

Differential association of HLA-DRB1 alleles in Japanese patients with early rheumatoid arthritis in relationship to autoantibodies to cyclic citrullinated peptide

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Abstract

Objective

To evaluate the role of HLA-DRB1 genotypes and antibodies to cyclic citrullinated peptides (anti-CCP antibodies) in the development and radiographic progression of Japanese patients with rheumatoid arthritis (RA).

Methods

One hundred and ten patients with early RA (88 female, 22 male) who visited our clinic within 1 year of symptom onset were examined for anti-CCP antibody levels and HLA-DRB1 genotypes. HLA-DRB1 genotypes were also determined in 265 healthy controls. Radiographic progression over a 2-year interval was evaluated using the Larsen's method in 66 patients.

Results

Among the 110 patients with early RA, 82 patients (74.5%) were anti-CCP positive. Carrier frequency of HLA-DRB1*0405 was significantly increased in RA patients with anti-CCP antibodies compared with controls and RA patients without anti-CCP antibodies (odds ratio [OR] 3.4, 95% confidence interval [95% CI] 2.0-5.7 and OR 3.3, 95% CI 1.3-8.6, respectively). Carriership of one or two SE alleles was significantly associated with production of anti-CCP antibodies (OR 2.7, 95% CI 1.1-6.7 and OR 9.3, 95% CI 1.1-78.2, respectively). On the other hand, allele frequency of HLA-DRB1*0901 was significantly increased in RA patients without anti-CCP antibodies compared with controls and RA patients with anti-CCP antibodies (OR 2.2, 95% CI 1.1-4.1 and OR 3.0, 95% CI 1.4-6.4, respectively).

Conclusions

In Japanese patients with RA, HLA-DRB1 SE alleles are associated with production of anti-CCP antibodies and HLA-DRB1 alleles appear to be differently associated with early RA depending on anti-CCP positivity as in Caucasian patients with RA.

Key words

Antibodies, cyclic citrullinated peptides, HLA-DRB1, Japanese, rheumatoid arthritis, shared epitope.

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Introduction

Rheumatoid arthritis (RA) is a chronic, autoimmune, inflammatory disease that mainly affects the joints of the hands and feet. Many studies have suggested that at least some of the genetic risk for RA can be explained on the basis of a 'shared epitope' (SE) in various class II *HLA-DR* molecules (1, 2). We previously reported that SE alleles were associated with susceptibility to the development of polyarthritis, but not with disease severity as the radiographic progression of 1 year in Japanese patients with early RA (3).

The peptidylarginine deiminase type 4 gene (*PADI4*) was recently reported to be a susceptibility locus for RA in Japanese (4, 5), Koreans (6), and Caucasians (7, 8). A haplotype of *PADI4* that affected stability of transcripts was associated with susceptibility to RA and with levels of antibody to citrullinated peptide in sera from individuals with RA (5). The IgG antibodies to cyclic citrullinated peptides (anti-CCP antibodies) have been reported to be highly specific and sensitive in Japanese and other ethnic patients with RA (9-11).

Several studies have shown significant correlation between anti-CCP antibody status and *HLA-DRB1* alleles (12-16), and anti-CCP positivity and radiographic progression (9, 10, 15, 17-21). Although previous studies showed the correlations between SE alleles and anti-CCP-positive RA (12-15), and between *HLA-DR3* and anti-CCP-negative RA (13, 16), and between anti-CCP positivity and the disease severity of RA (9, 10, 15, 17-21), no studies have examined these relations in Japanese patients with RA.

We examined in the present study the role of *HLA-DRB1* genotypes and anti-CCP antibodies in the development and radiographic progression of early RA in Japanese patients. Here, we showed significant associations of SE alleles with anti-CCP positive patients, and of *HLA-DRB1**0901 allele with anti-CCP negative patients.

Methods

Patients

One hundred and ten (88 female, 22 male) RA patients who visited the

outpatient clinic of the Institute of the Rheumatology, Tokyo Women's Medical University, within 1 year of symptom onset, were consecutively enrolled in the study as described previously (3). Their *HLA-DRB1* genotype and radiographic progression of 1 year have been previously reported (3). All the patients were diagnosed with RA according to the 1987 classification criteria for RA (22) at first (n = 45) or during the follow-up period (n = 65). They were enrolled in the study during 1991 through 1995 and each provided informed consent to participate. Each subject was under the care of an attending physician, and each had symptoms less than one year at the time of entry to the study. A standard diagnostic evaluation was performed at the first visit, which consisted of a patient history, physical and laboratory examinations, and radiographs of the hands and feet. Patients with active disease were offered treatment with disease modifying antirheumatic drugs (DMARDs) throughout the study of 2 years. Treatment during the first year was described as previously (3). During the 2 years, D-penicillamine (36%) was most commonly used among DMARDs; only a small portion of RA patients (14%) was treated with methotrexate during that period because the use of methotrexate for RA was officially approved in 1999 in Japan.

Controls

The control group consisted of 265 healthy personnel, including 148 male. All the controls were unrelated Japanese personnel living in the Tokyo area. *HLA-DRB1* genotypes of the control group have been reported (23, 24). The central part of Japan has been shown to be relatively homogeneous with respect to genetic background (25) permitting the case-control approach to be employed in this study.

Radiographic progression

Follow-up visits generally occurred at 4-week intervals. Radiographs of the hands-wrists and feet were taken at the first visit, 1 year later, and 2 years later. Radiographs of the hands and feet at baseline and 2 years were available for

66 (52 female, 14 male) of the 110 RA patients included in this cohort. The radiographs were assessed using Larsen's methods (26) by us (NI, KH) who were blinded to the clinical and genetic information but were aware of the temporal order of the radiographs as described in our previous study (3). Larsen score of grade 2 or more was the criterion for the presence of erosions. Progression of the radiographic damage, Δ Larsen score, was calculated by subtracting the baseline score from 2 years follow-up score for each 66 patient.

Biochemical analysis

Laboratory parameters were measured for each patient every 4 weeks, which included the erythrocyte sedimentation rate (ESR; in mm/hour, Westergren method) C-reactive protein (CRP) level (in mg/dl), and rheumatoid factor (RF). IgM RF status was assessed using a particle agglutination test (RAPA test; Fujizoki Pharmaceutical, Tokyo, Japan). For the analysis of anti-CCP antibodies, we analyzed the plasma samples obtained from 1992 to 1995, which had been stored at -20°C until this analysis. The second-generation anti-CCP ELISA kit (DIASSTAT Anti-CCP) was purchased from Axis-Shield, Dundee, UK. The assay was conducted according to the manufacturer's instruction. A cut-off value of >5 U/ml was used to indicate a positive result as manufacturer's instruction.

Genotyping

After obtaining the study subjects' informed consents, peripheral blood was drawn. Genomic DNA was extracted from the leukocytes, using a standard phenol-chloroform extraction procedure. The *HLA-DRB1* genotype was determined in 110 patients as well as 265 controls using the polymerase chain reaction (PCR)-restriction fragment length polymorphism method (27) or PCR-microtiter plate hybridization (MPH) technique (28).

Statistical analysis

Statistical significance of the differences between groups was determined using a Mann-Whitney U-test (continuous variables) and chi-square analysis or

Fisher's exact probability test (counts) as appropriate. For the comparison of *HLA-DRB1* genotypes, allele carrier frequencies (homozygotes and heterozygotes combined) and allele frequencies were compared. Corrected p (pc) were obtained by multiplying the observed p values by the number of alleles examined: 23 for *HLA-DRB1*. The odds ratio (OR) with 95% confidence interval (95% CI) was calculated.

Results

Baseline characteristics

The baseline characteristics of the patients are presented in Table I. The median age of the patients was 50.9 years; 80% were women; and the median disease duration was 5.4 months. Ninety-five of 110 patients (86.4%) were RF positive. Anti-CCP antibodies were present in 82 (74.5%) patients at the baseline. All patients had been diagnosed with RA according to the 1987 classification criteria for RA at baseline ($n = 45$) or during the follow-up period ($n = 65$). Disease progression was measured over a period of 2 years by scoring radiographs of the hands and feet using the Larsen's method in 66 patients. Among the 66 patients, 6 and 1 patients had received DMARDs (auranofin 3, D-penicillamine 1, bucillamine 1, gold sodium thiomalate 1) and prednisolone, respectively, before the first visit.

HLA-DRB1 and anti-CCP antibodies

Allele carrier frequency of *HLA-DRB1*

*0405 was significantly increased in patients with RA and patients with anti-CCP antibodies (52.4%) compared with controls (24.5%, OR 3.4 [95% CI 2.0 to 5.7], $P = 1.8 \times 10^{-6}$, $P_c = 4.1 \times 10^{-5}$) and RA patients without anti-CCP antibodies (25% OR 3.3 [95% CI 1.3 to 8.6], $P = 0.012$, P_c NS) (Table II). Compared with controls (19.2%), carrier frequency of *HLA-DRB1**1302 was significantly decreased in RA patients with anti-CCP antibodies (7.3%, OR 0.33 [95% CI 0.14 to 0.80], $P = 0.011$, P_c NS) and also in those without anti-CCP antibodies (3.6%, OR 0.16 [95% CI 0.021 to 1.2], $P = 0.039$, P_c NS). The frequency of *HLA-DRB1**0901 was significantly increased in RA patients without anti-CCP antibodies (46.4%) compared with RA patients with anti-CCP antibodies (22.0%, OR 3.1 [95% CI 1.2 to 7.6], $P = 0.013$, P_c NS). Although the difference was not significant ($P = 0.058$), the carrier frequency of *HLA-DRB1**0901 tended to be increased in RA patients without anti-CCP antibodies (46.4%) compared with controls (29.1%).

The allele frequency of *HLA-DRB1**0405 in RA patients with anti-CCP antibodies (30.5%) was significantly increased compared with controls (12.8%, OR 3.0 [95% CI 2.0 to 4.5], $P = 1.4 \times 10^{-7}$, $P_c = 3.2 \times 10^{-6}$) and RA patients without anti-CCP antibodies (12.5%, OR 3.1 [95% CI 1.3 to 7.2], $P = 0.0080$, P_c NS) (data not shown). The allele frequency of *HLA-DRB1**0901 was significantly increased

Table I. Baseline characteristics of the 110 Japanese patients with early rheumatoid arthritis (RA).

Patients characteristics	Value*
Age (years)	50.9 (41.0 to 58.3)
Sex (female)	88 (80.0%)
Age at disease onset (years)	50.1 (40.6 to 57.9)
Disease duration (months)	5.4 (3.2 to 8.8)
Rheumatoid factor positive	95 (86.4%)
ESR (mm/h)	46.0 (28.1 to 64.5)
CRP (mg/dl)	0.7 (0.4 to 2.9)
Anti-CCP present	82 (74.5%)
Larsen Score (0-180)	5.0 (2.0 to 13.8)
Number (%) of RA criteria positive at baseline	45 (40.9%)

*Values are median (25th to 75th centile) or n (%). ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; anti-CCP: antibodies to cyclic citrullinated peptides.

in RA patients without anti-CCP antibodies (26.8%) compared with controls (14.5%, OR 2.2 [95% CI 1.1 to 4.1], $P = 0.016$, P_c NS) and RA patients with anti-CCP antibodies (11.0%, OR 3.0 [95% CI 1.4 to 6.4], $P = 0.0042$, P_c NS) (data not shown).

Anti-CCP antibodies and SE alleles

Among previously reported RA-related alleles, *DRB1**0101, *0401, *0404, *0405, *0410, and *1001 were present in RA patients in the present study; these were designated as SE alleles (2). SE alleles were more common in RA patients with anti-CCP antibodies than in controls (Table II). Thus, the frequency of SE carriers was significantly increased in RA patients with anti-CCP antibodies (70.7%) compared with controls (37.7%, OR 4.0 [95% CI 2.3 to 6.8], $P = 1.6 \times 10^{-7}$) and RA patients without anti-CCP antibodies (42.9%, OR 3.2 [95% CI 1.3 to 7.8], $P = 0.0081$). In contrast, SE carriership did not significantly differ between RA patients without anti-CCP antibodies and controls.

In our study, 13.6% (15 of 110) had two SE alleles (SE/SE), 50% (55 of 110) had one SE allele (SE/x), and 36.4% (40 of 110) had no SE alleles (Table III). Anti-CCP antibodies were found in 93.3% of SE/SE patients (14 of 15), 80% of SE/x patients (44 of 55), and 60% of x/x patients (24 of 40). Compared with carriership in x/x patients, SE/SE and SE/x carriership was significantly associated with production of anti-CCP antibodies (OR 9.3 [95% CI 1.1 to 78.2], $P = 0.017$ and OR 2.7 [95% CI 1.1 to 6.7], $P = 0.033$, respectively).

Anti-CCP antibodies and SE alleles as disease progression markers

Although the differences were not significant, the patients who carry SE alleles ($n = 43$, $P = 0.10$) tended to have more joint destructions compared with those without SE ($n = 23$) (data not shown). We did not find any significant difference in radiographic progression of 2 years between the patients with and without anti-CCP antibodies (data not shown). We also evaluated the radiographic progression in the patients with anti-CCP antibody level more

Table II. Carrier frequencies of *HLA-DRB1* alleles in Japanese early rheumatoid arthritis patients with and without anti-CCP antibodies, and controls (Values are the number (%) of subjects carrying each *HLA-DRB1* alleles [homozygotes and heterozygotes combined]).

<i>DRB1</i>	Anti-CCP+ (n = 82)	Anti-CCP- (n = 28)	Controls (n = 265)
0101	14 (17.1%)	3 (10.7%)	26 (9.8%)
0401	4 (4.9%)	1 (3.6%)	6 (2.3%)
0403	4 (4.9%)	1 (3.6%)	13 (4.9%)
0404	2 (2.4%)	0	4 (1.5%)
0405	43 (52.4%)**	7 (25.0%)	65 (24.5%)
0406	3 (3.7%)	3 (10.7%)	19 (7.2%)
0407	0	0	6 (2.3%)
0410	1 (1.2%)	1 (3.6%)	5 (1.9%)
0802	5 (6.1%)	1 (3.6%)	18 (6.8%)
0803	8 (9.8%)	4 (14.3%)	37 (14.0%)
0901	18 (22.0%)	13 (46.4%)§	77 (29.1%)
1001	1 (1.2%)	0	2 (0.8%)
1101	5 (6.1%)	0	5 (1.9%)
1201	8 (9.8%)	4 (14.3%)	19 (7.2%)
1202	1 (1.2%)	0	11 (4.2%)
1301	0	1 (3.6%)	0
1302	6 (7.3%)§	1 (3.6%) [†]	51 (19.2%)
1401	2 (2.4%)	2 (7.1%)	13 (4.9%)
1403	1 (1.2%)	1 (3.6%)	10 (3.8%)
1405	3 (3.7%)	2 (7.1%)	17 (6.4%)
1406	2 (2.4%)	0	7 (2.6%)
1501	6 (7.3%)	3 (10.7%)	29 (10.9%)
1502	16 (19.5%)	5 (17.9%)	54 (20.4%)
1602	0	0	8 (3.0%)
Shared Epitope*	58 (70.7%)§	12 (42.9%)	100 (37.7%)

Anti-CCP = antibodies to cyclic citrullinated peptides. *Shared epitope alleles include *DRB1**0101, *0401, *0404, *0405, *0410, and *1001. **Odds Ratio (OR) 3.4 (95% confidence interval [95% CI] 2.0 to 5.7), $P = 1.8 \times 10^{-6}$, $P_c = 4.1 \times 10^{-5}$ vs controls and OR 3.3 (95% CI 1.3 to 8.6), $P = 0.012$, P_c not significant (NS) vs anti-CCP- patients, respectively; [†]OR 0.33 (95% CI 0.14 to 0.80), $P = 0.011$, P_c NS vs controls; [§]OR 3.1 (95% CI 1.2 to 7.6), $P = 0.013$, P_c NS vs anti-CCP+ patients; [§]OR 0.16 (95% CI 0.021 to 1.2), $P = 0.039$, P_c NS vs controls; [§]OR 4.0 (95% CI 2.3 to 6.8), $P = 1.6 \times 10^{-7}$ vs controls and OR 3.2 (95% CI 1.3 to 7.8), $P = 0.0081$ vs anti-CCP- patients, respectively.

than 100 U/ml ($n = 15$) but did not find any significant difference between the patients and RA patients without anti-CCP antibodies (data not shown).

Discussion

This is the first to report the correlations between *HLA-DRB1* alleles and anti-CCP antibodies in Japanese patients with RA. *HLA-DRB1* alleles

were differently associated with anti-CCP-positive and -negative patients with early RA.

Previous studies have shown significant correlations between the presence of SE alleles and the positivity of anti-CCP antibodies in RA patients (12–15). Van Gaalen *et al.* reported that the associations between the SE alleles and RA were mainly due to the under-

Table III. Associations of *HLA-DRB1* shared epitope (SE) alleles and anti-CCP antibodies in Japanese patients with early rheumatoid arthritis ($n = 110$)^{*}.

SE status**	Anti-CCP+ (n = 82)	Anti-CCP- (n = 28)	OR (95% CI) [†]	<i>P</i>
SE/SE	14	1	9.3 (1.1 to 78.2)	0.017
SE/x	44	11	2.7 (1.1 to 6.7)	0.033
x/x	24	16		

^{*}See Table II for other definitions. **SE alleles include *DRB1**0101, *0401, *0404, *0405, *0410, and *1001. [†]ORs were calculated comparing double-dose SE carries (SE/SE) and single-dose carries (SE/x) against carries of no SE alleles (x/x).

lying association between the SE and the production of anti-CCP antibodies found in RA patients (15). Recently, Huizinga *et al.* reported that SE alleles were specific for disease with anti-CCP antibodies, indicating that these alleles do not associate with RA, but rather with a particular phenotype in Caucasian patients with RA (12). In our study, the frequencies of SE carriers were significantly increased in RA patients with anti-CCP antibodies, but not in RA patients without anti-CCP antibodies, compared with controls (Table II). Thus, our data were consistent with the previous studies (12-15), suggesting that the associations between the SE alleles and RA found in Japanese RA patients (3, 24, 29, 30) may also be due to the underlying association between SE alleles and anti-CCP positivity. The allele frequency of *HLA-DRB1*0901* allele seemed to be higher in RA patients without anti-CCP antibodies compared with RA patients with anti-CCP antibodies and controls in this study (Table II). *HLA-DRB1*0901* has been reported to be associated with RA susceptibility in Japanese (31), Korean (32), and Ojibway and Cree populations (33). In Caucasian population, this allele appears to be associated with anti-CCP antibodies (15) although it is thought to be a neutral allele in RA susceptibility (34). Verpoort *et al.* found that HLA-DR3 was associated with RA patients without anti-CCP antibodies and not with RA patients with anti-CCP antibodies in Caucasian, indicating different pathogenic mechanisms between RA patients with and without anti-CCP antibodies (16). Recently, Irigoyen *et al.* reported that HLA-DR3 alleles were associated with anti-CCP negative disease and with lower levels of anti-CCP antibodies in patients with RA (13). Although HLA-DR3 is common (around 20%) in the Caucasian population, it is very rare (0.1-0.2%) in Japanese (35). Our data suggest a possibility that the genetic risk factors for RA patients without anti-CCP antibodies are different between Japanese (DR9, *DRB1*0901*) and Caucasian (DR3, *DRB1*0301*) although the number of the patients without anti-CCP antibodies was relatively small

in our study and larger sample studies will be needed.

*HLA-DRB1*0901* is also known for an association with several autoimmune diseases in Japan, including microscopic polyangiitis (36, 37), antineutrophil cytoplasmic antibody-associated vasculitis (37), antiphospholipid antibody production in patients with systemic lupus erythematosus (38), juvenile onset myasthenia gravis (39), and type I diabetes mellitus (40). These results suggest that *HLA-DRB1*0901* may be related to some autoimmune propensity in Japanese.

We did not find significant associations between anti-CCP antibodies and radiographic progression of 2 years although several studies have shown that the presence of anti-CCP antibodies appears to predict radiographic damage and progression in patients with RA (9, 10, 15, 17-21). A study with a much larger sample size than that in the current study will be needed to detect radiographic associations between Japanese patients with RA who had anti-CCP antibodies and patients who did not have anti-CCP antibodies. The current study did not have adequate power to assess these associations.

Despite the advantages of a prospective cohort study in a single institute, our study has some limitations. First, we did not analyze the association of disease activity score 28 (DAS28) (41), and health assessment questionnaire (HAQ) (42) with anti-CCP antibodies or SE since we did not collect data of DAS28 or HAQ at the baseline period (1991-1995) of this study. Second, we used Larsen methods (26) to evaluate radiographic progression of early RA as previous studies (3, 18, 20, 43, 44). However, this method is reported to be less sensitive than Sharp/van der Heijde methods (45, 46) that are commonly used in the recent years (15, 17, 19, 21). Third, although the use of methotrexate was not common in Japan during our study period, we did not evaluate the influence of treatments on the radiographic progression.

In summary, our study provides further evidence that *HLA-DRB1* SE alleles are associated with anti-CCP-positive RA. *HLA-DRB1* alleles appear to be

differently associated with early RA, depending on anti-CCP positivity in Japanese patients.

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