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著者（英）	Shomi Oka, Hiroshi Furukawa, Kota Shimada, Atsushi Hashimoto, Akiko Komiya, Shinichiro Tsunoda, Koichiro Saisho, Naoyuki TSUCHIYA, Masao Katayama, Satoshi Shinohara, Toshihiro Matsui, Naoshi Fukui, Hajime Sano, Kiyoshi Migita, Shigeto Tohma
journal or publication title	Medicine
volume	98
number	48
page range	e18218
year	2019
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URL	http://hdl.handle.net/2241/00161182

doi: 10.1097/MD.0000000000018218

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

Shomi Oka, PhD[a,b,c], Hiroshi Furukawa, MD[a,b,c,*], Kota Shimada, MD[d,e], Atsushi Hashimoto, MD[d], Akiko Komiya, MD[a,f], Shinichiro Tsunoda, MD[g,h], Koichiro Saisho, MD[i,j], Naoyuki Tsuchiya, MD[b], Masao Katayama, MD[k], Satoshi Shinohara, MD[l], Toshihiro Matsui, MD[a], Naoshi Fukui, MD[a], Hajime Sano, MD[g], Kiyoshi Migita, MD[m,n], Shigeto Tohma, MD[a,c]

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* ($P = .0204$, OR 7.69, 95%CI 1.39–42.72), *DRB1*04:05/DRB1*12:01* ($P = .0050$, 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) in YORA was

Editor: Chinnadurai Mani.

The work was supported by Grants-in-Aid for Scientific Research (B, C) (26293123, 22591090, 15K09543, 18K08402) and for Young Scientists (B) (24791018) from the Japan Society for the Promotion of Science, Health and Labour Science Research Grants from the Ministry of Health, Labour, and Welfare of Japan, Grants-in-Aid of the Practical Research Project for Allergic Diseases and Immunology (Research on Allergic Diseases and Immunology) from Japan Agency for Medical Research and Development, Grants-in-Aid for Clinical Research from National Hospital Organization, Research Grants from Daiwa Securities Health Foundation, Research Grants from Japan Research Foundation for Clinical Pharmacology, Research Grants from The Nakatomi Foundation, Research Grants from Takeda Science Foundation, Research Grants from Mitsui Sumitomo Insurance Welfare Foundation, Bristol-Myers K.K. RA Clinical Investigation Grant from Bristol-Myers Squibb Co., and research grants from the following pharmaceutical companies: Abbott Japan Co., Ltd., Astellas Pharma Inc., Chugai Pharmaceutical Co., Ltd., Eisai Co., Ltd., Mitsuibishi Tanabe Pharma Corporation, Merck Sharp and Dohme Inc., Pfizer Japan Inc., Takeda Pharmaceutical Company Limited, Teijin Pharma Limited. The funders had no role in study design, data collection and analysis, decision to publish, or preparing the manuscript.

Conflict of interest statement: HF has the following conflicts, and the following funders are supported wholly or in part by the indicated pharmaceutical companies. The Japan Research Foundation for Clinical Pharmacology is run by Daiichi Sankyo, the Takeda Science Foundation is supported by an endowment from Takeda Pharmaceutical Company and the Nakatomi Foundation was established by Hisamitsu Pharmaceutical Co., Inc. The Daiwa Securities Health Foundation was established by Daiwa Securities Group Inc. and Mitsui Sumitomo Insurance Welfare Foundation was established by Mitsui Sumitomo Insurance Co., Ltd. HF was supported by research grants from Bristol-Myers Squibb Co. HF received honoraria from Ajinomoto Co., Inc., Daiichi Sankyo Co., Ltd., Dainippon Sumitomo Pharma Co., Ltd., Pfizer Japan Inc., and Takeda Pharmaceutical Company, Luminex Japan Corporation Ltd., and Ayumi Pharmaceutical Corporation. ST was supported by research grants from 9 pharmaceutical companies: Abbott Japan Co., Ltd., Astellas Pharma Inc., Chugai Pharmaceutical Co., Ltd., Eisai Co., Ltd., Mitsubishi Tanabe Pharma Corporation, Merck Sharp and Dohme Inc., Pfizer Japan Inc., Takeda Pharmaceutical Company Limited, Teijin Pharma Limited. ST received honoraria from Asahi Kasei Pharma Corporation, Astellas Pharma Inc., AbbVie GK., Chugai Pharmaceutical Co., Ltd., Ono Pharmaceutical Co., Ltd., Mitsubishi Tanabe Pharma Corporation, Pfizer Japan Inc.

The other authors have no conflicts of interests to disclose.

Supplemental Digital Content is available for this article.

[a] *Clinical Research Center for Allergy and Rheumatology, National Hospital Organization Sagamihara National Hospital, Sagamihara,* [b] *Molecular and Genetic Epidemiology Laboratory, Faculty of Medicine, University of Tsukuba, Tsukuba,* [c] *Tokyo National Hospital, National Hospital Organization, Kiyose,* [d] *Department of Rheumatology, National Hospital Organization Sagamihara National Hospital, Sagamihara,* [e] *Department of Rheumatic Diseases, Tokyo Metropolitan Tama Medical Center, Fuchu,* [f] *Department of Clinical Laboratory, National Hospital Organization Sagamihara National Hospital, Sagamihara,* [g] *Division of Rheumatology, Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya,* [h] *Department of Rheumatology, Sumitomo Hospital, Osaka,* [i] *Department of Orthopedics/Rheumatology, Miyakonojo Medical Center, National Hospital Organization, Miyakonojo,* [j] *Tanimura Hospital, Nobeoka,* [k] *Department of Internal Medicine, Nagoya Medical Center, National Hospital Organization, Nagoya,* [l] *Tochigi Rheumatology Clinic, Utsunomiya,* [m] *Clinical Research Center, Nagasaki Medical Center, National Hospital Organization, Omura,* [n] *Department of Gastroenterology and Rheumatology, Fukushima Medical University School of Medicine, Fukushima, Japan.*

[*] *Correspondence: Hiroshi Furukawa, Department of Rheumatology, National Hospital Organization Tokyo National Hospital, 3-1-1 Takeoka, Kiyose 204–8585, Japan (e-mail: furukawa-tky@umin.org).*

How to cite this article: Oka S, Furukawa H, Shimada K, Hashimoto A, Komiya A, Tsunoda S, Saisho K, Tsuchiya N, Katayama M, Shinohara S, Matsui T, Fukui N, Sano H, Migita K, Tohma S. Association of HLA-DRB1 genotype with younger age onset and elder age onset rheumatoid arthritis in Japanese populations. Medicine 2019;98:48(e18218).

Received: 6 August 2019 / Received in final form: 16 September 2019 / Accepted: 3 November 2019

http://dx.doi.org/10.1097/MD.0000000000018218

higher than EORA. 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 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[1] 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 patents 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, München, 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, Wakunaga, 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.[9] Genotyping results for some of the RA patients and the healthy controls were reported in previous studies.[8,23]

2.3. Statistical analysis

Differences of demographic features of RA patients were analyzed by Fisher exact test using 2X2 contingency tables. Associations of *HLA-DRB1* allele carrier frequencies, genotype frequencies, or amino acid residue carrier frequencies were tested by Fisher exact test using 2X2 contingency tables. Simple linear regression analyses under the additive model were conducted to analyze whether each allele contributes to the age at onset of RA. The deviation from zero was tested for partial regression coefficient (PRC) and *P* values were calculated. Alleles with PRC more than 0 contribute to increase the age at onset of RA and alleles with PRC less than 0 decrease. Multiple linear regression analyses of an allele were performed to identify the independent association of the allele from another allele. The deviation from zero was tested for PRC of an allele and *P* values were calculated, when conditioned on another allele. Multiple comparisons were corrected by Bonferroni method; the corrected *P* (*Pc*) values were obtained by multiplying the *P* values by the number of alleles or amino acid residues tested.

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.

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 ($P=.0004$, $Pc=.0119$, odds ratio [OR] 3.11, 95% confidence interval [CI] 1.69–5.73). *DRB1**04:05* was associated with the susceptibility of YORA ($P=8.33\times10^{-14}$, $Pc=2.42\times10^{-12}$, OR 5.47, 95% CI 3.47–8.61), MORA ($P=1.02\times10^{-31}$, $Pc=3.15\times10^{-30}$, OR 3.35, 95% CI 2.73–4.12), and EORA ($P=2.45\times10^{-12}$, $Pc=7.11\times10^{-11}$, 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 2.12–262.47, EORA: $P=.0011$, OR 19.04, 95%CI 2.28–158.74) and *DRB1**04:05/DRB1**09:01* (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* ($P=.0064$, OR 3.20, 95%CI 1.42–7.19) and *DRB1**04:05/DRB1**04:05* ($P=.0047$, OR 2.71, 95% CI 1.38–5.33) 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 1.39–42.72), *DRB1**04:05/DRB1**12:01* ($P=.0050$, 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) in YORA was higher than EORA. 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/not SE: $P=2.65\text{X}10^{-14}$, OR 2.88, 95%CI 2.18–3.79, SE/SE: $P=2.18\text{X}10^{-13}$, OR 5.47, 95%CI 3.52–8.50), though this gene dosage effect was not observed in YORA (SE/not SE: $P=3.35\text{X}10^{-10}$, 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, $P=8.10\text{X}10^{-8}$, OR=4.07, $Pc=2.75\text{X}10^{-6}$, 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 (3.6)	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)	$5.93\text{X}10^{-18}$
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 2X2 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					EORA vs control					Control	YORA vs EORA				YORA vs MORA				MORA vs EORA			
	YORA (n = 89)	*P*	OR	*Pc*	95%CI	MORA (n = 714)	*P*	OR	*Pc*	95%CI	EORA (n = 329)	*P*	OR	*Pc*	95%CI	(n = 1026)	*P*	OR	*Pc*	95%CI	*P*	OR	*Pc*	95%CI	*P*	OR	*Pc*	95%CI
*DRB1*01:01*	4 (4.5)	.0676	0.39	NS	(0.14–1.09)	102 (14.3)	.0305	1.39	0.9468	(1.04–1.85)	54 (16.4)	.0085	1.64	0.2458	(1.15–2.33)	110 (10.7)	.0029	0.24	0.0669	(0.08–0.68)	.0074	0.28	0.2142	(0.10–0.79)	.4005	0.85	NS	(0.59–1.22)
*DRB1*04:01*	7 (7.9)	.0060	3.90	0.1726	(1.62–9.39)	52 (7.3)	$3.51X10^{-7}$	3.58	$1.09X10^{-5}$	(2.16–5.96)	21 (6.4)	.0004	3.11	0.0119	(1.69–5.73)	22 (2.1)	.6339	1.25	NS	(0.51–3.05)	.8293	1.09	NS	(0.48–2.47)	.6955	1.15	NS	(0.68–1.95)
*DRB1*04:05*	56 (62.9)	$8.33X10^{-14}$	5.47	$2.42X10^{-12}$	(3.47–8.61)	364 (51.0)	$1.02X10^{-31}$	3.35	$3.15X10^{-30}$	(2.73–4.12)	146 (44.4)	$2.45X10^{-12}$	2.57	$7.11X10^{-11}$	(1.98–3.34)	243 (23.7)	.0027	2.13	0.0615	(1.31–3.44)	.0423	1.63	NS	(1.04–2.57)	.0532	1.30	NS	(1.00–1.69)
*DRB1*09:01*	27 (30.3)	.5376	1.16	NS	(0.72–1.86)	188 (26.3)	.6607	0.95	NS	(0.77–1.18)	89 (27.1)	1.0000	0.99	NS	(0.75–1.31)	280 (27.3)	.5937	1.17	NS	(0.70–1.96)	.4466	1.22	NS	(0.75–1.97)	.8212	0.96	NS	(0.72–1.29)
*DRB1*12:01*	9 (10.1)	.3004	1.43	NS	(0.69–2.95)	38 (5.3)	.1134	0.71	NS	(0.48–1.07)	24 (7.3)	1.0000	1.00	NS	(0.62–1.61)	75 (7.3)	.3793	1.43	NS	(0.64–3.20)	.0890	2.00	NS	(0.93–4.29)	.2082	0.71	NS	(0.42–1.21)
*DRB1*15:01*	12 (13.5)	1.0000	0.99	NS	(0.53–1.87)	81 (11.3)	.1871	0.82	NS	(0.61–1.09)	43 (13.1)	.8532	0.96	NS	(0.66–1.38)	139 (13.5)	1.0000	1.04	NS	(0.52–2.06)	.5972	1.22	NS	(0.64–2.33)	.4712	0.85	NS	(0.57–1.26)
SE	68 (76.4)	$5.80X10^{-11}$	4.73		(2.85–7.84)	516 (72.3)	$1.45X10^{-39}$	3.81		(3.10–4.68)	226 (68.7)	$5.06X10^{-19}$	3.20		(2.46–4.17)	417 (40.6)	.1909	1.48		(0.86–2.54)	.4507	1.24		(0.74–2.08)	.2402	1.19		(0.89–1.58)

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, EORA = elder age onset RA, OR = odds ratio, CI = confidence interval, *Pc* = corrected *P* value, NS = not significant, SE = Shared epitope.

Table 3

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

	YORA	YORA vs control			MORA	MORA vs control			EORA	EORA vs control			Control	YORA vs EORA			YORA vs MORA			MORA vs EORA		
	(n = 89)	*P*	OR	95%CI	(n = 714)	*P*	OR	95%CI	(n = 329)	*P*	OR	95%CI	(n = 1026)	*P*	OR	95%CI	*P*	OR	95%CI	*P*	OR	95%CI
*DRB1*01:01/DRB1*04:05*	0 (0.0)	.6142	0.45	(0.03–7.72)	22 (3.1)	.0073	2.69	(1.32–5.46)	12 (3.6)	.0064	3.20	(1.42–7.19)	12 (1.2)	.0784	0.14	(0.01–2.42)	.1585	0.17	(0.01–2.86)	.7078	0.84	(0.41–1.72)
*DRB1*04:01/DRB1*04:05*	2 (2.2)	.0179	23.56	(2.12–262.47)	17 (2.4)	$2.63X10^{-6}$	25.00	(3.32–188.29)	6 (1.8)	.0011	19.04	(2.28–158.74)	1 (0.1)	.6803	1.24	(0.25–6.24)	1.0000	0.94	(0.21–4.15)	.6559	1.31	(0.51–3.36)
*DRB1*04:05/DRB1*04:05*	2 (2.2)	.6815	1.22	(0.28–5.32)	39 (5.5)	$5.82X10^{-5}$	3.06	(1.75–5.34)	16 (4.9)	.0047	2.71	(1.38–5.33)	19 (1.9)	.3852	0.45	(0.10–1.99)	.3034	0.40	(0.09–1.68)	.7667	1.13	(0.62–2.05)
*DRB1*04:05/DRB1*04:06*	4 (4.5)	.0155	5.32	(1.60–17.63)	12 (1.7)	.1793	1.93	(0.81–4.61)	2 (0.6)	1.0000	0.69	(0.15–3.22)	9 (0.9)	.0204	7.69	(1.39–42.72)	.0906	2.75	(0.87–8.73)	.2469	2.79	(0.62–12.56)
*DRB1*04:05/DRB1*09:01*	10 (11.2)	.0028	3.38	(1.62–7.06)	57 (8.0)	$9.14X10^{-5}$	2.32	(1.52–3.55)	27 (8.2)	0.0014	2.39	(1.43–3.99)	37 (3.6)	.4004	1.42	(0.66–3.05)	.3079	1.46	(0.72–2.97)	.9029	0.97	(0.60–1.56)
*DRB1*04:05/DRB1*12:01*	7 (7.9)	.0004	7.21	(2.76–18.82)	12 (1.7)	.4068	1.44	(0.65–3.23)	5 (1.5)	.5774	1.30	(0.46–3.73)	12 (1.2)	.0050	5.53	(1.71–17.88)	.0027	4.99	(1.91–13.04)	1.0000	1.11	(0.39–3.17)
*DRB1*04:05/DRB1*15:01*	10 (11.2)	$1.09X10^{-6}$	11.68	(4.81–28.34)	25 (3.5)	.0009	3.35	(1.64–6.85)	12 (3.6)	.0049	3.49	(1.53–7.99)	11 (1.1)	.0124	3.34	(1.39–8.02)	.0030	3.49	(1.62–7.53)	1.0000	0.96	(0.48–1.93)
SE/not SE	59 (66.3)	$3.35X10^{-10}$	4.70	(2.81–7.86)	392 (54.9)	$6.25X10^{-29}$	3.31	(2.67–4.11)	177 (53.8)	$2.65X10^{-14}$	2.88	(2.18–3.79)	364 (35.5)	.0845	1.63	(0.94–2.85)	.2061	1.42	(0.84–2.40)	0.3606	1.15	(0.86–1.55)
SE/SE	9 (10.1)	.0007	4.92	(2.15–11.29)	124 (17.4)	$9.95X10^{-30}$	7.20	(5.02–10.31)	49 (14.9)	$2.18X10^{-13}$	5.47	(3.52–8.50)	53 (5.2)	1.0000	0.90	(0.38–2.11)	.4334	0.68	(0.30–1.54)	.2199	1.32	(0.88–1.98)

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, EORA = elder age onset RA, MORA = moderate age onset RA, OR = odds ratio, RA = rheumatoid arthritis, SE = Shared epitope, YORA = younger age onset RA.

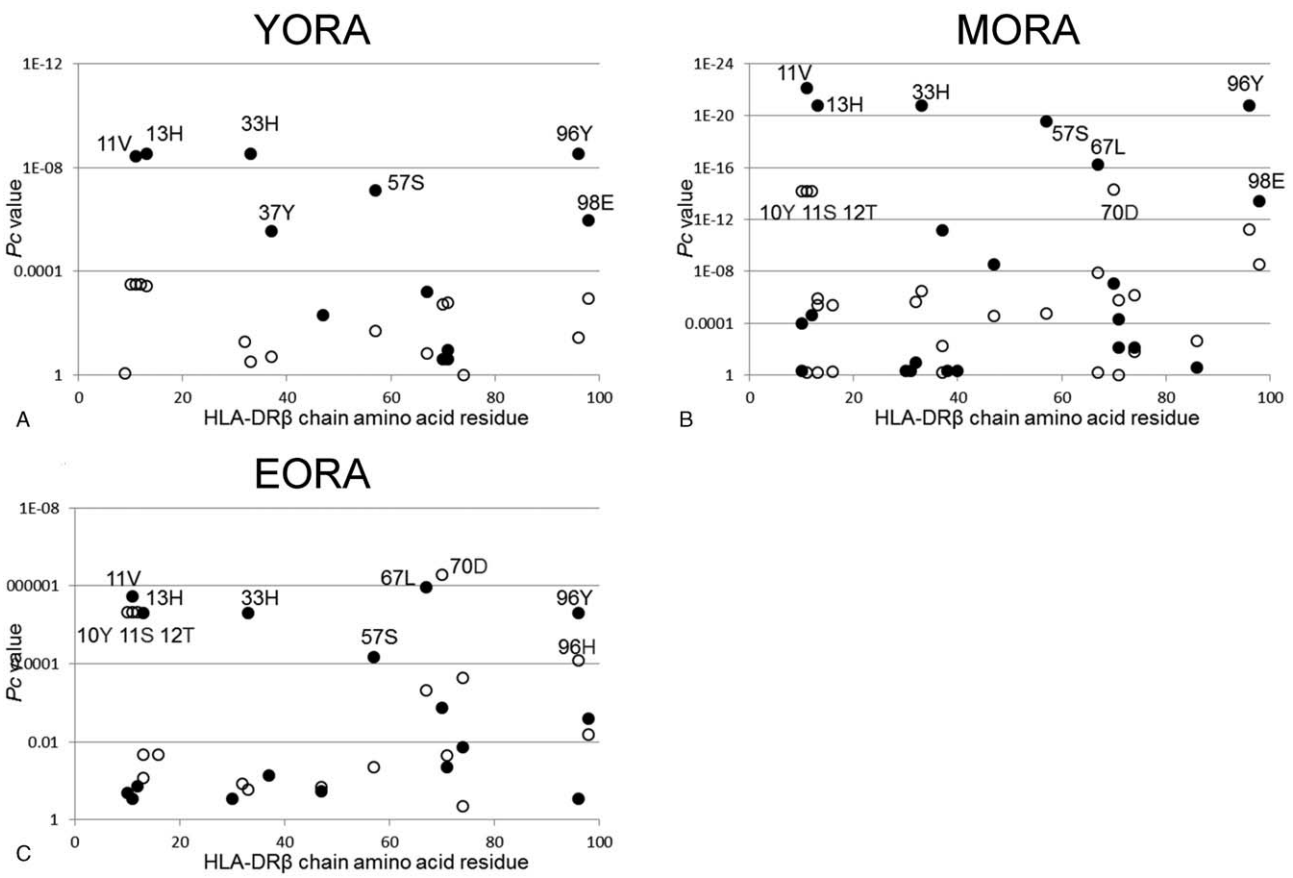

Figure 1. Associations of amino acid residues in the DRβ chain with YORA (A), MORA (B), and EORA (C). Associations were established by Fisher exact test using 2X2 contingency tables. Corrected *P* (*Pc*) values were calculated by multiplying the *P* value by the number of amino acid residues tested. Positive associations are indicated by filled circles and negative associations by open circles. RA = rheumatoid arthritis, YORA = younger age onset RA, MORA = moderate age onset RA, EORA = elder age onset RA.

with YORA (Fig. 1A), though these amino acid residues were associated with EORA (Fig. 1C, 67L: $P = 3.20\text{X}10^{-8}$, OR = 2.19, $Pc = 1.10\text{X}10^{-6}$, 95% CI 1.65–2.92, 70D: $P = 1.50\text{X}10^{-8}$, OR = 0.48, $Pc = 5.12\text{X}10^{-7}$, 95% CI 0.37–0.62). The association pattern of MORA (Fig. 1B) was similar to that of RA per se.[8] 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 ($P = .0005$, $Pc = 0.0079$, PRC –2.36), 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.[25] 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.

Table 4

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

HLA allele	PRC	95%CI	*P*	*Pc*
*B*15:07*	11.41	(2.16∼20.65)	.0156	0.5937
*B*44:03*	3.02	(0.35∼5.69)	.0265	NS
*B*67:01*	9.69	(3.61∼15.76)	.0018	0.0683
*DRB1*01:01*	2.44	(0.21∼4.67)	.0317	0.9205
*DRB1*03:01*	−26.56	(−46.10∼−7.02)	.0078	0.2251
*DRB1*04:05*	−1.90	(−3.29∼−0.51)	.0074	0.2151
*DRB1*04:06*	−4.83	(−8.82∼−0.83)	.0179	0.5202
*DRB1*13:02*	3.59	(0.62∼6.55)	.0177	0.5138
*DQB1*04:01*	−2.36	(−3.70∼−1.03)	.0005	0.0079
*DQB1*05:01*	2.48	(0.45∼4.50)	.0168	0.2520
*DQB1*06:04*	3.16	(0.12∼6.19)	.0416	0.6236
*DPB1*02:01*	−1.75	(−3.05∼−0.45)	.0085	0.1357
*DPB1*04:01*	3.15	(0.02∼6.28)	.0486	0.7781

Association for age at onset of RA was tested by linear regression analysis.
nnCI = confidence interval, NS = not significant, *Pc* = 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.[8] In the present study, the frequencies of some genotypes including *DRB1*04:05* were differently distributed between YORA and EORA, though the frequencies of some genotypes were reported to be increased in overall RA.[29–33] 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.[23,34] 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*04:01/DRB1*04:05*, *DRB1*04:05/DRB1*04:05,* *DRB1*04:05/DRB1*09:01,* and *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.[24,35] Analogically, 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 *DQA1* is a risk factor for RA in Chinese populations.[36] 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*04:05* were increased in EORA. The gene dosage effect of the SE alleles was detected in EORA, but not observed in YORA.

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.

Acknowledgments

We thank Ms. Mayumi Yokoyama and Ms. Satomi Hanawa (Clinical Research Center for Allergy and Rheumatology, Sagamihara National Hospital) for secretarial assistance.

Author contributions

Conceptualization: Hiroshi Furukawa, Shigeto Tohma.
Data curation: Shomi Oka, Hiroshi Furukawa.
Formal analysis: Shomi Oka, Hiroshi Furukawa.
Funding acquisition: Hiroshi Furukawa, Atsushi Hashimoto, Shigeto Tohma.
Investigation: Shomi Oka, Hiroshi Furukawa.
Methodology: Shomi Oka.
Resources: Kota Shimada, Atsushi Hashimoto, Akiko Komiya, Shinichiro Tsunoda, Koichiro Saisho, Naoyuki Tsuchiya, Masao Katayama, Satoshi Shinohara, Toshihiro Matsui, Naoshi Fukui, Hajime Sano, Kiyoshi Migita, Shigeto Tohma.
Supervision: Kiyoshi Migita, Shigeto Tohma.
Validation: Shomi Oka, Hiroshi Furukawa.
Visualization: Shomi Oka, Hiroshi Furukawa.
Writing – original draft: Shomi Oka.
Writing – review & editing: Hiroshi Furukawa, Shigeto Tohma.

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