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Anti-glucose-6-phosphate isomerase, anti-cyclic citrullinated peptide antibodies and HLA-DRB1 genotypes in Japanese patients with early rheumatoid arthritis

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ABSTRACT

Objective. *Our goal was to evaluate the associations of antibodies (Abs) to glucose-6-phosphate isomerase (GPI) with Abs to cyclic citrullinated peptide (CCP) and* HLA-DRB1 *genotypes in Japanese patients with early rheumatoid arthritis (RA).*

Methods. *One hundred and eight patients with early RA (85 female, 23 male) who visited our clinic within 1 year of symptom onset were examined for anti-GPI and anti-CCP Ab levels, and* HLA-DRB1 *genotype. Anti-GPI and anti-CCP Ab levels, and* HLA-DRB1 *genotypes were also determined in 63 controls and 265 healthy controls, respectively.*

Results. *Of the 108 patients with early RA and the 63 controls, 20 (18.5%) and 3 (4.8%) were anti-GPI Ab-positive, respectively. Of the 20 patients with anti-GPI Abs, 17 (85%) were positive for anti-CCP Abs. HLA-DRB1*0405 and shared epitope (SE) carrier frequencies were significantly increased not only in anti-GPI Ab-positive patients (p=0.00057, odds ratio [OR] 4.6, 95% CI 1.8-11.8; p=0.0011, OR 5.0, 95% CI 1.7-14.0), but also in anti-GPI Ab-negative patients (p=0.0017, OR 2.2, 95% CI 1.3-3.7; p=0.00011, OR 2.6, 95% CI 1.6-4.3), when compared with controls. In addition, the carrier frequency of HLA-DRB1*1201 was significantly increased in anti-GPI Ab-positive patients compared with controls (p=0.0056, OR 4.3, 95% CI 1.4-13.2).*

Conclusions. *The majority of anti-GPI Ab-positive RA patients constitute a subset of* HLA-DRB1* *SE-associated, anti-CCP Ab-positive RA patients.*

Introduction

Rheumatoid arthritis (RA) is a chronic, autoimmune, inflammatory disease that mainly affects the joints of the hands and feet. We previously reported that 'shared epitope' (SE) (1) and *HLA-DRB1*0405* are associated with susceptibility (2) in Japanese RA patients. Recently, we reported that SE and *HLA-DRB1*0405* correlated with anti-CCP Ab-positivity in RA patients, while *HLA-DRB1*0901* was significantly increased in anti-CCP Ab-negative RA patients in Japanese (3).

Anti-glucose-6-phosphate isomerase (GPI) antibodies (Abs) are detected as arthritogenic Abs in the serum of K/BxN T cell receptor transgenic mice (4). In human RA, anti-GPI Abs have been detected frequently in patients with aggressive forms of arthritis (5-7), and their levels significantly correlated with extra-articular manifestations such as rheumatoid nodules, rheumatoid vasculitis and Felty's syndrome (8). In other rheumatologic conditions, anti-GPI Abs are detected at a similar prevalence (12-25%) as in RA (6). An association between anti-CCP and anti-GPI Abs has not been reported. Although Kori *et al*. reported a suggestive association between GPI-reactive T cells and *HLA-DRB1 *0405* or **0901* within anti-GPI Ab-positive individuals in Japanese patients with RA (9), the correlation between anti-GPI Abs and *HLA-DRB1* haplotype has not been clearly defined.

In the present study, we examined the associations of anti-GPI Abs with anti-CCP Abs and *HLA-DRB1* genotypes in Japanese patients with early RA. Here, we show that the majority of anti-GPI Ab-positive patients were also positive for anti-CCP Abs. *HLA-DRB1* SE was associated not only with being anti-GPI Ab positive but also with anti-GPI Ab-negative RA patients, in contrast with anti-CCP Abs (3, 10, 11).

Materials and methods

Patients

One hundred and eight RA patients who visited the outpatient clinic of the Institute of Rheumatology, Tokyo Women's Medical University, within 1 year of symptom onset were consecutively enrolled in the study. Associations between their *HLA-DRB1* genotypes, RA susceptibility (2) and anti-CCP Ab status (3) were reported previously. The patients satisfied the 1987 classification criteria for RA either at presentation (n=45) or during the follow-up period (n=63).

Controls

The control group for *HLA-DRB1* genotypes consisted of 265 healthy personnel whose *HLA-DRB1* genotypes have been reported previously

Table I. Baseline characteristics of 108 Japanese patients with early rheumatoid arthritis.

Patient characteristics	Value*
Age, years (range)	50.5 (40.8 – 57.9)
Gender (% female)	85 (78.7%)
Age at disease onset, years (range)	49.9 (40.4 – 57.8)
Disease duration, months (range)	5.4 (3.2 – 8.9)
Rheumatoid factor positive (%)	93 (86.1%)
Erythrocyte sedimentation rate, mm/h (range)	46.5 (27.9 – 65.2)
C-reactive protein, mg/dL (range)	0.7 (0.4 – 3.0)
Anti-CCP present (%)	81 (75.0%)
Anti-GPI present (%)	20 (18.5%)
Both anti-CCP and anti-GPI present (%)	17 (15.7%)
Larsen score, 0-180 (range)	5.0 (2.0 – 14.0)
Number of positive RA criteria at baseline (%)	45 (41.7%)

*Values are median (25th to 75th percentile) or n (%). Anti-CCP, antibodies to cyclic citrullinated peptides; anti-GPI, antibodies to glucose-6-phosphate isomerase; RA: rheumatoid arthritis.

Table II. Carrier frequencies of *HLA-DRB1* alleles in Japanese early rheumatoid arthritis patients with and without anti-GPI antibodies, and controls.

DRB1	Anti-GPI+ (n=20)	Anti-GPI- (n=88)	Controls (n=265)
0101	2 (10.0%)	15 (17.0%)	26 (9.8%)
0401	2 (10.0%)	3 (3.4%)	6 (2.3%)
0403	2 (10.0%)	3 (3.4%)	13 (4.9%)
0404	1 (5.0%)	1 (1.1%)	4 (1.5%)
0405	12 (60.0%)†	37 (42.0%)¶	65 (24.5%)
0406	1 (5.0%)	5 (5.6%)	19 (7.2%)
0407	0	0	6 (2.3%)
0410	0	2 (2.3%)	5 (1.9%)
0802	0	6 (6.8%)	18 (6.8%)
0803	0	11 (12.5%)	37 (14.0%)
0901	5 (25.0%)	25 (28.4%)	77 (29.1%)
1001	0	1 (1.1%)	2 (0.8%)
1101	1 (5.0%)	4 (4.5%)	5 (1.9%)
1201	5 (25.0%)‡	10 (11.4%)	19 (7.2%)
1202	0	1 (1.1%)	11 (4.2%)
1301	0	1 (1.1%)	0
1302	1 (5.0%)	6 (6.8%)	51 (19.2%)
1401	0	4 (4.5%)	13 (4.9%)
1403	0	2 (2.3%)	10 (3.8%)
1405	0	4 (4.5%)	17 (6.4%)
1406	1 (5.0%)	1 (1.1%)	7 (2.6%)
1501	1 (5.0%)	8 (9.1%)	29 (10.9%)
1502	4 (16.0%)	17 (19.3%)	54 (20.4%)
1602	0	0	8 (3.0%)
Shared epitope*	15 (75.0%)#	54 (61.4%)**	100 (37.7%)

Values are the number (%) of subjects carrying each *HLA-DRB1* allele (homozygotes and heterozygotes combined). Anti-GPI = antibodies to anti-glucose-6-phospate isomerase. *Shared epitope alleles include *DRB1*0101, *0401, *0404, *0405, *0410*, and **1001*. †Odds Ratio (OR) 4.6 (95% confidence interval [95% CI] 1.8-11.8), p=0.00057, pc=0.013 vs. controls; ‡OR 4.3 (95% CI 1.4-13.2), p=0.0056, pc NS *vs.* controls; #OR 5.0 (95% CI 1. -14.0), p=0.0011 *vs.* controls; ¶OR 2.2 (95% CI 1.3-3.7), p=0.0017, pc=0.039 vs. controls; **OR 2.6 (95% CI 1.6-4.3), p=0.00011 vs. controls.

(3). The control group for anti-GPI and anti-CCP Ab levels consisted of plasma from 63 personnel, including 8 patients with gout, 40 patients with osteoarthritis, and 15 healthy co-workers in our institute.

Radiographic progression

Radiographs of the hands and feet at baseline and 2 years were available for 65 patients. Progression of the radiographic damage was calculated as described previously (2, 3).

Biochemical analysis and genotyping

Laboratory parameters were measured for each patient; these included the erythrocyte sedimentation rate (ESR; in mm/hour), C-reactive protein (CRP) level (in mg/dL), and rheumatoid factor (RF) as described previously (2, 3). Serum IgG, IgA, and IgM concentrations were also examined at baseline. For the analysis of anti-GPI and anti-CCP Abs, we obtained plasma samples from 1992 to 1995, which had been stored at -20°C and frozen and thawed several times over a period of ten years. For selecting anti-GPI Ab-positive patients, we used rabbit muscle GPI (raGPI) (Sigma, St Louis, MO) as described previously (5, 6). The cutoff OD was calculated from an ELISA reaction of 63 control Japanese personnel ; the mean value + two standard deviations was 1.64. The second generation anti-CCP ELISA kit was used as described previously (3). The *HLA-DRB1* genotype was determined in 108 patients and 265 controls as described previously (2, 3).

Statistical analysis

Statistical significance of the differences between groups was determined using a Mann-Whitney U-test (continuous variables) and chi-square analysis, Fisher's exact probability test or Mantel-Haenszel procedure (counts), as appropriate. For the comparison of *HLA-DRB1* genotypes, allele carrier frequencies (homozygotes and heterozygotes combined) and allele frequencies were compared. Corrected p-values (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 (CI) was calculated.

Results

Baseline characteristics and anti-GPI Ab detection

The baseline characteristics of the patients are presented in Table I. Among the patients with (n=20) and without anti-GPI Abs (n=88), 17 (85.0%) and 64 patients (72.7%) were positive for anti-CCP Abs, respectively. Among the patients with (n=81) and without anti-CCP Abs (n=27), 17 (21.0%) and 3 patients (11.1%) were positive for

anti-GPI Abs, respectively. Anti-GPI and anti-CCP antibodies occurred more commonly in the patients with early RA (n=20, 18.5%, OR 4.5 [95% CI 1.3 - 16.0] and n=81, 75.0%, OR 91.5 [95% CI 20.9 - 399.7]) than in controls (n=3, 4.8% and n=2, 3.2%), respectively.

HLA-DRB1 and anti-GPI Abs

*HLA-DRB1*0405* and SE carrier frequencies were significantly increased not only in anti-GPI Ab-positive patients, but also in anti-GPI Ab-negative patients, when compared with controls (Table II), although the association appeared to be stronger in anti-GPI Ab-positive patients. In addition, the allele frequency of *HLA-DRB1*1201* was significantly increased in anti-GPI Ab-positive patients compared with controls.

Radiographic progression, serum severity markers and anti-GPI Abs

The radiographic progression and serum markers were not significantly different between the patients with and without ant-GPI Abs (data not shown). Treatment strategy, mean age, and sex were approximately equally distributed between the patients with and without anti-GPI Abs.

Discussion

We examined the associations between anti-GPI and anti-CCP Abs, and between anti-GPI Abs and *HLA-DRB1* genotype in Japanese patients with early RA. The majority of anti-GPI Ab-positive patients were positive for anti-CCP Abs (Table I). SE and *HLA-DRB1*0405* were associated not only with being anti-GPI Ab-positive, but also in anti-GPI Ab-negative RA patients (Table II).

Kori *et al*. reported a possible link between GPI-reactive T cells and *HLA-DRB1 *0405* or **0901* within anti-GPI Ab-positive Japanese RA patients (9). However, the numbers examined by that report were small and further study is needed to evaluate the association between anti-GPI Abs and *HLA-DRB1* haplotypes. We confirmed the correlation of anti-GPI Ab production to *HLA-DRB1*0405,* but did not verify the associations of those with *HLA-DRB1*0901* in a different population of Japanese RA patients (Table II). Since SE has been shown to be strongly associated with anti-CCP Abs (3, 12), we analyzed the data to exclude the effect using the Mantel-Haenszel procedure but did not find any significant influence (data not shown). Our results demonstrated that since most of the anti-GPI Ab-positive RA patients were also positive for anti-CCP Abs, the genetic background was also shared with that of the anti-CCP Ab-positive RA patients.

The allele frequency of *HLA-DRB1*1201* was significantly higher in RA patients with anti-GPI Abs compared with controls in this study (Table II). Fujii *et al*. reported that *HLA-DRB1*1201* is associated with adult Still's disease in Japanese (13). These results suggest that *HLA-DRB1*1201* may be related to some autoimmune propensity in Japanese.

In our study, 18.5% of Japanese RA patients were positive for raGPI Abs. A recent report in a different population showed that Abs specific for raGPI were present in 17% of Japanese patients with RA (5). Thus, our results were consistent with the previous study (5). We attempted to detect anti-GPI Abs using recombinant human GPI (huGPI). However, we did not get consistent results, although all patients with anti-raGPI Abs were also positive for anti-huGPI Abs (data not shown). Since this is the first study to analyze plasma samples for anti-GPI Abs, and our samples had been frozen and thawed several times over a period of ten years, conditions may not have been suitable for the detection of anti-huGPI Abs in particular. Some cross-reactivity occurred with huGPI in our previous study (6). The specificity of raGPI was reported (14) and two papers have reported the results of anti-GPI Abs using raGPI only (7, 15).

Although the serum IgM levels were significantly higher in patients without anti-GPI Abs than those in patients with anti-GPI Abs, among the patients with anti-CCP Abs (p=0.04) (data not shown), we did not find significant associations between anti-GPI Abs and RA severity. Since several studies have shown that the presence of anti-GPI Abs has been detected in patients with aggressive forms of arthritis (5-7), a study with a much larger sample size will be needed to examine the differences between anti-GPI Ab-positive and -negative RA patients.

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 and health assessment questionnaire scores with anti-GPI Ab status since we did not collect the data during the baseline period (1991-1995). Second, we used Larsen methods to evaluate radiographic progression, as did previous studies (2, 3), although Sharp/van der Heijde methods are commonly used recently (10).

In conclusion, the majority of anti-GPI Ab-positive patients were also positive for anti-CCP Abs. *HLA-DRB1* SE was strongly associated with patients with anti-GPI Abs. These data suggest that the majority of anti-GPI Ab-positive RA patients constitute a subset of *HLA-DRB1** SE–associated, anti-CCP Ab-positive RA patients. We did not find any additive values in diagnosing/predicting the course of RA in this study.

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