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CASE REPORT

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# Human leukocyte antigen haplotype and autoantibodies of a family with systemic lupus erythematosus

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**Abstract** A search for HLA haplotypes of a family of five indicated that four members had the same haplotype. Systemic lupus erythematosus (SLE) had already developed in three of these four people. SLE has now developed in the remaining person, and the result is that all the members of the family having the same haplotype will develop SLE. Regarding these four SLE patients, the types of autoantibodies and the symptoms were different in each person, so the idea that this haplotype is strongly related to the onset of SLE but minimally related to the symptoms was suggested.

**Key words** Autoantibody · Family study · Human leukocyte antigen (HLA)-haplotype · Susceptibility · Systemic lupus erythematosus (SLE)

## Introduction

Systemic lupus erythematosus (SLE) is a representative autoimmune disease characterized by various immune abnormalities such as hypocomplementemia, hematological disorder, and multiple autoantibodies. The pathogenesis of SLE still remains unclear, although many arguments favor environmental factors, while others suggest genetic origins. Based on serological studies, associations between certain human leukocyte antigens (HLA) and SLE have long been suggested. Studies of MHC class II alleles have shown that the HLA haplotypes are associated with SLE,[1] although their roles in SLE remain controversial.

Important production of autoantibodies occurs in the course of SLE. Antinuclear antibodies (ANA), especially anti-Sm antibody (anti-Sm), and anti-double-stranded DNA antibodies (anti-dsDNA), are specific to this disease. Anti-Ro antibody (anti-SS-A) is also frequently present, although it is also found in other diseases such as Sjögren's syndrome. It has been suggested that these autoantibodies are associated with certain HLA antigens.[1]

We previously reported the case of a family which contained three SLE patients with identical HLA haplotype.[2] Here, we report the recent onset of SLE in another member of this family, and consider the association between the clinical features and the HLA haplotypes of this family.

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## Materials and methods

### Patients

Four family members who were diagnosed with SLE were followed in our hospital. Figure 1 shows the family tree. Of the eight family members, five (a 48-year-old woman, patient (Pt)-2; a 51-year-old man, her brother, Pt-1; a 26-year-old woman, her daughter, Pt-3; a 24-year-old man, her older son, Pt-4; a 22-year-old man, her younger son, Pt-5) agreed to participate in our study, two had died, and we were unable to contact one. Informed consent was obtained for participation in this study in accordance with the provisions of the Declaration of Helsinki. SLE was diagnosed according to the American College of Rheumatology 1982 revised criteria for SLE.[3]

### Laboratory investigations

All available members of the family were studied for autoantibodies, including ANA (by indirect immunofluorescence using against Hep-2 cells, positive at a dilution of $>1:80$), anti-Sm, anti-U1-RNP antibody (anti-RNP) (by enzyme-linked immunosorbent assay, positive at a dilution of $>1:100$), anti-dsDNA (by enzyme-based immunoassay, positive at $>40$ IU/ml), anti-SS-A, and anti-La antibody (anti-SS-B) (by double immunodiffusion and counter-

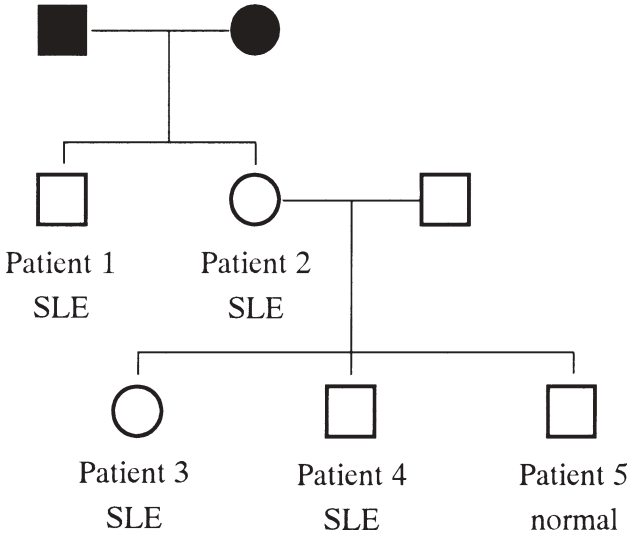


**Fig. 1.** Family pedigree. *Squares*, male; *circles*, female; *black symbols*, dead. *SLE*, systemic lupus erythematosus

immunoelectrophoresis using calf thymus extracts as the antigen source).

HLA typing for class I (A, B, and Cw) antigens was performed as described elsewhere.[4] HLA class II DNA typing (DRB1, DQB1, and DPB1) was performed by polymerase chain reaction–restriction fragment length polymorphism analysis (PCR–RFLP) with a Smitest HLA DNA typing system (Sumitomo Metal, Tokyo, Japan) according to the manufacturer's instructions.

## Results

Table 1 shows the clinical features as well as the results of studies for autoantibodies and of HLA typing of the members of the family. A 48-year-old woman (patient (Pt)-2), a 51-year-old man (her brother, Pt-1), a 26-year-old woman (her daughter, Pt-3), and a 24-year-old man (her son, Pt-4) each had a malar rash and polyarthritis. In addition, Pt-1 and Pt-3 had nephrotic syndrome, and Pt-4 had hemolytic anemia and hematological disorder (luekocytopenia and lymphopenia). Pt-3 had a convulsion in his clinical course. All the patients with SLE were positive for ANA and anti-SS-A. Pt-1 and Pt-3, who had nephrotic syndrome, were positive for anti-dsDNA, and all except Pt-1 had positive LE cells. These four patients had anti-SS-A, but they did not have sicca syndrome.

HLA typing of this family revealed that the four members who had SLE possessed the identical HLA haplotype A11 / DRB1 *1501 / DQB1 *0602 / DPB1 *0501.

## Discussion

Serological studies have long suggested an association between certain HLA antigens and many autoimmune diseases. With the development of the PCR technique, the identification of HLA alleles at the DNA level has become possible. This has allowed a more precise determination of the epitopes exhibiting a strong association with autoimmune disease.

In this study, we found that the family examined included four SLE patients who had identical HLA haplotype A11 / DRB1 *1501 / DQB1 *0601 / DPB1 *0501. We previously reported that three members of this family (Pt-1, Pt-2, and Pt-4) had SLE.[2] At that time, although Pt-3 possessed the identical haplotype, she did not meet the criteria for SLE. However, she has recently been diagnosed with SLE on the basis of the following criteria: malar rash, arthritis, nephrotic syndrome, ANA positivity, and immunological disorder (anti-dsDNA positivity, and positivity for LE cell preparation). Since HLA allele frequencies differ considerably among races, susceptibilities conferred by alleles to the development of diseases or autoantibodies have been studied in different races. Dong et al.[5] reported a positive association of SLE with DRB1 *1501, DRB5 *0101, and DQB1 *0602 in Japanese patients. DRB1 *1501 and DRB5 *0101 are in strong linkage disequilibrium with the DQA1 *0101 / DQB1 *0602 haplotype in the Japanese population. Thus, the DRB1 *1501 / DRB5 *0101 / DQA1 *0102 / DQB1 *0602 haplotype may confer susceptibility to the development of SLE.[5] Recently, it has been reported that the co-occurrence of HLA-DQA1 *0101 and -DRB1 *1501 amplifies the risk of lupus nephritis in Italian SLE patients.[6] Furthermore, Graham et al.[7] has reported that the haplotype containing DRB1 *1501 and DRB1 *0801 links to an ~500-kb region associated with SLE.

Although the production of anti-RNP and anti-Sm occurs frequently in SLE, these four SLE patients did not have these autoantibodies. However, they were positive for anti-SS-A. Associations between certain HLA alleles and autoantibodies (anti-RNP and anti-Sm) have been reported. In the Japanese population in particular, a significant genetic linkage between HLA DQB1 *0302 and anti-RNP has been shown to exist.[8] Miyagawa et al.[9] reported that the haplotype DRB1 *08032 / DQA1 *0103 / DQB1 *0601 exhibited associations with anti-SS-B (with anti-SS-A together), and in their study, all the Japanese patients who were positive for anti-SS-A possessed at least one DQB1 *06 or *03 subtypes that shared the same amino acid residues at positions 71–77 of the DQB1 chain. Pt-1 and Pt-3 were positive for anti-dsDNA and lupus nephritis (nephrotic syndrome). Anti-dsDNA in particular is considered to be an important mediator in the pathogenesis of lupus nephritis. A rise in titer of anti-dsDNA often precedes the onset or exacerbation of renal disease.[10] It has been reported that the combination of high anti-dsDNA and low C4 serum levels correlated with the absence of HLA-DR5 in the Greek population.[11] However, there has been no report concerning that genetic linkage

**Table 1.** Clinical information and HLA type of the family members

| Patient | No. | | 1 | | 2 | | 3 | | 4 | | 5 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sex / age | | M / 5 | | F / 48 | | F / 26 | | M / 24 | | M / 22 | |
| | Diagnosis | | SLE | | SLE | | SLE | | SLE | | Normal | |
| Haplotype | A | | 11 | 2 | 11 | 2 | 11 | 26 | 11 | 26 | 26 | 2 |
| | B | | 61 | – | 51 | – | 51 | 67 | 51 | 67 | 67 | – |
| | Cw | | 3 | – | 7 | – | 7 | – | 7 | – | – | – |
| | DRB1 | | *1501 | *0410 | *1501 | *0410 | *1501 | *1401 | *1501 | *1401 | *1401 | *0410 |
| | DQB1 | | *0602 | *0402 | *0602 | *0402 | *0602 | *0502 | *0602 | *0502 | *0502 | *0402 |
| | DPB1 | | *0501 | *0501 | *0501 | *0501 | *0501 | *0202 | *0501 | *0202 | *0202 | *0501 |
| Clinical features | | | | | | | | | | | | |
| | Malar rash | | + | | + | | + | | + | | – | |
| | Arthritis | | + | | + | | + | | + | | – | |
| | Renal disorder | | Nephritic synd. | | – | | Nephritic synd. | | – | | – | |
| | Neurological disorder | | – | | – | | – | | Seizures | | – | |
| | Hematological disorder | | – | | – | | – | | Leukopenia/ lymphopenia | | – | |
| | Immunological disorder | | dsDNA | | Positive LE cell | | dsDNA/positive | | Positive LE cell LE cell | | – | |
| Autoantibodies | | | | | | | | | | | | |
| | ANA | [normal range: <20] | ×640 | | ×320 | | ×640 | | ×160 | | <×20 | |
| | Anti-ssDNA (AU/ml) | [<25] | 65 | | >800 | | 124 | | 56 | | 0 | |
| | Anti-dsDNA (AU/ml) | [<25] | 325 | | 8 | | 370 | | 16 | | 0 | |
| | Anti-RNP | | – | | – | | – | | – | | – | |
| | Anti-Sm | | – | | – | | – | | – | | – | |
| | Anti-SS-A | | + | | + | | + | | + | | – | |
| | Anti-SS-B | | – | | – | | – | | – | | – | |
| CBC | WBC (/μl) [lymph (/μl)] | [30~85] | 4100 [1800] | | 4500 [1700] | | 5200 [1600] | | 2800 [600] | | 5800 [2000] | |
| | Hb (mg/dl) | [M: 12.9~17.2, F: 11.3~15.4] | 13.2 | | 15.1 | | 12.9 | | 13.0 | | 16.9 | |
| | Plt (×10^4/μl) | [14.0~34.0] | 15.0 | | 22.8 | | 18.8 | | 14.3 | | 29.1 | |
| s-ALB (g/dl) | | | 2.8 | | 4.2 | | 2.6 | | 3.9 | | 4.7 | |
| Complement | CH50 (U/ml) | [30~50] | <10 | | 20.2 | | <10 | | 29.2 | | 40 | |
| | C3 (ng/ml) | [M: 50~76, F: 51~71] | 28 | | 78 | | 16 | | 31 | | 92 | |
| | C4 (ng/ml) | [M: 17~29, F: 16~28] | 2 | | 8 | | 4 | | 11 | | 22 | |
| Urinary | Urinary protein | | 3+ | | – | | 3+ | | – | | – | |
| | Occult blood | | 3+ | | – | | + | | +/– | | – | |
| | Urinary WBC | | + | | – | | 2+ | | – | | – | |
| | Urinary cast | | Granular, tubular, red cell | | – | | Granular, red cell | | – | | – | |
| Vitamin D receptor genotype (*Bsm* I) | | | bb | | Bb | | bb | | Bb | | Bb | |

between a certain HLA allele and anti-dsDNA in the Japanese population.

Interestingly, Pt-3 and Pt-4 had the same haplotypes, but their clinical features were quite different. Recently, we reported that the *bb* genotype, an allelic variant of the gene encoding vitamin D receptor (VDR) detected by *Bsm* I, was associated with lupus nephritis.[12] Pt-1 and Pt-3, who had lupus nephritis, possessed the *bb* genotype, and Pt-2, -4 (SLE without renal dysfunction) and Pt-5 (normal) possessed the Bb genotype. 1,25(OH)2D3 is an immunosuppressive hormone that is reported to negatively regulate IL-12 production by the downregulation of NF-κB activation and binding to the p40-κB sequence. 1,25(OH)2D3 binds to a nuclear receptor, VDR, and this receptor can heterodimerize with a nuclear receptor bound to 9-*cis* retinoic acid, termed retinoid X receptor (RXR). It is reported that VDR–RXR heterodimer repression of IL-2 gene transcription acts via blocking NFATp/AP1 complex formation. The differentiation of VDR genotypes may trigger the breakdown of cytokine relationships directly or indirectly, and may be associated with the pathogenesis of lupus nephritis. Thus, these results suggest that the symptoms and signs of SLE might not be correlated with HLA status. As noted above, anti-dsDNAs are considered to be important mediators of lupus nephritis. So in other words, it could be said that being positive for anti-dsDNA might not be correlated with HLA status. At the very least, other symptoms of neurological and hematological disorders in Pt-3 and Pt-4 might not be correlated with HLA status. However, this family study suggested that the HLA allele and/or haplotype might control the development of SLE and the occurrence of certain antibodies that occur frequently in SLE.

## References


1. Tan FK, Arnett FC. The genetics of lupus. Curr Opinion Rheumatol 1998;10:399–408.
2. Kagawa H, Nomura S, Ozaki Y, Uehira K, Matsuzaki T, Ishida T, et al. HLA typing of a family with systemic lupus erythematosus [letter]. Autoimmunity 1996;24:265–6.
3. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271–7.
4. Terasaki PI, Bernoco D, Park MS, Ozturk G, Iwaki Y. Microdroplet testing for HLA-A, -B, -C, and -D antigens. Phillip Levine Award Lecture. Am J Clin Pathol 1978;69:103–20.
5. Dong RP, Kimura A, Hashimoto H, Akizuki M, Nishimura Y, Sasazuki T. Difference in HLA-linked genetic background between mixed connective tissue disease and systemic lupus erythematosus. Tissue Antigens 1993;41:20–5.
6. Marchini M, Antonioli R, Lleo A, Barili M, Caronni M, Origgi L, et al. HLA class II antigens associated with lupus nephritis in Italian SLE patients. Hum Immunol 2003;64:462–8.
7. Graham RR, Ortmann WA, Langefeld CD, Jawaheer D, Selby SA, Rodine PR, et al. Visualizing human leukocyte antigen class II risk haplotypes in human systemic lupus erythematosus. Am J Hum Genet 2002;71:543–53.
8. Kuwana M, Okano Y, Kaburaki J, Tsuji K, Inoko H. Major histocompatibility complex class II gene associations with anti-U1 small nuclear ribonucleoprotein antibody. Relationship to immunoreactivity with individual constituent proteins. Arthritis Rheum 1995; 38:396–405.
9. Miyagawa S, Shinohara K, Nakajima M, Kidoguchi K, Fujita T, Fukumoto T, et al. Polymorphisms of HLA class II genes and autoimmune responses to Ro/SS-A-La/SS-B among Japanese subjects. Arthritis Rheum 1998;41:927–34.
10. ter Borg EJ, Horst G, Hummel EJ, Limburg PC, Kallenberg CG. Measurement of increases in anti-double-stranded DNA antibody levels as a predictor of disease exacerbation in systemic lupus erythematosus. A long-term, prospective study. Arthritis Rheum 1990;33:634–43.
11. Andonopoulos AP, Papasteriades CA, Drosos AA, Dimou GS, Moutsopoulos HM. HLA alloantigens in Greek patients with systemic lupus erythematosus. Clin Exp Rheumatol 1990;8:47–50.
12. Ozaki Y, Nomura S, Nagahama M, Yoshimura C, Kagawa H, Fukuhara S. Vitamin D receptor genotype and renal disorder in Japanese patients with systemic lupus erythematosus. Nephron 2000;85:86–91.