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# Multicenter cooperative study of HLA class II alleles in Japanese patients with systemic lupus erythematosus

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**Abstract** The relation between HLA class II alleles and clinical findings were examined in Japanese patients with systemic lupus erythematosus (SLE). In 284 patients with multicenter SLE, HLA class II alleles were examined using the DNA typing method, and the results were compared with the clinical findings. The frequency of DRB1*0101 and DQB1*0501 significantly increased in male patients, and that of DRB1*0803 significantly increased in patients over 50 years of age. In relation to cutaneous manifestations, there were positive photosensitivity associations with DQA1*0101 and/or DQA1*0301, malar rash with DQA1*0101 and/or DRB1*0901, alopecia with DQA1*0101, skin ulcers with DRB1*0401, and oral ulcers with DQA1*0301. In addition, there were also positive associations of myalgia with DRB1*1406 and negative associations of aseptic bone necrosis with DQA1*0601, and hepatomegaly with DRB1*0405 and/or DQA1*0401. In relation to laboratory findings, there were positive associations of hemolytic anemia with DRB1*1402 and negative associations of leukopenia with DQA1*0601, lymphopenia with DQA1*0301, and proteinuria with DRB1*0901. Interestingly, DQA1*0101 and/or DQB1*0501 were significantly associated with WHO classification type II rather than type IV. In patients with SLE, some HLA types related to clinical or laboratory findings.

**Key words** SLE · HLA · Lupus nephritis

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## Introduction

Systemic lupus erythematosus (SLE) is a complex autoimmune disease characterized by clinical manifestations and autoantibody production. These characteristics are related to immunological abnormalities, genetic factors, or environmental factors. Among the genetic factors, the immune response gene and human leukocyte antigen (HLA) are most important. Previous studies which focused on HLA class II have revealed that HLA-DR3 and -DR2 were associated with SLE patients in Caucasians.[1] The association with DR2 was also recognized in other ethnic groups, such as blacks and Japanese.[2–4] This relationship to certain HLA class II alleles may suggest that these autoimmune reactions are mediated by genetically restricted antigen-specific T-helper cells interacting with specific HLA molecules,[5–7] and these HLA class II antigens may also influence susceptibility to several other autoimmune diseases.[8]

Serological methods have been used to examine HLA in early studies. However, some problems were encountered, such as the limitations of the reagents, and interference with HLA serotyping by autoantibodies or medication. Recently, DNA typing by polymerase chain reaction (PCR) has been used as the main method for HLA typing. Using these methods, we investigated HLA typing of Japanese SLE patients in a previous study, and the results showed an increased frequency of HLA-B39, DRB1*1501, DRB1*0101, and DQB1*0602.[9] This study also showed that

some HLA class II alleles related to specific clinical findings or laboratory data. However, it was possible that these relations were incidental results, since the number of cases was limited.

In order to resolve this problem, the present research examined HLA class II alleles using the DNA typing method in 284 Japanese patients with SLE in a multicenter study, and the results were related to clinical and laboratory findings, as previously described.

## Patients and methods

### Patients

Two hundred and eighty-four Japanese patients with SLE, from several different institutions, were used for this multicenter study. All of the patients fulfilled four or more diagnostic criteria for SLE.[10] Proteinuria was defined as a protein level of more than 0.5 g/day, renal failure with serum creatinine levels of more than 3.0 mg/dl, and anemia as a result of low hemoglobin levels (<11.0 g/dl) and/or a decrease in red blood cell count ($<380 \times 10^4$). Hemolytic anemia was defined as anemia, shortened erythrocyte survival, reticulocytosis, low haptogloburinemia, and a positive Coomb's test. Leukopenia or lymphopenia were defined as a white cell count of less than 4000, or a lymphocyte count of less than 1500. Renal histopathological findings were classified into six groups according to the World Health Organization (WHO) classification for lupus nephritis.[11]

After the relation between clinical findings and HLA typing had been examined, the clinical findings were used as data.

### DNA typing

DNA typing of the class II gene was performed by the PCR–SSOP method; all of the defined class II genes were studied. DNA was extracted from peripheral granulocytes from each subject, as described previously.[12] Genomic DNA was subjected to 30 cycles of PCR in a terminal cycler (Perkin Elmer Cetus, Norwalk, CA, USA) to amplify the second exon of the DRB1, DRB3, DRB4, DRB5, DQA1, DQB1, or DPB1 gene using thermostable DNA polymerase (Amply Taq, Perkin Elmer Cetus, obtained via Takara Shuzo Co. Ltd., Kyoto, Japan). The primers and SSOP were those used in the DNA component of the XI International HLA Workshop.[13] Conditions of the PCR and the procedures of hybridization with SSOP were as described previously.[12]

The presence of HLA typing was shown by allele positivity, and statistical analyses were performed as described below. Although there was a strong linkage disequilibrium, the results of each locus was analyzed separately.

### Statistical analysis

The strength of the statistical association between clinical findings and HLA class II alleles in the 284 patients with SLE was expressed by the relative risk (RR), as presented by Woolf,[14] and the statistical significance was examined by the $\chi^2$ test with Yates' continuity correction or Fisher's exact test. Linkage disequilibrium was estimated according to the method of Mittal.[15]

## Results

### Association with clinical findings

The results of the DNA typing of class II alleles performed in 284 patients were compared with the clinical findings. Some relations were recognized in age and sex. As shown in Table 1, the frequency of DRB1*0101 ($P < 0.05$) and DQB1*0501 ($P < 0.005$) was significantly increased in male patients, and, as shown in Table 2, the frequency of DRB1*0803 was significantly increased in patients over 50 years of age ($P < 0.025$),

There was significant relation between some of the cutaneous manifestations. As shown in Table 3, there were positive associations of photosensitivity with DQA1*0101 ($P < 0.05$) and/or DQA1*0301 ($P < 0.005$), malar rash with DQA1*0101 ($P < 0.025$) and/or DRB1*0901 ($P < 0.05$), alopecia with DQA1*0101, skin ulcers with DRB1*0401, and oral ulcers with DQA1*0301. On the other hand, a negative association of photosensitivity with DQB1*0402, and of alopecia with DRB1*0901, were also recognized.

The relations with other clinical findings were also examined, but they were significant in only a limited number of cases. As shown in Table 4, there were positive associations of myalgia with DRB1*1406 ($P < 0.005$), and negative associations of aseptic bone necrosis with DQA1*0601 ($P <$

**Table 1.** Class II alleles and sex

| | Male ($n = 20$) | Female ($n = 264$) |
|---|---|---|
| DRB1*0101 ($n = 12$) | 3 (15)* | 9 (3) |
| 1502 ($n = 28$) | 1 (5) | 27 (10) |
| DQA1*0101 ($n = 35$) | 6 (30) | 29 (11) |
| DQB1*0501 ($n = 25$) | 6 (30)** | 19 (7) |

Numbers in parentheses are percentages
The frequency of DRB1*0101 and DQB1*0501 was significantly higher in male patients
*$P < 0.05$; **$P < 0.005$

**Table 2.** Class II alleles and age of onset

| | Age at onset (years) | | |
|---|---|---|---|
| | ≦15 ($n = 7$) | 16–49 ($n = 238$) | ≧50 ($n = 39$) |
| DRB1*0803 ($n = 61$) | 1 (14) | 45 (19) | 15 (38)* |
| DQA1*0103 ($n = 112$) | 2 (29) | 90 (38) | 20 (51) |
| DQB1*0601 ($n = 105$) | 2 (29) | 83 (35) | 20 (51) |

Numbers in parentheses are percentages
The frequency of DRB1*0803 was significantly higher in the elderly
*$p < 0.025$

**Table 3.** Association of class II alleles with cutaneous manifestations

| | Cutaneous manifestations | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Photosensitivity | | Malar rash | | Alopecia | | Skin ulcers | | Oral ulcer | |
| Positive/negative | + | − | + | − | + | − | + | − | + | − |
| Cases | 107 | 177 | 187 | 94 | 107 | 177 | 17 | 267 | 104 | 180 |
| DRB1*0901 ($n = 67$) | 27 | 40 | 37* | 30 | 20 | 47** | 4 | 63 | 24 | 33 |
| DQA1*0101 ($n = 35$) | 19* | 16 | 30** | 5 | 22† | 13 | 1 | 34 | 16 | 19 |
| 0301 ($n = 52$) | 33† | 19 | 39 | 13 | 13 | 39 | 6 | 46 | 28** | 24 |
| 0501 ($n = 27$) | 11 | 16 | 16 | 11 | 8 | 19 | 3 | 24 | 12 | 15 |
| DQB1*0301 ($n = 43$) | 15 | 28 | 25 | 18 | 13 | 30 | 4 | 39 | 13 | 30 |
| 0401 ($n = 64$) | 4 | 23 | 40 | 24 | 26 | 38 | 8* | 56 | 27 | 37 |
| 0402 ($n = 26$) | 3 | 23*** | 18 | 8 | 12 | 14 | 0 | 26 | 11 | 15 |

The frequency of DRB1*0901 was significantly higher in patients with malar rash. That of DQA1*0101 was significantly higher in patients with photosensitivity, malar rash, and alopecia. DQA1*0301 was significantly higher in patients with photosensitivity and oral ulcers, and that of DQB1*0401 was significantly lower in patients with skin ulcers
* $P < 0.05$; ** $P < 0.025$; *** $P < 0.01$; † $P < 0.005$

**Table 4.** Association of class II alleles with clinical findings in SLE

| | Clinical findings | | | | | |
|---|---|---|---|---|---|---|
| | Myalgia | | Aseptic bone necrosis | | Hepatomegaly | |
| Positive/negative | + | − | + | − | + | − |
| Cases | $n = 13$ | $n = 271$ | $n = 15$ | $n = 269$ | $n = 11$ | $n = 273$ |
| DRB1*01 ($n = 18$) | 0 | 18 | 1 | 17 | 2 | 16 |
| 0405 ($n = 66$) | 5 | 61 | 5 | 61 | 6* | 60 |
| 1406 ($n = 3$) | 2** | 1 | 0 | 3 | 0 | 3 |
| DQA1*0301 ($n = 52$) | 2 | 50 | 1 | 51 | 2 | 50 |
| 0601 ($n = 11$) | 1 | 10 | 4** | 7 | 0 | 11 |
| DQB1*0301 ($n = 43$) | 2 | 41 | 4 | 39 | 0 | 43 |
| 0401 ($n = 64$) | 4 | 60 | 5 | 59 | 6* | 58 |
| 0402 ($n = 26$) | 2 | 24 | 1 | 25 | 1 | 25 |

The frequency of DQA1*0601 was significantly lower in patients with aseptic bone necrosis
* $P < 0.05$; ** $P < 0.005$

**Table 5.** Association of class II alleles with hematological findings

| | Clinical findings | | | | | |
|---|---|---|---|---|---|---|
| | Hemolytic anemia | | Leukopenia | | Lymphopenia | |
| Positive/negative | + | − | + | − | + | − |
| Cases | $n = 42$ | $n = 239$ | $n = 150$ | $n = 133$ | $n = 205$ | $n = 78$ |
| DRB1*1402 ($n = 2$) | 2* | 0 | 2 | 0 | 2 | 0 |
| DQA1*0301 ($n = 167$) | 25 | 142 | 81 | 86 | 109 | 58*** |
| 0601 ($n = 11$) | 0 | 11 | 1 | 10** | 5 | 6 |
| DQB1*0301 ($n = 43$) | 6 | 37 | 21 | 22 | 31 | 12 |
| 0601 ($n = 107$) | 14 | 93 | 57 | 50 | 82 | 25 |

The frequency of DQA1*0301 was significantly higher in patients with lymphopenia
* $P < 0.025$;** $P < 0.01$; *** $P < 0.005$

0.005), and of hepatomegaly with DRB1*0405 ($P < 0.005$) and/or DQB1*0401 ($P < 0.05$).

## Association with laboratory findings

There were some significant relations among hematological findings. As shown in Table 5, positive associations of hemolytic anemia with DRB1*1402 were observed ($P < 0.025$). There were also negative associations of leukopenia with DQA1*0601 ($P < 0.01$), and lymphopenia with DQA1*0301 ($P < 0.005$). There was no association of lupus erythematosus (LE) cells, anti-DNA antibodies, anti-U1-RNP antibodies, or anti-SS-A antibodies.

There were also significant findings among renal manifestations. Table 6 shows a negative association of proteinuria with DRB1*0901 ($P < 0.005$). The association with the WHO classification revealed further interesting results.

**Table 6.** Association of class II alleles with renal manifestations

| | Renal manifestation proteinuria | |
|---|---|---|
| Positive/negative | + | − |
| Cases | $n = 166$ | $n = 113$ |
| DRB1*0401 ($n = 7$) | 3 | 4 |
| 0405 ($n = 66$) | 38 | 28 |
| 1401 ($n = 13$) | 8 | 5 |
| 0901 ($n = 67$) | 28 | 39* |
| DQA1*03 ($n = 187$) | 104 | 83 |
| 0301 ($n = 53$) | 25 | 27 |
| DQB1*0401 ($n = 64$) | 38 | 26 |
| 0503 ($n = 13$) | 8 | 5 |

The frequency of DRB1*0901 was significantly lower in patients with proteinuria
* $P < 0.005$

**Table 7.** Association of class II alleles with renal histopathological findings

| Cases | WHO classification | | | | |
|---|---|---|---|---|---|
| | I $n = 7$ | II $n = 18$ | III $n = 8$ | IV $n = 26$ | V $n = 5$ |
| DRB1*0101 ($n = 1$) | 0 | 1 | 0 | 0 | 0 |
| 1502 ($n = 11$) | 1 | 1 | 1 | 6 | 2 |
| 0803 ($n = 12$) | 3 | 1 | 0 | 7 | 1 |
| 1001 ($n = 1$) | 0 | 1 | 0 | 0 | 0 |
| DQA1*0101 ($n = 10$) | 0 | 9* | 0 | 0 | 1 |
| 0103 ($n = 21$) | 4 | 2 | 1 | 12 | 2 |
| DQB1*0501 ($n = 7$) | 0 | 6* | 0 | 0 | 1 |
| 0601 ($n = 20$) | 4 | 2 | 1 | 11 | 2 |

The frequencies of DQA1*0101 and DQB1*0501 were significantly higher in patients with WHO type II alleles
I, normal; II, mesangial alteration; III, focal segmental GN; IV, diffuse proliferation GN; V, diffuse membranes GN
* $P < 0.01$ and $<0.005$ for classification II and IV, respectively

As shown in Table 7, DQA1*0101 ($P < 0.005$) and/or DQB1*0501 ($P < 0.01$) was significantly associated with WHO classification type II rather than type IV. Although there was no statistical significance, patients with WHO type IV were frequently associated with DQA1*0103 and/or DQB1*0601.

## Discussion

Recently, DNA typing with the PCR has become the main method of examining HLA. Our previous study,[9] which used this method in patients with SLE, showed the following results. First, the association of haplotypes HLA-DRB1*1501–DRB5*0101–DQA1*0102–DQB1*0602 with SLE was recognized. Second, there were some relations between HLA typing and clinical manifestations. The latter included the positive associations of photosensitivity with DRB1*0404 and/or DQB1*0401, malar rash with DPB1*0201, oral ulcers with DPB1*0901, alopecia with DPA1*0201, anemia with DRB1*1502, DRB5*0102, DPA1*0201, DPB1*0901, and DRB1*0410, and anti-U1-RNP antibody with DRB1*0410, and the negative association of lymphopenia with DRB3*0303, and of LE cells with DRB1*0901, DQA1*0301, and/or DQB1*0303. The relation between some HLA types and clinical manifestations may suggest that the heterogeneity of manifestations in SLE relate to the immune reaction mediated by the HLA molecule. However, since the number of patients with each manifestation was small, it may be possible that the results were accidental. Therefore, we re-examined this association by testing many patients in this multicenter study.

Unfortunately, the results did not confirm these associations. However, we found some different associations between HLA alleles and clinical or serological findings of SLE. For example, in sex and age, male patients had more frequent associations with DRB1*0101 and DQB1*0501, and elderly male patients with DRB1*0803, although the previous report did not show this result. As previously reported,[16] elderly or male patients with SLE had special clinical findings. Therefore, these results are interesting. In clinical manifestations, there were positive associations of photosensitivity with DQA1*0101 and/or DQA1*0301, malar rash with DRB1*0401 and/or DRB1*0901, and alopecia with DQA1*0101, although the previous report showed an association of photosensitivity with DRB1*0404 and/or DQB1*0401, malar rash with DPB1*0201, and alopecia with DPA1*0201. Thus, there were additional positive associations of skin ulcers with DRB1*0401 and of oral ulcers with DQA1*0301, although the previous report had shown an association of oral ulcers with DPB1*0901. There was also a negative association of aseptic bone necrosis with DQA1*0601, and this result supports the one in the previous study. As with the laboratory findings, there were positive associations of hemolytic anemia with DRB1*1402, and negative associations of lymphopenia with DQA1*0601, although the previous report showed negative associations of lymphopenia with DRB3*0303. Thus, although there were differences in HLA type, both the present and previous studies show that there are relations between some clinical findings and HLA DNA typing. It is difficult for us to determine whether these results relate to pathogenesis or not. Since our recent report showed that hemolytic anemia relates to Th1 cytokine,[17] the associations with hemolytic anemia may have some relation to the pathogenesis mediated by some antigen and cytokine. The association with lymphopenia is interesting when the relation to apoptosis is considered. However, to prove these relations is very difficult. We believe that other associations have no relation to pathogenesis, and that these associations may simply be coincidence, since we cannot find any factor which may be related to them.

On the other hand, some interesting findings were recognized in relation to nephropathy. In brief, there was a positive association of WHO class II HLA with DQA1*0101 and DQB1*0501. When some long-term follow-up patients were examined for HLA, WHO class II patients did not generally have nephropathy (unpublished data). On the other hand, WHO class IV patients showed a frequent association with DQA1*0103 and/or DQB1*0601, and patients with this type of HLA tended to develop nephropathy in

the long term, even though they did not have nephropathy at initial diagnosis (unpublished data). Furthermore, it is of interest that one patient with WHO type II nephropathy and DQA1*0103 and DQB1*0601 had progressed to WHO type IV in the second biopsy after 2 years. These results may suggest that HLA type relates to the severity of nephropathy. Although many previous studies have shown the relation between HLA type and the risk of nephropathy,[18] no study has shown a relation to WHO classification.

With respect to autoantibodies, although our previous reports showed an association between anti-DNA antibodies, anti-SS-A antibodies, anti-U1-RNP antibodies, and LE cells,[9] no associations between these factors were recognized in this study. Previous reports have also suggested an association with some autoantibodies, but the number of such cases is limited and the relative risk was not very high. Therefore, we believe that there is no direct association between HLA and autoantibody production, although we cannot neglect the relation between HLA and the epitopes of autoantigens.

This study and our previous one have shown various associations between HLA class and clinical findings. As previously stated, we do not yet fully understand the clinical or pathogenic significance of these results. However, we can never neglect any relation to the heterogeneity of SLE. Although it is well known that the disease is never established by one single factor, it is possible that HLA contributes to the onset or the development of particular types of disease as one of many factors.

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