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

Katsuji Nishimura, M.D., Ph.D., Masako Omori, M.D., Eri Sato, M.D., Yasuhiro Katsumata, M.D., Ph.D., Takahisa Gono, M.D., Ph.D., Yasushi Kawaguchi, M.D., Ph.D., Masayoshi Harigai, M.D., Ph.D., Hisashi Yamanaka, M.D., Ph.D., Jun Ishigooka, M.D., Ph.D.

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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From Department of Psychiatry, Tokyo Women's Medical University School of Medicine, Tokyo, Japan (KN, MO, JI); Institute of Rheumatology, Tokyo Women's Medical University, Tokyo, Japan (ES, YK, TG, YK, HY); Department of Pharmacovigilance, Graduate School, Tokyo Medical and Dental University, Tokyo, Japan (MH). Send correspondence and reprint requests to Katsuji Nishimura, M.D., Ph.D., Dept. of Psychiatry, Tokyo Women's Medical University School of Medicine, 8-1 Kawada-cho, Shinjuku-ku, Tokyo, 162-8666, Japan; e-mail: knishimura@psy.twmu.ac.jp

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					Duration[b] Days	Marked Adverse Effects of RPD	PSL Dose at Recovery, mg
						RPD Initial/Maximum Dose, mg	YMRS						
							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.

Results

Clinical characteristics and the course of CIMDs treated with risperidone in the present six patients with SLE are shown in Table 1. Corticosteroids were administrated at a mean dosage of 51.7 mg/d (range, 40–60 mg/d) as prednisolone. This was the peak dosage of corticosteroids in the course of CIMDs. New-onset mood disorders, accompanied by manic features in four patients and mixed features in two, developed in a mean of 12.7 days (range, 2–28 days) of corticosteroid administration.

The mean score of the Young Mania Rating Scale (YMRS)[17] at baseline (at the point that psychiatric consultants saw the patients and diagnosed CIMD, at which point a decision was made to start risperidone) was 28.2 points (range, 20–43 points). Risperidone was started at a mean of 1.8 mg/d (range, 1–4 mg/day) and was flexibly increased up to a mean of 3.0 mg/d (range, 1–9 mg/d). The YMRS score was decreased to a mean of 13.8 points (range, 6–18 points) in 1 week and 8.0 points (range, 0–14 points) in 2 weeks. During this period, the corticosteroid dosage was the same as that at baseline, except for case 4. Four weeks later, the YMRS score was further decreased to a mean of 2.7 points (range, 0–4 points). At this point, the tapering of corticosteroid dosage had already started in all patients. Subsequently, all CIMDs in these six patients were resolved completely during the reduction in corticosteroid dosage without additional immunosuppressive agents, and risperidone was discontinued. The mean dosage of corticosteroids at this time was 32.5 mg/d (range, 15–45 mg/d) given as prednisolone.

Cases 2 and 3, with mixed episodes, recovered via a major depressive episode. Case 2's mixed episode at baseline (YMRS 23; Hamilton Depression Rating Scale [HAM-D] 27) was ameliorated with risperidone in 56 days, but a severe major depressive episode developed (HAM-D 31). We used milnacipran, a serotonin-noradrenalin reuptake inhibitor, up to 100 mg/d, but it was not effective. Next, we switched to dosulepin (75 mg/d) with good results. She recovered in a total of 240 days. In case 3, the mixed episode at baseline (YMRS 30; HAM-D 28) was ameliorated with risperidone in 35 days but shifted to a mild major depressive episode (HAM-D 13) that improved gradually after the discontinuation of risperidone without using antidepressants. She recovered in a total of 90 days.

These six patients tolerated risperidone well, except for sedation in case 4 and mild Parkinsonism in case 5. Metabolic effects of risperidone in combination with corticosteroids are

TABLE 2. Metabolic Effects of Risperidone Treatment in Combination with Corticosteroids

Case	Duration of RPD Treatment, Days	Body Weight, kg		FPG, mg/dl		LDL-C, mg/dl		HDL-C, mg/dl		TG, mg/dl		Parallel Medication for	
		Baseline	Post-Treatment	Baseline	Post-Treatment	Baseline	Post-Treat	Baseline	Post-Treat	Baseline	Post-Treatment	Diabetes	Dyslipidemia
1	40	43.6	40.8	72	68	156*	116	74	96	239*	284*	No	Yes
2	56	53.2	51.0	94	93	170*	154*	49	58	259*	230*	No	Yes
3	35	58.4	56.4	98	75	139	130	80	84	142	153*	No	No
4	14	65.0	64.0	123*	119*	180*	154*	77	74	104	123	No	No
5	28	45.2	45.6	89	75	166*	188*	47	34*	149	173*	No	Yes
6	56	49.0	50.2	94	84	131	146*	61	66	113	245*	No	No

RPD = risperidone; FPG = fasting plasma glucose; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; TG = triglycerides.
* Abnormal; FPG ≥ 100 mg/dl; LDL-C ≥ 140 mg/dl; HDL-C < 40 mg/dl; TG ≥ 150 mg/dl.

shown in Table 2. Abnormalities of fasting plasma glucose (≥100 mg/dL) at baseline vs. post-risperidone-treatment were detected in 1 vs. 1 of the six patients; low-density lipoprotein (LDL) cholesterol (≥140 mg/dL) in 4 vs. 4; high-density lipoprotein cholesterol (<40 mg/dL) in 0 vs. 1; triglycerides (≥150 mg/dL) in 2 vs. 5. No patient took parallel medication for diabetes, while three patients (case 1, 2, and 5) took parallel medication for dyslipidemia during the risperidone treatment.

Discussion

In five of six patients in the present case series, the effectiveness of risperidone alone in the first 2 weeks was evident because the corticosteroids dosage was fixed in this period. In the following periods, recovery from CIMDs mirrored the tapering of the corticosteroids dosage, but risperidone may have assisted the recovery, although we could not clearly differentiate effects between the two.

As for adverse effects of risperidone treatment, sedation occurred in one patient, who had impaired renal function (serum creatinine, 1.19 mg/dL) caused by lupus nephritis. With lupus nephritis, a careful dose setting is required because the clearance of the active metabolite of risperidone is reduced in patients with renal diseases.[18] Parkinsonism occurred in one patient, when a high dose of risperidone was used (up to 9 mg/d), but it disappeared during the tapering of risperidone.

Combining an SGA such as risperidone with a corticosteroid could show an additive effect in terms of metabolic syndrome.[19] In our short-term observation, dyslipidemia, particularly abnormality of LDL cholesterol or triglycerides, was observed after the risperidone treatment in all cases, although the direct metabolic effect of risperidone could not be determined. Further controlled studies are necessary.

It is often difficult to distinguish CIMDs from CNS-SLE because no diagnostic gold standard for CNS-SLE exists[20] and because of the complicating fact that CNS-SLE manifestation can be triggered by corticosteroid therapy.[14] The present six patients could be diagnosed as definitely having CIMDs because of no findings suggesting CNS-SLE, as well as because of their complete recovery through a reduction in corticosteroid dosage without additional immunosuppressive agents. Strict definition of CIMDs should be required in the SLE population when extending the findings to other patient cohorts and medical groups.

Limitations of the present study include its small sample size, open-label design, and very specific cohort of patients: female Japanese, all having SLE. But our experience suggests risperidone is beneficial, especially in the first 2 weeks, in the treatment of CIMDs with manic or mixed features in patients with SLE, although the dosage reduction of corticosteroids contributes to the ultimate resolution of CIMDs. Further controlled studies are necessary.

Corticosteroids are the cornerstone of treatment for various inflammatory and immunologically mediated disorders, such as SLE. When CIMDs occur, corticosteroids cannot be rapidly tapered or discontinued in most cases given their risk/benefit rates. Our results may contribute to the guidance of clinicians with regard to the management of CIMDs in such cases.

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Original article

Up-regulated expression of *HLA-DRB5* transcripts and high frequency of the *HLA-DRB5*01:05* allele in scleroderma patients with interstitial lung disease

Toshio Odani[1], Shinsuke Yasuda[1], Yuko Ota[2], Yuichiro Fujieda[1], Yujiro Kon[1], Tetsuya Horita[1], Yasushi Kawaguchi[2], Tatsuya Atsumi[1], Hisashi Yamanaka[2] and Takao Koike[1]

Abstract

Objective. Interstitial lung disease (ILD) is a serious complication of SSc. We aimed to identify markers associated with SSc-related ILD.

Methods. RNA was prepared from the peripheral blood mononuclear cells of 14 SSc patients, divided into four different RNA pools according to the presence or absence of ILD and to the treatment, and subjected to microarray analysis. Real-time quantitative PCR was used to confirm the microarray results in 43 SSc patients, 42 autoimmune controls and 10 healthy controls. Genomic DNA samples were collected from 149 patients with SSc (70 in Hokkaido and 79 in Tokyo) who underwent a high-resolution CT for the evaluation of ILD and from 230 healthy controls. Genotyping was performed using sequence-specific primers.

Results. The microarray analysis revealed *HLA-DRB5* to be the only gene commonly up-regulated in patients with ILD compared with those without ILD in both comparison groups. High expression levels of *HLA-DRB5* in SSc patients with ILD were confirmed by real-time quantitative PCR. The prevalence of *HLA-DRB5* gene carriers increased in the SSc patients with ILD relative to those without ILD or to healthy controls in both cohorts. Among the four detected alleles, the *HLA-DRB5*01:05* allele was significantly more frequent in SSc patients with ILD than in SSc patients without ILD or in healthy controls. These associations were confirmed in the second cohort.

Conclusion. *HLA-DRB5* was highly expressed in PBMCs from patients with SSc-related ILD. The *HLA-DRB5*01:05* allele is a risk factor for ILD in patients with SSc.

Key words: SSC, human leucocyte antigen, pulmonary fibrosis, microarray, peripheral blood mononuclear cells, gene expression, risk factor.

Introduction

SSc is an autoimmune connective tissue disorder characterized by microvascular injury, skin fibrosis and distinctive visceral changes. SSc is a clinically heterogeneous disease, ranging from a mild form with less extensive involvement of the viscera to a more severe type with widespread visceral changes significantly affecting morbidity and mortality [1–4]. Interstitial lung disease (ILD), one of the most serious complications of SSc, develops in >50% of patients with SSc [5, 6]. In contrast to the recent improvements in the survival of patients with SSc complicated by renal crisis, the frequency of deaths due to ILD among SSc patients has increased significantly over the last 30 years, from 6 to 33% of SSc-related deaths [7]. Other studies have concurred that the presence of ILD is associated with a poorer prognosis in patients with SSc [8–10]. Although modest clinical efficacy of cyclophosphamide was reported in two high-quality randomized controlled trials [11, 12], and potentially effective treatments, such as mycophenolate mofetil,

[1]Department of Medicine II, Hokkaido University Graduate School of Medicine, Sapporo and [2]Institute of Rheumatology, Tokyo Women's Medical University, Tokyo, Japan.

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Correspondence to: Shinsuke Yasuda, Department of Medicine II, Hokkaido University Graduate School of Medicine, Sapporo 080-8638, N15, W7, Kita-ku, Japan. E-mail: syasuda@med.hokudai.ac.jp

imatinib mesylate or haematopoietic stem cell transplantation (HSCT), remain to be evaluated [13, 14], the available treatments targeting SSc-related ILD are still limited. Therefore the search for novel biomarkers or genetic predispositions specific for SSc-related ILD is critical to provide new insights into the disease process, potentially leading in the longer term to a better prognosis for this severe visceral complication. Genetic backgrounds, up-regulated cytokines/growth factors and signal molecules such as HLA, *PTPN22*, *CTGF*, *TGF-β* and *PDGF* have been implicated in the pathophysiology of SSc [15–17]. However, until recently, few studies have focused on specific biomarkers in SSc-related ILD.

In the present study we aimed to identify up-regulated molecule(s) in patients with SSc-related ILD based on an unbiased microarray analysis using pooled RNA samples. The analysis of four pools of RNA derived from peripheral blood mononuclear cells (PBMCs) identified *HLA-DRB5* as a candidate up-regulated gene in patients with ILD. We then confirmed its expression in individual patients and also genotyped the *HLA-DRB5* gene in two cohorts. Detailed genotyping led us to identify the specific *HLA-DRB5* allele **01:05* as a novel risk factor for SSc-related ILD.

Patients and methods

This study was approved by the ethical committees of the Hokkaido University Graduate School of Medicine and the Tokyo Women's Medical University. Informed patient consent was obtained for this study.

Global gene expression analysis using DNA microarray

We first evaluated global gene expression profiles in pooled RNA from the PBMCs of patients with SSc. Four sets of RNA pools were prepared from PBMCs from 14 SSc patients seen at the Hokkaido University Hospital. The first pool was prepared from four patients with ILD [three females, median age 46 (30–58) years, median disease duration 84 (70–119) months] who received only conventional therapies [prostaglandin and/or the following immunosuppressive agents: prednisolone ($n=1$), ciclosporin ($n=1$), tacrolimus ($n=1$) and CYC ($n=1$)]. The second pool was derived from four patients without ILD [three females, median age 60 (40–64) years, median disease duration 102 (60–234) months] who received conventional therapies [prostaglandin and/or immunosuppressive agents: ciclosporin ($n=1$)]. The third pool included four patients with ILD [three females, median age 39 (22–61) years, median disease duration 93 (66–103) months] who received HSCT. The last array comprised two patients without ILD [one female, median age 49 (33–65) years, median disease duration 93 (54–132) months] who received HSCT. The mixed RNA samples in each pool were subjected to a single DNA microarray (Affymetrix GeneChip Human Gene 1.0 ST Array, Affymetrix, Santa Clara, CA, USA). Data were analysed using GeneSpring GX version 10.0 (Agilent Technologies, Inc., Santa Clara, CA, USA). The fluorescence intensity results were defined as RAW normalized according to the Robust Multi-array Average algorithm. To normalize the treatment background of the patients, the first comparison performed was with the conventional therapy group, and the second was with the HSCT group. Up-regulated genes were defined as those for which the gene expression in the first or the third pool increased more than 3-fold relative to that in the second or the fourth pool, respectively.

Quantitative real-time PCR

All patients were observed at the Hokkaido University Hospital. Our subjects comprised 43 SSc patients {25 with ILD [19 females, median age 55 (18–77) years, median disease duration 74 (9–282) months] and 18 without ILD [15 females, median age 59 (28–83) years, median disease duration 84 (4–345) months]}. Among the patients with ILD, 5 were treated by HSCT and 20 were on immunosuppressive agents [prednisolone ($n=10$), ciclosporin ($n=6$), tacrolimus ($n=4$), CYC ($n=2$) and D-Pen ($n=3$)]. Among the patients without ILD, 3 were treated by HSCT, and 15 were on prostaglandin and/or immunosuppressive agents [prednisolone ($n=6$), ciclosporin ($n=2$) and D-Pen ($n=5$)]. As control groups, PBMCs were also collected from 42 patients with other autoimmune diseases [RA ($n=20$ and DM/PM ($n=22$)] and from 10 healthy controls. The expression levels of *HLA-DRB5* and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) in each individual were evaluated by real-time quantitative PCR using TaqMan Gene Expression assays (*HLA-DRB5* assay ID: Hs03046116_m1, GAPDH assay ID: Hs9999905_m1), TaqMan Universal PCR Master Mix and the ABI PRISM 7000 sequence detection system (Applied Biosystems, Foster City, CA, USA). Relative quantification was performed using the comparable cycle threshold (CT) method in which ΔCT is defined as the level of the HLA-DRB5 transcript in the RNA sample relative to that of the GAPDH transcript, as previously reported [18].

Genomic DNA extraction and genotyping of *HLA-DRB5* and *HLA-DRB1*

The first cohort comprised 70 Japanese patients with SSc who visited the Hokkaido University Hospital and fulfilled the 1980 criteria of the ACR [19] and 147 disease-free Japanese volunteers residing in the Hokkaido area as healthy controls. Patients with SSc were classified as either having ILD ($n=41$) or not having ILD ($n=29$) based on HRCT, as described in the next subsection. Of the 41 patients with ILD, 6 were treated with HSCT using autologous peripheral blood stem cells, and the other 35 patients received conventional therapy. HSCT was performed as previously described [20]. Of the six patients who received HSCT, three also received ciclosporin for recurrent or progressive scleroderma. Conventional therapy included prostaglandin and immunosuppressive agents [prednisolone ($n=16$), ciclosporin ($n=5$), tacrolimus ($n=7$), CYC ($n=2$) and/or D-Pen ($n=5$)]. Of the 29 patients without ILD, 4 were treated with HSCT and the other 25 with conventional therapy. Patients with other autoimmune diseases comprised 20 with RA and

29 with DM or PM. RA patients fulfilled the ACR criteria [21], and DM/PM patients fulfilled the Bohan and Peter criteria [22, 23]. The second cohort comprised 83 disease-free Japanese volunteers residing in the Tokyo area and 79 Japanese patients with SSc observed at the Tokyo Women's Medical University Hospital. These patients with SSc were classified as having ILD ($n = 40$) or as not having ILD ($n = 39$) (Table 1).

Genomic DNA samples were extracted using DNA Quick II (DS Pharma Biomedical Co. Ltd, Osaka, Japan), according to the manufacturer's instructions. *HLA-DRB5* genotyping was performed by PCR with sequence-specific primers (PCR-SSP) using Olerup SSP *HLA-DRB5* (Olerup SSP AB, Saltsjöbaden, Sweden). All of the 19 known alleles of *HLA-DRB5* are theoretically identifiable using this PCR-SSP method [24, 25]. The *HLA-DRB1* alleles were determined using the PCR-SSOP Luminex method with LABType SSO (One Lambda Inc., Canoga Park, CA, USA), a reverse SSO DNA typing system, according to the manufacturer's instructions.

Evaluation of pulmonary and other organ involvement

The presence or absence of ILD was evaluated using HRCT in all of our patients with SSc. The diagnosis of ILD was made when one or more of the following features were evident: isolated ground-glass opacities, honeycombing, ground-glass attenuation and/or traction bronchiectasis. After the diagnosis of ILD was made, the HRCT findings were classified into the following: non-specific interstitial pneumonia (NSIP), usual interstitial pneumonia (UIP), or organizing pneumonia (OP). Pulmonary hypertension (PH) was screened for by echocardiography and confirmed by right-heart catheterization [26]. Other organ involvement was defined as follows: renal (renal crisis in patients with SSc and nephritis in other autoimmune diseases), cardiac (arrhythmia), joint (arthritis) and muscle (continuous increase in serum creatinine kinase and/or serum aldolase levels, electromyogram and/or muscle biopsy).

Autoantibodies

ANA was detected with IIF. A titre of 1:80 or more was considered to be positive. Anti-topo-I antibody (anti-Topo-I), ACA, anti-U1-RNP antibody and anti-SS-A antibody levels were examined using enzyme-linked immunoassays (MESACUP, Medical & Biological Laboratories Co., Ltd, Japan), RF levels were evaluated by the latex turbidimetric assay (N-assay TIA RF Nittobo, Nittobo

Table 1 Clinical features of the patients with SSc and the autoimmune controls

Characteristics		First cohort			Second cohort
		SSc ($n = 70$)	RA ($n = 20$)	DM/PM ($n = 29$)	SSc ($n = 79$)
Gender (female/male)		55/15	16/4	22/7	73/6
Age, mean (s.d.), years		51.0 (13.6)	54.4 (13.8)	52.2 (14.2)	41.0 (12)
Disease duration, median (range), months		26.0 (2–516)	86.0 (4–408)	24.0 (1–380)	ND
Smokers, *n*		28	10	9	ND
Diffuse/limited		45/25			48/31
Organ involvement, *n* (%)					
ILD		41 (58.6)	7 (35)	17 (58.6)	40 (50.6)
HRCT findings					
NSIP		25	5	15	ND
UIP		16	1	0	ND
OP		0	1	2	ND
PH		11 (15.7)	0 (0)	1 (3.4)	8 (10.1)
Digital ulcer		6 (8.6)	0 (0)	0 (0)	14 (17.7)
Renal		7 (10.0)	0 (0)	3 (10.3)	0 (0)
Cardiac		7 (10.0)	0 (0)	8 (27.6)	ND
Joint		9 (12.9)	20 (100)	5 (17.2)	ND
Muscle		2 (2.9)	1 (5.0)	29 (100)	ND
Autoantibodies, *n* (%)					
ANA	⩾1:80	69 (98.6)	16 (80.0)	19 (65.5)	79 (100)
aTopo-I	>18.9 INDEX	22 (31.4)	NA	NA	29 (36.7)
ACA	>15.9 INDEX	18 (25.7)	NA	NA	16 (20.2)
aU1-RNP	>12.9 INDEX	11 (15.7)	NA	NA	NA
RF	>16.3 U/L	7 (10.0)	16 (80.0)	8 (27.6)	NA
aJo-1	>12.9 INDEX	0 (0)	NA	8 (27.6)	NA
aSS-A/Ro	>9.9 INDEX	17 (24.3)	NA	NA	NA
aDNA	>6 U/L	4 (5.7)	NA	NA	NA

Organ involvement was defined as follows: lung (interstitial pneumonia as shown by HRCT, with findings based on radiologists' interpretation), renal (renal crisis in patients with SSc and nephritis in patients with other diseases), cardiac (arrhythmia), joint (arthritis), muscle (continuously increased serum creatinine kinase or serum aldolase, or both). aTopo-I: anti-topoisomerase-I; aU1-RNP: anti-U1-RNP antibody; aSS-A/Ro: anti-SS-A antibody; NA: not available; ND: no data.

Medical Co. Ltd, Tokyo, Japan). Anti-DNA (aDNA) antibody levels were measured using radioimmunoassay (SRL, Inc., Japan).

Isolation of PBMCs and RNA extraction

PBMCs were obtained from heparinized venous blood using gradient centrifugation over Ficoll-Paque PLUS (Amersham Biosciences Corp., NJ, USA). Total RNA levels from PBMCs were isolated using the RNeasy Mini Kit (QIAGEN Science, Germantown, MD, USA).

Statistical analysis

Calculations were performed using the statistical software package SPSS statistics (version 19.0). Comparisons of mRNA expression in PBMCs were performed using the Mann–Whitney test. Univariate analyses were performed using the chi-squared test and Fisher's exact test. When a value was zero, this number was replaced by 0.5 to perform the chi-squared test. With regard to multiplicity, the Bonferroni correction was used to adjust the significance levels for the analysis of frequencies of the *HLA-DRB5* and *HLA-DRB1* alleles. Other analyses, namely the analysis of the prevalence of carriers of *HLA-DRB5* and the comparisons of demographic and clinical parameters of SSc patients with and without *HLA-DRB5*01:05*, were regarded as exploratory, and therefore no corrections for multiple testing were performed. The demographic variables, clinical independent variables and genetic risk factors were evaluated for their associations with ILD in multivariate logistic regression analyses.

Results

Evaluation of global gene expression

In the patients who received HSCT, two genes were up-regulated in patients with ILD relative to those without ILD. In the conventional treatment group, 17 genes were up-regulated in patients with ILD compared with those without ILD. Of these genes, *HLA-DRB5* was the only commonly up-regulated gene in both comparisons (Table 2).

Quantitative real-time PCR

HLA-DRB5 gene expression levels were higher in the PBMCs from SSc patients with ILD than in those patients without ILD (Fig. 1). *HLA-DRB5* expression levels were related neither to disease duration nor to the presence or absence of immunotherapy (data not shown). In the relatively small numbers of patients with other autoimmune disease, there were apparently no differences in the levels of *HLA-DRB5* gene expression between patients with ILD and those without ILD or healthy controls.

HLA-DRB5 genotyping (the first cohort)

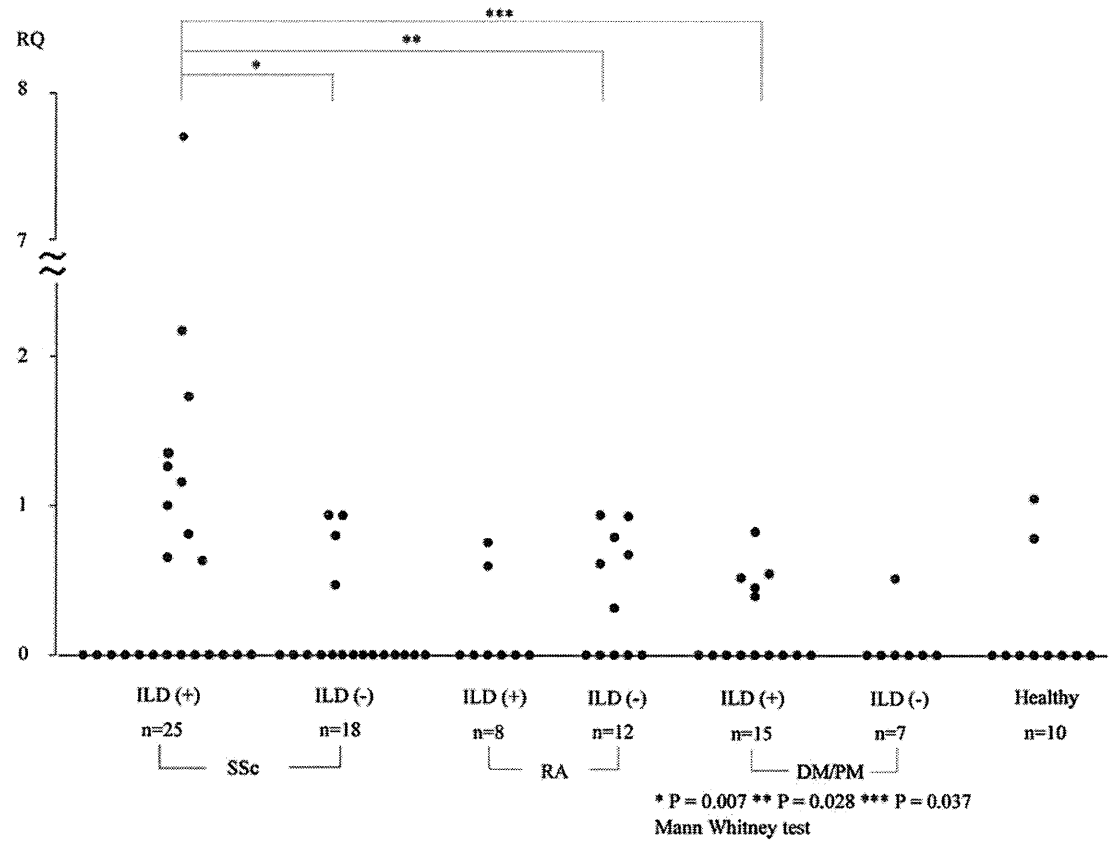
The prevalence of *HLA-DRB5* gene carriers among SSc patients was not significantly different from that in healthy controls. The prevalence of *HLA-DRB5* gene carriers in patients with RA or DM/PM was also not different from

Table 2 Up-regulated genes in PBMCs from SSc patients with ILD as evaluated by DNA microarray

Classification	Up-regulated genes	GenBank accession number	RAW data		Ratio (fold increase)
			ILD$^+$	ILD$^-$	
Conventional therapy group					
Human lymphocyte antigen	*HLADRB5*	M20429	652.54	202.01	3.23
Membrane receptors	IL8RA	L19591	1502.38	345.52	4.35
	IL8RB	L19593	1405.91	351.07	4.00
	FFAR2	BC096198	697.9	214.9	3.25
Signalling intermediates	CYP4F3	AB002454	746.32	150.11	4.97
	PTGS2	AY151286	1381.82	324.75	4.25
	MME	J03779	724.01	171.75	4.22
	PROK2	AF333025	1186.54	289.64	4.10
	KCNJ15	BC013327	285.81	89.85	3.18
	ALPL	BC090861	473.19	152.16	3.11
	CNTNAP3B	AL353791	102.19	33.30	3.05
	MGAM	AF016833	421.15	138.45	3.04
Translation initiation factor	EIF1AY	AF000987	331.90	81.58	4.07
snRNA	SNOAD13	X58062	4821.71	1184.11	4.07
	RNU5E	M77839	2884.99	759.51	3.80
	SNOAD60		605.15	193.12	3.13
	SNOAD41	X96640	866.85	283.01	3.06
HSCT group					
Human lymphocyte antigen	*HLADRB5*	M20429	1083.57	298.12	3.63
Signalling intermediates	ERAP2	AB163917	999.11	259.17	3.86

snRNA: small nuclear ribonucleic acid.

FIG. 1 Quantitative evaluation of *HLA-DRB5* transcripts in PBMCs.



PBMCs collected from 43 SSc patients (25 with ILD and18 without ILD), 42 patients with other autoimmune diseases [RA ($n = 20$), DM/PM ($n = 22$)] and 10 healthy controls were evaluated for the levels of *HLA-DRB5* transcripts using real-time qPCR. Relative quantification was performed using the C_T method, in which ΔC_T is defined as the level of *HLA-DRB5* transcript in the RNA sample relative to that of the GAPDH transcript.

that in healthy controls. However, the prevalence of *HLA-DRB5* gene carriers was higher in SSc patients with ILD than in those without ILD or in healthy controls. In contrast, *HLA-DRB5* was not associated with ILD in patients with RA or DM/PM, although the sample size for our autoimmune control group was relatively small (Table 3). In patients with SSc, *HLA-DRB5* was not associated with other clinical complications such as PH, renal crisis or digital ulcer in patients with SSc or in the presence or absence of the evaluated autoantibodies (data not shown).

Genotyping of the *HLA-DRB5* gene revealed 4 of the known 19 alleles in the SSc patients and healthy controls: **01:01*, **01:02*, **01:05* and **02:02*. Among the different *HLA-DRB5* alleles, the *HLA-DRB5*01:05* allele was significantly more frequent in SSc patients than in the controls. More specifically, *DRB5*01:05* was significantly more frequent in SSc patients with ILD, but not in SSc patients without ILD, compared with healthy controls (Table 4). In addition, the *DRB5*01:05* allele was more frequent in SSc patients with ILD than in those without ILD.

In the univariate analysis, the presence of the *DRB5*01:05* allele was associated with PH. However, no other clinical features, including age, gender, disease duration, renal crisis and all of the tested autoantibodies, were related to the presence of the *DRB5*01:05* allele (Table 5). The *DRB5*01:05* allele was not associated with ILD in patients with RA or PM/DM (data not shown).

HLA-DRB1 genotyping (the first cohort)

The frequencies of the *HLA-DRB1* alleles in the 70 SSc patients from the first cohort are shown in supplementary data Table 1, available as supplementary data at *Rheumatology* Online. A total of 20 *HLA-DRB1* alleles were identified. Of these alleles, the *HLA-DRB1**15:02 allele was more frequent in SSc patients with anti-Topo-I (50.0%) than in those patients without anti-Topo-I (20.8%, $P = 0.013$) but was not significantly increased in

TABLE 3 Prevalence (%) of *HLA-DRB5* carriers among patients with SSc, RA, DM/PM and in healthy controls

Patient group	*n*	Prevalence (%)	OR (95% CI) *vs* control	OR (95% CI) *vs* ILD (–)
First cohort				
SSc				
Total	70	41.4	1.66 (0.92, 2.99)	3.30** (1.16, 9.41)
ILD $^{+}$	42	52.4	2.58* (1.28, 5.19)	
ILD $^{-}$	28	25.0	0.78 (0.31, 1.97)	
RA				
Total	20	30.0	1.00 (0.36, 2.78)	0.90 (0.12, 6.78)
ILD $^{+}$	7	28.5	0.94 (0.17, 5.01)	
ILD $^{-}$	13	30.8	1.04 (0.30, 3.56)	
DM/PM				
Total	29	34.6	1.05 (0.44, 2.50)	0.83 (0.17, 4.09)
ILD +	17	29.4	0.98 (0.32, 2.93)	
ILD –	12	33.3	1.17 (0.33, 4.09)	
Controls	147	29.9		
Second cohort				
SSc				
Total	79	43.0	1.33 (0.71, 2.51)	2.75**** (1.09, 6.92)
ILD +	40	55.0	2.16*** (1.00, 4.65)	
ILD –	39	23.4	0.79 (0.35, 1.77)	
Controls	83	36.1		

*$P = 0.012$, **$P = 0.022$, ***$P = 0.047$, ****$P = 0.030$. The chi-squared test or Fisher's exact test was employed. OR: odds ratio.

SSc patients with ILD relative to those without ILD. The *HLA-DRB1*04:03* and *DRB1*08:02* alleles were less frequent in SSc patients with ILD than in those without ILD. The frequencies of the other *HLA-DRB1* alleles did not significantly differ between SSc patients with and without ILD.

Multivariate analysis (the first cohort)

In a multivariate analysis composed of clinical and demographic parameters after the inclusion of the HLA alleles, we identified *HLA-DRB5*01:05* and anti-Topo-I as independent risk factors for ILD ($P = 0.024$ and 0.011, respectively). Other parameters, such as *HLA-DRB1*15:02* or other *DRB5* alleles, were not significantly associated with ILD (supplementary data Table 2, available as supplementary data at *Rheumatology* Online).

HLA-DRB5 genotyping (the second cohort)

The prevalence of *HLA-DRB5* gene carriers in SSc patients was not significantly different from that in healthy controls. The prevalence of *HLA-DRB5* gene carriers was higher in SSc patients with ILD than in those without ILD or in healthy controls (Table 2). Neither clinical complications, such as PH, renal crisis or digital ulcer, nor measured autoantibody levels were associated with the *HLA-DRB5* allele (data not shown).

No association was found between SSc and the *DRB5*01:05* allele in the second cohort. The *HLA-DRB5*01:05* allele was significantly more frequent in patients with ILD than in healthy controls (Table 4). Thus the associations between SSc-related ILD and the *DRB5*01:05* allele were confirmed in the second cohort.

On univariate analysis, no clinical factors except ILD correlated with the presence of the *DRB5*01:05* allele (Table 5).

Discussion

The *HLA-DRB5* locus and its alleles have rarely been brought to researchers' attention in terms of disease susceptibility. Taking advantage of unbiased mRNA expression profiling using pooled PBMC samples from SSc patients with or without ILD, we focused on *HLA-DRB5* and confirmed the high expression of this gene in individual patients with SSc-related ILD compared with those without ILD. Furthermore, we identified the *HLA-DRB5*01:05* allele as a novel risk factor for ILD in SSc using a genotyping approach. Due to the limitations of our cohort in terms of relatively small size and uniform ethnicity, our results clearly need to be confirmed in a larger cohort or in other ethnicities. As many of the RNA samples were obtained from patients currently under treatment, prospective observation would also be desirable to determine whether *HLA-DRB5* expression in PBMCs or the *HLA-DRB5*01:05* allele has predictive value for the development of ILD in patients with early-stage SSc. However, because *HLA-DRB5* expression is genetically regulated and is unaffected by disease duration or immunotherapy, it is highly unlikely that there is any significant discrepancy between *HLA-DRB5* expression levels and genotype.

The first genome-wide association study concerning SSc in a sample of European ancestry confirmed the role of *HLA* gene regions as SSc genetic risk factors

TABLE 4 Frequency (%) of *HLA-DRB5* alleles in SSc patients with and without ILD and in healthy controls

Patient group	*n*	Frequency (%)	OR (95% CI) *vs* controls	OR (95% CI) *vs* ILD (–)
First cohort				
*B5*01*				
**01:01*				
SSc total	70	12.9	0.89 (0.38, 2.05)	0.52 (0.13, 2.13)
ILD +	41	9.8	0.65 (0.21, 2.01)	
ILD –	29	17.2	1.25 (0.43, 3.64)	
Controls	147	14.3		
**01:02*				
SSc total	70	18.6	1.30 (0.61, 2.75)	2.44 (0.60, 9.94)
ILD +	41	22.0	1.83 (0.79, 4.27)	
ILD –	29	10.3	0.66 (0.18, 2.35)	
Controls	147	14.9		
**01:05*				
SSc total	70	22.9	5.15§ (2.08, 12.73)	4.02‡ (1.03, 15.74)
ILD +	41	31.7	8.07§ (3.06, 21.28)	
ILD –	29	10.3	2.00 (0.50, 8.06)	
Controls	147	5.4		
*B5*02*				
**02:02*				
SSc total	70	4.3	2.15 (0.42, 10.93)	
ILD +	41	4.9	2.46 (0.40, 15.25)	
ILD –	29	0	1.43 (0.07, 28.41)	
Controls	147	2.0		
Second cohort				
*B5*01*				
**01:01*				
SSc total	79	10.3	0.74 (0.28, 1.94)	0.53 (0.14, 2.02)
ILD +	40	7.7	0.53 (0.14, 2.02)	
ILD –	39	12.8	0.96 (0.31, 2.99)	
Controls	83	14.3		
**01:02*				
SSc total	79	29.1	1.48 (0.73, 3.03)	3.08 (1.08, 8.57)
ILD +	40	40.0	2.41 (1.06, 5.47)	
ILD –	39	17.9	0.79 (0.30, 2.08)	
Controls	83	21.7		
**01:05*				
SSc total	79	8.9	7.97 (0.96, 66.36)	
ILD +	40	17.5	17.39† (2.06, 146.94)	
ILD –	39	0	1.44 (0.06, 36.06)	
Controls	83	1.2		

§$P < 0.001$, †$P = 0.009$, ‡$P = 0.036$. The chi-squared test or Fisher's exact test was used. *P*-values were adjusted using the Bonferroni correction. OR: odds ratio.

with the strongest association [27]. Previously, HLA studies in SSc have suggested that MHC genes exert their influence through the presentation of a specific self-antigen [28]. In the Japanese population, the frequencies of *DRB1*15:02*, *DQB1*06:01* and *DPB1*09:01* have been reported to be significantly increased in anti-Topo-I-positive patients [29]. In terms of pulmonary complications, an association between *DR3/DR52a* and pulmonary fibrosis has been documented in Caucasian patients with SSc [30]. However, *HLA-DR3* is quite rare in the Japanese population, and, in fact, none of the investigated individuals in our first cohort had a *DRB1* allele corresponding to this serotype. The correlation between the *HLA-DRB1*15:02* allele and anti-Topo-I antibodies was confirmed in our study, as well as the absence of a significant correlation between this allele and SSc or SSc-related ILD.

HLA-DRB5 belongs to the HLA class II beta chain paralogues. Currently 19 alleles have been identified for the *HLA-DRB5* gene, and several studies have reported the frequencies of *HLA-DRB5* alleles and the *DRB5-DRB1-DQB1* haplotype in healthy and diseased populations [31–33]. However, no report has referred to the frequencies of the *HLA-DRB5*01:05* allele or the *DRB5*01:05-DRB1* haplotype. Although the frequency of the *HLA-DRB5*01:05* allele in the healthy Japanese population

Table 5 Comparisons of demographic and clinical parameters between SSc patients with and without *HLA-DRB5*01:05*

		*HLA-DRB5 *01:05*		OR (95% CI)
First cohort		(+) $n=16$	(–) $n=54$	
Profile	*n* (%)			
Gender (male)		3 (18.7)	11 (20.3)	0.90 (0.22, 3.73)
Smokers		7 (43.8)	21 (38.9)	1.15 (0.37, 3.56)
Diffuse type		12 (75.0)	33 (61.1)	1.91 (0.54, 6.71)
Complication				
ILD		13 (81.2)	28 (51.9)	4.02* (1.03, 15.74)
PH		5 (31.2)	4 (7.4)	5.56** (1.24, 24.91)
SRC		0 (0)	2 (3.7)	
Digital ulcer		2 (12.5)	6 (11.1)	1.14 (0.21, 6.30)
Antibodies				
ACA		4 (25.0)	14 (25.9)	0.95 (0.26, 3.44)
aTopo-I		6 (37.5)	16 (29.6)	1.43 (0.44, 4.58)
anti-U1-RNP		1 (6.25)	10 (18.6)	0.29 (0.03, 2.49)
RF		2 (12.5)	5 (11.4)	1.11 (0.19, 6.41)
aSS-A/Ro		1 (7.1)	16 (34.8)	0.14 (0.02, 1.20)
aDNA		0 (0)	4 (9.3)	
Second cohort		(+) $n=7$	(–) $n=72$	
Profile	*n* (%)			
Gender (male)		0 (0)	6 (8.3)	
Diffuse type		6 (85.7)	37 (51.4)	5.68 (0.65, 49.56)
Complication				
ILD		7 (100)	0 (0)	***
PH		1 (14.3)	7 (9.7)	1.55 (0.16, 14.77)
SRC		0 (0)	0 (0)	
Digital ulcer		1 (14.3)	13 (18.1)	0.76 (0.08, 6.83)
Antibodies				
ACA		1 (14.3)	15 (20.8)	0.63 (0.07, 5.67)
aTopo-I		4 (57.1)	25 (34.7)	2.51 (0.52, 12.09)

*$P=0.036$, **$P=0.030$, ***$P<0.001$. The chi-squared test or Fisher's exact test was used. OR: odds ratio; SRC: scleroderma renal crisis; aTopo-I: anti-topoisomerase-I antibody; aU1-RNP: anti-U1-RNP antibody; aSS-A/Ro: anti-SS-A antibody.

has not yet been reported, we determined, using our own healthy cohorts, that *HLA-DRB5*01:05* is associated with SSc-related ILD, with odds ratios ranging from 8.07 to 17.39 (Table 4). Our multivariate analysis also identified *HLA-DRB5*01:02* and anti-Topo-I as risk factors for SSc-related ILD (supplementary data Table 2, available as supplementary data at *Rheumatology* Online). Although the *HLA-DRB5* locus is almost exclusively carried by *HLA-DRB1*15* and *HLA-DRB1*16* haplotypes, neither of these alleles were related to ILD (supplementary data Table 1, available as supplementary data at *Rheumatology* Online). The lack of correlation between *HLA-DRB5*01:05* and any of the investigated autoantibodies suggests the existence of unknown or unstudied autoantibodies strongly related to ILD in SSc. Further investigation is necessary to identify the lung-related self antigen(s) recognized by HLA haplotypes, including this DRB5 allele. Of note, the *HLA-DRB5*01:05* allele is not more frequent in SSc patients without ILD relative to healthy controls. Although the possibility remains that another molecule in linkage disequilibrium with this *HLA-DRB5* allele is responsible for the development of ILD in SSc patients, the increase in the *HLA-DRB5* transcripts in SSc-ILD patients reveals that *HLA-DRB5* itself is a risk for this visceral complication.

In conclusion, we determined in two Japanese cohorts that *HLA-DRB5* gene carriers were more frequent in SSc patients with ILD than in those without ILD or in healthy controls. In particular, the *HLA-DRB5*01:05* allele was more frequent in SSc patients with ILD than in those without, with stronger statistical significance. *HLA-DRB5*01:05* is a novel candidate for a risk factor for developing ILD in patients with SSc.

Rheumatology key messages

- *HLA-DRB5* transcripts were up-regulated in peripheral blood of SSc patients with ILD.
- *HLA-DRB5* carriers were more prevalent in SSc patients with ILD than in those without.
- The *HLA-DRB5*01:05* allele is a risk for the development of ILD in patients with SSc.

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Supplementary data

Supplementary data are available at *Rheumatology* Online.

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