Association of NXP2 autoantibodies with a more severe clinical phenotype of juvenile dermatomyositis

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Abstract

Objective

Myositis specific autoantibodies (MSAs) correlate with a distinct clinical phenotype of juvenile dermatomyositis (JDM). This study aims to compare the clinical features of JDM patients with positive anti-NXP2, anti-MDA5 and anti-TIF1γ autoantibodies, and differences in juvenile (JDM) and adult dermatomyositis.

Methods

This study included 18 NXP2$^+$ JDM patients, 12 MDA5$^+$ JDM patients, 12 TIF1γ$^+$ JDM patients, and 20 NXP2$^+$ adult DM patients. Repeated measures analysis was performed to track longitudinal changes in creatine kinase (CK) levels, Childhood Myositis Assessment Scale (CMAS) scores, and IL-10/γ-interferon ratios. Kaplan-Meier survival and Cox regression analysed relapse rates and recurrence factors.

Results

NXP2$^+$ JDM patients exhibited significantly elevated serum creatine kinase levels compared to MDA5$^+$ JDM (3792 vs. 180; p<0.001), adult NXP2$^+$DM (3792 vs. 437.5; p=0.003), and TIF1-γ$^+$ JDM (3792 vs. 189; p<0.001). Concurrently, NXP2$^+$ JDM patients also showed lower CMAS scores compared to MDA5$^+$ (31.33 vs. 43.25; p<0.001) and TIF1-γ$^+$ JDM groups (31.33 vs. 42.67; p=0.004). The NXP2$^+$ JDM patients presented with a high frequency of macrophage activation syndrome (MAS), myocardial damage, dysphagia, and calcinosis. Repeated measures analysis showed that NXP2$^+$ JDM patients had more severe muscle damage throughout the disease course compared with MDA5$^+$ JDM patients and TIF1γ$^+$ JDM patients. Furthermore, significant interaction effects of Group and Time on CK were observed in JDM patients. A three-year follow-up study revealed a higher relapse risk in NXP2$^+$ JDM patients compared to MDA5$^+$ JDM, TIF1γ$^+$ JDM and NXP2$^+$ adult DM patients.

Conclusion

The NXP2$^+$ JDM patients experience more severe muscle damage, systemic complications, and higher relapse risks. Monitoring dynamic changes in CK and CMAS is essential for predicting disease progression and relapse risk.

Key words

clinical phenotype, juvenile dermatomyositis, NXP2 autoantibody, MDA5 autoantibody, recurrence risk

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Introduction

Juvenile dermatomyositis (JDM) is the most common idiopathic inflammatory myopathy in children, primarily affecting the skin and muscles, and represents a multi-system autoimmune disorder (1). In recent years, a number of myositis-specific autoantibodies (MSAs) and myositis-associated autoantibodies (MAAs) have been identified and well characterised, and commercial assays for their detection have become available (2). MSAs and MAAs are identified in about 70% of patients with DM (3). Numerous studies have demonstrated that each MSA including anti-nuclear matrix protein 2 (anti-NXP2), anti-melanoma differentiation-associated gene 5 (anti-MDA5), anti-Mi2, anti-transcription intermediary factor 1 gamma (anti-TIF1), anti-small ubiquitin-like modifier activating enzyme (anti-SAE), anti-signal recognition peptide (anti-SRP) and anti-synthetase autoantibodies correlates with a distinct clinical phenotype, making MSAs important diagnostic biomarkers (2, 4-6).

Anti-NXP2 autoantibodies are frequently detected in JDM. They were first observed with a high frequency in patients with JDM and associated with calcinosis and a severe phenotype characterised by muscle contractures and atrophy and significant compromise of functional status (7, 8). Different from adult dermatomyositis patients with positive anti-NXP2 antibodies, paediatric patients are more likely to be at higher risk of complications such as macrophage activation syndrome (MAS) and intestinal perforation (8-10). In contrast, anti-MDA5-positive patients are characterised by skin ulcerations and rapidly progressive interstitial lung disease (RP-ILD), with minimal or no muscle involvement (11). Widespread, severe cutaneous manifestations, including photosensitive rashes, skin ulcers, and lipodystrophy usually characterise anti-TIF1γ-positive patients (12).

The present study aimed to analyse the clinical features of JDM with positive anti-NXP2 autoantibodies and investigate the differences between paediatric and adult anti-NXP2-positive patients. Dynamic changes in serum creatine kinase (CK) levels and muscle function scores (CMAS) were analysed through repeated measurements to assess disease progression. Furthermore, survival analysis was employed to explore relapse risks between different patient groups.

Patients and methods

Patients

This is a retrospective study that included 18 JDM patients with anti-NXP2 autoantibodies, 12 JDM patients with anti-MDA5 autoantibodies and 12 JDM patients with anti-TIF1γ autoantibodies from the Children's Hospital of Zhejiang University School of Medicine, and 20 adult dermatomyositis patients with anti-NXP2 antibodies from the First Affiliated Hospital of Zhejiang University School of Medicine. All patients were newly diagnosed and treated at the above hospitals and were followed up for at least 3 years. The diagnosis of JDM was based on the Bohan and Peter criteria (13), which include:

1. symmetrical proximal muscle weakness; 2. characteristic rash; 3. elevated muscle enzymes; 4. myopathic changes on electromyography; 5. biopsy-proven myositis. Definite JDM was defined as the presence of a characteristic rash plus three additional criteria, probable JDM as a rash plus two criteria, and possible JDM as a rash plus one criterion. The study was approved by the Ethic Review Board of Children's Hospital, Zhejiang University School of Medicine. In accordance with the Helsinki Declaration, informed consent was waived as the data were anonymised and de-identified prior to analysis, and the study was determined to pose no additional risk to patients.

Definitions and assessment of clinical outcomes

Skin involvement includes skin calcifications, Gottron's papules, heliotrope rash, and rashes in other areas (such as the shoulders/neck, chest/back, and lower limbs). Cardiac damage was defined as the presence of clinical symptoms (*e.g.* chest pain, arrhythmia, or heart failure) accompanied by objective findings such as elevated cardiac troponin levels, abnormal electrocardiogram (ECG), or echocardiographic evidence of myocardial dysfunction. Gas-

Table I. Comparison of clinical features between NXP2+ JDM, MDA5+ JDM and TIF1γ+JDM.

Variables	NXP2+ JDM (n=18)	MDA5+ JDM (n=12)	*p*-value	TIF1-γ+JDM (n=12)	*p*-value
CMAS	31.33 ± 11.80	43.25 ± 4.67	<0.001***	42.67 ± 8.06	0.004**
Age at onset (years)	8.01 ± 3.80	7.50 ± 3.84	0.720	5.04 ± 3.19	0.034
Sex, F (%)	7 (38.89)	6 (50.00)	0.711	4 (33.33)	1.000
Course of diseases (months)	1.17 (1.00, 2.00)	3.00 (2.00, 6.00)	0.056	0.40 (0.20,0.77)	0.006**
WBC (×10^9/L)	7.76 ± 3.23	5.86 ± 3.3	0.132	7.76 ±1.93	1.000
HB (g/L)	110.28 ± 30.15	115.08 ± 9.87	0.600	125.17 ± 12.19	0.117
PLT (×10^9/L)	264.78 ± 93.90	266.67 ± 109.98	0.960	309.50 ± 70.91	0.172
CRP (mg/L)	0.75 (0.35, 1.47)	0.7 (0.5, 1.21)	0.636	0.5 (0.5, 1.09)	0.812
ESR (mm/h)	20.04 ± 12.14	21.77 ± 14.61	0.731	12.71 ± 10.25	0.099
CK (U/L)	3792 (893, 5126)	180 (59, 284)	<0.001***	189 (63,301)	<0.001***
CK-MB (U/L)	50 (30.1, 169)	25 (23, 27.5)	0.027*	29 (26.75,36.75)	0.032*
ALT (U/L)	81 (44, 97)	63 (27, 109)	0.626	24 (19.5, 26.5)	0.001**
AST (U/L)	147 (66, 232)	99.5 (61.5, 174)	0.413	49 (34.25, 64)	<0.001***
LDH(U/L)	755.29±332.26	543.50 ± 251.77	0.074	479.09 ± 191.55	0.019*
Creatinine (μmol/L)	31.75 ± 11.29	43.58 ± 13.30	0.021*	36.45 ± 13.13	0.329
Urea (mmol/L)	4.23 ± 0.97	3.52 ± 0.88	0.065	4.43 ± 0.92	0.600
Ferritin (μg/L)	345.1 (249.6,595.2)	434.35 (250.3,490.17)	0.776	112.7 (32.5,125.75)	0.001**
IgG (g/L)	12.39 ± 4.98	15.26 ± 5.94	0.177	11.78 ± 3.13	0.722
IgA (g/L)	1.39 ± 0.70	1.56 ± 0.66	0.515	0.92 ± 0.37	0.050
IgM (g/L)	0.87 (0.72, 1.19)	1.12 (0.93, 1.72)	0.097	1.31 (1.13, 1.38)	0.024*
C3 (g/L)	0.98 ± 0.22	1.10 ± 0.26	0.216	1.13 ± 0.19	0.086
C4 (g/L)	0.29 ± 0.08	0.36 ± 0.10	0.092	0.33 ± 0.09	0.288
CD19 (%)	20.59 ± 12.47	15.34 ± 7.73	0.269	23.00 ± 10.29	0.607
CD3 (%)	66.19 ± 13.50	73.88 ± 12.53	0.188	66.19 ± 10.78	0.999
CD4 (%)	37.14 ± 12.99	40.90 ± 6.80	0.362	41.90 ± 8.74	0.304
CD8 (%)	25.26 ± 8.37	29.86 ± 9.20	0.239	19.83 ± 8.72	0.121
NK (%)	5.39 ± 3.88	6.37 ± 3.63	0.532	6.92 ± 3.49	0.310
CD4/CD8	1.69 ± 1.00	1.47 ± 0.49	0.568	2.30 ± 0.90	0.131
IL-2 (pg/ml)	2.90 (1.95, 3.65)	2.00 (1.90, 2.80)	0.207	2.70 (1.95,3.55)	0.815
IL-4 (pg/ml)	2.90 (2.40, 3.20)	2.20 (1.80, 2.40)	0.014*	2.10 (1.80,2.80)	0.058
IL-6 (pg/ml)	11.80 (9.70,30.95)	17.60 (13.55,22.22)	0.292	5.60 (2.75,8.20)	0.007**
IL-10 (pg/ml)	5.50 (4.00, 8.00)	6.50 (4.40, 9.80)	0.244	5.30 (3.05,5.85)	0.350
TNF-α (pg/ml)	1.40 (1.05, 2.30)	1.50 (1.20, 2.40)	0.674	1.20 (1.00,2.00)	0.580
γ-IFN (pg/ml)	2.90 (1.85, 3.55)	1.50 (1.40, 3.20)	0.420	1.60 (1.10,4.20)	0.483
IL-10/γ-IFN	1.90 (1.39, 3.53)	4.20 (3.40, 5.13)	0.041*	2.18 (1.17,3.66)	1.000
MAS, n (%)	4 (22.22)	1 (8.33)	0.622	0 (0.00)	0.130
Myocardial damage, n (%)	6 (33.33)	1 (8.33)	0.193	1 (8.33)	0.193
Dysphagia, n (%)	5 (27.78)	0 (0.00)	0.066	0 (0.00)	0.066
Mouth ulcer, n (%)	3 (16.67)	1 (8.33)	0.632	1 (8.33)	0.632
Calcinosis cutis, n (%)	3 (16.67)	0 (0.00)	0.255	0 (0.00)	0.255
Gottron papules, n (%)	13 (72.22)	12 (100.00)	0.066	12 (100.00)	0.066
Heliotrope rash, n (%)	17 (94.44)	12 (100.00)	1.000	12 (100.00)	1.000
Skin rash on other parts, n (%)	5 (27.78)	4 (33.33)	1.000	3 (25.00)	1.000
ILD, n (%)	5 (27.78)	2 (16.67)	0.669	2 (16.67)	0.669
Gastrointestinal involvement, n (%)	2 (11.11)	1 (8.33)	1.000	0 (0.00)	0.503
Other complications, n (%)	9 (50.00)	2 (16.67)	0.121	0 (0.00)	0.004**
Recurrence, n (%)	9 (60.00)	1 (8.33)	0.014*	6 (50.00)	0.707

Continuous variables as median [interquartile range (IQR)] or mean ± SD, and dichotomous variables were represented as count (percentage).
NXP2: nuclear matrix protein 2; MDA5: melanoma differentiation-associated protein 5; TIF1-γ: transcription intermediary factor 1-gamma; JDM: juvenile dermatomyositis; CMAS: Childhood Myositis Assessment Scale; WBC: white blood cell; HB: haemoglobin; PLT: platelet; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; CK: creatine kinase; CK-MB: creatine kinase-MB; ALT: alanine aminotransferase; AST: aspartate aminotransferase; LDH: lactate dehydrogenase; IL: interleukin; TNF-α: tumour necrosis factor-alpha; γ-IFN: gamma interferon; IL-10/γ-IFN: interleukin-10 to gamma interferon ratio; MAS: macrophage activation syndrome; ILD: interstitial lung disease; Ig: immunoglobulin; C3: complement 3; CD: cluster of differentiation.
Other complications: sepsis, pleural effusion, anaemia, joint contracture.
*p<0.05, ** p<0.01, *** p<0.001.

trointestinal involvement was identified based on symptoms such as abdominal pain, vomiting, diarrhoea, or gastrointestinal bleeding, with supporting imaging or endoscopic findings. Dysphagia was assessed through symptoms of difficulty swallowing alongside clinical evaluation.

Statistical analysis

Continuous variables were described as means and standard deviations or median [interquartile range (IQR)], with group comparisons made using the non-parametric Mann-Whitney U-test. Categorical variables were presented as absolute frequencies (percentages) and compared using Fisher's exact test. Significant variables were analysed using repeated measures ANOVA and linear mixed-effects models. Fixed effects included group, quadratic time effects, and group: time interactions, where statistical significance would indicate a group effect. Random effects

allowed time slopes and intercepts to vary. Between-participant correlations were modelled as a first-order autoregressive error. Missing data were handled using mixed-effects models. Survival analysis was estimated using the Kaplan-Meier method, and subgroup differences were assessed using the log-rank test. A *p*-value less than 0.05 was considered statistically significant. All analyses were conducted using the R Project for Statistical Computing (R, version 3.4).

Results

Comparison of clinical features between positive anti-NXP2 JDM, positive anti-MDA5 JDM, positive anti- TIF1γ JDM, and positive anti-NXP2 adult DM on admission

We collected the initial clinical data of 42 patients with juvenile dermatomyositis (JDM) on admission, including 18 patients with anti-NXP2 autoantibodies, 12 patients with anti-MDA5 autoantibodies, and an additional 12 patients with anti-TIF1γ autoantibodies (Table I). The NXP2$^+$ JDM patients had higher serum creatine kinase (CK) levels (3792 *vs*. 180 IU/L, $p<0.001$) and lower CMAS (Childhood Myositis Assessment Scale, 31.33 *vs*. 43.25, $p<0.001$) compared with those MDA5$^+$ JDM patients, indicating a more severe muscle involvement in the NXP2$^+$ JDM. Notably, when compared to the TIF1γ$^+$ JDM, NXP2$^+$ patients also exhibited higher CK levels (3792 *vs*. 189 IU/L, $p<0.001$) and lower CMAS scores (31.33 *vs*. 42.67, $p=0.004$), further supporting the association of NXP2 antibodies with aggressive myopathy. The NXP2$^+$ JDM patients had significantly higher serum levels of ALT (81 *vs*. 24 U/L, $p=0.001$), AST (147 *vs*. 49 U/L, $p<0.001$), and Ferritin (345.1 *vs*. 112.7 μg/L, $p=0.001$) compared with the TIF1γ$^+$ JDM patients. There were no significant differences in serum IL-10 and interferon-γ (IFN-γ) levels between the NXP2$^+$ and MDA5$^+$ JDM patients. However, the IL-10/γ-IFN ratio was significantly higher in the MDA5$^+$ group ($p=0.041$), suggesting a potentially stronger anti-inflammatory response. Additionally, compared to TIF1γ$^+$ and MDA5$^+$ JDM subgroups, the NXP2$^+$ JDM patients presented with a high frequency of macrophage activation syndrome (MAS), myocardial damage, dysphagia, and calcinosis. Beyond classic dermatomyositis-related complications, NXP2$^+$ patients exhibited an elevated incidence of systemic comorbidities (9(50.00) *vs*. 0(0.00), $p=0.004$, including encephalopathy and sepsis, suggesting a broader spectrum of end-organ involvement in this autoantibody-defined phenotype.

Table II. Comparison of clinical features between NXP2$^+$ JDM and NXP2$^+$ adult DM.

Variables	NXP2+ JDM (n=18)	NXP2+adult DM (n=20)	*p*-value
Sex, F (%)	7 (38.89)	5 (25.00)	0.307
Course of diseases (months)	1.17(1.00, 2.00)	3.00 (2.75, 12.00)	0.008**
WBC (×10^9/L)	7.76 ± 3.23	6.62 ± 1.79	0.180
HB (g/L)	110.28 ± 30.15	115.65 ± 15.55	0.488
PLT (×10^9/L)	264.78 ± 93.90	235.55 ± 79.94	0.307
CRP (mg/L)	0.75 (0.35, 1.47)	3.1 (3, 6.16)	0.002**
ESR (mm/h)	13.65 (11, 28)	8 (5, 19.25)	0.045*
CK (U/L)	3792 (893, 5126)	437.5 (216.25, 765)	0.003**
CK-MB (U/L)	50 (30.1, 169)	22.5 (16.5, 40.75)	0.002**
ALT (U/L)	81 (44, 97)	43.5 (23.75, 56.75)	0.023*
AST (U/L)	175.06±126.63	71.55 ± 69.71	0.006**
LDH (U/L)	755.29±332.26	394.40 ± 169.14	<0.001***
Creatinine (μmol/L)	31.75 ± 11.29	50.25 ± 10.12	<0.001***
Urea (mmol/L)	4.23 ± 0.97	5.84 ± 2.10	0.005**
Ferritin (μg/L)	345.1 (249.6,595.2)	339.95 (261.55,635.28)	1.000
IgG (g/L)	12.39 ± 4.98	12.84 ± 4.05	0.768
IgA (g/L)	1.39 ± 0.70	2.12 ± 1.11	0.023*
IgM (g/L)	1.15 ± 0.64	1.05 ± 0.49	0.416
C3 (g/L)	0.98 ± 0.22	1.11 ± 0.14	0.039*
C4 (g/L)	0.29 ± 0.08	0.28 ± 0.09	0.633
CD19 (%)	20.59 ± 12.47	20.85 ± 11.22	0.949
CD3 (%)	66.19 ± 13.50	68.68 ± 13.48	0.586
CD4(%)	37.14 ± 12.99	47.01 ± 14.83	0.044*
CD8 (%)	25.26 ± 8.37	20.77 ± 7.98	0.116
NK (%)	4.1 (2.71, 7.1)	6.9 (3.03, 11.57)	0.217
CD4/CD8	1.69 ± 1.00	3.17 ± 2.72	0.053
IL-2 (pg/ml)	2.90 (1.95, 3.65)	0.32 (0.10, 1.29)	0.001***
IL-4 (pg/ml)	2.90 (2.40, 3.20)	0.10 (0.10, 1.18)	0.001***
IL-6 (pg/ml)	11.80 (9.70,30.95)	4.26 (2.68,10.33)	0.017*
IL-10 (pg/ml)	5.50 (4.00, 8.00)	2.75 (1.94, 4.15)	0.027*
TNF-α (pg/ml)	1.40 (1.05, 2.30)	0.86 (0.10, 1.92)	0.091
γ-IFN (pg/ml)	2.90 (1.85, 3.55)	0.97 (0.10, 2.81)	0.048*
IL-10/γ-IFN	1.90 (1.39, 3.53)	2.56 (0.88,26.35)	0.521
MAS, n (%)	4 (22.22)	0 (0.00)	0.041*
Myocardial damage, n (%)	6 (33.33)	0 (0.00)	0.007**
Dysphagia, n (%)	5 (27.78)	2 (10.00)	0.222
Mouth ulcer, n (%)	3 (16.67)	0 (0.00)	0.097
Calcinosis cutis, n (%)	3 (16.67)	0 (0.00)	0.097
Gottron papules, n (%)	13 (72.22)	14 (70.00)	1.000
Heliotrope rash, n (%)	17 (94.44)	15 (75.00)	0.184
Skin rash on other parts, n (%)	5 (27.78)	0 (0.00)	0.017*
Malignancy, n (%)	0 (0.00)	3 (15.00)	0.232
ILD, n (%)	5 (27.78)	16 (80.00)	0.003**
Gastrointestinal involvement, n (%)	2 (11.11)	0 (0.00)	0.218
Other complications, n (%)	9 (50.00)	0 (0.00)	<0.001***
Recurrence, n (%)	9 (60.00)	1 (5.00)	<0.001***

Continuous variables as median [interquartile range (IQR)] or mean ± SD, and dichotomous variables were represented as count (percentage).

NXP2: nuclear matrix protein 2; JDM: juvenile dermatomyositis; CMAS: Childhood Myositis Assessment Scale; WBC: white blood cell; HB: haemoglobin; PLT: platelet; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; CK: creatine kinase; CK-MB: creatine kinase-MB; ALT: alanine aminotransferase; AST: aspartate aminotransferase; LDH: lactate dehydrogenase; IL: interleukin; TNF-α: tumour necrosis factor-alpha; γ-IFN: gamma interferon; IL-10/γ-IFN: interleukin-10 to gamma interferon ratio; MAS: macrophage activation syndrome; ILD: interstitial lung disease; Ig: immunoglobulin; C3: complement 3; CD: cluster of differentiation.

Other complications: sepsis, pleural effusion, anaemia, joint contracture.

*$p<0.05$, **$p<0.01$, ***$p<0.001$.

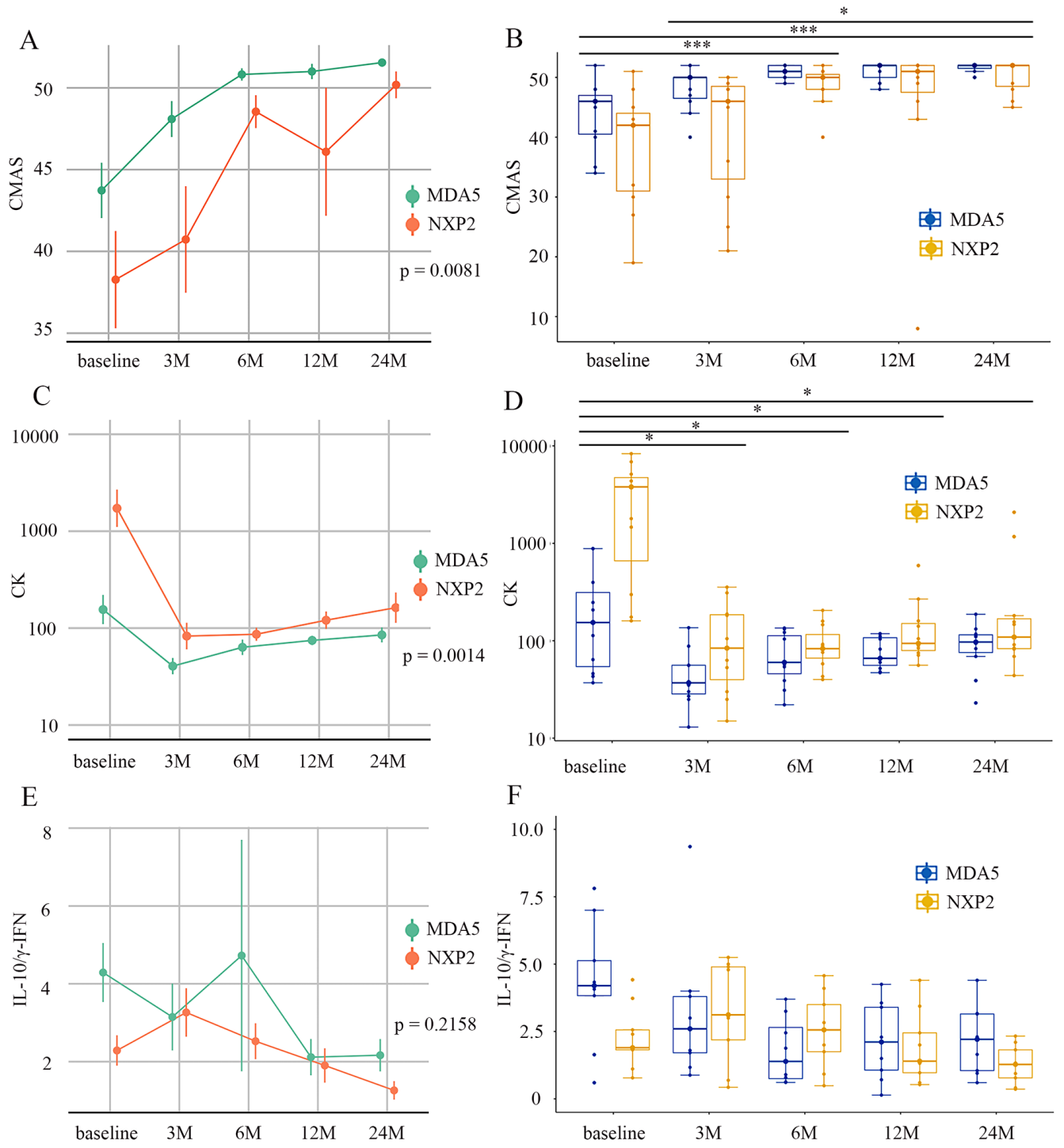

Fig. 1. Comparison of CK, CMAS, and IL-10/γ-IFN in NXP2 and MDA5 autoantibody-positive juvenile dermatomyositis patients.
A-**B**: Longitudinal CMAS scores in NXP2+ and MDA5⁺ JDM patients over time. **C**-**D**: Longitudinal serum CK levels in NXP2+ and MDA5⁺ JDM patients over time. (**E**-**F**: Longitudinal IL-10/γ-IFN in NXP2⁺ and MDA5⁺ JDM patients over time.

Notably, the relapse rate was significantly higher in the NXP2⁺ JDM group compared with the MDA5⁺JDM group (60% *vs*. 40%, *p*=0.014). Overall, the NXP2⁺ JDM patients exhibited more severe impairment of muscle function, higher disease activity, and a higher risk of relapse.

We also collected data from adult DM patients with anti-NXP2 autoantibodies and found significant differences in several clinical parameters between the juvenile and adult groups (Table II). The NXP2⁺ JDM patients had significantly higher serum levels of CK (3792 *vs*. 437.5 IU/L, *p*=0.003), CK-MB (50 *vs*. 22.5 IU/L, *p*=0.002), ALT (81 *vs*. 43.5 U/L, *p*=0.023), AST (175.06 *vs*. 71.55 U/L, *p*=0.006), LDH (755.29 *vs*. 394.40 U/L, *p*<0.001), and Ferritin (345.1 *vs*. 333.95 μg/L, *p*=1.000) compared with the NXP2⁺ adult DM patients. Furthermore, JDM patients were more likely to develop MAS (22.22%

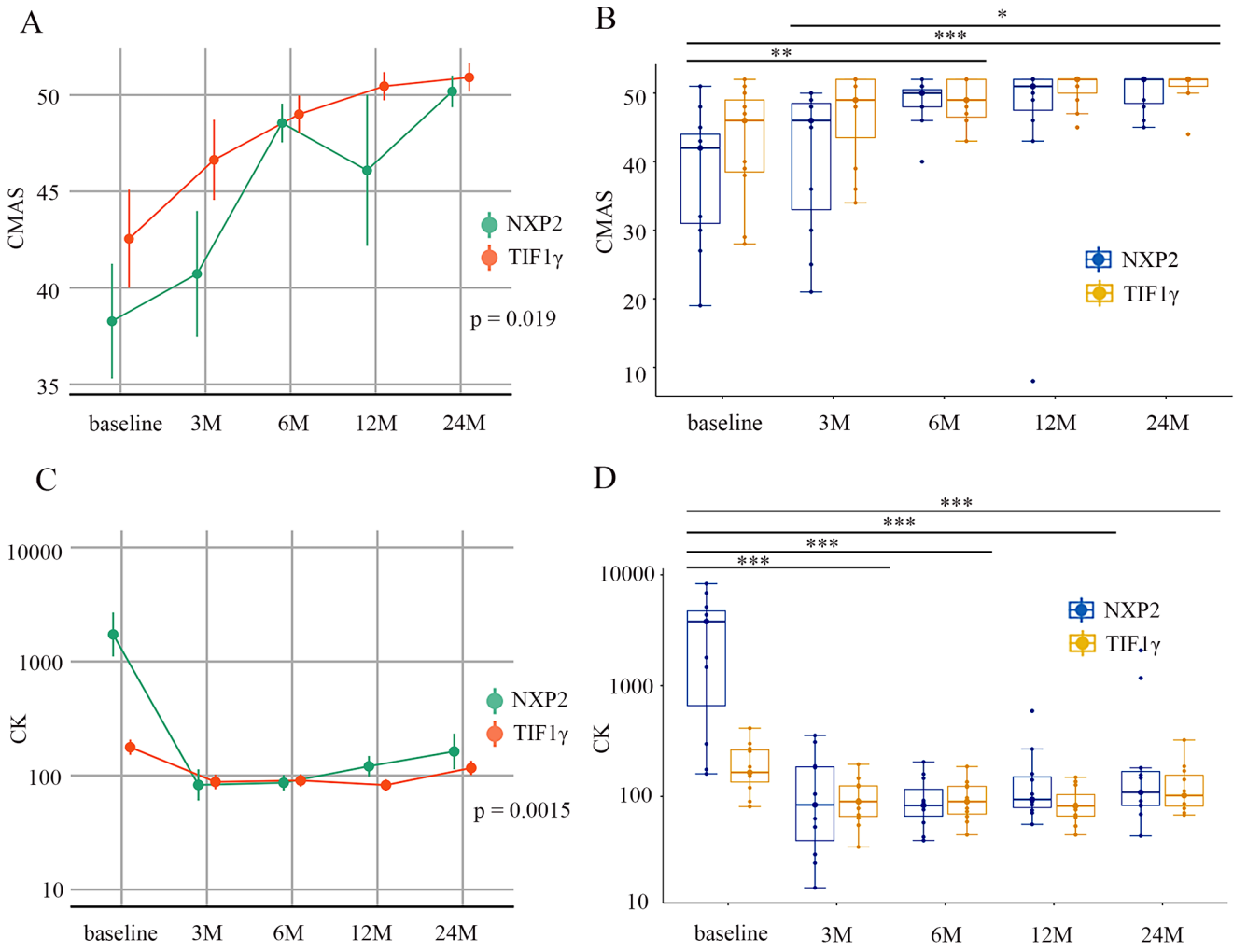

Fig. 2. Comparison of CK and CMAS in NXP2 and TIF1γ autoantibody-positive juvenile dermatomyositis patients.
A-**B**: Longitudinal CMAS scores in NXP2$^+$ and TIF1γ$^+$ JDM patients over time. **C**-**D**: Longitudinal serum CK levels in NXP2$^+$ and TIF1γ$^+$ JDM patients over time.

vs. 0%, p=0.041), cardiac injury (33.33% *vs*. 0%, p=0.007), and various skin rashes and other complications, indicating a more pronounced systemic inflammatory response compared with adult DM patients. In contrast, adults were more prone to developing interstitial lung disease (ILD, 16 (80.00) *vs*. 5(27.78), p=0.003). Additionally, the time from disease onset to diagnosis was significantly shorter in JDM patients compared to adult DM patients (1.17(1.00, 2.00) *vs*. 3.00 (2.00, 6.00), p=0.008), suggesting a more acute disease onset and rapid progression in children. Overall, JDM patients demonstrated more severe clinical involvement, including greater muscle and cardiac damage and a more robust systemic inflammatory response relative to the adult DM patients.

Changes in serum CK levels, CMAS activity, and IL-10/γ-IFN ratio during the follow-up of the JDM patients

We collected two-year of clinical data from JDM patients and conducted repeated measures analyses for CK, CMAS, and IL-10/γ-IFN due to the differences observed between the groups. Repeated measures ANOVA and linear mixed models (LMM) were used to evaluate the effects of MSAs status, time, and their interactions on these variables. The model accounted for individual differences as random effects. The main effects of MSAs status on CK (p=0.0081) and CMAS (p=0.0014) were significant, indicating that anti-NXP2 positive patients had more severe muscle involvement throughout the disease course compared with anti-MDA5 positive patients (Fig. 1A and C). We found that CMAS levels in the NXP2$^+$ JDM patients remained low at 3 months post-treatment, although they had improved from baseline (Fig. 1B). This further suggests that despite effective treatment, the initial severity of muscle involvement in NXP2$^+$ JDM patients may necessitate a longer period for full recovery. In contrast, CK levels in the MDA5$^+$ JDM patients quickly returned to normal within 3 months post-treatment, accompanied by significant improvement in muscle function. (Fig. 1D). However, no significant difference was found between the NXP2$^+$ and MDA5$^+$ groups in terms of the IL-10/γ-IFN ratio (Fig. 1E-F).

At all observation time points, the CMAS scores of the NXP2$^+$ JDM group were lower than those of the

TIF1γ$^+$ group (Fig. 2A). Although the muscle function of both groups improved over time, the CMAS score of the NXP2$^+$ group was significantly lower at baseline, suggesting a more severe degree of muscle weakness. Despite the increase in the CMAS score after treatment, at 24 months, the score of the NXP2$^+$ group was still significantly lower than that of the TIF1γ$^+$ group (Fig. 2B).

In contrast, NXP2$^+$ JDM patients showed elevated considerably creatine kinase (CK) levels at baseline, which gradually decreased after treatment, while the CK levels of TIF1γ$^+$ patients remained relatively stable throughout the disease course (Fig. 2C). Three months after treatment, the CK levels of TIF1γ$^+$ patients had stabilised and remained at a low level. In comparison, the CK levels of NXP2$^+$ patients remained high even at 24 months, suggesting a slower recovery process of muscle damage and possibly a more prolonged disease course (Fig. 2D).

Additionally, we found that the time factor had a significant effect on both CMAS and CK (χ^2=27.386, p<0.001; χ^2=107.045, p<0.001, respectively), indicating notable changes over time for these variables. Moreover, for CK, the Group effect was significant (χ^2=60.078, p<0.001), as was the group x time interactions (χ^2=46.659, p<0.001). This suggests that the CK levels changed differently over time between groups, highlighting a significant dynamic difference in CK trends across the groups. In contrast, the group × time interactions for CMAS did not reach statistical significance (χ^2=3.141, p=0.535), implying similar time-related changes across groups. For IL-10/γ-IFN, no significant effects were observed for group, time, or their interaction (χ^2=1.737, p=0.188; χ^2=5.531, p=0.237; χ^2=2.083, p=0.721, respectively), suggesting consistent trends across groups and time points for this variable.

Thus, these results underscore a unique, significant interaction effect of group and time on CK in JDM patients, emphasising distinct group-specific temporal patterns not seen in CMAS or IL-10/γ-IFN (Table III).

Table III. Linear mixed-effects models for group, time and group × time interactions effects on CMAS, CK, and IL-10/γ-IFN responses.

Analysis of deviance table (type III Wald χ^2 tests)	χ^2 value	p-value
Response: CMAS (Intercept)	358.135	< 0.001***
Group	3.6371	0.05651
Time	27.3862	< 0.001***
Group × time interactions	3.1407	0.53456
Response: CK (Intercept)	143.511	< 0.001***
Group	60.078	< 0.001***
Time	107.045	< 0.001***
Group × time interactions	46.659	< 0.001***
Response: IL-10/γ-IFN (Intercept)	15.9349	< 0.001***
Group	1.7374	0.1875
Time	5.5306	0.2371
Group × time interactions	2.0829	0.7205

***p<0.001.

Higher recurrence rate in the NXP2$^+$ JDM patients

We conducted a three-year recurrence regression analysis between the NXP2$^+$ JDM, MDA5$^+$ JDM, TIF1γ$^+$ JDM, and NXP2$^+$ adult DM groups. We define recurrence as the reappearance or aggravation of rash, muscle strength decline, or the emergence of some systemic symptoms while taking medication regularly and excluding the influence of other diseases. The overall analysis showed a significant difference in relapse rates between the three groups (p=0.00026) (Fig. 3). Pairwise comparisons revealed a significant difference in relapse rates between the NXP2$^+$ JDM and MDA5$^+$ JDM groups (p=0.03) and an even more significant difference between the NXP2$^+$ JDM and NXP2$^+$ adult DM groups (p<0.0001). Reviewing the history further, we found that patients with other antibody-positive dermatomyositis relapsed more often with a reappearance of the rash, whereas those with anti-NXP2 antibody-positive patients relapsed mainly due to a decrease in muscle strength or some systemic reaction. These findings suggest that the NXP2$^+$ JDM patients have a significantly higher risk of relapse compared with the MDA5$^+$ JDM, TIF1γ$^+$ JDM and NXP2$^+$ adult DM patients, indicating that age could play an important role in relapse risk in anti-NXP2 autoantibody-associated dermatomyositis. Additionally, the difference in relapse rates between the NXP2$^+$ JDM and non-NXP2$^+$ JDM patients highlighted the heterogeneity in relapse patterns between different MSAs types. These results further support the need for personalised management based on autoantibody type, with tailored long-term monitoring and treatment strategies.

Discussion

Anti-NXP2 autoantibodies were first discovered in JDM and later reported in adult dermatomyositis (14, 15). Anti-NXP2 antibodies primarily bind to nuclear matrix protein 2 (NXP2), a protein that plays various regulatory roles in the nucleus, including the regulation of RNA metabolism and gene expression (16). Deficiencies or dysfunctions in NXP2 are closely associated with degenerative changes in muscle, which may explain the more severe muscle damage observed in anti-NXP2 positive patients (17). Our study found that patients positive for anti-NXP2 antibodies had significantly elevated creatine kinase (CK) levels and lower CMAS scores, suggesting greater impairment of muscle function. This aligns with previous studies noting that anti-NXP2 positive patients often exhibit severe symmetrical muscle weakness and generalised oedema (18, 19). Additionally in the present study, a higher recurrence rate in anti-NXP2 positive patients was observed, particularly in the paediatric group, indicating greater disease fluctuations. Previous studies have shown that children with anti-NXP2 positivity are more

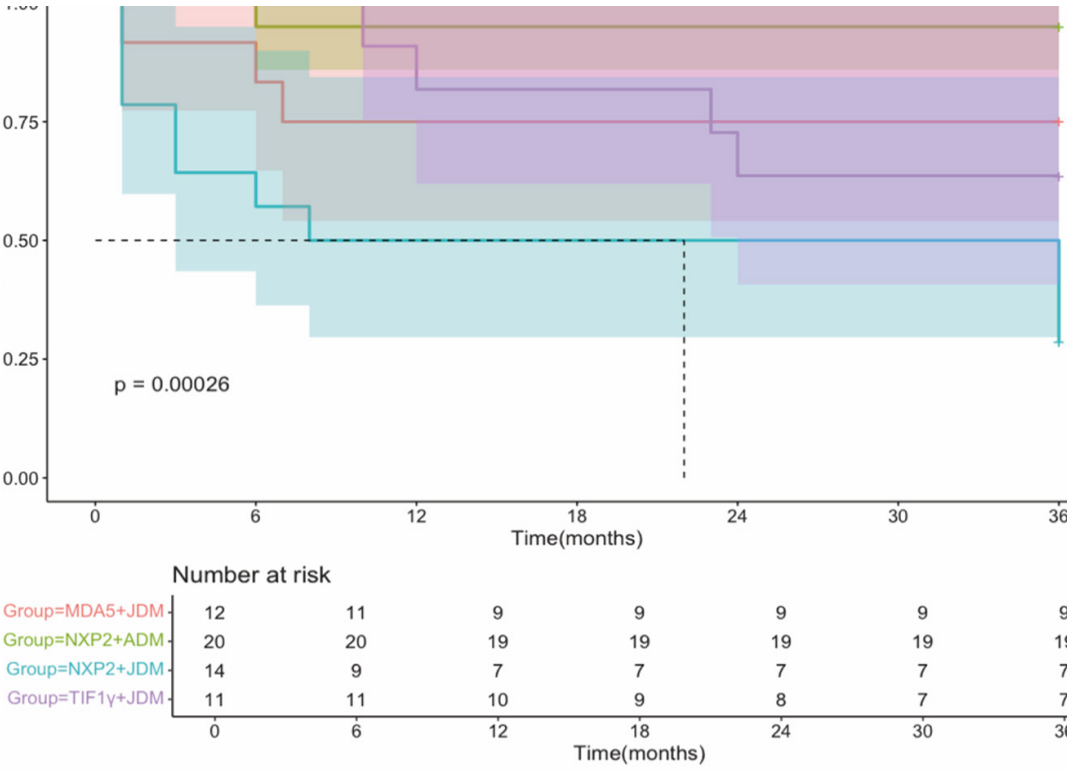

Fig. 3. Kaplan-Meier survival analysis comparing relapse regression analysis between the NXP2+ JDM, MDA5+ JDM, TIF1γ+ JDM, and NXP2+ adult DM patients.

prone to calcinosis and macrophage activation syndrome (MAS) (20), which is consistent with our observations of a higher recurrence rate and stronger systemic inflammatory response.

MDA5 is a nucleic acid recognition receptor that plays a role in antiviral immune responses. When MDA5 recognises viral RNA within cells, it activates the innate immune response via the interferon pathway (21).The presence of anti-MDA5 antibodies may interfere with the normal function of MDA5, leading to an excessive inflammatory response, particularly in the lungs, which explains the high incidence of RP-ILD in these patients (22, 23). Additionally, the elevated IL-10/γ-IFN ratio in anti-MDA5 positive patients suggests a potentially stronger anti-inflammatory response (24), which may help explain why these patients experience relatively mild muscle damage despite severe pulmonary complications.

TIF-1γ, also known as TRIM33, or p155/140, belongs to the larger tripartite motif (TRIM) family of proteins involved in many important biological processes. For example, it plays a role in transcription elongation, DNA repair, cell differentiation, embryonic development and mitosis (25, 26). TIF1-γ is a key regulator of the TGF-β signalling pathway and is highly expressed in the skin. Its aberrant expression or antibody attack may lead to cellular dysfunction, triggering skin inflammation and injury (27). This is also consistent with our finding that most of the TIF1-γ-positive patients showed skin rashes.

In addition, the NXP2+ JDM patients had significantly higher CK, ALT, AST, and LDH levels than the NXP2+ adult DM patients, indicating more severe muscle involvement and metabolic disturbances. Furthermore, paediatric patients were more prone to developing MAS and myocardial injury, suggesting a more intense systemic inflammatory response compared to matched adult patients. MAS is a severe inflammatory syndrome observed in children with rheumatic diseases, characterised by the presence of bone marrow macrophages engulfing other haematopoietic cells (28). Currently, no diagnostic criteria exist for JDM-associated MAS. Therefore, we referenced the 2016 sJIA-MAS classification criteria: fever (unrelated to sJIA activity), ferritin ≥684 μg/L, and any two of the following: platelets ≤181 x 10^9/L; aspartate aminotransferase (AST) >48 U/L; triglycerides ≥156 mg/dL; fibrinogen ≤360 mg/dL (29). The diagnosis of myocardial involvement has been outlined in the methodology section. Patients presenting with myocardial involvement primarily exhibit chest pain, elevated myocardial injury markers (troponin, myoglobin, creatine kinase MB isoenzyme, B-type natriuretic peptide, etc.), and abnormal findings on cardiac echocardiography. In contrast, adult patients were more likely to develop interstitial lung disease, which aligns with existing literature indicating that adult patients with anti-NXP2 positivity are at a higher risk for malignancies and pulmonary diseases. While adult patients exhibit milder muscle damage, their higher risk for complications, especially pulmonary issues, may be related to age, immune status, and environmental factors.

Survival analysis indicated that the NXP2+ JDM patients had a significantly higher risk of recurrence compared to the MDA5+ JDM patients and the

NXP2+ adult DM patients. The marked difference in recurrence rates between NXP2+JDM and NXP2+ adult DM patients highlights the important role of age in disease recurrence. These findings facilitate clinical efforts to devise personalised, long-term management strategies according to patients' ages and autoantibody types. For anti-NXP2 positive patients, especially children, prolonged monitoring and more customised treatment plans are recommended to reduce recurrence risks. Nevertheless, while the recurrence rate in anti-MDA5 positive patients is relatively low, the high risk of pulmonary complications still necessitates close monitoring, particularly in the early stages of the disease.

Notably, the dynamic changes in CK levels are closely associated with relapse risk. Among the NXP2+ JDM patients, those with smaller declines in CK levels post-treatment had a significantly higher risk of relapse, suggesting that CK could serve as an important predictor of relapse. Additionally, changes in CMAS scores were linked to relapse risk, with lower CMAS scores associated with higher relapse risk. This suggests that residual muscle damage after short-term treatment may increase the long-term risk of relapse.

There are several limitations to the current study. First, due to the rarity of JDM, the study was constrained by a limited sample size. Further research benefiting from a larger sample size and multicentre study design, would enhance the robustness and generalisability of the findings. Second, the present study focuses on the anti-NXP2 and anti-MDA5 autoantibodies, and it is necessary to conduct comparison analysis on the other antibodies as well. Finally, the recurrence data were acquired through three-year outpatient follow-up. Nonetheless, owing to the restricted follow-up duration for certain detection indicators including CK, CMAS, and cytokines, numerous patients possess only two years of follow-up data. This discrepancy may potentially impact the comparison and interpretation between various indicators.

Furthermore, our study exclusively included Chinese patients, which may limit the applicability of our findings to other populations. Previous studies have suggested that myositis-specific antibodies (MSAs), such as MDA5, exhibit distinct prevalence and clinical associations across different racial and ethnic groups. For instance, MDA5+ dermatomyositis is notably more common and severe in East Asian populations, while its clinical spectrum may vary in other ethnic groups (30). It remains uncertain whether the observed associations in this study, such as the relationship between NXP2 positivity and disease severity or MAS, are unique to Chinese patients or represent broader patterns. Future studies involving more diverse populations are necessary to validate these findings and investigate potential genetic, environmental, or immunological factors contributing to these differences. A larger, prospective, and multicentre study involving diverse populations will be necessary to further explore the potential role of MSAs in JDM and to address the limitations identified in this study.

Conclusion

This study revealed that the anti-NXP2 positive JDM patients experience more severe muscle damage, systemic complications, and higher relapse risks. Monitoring dynamic changes in CK and CMAS can better predict disease progression and recurrence. Therefore, future treatments should be personalised based on autoantibody types and changes in muscle enzymes to improve therapeutic outcomes and reduce recurrence rates.

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