*04:01*) in the DQβ chain were associated with predisposition to PMR. However, the patterns of amino acid residues associated with PMR were different from those in RA. Amino acid residues at positions 11 and 13 in the DRβ chain were strongly associated with susceptibility to RA,[33 50] whereas amino acid residues at positions 70 and 74 were associated with predisposition to PMR, suggesting that amino acid sequences at positions 70–74 in SE are important for predisposition to PMR, but not to RA. Different autoantigens might be presented by SE alleles in the pathogenesis of PMR and RA. Thus, these data suggest PMR and RA have a different pathogenesis.

This study had some limitations. This study did not contain replication analyses and the sample size of the study was modest. Thus, further larger-scale studies including other populations should be performed to verify the results of this study.

This is the first report of the predisposing associations of *DRB1*04:05*, SE and *DQB1*04:01*, and protective associations of *DRB1*09:01* and *DQB1*03:03* with PMR in Japanese patients. A gene dosage effect was observed for *DRB1*09:01* and *DQB1*03:03*, but not for *DRB1*04:05*, SE or *DQB1*04:01*. Genotypes of *DRB1*04:05/DRB1*04:10*, *DRB1*04:05/DRB1*12:01* and *DRB1*04:05/DRB1*15:01* were associated with PMR. Regarding haplotype frequencies, *DRB1*04:05-DQB1*04:01* was increased, and *DRB1*09:01-DQB1*03:03* was decreased in PMR. These data indicate the predisposing and protective roles of *HLA* molecules in the pathogenesis of PMR.

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**Ethics approval** This study was conducted in accordance with the principles expressed in the Declaration of Helsinki and was reviewed and approved by the NHO Central IRB (R4-0120007) and the Research Ethics Committees of Tama Medical Center, Sagamihara National Hospital, Tokyo National Hospital and Fukushima Medical University. Written informed consent was obtained from each individual.

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**Data availability statement** Data are available on reasonable request. Data supporting the findings of this study are presented and other data are available from the authors on reasonable request. The clinical and genotyping data of each participant are not available under the conditions of obtained informed consent mandated by the Act on the Protection of Personal Information in Japan.



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