

Association of Clinical Features with Human Leukocyte Antigen in Japanese Patients with Ulcerative Colitis

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ABSTRACT

Background The human leukocyte antigen (HLA) region has been found to be involved in the pathogenesis of inflammatory bowel disease (IBD), which is classified into ulcerative colitis (UC) and Crohn's disease (CD), by genome-wide association studies. The aim of this study was to confirm whether HLA-alleles confer susceptibility to UC and to determine whether HLA-alleles are associated with the clinical phenotypes in Japanese patients with UC.

Methods In this study, HLA typing was performed by PCR-sequence-specific oligonucleotides (PCR-SSO) to confirm the correlation between UC and HLA alleles (for HLA-A, B, DRB1) in 45 Japanese UC patients. In addition, whether the HLA alleles are related to patient and clinical background characteristics was examined.

Results Overall, 62.2%, and 66.7% of the 45 UC patients had HLA-B*52 and HLA-DRB1*15, respectively. These allele frequencies were significantly higher than in previously reported Japanese control persons ($P < 0.0001$). The frequencies of extraintestinal manifestations [odds ratio (OR) = 0.12, $P = 0.039$] and a history of colectomy (OR = 0.18, $P = 0.046$) were lower in HLA-B*52-positive UC patients than in HLA-B*52 negative UC patients. The white blood cell (WBC) count was significantly higher in HLA-DRB1*15-positive patients ($9430 \pm 4592/\mu\text{L}$) than in HLA-DRB1*15-negative patients ($6729 \pm 2160/\mu\text{L}$). Thus, HLA-B*52 and DRB1*15 appear to be associated with disease features and severity in Japanese UC patients.

Conclusion These results indicate that HLA-B*52 and DRB1*15 are not only associated with overall UC susceptibility, but also with the clinical phenotypes in Japanese patients.

Key words HLA; inflammatory bowel disease; Japanese; ulcerative colitis

Inflammatory bowel disease (IBD), an autoimmune disease (AD) of the gastrointestinal tract, consists of two clinical forms, Crohn's disease (CD) and ulcerative colitis (UC). IBD has a complex etiology involving multiple genetic and environmental factors.¹ The past three de-

acades have seen an exponential increase in the incidence and prevalence of both CD and UC in Japan.² Several genome-wide studies have confirmed that UC and CD are connected to chromosome 6p21.1-23, which has been named IBD3 and overlaps with the human leukocyte antigen (HLA) region.³⁻⁶

The human HLA region is highly polymorphic,⁷ some of which may predispose to particular ADs.⁸ Class-I molecules (HLA-A and -B) are recognized by receptors on CD8-positive suppressor T cells, while class-II molecules (HLA-DRB1) are recognized by receptors on CD4-positive helper T cells when attacking and eliminating foreign substances. Through this mechanism, HLA molecules are involved in not only the recognition of self or non-self, but also self-defense and pathogenesis of diseases. Previous studies have reported that HLA alleles were associated with UC susceptibility and phenotype. However, only a few reports evaluated the contribution of HLA-alleles to the clinical phenotype of Japanese UC patients.^{9, 10} In this study, whether HLA-alleles confer susceptibility to UC was confirmed, and whether HLA-alleles are associated with the clinical phenotypes in Japanese patients with UC was investigated.

SUBJECTS AND METHODS

Patients

A total of 45 Japanese patients [20 men, 25 women, mean age (range) 45.8 ± 14.5 (range 22–74) years] with UC from the Tottori University Hospital were included between January 2015 and December 2016. The diagnosis of UC in all subjects was made by gastroenterologists based on clinical, radiological, endoscopic, and histological features according to the 2010 criteria of the

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Abbreviations: AD, autoimmune disease; CD, Crohn's disease; CI, confidence interval; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; GI, gastrointestinal; HLA, human leukocyte antigen; IBD, Inflammatory bowel disease; NLR, neutrophil-lymphocyte ratio; OR, odds ratio; UC, ulcerative colitis; WBC, white blood cell

Japanese Research Committee of Inflammatory Bowel Disease.¹¹

Clinical characteristics

The following characteristics of the 45 patients were examined: sex, age at disease onset, smoking, disease extent, gastrointestinal (GI) complications and extraintestinal manifestations. The following laboratory data were also examined: WBC count, hemoglobin, platelets, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). The treatments administered to the patients were also analyzed. The data at diagnosis or the first visit were used in this study.

HLA typing

HLA allele typing was performed for the 45 UC patients (44 patients for HLA-DRB1) using PCR-sequence-specific oligonucleotide (BIOMEDICAL LABORATORIES, Tokyo, Japan). Frequencies of HLA alleles (HLA-B, DRB1) in the patients were compared with those in the previously reported Japanese controls.¹² Clinical characteristics were compared between the subgroups with or without the disease-associated HLA alleles. Approval was obtained from the Ethics Committee of the institute in accordance with the provisions of the World Medical Association's Declaration of Helsinki. All patients gave their informed consent to be involved in the protocol, which was approved by the Institutional Review Board of Tottori University (1701A175).

Statistical analysis

The chi-square test was used for categorical variables. Mann-Whitney's *U*-test was used to compare patients' ages at onset and laboratory data. *P*-values < 0.05 were considered significant. All statistical analyses were performed using Stat Flex (ver. 6.0; Artech, Osaka, Japan).

RESULTS

Patient and clinical characteristics

The characteristics of the patients with UC are shown in Table 1. The average age of the 45 UC patients was 45.8 ± 14.5 years; 25 patients (55.6%) were female. The average age of disease onset was 32.1 ± 13.7 years, and 34 patients (75.6%) were younger than 40 years at onset. Five patients had a family history of UC. Eight patients (17.8%) were ex- and current smokers. The most common disease extent was total colitis, followed by left-sided colitis and proctitis. Seven patients had a history of rectocolectomy for UC. No patients had a history of appendectomy.

Initial laboratory data showed the following: WBC count $8527 \pm 4117/\mu\text{L}$, hemoglobin 12.8 ± 2.0 g/dL,

Table 1. Clinical characteristics of 45 patients with UC

Male:Female (<i>n</i>)	20:25
Age at entry (y)	45.8 ± 14.5
Age at onset (y)	32.1 ± 13.7
≤ 40 y	34 (75.6%)
> 40 y	11 (24.4%)
Family history of UC	5 (11.1%)
Smoking habit	
Current smoker	3 (6.7%)
Ex-smoker	5 (11.1%)
Disease extent	
Proctitis	9 (20.0%)
Left-sided colitis	12 (26.7%)
Total colitis	24 (53.3%)
GI complications	4 (8.9%)
Extraintestinal manifestations	5 (11.1%)
Articular	1 (2.2%)
Dermatological	2 (4.4%)
Other	2 (4.4%)

Values are means \pm SD or numbers (percentage) unless indicated otherwise. GI, gastrointestinal; UC, ulcerative colitis; y, years.

Table 2. Laboratory data and treatments of 45 UC patients

Laboratory data	
WBC (μL)	8527 ± 4117
Hemoglobin (g/dL)	12.8 ± 2.0
Platelets ($\times 10^4/\text{mm}^3$)	31.0 ± 12.4
ESR (mm/hour)	31.0 ± 30.1
CRP (mg/dL)	1.2 ± 2.5
Treatment	
Corticosteroids	2 (4.4%)
Ever corticosteroids	32 (71.1%)
5-aminosalicylic acid	36 (80.0%)
Immunosuppressants	15 (33.3%)
Biologics	9 (20.0%)
Surgical treatment (rectocolectomy)	7 (15.6%)

Values are means \pm SD or numbers (percentage) unless indicated otherwise. CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; UC, ulcerative colitis; WBC, white blood cell.

platelets $31.0 \pm 12.4 \times 10^4/\text{mm}^3$, ESR 31.0 ± 30.1 mm/hour and CRP 1.2 ± 2.5 mg/dL (Table 2). A total of 32 patients (71.1%) had been treated with glucocorticoids, whereas immunosuppressive agents were prescribed in 15 patients (33.3%) and biologics in 9 patients (20.0%). Surgical treatment (rectocolectomy) was performed in 7 patients (15.6%), as described above.

HLA phenotype and allele frequencies

Table 3 summarizes the results of the HLA analysis. Only the alleles showing significant associations with UC are shown. As for the class I alleles, the frequency

Table 3. Association of HLA with Ulcerative colitis

	Patients			Controls		P value	Odds ratio
	n	PF	AF	n	AF		
HLA-B*52	45	62.2%	31.1%	1023	10.7%	< 0.0001	3.79
HLA-DRB1*15	44	68.2%	36.4%	898	17.4%	< 0.0001	2.72

AF, allele frequency; HLA, human leukocyte antigen; PF, phenotype frequency.

Table 4. Associations of the HLA-B*52 allele with clinical characteristics in 45 UC patients

	HLA-B*52 (+) (n = 28)	HLA-B*52 (-) (n = 17)	P value	Odds ratio	95% CI
Male:Female (n)	13:15	7:10	0.73	0.81	0.24–2.73
Age at onset (y)	34.1 ± 13.8	28.8 ± 13.4	0.19	–	–
> 40 y	9 (32.1%)	2 (11.8%)	0.12	3.55	0.71–17.79
Family history of UC	4 (14.3%)	1 (5.9%)	0.38	2.67	0.29–24.32
Smoking					
Never smoker	24 (85.7%)	13 (76.5%)	0.43	1.85	0.40–8.51
Current smoker	1 (3.6%)	2 (11.8%)	0.29	0.28	0.026–2.91
Former smoker	3 (10.7%)	2 (11.8%)	0.91	0.90	0.13–6.02
Disease extent					
Proctitis	6 (21.4%)	3 (17.6%)	0.76	1.27	0.27–5.92
Left-sided colitis	8 (28.6%)	4 (23.5%)	0.71	1.30	0.32–5.20
Total colitis	14 (50.0%)	10 (58.8%)	0.57	0.70	0.21–2.36
GI complications	1 (3.6%)	3 (17.6%)	0.11	0.17	0.020–1.47
Extraintestinal manifestations	1 (3.6%)	4 (25.5%)	0.039*	0.12	0.016–0.90
WBC (μL)	8421 ± 3655	8700 ± 4900	0.93	–	–
Hemoglobin (g/dL)	13.2 ± 2.0	12.1 ± 2.0	0.068	–	–
Platelets (× 10 ⁴ /mm ³)	30.2 ± 12.2	32.3 ± 13.0	0.36	–	–
ESR (mm/hour)	30.1 ± 30.5	32.5 ± 30.3	0.96	–	–
CRP (mg/dL)	1.39 ± 2.84	1.00 ± 2.03	0.62	–	–
5-aminosalicylic acid	24 (85.7%)	12 (70.6%)	0.22	2.50	0.58–10.77
Corticosteroids	1 (3.6%)	1 (5.9%)	0.72	0.59	0.036–9.86
Ever corticosteroids	20 (71.4%)	12 (70.6%)	0.95	1.04	0.28–3.93
Immunosuppressants	10 (35.7%)	5 (29.4%)	0.66	1.33	0.36–4.88
Biologics	6 (21.4%)	3 (17.6%)	0.76	1.27	0.27–5.92
Surgical treatment	2 (7.1%)	5 (29.4%)	0.046*	0.18	0.035–0.97

Values are means ± SD or numbers (percentage) unless indicated otherwise. **P* < 0.05. CI, confidence interval; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; GI, gastrointestinal; HLA, human leukocyte antigen; UC, ulcerative colitis; WBC, white blood cell; y, years.

of HLA-B*52 was significantly higher in patients than in controls [31.1% versus 10.7%, odds ratio (OR) 3.79, *P* < 0.0001]. As for the class II alleles, there was a significant positive association between UC and HLA-DRB1*15 (36.4% versus 17.4%, OR 2.72, *P* < 0.0001).

HLA typing and comparison with patient and clinical characteristics

The results of HLA typing in 45 UC patients were compared with patient and clinical characteristics. Patient and clinical characteristics were compared between the HLA-B* 52-positive and -negative patients, as shown in

Table 4. The incidence of extraintestinal manifestations was lower in HLA-B*52-positive UC patients (3.6%) than in HLA-B*52-negative UC patients (25.5%) (*P* = 0.039, OR 0.12). The frequency of a history of colectomy was lower in HLA-B*52-positive patients (7.1%) than in HLA-B*52-negative patients (29.4%) (*P* = 0.046, OR 0.18). The clinical characteristics were also compared between the HLA-DRB1*15-positive and -negative groups, as shown in Table 5. The WBC level was significantly higher in the HLA-DRB1*15-positive patients (9430 ± 4592/μL) than in the HLA-DRB1*15-negative patients (6729 ± 2160/μL). The lymphocyte level was sig-

Table 5. Associations of the HLA-DRB1*15 allele with clinical characteristics in 44 UC patients

	HLA-DRB1*15 (+)(n = 30)	HLA-DRB1*15 (-)(n = 14)	P value	Odds ratio	95% CI
Male:Female (n)	13:17	7:7	0.68	1.31	0.37–4.66
Age at onset (y)	34.5 ± 14.5	27.1 ± 11.3	0.11	–	–
≤ 16 y	4 (13.3%)	3 (21.4%)	0.49	0.56	0.11–2.91
17–40 y	16 (53.3%)	10 (71.4%)	0.26	0.46	0.12–1.76
> 40 y	10 (33.3%)	1 (7.1%)	0.061	6.50	0.91–46.29
Family history of UC	3 (10.0%)	1 (7.1%)	0.76	1.44	0.14–15.10
Smoking					
Never smoker	24 (80.0%)	12 (85.7%)	0.65	0.67	0.12–3.78
Current smoker	2 (6.7%)	1 (7.1%)	0.95	0.93	0.077–11.18
Former smoker	4 (13.3%)	1 (7.1%)	0.55	2.00	0.21–19.06
Disease extent					
Proctitis	4 (13.3%)	5 (35.7%)	0.087	0.28	0.064–1.20
Left-sided colitis	10 (33.3%)	2 (14.3%)	0.19	3.00	0.59–15.30
Total colitis	16 (53.3%)	7 (50.0%)	0.84	1.14	0.32–4.07
GI complications	1 (3.3%)	3 (21.4%)	0.052	0.13	0.016–1.02
Extraintestinal manifestations	2 (6.7%)	3 (21.4%)	0.15	0.26	0.042–1.63
WBC (μL)	9430 ± 4592	6729 ± 2160	0.049*	–	–
Neutrophil (μL)	6611 ± 4237	4468 ± 1849	0.15	–	–
Lymphocyte (μL)	1958 ± 745	1371 ± 428	0.006*	–	–
Monocyte (μL)	579 ± 244	625 ± 350	0.84	–	–
Eosinophil (μL)	263 ± 248	238 ± 252	0.45	–	–
Basophil (μL)	55 ± 85	36 ± 37	0.95	–	–
NLR	3.9 ± 3.5	3.7 ± 2.2	0.71	–	–
Hemoglobin (g/dL)	12.7 ± 2.0	13.1 ± 2.2	0.75	–	–
Platelets (× 10 ⁴ /mm ³)	31.6 ± 13.2	29.3 ± 11.1	0.88	–	–
ESR (mm/hour)	31.6 ± 29.6	30.7 ± 33.2	0.39	–	–
CRP (mg/dL)	1.32 ± 2.75	1.18 ± 2.21	0.94	–	–
5-aminosalicylic acid	25 (83.3%)	10 (71.4%)	0.36	2.00	0.45–8.87
Corticosteroids	1 (3.3%)	1 (7.1%)	0.57	0.45	0.028–7.25
Ever corticosteroids	24 (80.0%)	8 (57.1%)	0.11	3.00	0.77–11.66
Immunosuppressants	11 (36.7%)	4 (28.6%)	0.60	1.45	0.27–5.72
Biologics	7 (23.3%)	2 (14.3%)	0.49	1.83	0.33–10.03
Surgical treatment	3 (10.0%)	4 (28.6%)	0.12	0.28	0.056–1.38

Values are means ± SD or numbers (percentage) unless indicated otherwise. * $P < 0.05$. CI, confidence interval; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; GI, gastrointestinal; HLA, human leukocyte antigen; NLR, neutrophil–lymphocyte ratio; UC, ulcerative colitis; WBC, white blood cell.

nificantly higher in the HLA-DRB1*15-positive patients ($1958 \pm 745/\mu\text{L}$) than in the HLA-DRB1*15-negative patients ($1371 \pm 428/\mu\text{L}$), but no significant difference was observed with respect to neutrophil-lymphocyte ratio (NLR) between these two groups. No significant differences were found in the allele frequencies for other clinical background characteristics.

DISCUSSION

Recently, several linkage and association studies for IBD including UC were reported, and several susceptibility loci, including the HLA region, were identified.^{13–18} In the Japanese population, a previous study suggested an association between UC and HLA.^{19–23} In the present

study, whether HLA-A/B/DRB1 alleles confer susceptibility to UC or determine the clinical phenotype of UC was examined. It was found that HLA-B*52, and DRB1*15 contribute to susceptibility and the clinical phenotype in Japanese UC patients.

Previous association studies of HLA showed several associated alleles and haplotypes of HLA in patients with UC, although HLA typing differs by race and region.²⁴ Among the associated alleles, HLA-B*52 is well known to be strongly associated with UC in Japanese patients.^{10, 19–21} According to Arimura and others' 2014 meta-analysis of Japanese IBD genetics,¹⁴ HLA-DRB1*1502 was confirmed to have a positive association with UC. The results of the present study showed

that the frequencies of HLA-B*52 and DRB1*15 alleles in UC patients were higher than in previously reported Japanese controls.¹² Therefore, these results were compatible with the previously reported associations of HLA-B*5201 and DRB1*1502 with UC.^{10, 21, 25, 26}

Asakura and others reported significant associations between UC and HLA-B*52 and DRB1*15 haplotypes in Japanese populations.¹⁹ Furthermore, it is well known that a conserved haplotype of HLA A*24-B*52-DRB1*1502-DQB1*0601-DPBI*0901 is strongly associated with UC in the Japanese population.²⁰ Interestingly, HLA-A*24-B*52-DRB1*15 haplotype is a characteristic of the Japanese population (8.7%).²⁷ Thus, it appears that a genetic factor plays an important role in the pathogenesis of UC. Okada and others reported that a particular HLA haplotype, HLA-Cw*1202-B*5201-DRB1*1502, increases susceptibility to UC but reduces the risk for CD.¹³ Thus, use of the information about HLA alleles might contribute to improvements in the diagnostic approaches to UC and CD. In addition to prediction of UC susceptibility, HLA typing could assist in the discrimination of UC from CD. Moreover, the pathological relevance of the HLA haplotype requires further investigation. Indeed, advancing microbiome technology has recently demonstrated the interaction of HLA genotypes with microbiota as a representative environmental factor. For example, T cell responsiveness to specific microbiota depends on HLA genotypes.²⁸

In the present study, whether the HLA alleles were associated with the characteristics of UC was also investigated. In prior studies, it was reported that the HLA-B*52 allele is associated with younger age at onset of UC in Japan.¹⁰ In contrast, Matsumura and others reported that HLA-DRB1*09 was associated with older age of onset.⁹ However, similar results were not obtained in this study. Monsen and others raised the possibility that a major gene may be associated with a separate type of UC with more extensive involvement, younger age of onset, and more immunologic side effects, such as extraintestinal manifestations.^{29, 30} Surprisingly, in the present study, the HLA-B*52 allele frequency was lower in the group with extraintestinal manifestations and with a history of colectomy than in those without. These results may be due to the improvement of UC treatment, as with biologics. The WBC count was higher in the present HLA-DRB1*15-allele-positive group than in the HLA-DRB1*15-allele-negative group. Matsumura and others reported that HLA-DRB1*08 is associated with disease extent in Japanese UC patients,⁹ which might suggest an association of the HLA-DRB1 allele with more active and severe disease. Similarly, Futami and others reported that HLA-DRB1*1502 was

associated with disease severity in patients with UC.³¹ Thus, HLA typing is likely to be useful in the prediction of the disease course in patients with an established diagnosis of UC. Whereas, the lymphocyte count was higher in the HLA-DRB1*15-allele-positives than in the HLA-DRB1*15-allele-negatives, but no significant difference was observed with respect to NLR between these two groups. Recent studies demonstrated that NLR was higher in patients with active UC.^{32,33} NLR may be affected by the use of corticosteroids, hematological or neoplastic disorders, chronic liver disease, and clinical evidence of active infection. In this study, the data were obtained at diagnosis or the first visit. From this point of view, HLA-DRB1*15 may not affect the disease activity in UC.

Contradictory results on the association between HLA alleles and UC phenotypes compared with previous studies may be due to the limited size, methods used, clinical courses of the UC patients selected, and heterogeneity of the populations studied. Moreover, since the present study involved only a small number of patients and was limited by its retrospective nature, more cases are required in future studies to confirm the involvement of the HLA alleles in susceptibility and clinical characteristics of UC patients.

In conclusion, the results of the present study showed that HLA-B*52 and HLA-DRB1*15 are not only associated with overall UC susceptibility, but also with the clinical phenotypes in Japanese patients. These observations suggest that both susceptibility and the clinical characteristics of UC patients were controlled in part by the HLA-alleles. Further analysis of these phenotypes and subgroup analysis may elucidate how these alleles contribute to screening, susceptibility, and treatment in UC patients

The authors declare no conflict of interest.

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