Interestingly, six patients with cardiac involvement and PBC have been diagnosed as having asymptomatic PBC.

From the view-point of cardiovascular system functions, abnormal autonomic nervous system regulation in patients with PBC was reported (Selmi *et al.*, 2011), and other reports show that autonomic dysfunction in PBC is associated with an increased cardiac mortality risk in non-liver chronic disease states (Neubauer *et al.*, 1997; Jackson *et al.*, 2007). Furthermore, a recent study showed impaired cardiovascular function in PBC using impedance cardiography and magnetic resonance methodologies (Jones *et al.*, 2010). Considering the above previous reports, in addition to our present results, it is possible that the frequent association of cardiac complications is a characteristic feature in inflammatory myopathies with PBC. Further study should be carried out to clarify the association between arrhythmia and AMAs, and myositis patients with AMA should be followed up carefully for cardiac complications, especially arrhythmia.

From the diagnostic view-point of myopathies, it should be noted that the 10 patients in our series showed a clinically chronic course with muscle atrophy, the findings of which are common to those of muscular dystrophy. In patients with muscle atrophy, three had paraspinal muscle involvement with lordotic posture, which is an atypical feature of inflammatory myopathies. Since myositis patients with AMA respond to treatment, as a diagnostic approach to chronic myopathies, AMAs should be evaluated particularly in patients with chronic myopathies and muscle atrophy associated with or without lordortic posture, or cardiopulmonary involvement. Moreover, the patients presenting PBC associated with AMAs with increased serum creatine kinase levels should be evaluated if they show muscle involvement or cardiomyopathy.

It has been reported that granulomatous inflammation with bile duct injury is a characteristic liver histopathological change in PBC (Ludwig *et al.*, 1978). Thus, it is interesting that six of the patients with a clinically chronic disease course showed granulomatous inflammation in muscle histopathology. In association with PBC, granulomatous extrahepatic lesions have been described in other organs such as the skin (Kishor *et al.*, 2008) and lungs (Fagan *et al.*, 1983). The presence of granulomatous inflammation in the patients with such characteristic clinical features also suggests that a pathogenic mechanism may be related to that of PBC. Further study should be carried out to clarify the exact background mechanism underlying this association.

AMAs in serum are highly sensitive and specific for PBC; they are detected in nearly 95% of patients with PBC, with specificity close to 100% when tested with recombinant antigens (Selmi *et al.*, 2011). In our study, we used an enzyme-linked immunosorbent assay for the detection of immunoglobulin G, M or A class antibodies against at least one of the 2-OADC enzymes. When performed in accordance with the manufacturer's protocol (Kadokawa *et al.*, 2003), the specificity of the detection method is 98%. In our series, seven patients were diagnosed as having inflammatory myopathies with PBC and 17 patients were diagnosed as having inflammatory myopathies with AMAs without any clinical features of PBC. In comparison of the two groups, associated autoimmune diseases and autoantibodies were observed in both groups, and no significant difference in clinical features was found between the groups except for cardiac involvement being more frequently observed in patients with PBC than in those without PBC (Table 2).

Although AMAs serve as highly sensitive markers for the diagnosis of PBC, AMAs can frequently be detected in patients with other diseases, such as primary systemic sclerosis, Sjögren's syndrome, rheumatoid arthritis and autoimmune hepatitis (Hu *et al.*, 2010). It has also been reported that other autoantibodies associated with PBC are rheumatoid factors (70%), anti-smooth muscle antibodies (66%), anti-thyroid (anti-microsomal, anti-thyroglobulin) antibodies (40%) and anti-nuclear antibodies (35–50%) (Talwalkar and Lindor, 2003; Selmi *et al.*, 2011). It has also been reported that AMA-positive individuals, even those without signs of cholestasis or liver inflammation, are very likely to develop PBC (Metcalf *et al.*, 1996; Hohenester *et al.*, 2009). It is not clear whether the AMA-positive patients without PBC in our series will develop PBC several years later. However, considering that inflammatory myopathies associated with AMAs are frequently observed in patients with a clinically chronic disease course, muscle atrophy, cardiopulmonary involvement and granulomatous inflammation regardless of the presence or absence of PBC, we believe that inflammatory myopathies associated with AMAs form a characteristic subgroup.

Further study, including the study about the roles of autoantibodies against antigens other than AMAs in PBC, should be carried out to clarify the exact background mechanism underlying this association.

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Original article

Anti-MDA5 antibody, ferritin and IL-18 are useful for the evaluation of response to treatment in interstitial lung disease with anti-MDA5 antibody-positive dermatomyositis

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Abstract

Objective. The aim of this study was to investigate the precise clinical characteristics and to analyse the association between the anti-MDA5 antibody (anti-MDA5ab) titre and disease status in patients with anti-MDA5ab-positive DM.

Methods. Twenty-seven patients who presented with DM and were positive for the anti-MDA5ab were enrolled. The association between the clinical manifestations and the clinical parameters, including the anti-MDA5ab, was analysed.

Results. The complication of rapidly progressive interstitial lung disease (RP-ILD) occurred in 20 (74%) patients. The frequencies of fatal outcome, relapse and malignancy were 33, 4 and 4%, respectively. Remarkably, a fatal outcome occurred within the first 6 months. Compared with six non-RP-ILD patients, elderly age at onset, severely involved pulmonary function and high levels of serum ferritin were present in 20 RP-ILD patients with anti-MDA5ab. Alveolar–arterial oxygen difference ($AaDO_2$) $\geqslant$32 mmHg and ferritin $\geqslant$828 ng/ml at admission were poor prognostic factors in RP-ILD patients with anti-MDA5ab-positive DM. The median value of the anti-MDA5ab titre on admission was higher in patients who later died than in those who survived. The efficacy of treatment was indicated by the anti-MDA5ab, ferritin and IL-18 concentrations. The decline index of the anti-MDA5ab titre after treatment was lower in the subset of patients who died than in the subset of patients who lived. Sustained high levels of anti-MDA5ab, ferritin and IL-18 were present in the patients who died.

Conclusion. Anti-MDA5ab titre and ferritin and IL-18 concentrations are useful for the evaluation of the response to treatment and the status of ILD in patients with anti-MAD5ab-positive DM.

Key words: dermatomyositis, interstitial lung disease, anti-MDA5 antibody, ferritin, interleukin-18.

Introduction

DM is characterized by inflammation of the skin and muscles [1]. Rapidly progressive interstitial lung disease (RP-ILD) in particular is of prime importance in the clinical management of patients with DM because it is an intractable and life-threatening complication [2–5]. Clinically amyopathic DM (CADM) includes typical skin lesions with amyopathy or hypomyopathy [6]. CADM patients with the anti-MDA5 antibody (anti-MDA5ab) frequently develop the complication of RP-ILD, especially in Japan [7–10]. Sato *et al.* [7] identified melanoma differentiation-associated gene 5 (MDA5) as the CADM-140 antigen. The MDA5 protein plays a role in the innate immune response. MDA5 initially recognizes picornaviruses and evokes antiviral responses by eliciting the production of type I IFNs

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and TNF-α [11]. We previously reported that high levels of ferritin are associated with the development and prognosis of RP-ILD with DM [9, 12]. In addition, IL-18 is a potential contributor to ILD with DM [13]. High levels of ferritin and IL-18 are also implicated in macrophage activation syndrome (MAS) [14, 15]. Although a cytokine storm may contribute to the pathogenesis of RP-ILD with anti-MDA5ab-positive DM, especially in the skin and lungs, the precise pathogenesis remains unknown. Moreover, long-term prognosis, frequency of recurrence, complication with malignancy and the association between the anti-MDA5ab titre and the clinical course remain unclear in anti-MDA5ab-positive DM.

Thus we investigated the clinical characteristics and the correlation between the anti-MDA5ab titre and clinical parameters, such as ferritin and IL-18 levels, in patients with anti-MDA5ab-positive DM. In addition, we analysed the association between the anti-MDA5ab titre and the clinical course in these patients.

Patients and methods

Patients

The present retrospective study included patients with idiopathic inflammatory myopathy who were admitted to the Tokyo Women's Medical University Aoyama Hospital or Keio University Hospital from August 1992 to December 2009. All of the enrolled patients suffered from skin rash, myopathy or respiratory symptoms (or a combination thereof) at admission. These patients were diagnosed with DM or CADM based on the criteria of Bohan and Peter [16] or Sontheimer [17], respectively. In general, CADM presents with typical skin lesions and either amyopathy or hypomyopathy for >6 months. A subset of the CADM group included patients who developed fatal ILD within the first 6 months of this study. Medical records were obtained from 142 and 53 patients who were diagnosed with DM and CADM, respectively. In the present study, 5 DM patients and 22 CADM patients who were positive for the anti-MDA5ab were enrolled. The frequencies of anti-MDA5ab positivity were 4 and 42% in the DM patients and in the CADM patients, respectively. Clinical data were obtained from medical records on admission. The study was approved by the ethical committee of the Institute of Rheumatology, Tokyo Women's Medical University, and the study complied with the Declaration of Helsinki guidelines. Disease duration was defined as the time between the appearance of symptoms, such as skin rash, myopathy or respiratory symptoms, and the initiation of treatment.

Evaluation of clinical laboratory parameters and the anti-MDA5ab

Blood tests evaluated creatine kinase (CK), lactate dehydrogenase (LD), KL-6, CRP, ferritin and ANA. Serum IL-18 was measured with an ELISA (R&D Systems, Minneapolis, MN, USA). The median level (range) of IL-18 was 50.5 (18–121) pg/ml in 30 healthy controls. Anti-MDA5ab was detected with an ELISA using recombinant MDA-5 as an antigen, as described previously [7]. The normal value for the anti-MDA5ab titre was ⩽8 U/ml.

Evaluation of pulmonary function and classification of ILD

The PaO_2/F_iO_2 (P/F ratio), pulse oximeter oxygen saturation/FiO_2 (S/F ratio), alveolar–arterial oxygen difference ($AaDO_2$), forced expiratory volume in 1 s (FEV1)/forced vital capacity (FVC) ratio, VC percentage (%VC) and diffusing capacity of the lung for carbon monoxide (DLco) were used to evaluate pulmonary function. The normal values are defined as >380 for the P/F ratio, >450 for the S/F ratio, <10 mmHg for $AaDO_2$, >70% for the FEV1/FVC ratio, >80% for %VC and >20 ml/min/mmHg for the DLco. The ILD was assessed with chest radiography and CT or high-resolution CT of the chest. RP-ILD is defined as a progressive ILD within 3 months of the onset of respiratory symptoms. Chronic ILD is defined as an asymptomatic, non-rapidly progressive ILD or slowly progressive ILD over 3 months by the International Consensus Statement of Idiopathic Pulmonary Fibrosis of the American Thoracic Society and the European Respiratory Society [18].

Statistical analysis

Statistical analyses were performed using the Student's *t*-test to compare mean values, the Mann–Whitney U-test to compare median values and Fisher's exact test to compare frequencies. Correlation coefficients were established by employing Spearman's correlation coefficients. The cumulative survival rate was calculated using the Kaplan–Meier test. The Wilcoxon signed-rank test was performed when comparing clinical parameters upon admission with those parameters after treatment in each patient. The data were analysed using JMP® software (SAS Institute, Cary, NC, USA). A value of $P < 0.05$ indicated statistical significance.

Results

Clinical characteristics in patients with anti-MDA5ab-positive DM

The clinical characteristics of 27 patients with anti-MDA5ab-positive DM are shown in Table 1. The laboratory data were obtained at the first examination upon admission. The frequency of CADM was 81%. The median value of CK was 92 IU/l (interquartile range: 67–271). The complication of RP-ILD was present in 20 (74%) patients. Six additional patients had the complication of chronic ILD, and one patient had neither complication. Although the values of FEV1/FVC ratio and %VC were normal in almost all patients, the DLco levels were decreased. The median values of KL-6, CRP, ferritin and IL-18 were high. ANA positivity was found in four patients (homogeneous and speckled pattern in two patients, homogeneous pattern in one patient and nucleolar pattern in one patient). In 9 of the other 23 patients without ANA positivity, a cytoplasmic pattern was revealed. The frequencies of the fatal

TABLE 1 Clinical characteristics of patients with anti-MDA5ab-positive DM ($n=27$)

Characteristic	Value
Age, years	48 (13)
Female, *n* (%)	20 (74)
Disease duration, weeks	6 (3-8)
CADM, *n* (%)	22 (81)
RP-ILD, *n* (%)	20 (74)
P/F ratio	348 (324-438)
$AaDO_2$, mmHg	26.2 (10.2-41.5)
FEV1/FVC ratio ($n=18$)	82 (78-89)
%VC ($n=22$)	76 (71-84)
DLco, ml/min/mmHg ($n=9$)	10 (9.1-13.6)
LD, IU/l	382 (253-512)
KL-6, U/ml (normal value $\leqslant$ 500) ($n=23$)	735 (570-985)
CRP, mg/dl	0.63 (0.13-1.37)
Ferritin, ng/ml	642 (217-1120)
IL-18, pg/ml (normal range 18-121) ($n=21$)	550 (328-746)
ANA $\geqslant$160×, *n* (%)	4 (15)
Fatal outcome, *n* (%)	9 (33)
Relapse, *n* (%)	1 (4)
Malignancy, *n* (%)	1 (4)

The values of age indicate the mean (S.D.), and the laboratory markers and pulmonary function tests are presented as the median (interquartile range).

outcome, relapse and malignancy were 33, 4 and 4%, respectively.

Comparison of clinical manifestations between patients with anti-MDA5ab-positive DM with and without RP-ILD

Clinical manifestations were compared between patients who had anti-MDA5ab-positive DM with and without RP-ILD (Table 2). The following information indicates the significant results for the patients with RP-ILD: elderly age at onset ($P=0.0021$), decreased P/F ratio ($P=0.0079$), increased $AaDO_2$ ($P=0.0031$), increased ferritin ($P=0.036$) and high frequency of fatal outcome ($P=0.036$). The median values of %VC and DLco were lower in patients with RP-ILD than in those without RP-ILD, although the difference was not statistically significant. The ferritin level was significantly higher in the patients with RP-ILD. The frequency of fatal outcome was high: 45% in the patients with RP-ILD. The cut-off value as a predictor for RP-ILD was estimated by a receiver operating characteristic (ROC) curve of age at onset, P/F ratio, $AaDO_2$ and ferritin. The following parameters can be used as cut-off values (odds ratio, *P*-value): age $\geqslant$46 years (14, 0.011), P/F ratio <438 torr (23, 0.0047), Aa DO_2 $\geqslant$22 mmHg (34, 0.0017) and ferritin $\geqslant$217 ng/ml (48, 0.0014).

No association between anti-MDA5ab titre and clinical parameters

Correlation coefficients between the anti-MDA5ab titre and clinical parameters were established in patients with anti-MDA5ab-positive DM. The clinical parameters included $AaDO_2$, %VC and laboratory markers (KL-6, CRP, ferritin and IL-18). All of these clinical parameters were obtained from 18 patients at the first examination upon admission. There was no significant correlation between anti-MDA5ab titre and other clinical parameters. Significant correlations were only found between $AaDO_2$ and ferritin ($r_s=0.47$, $P=0.014$) in patients with anti-MDA5ab-positive DM.

Comparison of clinical manifestations in living patients and patients who died with RP-ILD with anti-MDA5ab-positive DM

We analysed the clinical manifestations of the patients who had anti-MDA5ab-positive DM and died, and compared them with the manifestations of the surviving anti-MDA5ab-positive DM patients with RP-ILD (Table 3). The P/F ratio and $AaDO_2$ on admission were significantly worse and the ferritin levels were significantly higher ($P=0.017$) in the patients who died. The median anti-MDA5ab titre was higher, although not significantly ($P=0.099$), in patients who died than in those who survived. RP-ILD was refractory and progressive in the patients who died, although almost all of these patients received combination therapy, including prednisolone (PSL), i.v. CYC therapy (IVCY) and calcineurin inhibitor (CNI). The cut-off values as a predictor of fatal outcome in RP-ILD were estimated by the ROC curve of the P/F ratio, $AaDO_2$ and ferritin and are as follows (odds ratio, *P*-value): P/F ratio < 324 torr (9.3, 0.035), Aa DO_2 $\geqslant$32 mmHg (9.3, 0.035) and ferritin $\geqslant$828 ng/ml (14, 0.025).

Survival rates of patients with anti-MDA5ab-positive DM

The cumulative 100-month survival rate was 66% for the entire anti-MDA5ab-positive DM patient group (Fig. 1A). Fatal outcome occurred remarkably often within the first 6 months. The median survival duration was 2 months in the nine patients who died. In contrast, the median survival duration was 29 months in the 18 surviving patients. Next, the patients with anti-MDA5ab-positive DM were divided into an RP-ILD subset and a non-RP-ILD subset. As shown in Fig. 1B, the cumulative 100-month survival rates were significantly lower in the RP-ILD subset than in the non-RP-ILD subset (log-rank test, $P=0.039$).

Association between the anti-MDA5ab titre and the clinical course in patients with anti-MDA5ab-positive DM

We investigated the association between the clinical parameters and the clinical course. Clinical parameters included the anti-MDA5ab titre, the S/F ratio, KL-6, ferritin and IL-18 concentrations. Seventeen patients with anti-MDA5ab-positive DM, including 15 patients with RP-ILD and 2 patients with chronic ILD, were enrolled. Eleven patients were categorized as the living subset and the remaining six patients formed the dead subset. All six patients in the dead subset had the complication of

TABLE 2 *Comparison of the clinical manifestations between patients with anti-MDA5ab-positive DM with and without RP-ILD*

Variable	RP-ILD (−) ($n=7$)	RP-ILD (+) ($n=20$)	*P*
Age, years	35 (4)	52 (2)	0.0021
Female, *n* (%)	6 (86)	14 (70)	0.63
Disease duration, weeks	8 (6–16)	4 (2–8)	0.098
CADM, *n* (%)	6 (86)	16 (80)	1
CK, IU/l	165 (84–271)	85 (47–345)	0.36
LD, IU/l	472 (221–643)	373 (267–500)	0.51
P/F ratio	448 (348–522)	339 (308–388)	0.0079
$AaDO_2$, mmHg	4 (0–18)	30 (24–54)	0.0031
%VC	82 (74–98) ($n=6$)	76 (67–82) ($n=16$)	0.21
DLco, ml/min/mmHg	14.7 (12.5–16.9) ($n=2$)	9.6 (8.9–12.7) ($n=7$)	–
KL-6, U/ml (normal value $\leqslant$500)	346 (278–1104) ($n=5$)	801 (675–1009) ($n=18$)	0.1
CRP, mg/dl	0.46 (0.02–0.80)	0.72 (0.15–1.85)	0.22
Ferritin, ng/ml	186 (120–626)	835 (285–1480)	0.036
IL-18, pg/ml (normal range 18–121)	550 (216–736) ($n=5$)	552 (243–765) ($n=16$)	0.65
Anti-MDA5ab, U/ml	258.8 (217.1–542.7)	152.3 (56.7–376.8)	0.21
Fatal outcome, *n* (%)	0 (0)	9 (45)	0.036

The values of age indicate the mean (S.D.), and laboratory markers and pulmonary function tests are presented as the median (interquartile range).

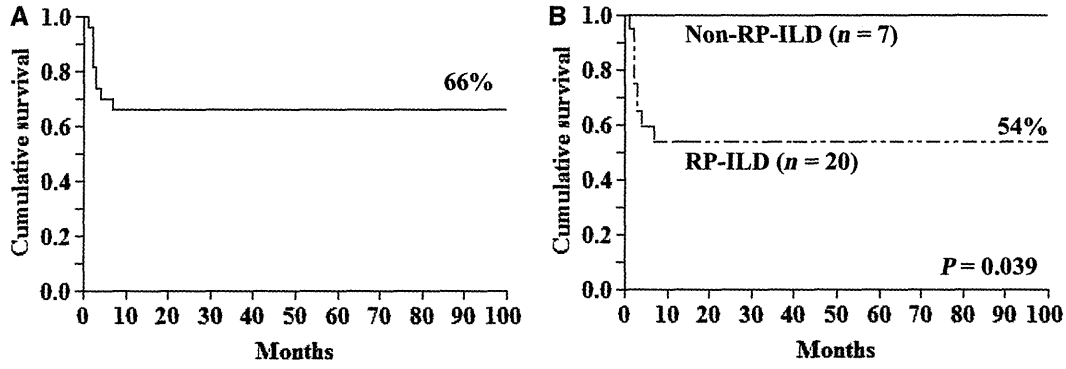
TABLE 3 Comparison of the clinical manifestations between living patients (alive) and patients who died (dead) with RP-ILD and anti-MDA5ab-positive DM upon admission

Variable	Alive ($n=11$)	Dead ($n=9$)	*P*
Age, years	50 (3)	54 (3)	0.29
Female, *n* (%)	9 (82)	5 (56)	0.34
Disease duration, weeks	8 (2–12)	4 (3–7)	0.76
CADM, *n* (%)	9 (82)	7 (78)	1
CK, IU/l	95 (38–383)	77 (62–324)	0.91
P/F ratio	369 (331–403)	319 (246–352)	0.03
$AaDO_2$, mmHg	26 (22–34)	41 (30–102)	0.044
%VC	76 (71–90) ($n=10$)	71 (62–78) ($n=6$)	0.25
DLco, ml/min/mmHg	9.5 (7.8–13.2) ($n=4$)	10 (8.9–12.7) ($n=3$)	–
LD, IU/l	364 (243–488)	460 (308–518)	0.3
KL-6, U/ml (normal value $\leqslant$500)	842 (678–1009) ($n=10$)	731 (602–1099) ($n=8$)	0.59
CRP, mg/dl	0.63 (0.10–1.96)	1.06 (0.17–2.16)	0.7
Ferritin, ng/ml	409 (248–843)	1600 (835–1935)	0.017
IL-18, pg/ml (normal range 18–121)	503 (343–727) ($n=10$)	540 (338–798) ($n=7$)	0.70
Anti-MDA5ab, U/ml	129.3 (44.6–254.0)	332.1 (92.0–599.8)	0.099
Treatment			
PSL + IVCY + CNI	5 (46)	7 (78)	0.2
PSL ± IVCY or CNI	6 (54)	2 (22)	
Improvement of ILD	11 (100)	0 (0)	<0.0001

The age values are presented as the mean (S.D.), and laboratory markers and pulmonary function tests are presented as the median (interquartile range).

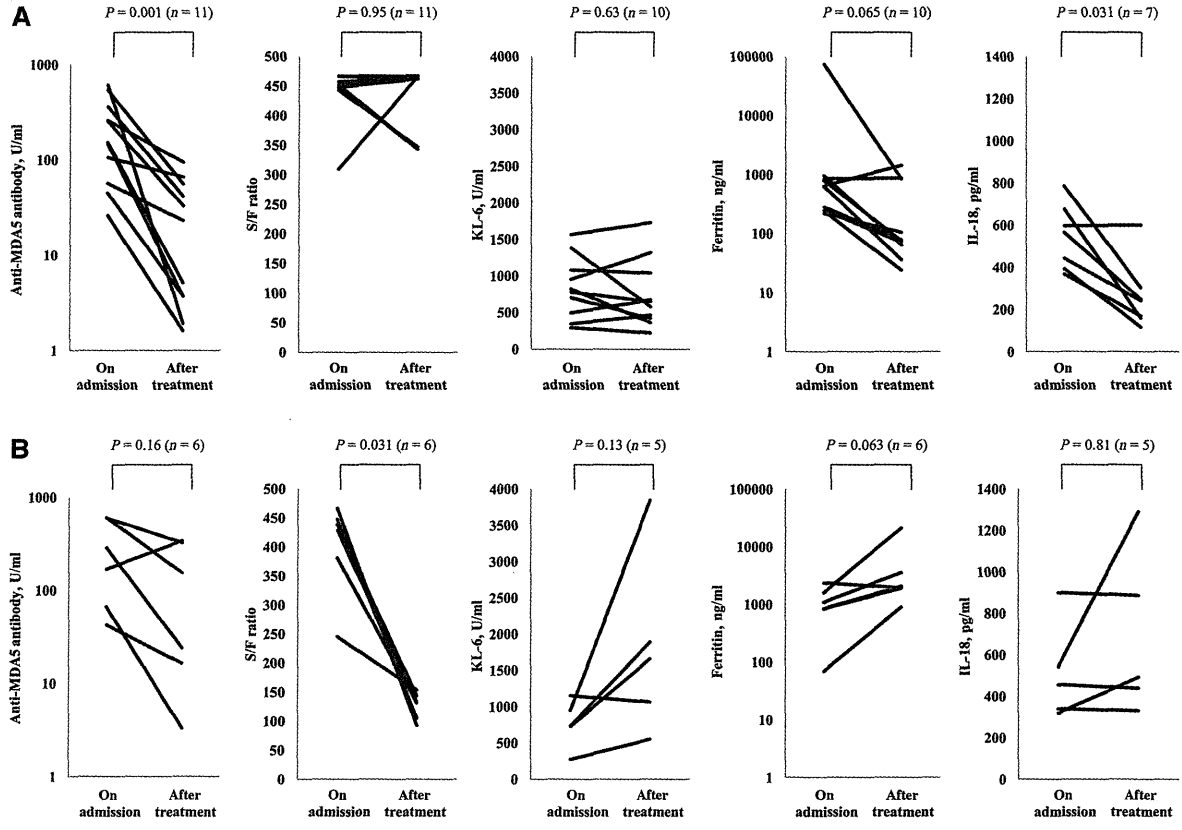
refractory RP-ILD and died within 6 months of treatment because of respiratory failure resulting from RP-ILD. We compared the clinical parameters upon admission with the parameters after treatment in each subset (Fig. 2). The median duration of evaluation after treatment was 3 months in the living subset and 2 months in the dead subset. There was no significant difference ($P=0.21$) between the two subsets in terms of the duration of evaluation after treatment. The data for each clinical parameter could only be partially obtained in some patients. The number of patients for whom data were obtained is indicated in each figure panel.

Fig. 1 Cumulative 100-month survival rates for all patients with anti-MDA5ab-positive DM (**A**), and the RP-ILD and non-RP-ILD subsets of anti-MDA5ab-positive DM patients (**B**).



The cumulative 100-month survival rates were calculated using the Kaplan–Meier test. The log-rank test was also used to compare survival rates. Survival rates and *P*-values are indicated in each figure panel.

Fig. 2 Comparison between clinical parameters upon admission and after treatment in patients with anti-MDA5ab-positive DM.



Based on patient survival, we analysed clinical parameters in two subsets: the living subset and the dead subset (**A** and **B**). The number of patients for whom data were obtained is indicated in each figure panel. Statistical analyses were performed with the Wilcoxon signed-rank test for comparisons of median values.

Anti-MDA5ab titre was significantly lower ($P = 0.001$) after treatment than on admission in the living subset (Fig. 2A). Anti-MDA5ab disappeared after treatment in 5 (45%) of the 11 living patients. On the other hand, there was no statistically significant difference ($P = 0.16$) in the dead subset between the anti-MDA5ab titre upon admission compared with the antibody titre after treatment (Fig. 2B). Anti-MDA5ab was still present after treatment in all dead patients except one. Moreover, the decline index of the anti-MDA5ab titre after treatment was analysed and compared among each subset. The decline index of the anti-MDA5ab was calculated as follows: (the antibody titre after treatment—the antibody titre upon admission) × 100/(the antibody titre upon admission). The median decline indices of the anti-MDA5ab titre (interquartile range) were 90% (63–97%) and 68% (9–92%) in the living and dead subsets, respectively.

In the dead subset, the S/F ratio was significantly lower after treatment ($P = 0.031$). The levels of KL-6 tended to decrease in the living subset and increase in the dead subset. On the other hand, the levels of ferritin more sensitively reflected the response to treatment than the levels of KL-6. The median values of ferritin after treatment were 76 ng/ml and 1987 ng/ml in the living and dead subsets, respectively ($P = 0.0017$). Moreover, the levels of IL-18 were significantly lower ($P = 0.031$) after treatment in the living subset. In the dead subset, the levels of IL-18 were not significantly lower after treatment.

Discussion

We have measured the clinical characteristics of disease and have demonstrated an association between clinical parameters and clinical course in patients with anti-MDA5ab-positive DM. The clinical manifestations of anti-MDA5ab-positive DM have been reported, mainly in Japanese studies [7–10]. Two different subsets of ILD with CADM patients are those with RP-ILD or with chronic ILD [19]. Fathi *et al.* [20] have reported that patients with inflammatory myopathy with ILD require careful evaluation of their clinical features because the course of ILD cannot be predicted at the first examination. However, we determined that investigation of both the anti-MDA5ab and the serum ferritin concentration are useful for predicting the onset of RP-ILD in DM [7, 21]. On the other hand, the serum ferritin level was <500 ng/ml in some patients with DM-associated RP-ILD [12]. These patients with RP-ILD were occasionally positive for the anti-aminoacyl-tRNA synthetase antibody and appeared to be well controlled with CSs and immunosuppressant agents compared with patients with DM-associated RP-ILD having anti-MDA5ab and/or hyperferritinaemia. This distinction in response to treatments might be responsible for the cellular phenotypes affecting the pathogenesis of ILD. Taken together, if the serum ferritin level is high in patients with DM, it should be considered that these patients may have anti-MDA5ab, and their clinical course may be complicated by RP-ILD.

CADM with RP-ILD showed a rapidly progressive pattern with a 6-month survival rate of 40.8–45%, which corresponded to the results in our study [22, 23]. In our study, $AaDO_2$ levels ⩾32 mmHg and ferritin levels ⩾828 ng/ml on admission were poor prognosis factors for RP-ILD with anti-MDA5ab-positive DM. The median anti-MDA5ab titre on admission was higher in the patients who died than in the living patients, although the difference between the two subsets was not statistically significant. However, the median anti-MDA5ab titre on admission was higher in the patients without RP-ILD than in those with RP-ILD. The anti-MDA5ab titre before treatment was not predictive of the prognosis of RP-ILD in anti-MDA5ab-positive DM. Measuring levels of serum ferritin and $AaDO_2$ before treatment is useful for predicting the prognosis of RP-ILD in DM.

We analysed the association between the anti-MDA5ab titre and the clinical course. We confirmed that the anti-MDA5ab titre has disappeared in improving surviving patients in our longitudinal observation (data not shown). Relapse has not occurred in any of the improving surviving patients except one. In the future we will investigate whether the anti-MDA5ab titre is increased again in either a pulmonary flare or skin exacerbation. Moreover, we have analysed several patients, in whom the serum ferritin level and IL-18 level were high, and were correlated with the clinical course in patients with RP-ILD with DM [24]. Immunosuppressive therapy had some effect on clinical parameters such as cytokines and antibodies regardless of clinical course. In the present study, however, immunosuppressive therapy was received more intensively in the dead subset than in the living subset of patients with anti-MDA5ab-positive DM. The frequency of receiving PSL + CNI + IVCY was higher in the dead subset than in the living subset. Moreover, there was no significant difference between the two subsets in terms of the duration of evaluation after treatment. Taken together, the sustained high levels of anti-MDA5ab, ferritin and IL-18 could be attributed to the poor response to treatment in the dead subset. Investigations of the anti-MDA5ab titre, ferritin level and IL-18 level after treatment are useful for predicting the clinical course and evaluating the response to treatment in patients with ILD with anti-MDA5ab-positive DM.

The levels of serum ferritin and IL-18 were associated with the status of ILD with anti-MDA5ab in the present study, as shown in previous reports [9, 12, 24]. Serum ferritin is an important laboratory finding of MAS [14]. MAS is now an accepted term that is used to refer to a form of secondary haemophagocytic lymphohistiocytosis observed in the context of rheumatic disorders [14, 15]. The pathophysiology of MAS involves a lack of T lymphocyte regulation and the excessive production of cytokines, such as TNF-α, IL-1β, IL-6 and IL-18, resulting in the activation of macrophages [15, 25]. The mRNA for IL-18 and IL-12 is readily detected in Kupffer cells and activated macrophages, and dendritic cells produce IL-18 in active inflammatory myopathies [26, 27]. We also reported that IL-18 is a key mediator in ILD with DM [13]. Moreover,

alveolar macrophages activated by some antigens, microbes and autoimmune stimuli are induced to produce leukotriene B4 and IL-8. These factors stimulate neutrophils to induce fibrosis in the lungs [28]. The MDA5 protein initially recognizes picornaviruses, such as the Coxsackie virus, and evokes antiviral responses by producing type I IFNs and TNF-α [11]. Previously, Coxsackie virus infection was reported to be one of the contributing factors in the pathogenesis of JDM [29]. Anti-MDA5ab-positive DM may be a type of MAS mainly in the skin and lungs that contributes to infections such as those caused by the Coxsackie virus. In conclusion, anti-MDA5ab titre, serum ferritin and IL-18 are useful for the evaluation of the response to treatment of RP-ILD with anti-MDA5ab-positive DM.

Rheumatology key messages

- Anti-MDA5ab is a disease-specific marker in DM with RP-ILD.
- Anti-MDA5ab titre, ferritin and IL-18 are useful for evaluation of response to treatment in DM with RP-ILD.

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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 α (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†	–	4.5×10^{-3}	4.4×10^{-2}	1.1×10^{-5}	2.0×10^{-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 DRβ 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 DRβ 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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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)	4.0×10^{-4}	0.0012	83 (0.18)	1.87 (1.33 to 2.62)	3.9×10^{-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 χ^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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