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Original article

Anti-MDA5 autoantibodies associated with juvenile dermatomyositis constitute a distinct phenotype in North America

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Abstract

Objective. Myositis-specific autoantibodies have defined distinct phenotypes of patients with juvenile myositis (JIIM). We assessed the frequency and clinical significance of anti-melanoma differentiation-associated gene 5 (MDA5) autoantibody-associated JIIM in a North American registry.

Methods. Retrospective examination of the characteristics of 35 JIIM patients with anti-MDA5 autoantibodies was performed, and differences from other myositis-specific autoantibody groups were evaluated.

Results. Anti-MDA5 autoantibodies were present in 35/453 (7.7%) of JIIM patients and associated with older age at diagnosis, and lower serum creatine kinase and aldolase levels. Patients with anti-MDA5 autoantibodies had more frequent weight loss, adenopathy, arthritis, interstitial lung disease (ILD), and less frequent falling compared with anti-transcriptional intermediary factor 1 (TIF1), anti-nuclear matrix protein 2 (NXP2) and myositis-specific autoantibody/myositis-associated autoantibody-negative patients. They had a different season of diagnosis and less frequent mechanic's hands and ILD compared with those with anti-synthetase autoantibodies. Anti-MDA5 patients received fewer medications compared with anti-TIF1, and corticosteroid treatment was shorter compared with anti-TIF1 and anti-nuclear matrix protein 2 autoantibody groups. The frequency of remission was higher in anti-MDA5 than anti-synthetase autoantibody-positive JIIM. In multivariable analyses, weight loss, arthritis and arthralgia were most strongly associated with anti-MDA5 autoantibody-positive JIIM.

Conclusion. Anti-MDA5 JIIM is a distinct subset, with frequent arthritis, weight loss, adenopathy and less severe myositis, and is also associated with ILD. Anti-MDA5 is distinguished from anti-synthetase autoantibody-positive JIIM by less frequent ILD, lower creatine kinase levels and differing seasons of diagnosis. Anti-MDA5 has comparable outcomes, but with the ability to discontinue steroids more rapidly and less frequent flares compared with anti-TIF1 autoantibodies, and more frequent remission compared with anti-synthetase JIIM patients.

Key words: juvenile dermatomyositis, myositis specific autoantibodies, MDA5, clinical features, treatment, outcome

CLINICAL SCIENCE

Rheumatology key messages

- Anti-MDA5 JIIM is distinguished by frequent skeletal and constitutional features, ILD and less severe myositis.
- Anti-MDA5 differs from anti-synthetase JIIM by less frequent ILD, lower serum CK and season of diagnosis.
- Anti-MDA5 has comparable outcomes with other MSAs in JIIM, and shorter duration of corticosteroid therapy.

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*See supplementary data available at *Rheumatology* online for a list of the Members of the Childhood Myositis Heterogeneity Collaborative Study Group.

Introduction

The juvenile-onset idiopathic inflammatory myopathies (JIIM) are rare systemic autoimmune diseases with childhood onset characterized by chronic muscle inflammation. Myositis-specific autoantibodies (MSAs) have defined subgroups of patients with shared clinical features and outcomes [1–3]. A recently-described MSA associated with dermatomyositis (DM) is targeted against melanoma differentiation-associated gene 5 (MDA5) [4]. MDA5 is also known as interferon-induced helicase C domain-contacting protein I (IFIH1), a cytoplasmic retinoic acid-inducible gene I-like receptor functioning in innate immunity by censoring viral nucleic acids [5, 6]. Anti-MDA5 autoantibodies have been associated with clinically amyopathic and classic adult and juvenile DM/JDM, with distinct cutaneous features, severe rapidly-progressive interstitial lung disease (RP-ILD), and a poor prognosis in Asian patients [7–10]. Anti-MDA5 autoantibodies have been observed in North American and European DM and JDM populations in lower frequency (7–13%) and found to be associated with cutaneous ulceration, mucus membrane ulcers, arthritis, milder muscle disease, and chronic and RP-ILD [11–17]. The objective of this study was to examine the distinct phenotypic features and clinical significance of anti-MDA5 autoantibodies associated with JIIM in North America.

Methods

Patients

Thirty-five patients with JDM or connective tissue disease-associated (JCTM) diagnosed before age 18 years with anti-MDA5 autoantibodies were compared with 157 JDM/JCTM patients with anti-transcriptional intermediary factor 1 (TIF1) autoantibodies, 116 with anti-nuclear matrix protein 2 (NXP2) autoantibodies, 15 with anti-synthetase (aminoacyl-tRNA synthetase) autoantibodies (eight patients with anti-histidyl, five patients with anti-alanyl, one patient each with anti-glycyl and anti-asparaginyl aminoacyl-tRNA synthetase autoantibodies), and 60 MSA/myositis-associated autoantibody (MAA)-negative JIIM patients. All patients met probable or definite Bohan and Peter criteria [18] from a cohort of 453 JIIM patients who enrolled from 1994 to 2015 in investigational review board-approved myositis natural history studies approved by institutional review boards of the National Institutes of Health and the George Washington University Office of Human Research [1, 3, 19]. Patients provided written consent/assent according to standards of the Declaration of Helsinki. A standardized physician questionnaire was completed, including demographics, environmental exposures within 6 months of illness onset, clinical and laboratory features ever present, symptom scores at diagnosis, and outcomes [1, 3, 19]. In total, 349 of 383 (91%) patients had at least 6 months of treatment data, including the courses of medications received, medications doses, as well as start and end dates. A treatment trial was defined as beginning at administration of a medication or combination of medications to termination [20]. Complete clinical response and remission were evaluated according to the consensus definitions of the International Myositis Assessment and Clinical Studies Group [21].

Environmental factors, including documented infections, medications, vaccines, and stressful life events within 6 months of illness onset were based on questionnaire data and medical record review [22]. Stressful life events were classified into major or minor life stressors based on the Adolescent Perceived Event Scale of Compas [23]. Average daily and maximum ultraviolet (UV) index based on residential location 30 days prior to date of myositis diagnosis were determined using the National Weather Service UV Index Cities Forecast Archive [24].

Sera were tested for MSA and MAA using validated immunoprecipitation (IP) and IP-immunoblotting methods at the Oklahoma Medical Research Foundation laboratory [19]. Low-to high resolution genotyping of HLA Class II (HLA-DRB1 and DQA1) alleles was performed in Caucasian anti-MDA5 autoantibody-positive JIIM [25].

Statistical analysis

GraphPad Prism version 8.0 for Windows (GraphPad Software, San Diego, CA, USA), SAS Enterprise Guide version 5.1, JMP for Windows version 11.0.0 (SAS Institute Inc., Cary, NC, USA), and The statistical program R version 3.5.0 (R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, http://www.r-project.org) were used for analyses. Summary data were expressed as median and interquartile ranges, and differences between patient groups were obtained by the Mann–Whitney U or Fisher exact tests, and Kaplan–Meier and log-rank tests were used for time-to-event treatment analysis. Rayleigh and Watson's two sample tests assessed possible seasonal clustering in the diagnosis of anti-MDA5 autoantibody-associated JIIM and compared with those of anti-TIF1, anti-NXP2, and anti-synthetase autoantibodies, and MSA/MAA-negative patients [26]. The proportion trend test was used to assess the trend of anti-MDA5 autoantibodies observed over time, compared with anti-TIF1 and anti-NXP2 autoantibodies [27]. A P-value ≤ 0.05 was considered significant. Random forests classification and multivariable logistic regression analyses were performed to further evaluate significant univariable differences between JIIM patients with anti-MDA5 autoantibodies and those with anti-TIF1, anti-NXP2 and anti-synthetase autoantibodies, and MSA/MAA-negative patients. The Random Forests classification algorithm was performed using the learning machine RandomForests in R (http://stat-www.berkeley.edu/users/breiman/RandomForests/) [28].

Results

Anti-MDA5 autoantibodies were identified in 35 (7.7%) of 453 JIIM patients: 31 patients had JIIM and four had JCTM. Of the patients with overlap JIIM, two had juvenile idiopathic arthritis, and one each had systemic lupus erythematosus and autoimmune hepatitis. Anti-MDA5 autoantibody-positive JIIM patients were older at diagnosis (median age 8.7 years) compared with anti-TIF1 (7.2 years) and anti-NXP2 (6.3 years) patients, and they had a shorter delay to diagnosis compared with anti-synthetase-positive patients (median 4 *vs* 8 months). In total, 60% of anti-MDA5 autoantibody positive patients were Caucasian. There were no other differences in demographic or onset features between anti-MDA5 patients and those with other MSAs or MSA/MAA-negative patients (Supplementary Table S1, available at *Rheumatology* online).

Clinical features

Differences in signs and symptoms of illness present throughout the course of illness, as well as organ system scores at diagnosis [3] were observed in anti-MDA5 autoantibody-positive JIIM patients compared with those with other MSAs and MSA/MAA-negative patients (Table 1). The overall total clinical system score at diagnosis was higher in anti-MDA5 compared with anti-TIF1 and anti-NXP2 autoantibody-positive, and MSA/MAA-negative JIIM. The cutaneous system score at diagnosis was higher in JIIM patients with anti-MDA5 autoantibodies *vs* those with anti-NXP2 and anti-synthetase autoantibodies. Some cutaneous features were more common in anti-MDA5 patients compared with those with the other autoantibodies: Gottron's papules (97%) and photosensitivity (47%) were more frequent and mechanic's hands (9%) less frequently present in anti-MDA5 patients than those with anti-synthetase autoantibodies. Alopecia (23%), palmar papules (9%), and digital infarcts (9%) were more characteristic of anti-MDA5 autoantibodies, in contrast to anti-NXP2 JIIM. Periungual capillary changes (88%) and alopecia (23%) were more often present in anti-MDA5 *vs* MSA/MAA-negative patients. However, anti-MDA5 patients less frequently had malar rash, photosensitivity, V-sign rash, cuticular overgrowth, and lipodystrophy compared with those with anti-TIF1autoantibodies. There was no difference in the frequency of mucous membrane lesions, cutaneous ulceration or calcinosis in anti-MDA5 patients compared with the other groups.

The muscle system score at diagnosis was lower in anti-MDA5 autoantibody-positive in contrast to anti-NXP2, while the skeletal system score at diagnosis was higher than in anti-TIF1 and anti-NXP2 autoantibody-positive and MSA/MAA-negative patients. Falling episodes were less frequent in anti-MDA5 patients (17%) *vs* those with anti-TIF1 and anti-NXP2 autoantibodies. Arthritis (89%) and arthralgias (88%) were more characteristic of anti-MDA5 autoantibodies, compared with anti-TIF1, anti-NXP2 and MSA/MAA-negative patients. No differences in the frequency of muscle and skeletal symptoms in anti-MDA5 and anti-synthetase JIIM patients were observed.

Anti-MDA5 JIIM patients had higher median constitutional system scores at diagnosis compared with those with anti-TIF1 and anti-NXP2 autoantibodies and MSA/MAA-negative patients. Weight loss (80%) and adenopathy (43%), which were primarily present prior to or at diagnosis, were more frequent in anti-MDA5 patients compared with those with anti-TIF1 and anti-NXP2 autoantibodies and MSA/MAA-negative patients. Fever (63%) was another characteristic constitutional symptom in anti-MDA5 patients, in contrast to those with anti-TIF1 and anti-NXP2 autoantibodies, and the majority developed fever prior to or at diagnosis.

The pulmonary system score at diagnosis was higher in patients with anti-MDA5 autoantibodies compared with anti-TIF1 and MSA/MAA-negative patients. Dyspnea on exertion (46 *vs* 15–25%) and ILD (26 *vs* 1.7–2.6%) were more frequent in anti-MDA5 autoantibody-positive patients *vs* those with anti-TIF1 and anti-NXP2 autoantibodies and MSA/MAA-negative (Table 1). This is in contrast to patients with anti-synthetase autoantibodies, in which 67% had documented ILD. Of the nine anti-MDA5 autoantibody-positive patients with ILD, two had RP-ILD. In one patient with RP-ILD, lung biopsy showed peribronchiolar lymphoid infiltrates and focal interstitial fibrosis with an obstructive pneumonitis. The second patient with RP-ILD died from respiratory failure. The postmortem lung histopathology showed diffuse alveolar damage with extensive regenerative squamous metaplasia, severe fibrosis and interstitial thickening, multiple areas of hemorrhage, and focal areas of congestion with neutrophils and plasma cells.

In the gastrointestinal system (Table 1), dysphagia and regurgitation were lower in frequency in anti-MDA5 patients compared with some of the other autoantibody groups (Table 1). Three anti-MDA5 patients had hepatic findings, including steatohepatitis, steatosis and autoimmune hepatitis in one patient each, confirmed by ultrasound and liver biopsy.

There were no differences in cardiac system score at diagnosis and cardiac clinical manifestations during the illness course between the anti-MDA5 patients and each of the other four groups (data not shown).

Laboratory investigations

Maximum serum muscle enzyme levels, including creatine kinase (CK), aldolase and aspartate aminotransferase were lower in anti-MDA5 patients compared with those with other MSAs and MSA-negative patients. Serum CK levels were less frequently elevated in anti-MDA5 patients (30%), in contrast to those with other MSAs (70–93%). Anti-MDA5 patients less frequently had an elevated ANA compared with those with anti-TIF1 (52 *vs* 82%), and the median ANA titre was lower (1:40 *vs* 1:320 respectively) (Table 2).

The frequency of DRB1 and DQA1 HLA alleles in Caucasian anti-MDA5 autoantibody-positive JIIM

Table 1 Signs and symptoms in anti-MDA5 autoantibody-associated juvenile myositis patients compared with other myositis autoantibody groups

Organ system score at diagnosis or symptom/sign ever present[a]	Anti-MDA5 *n* = 35 Median (IQR) or *n* (%)	Anti-TIF1 *n* = 157 Median (IQR) or *n* (%)	Anti-NXP2 *n* = 116 Median (IQR) or *n* (%)	Anti-ARS *n* = 15 Median (IQR) or *n* (%)	MSA/MAA-negative *n* = 60 Median (IQR) or *n* (%)
Overall/total clinical system score at diagnosis	0.28 (0.22–0.35)	0.21 (0.13–0.28)****	0.22 (0.16–0.32)**	0.32 (0.23–0.37)	0.17 (0.12–0.26)****
Cutaneous					
Cutaneous system score at diagnosis	0.29 (0.22–0.38)	0.33 (0.22–0.39)	0.22 (0.14–0.31)**	0.22 (0.11–0.29)*	0.24 (0.14–0.33)
Gottron papules	34 (97.1)	153 (97.5)	98 (84.5)	10 (66.7)**	53 (89.8)
Malar rash	23 (65.7)	145 (92.4)***	81 (69.8)	5 (33.3)	39 (65.0)
Heliotrope	29 (82.9)	141 (89.8)	99 (85.3)	13 (86.7)	47 (79.7)
Photosensitivity	16 (47.1)	102 (66.7)*	40 (35.7)	2 (13.3)*	28 (47.5)
Raynaud's phenomenon	4 (11.4)	12 (7.6)	4 (3.5)	5 (33.3)	6 (10.0)
Periungual capillary abnormalities	30 (88.2)	137 (88.4)	90 (79.6)	11 (73.3)	36 (63.2)*
Cuticular overgrowth	10 (28.6)	73 (48.0)*	38 (33.9)	3 (21.3)	10 (17.2)
Mucous membrane lesions	17 (48.6)	59 (37.8)	37 (32.2)	3 (20.0)	18 (30.0)
'V'-sign	6 (17.1)	67 (42.9)**	25 (21.6)	3 (20.0)	15 (25.4)
Cutaneous ulceration	11 (31.4)	31 (19.7)	26 (22.4)	2 (13.3)	10 (16.7)
Digital infarcts	3 (8.6)	7 (4.5)	0 (0)*	1 (6.7)	1 (1.7)
Mechanic's hands	3 (8.8)	7 (4.5)	2 (1.8)	5 (33.3)*	3 (5.1)
Lipodystrophy	0 (0)	26 (16.6)**	9 (7.8)	0 (0)	3 (5.1)
Calcinosis	13 (37.1)	48 (30.6)	41 (35.3)	2 (13.3)	25 (48.3)
Alopecia	8 (22.9)	17 (10.8)	5 (4.3)**	3 (20.0)	2 (3.3)**
Musculoskeletal					
Muscle system score at diagnosis	0.28 (0.14–0.43)	0.29 (0.17–0.43)	0.43 (0.29–0.57)*	0.43 (0.14–0.43)	0.29 (0.15–0.48)
Proximal muscle weakness	35 (100.0)	157 (100.0)	115 (99.1)	15 (100.0)	60 (100.0)
Distal muscle weakness	16 (45.7)	74 (48.4)	53 (47.7)	5 (33.3)	28 (46.7)
Asymmetric weakness	5 (14.3)	18 (11.5)	19 (16.5)	1 (6.7)	5 (8.5)
Falling episodes	6 (17.1)	60 (38.5)*	56 (48.7)***	3 (21.4)	21 (35.0)
Muscle atrophy	9 (25.7)	65 (41.7)	40 (34.8)	5 (35.7)	15 (25.0)
Myalgia	16 (45.7)	90 (59.2)	84 (73.7)**	9 (64.3)	32 (55.2)
Skeletal system score at diagnosis	0.5 (0.5–1.0)	0.5 (0.0–0.5)****	0.5 (0.0–1.0)**	0.5 (0.0–1.0)	0.0 (0.0–0.5)****
Arthralgia	30 (88.2)	91 (58.0)**	76 (65.5)*	12 (80.0)	28 (46.7)****
Arthritis	31 (88.6)	68 (43.3)****	54 (47.0)****	10 (66.7)	25 (41.7)****
Contractures	16 (45.7)	94 (59.9)	73 (63.5)	7 (50.0)	29 (48.3)
Constitutional					
Constitutional system score at diagnosis	0.5 (0.5–0.75)	0.25 (0.25–0.5)****	0.25 (0.25–0.5)****	0.5 (0.25–0.75)	0.25 (0.25–0.5)****
Fatigue	33 (94.3)	136 (86.6)	103 (89.6)	15 (100.0)	48 (80.0)
Weight loss	28 (80.0)	54 (34.4)****	36 (31.3)****	10 (66.7)	17 (28.8)****
Fever	22 (62.9)	51 (32.5)***	47 (40.5)*	9 (60.0)	26 (43.3)
Adenopathy	15 (42.9)	34 (21.9)**	23 (20.2)*	3 (20.0)	9 (15.3)**
Pulmonary					
Pulmonary system score at diagnosis	0.0 (0.0–0.2)	0.0 (0.0–0.0)*	0.0 (0.0–0.17)	0 (0–0.5)	0.0 (0.0–0.0)*
Dysphonia	7 (20.0)	47 (30.1)	52 (45.2)***	1 (6.7)	18 (30.0)
Dyspnea on exertion	16 (45.7)	29 (18.7)**	29 (25.0)*	5 (33.3)	9 (15.0)**
Interstitial lung disease	9 (25.7)	4 (2.6)****	2 (1.8)****	10 (66.7)*	1 (1.7)**
Pneumothorax	2 (5.9)	0*	1 (0.9)	1 (6.7)	0

(continued)

TABLE 1 Continued

Organ system score at diagnosis or symptom/sign ever present[a]	Anti-MDA5 $n = 35$ Median (IQR) or n (%)	Anti-TIF1 $n = 157$ Median (IQR) or n (%)	Anti-NXP2 $n = 116$ Median (IQR) or n (%)	Anti-ARS $n = 15$ Median (IQR) or n (%)	MSA/MAA-negative $n = 60$ Median (IQR) or n (%)
Gastrointestinal					
Gastrointestinal system score at diagnosis	0.0 (0.0–0.11)	0.0 (0.0–0.11)	0.0 (0.0–0.13)	0.0 (0–0.22)	0.0 (0.0–0.11)
Dysphagia	6 (17.1)	61 (38.9)*	56 (48.3)***	3 (20.0)	21 (35.6)
Regurgitation	3 (8.6)	34 (21.7)	32 (27.8)*	2 (13.3)	8 (13.3)

Anti-MDA5 autoantibody positive group was compared with anti- TIF1, anti-NXP2, and anti-ARS autoantibody positive and MSA/MAA negative JIIM groups. Note that 70 juvenile myositis patients with other autoantibodies were not included in this analysis. Note that percentages may not reflect the number divided by the total number of subjects, if data are missing. [a]A score for each individual organ system was defined as the number of signs/symptoms present related to that system at diagnosis, divided by the number of items assessed; values ranged from 0 to 1. The overall clinical symptom score was calculated by averaging the clinical symptom scores of the seven individual organ systems [3]. The symptoms recorded were present at any time before or after diagnosis. Significant differences from anti-MDA5 autoantibody-positive: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$. ARS: aminoacyl-tRNA synthetase; IQR: interquartile range; JIIM: juvenile myositis; MAA: myositis associated autoantibody; MDA5: melanoma differentiation-associated gene 5; MSA: myositis specific autoantibody; NXP2: nuclear matrix protein 2; TIF1: transcriptional intermediary factor 1.

patients did not differ from race-matched healthy controls. DRB1*0301 (29 *vs* 23%) and DQA1*0501 (40 *vs* 51%) were also not increased in the anti-MDA5 JIIM patients *vs* controls. There was no difference in the frequency of DRB1*0101 in anti-MDA5 JIIM patients and control subjects. No anti-MDA5 JIIM patients had HLA DRB1*0405 allele.

Environmental factors

Infection was the most frequent documented exposure within 6 months of illness onset in anti-MDA5 group and was reported in 17 patients (49%), which did not differ from the other groups (21–41%). Anti-MDA5 autoantibody-positive patients more frequently reported stressful life events prior to illness onset, in contrast to anti-NXP2 patients (17 *vs* 5.4%, $P = 0.04$). Residential UV radiation exposure within 30 days of diagnosis was higher in anti-MDA5 patients compared with the MSA/MAA-negative group (average UV index 5.1 *vs* 3.7, $P = 0.04$). The frequency of medications and immunizations received in the 6 months prior to diagnosis did not differ between the anti-MDA5 patients and the other groups.

There was no seasonal clustering of the month of diagnosis in anti-MDA5, anti-TIF1, anti-NXP2 autoantibody-positive and MSA/MAA-negative JIIM patients (Supplementary Fig. S1A–C and E, available at *Rheumatology* online). In contrast, a seasonality in month of diagnosis was observed in anti-synthetase JIIM patients, with peak months of diagnosis ranging from May to July, and no patients with anti-synthetase autoantibodies were diagnosed from January to April ($P = 0.03$, Supplementary Fig. S1D, available at *Rheumatology* online). The two-sample comparison between the seasonal patterns of anti-MDA5 *vs* anti-TIF1, anti-NXP2, anti-synthetase-autoantibody-positive, and MSA/MAA-negative JIIM patients did not reveal differences. There was also no variation in the temporal trend in the frequency of anti-MDA5 autoantibody-positive patients compared with anti-TIF1 and anti-NXP2 autoantibody-positive patients over time, from 1988 to 2015, based on year of symptom onset or year of diagnosis of JIIM. The median population density of the residential location at diagnosis did not differ between anti-MDA5 and the other groups.

Medication history

Anti-MDA5 autoantibody-positive patients received fewer medications, and the corticosteroid treatment duration was shorter compared with those with anti-TIF1 and anti-NXP2 autoantibodies (Table 3), despite similar overall treatment duration among groups. Patients with anti-MDA5 autoantibodies less frequently received antimalarial therapy *vs* those with anti-TIF1. Usage of other medications was similar among patients with anti-MDA5 and the other groups. Anti-MDA5 patients less frequently increased therapy (46%), in contrast to anti-TIF1 (71%). The frequency of complete clinical response and remission did not differ from the other groups, whereas the frequency of clinical remission was higher in anti-MDA5 patients (27%) compared with those with anti-synthetase autoantibodies (0%).

Anti-MDA5 autoantibody-positive JIIM patients with ILD received corticosteroids for a longer duration [median 4.0 years (interquartile range 1.2–10.2)] compared with those without ILD [0.9 (0.4–2.0) years, $P = 0.04$], more frequently received cytotoxic/biologic medications (50 *vs* 4%, $P = 0.008$), and had no documented time off therapy. In addition, methotrexate was less frequently

TABLE 2 Laboratory investigations in anti-MDA5 autoantibody-associated juvenile myositis patients compared with other autoantibody groups

	Anti-MDA5	Frequency abnormal	Anti-TIF1	Frequency abnormal	Anti-NXP2	Frequency abnormal	Anti-ARS	Frequency abnormal	MSA/MAA-negative	Frequency abnormal
	n = 35	*n* (%)	*n* = 157	*n* (%)	*n* = 116	*n* (%)	*n* = 15	*n*(%)	*n* = 60	*n*(%)
	Median (IQR)		Median (IQR		Median (IQR)		Median (IQR)		Median (IQR)	
CK	184.0 (85.5–257.0)	10 (30)	447.5 (196.0–1602.0)****	103 (70)	1669.0 (438.0–5280.0)****	99 (89)	3205.0 (1121.0–6861.0)****	14 (93)	745.5 (293.0–3029.0)****	49 (82)
Aldolase	8.1 (6.9–12.1)	26 (76)	10.1 (7.3–16.8)*	112 (85)	12.0 (9.2–21.8)***	85 (92)	13.8 (8.7–38.9)***	12 (100)	11.8 (7.2–21.0)*	44 (88)
AST	75.0 (43.0–108.0)	30 (88)	64.0 (38.0–128.0)	112 (84)	105.0 (58.0–213.0)*	95 (92)	79 (46.0–222.0)	14 (93)	71 (40.0–181.0)	41 (79)
LDH	351.0 (258.0–577.0)	27 (87)	367 (267.0–591.0)	100 (84)	484.0 (292.0–674.0)	77 (89)	647.0 (341.0–807.0)	11 (92)	405 (300.0–830.0)	35 (79)
ANA titre	1:40 (0–320)	11 (52)	1:320 (80–940)****	101 (84)	1:80 (0–320)	68 (64)	1:320 (0–1080)	9 (60)	1:40 (0–320)	24 (46)

Anti-MDA5 autoantibody-positive group was compared with anti-TIF1, anti-NXP2, anti-ARS autoantibody positive, and MSA/MAA negative juvenile myositis groups. Note that 70 juvenile myositis patients with other autoantibodies were not included in this analysis. Significant differences from anti-MDA5 autoantibody-positive: *P <0.5; **P <0.01; ***P <0.001; ****P <0.0001. Note that percentage may not reflect the numbers divided by the total number of subjects, if data are missing. ANA: antinuclear autoantibodies; ARS: aminoacyl-tRNA synthetase; AST: aspartate aminotransferase; CK: creatine kinase; IQR: interquartile range; JIIM: juvenile myositis; LDH: lactate dehydrogenase; MAA: myositis associated autoantibody; MDA5: melanoma differentiation-associated gene 5; MSA: myositis specific autoantibody; NXP2: nuclear matrix protein 2; TIF1: transcriptional intermediary factor 1; U/L: units/litre; upper limit normal values: CK <252 U/L, aldolase <6 U/L, AST <34 U/L, LDH <226 U/L, positive ANA titre is >40.

received by anti-MDA5 patients without ILD (56 *vs* 76%, $P = 0.049$). They also had fewer treatment courses [median 3 (2.5–6) *vs* 5 (3–8), $P = 0.035$], and less frequent treatment escalation (40 *vs* 71%, $P = 0.005$) compared with anti-TIF1 patients.

Disease course

Patients with anti-MDA5 autoantibodies less frequently had a chronic illness course (29%) compared with those with anti-TIF1 and anti-synthetase (52–67%), and more frequently had a monocyclic course (26%) compared with those with anti-TIF1 autoantibodies (11%) (Table 4). Mortality was ≤3% and similar to the other autoantibody groups, except higher in anti-synthetase patients (13%). At the most recent evaluation, anti-MDA5 patients more frequently had active disease (69%) and periungual capillary changes (39%) *vs* those with anti-NXP2 autoantibodies, and more frequent skin rashes (57%) *vs* MSA/MAA-negative patients.

Multivariable analysis results

Random Forests analysis (Supplementary Table S2, available at *Rheumatology* online) followed by multivariable logistic regression (Table 5) revealed weight loss [odds ratio (OR) range 5.6–13.6] and arthritis (OR range 6.3–9.5) to be the top factors in differentiating anti-MDA5 from anti-TIF1 and anti-NXP2 autoantibody-positive and MSA/MAA-negative patients. Arthralgia (OR 4.2 and 6.1) was next in importance in differentiating anti-MDA5 patients from those with anti-TIF1 autoantibodies and MSA/MAA-negative. Less frequent dysphagia in the anti-MDA5 group was also an important distinction from anti-TIF1 and anti-NXP2 patients (OR 0.12 and 0.19). Less frequent falling episodes (OR 0.26) distinguished anti-MDA5 from anti-TIF1 group, and periungual capillary changes (OR 5.2) were helpful in discriminating from MSA/MAA-negative patients. Less frequent ILD, lower serum CK levels, and shorter delay to diagnosis (OR each 0.06) were the most important factors in distinguishing anti-MDA5 from anti-synthetase group (Table 5).

Discussion

Anti-MDA5 autoantibody-associated JIIM is an unique phenotype distinguished from other MSA groups by the presence of frequent arthritis, weight loss, adenopathy, ILD, and less severe myositis. However, anti-MDA5 is distinguished from anti-synthetase autoantibody-positive JIIM by less frequent ILD, lower CK levels, and differing seasons of diagnosis. Anti-MDA5 has comparable outcomes, but with the ability to discontinue steroids more rapidly and less frequent flares compared with anti-TIF1 autoantibodies, and more frequent remission compared with anti-synthetase JIIM patients.

The frequency of anti-MDA5 autoantibodies in our JIIM cohort was close to 8%, comparable to reports of JDM and adult DM in North America and Europe [11–

TABLE 3 Treatment characteristics in anti-MDA5 autoantibody-associated juvenile myositis patients compared with other myositis autoantibody groups

Variable	Anti-MDA5 *n* = 33 Median (IQR) or *n* (%)	Anti-TIF1 *n* = 143 Median (IQR) or *n* (%)	Anti-NXP2 *n* = 105 Median (IQR) or *n* (%)	Anti-ARS *n* = 14 Median (IQR) or *n* (%)	MSA/MAA-negative *n* = 54 Median (IQR) or *n* (%)
Median disease duration (years)	3.1 (1.0–7.7)	4.5 (2.0–8.7)	3.8 (2.3–7.5)	2.3 (0.74–4.2)	4.3 (2.1–7.4)
Treatment characteristics					
Total number of medication trials	4 (3–6)	5 (3–8)*	5 (3–8)	4 (2–5)	3 (2–6)
Number of major medications	2 (2–4)	3 (2–4)	3 (2–4)	3 (2–4)	2 (1–3)
Median steroid treatment duration, months	17.0 (8.1–36.0)	27.0 (15.6–53.6)*	27.0 (16.4–48.0)*	15.9 (6.2–32.6)	21.8 (10.9–47.8)
Medication usage					
Oral prednisone	33 (100.0)	141 (98.6)	104 (99.1)	14 (100.0)	53 (98.2)
IV methylprednisolone	18 (54.6)	86 (60.1)	61 (58.1)	9 (64.3)	21 (38.9)
Methotrexate	20 (60.6)	109 (76.2)	78 (74.3)	10 (71.4)	28 (51.9)
IV Immunoglobulin	11 (33.3)	53 (37.1)	41 (39.1)	2 (14.3)	13 (24.1)
Other DMARDs	7 (21.2)	37 (26.8)	23 (21.9)	3 (21.4)	8 (14.8)
Cytotoxic drugs/biologics[a]	5 (15.2)	19 (13.3)	12 (11.4)	2 (14.3)	5 (9.3)
Antimalarial drugs	15 (45.5)	89 (62.2)*	37 (35.2)	4 (28.6)	16 (29.6)
Combination of 2 major medications	21 (63.6)	110 (76.9)	74 (70.5)	10 (71.4)	32 (59.3)
Combination of 3 major medications	10 (30.3)	60 (42.0)	43 (41.0)	7 (50.0)	13 (24.1)
Combination of ≥4 major medications	6 (18.2)	30 (21.0)	23 (21.9)	1 (7.1)	6 (11.1)
Therapeutic outcomes					
Treatment escalation[b]	15 (45.5)	101 (70.6)*	64 (59.1)	8 (57.1)	27 (50.0)
Complete clinical response	8 (25.0)	38 (27.9)	41 (40.2)	3 (21.4)	14 (25.9)
Remission	9 (27.3)	31 (21.7)	36 (34.3)	0 (0.0)*	16 (29.6)

Anti-MDA5 autoantibody positive group was compared with anti-TIF1, anti-NXP2, and anti-ARS autoantibody-positive and MSA/MAA-negative JIIM groups. Note that 70 juvenile myositis patients with other autoantibodies were not included in this analysis. Note that percentages may not reflect the number divided by the total number of subjects, if data are missing. [a]Cytotoxic drugs/biologics received by anti-MDA5 group included: cyclophosphamide (oral and/or intravenous), etanercept, abatacept. [b]Treatment escalation, an increase of therapy due to clinical or laboratory evidence of increased disease activity resulting in the addition of a new medication, or an increase in the dose of an existing medication by ≥25%. Significant differences from anti-MDA5 autoantibody-positive: *P <0.05. ARS: aminoacyl-tRNA synthetase; IQR: interquartile range; JIIM: juvenile myositis; MAA: myositis associated autoantibody; MDA5: melanoma differentiation-associated gene 5; MSA: myositis specific autoantibody; NXP2: nuclear matrix protein 2; TIF1: transcriptional intermediary factor 1.

17], although less frequent than in Asian population [7–10]. Of note, the distribution of the background of origin of children who developed MDA5-autoantibody positive disease in North America was not primarily Asian, which may account for some of the clinical differences compared with those from Japan [7–10]. In contrast, a higher proportion of Asian patients (33% and 71%) was reported among Canadian anti-MDA5 DM patients, and they also had a higher frequency of RP-ILD [16, 17].

The clinical phenotype of anti-MDA5 autoantibody-associated JIIM in our cohort was comparable to other reports of anti-MDA5 autoantibodies in adult DM and JDM from the United States and Europe [11–15, 29]. However, we have shown some specific characteristics that distinguish anti-MDA5 JIIM from the other major MSAs and MSA/MAA negative groups. These data may help physicians identify JIIM patients with anti-MDA5 autoantibodies among other JIIM patients.

Specifically, the cutaneous disease in DM/JDM patients with anti-MDA5 autoantibodies presents with cutaneous and mucosal ulcerations and palmar papules [11, 12, 14–17, 29], in addition to the characteristic DM rashes. In our MDA5 JIIM group, however, alopecia and mechanic's hands were less frequent than in adult DM patients with MDA5 autoantibodies [11, 14–16, 29]. In our study, 31% of MDA5 patients had cutaneous ulcerations, which is less frequent than the 38–80% reported in other cohorts [11, 13, 14]. Moreover, we did find certain cutaneous features (alopecia, digital infarcts and periungual capillary changes) present more frequently in anti-MDA5 JIIM patients compared with those with anti-NXP2 autoantibodies and MSA/MAA-negative patients.

The muscle disease was mild in anti-MDA5 autoantibody-positive JIIM patients, consistent with other reports [12, 14–17]. Unlike adults, in which MDA5 autoantibodies can be associated with clinically-amyopathic DM, MDA5 autoantibodies were uncommonly present in clinically-amyopathic JDM [30]. Similar to previous studies in JDM and DM, arthritis and arthralgia (86–88%) were common, and occurred more frequently in anti-

TABLE 4 Outcomes in anti-MDA5 autoantibody-associated juvenile myositis patients compared with other myositis autoantibody groups

Variable	Anti-MDA5 *n* = 35 Median (IQR) or *n* (%)	Anti-TIF1 *n* = 157 Median (IQR) or *n* (%)	Anti-NXP2 *n* = 116 Median (IQR) or *n* (%)	Anti-ARS *n* = 15 Median (IQR) or *n* (%)	MSA/MAA-negative *n* = 60 Median (IQR) or *n* (%)
Median disease duration, years	3.1 (1.0–7.7)	4.5 (2.0–8.7)	3.8 (2.3–7.5)	2.3 (0.74–4.2)	4.3 (2.1–7.4)
Disease course[a]					
Chronic	10 (28.6)	82 (52.2)*	45 (45.9)	10 (66.7)*	19 (31.7)
Polycyclic	3 (8.6)	25 (15.9)	26 (22.4)	1 (6.7)	14 (23.3)
Monocyclic	9 (25.7)	17 (10.8)*	27 (23.3)	2 (13.3)	19 (31.7)
Mortality	1 (2.8)	3 (1.9)	2 (1.7)	2 (13.3)	2 (3.3)
Wheelchair use	1 (2.9)	8 (5.1)	8 (6.9)	1 (6.7)	0 (0.0)
Ever hospitalized	17 (50.0)	74 (49.3)	71 (63.4)	10 (66.7)	23 (42.6)
Median number of hospitalizations	0.25 (0–1)	0 (0–1)	1 (0–2)	1 (0–2)	0 (0–1)
Outcome at last evaluation					
Frequency of active disease	24 (68.6)	110 (71.0)	55 (47.8)*	12 (80.0)	31 (51.7)
Median ACR functional class	1 (1–2)	1 (1–2)	1 (1–1)	1 (1–2)	1 (1–1)
Muscle enzymes elevated	11 (34.4)	39 (26.7)	33 (29.2)	7 (50.0)	20 (34.5)
Muscle weakness	16 (45.7)	81 (51.9)	48 (41.7)	6 (40.0)	22 (37.3)
Muscle atrophy	5 (14.3)	40 (25.6)	23 (20.4)	2 (13.3)	7 (11.9)
Periungual capillary changes	13 (39.4)	65 (44.2)	21 (20.0)*	3 (20.0)	13 (22.8)
Skin rash	20 (57.1)	102 (65.4)	48 (41.7)	6 (40.0)	20 (33.9)*
Skin atrophy	10 (30.3)	51 (34.2)	20 (18.3)	1 (7.1)	10 (16.9)
Calcinosis	8 (22.9)	38 (24.5)	29 (25.2)	2 (13.3)	17 (28.8)

Anti-MDA5 autoantibody-positive group was compared with anti-TIF1, anti-NXP2, and anti-ARS autoantibody positive and MSA/MAA-negative JIIM groups. Note that 70 juvenile myositis patients with other autoantibodies were not included in this analysis. [a]See [3]. Significant differences from anti-MDA5 autoantibody-positive: *$P < 0.05$. Note that percentages may not reflect the number divided by the total number of subjects, if data are missing. ARS: aminoacyl-tRNA synthetase; IQR: interquartile range; JIIM: juvenile myositis; MAA: myositis associated autoantibody; MSA: myositis specific autoantibody; MDA5: melanoma differentiation-associated gene 5; NXP2: nuclear matrix protein 2; TIF1: transcriptional intermediary factor 1.

MDA5 compared with anti-TIF1 and anti-NXP2 autoantibody-positive, and the MSA/MAA-negative groups [8, 11, 12, 14–17, 31].

The anti-MDA5 autoantibody JIIM phenotype was also associated with a high frequency of constitutional symptoms (weight loss, fever and adenopathy), as previously reported [15, 16, 31]. Fever has been described in 25–67% of anti-MDA5 JDM and DM patients, and furthermore, as a single presenting symptom in the absence of cutaneous or muscular manifestations of DM [32].

Pulmonary manifestations are the most important features determining the clinical course and prognosis in anti-MDA5 autoantibody-positive DM and JDM, and have been reported in 67–100% of patients, with the highest frequency in Asian patients [7–17, 29, 33, 34]. We documented ILD in 26% of anti-MDA5 JIIM, similar to a report of 20% of JDM patients with anti-MDA5 autoantibodies in the United Kingdom [12]. However, ILD was less frequent compared with the anti-synthetase group (67%), but more frequent compared with other MSAs and the MSA/MAA-negative groups. Although prior reports found MDA5 autoantibodies to be frequently associated with RP-ILD or chronic ILD [7–10, 13, 15–17], we observed RP-ILD in only 6% of the anti-MDA5 JIIM patients. A lower percentage of RP-ILD may be related to enrolment bias in our studies, as a number of patients with RP-ILD were too ill or medically unstable to enrol (unpublished).

Anti-MDA5 and anti-synthetase autoantibody-associated myositis share a number of overlapping clinical features [15]. Through multivariable analysis we were able to determine several features distinguishing these two groups, including less frequent ILD, lower serum CK levels, and shorter delay to diagnosis as the features best differentiating MDA5 from anti-synthetase group. In contrast to other studies, we found the MDA5 patients had less frequent mechanic's hands *vs* anti-synthetase patients [15]. A shorter delay to diagnosis in anti-MDA5 patients compared with those with anti-synthetase autoantibodies is possibly related to the presence of more prominent cutaneous and systemic features at illness onset. Anti-synthetase JIIM patients also more frequently had a chronic illness course and less frequently entered remission, similar to adult patients [1].

Environmental and genetic factors may contribute to illness onset in JIIM [22, 35]. The associations of these with anti-MDA5 autoantibodies in JIIM in North America appeared to differ from studies in Japan. We found

TABLE 5 Multivariable logistic regression model for anti-MDA5 autoantibody-associated juvenile myositis compared with other myositis autoantibody groups

Variable[a]	Odds ratio	95% confidence interval	*P*-value
Anti-MDA5 (n =34) autoantibody-positive *vs* anti-TIF1 autoantibody-positive (n =155) JIIM			
Weight loss	9.2	1.3, 17.2	<0.0001
Arthritis	6.5	2.2, 24.6	0.002
Arthralgia	4.2	3.9, 17.2	0.026
Dysphagia	0.19	0.06, 0.55	0.004
Falling episodes	0.26	0.08, 0.74	0.017
Likelihood ratio chi-square 64.9, P<0.0001, percent concordant 86.9, c statistic 0.884			
Anti-MDA5 autoantibody-positive (n =31) *vs* anti-NXP2 autoantibody-positive (n =74) JIIM			
Weight loss	13.6	3.9, 58.8	0.0001
Arthritis	9.5	2.5, 46.2	0.002
Dysphagia	0.12	0.02, 0.61	0.018
Likelihood ratio chi-square 58.4, P<0.0001, percent concordant 88.8, c statistic 0.893			
Anti-MDA5 autoantibody-positive (n =23) *vs* anti-synthetase autoantibody-positive (n =14) JIIM			
Interstitial lung disease	0.058	0.002, 0.57	0.032
Serum creatine kinase level	0.063	0.003, 0.54	0.027
Delay to diagnosis	0.063	0.003, 0.54	0.029
Likelihood ratio chi-square 25.9, P<0.0001, percent concordant 88.8, c statistic 0.916			
Anti-MDA5 autoantibody-positive (n =33) *vs* MSA/MAA-negative (n =54) JIIM			
Arthritis	6.3	1.4, 35.6	0.023
Arthralgia	6.1	1.4, 34.9	0.025
Weight loss	5.6	1.6, 21.7	0.008
Periungual capillary changes	5.2	1.2, 27.9	0.039
Likelihood ratio chi-square 49.2, P<0.0001, percent concordant 88.8, c statistic 0.896			

[a]The top variables from the pruned Random Forests models were used in the logistic regression models. Only subjects with complete data were used in the analysis. Note that 70 juvenile myositis patients with other autoantibodies were not included in this analysis. ARS: aminoacyl-tRNA synthetase; IQR: interquartile range; JIIM: juvenile myositis; MAA: myositis associated autoantibody; MSA: myositis specific autoantibody; MDA5: melanoma differentiation-associated gene 5; NXP2: nuclear matrix protein 2; TIF1: transcriptional intermediary factor 1.

stressful life events and UV radiation exposure within 6 months of diagnosis were increased in anti-MDA5 autoantibody-positive patients compared with anti-NXP2 and MSA/MAA-negative groups. We did not observe an increased association with infections in anti-MDA5 JIIM, although could not differentiate the specific types of infection prior to disease onset. This finding may differ from the hypothesis that certain viral infections may activate the retinoic acid-inducible gene I viral sensing pathway and the expression of anti-MDA5 autoantibodies [35, 36]. There was no seasonal pattern of diagnosis in the anti-MDA5 group, in contrast to patients with anti-synthetase autoantibodies, who had a late spring to early summer seasonal disease onset. A peak season of onset in autumn and winter in DM patients with anti-MDA5 autoantibodies has been observed in Japan, particularly in less dense population areas [37, 38]. The Japanese studies also found geographic clustering of cases associated with lower population density, which we did not observe, and with proximity to bodies of water [37, 38]. Unlike the Muro *et al*. [37] study, we did not see an increase in the prevalence of anti-MDA5 autoantibodies over time, especially notable as we compared this subgroup to the prevalence of anti-TIF1 and anti-NXP2 autoantibodies, which were discovered in a similar time period. We also did not identify a HLA risk factor, in contrast to the association with HLA-DRB1*0101/*0405 alleles in Japanese DM patients with anti-MDA5 autoantibodies [39]. Further larger studies of environmental and genetic factors associated with the anti-MDA5 autoantibodies are needed and may account for some of the phenotypic differences between North America and Japan in this subgroup.

Medication therapy in anti-MDA5 patients was similar to the other autoantibody groups, except for a shorter duration of corticosteroid treatment and less frequent usage of antimalarial therapy compared with the anti-TIF1 group. Similarly, Tansley *et al.* [12] reported no significant difference in the frequency of patients with and without anti-MDA5 autoantibodies treated with methotrexate and cyclophosphamide. We did note a longer duration of corticosteroid treatment and increased use of cytotoxic and biologic therapies in the anti-MDA5-positive JIIM patients with ILD. In the present study, anti-MDA5 JIIM patients had a low mortality, which was a comparable outcome to the other

autoantibody groups, in contrast to high mortality reported in anti-MDA5 DM and JDM patients with RP-ILD [7–10].

Limitations of the present study include the retrospective nature of the data, and relatively small sample size of the anti-MDA5 autoantibody group, although our group is larger than most previous reports. Future studies are needed, especially of the subgroup of anti-MDA5 autoantibody patients with RP-ILD.

In conclusion, we have described the anti-MDA5 autoantibody-associated JIIM phenotype in North America as a distinct subset with frequent musculoskeletal and constitutional characteristics, ILD, and specific cutaneous features, but with less severe muscle involvement. MDA5 patients have comparable outcomes to other major MSAs and MSA/MAA-negative JIIM, although with the ability to discontinue steroids more rapidly and with less frequent disease flares compared with patients with anti-TIF1 autoantibodies. Anti-MDA5 autoantibody-positive JIIM patients also have less frequent ILD and more frequent remission compared with those with anti-synthetase autoantibodies.

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Supplementary data

Supplementary data are available at *Rheumatology* online.

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