Journal of Autoimmunity xxx (2016) 1–7

Contents lists available at ScienceDirect

Journal of Autoimmunity

journal homepage: www.elsevier.com/locate/jautimm

Splicing factor proline/glutamine-rich is a novel autoantigen of dermatomyositis and associated with anti-melanoma differentiation-associated gene 5 antibody

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ARTICLE INFO

Article history:
Received 10 October 2016
Received in revised form
22 November 2016
Accepted 25 November 2016
Available online xxx

Keywords:
SFPQ
MDA5
Myositis
Antibody
CADM
Virus

ABSTRACT

Objective: Anti-MDA5 antibody positive dermatomyositis (DM) and clinically amyopathic DM (CADM) often develop into rapidly progressive interstitial lung disease, but their pathogenesis remains unclear. We observed that sera from DM/CADM patients immunoprecipitated a common 110 kDa polypeptide. We investigated this autoantigen and its clinical significance.

Methods: Autoantibodies were screened in 333 patients with various connective tissue diseases (CTDs) and 20 healthy controls (HCs) by immunoprecipitation with [^{35}S]methionine-labeled HeLa cells. Immunoabsorbent column chromatography was used to purify the reactive autoantigen which was subsequently analyzed by peptide mass fingerprinting.

Results: Anti-110 kDa antibody was detected in sera from 27 DM/CADM patients, but not in sera from other CTD patients or HCs. All patients with anti-110 kDa antibody had anti-MDA5 antibody. The maximum KL-6 levels in anti-110 kDa antibody-positive patients were higher than in anti-110 kDa antibody-negative patients, and all anti-MDA5-antibody-positive patients who showed the recurrence of DM/CADM were anti-110 kDa antibody-positive. The corresponding autoantigen was identified as splicing factor proline/glutamine-rich protein (SFPQ). In some cases, anti-SFPQ antibody was detected at diagnosis (early-detected group), but in other cases, it appeared during the disease course (delayed-detected group). The diagnosis timing of DM/CADM showed seasonal patterns according to the timing of anti-SFPQ antibody appearance. Specifically, 77% (10/13) of patients were diagnosed between August and October in the early-detected group, while 57% (8/14) of patients were diagnosed between January and March in the delayed-detected group.

Conclusions: Some anti-MDA5 antibody-positive patients had an antibody to SFPQ, which is known to play a role in innate immune responses. Anti-SFPQ antibody may be involved in the chronic disease course of DM/CADM. The diagnosis timing of DM/CADM in anti-MDA5 antibody-positive patients showed seasonal patterns according to the timing of anti-SFPQ antibody appearance. These findings may provide new insights into the pathogenesis of DM/CADM.

1. Introduction

Polymyositis (PM) and dermatomyositis (DM) are systemic autoimmune diseases that involve muscle, skin and other organs including the lung, heart, and joints. Myositis-specific autoantibodies (MSAs) and myositis-associated autoantibodies (MAAs) are often detected in sera from PM/DM patients. These MSAs and MAAs are closely associated with clinical features. Therefore, detection of MSAs and MAAs facilitates disease diagnosis, the clinical course prediction, and aids therapeutic decisions in the early stages of disease. Among the MSAs, anti-melanoma differentiation-associated protein 5 (MDA5) autoantibody is specifically associated with DM and clinically amyopathic DM (CADM) [1,2]. Anti-MDA5 antibody-positive patients with interstitial lung disease (ILD) often

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http://dx.doi.org/10.1016/j.jaut.2016.11.006
0896-8411/

develop acute progressive ILD with poor responses to conventional therapies and fatal prognosis [3–5]. The autoantigen MDA5, also known as interferon induced with helicase C domain protein 1 (IFIH1), is a member of the retinoic acid-inducible gene-I (RIG-I)-like receptor family and is involved in the recognition of cytoplasmic RNA viral infection [6,7]. MDA5 is one of the autoantigens for DM-specific antibodies, suggesting an association between viral infection and DM. The pathophysiological background of anti-MDA5 antibody-positive DM/CADM is poorly understood. A recent study suggested that nuclear-enriched abundant transcript 1 (NEAT1) regulates the genes of viral sensors, such as MDA5 and RIG-I [8]. NEAT1 is a long noncoding RNA that regulates gene expression [9], and is an essential scaffolding factor for nuclear paraspeckle formation [10]. NEAT1 is induced by viral infection and binds directly to splicing factor proline/glutamine-rich (SFPQ) [8], which regulates anti-viral genes.

Here, we report a novel autoantibody against SFPQ with high specificity for anti-MDA5-antibody-positive DM/CADM and demonstrate a relationship with the seasonality of DM/CADM onset and the timing of anti-SFPQ antibody appearance.

2. Materials and methods

2.1. Patients

Serum samples were collected from 333 patients with various CTDs in Kyoto University Hospital and 20 healthy controls (HCs). The selected patients had a certain variety of diseases, complising DM (n = 60), CADM (n = 34), PM (n = 49), inclusion body myositis (n = 5), systemic lupus erythematosus (n = 88), idiopathic pulmonary fibrosis (n = 21), IgG4 related-disease (n = 20), rheumatoid arthritis (n = 19), scleroderma (n = 19), Sjögren's syndrome (n = 10), anti-neutrophil cytoplasmic antibody-associated vasculitis syndrome (n = 3), psoriatic arthritis (n = 3) and ankylosing spondylitis (n = 2). All CTDs were diagnosed according to indivisual criteria [11–21]. We diagnosed patients as CADM when they showed the typical skin lesions of DM but little or no evidence of clinical myositis, indicated by low serum creatinine kinase (CK) levels of less than 300 IU/l. The presence of ILD was diagnosed based on chest radiography, computed tomography, and pulmonary function tests. Rapidly progressive ILD was defined as progressive dyspnea, hypoxemia, and interstitial lesions within a few months from the onset of respiratory symptoms.

All patients and HCs provided informed consent, in accordance with the Declaration of Helsinki, before serum sample collection. This study was approved by the Medical Ethics Committee of Kyoto University Graduate School of Medicine (E472).

2.2. Immunoprecipitation

Immunoprecipitation (IPP) was performed using HeLa cell extracts as previously described [22]. For polypeptide studies, 2×10^7 HeLa cells in 100 ml of methionine-free minimal essential medium were labeled with 18.5 MBq [^{35}S]methionine (Perkin Elmer, Waltham, MA, USA) and incubated at 37 °C for 18 h. After four washes in IPP buffer (10 mM Tris-HCl, 500 mM NaCl, 0.1% Nonidet P-40, pH 8.0) and resuspension in 4 ml of IPP buffer, [^{35}S]methionine-labeled HeLa cells were sonicated using a Misonix Microson (Misonix, Farmingdale, NY, USA). The soluble supernatant was recovered by centrifugation (10,000 × g for 10 min).

Ten microliters of serum was mixed with 2 mg of protein A CL-4B Sepharose beads (GE Healthcare, Uppsala, Sweden) in IPP buffer on a rotator for 2 h at room temperature. The IgG-coated Sepharose beads were washed four times and mixed with [^{35}S]methionine-labeled HeLa cell extracts for 2 h at 4 °C. After washing in 500 μl of IPP buffer four times and 500 μl of distilled water once, the Sepharose beads were resuspended in sodium dodecyl sulfate (SDS) sample buffer. Then, polypeptides were then fractionated by 8% SDS–polyacrylamide gel electrophoresis (PAGE). Radiolabeled polypeptide components were analyzed by autoradiography using a Fujix Bio-Imaging Analyzer System-5000 (Fuji Photo-Film, Tokyo, Japan).

2.3. Immunoabsorbent column chromatography and immunoblotting

IgG was derived from 5 ml of patient sera containing anti-110 kDa antibody using an IgG purification kit (ImmunoPure (G) IgG Purification Kit, Pierce, Rockford, IL, USA). Purified IgG (10 mg/g of gel) was coupled with cyanogen bromide (CNBr)-activated Sepharose 4B beads (GE Healthcare) according to the manufacturer's instructions. The IgG-coupled Sepharose 4B beads were poured into a glass column (Bio Rad, Hercules, CA, USA) and extracts of 6×10^8 HeLa cells were circulated through the column at 4 °C overnight by a peristaltic pump. After washing the column with 200 ml of Tris-buffered saline (TBS, 10 mM Tris–HCl, 150 mM NaCl, pH 7.5) containing 0.1% NP-40, the antigen was eluted in a step-wise manner with 1M NaCl, 1M $MgCl_2$ and 3M $MgCl_2$ (adjusted to pH 7.0 with Tris base). The eluates were dialyzed against TBS containing 0.05% NP-40, concentrated to 0.5 mg/ml using an Amicon Centriprep concentrator (Millipore, Billerica, MA, USA) and subjected to electrophoresis to analyze the antigens. The eluted proteins were analyzed by western blotting using sera from anti-110 kDa-antibody-positive patients. Western blotting was performed using a modified procedure [23].

2.4. Peptide mass fingerprinting (PMF)

After SDS-PAGE of eluates from the immunoabsorbent columns, the protein bands were visualized by silver staining, separately excised, digested with trypsin (Promega, Madison, WI, USA), mixed with α-cyano-4-hydroxycinnamic acid in 50% acetonitrile/0.1% TFA, and subjected to MALDI-TOF analysis (Microflex LRF 20, Flex Analysis 3.0 software, Bruker Daltonics, Billerica, MA, USA) for protein identification by PMF as described [24]. Spectra were collected from 300 shots per spectrum over m/z range 600–3000 and calibrated by two-point internal calibration using trypsin auto-digestion peaks (m/z 842.5099, 2211.1046). A peak list was generated using Flex Analysis 3.0 software. The threshold used for peak-picking was 500 for minimum resolution of monoisotopic mass, and 5 for S/N. The search program MASCOT, developed by The Matrixscience (http://www.matrixscience.com/), was used for protein identification by PMF. The following parameters were used for database searches: trypsin as the cleaving enzyme, maximum of one missed cleavage; iodoacetamide (Cys) as a complete modification, oxidation (Met) as a partial modification; monoisotopic masses; and mass tolerance of ±0.1 Da. The PMF acceptance criterion was probability scoring.

2.5. Expression and identification of SFPQ

Overexpression of SFPQ protein was achieved with plasmids encoding SFPQ cDNA (pReceiver-B01; GeneCopoeia Inc., MD, USA) using a TnT® Quick Coupled Transcription/Translation system according to the manufacturer's protocol. We examined the reactivity of patient sera with anti-110 kDa antibody and anti-SFPQ antibodies (ab76955 and ab177149; Abcam, Cambridge, MA, USA) against *in vitro* translated SFPQ by immunoprecipitation.

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2.6. Statistical analysis

Statistical analyses were performed using JMP® Pro 11.2.0 software (SAS Institute Inc., Cary, NC, USA). The clinical characteristics and prognosis in the two groups (anti-MDA5 antibody-positive patients with or without anti-SFPQ antibody) were compared and analyzed by Fisher's extract-test and Student's t-test. Values of $P < 0.05$ were considered statistically significant. Seasonal onset patterns were compared by chi-square analysis of 6-month clusters (January to June versus July to December).

3. Results

3.1. Screening for anti-110 kDa-antibody-positive patients

We noticed that sera from anti-MDA5-antibody-positive patients often immunoprecipitated a common 110 kDa protein. Therefore, we investigated the prevalence of these autoantibodies. Sera from 333 patients with various CTDs and 20 HCs were screened by IPP with [^{35}S]methionine-labeled HeLa cells. Sera from 27 patients immunoprecipitated the 110-kDa polypeptide from [^{35}S]methionine-labeled HeLa cells (Fig. 1A). All of these patients were also positive for anti-MDA5 antibody and diagnosed as either DM or CADM. No MSA other than anti-MDA5 antibody or MAA other than anti-SS-A/Ro antibody were observed in the patients with anti-110 kDa antibody. No sera from other CTD patients or HCs showed the same IPP pattern as the 110-kDa polypeptide. After absorption of an [^{35}S]methionine-labeled HeLa cell extract using serum from a patient with anti-MDA5 antibody, but not anti-110 kDa antibody, the 110 kDa polypeptide but not MDA5 was immunoprecipitated by serum from the same patient with both anti-110 kDa and MDA5 antibodies (Supplement 1). Therefore, the anti-110 kDa antibody was specific to anti-MDA5-antibody-positive patients and the precipitated 110-kDa polypeptide was not directly reactive with the anti-MDA5 antibody.

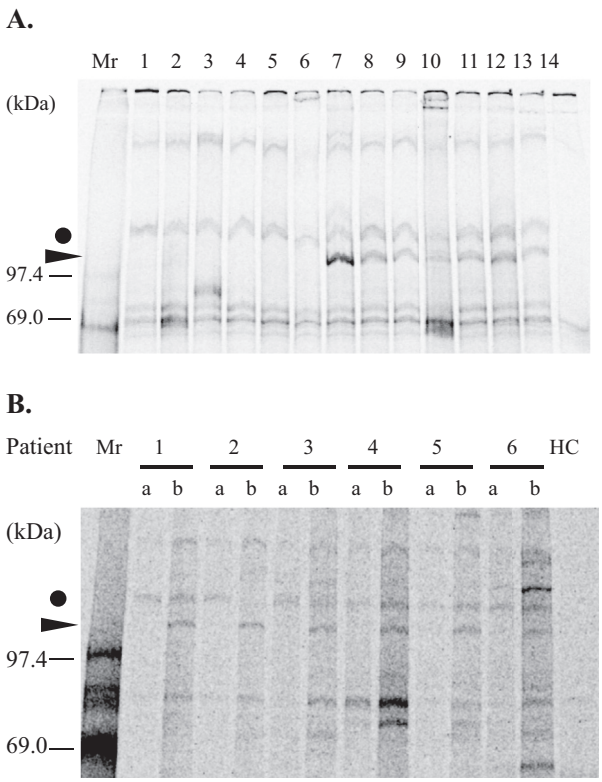

Fig. 1. Immunoprecipitated polypeptides with patient sera. Using [^{35}S] methionine-labeled HeLa cell extracts, 140 kDa (dot) and 110 kDa (arrowhead) polypeptides were immunoprecipitated. Lanes 1–6, anti-MDA5 antibody single-positive; lanes 7–13, anti-MDA5, and anti-110 kDa antibody double-positive sera, and lane 14, healthy control (HC). Mr: Molecular range (A). In some patients, Anti-110 kDa antibody was newly appeared during the disease course (B). Sera from patients (No. 1–6) were compared between time of diagnosis (lane a) and after several months (lane b). Although only the 140-kDa polypeptide (MDA5) was precipitated at diagnosis, the 110-kDa polypeptide (arrowhead) was precipitated after several months. Patient No. 2 showed the disappearance of anti-MDA5 antibody and appearance of anti-110 kDa antibody during disease course.

3.2. Anti-110 kDa antibody appears during the DM disease course

Among the 27 patients with anti-110 kDa antibody, only 13 (48%) were positive for anti-110 kDa antibody at the time of diagnosis of DM. Four of these 13 patients did not receive glucocorticoids or any immunosuppressants at the time of anti-110 kDa antibody detection. Meanwhile, the remaining 14 of 27 (52%) patients were negative for anti-110 kDa antibody at the time of diagnosis of DM/CADM, and converted to antibody-positive cases during the disease course, even though the disease was gradually improved by intensive immunosuppressive therapies (Fig. 1B). In one patient, anti-110 kDa antibody became positive despite the disappearance of anti-MDA5 antibody (Fig. 1B, Patient No. 2). Anti-110 kDa antibody was present even after the disease activity was stabilized by intensive treatment including plasma exchange (Supplement 2). All patients were positive for anti-MDA5 antibody at diagnosis, but anti-110 kDa antibody was detected at diagnosis or during the disease course. In addition, anti-110 kDa antibody was not related to anti-MDA5 antibody titers, or therapeutic agents.

3.3. Identification of the autoantigen recognized by the anti-110 kDa antibody

To determine the autoantigen required for analysis, we partially purified the target autoantigen by immunoaffinity chromatography. The polypeptides in each eluate from the charged anti-110 kDa antibody affinity column are shown in Fig. 2A. To determine which polypeptide was specifically recognized by the anti-110 kDa antibody, we employed immunoblot analysis using the same sera used for immunoaffinity chromatography (Fig. 2B). The 110-kDa polypeptide from the 3M $MgCl_2$ eluate reacted with anti-110-kDa-antibody-positive sera. A silver-stained gel band corresponding to the 110-kDa polypeptide was excised, subjected to PMF and identified as SFPQ (percentage sequence coverage of SFPQ was 38%; Fig. 3).

3.4. Confirmation of SFPQ protein as the 110 kDa antigen

To confirm that SFPQ was specifically recognized by the anti-110 kDa antibody, we prepared human SFPQ protein from SFPQ cDNA using an *in vitro* transcription/translation system. [^{35}S] methionine-labeled SFPQ was immunoprecipitated by sera from anti-110 kDa and anti-MDA5 antibody double-positive patients as well as monoclonal anti-SFPQ antibodies, but was not recognized by sera from anti-MDA5 antibody single-positive patients (Fig. 4). Thus, we confirmed that SFPQ was the target antigen recognized by the anti-110 kDa antibody in DM/CADM patients.

3.5. Clinical and laboratory features of anti-SFPQ antibody-positive patients

Among the anti-MDA5-positive patients, 53% (27/51) were positive for anti-SFPQ antibody. We compared clinical characteristics and prognosis between the anti-SFPQ-positive and -negative

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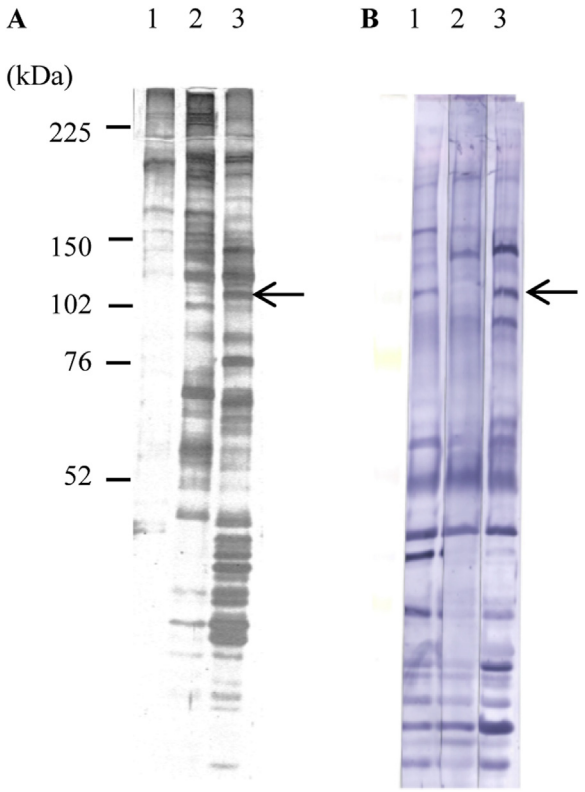

Fig. 2. Purification of antigen using immunoaffinity chromatography. (A) IgG from anti-110-kDa-antibody-positive serum was coupled with CNBr-activated Sepharose 4B beads and reacted with HeLa cell extracts. Autoantigens were eluted using buffers with increasing ionic strength in step-wise gradients (1 M NaCl: lane 1, 1 M $MgCl_2$: lane 2, and 3 M $MgCl_2$: lane 3). The eluates were fractionated on an 8% SDS–polyacrylamide gel and visualized by silver staining. Corresponding bands in the immunoblot analysis were cut and analyzed by PMF (indicated by the arrow head in lane 3). (B) Immunoblot analysis of the eluted antigen. Polypeptides eluted from immunoaffinity chromatography (1 M NaCl: lane 1, 1 M $MgCl_2$: lane 2, and 3 M $MgCl_2$: lane 3) were reacted with the same anti-110 kDa positive serum as for the immunoaffinity chromatography. The corresponding band was detected in polypeptides eluted by 3 M $MgCl_2$ (indicated by the arrow head in lane 3).

patients (Table 1). There were no significant differences in sex, frequencies of ILD including rapidly progressive ILD, muscle weakness, skin manifestations other than mechanic's hand, MAA or maximum CK level. However, the mean age at diagnosis of anti-SFPQ-positive patients was significantly higher than that of anti-SFPQ-negative patients (50.1 ± 12.38 vs 41.3 ± 13.37, P = 0.0204).

1 MSRDRFRSRGGGGGGFHRRGGGGGRGGLHDFR**SPPPGMGLNQNR**GPMGPG
51 PGQSGPKPPIPPPPPHQQQQPPPQQPPPQQPPPHQPPPHPQPHQQQQPP
101 PPPQDSSKPVVAQGPGPAPGVGSAPPASSSAPPATPPTSGAPPGSGPGPT
151 PTPPPAVTSAPPGAPPPTPPSSGVPTTPPQAGGPPPPPAAVPGPGPGPKQ
201 GPGPGGPKGGK**MPGGPKPGGGPGLSTPGGHPKPPHRGGGEPRGGRQHHPP**
251 **YHQQHHQGPPPGGPGGR**SEEK**ISDSEGFKANLSLLR**RPGEKTYTQRCR**LF**
301 **VGNLPADITEDEFKR**LFAK**YGEPGEVFINKGKGFGFIK**LESRALAEIAKA
351 ELDDTPMRGRQLRVR**FATHAAALSVRNLSPYVSNELLEEAFSQFGPIER**A
401 VVIVDDRGRSTGK**GIVEFASKPAAR**KAFERCSEGVFLLTTTPRPVIVEPL
451 EQLDDEDGLPEKLAQKNPMYQKERETPPR**FAQHGTFEYEYSQRWKSLDEM**
501 **EK**QQREQVEKNMKDAK**DKLESEMEDAYHEHQANLLR**QDLMRRQEELRR**ME**
551 **ELHNQEMQK**RK**EMQLRQEEER**RRREEEMMIRQREMEEQMRRQREESYSR**M**
601 **GYMDPR**ERDMRMGGGGAMNMGDPYGSGGQKFPPLGGGGGIGYEANPGVPP
651 ATMSGSMMGSDMRTER**FGQGGAGPVGGQGPRGMGPGTPAGYGR**GR**EEYEG**
701 **PNKKPR**F

Fig. 3. Identification of SFPQ as an autoantigen recognized by the anti-110 kDa antibody. In the MS analysis of the excised protein in Fig. 3A, the bold typed polypeptides were identified. Percentage sequence coverage of SFPQ was 38% (shown in bold type and underlined).

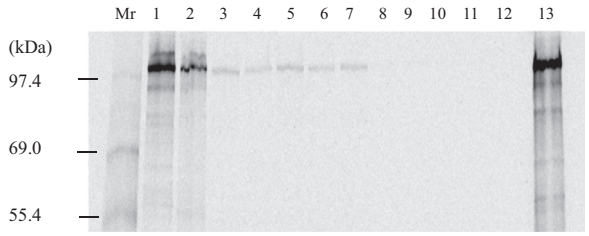

Fig. 4. Immunoprecipitation analysis of *in vitro* translated SFPQ. SFPQ protein from a plasmid encoding SFPQ cDNA was expressed *in vitro* and immunoprecipitated using sera from anti-110-kDa-positive patients, anti-110-kDa-negative/anti-MDA5-positive patients and healthy controls (HC). Lanes 1, 2: monoclonal anti-SFPQ antibody (lane 1: anti-SFPQ: ab76955, lane 2: ab177149); Lanes 3–7: sera from different patients with anti-110 kDa antibody; lanes 8–11: sera from anti-110 kDa-negative and anti-MDA5-positive patients; lane 12: HC, and lane 13; *in vitro* translated SFPQ protein. Mr; Molecular range.

In addition, the frequency of mechanic's hand was significantly higher in anti-SFPQ-positive patients than in anti-SFPQ-negative patients (89% vs 50%, P = 0.0064), whereas arthritis was lower in anti-SFPQ-positive patients (59% vs 88%, P = 0.0309). Moreover, the mean max KL-6 level of anti-SFPQ-positive patients was significantly higher than in anti-SFPQ-negative patients (1912.87 ± 1672.22 vs 1047.71 ± 770.69 U/ml, P = 0.0340). There was no significant difference in prognosis between the two groups. Among MAAs, only SS-A/Ro antibody was detected in anti-SFPQ-positive patients. The frequency of anti-SS-A/Ro antibody in anti-SFPQ-positive patients was not significantly different from that of anti-SFPQ-negative patients or controls. In addition, there was no clinical difference between anti-SS-A/Ro-positive and -negative patients with anti-SFPQ antibody.

We compared the clinical characteristics of anti-SFPQ-positive patients according to the temporal appearance of anti-SFPQ antibody. We divided the anti-SFPQ-positive patients into two groups: early-detected group, those positive for anti-SFPQ antibody at diagnosis (48%, 13/27); and delayed-detected group, those positive for anti-SFPQ antibody during the disease course (52%, 14/27). There was no significant difference in the clinical features and prognosis between the two groups (data not shown). However, the seasonal patterns of diagnosis time differed between the early-detected and delayed-detected groups (Fig. 5). Specifically, 71% (10/14) of the delayed-detected group were diagnosed as DM/CADM between January and June, compared with only 15% (2/13) of the early-detected group (P < 0.05). The early-detected group had a tendency for diagnosis time in August to October, being summer to autumn season in Japan (77%, 10/13), and the delayed-detected group had disease onset in January to March, the winter season (57%, 8/14). No patients in either group had disease onset between June and July.

4. Discussion

We have described three novel findings. First, anti-SFPQ antibody is a new DM-specific autoantibody, that is particularly specific for anti-MDA5-antibody-positive DM/CADM. Second, there were two patterns of timing when anti-SFPQ antibody could be detected, at the diagnosis of DM/CADM or during the disease course. Third, the diagnosis time of anti-SFPQ-antibody-positive DM/CADM had distinct seasonal patterns according to the timing of anti-SFPQ antibody appearance.

SFPQ, also known as polypyrimidine tract-binding protein-associated splicing factor [25], is a multifunctional nuclear protein involved in the human gene expression pathway [26], RNA production and processing [25–28] and viral infection including

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Table 1
Comparison of clinical features between anti-SFPQ-positive and negative patients with anti-MDA5 antibody.

	Anti-SFPQ antibody (+) (N = 27)	Anti-SFPQ antibody (−) (N = 24)	P value
Age at diagnosis, mean (±S.D.), years	50.1 ± 12.38	41.3 ± 13.37	**0.0204**
No. of males/females	4/23	8/16	>0.05
CADM	78% (21/27)	67% (16/24)	>0.05
ILD	100% (27/27)	88% (21/24)	>0.05
RP-ILD	93% (25/27)	79% (19/24)	>0.05
Arthritis	59% (16/27)	88% (21/24)	**0.0309**
Muscle weakness	56% (15/27)	69% (18/24)	>0.05
Gottron's sign	96% (26/27)	83% (20/24)	>0.05
Heliotrope rash	33% (9/27)	54% (13/24)	>0.05
Mechanics hand	89% (24/27)	50% (10/24)	**0.0064**
Periungual Erythema	96% (26/27)	88% (21/24)	>0.05
Skin ulcer	26% (7/27)	23% (6/24)	>0.05
Mortality rate	16% (4/27)	31% (6/24)	>0.05
MAA	[a]7% (2/27)	[a]16% (4/24)	>0.05
Max CK level (U/L)	289.160 ± 105.33	403.654 ± 103.28	>0.05
Max KL-6 level (U/ml)	1912.87 ± 1672.22	1047.71 ± 770.69	**0.0340**
Recurrence of DM/CADM	19% (5/27)	0% (0/24)	>0.05

MAA: myositis-associated antibody, KL-6: Krebs von den Lungen-6.
[a] All detected MAA was anti-SS-A/Ro antibody.

influenza virus infection [29]. In addition, SFPQ binds to NEAT1 and acts as a repressor of IL-8 transcription under normal conditions [8]. Once viral infection occurs, transcriptional activation of the NEAT1 gene family through the TLR3-p38 pathway is initiated, and SFPQ is relocated from the IL-8 promoter to activate IL-8 transcription [8,30]. IL-8 is secreted by alveolar epithelial cells during influenza virus infection [31], and acts as a neutrophil chemotactic factor [32,33]. Recent studies showed that serum IL-8 levels were correlated with disease activity in PM/DM [34], especially in anti-MDA5 positive ILD patients [35] in addition to serum ferritin levels [4,36–38]. Thus, the dynamics of SFPQ may be partly involved in the pathophysiology of anti-MDA5-positive DM-ILD, resulting in the elevation of IL-8.

All patients with anti-SFPQ antibody were also positive for anti-MDA5 antibody. MDA5 is a RIG-I-like receptor involved in the recognition of viral RNAs and plays important roles in innate immune responses [6,7]. Because SFPQ and MDA5 both have important roles in RNA viral infection, the identification of SFPQ as an autoantigen in the sera from anti-MDA5 antibody positive DM/CADM patients may suggest an association between DM and viral infection. In this study, we investigated the seasonal patterns of the diagnosis timing of DM/CADM patients with both anti-SFPQ and anti-MDA5 antibodies according to the timing of anti-SFPQ antibody appearance. The diagnosis time of the early-detected group (anti-SFPQ antibody detected at diagnosis) showed a peak between August and October (summer to autumn season), while that of the delayed-detected group (anti-SFPQ antibody newly appearing during the disease course) showed a peak between January and March (winter season). Several reports have indicated an association between the seasonality and the onset of PM/DM. Leff et al. [39] reported that onset of PM/DM with anti-signal recognition particle (SRP) antibody was predominant between September and February, with the average being November. Miller et al. [40] also reported that onset of PM/DM with anti-SRP antibody had seasonality (August to January), and Sato et al. [41] reported a similar result. Muro et al. [42] reported that the patients with anti-MDA5 antibody most often developed DM/CADM during the autumn season. Our results indicated the possibility of the same predominant pattern of disease onset. These differences in seasonal disease onset lead us to speculate that not only disease onset, but also autoantibody production may have some association with environmental factors such as viral infections. Viral infections that stimulate the MDA5 signaling pathway might increase nuclear NEAT1 and intracellular SFPQ expression, which may explain the production of anti-SFPQ antibody before disease onset.

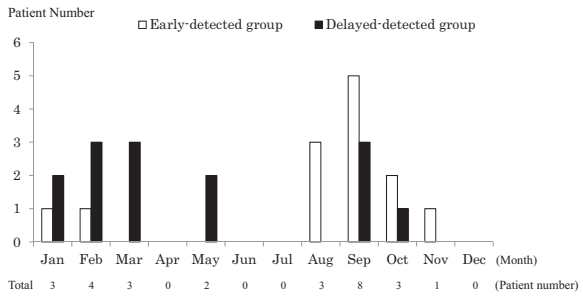

Fig. 5. Onset month of disease in anti-SFPQ antibody-positive patients. The onset of anti-MDA5-positive DM/CADM had two peaks. Early-detected group was concentrated between August and October (67%, 8/12), whereas that of the delayed-detected group was between January and March (69%, 9/13). None cases in each group had an onset at June or July.

As mentioned above, anti-SFPQ antibody was not always detected at diagnosis, and often newly appeared during the disease course in some patients. In one patient, anti-SFPQ antibody appeared even after anti-MDA5 antibody disappeared. These results indicate that the appearance of anti-SFPQ antibody is independent of treatment and that the mechanism involved in the production of anti-SFPQ antibody may be different from that of anti-MDA5 antibody. In addition, the maximum KL-6 levels in anti-SFPQ antibody positive patients were significantly higher than those in anti-SFPQ antibody negative patients. Moreover, all anti-MDA5 antibody-positive patients who showed recurrence of DM/CADM symptoms were positive for anti-SFPQ antibody. Because serum KL-6 levels are elevated in patients with ILD complicated with PM and DM [43,44], and may reflect the presence of fibrotic lung lesions accompanied by destructing lung epithelial cells [45], anti-SFPQ antibody production may be involved in the chronic disease course.

There were several limitations to our study. First, the detection of anti-SFPQ antibody was not available for all patients in the time series; thus, the definite initial appearance of anti-SFPQ antibody remains unclear. Second, it was difficult to evaluate the transition of the anti-SFPQ antibody titers because we used IPP for anti-SFPQ antibody detection and the protein being detected is sometimes present in very small amounts. Third, there may be a time lag between the onsets of DM/CADM and diagnosis. The onset is largely

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based on the subjective symptoms of the patients, and it is thus difficult to clarify the correct onset time. Further studies are needed to clarify the associations between DM/CADM and anti-SFPQ antibody.

In conclusion, we identified a novel autoantibody, anti-SFPQ, in anti-MDA5-antibody-positive DM/CADM patients, that might be associated with certain clinical characteristics. Although further studies are needed to clarify the mechanism of anti-SFPQ antibody production, our data have the possibility to provide new insights for the pathophysiology of DM/CADM with anti-MDA5 antibody.

Funding

Part of this study was supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (Grant Number JP25293222), and a grant from the Practical Research Project for Rare/Intractable Disease in the Japan Agency for Medical Research and Development (AMED).

Competing interests

None declared.

Conflicts of interest

None.

Acknowledgements

We thank Ms. S. Ibuki for technical assistance.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jaut.2016.11.006.

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