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EXTENDED REPORT

# Positive association between *STAT4* polymorphisms and polymyositis/dermatomyositis in a Japanese population

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## ABSTRACT

**Objectives** To investigate associations between signal transducer and activator of transcription 4 (STAT4), one of the most commonly acknowledged genes for the risk of multiple autoimmune diseases, with susceptibility to adult-onset polymyositis/dermatomyositis among Japanese individuals.

**Methods** A single nucleotide polymorphism of STAT4, rs7574865, was genotyped using TaqMan assay in 1143 Japanese individuals. The first set comprised 138 polymyositis/dermatomyositis patients and 289 controls and the second set comprised 322 patients and 394 controls. 460 patients (273 polymyositis and 187 dermatomyositis patients) and 683 controls were genotyped.

**Results** rs7574865T conferred a risk of polymyositis/dermatomyositis with an OR of 1.37 (95% CI 1.16 to 1.64; $p=4x10^{-4}$; $p_{corr}=0.0012$). Both polymyositis and dermatomyositis exhibited high associations with the rs7574865T allele (polymyositis: OR=1.36, 95% CI 1.11 to 1.67; $p=0.0039$; $p_{corr}=0.012$; dermatomyositis: OR=1.40, 95% CI 1.10 to 1.78; $p=0.0054$; $p_{corr}=0.016$). The association between this STAT4 polymorphism and interstitial lung disease (ILD) was also investigated in the first set of polymyositis/dermatomyositis patients (n=138); those with ILD (n=79) bore rs7574865T more frequently compared with controls (OR 1.59, 95% CI 1.10 to 2.28; $p=0.013$; $p_{corr}=0.039$).

**Conclusion** This is the first study to show a positive association between a STAT4 polymorphism and polymyositis/dermatomyositis, suggesting that polymyositis/dermatomyositis shares a gene commonly associated with the risk of other autoimmune diseases.

Idiopathic inflammatory myopathies (IIM) are a heterogeneous group of diseases that affect skeletal muscles. Their common clinical feature is muscle weakness, and muscle biopsies typically show inflammatory cell infiltrates. IIM are clinically subdivided into several subgroups, including polymyositis, dermatomyositis, inclusion body myositis, myositis overlapping with another connective tissue disease (CTD), and cancer-associated myositis. Although the pathogenesis of IIM remains unclear, some environmental factors, such as viral infections, might trigger disease onset in genetically susceptible individuals, as is often the case with other autoimmune diseases.

Several studies have attempted to clarify the contributions of genetic factors for IIM susceptibility. Among possible candidate genes, major histocompatibility complex (human leucocyte antigen (HLA)) genes have been investigated most frequently.[1] In North American Caucasian patients, HLA alleles of the 8.1 ancestral haplotype (particularly HLA-B*0801 and DRB1*0301) are the principal HLA risk loci.[2] Among Japanese, HLA-DRB1*0803 was found to be associated with IIM and anti-aminoacyl-tRNA synthetase antibody.[3] Several genes outside the HLA regions, including the proinflammatory cytokines tumour necrosis factor alpha, interleukin (IL)-1α, IL-1β and interferon (IFN) γ, and an immunoglobulin gene[4–7] were found to be associated with specific IIM subgroups, particularly juvenile dermatomyositis.

However, because these diseases are rare and there is a broad spectrum of disease entities, genetic risk factors for IIM have not been thoroughly investigated. A functional variant of the protein tyrosine phosphatase N22 gene (*PTPN22*), an R620W polymorphism, was recently found to be associated with adult and juvenile IIM in British Caucasian patients.[8] This suggested that IIM might share a common genetic background with other autoimmune diseases.

Most susceptibility genes common to autoimmune diseases were originally identified in systemic lupus erythematosus (SLE) patients using genome-wide association studies. Among these, the following genes contributed most prominently: IFN regulatory factor 5 (*IRF5*), signal transducer and activator of transcription 4 (*STAT4*), *PTPN22*, B-lymphoid tyrosine kinase (*BLK*), B-cell scaffold protein with ankyrin repeats (*BANK1*) and tumour necrosis factor alpha-induced protein 3 (*TNFAIP3*). Their involvement has been replicated in different ethnic groups.[9] Furthermore, these genes were also found to be associated with the risk of several other autoimmune diseases including rheumatoid arthritis (RA),[10] systemic sclerosis (SSc),[11 12] and type I diabetes mellitus.[13]

The present study is the first to investigate the possible involvement of *STAT4*, the best established gene for susceptibility to autoimmune diseases across different ethnic groups, in the susceptibility to adult-onset polymyositis/dermatomyositis among Japanese individuals.

## METHODS

### Subjects

We enrolled polymyositis or dermatomyositis patients who were 18 years or older at disease onset and who had probable or definite myositis based on

**Table 1** Associations between *STAT4* rs7574865 and polymyositis/dermatomyositis

| | T allele | Allelic association | | | T/T genotype | Genotype association | | |
|---|---|---|---|---|---|---|---|---|
| Subjects (n) | (frequency) | OR (95% CI) | p Value | Corrected p value | (frequency) | OR (95% CI) | p Value | Corrected p value |
| First set of study | | | | | | | | |
| Polymyositis (46) | 32 (0.35) | ND | 0.47 | – | 8 (0.17) | ND | 0.11 | – |
| Dermatomyositis (92) | 74 (0.40) | 1.48 (1.06 to 2.10) | 0.025 | – | 18 (0.19) | 2.36 (1.23 to 4.52) | 0.015 | – |
| Controls (289) | 180 (0.31) | | | – | 27 (0.093) | | | |
| Second set of study | | | | | | | | |
| Polymyositis (227) | 180 (0.40) | 1.37 (1.08 to 1.74) | 0.011 | – | 41 (0.18) | 1.71 (1.08 to 2.71) | 0.029 | – |
| Dermatomyositis (95) | 75 (0.39) | ND | 0.072 | – | 16 (0.17) | ND | 0.17 | – |
| Controls (394) | 255 (0.32) | | | | 45 (0.11) | | | |
| Polymyositis + dermatomyositis (460) | 360 (0.39) | 1.37 (1.16 to 1.64) | $4.0\times10^{-4}$ | 0.0012 | 83 (0.18) | 1.87 (1.33 to 2.62) | $3.9\times10^{-4}$ | 0.0012 |
| Polymyositis (273) | 212 (0.39) | 1.36 (1.11 to 1.67) | 0.0039 | 0.012 | 49 (0.18) | 1.86 (1.25 to 2.75 | 0.0025 | 0.0075 |
| Dermatomyositis (187) | 148 (0.40) | 1.40 (1.10 to 1.78) | 0.0054 | 0.016 | 34 (0.18) | 1.88 (1.21 to 2.94) | 0.0076 | 0.023 |
| Total controls (683) | 435 (0.32) | | | | 72 (0.11) | | | |

ND, not determined; STAT4, signal transducer and activator of transcription 4.

the criteria of Bohan and Peter.[14] All patients underwent muscle biopsy. For our study group, dermatomyositis patients included those with clinically defined amyopathic dermatomyositis who fulfilled the traditional criteria of Sontheimer.[15] We excluded patients with myositis overlapping with other CTD, who met either the following published criteria (American College of Rheumatology (ACR) criteria for systemic sclerosis,[16] ACR criteria for systemic lupus erythematosus,[17] ACR criteria for rheumatoid arthritis[18] and American and European consensus criteria for Sjögren's syndrome)[19] or the criteria for mixed CTD by Sharp *et al*.[20] Inclusion body myositis is much less prevalent among Japanese than among European individuals, and was excluded on the basis of careful pathological examinations, clinical features, age of onset and response to immunosuppressive therapy. Patients with inherited, metabolic, or infectious myopathies, or muscle diseases caused by other factors were systematically excluded.

Polymyositis/dermatomyositis patients were recruited from two different institutions. For the first set for analysis, 138 patients (46 polymyositis patients and 92 dermatomyositis patients) were recruited from the Institute of Rheumatology, Tokyo Women's Medical University (TWMU), Tokyo, Japan, along with 289 healthy unrelated Japanese subjects as controls (60.4% women; mean age 42.3±12.3 years). For the second set for analysis, 322 patients (227 polymyositis patients and 95 dermatomyositis patients) were recruited from the National Center of Neurology and Psychiatry (NCNP), Kodaira, Tokyo, Japan, along with 331 control subjects (54.9% women; mean age 36.0±10.9 years).

Finally, a total of 460 adult-onset polymyositis/dermatomyositis patients (69.8% women) was enrolled, including 273 polymyositis patients (68.8% women) and 187 dermatomyositis patients (71.1% women). These included five polymyositis patients and 13 dermatomyositis patients with malignancies. The mean ages for polymyositis patients and dermatomyositis patients were 51.2±16.9 and 52.1±16.7 years, respectively. The combined control group included 683 subjects (57.1% women; mean age 38.6±11.9 years). All patients and controls were Japanese individuals.

For a subanalysis regarding the association between *STAT4* polymorphisms and the presence or absence of interstitial lung disease (ILD), 138 polymyositis/dermatomyositis patients recruited from TWMU were evaluated. Of these patients, data on ILD for six patients were missing; therefore, 132 polymyositis/dermatomyositis patients (43 polymyositis patients and 89 dermatomyositis patients) were investigated and their allele and genotype frequencies were compared with those of the 289 control subjects who were included in the first study set. The presence of ILD was confirmed or excluded by CT, high-resolution CT, if available and spirometry.

This study was reviewed and approved by the research ethics committees of TWMU and NCNP.

### Selection of single nucleotide polymorphisms

To date, rs7574865 of *STAT4* and related single nucleotide polymorphisms (SNP) have shown the strongest associations with autoimmune disease susceptibility. Therefore, we investigated rs7574865 and rs11889341, which are in strong linkage disequilibrium.

### Genotyping

Genotyping for each SNP site was performed using the TaqMan fluorogenic 5′ nuclease assay according to the manufacturer's instructions (Applied Biosystems, Tokyo, Japan). Endpoint fluorescence readings were made with an ABI Prism 7900 HT sequence detection system (Applied Biosystems).

### Statistical analysis

Association analysis used $\chi^2$ tests for 2×2 contingency tables. For the association analysis between *STAT4* polymorphisms and the three clinical subsets (all polymyositis/dermatomyositis patients, polymyositis patients and dermatomyositis patients vs control subjects); Bonferroni's correction was applied. Corrected p values ($p_{corr}$) were calculated by multiplying the p values by the results of these three comparisons. OR and 95% CI were also determined. For the subanalysis regarding the association study for *STAT4* polymorphisms and the presence of ILD, $p_{corr}$ values were also calculated by multiplying p values by the results of the following three comparisons: all polymyositis/dermatomyositis patients, those with ILD and those without ILD versus control subjects.

## RESULTS

### *STAT4* polymorphisms and polymyositis/dermatomyositis susceptibility

Because the patients in the first set for analysis were recruited from the Department of Rheumatology, whereas those in the second set for analysis were from neurology, the patient compositions in

**Table 2** Associations between *STAT4* rs7574865 and interstitial lung disease in the first polymyositis/dermatomyositis set

| | T allele | Allelic association | | | T/T genotype | Genotype association | | |
|---|---|---|---|---|---|---|---|---|
| Subjects (n) | (frequency) | OR (95% CI) | p Value | Corrected p value | (frequency) | OR (95% CI) | p Value | Corrected p value |
| Polymyositis + dermatomyositis (138*) | 106 (0.38) | 1.38 (1.02 to 1.86) | 0.037 | 0.11 | 26 (0.19) | 2.25 (1.26 to 4.03) | 0.0074 | 0.022 |
| Polymyositis + dermatomyositis with ILD (79) | 66 (0.41) | 1.59 (1.10 to 2.28) | 0.013 | 0.039 | 16 (0.20) | 2.46 (1.25 to 4.84) | 0.016 | 0.048 |
| Polymyositis + dermatomyositis without ILD (53) | 36 (0.34) | ND | 0.57 | ND | 9 (0.17) | ND | 0.13 | ND |
| Controls (289) | 180 (0.31) | | | | 27 (0.093) | | | |

*Among 138 patients in the first set of polymyositis/dermatomyositis patients, data for six patients were missing.
ILD, interstitial lung disease; ND, not determined; STAT4, signal transducer and activator of transcription 4.

these two groups were different: the first set included predominantly dermatomyositis patients (polymyositis:dermatomyositis, 1:2), whereas the second set included predominantly polymyositis patients (polymyositis:dermatomyositis, 2.4:1). Therefore, the polymyositis and dermatomyositis patients in each set were separately compared with their corresponding control groups. Finally, all patients and control subjects were combined; the risk allele frequencies among polymyositis/dermatomyositis, polymyositis and dermatomyositis patients were compared with those among the combined control subjects (table 1), from which corrected p values were calculated.

In the first set for analysis, only dermatomyositis patients showed higher rs7574865T allele and T/T genotype frequencies compared with those in the first set of control subjects (rs7574865T allele: OR 1.48, 95% CI 1.06 to 2.10; p=0.025; T/T genotype: OR 2.36, 95% CI 1.23 to 4.52; p=0.015). In the second study set, increased T allele and T/T genotype frequencies were observed only for polymyositis patients compared with those in the second set of control subjects (rs7574865T allele: OR 1.37, 95% CI 1.08 to 1.74; p=0.011; T/T genotype: OR 1.71, 95% CI 1.08 to 2.71; p=0.029).

When the data for the first and second sets of patients were combined, the risk allele frequencies achieved statistically significant levels for both polymyositis patients (OR 1.36, 95% CI 1.11 to 1.67; p=0.0039; $p_{corr}$=0.012) and dermatomyositis patients (OR 1.40, 95% CI 1.10 to 1.78; p=0.0054; $p_{corr}$=0.016) compared with those of the combined control subjects. The comparison between all polymyositis/dermatomyositis patients and the control subjects gave the lowest p values (rs7574865T allele: OR 1.37, 95% CI 1.16 to 1.64; p=4 ($10^{-4}$ $p_{corr}$=0.0012; T/T genotype: OR 1.87, 95% CI 1.33 to 2.62; p=3.9 ($10^{-4}$; $p_{corr}$=0.0012).

Because of the strong linkage disequilibrium between rs7574865 and rs11889341 ($R^2$=0.76 and D'=0.93), very similar results were observed for rs11885361 (see supplementary table S1, available online only). None of the SNP deviated from Hardy–Weinberg equilibrium in both the disease subgroups and the control groups.

To investigate a gene dose effect for a *STAT4* risk allele, patients and controls were divided into three groups: carriers of one risk allele for *STAT4*, carriers of two risk alleles, and carriers of no risk alleles. OR for polymyositis/dermatomyositis susceptibility were compared using individuals with no risk alleles as reference. The OR was 1.2 (95% CI 0.9 to 1.5) for carriers of one risk allele (rs7574865) and increased to 2.0 (95% CI 1.4 to 2.9) for two risk alleles. Similar results were observed for rs11889341; OR for disease susceptibility increased to 2.2 (95% CI 1.5 to 3.2) in carriers of two risk alleles, whereas that in carriers of one risk allele was 1.1 (95% CI 0.9 to 1.5).

### Association between *STAT4* polymorphisms and the ILD phenotype

We next investigated whether *STAT4* polymorphisms were associated with a particular disease phenotype (ILD) by evaluating the polymyositis/dermatomyositis patients recruited from TWMU (first study set). For 138 polymyositis/dermatomyositis patients, data on ILD were missing for six patients. For the remaining 132 patients whose data were available, 20 of 43 polymyositis patients (46.5%) and 59 of 89 dermatomyositis patients (66.3%) had ILD. After combining these patients, 79 patients had polymyositis/dermatomyositis complicated with ILD and 53 patients did not.

As shown in table 2, the frequencies for the rs7574865T allele and T/T genotype among the polymyositis/dermatomyositis patients from the first set group showed only borderline significant differences compared with the control subjects (T allele: OR 1.38, 95% CI 1.02 to 1.86; p=0.037; $p_{corr}$=0.11; T/T genotype: OR 2.25, 95% CI 1.26 to 4.03; p=0.0074; $p_{corr}$=0.022). These differences remained significant among the 79 ILD patients (T allele: OR 1.59, 95% CI 1.10 to 2.28; p=0.013; $p_{corr}$=0.039; T/T genotype: OR 2.46, 95% CI 1.25 to 4.84; p=0.016; $p_{corr}$=0.048). In contrast, no association was observed between patients without ILD and control subjects.

Nevertheless, the results of this intrasubgroup analysis failed to identify a significant difference in the risk allele frequencies between the polymyositis/dermatomyositis patients with ILD and those without ILD. Similar results were obtained for rs11889361 (see supplementary table S2, available online only).

## DISCUSSION

This is the first study to report that *STAT4* polymorphisms are involved in susceptibility to adult-onset polymyositis/dermatomyositis among Japanese individuals, regardless of the polymyositis or dermatomyositis disease phenotype. This suggests that these disorders share a genetic background common with other autoimmune diseases. The *STAT4* variant rs7574865, located in the third intron, has previously been implicated in the susceptibility to autoimmune diseases;[9 10 12 13] our results are consistent with these observations. There was also a gene dose effect for rs7574865 for polymyositis/dermatomyositis susceptibility. Although several studies have suggested that rs7574865 or a related haplotype was associated with high transcriptional levels of STAT4,[21 22] the functional consequence of rs7574865 remains unclear.

The STAT4 protein is activated on stimulation with IL-12, IL-23 and IL-17,[23] and drives T-helper (Th)1 and Th17-type immune responses. STAT4 is also activated by type I IFN cytokine signals (ie, IFNα and IFNβ), which results in a spike in IFNγ secretion by CD4 cells and natural killer cells without leading to Th1 development. Activation of a type I IFN pathway is a shared

pathological phenomenon among several autoimmune diseases, and the expression of type I IFN-inducible proteins in affected muscle tissues was reported predominantly for dermatomyositis.[24] Interestingly, SLE patients who carry the risk variant of *STAT4* show increased sensitivity to IFNα. [25] It is plausible that *STAT4* variations, which can cause increased and/or prolonged STAT4 protein activity, may trigger autoimmune disease pathology because of its impact on the immune system.

In Asian populations, in which the *STAT4* risk allele is more prevalent than in Caucasians, the contribution of *STAT4* to disease susceptibility has been considered to be greater.[26] This is in sharp contrast to *PTPN22*. Because of the extremely low frequency of this risk allele, *PTPN22* polymorphisms are not involved in autoimmune diseases among Asians.[27] In the present study, the observed frequency of the risk allele among polymyositis/dermatomyositis patients was 0.39 with an OR of 1.37. Kobayashi *et al*[28] investigated a total of 3567 Japanese RA patients from three independent Japanese populations, and reported that the rs7574865 allele frequency was 0.37, with a combined OR of 1.27 for RA, whereas the frequency for SLE patients (n=591) ranged from 0.39 to 0.44 with an OR of 1.61. Given the reported OR for Japanese RA and SLE patients, the contribution of the *STAT4* allele in polymyositis/dermatomyositis seems strong.

In addition to their influence on autoimmune disease susceptibility, *STAT4* polymorphisms can also influence disease phenotypes. For example, rs7574865 in SLE patients was associated with severe disease manifestations, such as nephritis, high double-stranded DNA antibody production and younger age of disease onset.[26 29] For SSc patients, this polymorphism was associated with the presence of ILD.[12] Therefore, we examined possible associations between *STAT4* and the clinical manifestation of ILD in polymyositis/dermatomyositis patients.

The rs7574865T allele and T/T genotype frequencies remained high when polymyositis/dermatomyositis patients were limited to those with ILD, whereas these were not significant for patients without ILD. Although a total of 460 polymyositis/dermatomyositis patients was genotyped in the present study, our subanalysis only tested 138 patients in our first set. This was because clinically equivalent data were not available between the collaborating institutions. The statistical power regarding the association between *STAT4* and the predisposition to ILD in polymyositis/dermatomyositis was thus rather limited.

In addition to shared susceptibility genes, it is plausible that each autoimmune disease has disease-specific risk genes that can influence each unique disease phenotype. For example, Asian RA patients who had a certain functional haplotype for *PADI4* (encoding for citrullinating enzyme of peptidyl arginie deaminase type 4) had high serum titres of an autoantibody to citrullinated proteins.[30 31] SSc patients, regardless of ethnicity, are likely to have genetic variants of *CTGF* that encode for a connective tissue growth factor,[32 33] which contributes to tissue fibrosis. A unique phenotypical feature of polymyositis/dermatomyositis is that skeletal muscle is targeted. Unfortunately, a risk gene(s) specific for polymyositis/dermatomyositis remains to be determined, and genome-wide association studies might help in its (their) discovery.

Our results established *STAT4* as a new polymyositis/dermatomyositis susceptibility gene. One study limitation was an insufficient association analysis regarding clinical subsets, including serological phenotypes (autoantibody profiles). However, in spite of the rarity of these diseases, we managed to obtain a large sample size, which provided sufficient statistical power for this case–control study. Further investigations will be needed to replicate the positive association between *STAT4* polymorphisms and polymyositis/dermatomyositis in Asian populations, as well as in different ethnic groups.

**Contributors** TS conceived the study and drafted the manuscript. YK was responsible for the design and coordination of the study and helped to draft the manuscript. KG participated in the genotyping and study design. YH and RT recruited a subset of patients from NCNP and participated in the coordination of the study. TF and TG recruited a subset of patients from TWMU and participated in the coordination of the study. IN and HY conceived the study and participated in the design of the study.

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**Competing interests** None.

**Patient consent** Obtained.

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BRIEF REPORT

# Association of HLA–DRB1*0101/*0405 With Susceptibility to Anti–Melanoma Differentiation–Associated Gene 5 Antibody–Positive Dermatomyositis in the Japanese Population

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***Objective.*** **The complication of interstitial lung disease (ILD) in polymyositis/dermatomyositis (PM/DM) is associated with anti–aminoacyl–transfer RNA synthetase (anti-aaRS) antibody or anti–melanoma differentiation–associated gene 5 (anti–MDA-5) antibody positivity. Anti–MDA-5 antibody is associated with clinically amyopathic DM and fatal outcome due to rapidly progressive ILD in Asian populations. The association between genetic factors and anti–MDA-5 antibody–positive DM is unclear. This study was undertaken to investigate the HLA–DRB1 genotype in patients with anti–MDA-5 antibody–positive DM.**

***Methods.*** **We examined genetic differences among 17 patients with anti–MDA-5 antibody–positive DM, 33 patients with anti-aaRS antibody–positive PM/DM, 33 patients with PM/DM without anti-aaRS antibody or ILD, and 265 healthy controls.**

***Results.*** **The frequencies of HLA–DRB1*0101 and DRB1*0405 were 29% and 71%, respectively, in patients with anti–MDA-5 antibody–positive DM, which were higher than the frequencies in healthy controls (10% and 25%, respectively). Among the 17 patients with anti–MDA-5 antibody–positive DM, 16 (94%) harbored either the DRB1*0101 or DRB1*0405 allele. The combined frequency of the DRB1*0101 allele and the DRB1*0405 allele was significantly higher in patients with anti–MDA-5 antibody–positive DM than in patients with PM/DM without anti-aaRS antibody or ILD, with an odds ratio (OR) of 42.7 (95% confidence interval [95% CI] 4.9–370.2) ($P = 1.1 \times 10^{-5}$), or in patients with anti-aaRS antibody–positive PM/DM (OR 13.3 [95% CI 1.6–112.6], $P = 4.5 \times 10^{-3}$).**

***Conclusion.*** **Our findings indicate that HLA–DRB1*0101/*0405 is associated with susceptibility to anti–MDA-5 antibody–positive DM in the Japanese population.**

Dermatomyositis (DM) is characterized by inflammation of the skin and muscle (1) and is occasionally complicated by interstitial lung disease (ILD). In particular, rapidly progressive ILD is an intractable and life-threatening complication. Clinically amyopathic DM (CADM) includes typical skin lesions with amyopathy or hypomyopathy (2). It has recently been reported that patients with CADM who are positive for the anti–melanoma differentiation–associated gene 5 (MDA-5) antibody frequently have complications with rapidly progressive ILD, especially in the Japanese population (3–5). In general, anti–MDA-5 antibody is specific for rapidly progressive ILD associated with CADM and is not detected in patients with CADM or DM without ILD or in patients with polymyositis (PM). The MDA-5 protein plays a role in the innate immune system. MDA-5 initially recognizes picornaviruses, such as coxsackievirus, and induces antiviral responses by producing type I interferons and tumor necrosis factor $\alpha$ (6). Hyperferritinemia is complicated by rapidly progressive ILD in anti–MDA-5 antibody–positive DM (4,5). Although the pathogenesis of rapidly progressive ILD associated with anti–MDA-5 antibody–positive DM has been tentatively attributed to a cytokine storm triggered

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Dr. Kuwana holds a patent on an anti–MDA-5 antibody measuring kit.

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by viral infection, especially in the skin and lungs, its exact mechanism is unknown.

In PM/DM, complication with ILD is associated with the anti–aminoacyl–transfer RNA synthetase (anti-aaRS) antibody or anti–MDA-5 antibody. It has been reported that 90% of Caucasian patients with the anti-aaRS antibody are carriers of HLA–DRB1*03 (7). In the Japanese population, HLA–DRB1*0405 is associated with susceptibility to anti-aaRS antibody–positive PM/DM (8). However, associations between genetic factors and anti–MDA-5 antibody–positive DM have remained unclear.

Therefore, we investigated the HLA–DRB1 gene in patients with anti–MDA-5 antibody–positive DM. In addition, we compared genetic differences in HLA among patients with anti–MDA-5 antibody–positive DM, patients with anti-aaRS antibody–positive PM/DM, and patients with PM/DM without anti-aaRS antibody or ILD.

**Table 1.** Clinical characteristics and HLA–DRB1 genotype of the patients with anti–MDA-5 antibody–positive DM*

| Patient/age/sex | Genotype | Phenotype | ILD type |
|---|---|---|---|
| 1/48/M | DRB1*0101/1602 | CADM | Rapidly progressive |
| 2/25/F | DRB1*0101/1501 | CADM | Chronic |
| 3/53/F | DRB1*0101/0803 | CADM | Rapidly progressive |
| 4/18/M | DRB1*0101/1502 | CADM | Rapidly progressive |
| 5/47/F | DRB1*0101/0405 | DM | Rapidly progressive |
| 6/58/M | DRB1*0405/1406 | CADM | Rapidly progressive |
| 7/16/F | DRB1*0405/0401 | CADM | Rapidly progressive |
| 8/53/F | DRB1*0405/1501 | CADM | Rapidly progressive |
| 9/53/F | DRB1*0405/0410 | CADM | Rapidly progressive |
| 10/44/F | DRB1*0405/1406 | CADM | Chronic |
| 11/45/F | DRB1*0405/1202 | CADM | Chronic |
| 12/39/M | DRB1*0405/0401 | CADM | Chronic |
| 13/47/F | DRB1*0405/1201 | CADM | Chronic |
| 14/76/F | DRB1*0405/0802 | CADM | Rapidly progressive |
| 15/56/F | DRB1*0405/1502 | CADM | Rapidly progressive |
| 16/43/M | DRB1*0405/0901 | CADM | Chronic |
| 17/66/F | DRB1*0901/1502 | CADM | Rapidly progressive |

* Anti–MDA-5 = anti–melanoma differentiation–associated gene 5; DM = dermatomyositis; ILD = interstitial lung disease; CADM = clinically amyopathic DM.

## PATIENTS AND METHODS

**Patients.** This retrospective study included patients admitted to Tokyo Women's Medical University Aoyama Hospital or Keio University Hospital from August 1992 to February 2010. Medical records were obtained for 142 and 57 patients diagnosed as having DM and CADM, respectively. The anti–MDA-5 antibody was detected in 31 patients. DNA samples were available for 17 patients with the anti–MDA-5 antibody, and all of these patients were enrolled in the study. All of the enrolled patients had skin rashes, myopathy, or respiratory symptoms (or a combination thereof) at admission. The patients were diagnosed as having DM or CADM based on the criteria of Bohan and Peter (9) or Sontheimer (10), respectively. Specific rashes, including heliotrope rash, Gottron's sign, or Gottron's papules, were used to define DM or CADM. In general, CADM patients present with typical skin lesions and amyopathy or hypomyopathy with a duration of >6 months. A subset of the CADM group included patients who developed fatal ILD within the first 6 months of this study. Clinical data were obtained from hospital admission records.

To investigate the characteristics of the HLA–DRB1 genotype in anti–MDA-5 antibody–positive DM, HLA data were obtained in patients with anti-aaRS antibody–positive PM/DM, patients without anti-aaRS antibody or ILD, and healthy controls. These HLA genotype databases have been described previously (8). All of the subjects in the present study were Japanese. None of the subjects had rheumatoid arthritis (RA) or other connective tissue diseases. This study was approved by the ethics committee of Tokyo Women's Medical University and was performed in accordance with the Declaration of Helsinki.

**Evaluation of autoantibodies.** Anti–MDA-5 antibody was detected by immunoprecipitation (IP) assay and enzyme-linked immunosorbent assay using recombinant MDA-5 as an antigen, as previously described (3). Anti-aaRS antibodies, including Jo-1, EJ, PL-7, PL-12, and OJ; anti–signal recognition particle (anti-SRP) antibody; anti-Ku antibody; and anti–U1 small nuclear RNP (anti-U1 snRNP) antibody were assessed by RNA IP assays.

**Classification of ILD.** Patients were evaluated for ILD by chest radiography and computed tomography (CT) or high-resolution CT of the chest. Rapidly progressive ILD was defined as a progressive ILD within 3 months of the onset of respiratory symptoms. Chronic ILD was defined as ILD that was asymptomatic and non–rapidly progressive or slowly progressive over 3 months (11).

**HLA–DRB1 genotyping.** HLA–DRB1 genotyping was performed using polymerase chain reaction–reverse sequence-specific oligonucleotide techniques and standard methods. The DNA for the HLA–DRB1 genotyping of the patients was extracted from peripheral blood mononuclear cells using standard methods.

**Statistical analysis.** The chi-square test was used for the comparison of frequencies, and Fisher's exact test was used when appropriate. Data were analyzed using JMP software (SAS Institute). *P* values were adjusted by Bonferroni correction when appropriate.

## RESULTS

**Clinical characteristics and HLA–DRB1 genotype of patients with anti–MDA-5 antibody–positive DM.** As shown in Table 1, 17 patients with anti–MDA-5 antibody–positive DM were enrolled in the study. Their mean ± SD age was 46 ± 16 years. Seventy-one percent were women. The HLA–DRB1*0101 and DRB1*0405 alleles were identified in 5 patients (29%) and 12 patients (71%), respectively. The HLA–DRB1*0101 or *0405 allele was identified in 16 (94%) of the 17

**Table 2.** Comparison of HLA–DRB1 genotypes among patients with anti–MDA-5 antibody–positive DM, patients with anti-aaRS antibody–positive PM/DM, and patients with PM/DM without anti-aaRS antibody or ILD*

| Genotype | Patients with anti–MDA-5 antibody–positive DM (n = 17) | Patients with anti-aaRS antibody–positive PM/DM | | Patients with PM/DM without anti-aaRS antibody or ILD | | Healthy controls (n = 265) |
|---|---|---|---|---|---|---|
| | | PM/DM (n = 33) | DM (n = 19) | PM/DM (n = 33) | DM (n = 21) | |
| DRB1*0101 | 29 | 12 | 11 | 12 | 14 | 10 |
| DRB1*0401 | 12 | 0 | 0 | 3 | 5 | 2 |
| DRB1*0403 | 0 | 9 | 5 | 6 | 5 | 5 |
| DRB1*0405 | 71† | 42 | 53 | 18 | 24 | 25 |
| DRB1*0406 | 0 | 6 | 5 | 3 | 5 | 7 |
| DRB1*0407 | 0 | 0 | 0 | 0 | 0 | 2 |
| DRB1*0410 | 6 | 3 | 5 | 9 | 5 | 2 |
| DRB1*0802 | 6 | 18 | 21 | 9 | 10 | 7 |
| DRB1*0803 | 6 | 24 | 21 | 27 | 19 | 14 |
| DRB1*0901 | 12 | 24 | 21 | 18 | 19 | 30 |
| DRB1*1101 | 0 | 3 | 0 | 0 | 0 | 2 |
| DRB1*1201 | 6 | 9 | 16 | 3 | 5 | 7 |
| DRB1*1202 | 6 | 6 | 0 | 0 | 0 | 4 |
| DRB1*1301 | 0 | 0 | 0 | 3 | 0 | 0 |
| DRB1*1302 | 0 | 6 | 5 | 21 | 29 | 19 |
| DRB1*1401 | 0 | 3 | 5 | 9 | 10 | 5 |
| DRB1*1403 | 0 | 3 | 0 | 6 | 10 | 4 |
| DRB1*1405 | 0 | 3 | 5 | 3 | 5 | 6 |
| DRB1*1406 | 12 | 0 | 0 | 3 | 5 | 3 |
| DRB1*1501 | 12 | 12 | 16 | 9 | 5 | 11 |
| DRB1*1502 | 18 | 6 | 11 | 21 | 24 | 20 |
| DRB1*1602 | 6 | 0 | 0 | 6 | 0 | 3 |
| Other | 0 | 0 | 0 | 0 | 0 | 5 |

* Values are the percent of subjects. Anti-aaRS = anti–aminoacyl–transfer RNA synthetase; PM = polymyositis (see Table 1 for other definitions).
† $P = 0.0003$ versus patients with PM/DM without anti-aaRS antibody or ILD; $P = 0.00018$ versus healthy controls.

patients. No patients had homoalleles of HLA–DRB1*0101 or DRB1*0405. One patient had both DRB1*0101 and DRB1*0405. With respect to the clinical phenotype, 16 patients had CADM. ILD complication was observed in all of the patients. Moreover, the frequency of rapidly progressive ILD was high (65%). No patients had RA or other connective tissue diseases as complications.

**Comparison of the HLA–DRB1 genotype in patients with anti–MDA-5 antibody–positive DM, patients with anti-aaRS antibody–positive PM/DM, and patients with PM/DM without anti-aaRS antibody or ILD.** To investigate the characteristics of the HLA–DRB1 genotype in anti–MDA-5 antibody–positive DM, the frequency of the HLA–DRB1 genotype was compared among patients with anti–MDA-5 antibody–positive DM, patients with anti-aaRS antibody–positive PM/DM, patients with PM/DM without anti-aaRS antibody or ILD, and healthy controls (Table 2).

Data previously obtained at our institution indicated that 33 PM/DM patients (14 patients with PM and 19 with DM) exhibited anti-aaRS antibody, as follows: 8 PM patients and 8 DM patients had anti–Jo-1; 4 PM patients and 6 DM patients had anti-EJ; 2 PM patients and 2 DM patients had anti–PL-7; 0 PM patients and 3 DM patients had anti–PL-12; and none of the patients had anti-OJ. Of the 33 patients with anti-aaRS antibody–positive PM/DM, 24 (73%) had ILD. Moreover, 33 PM/DM patients (12 PM patients and 21 DM patients) had neither anti-aaRS antibody nor ILD, and in all 21 of these DM patients, the clinical phenotype was classic DM, not CADM. In patients with PM/DM without anti-aaRS antibody or ILD, anti-SRP antibody, anti-U1 snRNP antibody, and anti-Ku antibody were detected in 3 PM patients, 1 DM patient, and 0 patients, respectively.

As shown in Table 2, the frequency of HLA–DRB1*0101 was ~30% in anti–MDA-5 antibody–positive DM and ~10% in the other subsets, although the difference was not significant ($P = 0.012$ versus

**Table 3.** Frequency of the HLA–DRB1*0101/0405 alleles in patients with anti–MDA-5 antibody–positive DM*

| | Patients with anti–MDA-5 antibody–positive DM (n = 17) | Patients with anti-aaRS antibody–positive PM/DM | | Patients with PM/DM without anti-aaRS antibody or ILD | |
|---|---|---|---|---|---|
| | | PM/DM (n = 33) | DM (n = 19) | PM/DM (n = 33) | DM (n = 21) |
| DRB1*0101 or DRB1*0405, % | 94 | 55 | 63 | 27 | 33 |
| $P$† | – | $4.5 \times 10^{-3}$ | $4.4 \times 10^{-2}$ | $1.1 \times 10^{-5}$ | $2.0 \times 10^{-4}$ |
| OR (95% CI) | – | 13.3 (1.6–112.6) | 9.3 (1.0–86.4) | 42.7 (4.9–370.2) | 32 (3.5–293.1) |

* Anti-aaRS = anti–aminoacyl–transfer RNA synthetase; PM = polymyositis; OR = odds ratio; 95% CI = 95% confidence interval (see Table 1 for other definitions).
† Versus patients with anti–MDA-5 antibody–positive DM.

healthy controls, adjusted $P$ value not significant). $P$ values with Bonferroni correction for multiple comparisons less than 0.0023 were considered significant; this was determined by dividing the $P$ value of 0.05 by 22 (the number of HLA genotypes). The inadequate statistical power may be attributed to small sample sizes. Moreover, the frequency of HLA–DRB1*0405 was significantly higher in the patients with anti–MDA-5 antibody–positive DM than in the patients with PM/DM without anti-aaRS antibody or ILD ($P = 0.0003$) or in the healthy controls ($P = 0.00018$). The frequency of HLA–DRB1*0405 was also high in patients with anti-aaRS antibody–positive PM/DM, although it was not significantly different from that in the other subsets. No significant differences were found regarding the frequencies of the other alleles.

**Frequency of HLA–DRB1*0101/*0405 in patients with anti–MDA-5 antibody–positive DM compared with other PM/DM patient subsets.** In this study, the HLA–DRB1*0101 or *0405 allele was identified in all but 1 of the 17 anti–MDA-5 antibody–positive patients. In the HLA–DRB1 alleles, residues 70–74 of the DR$\beta$ chain form the third hypervariable region, an important region for antigen presentation. This amino acid sequence is QRRAA, which is a shared epitope motif in both DRB1*0101 and DRB1*0405. We speculated that QRRAA may be a critical sequence in the pathophysiology of anti–MDA-5 antibody–positive DM. We considered the role of both DRB1*0101 and DRB1*0405 in anti–MDA-5 antibody–positive DM. Therefore, the combined frequency of the DRB1*0101 allele and the DRB1*0405 allele was compared among patients with anti–MDA-5 antibody–positive DM, patients with anti-aaRS antibody–positive PM/DM, and patients with PM/DM without anti-aaRS antibody or ILD.

As shown in Table 3, the combined frequency of DRB1*0101 and *0405 was significantly higher in patients with anti–MDA-5 antibody–positive DM than in patients with PM/DM without anti-aaRS antibody or ILD, with an odds ratio (OR) of 42.7 (95% confidence interval [95% CI] 4.9–370.2, $P = 1.1 \times 10^{-5}$), or in patients with DM without anti-aaRS antibody or ILD (OR 32 [95% CI 3.5–293.1], $P = 2 \times 10^{-4}$). The combined frequency of DRB1*0101 and *0405 was also higher in patients with anti–MDA-5 antibody–positive DM than in patients with anti-aaRS antibody–positive PM/DM (OR 13.3 [95% CI 1.6–112.6], $P = 4.5 \times 10^{-3}$) and patients with anti-aaRS antibody–positive DM (OR 9.3 [95% CI 1.0–86.4], $P = 4.4 \times 10^{-2}$). Moreover, the frequency of these alleles was higher in patients with anti-aaRS antibody–positive PM/DM than in patients with PM/DM without anti-aaRS antibody or ILD (OR 3.2 [95% CI 1.1–8.9], $P = 2.4 \times 10^{-2}$).

## DISCUSSION

We have demonstrated an association between a genetic factor and anti–MDA-5 antibody–positive DM. Specifically, this study shows that HLA–DRB1*0101/*0405 is associated with susceptibility to anti–MDA-5 antibody–positive DM. HLA–DRB1*0301 is associated with susceptibility to anti-aaRS antibody–positive PM/DM in Caucasians. In contrast, the frequency of HLA–DRB1*0301 is low, but the frequency of HLA–DRB1*0405 is relatively high, at ~20%, in the Japanese population. HLA–DRB1*0405 is associated with susceptibility to anti-aaRS antibody–positive PM/DM in the Japanese population, whereas HLA–DRB1*0101 is not (8). In the present study, the frequency of HLA–DRB1*0405 was high in both anti–MDA-5 antibody–positive DM and anti-aaRS antibody–positive PM/DM. In contrast, the frequency of HLA–DRB1*0405 among patients with PM/DM without anti-aaRS antibody or ILD was similar to that in healthy controls. Type 1

diabetes mellitus, Vogt-Koyanagi-Harada disease, and autoimmune hepatitis have also been associated with HLA–DRB1*0405 in the Japanese population (12–14). HLA–DRB1*0405 may contribute to the pathophysiology of several autoimmune diseases.

In addition, this study revealed that the frequency of HLA–DRB1*0101 was higher in patients with anti–MDA-5 antibody–positive DM than in patients with anti-aaRS antibody–positive PM/DM or patients with PM/DM without anti-aaRS antibody or ILD, although the number of enrolled patients was small. Previously, the HLA–DRB1*01 and *04 alleles were shown to play roles in the susceptibility to and progression of RA (15). Specifically, these alleles are associated with anti–citrullinated protein antibody (ACPA)–positive RA. Residues 70–74 of the DR$\beta$ chain (QRRAA) in both HLA–DRB1*0101 and DRB1*0405 constitute an important region for antigen presentation. QRRAA may indirectly influence outcome via ACPA production (15). Among PM/DM patients in the Japanese population, HLA–DRB1*0101 or *0405 can also be associated with the production of autoantibodies against MDA-5 or aaRS. QRRAA may be a critical sequence in the pathophysiology of anti–MDA-5 antibody–positive DM and anti-aaRS antibody–positive PM/DM. These antibodies are strongly associated with the development of ILD in PM/DM.

The HLA class II haplotypes are more important than individual alleles. DQB1 and DPB1 should be investigated in all of the patients and healthy donors included in this study. However, DQB1 and DPB1 alleles were not sufficiently investigated in all samples. This was a limitation of the present study. We plan to analyze the HLA class II haplotypes in patients with anti–MDA-5 antibody–positive DM in a future study.

In conclusion, HLA–DRB1*0101/*0405 is associated with susceptibility to anti–MDA-5 antibody–positive DM in the Japanese population. These alleles were also associated with ILD in patients with PM/DM.

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## AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Kawaguchi had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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# Frequency of Class III and IV Nephritis in Systemic Lupus Erythematosus Without Clinical Renal Involvement: An Analysis of Predictive Measures

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*ABSTRACT.* ***Objective.*** To determine the frequency of International Society of Nephrology/Renal Pathology Society (ISN/RPS) class III or IV lupus nephritis in patients with systemic lupus erythematosus (SLE) without clinical renal involvement.

***Methods.*** We investigated the renal pathology of 195 patients with SLE, including 86 patients without clinical renal involvement.

***Results.*** Lupus nephritis other than class I was found in 58% of the patients without clinical renal involvement, and class III and IV nephritis was found in 15% of these patients. To reveal the predictive measures involved in class III or IV lupus nephritis, we explored the clinical measures in patients with SLE who did not have clinical renal involvement. Anti-dsDNA antibody titers were significantly higher ($p = 0.0266$) and C3 values were significantly lower ($p = 0.0073$) in patients with class III or IV lupus nephritis than in patients without class III or IV lupus nephritis. The sensitivity and specificity values were 77% and 73%, respectively, for cutoff levels of both 40 IU/ml for anti-dsDNA antibodies and 55 mg/dl for C3 (OR 8.8, $p = 0.0011$).

***Conclusion.*** The frequency of nephritis, including ISN/RPS class III and IV, was unexpectedly high in SLE patients without clinical renal involvement. ISN/RPS class III or IV lupus nephritis could be hidden in patients with SLE who present both a high titer of anti-dsDNA antibody and a low concentration of C3, even when they have clinically normal urinary findings and renal function. (First Release Nov 15 2011; J Rheumatol 2012;39:79–85; doi:10.3899/jrheum.110532)

*Key Indexing Terms:*
SYSTEMIC LUPUS ERYTHEMATOSUS
SILENT LUPUS NEPHRITIS
COMPLEMENT
ANTI-dsDNA ANTIBODY

Systemic lupus erythematosus (SLE) is an autoimmune disease with multiple organ manifestations, including skin lesions, arthritis, serositis, nephritis, and neuropsychiatric and hematological disorders. In the 1950s, the 5-year survival rate in patients with SLE who had World Health Organization (WHO) class IV nephritis was 17%; more recently, however, therapy with corticosteroids and immunosuppressive agents (IA) has improved the prognosis of patients with SLE. The 5-year survival rate increased to 82% in the 1990s[1]. However, WHO class IV lupus nephritis is one of the most common manifestations that contribute to endstage renal failure (ESRF). The frequency of ESRF was 40.9% in patients with WHO class IV nephritis, higher than the 2.6% frequency in those with non-class IV lupus nephritis[2]. In general, combination therapy with corticosteroids and IA, such as cyclophosphamide and mycophenolate mofetil, should be recommended in active lupus nephritis with International Society of Nephrology/Renal Pathology Society (ISN/RPS) class III or IV. The early diagnosis and treatment of ISN/RPS class III or IV lupus nephritis is important to improve renal and overall survival in patients with SLE.

The renal manifestations of SLE range from asymptomatic urinary findings, such as microhematuria and proteinuria, to nephrotic syndrome or progressive renal impairment[3]; these manifestations are observed in 31% to 65% of patients with SLE[4]. Although renal biopsy is the "gold standard" for diagnosing and classifying lupus nephritis, it is invasive and has potential complications. Renal biopsy is not always performed on patients with SLE because some of them have normal renal findings or severe manifestations, such as thrombocytopenia, infections, or neuropsychiatric involvement. Thus, it would be beneficial if noninvasive examination could predict the development and severity of lupus nephritis when renal biopsy cannot be performed. Some markers, such as ß-1 integrin in peripheral blood T cells, urinary chemokine, and growth fac-

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tor, have been reported to predict active lupus nephritis, such as ISN/RPS class IV[5,6], although these markers are not available in clinical practice. Clinical measures such as urine sediment, proteinuria, serum complements, and anti-dsDNA antibody are considered conventional and useful predictors for the disease activity of lupus nephritis[7,8,9]. However, some patients show renal histological changes despite normal urinary findings and renal function. This condition is called silent lupus nephritis (SLN)[10,11]. Although most patients with SLN show mild lupus nephritis (i.e., ISN/RPS class II), it is believed that ISN/RPS class III or IV lupus nephritis is rare in patients with SLN[10,11]. There is a notable difference between the therapeutic strategies used for patients with SLE with or without ISN/RPS class III or IV lupus nephritis. However, the characteristics and predictive factors of ISN/RPS class III or IV lupus nephritis have not been revealed in the literature because previous studies have described only a small number of patients with SLN.

We investigated the frequency and predictive factors of ISN/RPS class III or IV lupus nephritis in SLE patients without clinical renal involvement. We analyzed the association between pathohistological renal changes and conventional clinical measures among 195 patients with SLE. We also compared patients with ISN/RPS class III or IV lupus nephritis with those with other ISN/RPS classes (I, II, or V) of lupus nephritis in patients with SLE who did not have clinical renal involvement.

## MATERIALS AND METHODS

*Patients.* We studied 467 consecutive patients who were hospitalized at our institution between 1994 and 2005. These patients were diagnosed with SLE based on the American College of Rheumatology classification criteria[12]. Of 467 patients, 296 (63%) had a renal biopsy (276 women, 20 men). To clarify precisely the degree of pathohistological renal involvement and disease activity in SLE, renal biopsy was performed in both patients with and those without clinical renal involvement. Written informed consent was obtained from each patient. Renal biopsies could not be performed in 171 of 467 patients who did not consent to a renal biopsy or who had a poor condition for examination. The renal biopsies of 31 patients could not be confirmed based on their clinical records; these patients were excluded. We also excluded 57 patients whose renal specimens contained fewer than 10 glomeruli because they were not diagnosed accurately[13]. Other patients excluded were 1 patient with diabetic nephropathy, 1 with IgA nephropathy, 6 with antiphospholipid antibody-related microangiopathy, and 5 with interstitial nephritis. Ultimately, 195 patients were enrolled. In addition, 7 patients were counted twice because re-biopsies were performed among 195 patients. All patients were Japanese except 3, including 2 non-Japanese Asians and 1 African Canadian. The ethics committee of our institution, in accord with the Declaration of Helsinki, approved our study.

*Evaluation of clinical measures.* Urinary tests, including proteinuria and hematuria on a dipstick, urinary sediment and quantitative proteinuria measured by 24-h urine, serum creatinine, complement hemolytic activity (CH50), complement components (C3 and C4), and anti-dsDNA antibody, were evaluated upon admission before renal biopsy. CH50, C3, and C4 were measured by the standard method. Anti-dsDNA antibody was detected by radioimmunoassay (normal value < 6 IU/ml). The estimated glomerular filtration rate (eGFR) was calculated according to the described method using variables that included serum creatinine, age, and sex[14].

*Evaluation of renal pathohistology.* Renal pathohistology was classified according to the 2003 ISN/RPS classification[13]. Biopsy results obtained prior to 2003 were reviewed and reclassified according to the 2003 ISN/RPS classification. Immunohistological pathology was tested by direct immunofluorescence and/or the enzyme-labeled antibody method (streptavidin-biotin). Positive results for glomerular immune deposits were defined as (1+) or more. Cases with minor glomerular abnormalities observed by light microscopy and no evidence of immune deposits were classified as "Nil" because they could not be classified as lupus nephritis according to the 2003 ISN/RPS classification[13,15].

*Definition of clinical renal involvement.* Clinical renal involvement was indicated for patients when 1 or more of the following criteria were satisfied: (1) proteinuria > 400 mg per day; (2) presence of active urinary sediments (> 5 red blood cells and/or 5 white blood cells per high power field and/or cellular cast); or (3) eGFR < 67 ml/min per 1.73 $m^2$. We determined these cutoff levels using a receiver-operating characteristic curve to predict class III or IV among our 195 patients with SLE. Our definitions were similar to those described in other reports[10,15].

*Statistical analysis.* Statistical analyses was performed using the chi-square test to compare frequencies, the t test to compare mean values, and the Mann-Whitney U test to compare median values. The data were analyzed using JMP software (SAS Institute, Cary, NC, USA). P values < 0.05 indicated statistical significance.

## RESULTS

*Clinical features of 195 patients with SLE.* The laboratory and pathohistological features of 195 patients with SLE enrolled in our study are summarized in Tables 1 and 2. The 195 patients enrolled included 109 patients with clinical renal involvement (overt subset) and 86 patients without clinical renal involvement (silent subset). Fifteen patients (8%) had no evidence of lupus nephritis as determined by light microscopy and immunofluorescence (Nil). The remaining 180 patients were classified based on the 2003 ISN/RPS classification. As shown in Table 2, the frequencies of ISN/RPS class I-V lupus nephritis were 28 (14%), 44 (23%), 36 (19%), 47 (24%), and 25 (13%), respectively. There were no patients with class VI lupus nephritis. Of the 180 patients excluded as Nil, immunohistological findings could be assessed in 169 patients. The positive frequencies of glomerular immune deposits with IgG, IgM, IgA, C3, and C1q were 131 (77.5%), 137 (81.1%), 122 (72.2%), 144 (85.2%), and 144 (85.2%), respectively.

*Comparison of clinical features and ISN/RPS classification between patients with and without clinical renal involvement.* As shown in Table 1, we compared the overt subset with the silent subset. The disease duration after SLE diagnosis was significantly shorter (p = 0.008) in the silent subset than in the overt subset. No significant differences were found in the frequency of treatment with and dosage of prednisolone (PSL) between the 2 subsets, although the frequency of treatment with IA was higher in the overt subset. Cyclophosphamide was administered by intravenous pulse therapy in only 2 patients of the overt subset. The remaining 3 patients were given a daily dose of cyclophosphamide orally. As expected, proteinuria was significantly increased (p < 0.0001) and serum creatinine was significantly higher (p < 0.0001) in the overt subset than in the silent subset. Although there was no significant difference between the 2 subsets in terms of anti-dsDNA antibody titer and C4, a slight difference was

*Table 1.* Clinical characteristics of 195 patients with SLE and comparison between patients with and without clinical renal involvement. Except for the percentages, data represent the median value and range.

| Characteristics | Total, n = 195 | Overt Subset, n = 109 | Silent Subset, n = 86 | p |
|---|---|---|---|---|
| Age at renal biopsy, yrs | 31 (11–69) | 32 (15–68) | 29 (11–69) | 0.10 |
| Women, n (%) | 181 (93) | 100 (92) | 81 (94) | 0.59 |
| Disease duration, yrs | 0 (0–23) | 1 (0–23) | 0 (0–19) | 0.008 |
| Patients received PSL, n (%) | 118 (61) | 69 (63) | 49 (57) | 0.37 |
| Dosage of PSL, mg/day | 9 (0–80) | 10 (0–80) | 5 (0–60) | 0.29 |
| Patients who received IA, n (%) | 20 (10) | 16 (15) | 4 (5) | 0.03 |
| Azathioprine, n | 4 | 4 | 0 | |
| Mizoribine, n | 11 | 8 | 3 | |
| Cyclophosphamide, n | 5 | 4 | 1 | |
| Proteinuria, mg/day | 398 (0–29000) | 886 (0–29000) | 0 (0–350) | < 0.0001 |
| Presence of active urinary sediments, n (%) | 67 (61) | 67 (61) | 0 (0) | < 0.0001 |
| Serum creatinine, mg/dl | 0.7 (0.3–4.0) | 0.8 (0.4–4.0) | 0.6 (0.3–1.0) | < 0.0001 |
| eGFR, ml/min/1.73 $m^2$ | 82 (12–206) | 70 (12–151) | 91 (68–206) | < 0.0001 |
| Anti-dsDNA, IU/ml | 41 (0–9635) | 39 (0–9635) | 44 (0–2180) | 0.74 |
| Anti-Sm positivity, n (%) | 27 (14) | 16 (15) | 11 (13) | 0.70 |
| CH50, U/ml | 22.6 (0–50.7) | 20.9 (0–50.7) | 24.5 (0–49.8) | 0.049 |
| C3, mg/dl | 52 (10–150) | 46 (10–150) | 56 (21–129) | 0.037 |
| C4, mg/dl | 11 (1–52) | 11 (1–52) | 11 (1–40) | 0.81 |

P values were estimated to allow comparisons between patients with and without clinical renal involvement; SLE: systemic lupus erythematosus; overt subset: patients with clinical renal involvement; silent subset: patients without clinical renal involvement; PSL: prednisolone; IA: immunosuppressive agents; eGFR: estimated glomerular filtration rate.

*Table 2.* ISN/RPS classification of 195 patients with SLE and comparison between patients with and without clinical renal involvement. Data are number (%) unless otherwise indicated.

| | Total, n = 195 | Overt Subset, n = 109 | Silent Subset, n = 86 | p |
|---|---|---|---|---|
| ISN/RPS classification | | | | |
| Nil | 15 (8) | 4 (4) | 11 (13) | |
| Class I | 28 (14) | 3 (3) | 25 (29) | |
| Class II | 44 (23) | 16 (15) | 28 (33) | |
| Class III | 36 (19) | 28 (26) | 8 (9) | |
| III, n | 23 | 16 | 7 | |
| III + V, n | 13 | 12 | 1 | |
| Class IV | 47 (24) | 42 (39) | 5 (6) | |
| IV, n | 34 | 31 | 3 | |
| IV + V, n | 13 | 11 | 2 | |
| Class V | 25 (13) | 16 (15) | 9 (10) | |
| Class VI | 0 (0) | 0 (0) | 0 (0) | |
| Immune deposits | n = 169 | n = 103 | n = 66 | |
| IgG | 131 (78) | 88 (85) | 43 (65) | 0.002 |
| IgM | 137 (81) | 88 (85) | 49 (74) | 0.07 |
| IgA | 122 (72) | 84 (82) | 38 (58) | 0.0007 |
| C3 | 144 (85) | 88 (85) | 46 (70) | 0.014 |
| C1q | 144 (85) | 92 (89) | 52 (79) | 0.06 |

P values were estimated by the chi-square test to allow comparisons between patients with and without renal involvement. ISN/RPS: International Society of Nephrology/Renal Pathology Society; SLE: systemic lupus erythematosus; overt subset: patients with clinical renal involvement; silent subset: patients without clinical renal involvement.

found in CH50 and C3 values (p = 0.0499 and 0.0365). As shown in Table 2, nephritis other than ISN/RPS class I was found in 58% of the silent subset. ISN/RPS class III and IV lupus nephritis was found in 15% of the silent subset, although the frequency of these classes was significantly higher (p < 0.0001) in the overt subset than in the silent subset. The positive frequencies of glomerular immune deposits with IgG and IgA were higher in the overt subset than in the silent subset, although no significant difference was found in the positive frequencies of glomerular immune deposits with IgM and C1q between the 2 subsets.

In 7 patients, renal biopsy was performed twice because of the deterioration of proteinuria. Among 3 patients of the overt subset, ISN/RPS class was not transformed in 2 patients with ISN/RPS class V, although the ISN/RPS class was transformed from class II to class III in 1 patient. In contrast, among 4 patients of the silent subset, ISN/RPS class was transformed from class II to class V in 2 patients. ISN/RPS class was not transformed in the remaining 1 patient with class IV and another one with class V. Clinical renal involvement became overt in all 4 patients of the silent subset after the first renal biopsy.

*Comparison of active and chronic lesions in ISN/RPS class III or IV between patients with and without clinical renal involvement.* We assessed active lesions and chronic lesions in 83 patients with ISN/RPS class III or IV lupus nephritis (Table 3). Although the frequency of endocapillary proliferation and wire-loops lesion were common occurrences in both subsets, the frequency of cellular/fibrocellular crescents was significantly higher (p = 0.003) in the overt subset than in the silent subset. In addition, the rupture of glomerular basement membrane (GBM) occurred more frequently in the overt subset,

*Table 3.* Comparison of active and chronic lesions in ISN/RPS class III and class IV between patients with and without clinical renal involvement.

| Lesion Type | Overt Subset, n = 70 | Silent Subset, n = 13 | p |
|---|---|---|---|
| Active lesions, n (%) | | | |
| Endocapillary proliferation | 66 (94) | 11 (85) | 0.24 |
| Wire loops | 19 (27) | 6 (46) | 0.17 |
| Cellular/fibrocellular crescents | 29 (41) | 0 (0) | 0.003 |
| Fibrinoid necrosis | 5 (7) | 1 (8) | 1 |
| Rupture of GBM | 9 (13) | 0 (0) | 0.34 |
| Karyorrhexis | 6 (9) | 2 (15) | 0.60 |
| Chronic lesions, n (%) | | | |
| Global sclerosis | 30 (43) | 4 (30) | 0.54 |
| Fibrous crescents/adhesions | 13 (19) | 1 (8) | 0.44 |

ISN/RPS: International Society of Nephrology/Renal Pathology Society; overt subset: patients with clinical renal involvement; silent subset: patients without clinical renal involvement; GBM: glomerular basement membrane.

although there was no statistically significant difference between the 2 subsets. On the other hand, chronic lesions, such as those due to global sclerosis, were observed more frequently than expected in the silent subset as well.

*Comparison of clinical features between patients with and without ISN/RPS class III or IV lupus nephritis among SLE cases with clinical renal involvement.* In the 109 patients of the overt subset, the clinical features of ISN/RPS class III or IV lupus nephritis subgroup were compared with those of others (Nil, class I, II, or V; Table 4). There was no significant difference between the 2 subsets in the disease duration after SLE diagnosis. The frequency of treatment with PSL was higher in patients with ISN/RPS class III or IV lupus nephritis than in patients without ISN/RPS class III or IV lupus nephritis, although no difference was found in the frequency of treatment with IA between the 2 subsets. Renal function was significantly worse in patients with ISN/RPS class III or IV. Complement values were lower in patients with ISN/RPS class III or IV, although there was no statistically significant difference between the 2 subsets. The anti-dsDNA antibody titer was significantly higher (p = 0.0012) in patients with ISN/RPS class III or IV.

*Comparison of clinical features between patients with and without ISN/RPS class III or IV lupus nephritis among SLE cases without clinical renal involvement.* We analyzed the 86 patients of the silent subset in the manner described above; the results are shown in Table 5. The disease duration was significantly longer (p = 0.0184) in patients with ISN/RPS class III or IV than in those with other classes (Nil, class I, II, or V). The frequency of treatment with PSL or IA was statistically identical between the 2 subsets. Although there was no significant difference in renal function, the anti-dsDNA antibody titer was significantly higher (p = 0.0266) and the C3 value was significantly lower (p = 0.0073) in patients in ISN/RPS class III or IV than in those in other classes.

Among the silent subset, 13 patients with ISN/RPS class III or IV were followed for a median of 30 months (range 14–178 mo). Although only 2 patients in ISN/RPS class III experienced exacerbated nephritis accompanying malignancy or pregnancy, the remaining 11 patients had no exacerbation of nephritis and good prognosis with PSL therapy alone, including both induction and maintenance therapy.

*Predictors for ISN/RPS class III or IV lupus nephritis in patients with or without clinical renal involvement.* To predict

*Table 4.* Comparison of clinical characteristics between ISN/RPS class III or IV and others (Nil, class I, II, V) among patients with clinical renal involvement. Except for the percentages, data represent the median value and range.

| Characteristics | Class II, IV | Nil, Class I, II, V | p |
|---|---|---|---|
| Enrolled patients, n | 70 | 39 | — |
| Age at renal biopsy, yrs | 34 (15–64) | 31 (17–68) | 0.44 |
| Women, n (%) | 64 (91) | 36 (92) | 0.87 |
| Disease duration, yrs | 2 (0–23) | 1 (0–14) | 0.20 |
| Patients who received PSL, n (%) | 55 (79) | 14 (36) | < 0.0001 |
| Dosage of PSL, mg/day | 10 (0–80) | 10 (0–70) | 0.52 |
| Patients who received IA, n (%) | 11 (16) | 5 (13) | 0.68 |
| Proteinuria, mg/day | 1464 (0–2900) | 689 (0–7995) | 0.001 |
| Presence of active urinary sediments, n (%) | 47 (67) | 20 (51) | 0.15 |
| Serum creatinine, mg/dl | 0.9 (0.4–4.0) | 0.7 (0.5–1.3) | 0.002 |
| eGFR, ml/min/1.73 $m^2$ | 61 (12–151) | 85 (35–136) | 0.002 |
| Anti-dsDNA, IU/ml | 67 (0–9635) | 17 (0–354) | 0.001 |
| Anti-SM positivity, n (%) | 9 (13) | 7 (18) | 0.47 |
| CH50, U/ml | 15.8 (0–50.7) | 25.3 (0–46.3) | 0.091 |
| C3, mg/dl | 44 (10–136) | 54 (19–150) | 0.08 |
| C4, mg/dl | 9 (1–52) | 13 (2–31) | 0.32 |

P values were estimated to allow comparisons between ISN/RPS class III or IV and others. ISN/RPS: International Society of Nephrology/Renal Pathology Society; PSL: prednisolone; IA: immunosuppressive agents; eGFR: estimated glomerular filtration rate.

*Table 5.* Comparison of clinical features between ISN/RPS class III or IV and others (Nil, class I, II, V) among patients without clinical renal involvement. Except for percentages, data represent the median value and range.

| Characteristics | Class III, IV | Nil, Class I, II, V | p |
|---|---|---|---|
| Enrolled patients, n | 13 | 73 | — |
| Age at renal biopsy, yrs | 27 (22–56) | 29 (11–69) | 0.80 |
| Women, n (%) | 13 (100) | 68 (93) | 1 |
| Disease duration, yrs | 5 (0–9) | 0 (0–19) | 0.018 |
| Patients received PSL, n (%) | 9 (69) | 40 (55) | 0.38 |
| Dosage of PSL, mg/day | 10 (0–30) | 5 (0–60) | 0.35 |
| Patients who received IA, n (%) | 1 (8) | 3 (4) | 0.49 |
| Proteinuria, mg/day | 108 (0–300) | 0 (0–350) | 0.45 |
| Serum creatinine, mg/dl | 0.7 (0.5–0.8) | 0.6 (0.3–1.0) | 0.54 |
| eGFR, ml/min/1.73 $m^2$ | 83 (73–133) | 91 (68–206) | 0.34 |
| Anti-dsDNA, IU/ml | 97 (4–2180) | 35 (0–1280) | 0.03 |
| Anti-Sm positivity, n (%) | 2 (15) | 9 (12) | 0.76 |
| CH50, U/ml | 14.5 (0–46.6) | 25.5 (0–49.8) | 0.02 |
| C3, mg/dl | 40 (22–99) | 59 (21–129) | 0.007 |
| C4, mg/dl | 10 (2–19) | 13 (1–40) | 0.08 |

P values were estimated to allow comparisons between ISN/RPS class III or IV and others. ISN/RPS: International Society of Nephrology/Renal Pathology Society; PSL: prednisolone; IA: immunosuppressive agents; eGFR: estimated glomerular filtration rate.

the development of ISN/RPS class III or IV lupus nephritis, a cutoff level for the clinical measures was estimated by calculating the receiver-operating characteristic curve. Sensitivities, specificities, positive predictive value (PPV), and negative predictive value (NPV) are shown in Table 6. In the patients of the overt subset, sensitivities and specificities were 61% and 74%, for a cutoff level of 1120 mg/day for proteinuria (OR 4.6, p = 0.0003); 56% and 80%, for a cutoff level of 63.8 ml/min/1.73 $m^2$ for eGFR (OR 4.9, p = 0.0004); and 47% and 87%, for a cutoff level of 75 IU/ml for the anti-dsDNA antibody (OR 6.1, p = 0.0003), respectively. PPV and NPV were about 80% and 50%, respectively, for each clinical measure within the patients of the overt subset. In contrast, in the patients of the silent subset, the sensitivities and specificities were 85% and 53%, for a cutoff level of 40 IU/ml for anti-dsDNA antibody (OR 6.3, p = 0.015); 85% and 58%, for a cutoff level of 55 mg/dl for C3 (OR 7.5, p = 0.0063); and 77% and 73%, for cutoff levels of both 40 IU/ml for anti-dsDNA antibodies and 55 mg/dl for C3 (OR 8.8, p = 0.0011). PPV and NPV were about 20%–30% and 95%, respectively, for each clinical measure among the patients of the silent subset.

## DISCUSSION

We have demonstrated for the first time, to our knowledge, the frequency and predictive factors for ISN/RPS class III or IV lupus nephritis in patients with SLE without clinical renal involvement. Numerous studies have indicated that proteinuria (> 0.5 g daily) might be indispensable for active nephritis confirmed by renal biopsy. However, our data reveal that 15% of patients without clinical renal involvement showed ISN/RPS class III or IV lupus nephritis pathohistologically — a surprisingly high percentage. In the patients without clinical renal involvement, the factors predicting ISN/RPS class III or IV lupus nephritis may include long disease duration, high anti-dsDNA antibody titer, and low concentration of C3. These results suggest that the duration and intensity of immune complex-associated inflammation could contribute to the development of ISN/RPS class III or IV lupus nephritis.

It has been reported that the majority of patients with SLE had immune deposits in their kidneys, which were revealed by immunofluorescence or electron microscopy[11,16,17]. Additionally, our study showed that the disease duration was longer and the frequency of class III or IV was higher in patients with clinical renal involvement than in those without clinical renal involvement. These results indicate that disease duration is important in the development and severity of lupus nephritis. Renal disease develops within the first 3 years following the SLE diagnosis[18,19]. In our study, the renal pathohistological findings were normal in some patients, although some had elevated anti-dsDNA antibodies (up to 270 IU/ml) or decreased complement components (C3 down to 42 mg/dl). In these patients, the disease duration was short (< 1 year). These results may reflect the existence of an early phase of SLE before clinically apparent renal disease is detectable. In contrast, our study showed that the median of disease duration was 5 years in ISN/RPS class III or IV lupus nephritis without clinical renal involvement. The disease duration was signifi-

*Table 6.* Predictors for ISN/RPS class III or IV lupus nephritis.

| Patient Group | Sensitivity, % | Specificity, % | PPV, % | NPV, % | OR (95% CI) | p |
|---|---|---|---|---|---|---|
| Patients of the overt subset, n = 109 | | | | | | |
| Proteinuria, ≥ 1120 mg/day | 61 | 74 | 81 | 52 | 4.6 (1.9–11.0) | 0.0003 |
| eGFR, ≤ 63.8 ml/min/1.73 $m^2$ | 56 | 80 | 83 | 50 | 4.9 (2.0–12.1) | 0.0004 |
| Anti-dsDNA, ≥ 75 IU/ml | 47 | 87 | 87 | 48 | 6.1 (2.2–17.3) | 0.0003 |
| Patients of the silent subset, n = 86 | | | | | | |
| Anti-dsDNA, ≥ 40 IU/ml | 85 | 53 | 24 | 95 | 6.3 (1.3–30.5) | 0.015 |
| C3 ≤ 55 mg/dl | 85 | 58 | 24 | 95 | 7.5 (1.5–36.1) | 0.0063 |
| Anti-dsDNA, ≥ 40 IU/ml and C3 ≤ 55 mg/dl | 77 | 73 | 33 | 95 | 8.8 (2.2–35.4) | 0.0011 |

ISN/RPS: International Society of Nephrology/Renal Pathology Society; PPV: positive predictive value; NPV: negative predictive value; eGFR: estimated glomerular filtration rate.

cantly longer (p = 0.0184) in patients with ISN/RPS class III or IV nephritis than in those without ISN/RPS class III or IV, among patients without clinical renal involvement. Chronic lesions, such as those due to global sclerosis, were observed more frequently than expected in patients without clinical renal involvement. These findings indicate that chronic inflammation can occur latently over several years, even in patients without clinical renal involvement. Renal function and urinary findings should be observed regularly in patients without clinical renal involvement, especially in those with long disease duration, such as > 5 years following SLE diagnosis. These careful observations can determine the appropriate period for performing renal biopsy and treatment and help prevent the development of ISN/RPS class III or IV lupus nephritis.

Anti-dsDNA antibody titers and complement fractions are useful in assessing SLE disease and renal activity[7,8,9]. The prognostic factors for lupus nephritis were divided into renal and nonrenal factors[19]. Renal dysfunction at presentation is associated with a poor prognosis, and a delay in starting immunosuppressive therapy significantly predicts renal failure and death from renal disease[20,21]. Nonrenal prognostic factors include male sex, hematological features such as thrombocytopenia and leukopenia, a younger age at diagnosis, persistent hypocomplementemia, increased anti-dsDNA antibody after treatment, and antiphospholipid antibody[18,19]. In particular, disease vintage, persistent hypocomplementemia, and high anti-dsDNA antibody after treatment have been found to predict renal relapse and mortality[22,23]. Additionally, the persistent elevation of anti-dsDNA antibody and low levels of complement components contributed to the development of overt lupus nephritis in patients with silent lupus nephritis for at least 24 months[15]. Although our study showed that the frequency of ISN/RPS class III or IV lupus nephritis was higher in patients with clinical renal involvement than in those without clinical renal involvement, hypocomplementemia and high anti-dsDNA antibody titers were revealed in both subsets. ISN/RPS class III or IV lupus nephritis without clinical renal involvement was associated with a decrease in C3 and an increase in anti-dsDNA antibody titer, suggesting that hypocomplementemia and high anti-dsDNA antibody titers are correlated with ISN/RPS class III or IV lupus nephritis. Clinical measures, including complement and anti-dsDNA antibody, should be monitored carefully in patients with SLE who do not have clinical renal involvement.

We also investigated the predictive factors of ISN/RPS class III or IV lupus nephritis in patients with SLE and without findings of clinical renal involvement, such as renal dysfunction, proteinuria, and active urinary sediments. First, in patients with SLE who have findings of clinical renal involvement, our study demonstrates that renal biopsy is recommended to confirm ISN/RPS class III or IV lupus nephritis in patients with proteinuria ≥ 1120 mg/day, eGFR ≤ 63.8 ml/min/1.73 $m^2$, or anti-dsDNA antibody > 75 IU/ml. On the other hand, our study reveals that the nephritis was found in 58% of the SLN subset. Additionally, ISN/RPS class III or IV was found in 15% of patients without clinical renal involvement. We performed further analysis to distinguish patients with ISN/RPS class III or IV lupus nephritis from those with other classes (Nil, class I, II, or V) using cutoff values for anti-dsDNA antibodies and C3. Our study shows that the PPV and NPV for ISN/RPS class III or IV lupus nephritis were about 20%–30% and 95% for each clinical measure in patients without clinical renal involvement. These results indicate that renal biopsy should not be recommended in patients with SLE without clinical renal involvement if they have anti-dsDNA antibody < 40 IU/ml and C3 > 55 mg/dl. However, it is difficult to decide whether renal biopsy should be performed in patients with SLE who do not have clinical renal involvement if they have anti-dsDNA antibody ≥ 40 IU/ml and/or C3 ≤ 55 mg/dl, because the PPV is low. Some believe that performing a renal biopsy to predict development of overt lupus nephritis (OLN) makes no sense in patients with SLE without findings of clinical renal involvement because almost all patients with SLN showed mild histological changes and a good prognosis[24]. It has been reported that endstage renal failure in patients with SLN is rare regardless of the histopathological renal lesions and that it is prudent to do a biopsy on patients with SLE in the absence of overt renal involvement, and to treat those with diffuse proliferative glomerulonephritis[25,26]. However, it remains unknown whether renal biopsy should be performed in SLE without clinical renal involvement and whether cytotoxic therapy, such as intravenous cyclophosphamide, should be used in patients with SLN, as in OLN ISN/RPS class III or IV patients with SLE. In our study, 13 ISN/RPS class III or IV patients with SLE without clinical renal involvement received PSL alone as both induction therapy and maintenance therapy. There was no recurrence during observation (median 30 mo, range 14–178 mo) in all but 2 patients. Our result is compatible with the results of previous reports. These findings indicate that the degree of progression and severity of renal dysfunction was relatively mild in ISN/RPS class III or IV SLE patients without clinical renal involvement. The reason may be that cellular/fibrocellular crescents and GBM rupture were not detected in all patients without clinical renal involvement. This finding may mean that urinary findings and renal function reflect whether these lesions that extend inflammation to extracapillary spaces coexist. IA, such as cyclophosphamide, may not need to be administered to patients without clinical renal involvement when cellular/fibrocellular crescents and GBM rupture were not revealed in kidney specimens.

There was a patient selection bias in our study because the study subjects were not consecutive. Renal biopsies were not performed in 171 of 467 patients, and an additional 101 patients were excluded for several reasons. Therefore, the frequency of lupus nephritis was not reported accurately. On the other hand, 118 patients (61%) received corticosteroids and/or immunosuppressants at renal biopsy. These treatments may

mask clinical findings indicating lupus nephritis. These potential inaccuracies represent limitations in our study.

The actual frequency of nephritis was higher than expected in patients with SLE without clinical renal involvement. ISN/RPS class III or IV lupus nephritis could be hidden in patients with SLE who present both a high titer of anti-dsDNA antibody and a low concentration of C3, even when they exhibit clinically normal urinary findings and renal function.

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## Case Reports

# Risperidone in the Treatment of Corticosteroid-Induced Mood Disorders, Manic/Mixed Episodes, in Systemic Lupus Erythematosus: A Case Series

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Corticosteroid therapy is frequently associated with adverse psychiatric effects, including dysregulated mood psychosis and delirium.[1,2] Recent reports have shown that the effects of short-term, high-dose corticosteroid therapy appear to be primarily associated with manic rather than depressive or psychotic symptoms, or delirium, while depressive symptoms may be more common and severe than manic symptoms during long-term corticosteroid therapy at relatively low dosages.[1] Management includes tapering corticosteroids, with or without the addition of medications to treat acute psychiatric symptoms. Use of adjunctive psychotropic medications may be necessary, particularly if corticosteroids cannot be tapered or discontinued.[1,2]

However, little information is available on the treatment of corticosteroid-induced mood disorders (CIMDs). One controlled study suggested lithium therapy may prevent CIMDs. None of the 27 patients given open-label lithium developed CIMDs while receiving corticosteroids, whereas 6 (14%) of 44 retrospectively reviewed patients not receiving lithium developed CIMDs.[3] One open-label trial on olanzapine has been conducted in 12 patients with multiple medical diagnoses.[4] In several case reports/series, various classes of psychotropic medications, including second-generation antipsychotics (SGAs), e.g., olanzapine,[5] risperidone,[6–9] and quetiapine,[10] have been successfully used to treat CIMDs, mainly for manic symptoms. Mood stabilizers, e.g., lithium,[3] valproate,[11,12] and carbamazepine,[11] have also been used to treat CIMDs.

Overall, these reports have had methodical limitations that complicate the assessment of the effects of psychotropic medications, namely multiple medical diagnoses,[4] as well as the parallel effects of tapering the corticosteroids dosage during the study because of CIMDs dose-dependency.[13] To overcome these limitations, we describe the use of risperidone in the treatment of CIMDs with manic or mixed episodes in six female inpatients with systemic lupus erythematosus (SLE). Although SLE may be one of the major risk factors for the development of CIMDs,[2] few reports have been published on the treatment of CIMDs in SLE patients.[8,11,12]

*Case Report*

This case series is, in part, a subset of a previously reported cohort of consecutive inpatients with an SLE flare who were treated with corticosteroids in our rheumatologic unit from August 1999 to December 2004.[14] In the cohort, corticosteroid-induced psychiatric disorders (CIPDs) were defined as new-onset psychiatric disorders that developed within 8 weeks of corticosteroids administration and that resolved completely through a reduction in corticosteroid dosage and without additional immunosuppressive agents.[14] The psychiatric events were evaluated at regular intervals (once a week), as well as on the request

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of the rheumatologic team, by experienced psychiatrists (KN and MO) using the DSM-IV criteria for substance-induced disorders. In the 139 episodes in 135 SLE inpatients without current overt central nervous system manifestations of SLE (CNS-SLE), the above-defined CIPDs occurred in 14 (10.1%) episodes in 14 inpatients. Psychotic disorders occurred in one (7%) of the 14 events, and mood disorders (CIMDs) occurred in 13 events (93%), including major depressive episodes in two (14%), manic episodes in nine (64%), and mixed episodes in two (14%).[14]

In the first half of the cohort, we primarily used haloperidol to treat CIMDs with manic or mixed episodes. To avoid adverse effects, such as the extrapyramidal symptoms associated with haloperidol, we used risperidone to treat six consecutive inpatients with these episodes, including five inpatients who were enrolled in the second half of the cohort from January 2002 to December 2004 (case 1, 2, 3, 4, and 5) and one inpatient, the first CIMD case in our rheumatologic unit after the above cohort periods (case 6). Risperidone was selected because other SGAs, e.g., olanzapine and quetiapine, were both contraindicated in patients with diabetes mellitus, a condition that corticosteroids induce with high frequency in Japan. Lithium was also avoided because lupus can also induce nephritis.

All patients provided written informed consent about its potential risks and the off-label use of risperidone for the treatment of CIMDs. The psychiatric follow-up was conducted at regular intervals, once a week, as well as on the request of the rheumatologic team. If patients were discharged from the hospital, the follow-up was continued at the outpatient clinic. Concerning additional psychotropic medications given, only benzodiazepines were used as needed.

The results of laboratory and neurologic tests, (including computed tomography/magnetic resonance imaging in the brain, electroencephalography, and cerebrospinal fluid analysis), showed no evidence of CNS-SLE or secondary causes in our six patients. All patients were Japanese, without any previous history of psychiatric or neurologic illness, and they fulfilled the American College of Rheumatology 1982 revised criteria for SLE.[15] The mean score of the SLE Disease Activity Index[16] at baseline was 16.3 points (range, 10–22 points), indicating active SLE episodes that required high-dose corticosteroid therapy. Case 2 had a history of Hashimoto's thyroiditis, and case 4 had a history of a dilated cardiomyopathy. Both comorbid diseases were under good control during this study.

**TABLE 1. Clinical Characteristics and Course of Corticosteroid-Induced Mood Disorders Treated with Risperidone in Patients with Systemic Lupus Erythematosus**

| Case | Age, Years/ Gender | SLEDAI | PSL Initial Dose mg | Interval[a] Days | Psychiatric Features | Course of RPD Treatment | | | | | Marked Adverse Effects of RPD | PSL Dose at Recovery, mg |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | RPD Initial/Maximum Dose, mg | YMRS | | | Duration[b] Days | | |
| | | | | | | | Baseline | 7 Days | 14 Days | 28 Days | | |
| 1 | 36/F | 22 | 50[c] | 5 | Manic | 2/2 | 20 | 12 | 8 | 4[d] | 40/40 | None | 40 |
| 2 | 19/F | 12 | 50 | 2 | Mixed | 2/2 | 23 | 18 | 14 | 7[d] | 56/240[e] | None | 15 |
| 3 | 22/F | 10 | 60 | 28 | Mixed | 1/2 | 30 | 15 | 8 | 1[d] | 35/90[e] | None | 27.5 |
| 4 | 62/F | 22 | 60 | 18 | Manic | 1/2 | 27 | 6 | 0[d] | 0[d] | 14/14 | Sedation | 45 |
| 5 | 25/F | 22 | 40 | 13 | Manic | 4/9 | 43 | 16 | 8 | 0[d] | 28/28 | Parkinsonism | 32.5 |
| 6 | 35/F | 10 | 50 | 10 | Manic | 1/1 | 26 | 16 | 10 | 4[d] | 56/56 | None | 35 |

SLEDAI = systemic lupus erythematosus disease activity index; PSL = prednisolone; RPD = risperidone; YMRS = Young mania rating scale.

[a] Interval between steroid administration and psychiatric manifestation.

[b] Duration of RPD treatment/duration of psychiatric events.

[c] Methylprednisolone (1 g/day) for 3 days initially administrated.

[d] On an additional effect of PSL dose tapering.

[e] Recovered via major depressive episodes. In *case 2*, the mixed episode was ameliorated with RPD in 56 days and transitioned to a severe major depressive episode that required antidepressants. In *case 3*, the mixed episode was ameliorated with RPD in 35 days and transitioned to a mild major depressive episode that improved after the discontinuation of RPD without using antidepressants.