Rheumatology 2008;47:324–328
Advance Access publication 30 January 2008
doi:10.1093/rheumatology/kem359

Clinical associations of autoantibodies to a p155/140 kDa doublet protein in juvenile dermatomyositis

H. Gunawardena[1,2], L. R. Wedderburn[3], J. North[2], Z. Betteridge[2], J. Dunphy[1], H. Chinoy[4,5], J. E. Davidson[6], R. G. Cooper[4] and N. J. McHugh[1,2] for the Juvenile Dermatomyositis Research Group UK

Objective. Myositis-specific autoantibodies (MSAs) may define homogeneous clinical subsets of adult patients with dermatomyositis (DM). Recently, there have been descriptions of novel autoantibodies in DM. This study was conducted to establish the clinical significance of anti-p155/140 autoantibodies in juvenile DM (JDM).

Methods. The first 116 children recruited to the JDM National Registry and Repository (UK and Ireland) were studied. Comprehensive clinical features were recorded and sera screened for anti-p155/140 autoantibodies using radio-immunoprecipitation. Sera from adults with DM ($n=20$), PM ($n=25$), SSc ($n=150$), SLE ($n=40$) and healthy subjects ($n=50$) were used for comparison. Immunodepletion experiments were used to establish whether the p155/140 kDa targets recognized by JDM sera were the same as adult DM sera.

Results. Twenty-seven out of 116 (23%) JDM cases were positive for anti-p155/140 in comparison with 6/20 (30%) adults with DM. Immunodepletion confirmed that the 155/140 kDa proteins recognized by JDM and adult DM sera were the same targets. All other adult control sera were negative for anti-p155/140 autoantibodies. There was a higher frequency of males in the anti-p155/140-positive JDM group ($P=0.02$). JDM patients with anti-p155/140 autoantibodies had significantly more cutaneous involvement including Gottron's papules ($P=0.003$), ulceration ($P=0.005$) and oedema ($P=0.013$). The distribution of skin lesions was more extensive particularly periorbitally ($P=0.014$) and over the small ($P<0.001$) and large joints ($P=0.003$).

Conclusion. Anti-p155/140 autoantibodies are clinically significant in JDM and may define a clinical subset in terms of disease severity and outcome. The same autoantigen target is detected in adult DM patients.

Key words: Inflammatory myopathy, Dermatomyositis, Juvenile dermatomyositis, Autoantibodies.

Introduction

Juvenile dermatomyositis (JDM) is the most common of the idiopathic inflammatory myopathies (IIMs) occurring in children. The reported incidence ranges from 0.8 to 4.1 per million children per year [1–3]. JDM is a chronic, potentially debilitating disease and despite improvements in multi-disciplinary treatment approaches, the condition is associated with significant morbidity and mortality [4]. Clinical outcomes and prognosis are difficult to predict due to the heterogeneity of the condition. Children with JDM share some clinical features with adult DM patients in terms of muscle disease and characteristic skin lesions. However, certain cutaneous manifestations are more characteristic in JDM including calcinosis and skin ulceration. As these features can cause permanent scarring, including contractures, they act as predictors of a more severe disease course in JDM [4–6]. In contrast to adults with DM, both interstitial lung disease (ILD) and cancer-associated myositis are very rare in JDM [7–9].

Classifying patients using a clinico-serological approach may lead to the identification of more homogeneous subsets within the JDM spectrum and therefore have prognostic implications. In adult IIM, distinct serological markers are well described and myositis-specific autoantibodies (MSAs) are associated with homogeneous clinical subsets [10, 11]. However, MSAs in juvenile myositis including JDM are less well characterized. Previous reports have described a low frequency of anti-aminoacyl-tRNA synthetase and anti-SRP autoantibodies in JDM, but anti-Mi-2 autoantibodies are described more frequently [12, 13]. In contrast, myositis-associated autoantibodies (MAAs) including anti-Pm-Scl, anti-Ku and anti-U1RNP autoantibodies are found in children with myositis overlap syndromes.

More recently, a number of less well-characterized autoantibodies have been described in JDM and particularly adult DM. There have been preliminary reports of autoantibodies to a 140 kDa protein (anti-MJ) and a 155 kDa protein in JDM [14, 15]. Sato *et al.* [16] have described autoantibodies to a 140 kDa polypeptide in adult patients with clinically amyopathic DM (CADM) and ILD. Furthermore, three studies have reported novel autoantibodies to 155 and 140 kDa nuclear polypeptides in adult DM patients [17–19]. In the study by Targoff *et al.* [18], the autoantibody to a 155 kDa protein was also detected in their JDM cohort.

The purpose of this study was to establish the frequency and to define the clinical significance of anti-p155/140 autoantibodies in children recruited to the UK JDM Registry. A secondary aim was to confirm whether the same autoantigen is targeted in adult DM in order to understand whether this autoantibody has any future predictive value in respect of clinical features.

Patients and methods

Patients and sera (JDM)

The first 116 patients recruited to the JDM National Registry and Repository (UK and Ireland) were studied [20]. The diagnosis of probable or definite myositis was based on the Bohan and Peter criteria [21, 22]. Children were recruited consecutively on visits to paediatric rheumatology departments if they had a diagnosis of JDM or JDM with overlap features of another connective tissue disease but where myositis was the predominant manifestation. Serial clinical data were collected prospectively using standardized proformas and stored using anonymous codes in a central database. The median age at symptom onset was 6 yrs

[1]Royal National Hospital for Rheumatic Diseases, [2]University of Bath, Bath, [3]Rheumatology Unit, Institute for Child Health, University College London, [4]Rheumatic Diseases Centre, Hope Hospital, Salford, [5]Centre for Integrated Genomic Medical Research, University of Manchester and [6]Royal Hospital for Sick Children, Glasgow, UK.

Submitted 16 July 2007; revised version accepted 6 December 2007.

Correspondence to: N. J. McHugh, Royal National Hospital for Rheumatic Diseases, Upper Borough Walls, Bath BA1 1RL, UK.
E-mail: neil.mchugh@rnhrd-tr.swest.nhs.uk

[inter-quartile range (IQR) 3–9] and at diagnosis was 7 yrs (IQR 4–10). The median follow-up from disease onset to time of this study was 69.5 months (IQR 47.5–105.2) and 44.2 months (IQR 35.7–65.8) from date of entry into the registry to time of this study. Clinical information included the degree of skin involvement, muscle strength tested by the Childhood Myositis Assessment Scale (CMAS) [23], physician's 10-point global assessment (PGA) (visual analogue scale) and serum muscle enzymes. Results of MRI and muscle biopsies were also recorded where available. When evaluating muscle involvement, only those JDM patients with documented muscle enzymes at disease onset/diagnosis (with subsequent maximum CK or LDH during follow-up) were included for analysis. Similarly, those with a baseline CMAS or PGA at the time of entry to the registry (plus subsequent serial measures) were included. The type of skin lesion, in particular, skin ulceration, oedema and Gottron's lesions combined with the distribution (extent) of skin involvement plus CMAS/PGA score was defined as a marker of disease severity for the purpose of this study. Serum samples were taken at the time of entry onto the JDM Registry and stored at −80°C until analysis.

Patients and sera (adults)

For adults, a diagnosis of probable or definite DM or PM was based on the Bohan and Peter criteria [21, 22]. Clinical information on patients with IIM attending the Royal National Hospital for Rheumatic Diseases, Bath, UK has been recorded prospectively and for the purpose of this study; patient notes were re-reviewed to confirm clinical details. Sera was taken at the time of diagnosis and stored at −80°C until required. Adult serum samples were analysed from 20 DM, 25 PM, 150 SSc and 40 SLE patients. All patients with SSc and SLE fulfilled the published criteria for those conditions [24, 25]. Sera from 50 age-matched healthy individuals (blood donors) were taken from our Bath biomarkers repository. The adult IIM patients reported here are separate to the UK-wide AOMIC study coordinated by two of the co-authors (H.C., R.G.C.) [19].

The study had both multi-centre and local regional ethics committee approval. All subjects gave written parental consent or full informed written consent before recruitment to the study.

Indirect immunofluorescence

Indirect immunofluorescence (IIF) was performed using Hep-2 cells as substrate and FITC-conjugated anti-human IgG (Sigma, UK).

Immunoprecipitation

Immunoprecipitation (IIP) was used to detect anti-p155/140 autoantibodies and all other known MSAs/MAAs (including the anti-synthetases, anti-Mi-2, anti-SRP, anti-CADM-140, anti-Pm-Scl, anti-Ku, anti-U1RNP, anti-U3RNP and anti-Ro autoantibodies). IPP from K562 cell extracts was performed as previously described [26]. Briefly, 10 µl of sera was mixed with 2 mg protein-A-Sepharose beads (Sigma) in IPP buffer (10 mM Tris–Cl pH 8.0, 500 mM NaCl, 0.1% v/v Igepal) at room temperature for 30 min. Beads were washed in IPP buffer prior to the addition of 120 µl [^{35}S]-methionine-labelled K562 cell extract. Samples were mixed at 4°C for 2 h. Beads were washed in IPP buffer followed by TBS buffer (10 mM Tris–Cl pH 7.4, 150 mM NaCl) before being resuspended in 50 µl SDS sample buffer (Sigma). After heating, proteins were fractionated by 10% SDS–PAGE, enhanced, fixed and dried at 70°C for 80 min. Labelled proteins were analysed by autoradiography.

Immunodepletion experiments

The immunodepletion studies were undertaken in order to ascertain whether the IPP pattern seen in JDM and adult DM sera was due to precipitation of the same autoantigens. Cell extracts were depleted of autoantibody targets using anti-p155/140 JDM-positive serum or adult DM anti-p155/140-positive serum and normal serum (NS) as a negative control. These extracts were then used in further immunoprecipitations using both juvenile and adult anti-p155/140-positive serum. In brief, duplicate samples each containing 10 mg protein A sepharose beads in 1 ml IPP buffer and 50 µl patient serum were mixed with end-over-end rotation at room temperature for 30 min. The beads were washed four times in 1 ml IPP buffer and 1 tube (A) was placed on ice whilst 150 µl [^{35}S]-methionine-labelled K562 cell extract and 350 µl IPP buffer was added to the remaining tube (B). Tube B was mixed with end-over-end rotation at 4°C for 2 h after which the supernatant was transferred to tube A, which was mixed with end-over-end rotation at 4°C for a further 2 h. The supernatant from tube A was then transferred to a fresh tube (C) and stored at −80°C. IPPs using JDM or adult DM serum and either 150 µl control [^{35}S]-methionine-labelled cell extract or the immunodepleted supernatants (C) were completed as described in the paragraph above.

Statistical analysis

Statistics were conducted using SPSS for Windows (version 12) software. The frequencies of clinical features were compared using the chi-squared test with Yates' continuity correction or the Fisher's exact test for groups with small numbers. Where data was not normally distributed the Mann–Whitney U-test was used to compare continuous data. Median values (IQR) were expressed where appropriate and P-values <0.05 were considered statistically significant.

Results

Following IPP, sera from a number of JDM patients recognized two distinct proteins forming a doublet with molecular weights of 155 and 140 kDa. The same pattern was observed in a subset of adult DM patients (Fig. 1). Non-specific weak nuclear patterns were observed on IIF between anti-p155/140 patients (data not shown).

The immunodepletion results support the co-identity of the p155/140 kDa doublet precipitated by sera from both JDM and

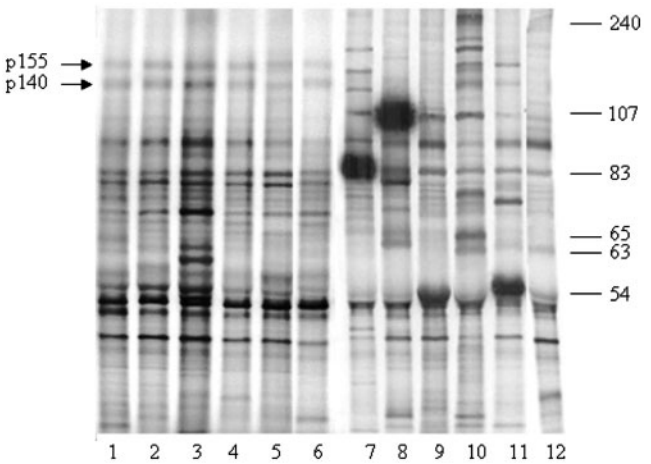

FIG. 1. Immunoprecipitation of p155/140 kDa autoantigens and other selected MSAs. 10% SDS–PAGE of immunoprecipitates of [^{35}S]-methionine-labelled K562 cell extract. Sera used for immunoprecipitation include lanes 1–3: adult anti-p155/140-positive serum, lanes 4–6: juvenile anti-p155/140-positive serum, lane 7: threonyl tRNA synthetase, lane 8: alanyl tRNA synthetase, lane 9: histidyl tRNA synthetase, lane 10: Mi-2, lane 11: signal recognition particle and lane 12: NS. Positions of the p155 and p140 antigens are indicated on the left. Positions of the Mi-2 bands at 240, 65 and 63 kDa are indicated. The 54, 83 and 108 kDa molecular weight markers correspond to signal recognition particle, threonyl tRNA synthetase and alanyl tRNA synthetase, respectively.

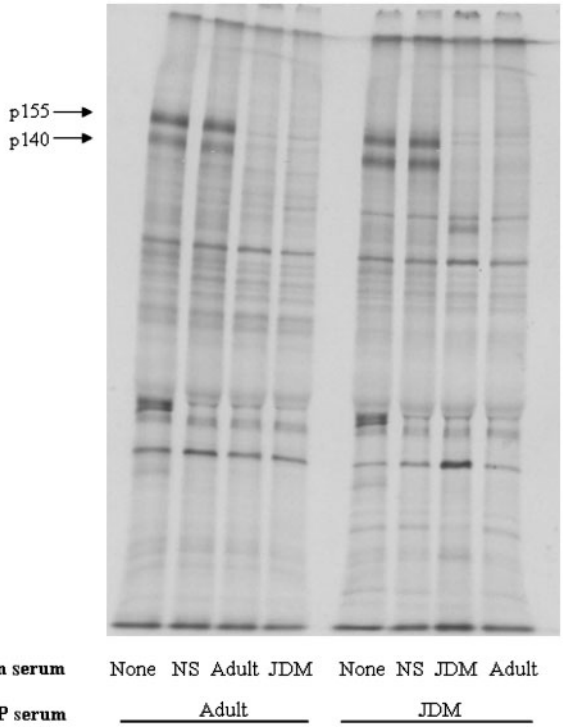

FIG. 2. Immunodepletion experiments—autoradiogram of 10% SDS–PAGE of immunoprecipitates using either adult-positive anti-p155/140 serum or JDM-positive anti-p155/140 serum. Immunoprecipitation was performed with control [^{35}S]-methionine-labelled cell extract or [^{35}S]-methionine-labelled cell extract depleted with either NS, adult anti-p155/140-positive serum or JDM anti-p155/140-positive serum. The bands corresponding to the p155 and p140 autoantigens are indicated.

adult DM groups (Fig. 2). When the cell extracts were pre-depleted with NS, no targets were removed from the extract and the 155 and 140 kDa autoantigens were still precipitated by juvenile and adult sera. However, when the cell extract was pre-depleted with either juvenile or adult anti-p155/140-positive sera, the autoantigens were no longer detectable in juvenile or adult anti-p155/140-positive sera, respectively. This provides good evidence that the sera from JDM and adult DM contained the same autoantibody specificity.

Frequency of anti-p155/140 autoantibodies

From 116 juvenile myositis sera, 27 (23%) had anti-p155/140 autoantibodies and of this group 26 children had JDM and one had JDM with overlap features of scleroderma. Two children with anti-p155/140 autoantibodies were also positive for anti-Mi-2 and one anti-p155/140-positive child had anti-Ku autoantibodies. In the anti-p155/140-negative group, 69 had JDM and 20 had JDM with overlap features. The overall autoantibody specificities in this JDM cohort have been described in a previous study [27]. In comparison, 6 out of 20 (30%) adult DM patients were positive for autoantibodies to the p155/140 kDa doublet. Other MSAs or MAAs were not detected in adults with anti-p155/140. Anti-p155/140 was not detected in any of the adult PM, SSc, SLE patients or normal adult sera.

Clinical features of the JDM patients with anti-p155/140 autoantibodies

Information on the degree of skin involvement and other selected clinical features are outlined in Table 1. There was a higher frequency of males in the anti-p155/140-positive children compared with anti-p155/140-negative children ($P = 0.02$). Anti-p155/140-positive JDM patients had an increased frequency of skin lesions (Gottron's papules $P = 0.003$, ulceration $P = 0.005$, oedema $P = 0.013$) with a wider distribution of cutaneous involvement, particularly periorbitally ($P = 0.014$) and over the small joints ($P < 0.001$) and large joints ($P = 0.003$). Overall, there was no significant difference in those with elevated muscle enzymes at diagnosis/during disease course or those with an abnormal MRI/muscle biopsy between anti-p155/140-positive and -negative groups (data not shown). However, not all children had data on this, in particular, some did not have an MRI or biopsy performed. There was a trend towards lower CMAS (lower values indicate more severe weakness) and higher PGA in anti-p155/140-positive children at baseline and during follow-up, although this did not reach statistical significance. The frequency of other clinical signs including lipoatrophy, arthritis, Raynaud's phenomenon, sclerodermatous skin changes, dysphagia, mouth ulcers and alopecia was not significantly different between children with or without anti-p155/140 (data not shown). There was no history of malignancy in the entire JDM cohort during the follow-up period.

TABLE 1. Clinical associations of anti-p155/140 autoantibodies in JDM patients

	Anti-p155/140		
	Positive ($n = 27$)	Negative ($n = 89$)	*P*-value
Age at diagnosis, median (IQR)	6 yrs (4–10)	7 yrs (5–10)	
Male:female	12:15 (44.4:55.6)	18:71 (20.2:79.8)	0.02
Type of skin lesion			
Gottron's papules	27 (100)	67[a] (76.1)	0.003
Ulceration	14 (51.9)	19 (21.3)	0.005
Oedema	17 (63)	30 (33.7)	0.013
Calcinosis	3 (11.1)	21 (23.6)	0.258
Distribution of skin lesion			
Periorbital	25 (92.6)	58[a] (65.9)	0.014
Periungal	22 (81.5)	52[a] (59.1)	0.058
Trunk	6 (22.2)	10[a] (11.4)	0.27
Small joints	27 (100)	55[a] (62.5)	<0.001
Large joints	21 (77.8)	38[a] (43.2)	0.003
Muscle disease[b]			
Baseline CMAS, median (IQR)	36 (13.8–48)	44 (35–50.5)	0.07
Lowest CMAS, median (IQR)	36 (13.8–46.8)	43 (29–49.0)	0.07
Baseline PGA, median (IQR)	4.7 (2.0–7.1)	2.8 (1.1–5.0)	0.07
Highest PGA, median (IQR)	5.5 (2.5–7.1)	3 (1.2–5.1)	0.07

Absolute values with percentages in brackets unless otherwise indicated. [a]Data on 88 children. [b]Not all patients had data available for each clinical feature. CMAS (childhood myositis assessment scale: 0–53) at baseline and lowest (worst) score during follow-up. PGA (physician's global assessment: 0–10) at baseline and highest (worst) score during follow-up.

Clinical features of the adult DM patients with anti-p155/140

Similar to the anti-p155/140 JDM cohort, adult patients with anti-p155/140 had more skin involvement. In comparison with anti-p155/140-negative adult DM patients, there was a significantly higher frequency of the V-sign ($P < 0.05$) and Shawl-sign rash ($P < 0.05$). Other cutaneous features including Gottron's papules, heliotrope rash and periungal changes tended to be more frequent in anti-p155/140 patients, although none of these findings reached statistical significance. Three adults with anti-p155/140 had a history of malignancy that was diagnosed either at onset or within 3 months of their presentation of classic DM. The remaining three adult patients with anti-p155/140 had CADM with no history of malignancy. There was no history of malignancy in the remainder of the adult IIM patients.

Discussion

Knowledge of an autoantibody profile is an important cornerstone in the diagnosis of patients with a wide variety of autoimmune connective tissue disorders, to the extent that certain autoantibodies form part of the diagnostic criteria. MSAs are

being detected with increasing frequency and appear to be associated with homogeneous subsets within the IIM disease spectrum [10, 11]. There are a number of well-described autoantigen targets particularly in adult IIM including Mi-2 and the aminoacyl–tRNA complex. To date, myositis autoantibodies are detected infrequently in JDM with anti-Mi-2 having the strongest association [12, 13]. Therefore, detection and characterization of novel MSAs in JDM may identify distinct clinical subsets within this disease group. Identification of new autoimmune markers may help clinicians predict clinical outcomes and lead to further insights in disease pathogenesis.

There have been reports of novel protein targets including p140 and p155 kDa polypeptides in both adult and juvenile DM. Two preliminary reports have described autoantibodies targeting a 140 kDa protein (anti-MJ) and a 155 kDa protein in US JDM patients [14, 15]. Further studies have reported the presence of autoantibodies reactive with 155 and 140 kDa nuclear proteins in adult DM. In adult DM, anti-p155/140 and anti-p155 is associated with a history of malignancy and this finding is confirmed in our adult DM cohort [17–19]. In addition, we have also found anti-p155/140 in adults with CADM with no history of cancer and although the numbers are small, this group of adults tended to be younger. In this cohort of children with JDM, no malignancy has been reported to-date in those with anti-p155/140; long-term follow-up studies will be required to ascertain whether there is any association with malignancy later in life in this group. In addition, validation of anti-p155/140 in larger groups is required. We acknowledge that one limitation of this study is that it has not included other juvenile disease groups or healthy children as controls, and this represents ethical issues in studies of this nature. However, an important finding of this study is the demonstration by immunodepletion that anti-p155/140 autoantibodies appear to target the same autoantigen in both adult and juvenile DM and work is required to investigate this further.

Targoff *et al*. [18] have described the presence of autoantibodies to 155 kDa protein in ~30% of their JDM population. This study also indicated that immunoprecipitation demonstrated in most cases a second weaker band at 140 kDa, although the actual frequency of this combination was not described. The clinical specificity of this cohort was not described. In contrast, our study describes detailed clinical features of JDM patients with autoantibodies targeting p155/140 and confirms they have similar cutaneous clinical associations to adults with the same autoantibody specificity. Of interest, there was a significantly higher frequency of boys in the anti-p155/140 group. In addition, like the adults with anti-p155/140 these children have more extensive skin involvement including Gottron's papules over a wider distribution. Anti-p155/140 autoantibodies also appear to define a subset of JDM with significantly more peripheral oedema and skin ulceration. There was a trend towards lower baseline and worst ever CMAS and higher PGA in doublet-positive JDM compared with doublet-negative patients, although this was not statistically significant.

In summary, anti-p155/140 autoantibodies occur frequently in JDM and our findings suggest that they may identify patients with more severe disease. Identification of this autoantibody at diagnosis may help predict the clinical course and outcome. The relationship of the p155 and p140 kDa proteins to each other needs addressing further. We believe anti-p155/140 described in our study is the same as those autoantibodies to p155 and p140 kDa proteins identified recently [17–19]. A preliminary report has identified the target of the anti-p155 autoantibodies as transcriptional intermediary factor 1-γ (TIF1-γ) [28]. Augmented expression of myositis-specific autoantigens in diseased muscle suggests a new paradigm for the pathogenesis of IIM [29]. Therefore, further characterization of p155/140 proteins; their expression in lesional tissue and how they relate to other autoantigens in DM may provide further insights into pathogenic mechanisms. It is not clear why what appears to be a cancer-associated autoantigen in adult DM should be recognized in JDM. Longitudinal studies are required to see whether these autoantibodies persist into adulthood and if so, are they clinically significant. Perhaps the observation that the same autoantigen system is expressed in younger DM adults without cancer as well as JDM suggests that some perturbation of p155/140 expression in proliferating cells may be a unifying mechanism to explain why it is targeted by an autoimmune response in children and adults with DM.

Rheumatology key messages

- Autoantibodies directed against a p155/140 kDa protein are a major autoimmune target in JDM.
- The clinical specificity of anti-p155/140 autoantibodies is distinct and identifies children with more severe cutaneous disease.

Acknowledgements

The authors would like to thank Miss Charlotte Carmichael for her advice on statistical analysis and for her assistance in collecting informed consent from the adult patients in the study. The Juvenile Dermatomyositis Research Group would like to thank all local research coordinators and principal investigators. The members who contributed were: Mr Ian Roberts, The Royal Liverpool Children's Hospital, Alder Hey, Liverpool and Booth Hall Children's Hospital, Manchester; Dr Eileen Baildam, Booth Hall Children's Hospital, Manchester; Mrs Janis Scott and Dr Clive Ryder, Birmingham Children's Hospital, Birmingham; Mrs Gillian Jackson and Dr Sue Wyatt, Leeds General Infirmary, Leeds; Ms Elizabeth Camp and Dr Janet Gardner-Medwin, The Royal Hospital for Sick Children, Yorkhill, Glasgow; Mrs Alison Swift, Dr Helen Foster and Dr Mark Friswell, The Royal Victoria Infirmary, Newcastle; Mrs Elizabeth Hutchinson and Dr Helen Venning, Queens Medical Centre, Nottingham; and Dr Clarissa Pilkington and Ms Sue Maillard, Great Ormond Street Hospital, London, UK.

Funding: The UK Raynaud's and Scleroderma Association, the Cathal Hayes Research Foundation and Myositis Support Group, UK supported this work.

Disclosure statement: The authors have declared no conflicts of interest.

References

1 Mendez EP, Lipton R, Ramsey-Goldman R *et al.* US incidence of juvenile dermatomyositis, 1995-1998: results from the National Institute of Arthritis and Musculoskeletal and Skin Diseases Registry. Arthritis Rheum 2003;49:300–5.
2 Oddis CV, Conte CG, Steen VD, Medsger TA Jr. Incidence of polymyositis-dermatomyositis: a 20-year study of hospital diagnosed cases in Allegheny County, PA 1963-1982. J Rheumatol 1990;17:1329–34.
3 Symmons DP, Sills JA, Davis SM. The incidence of juvenile dermatomyositis: results from a nation-wide study. Br J Rheumatol 1995;34:732–6.
4 Huber AM, Lang B, LeBlanc CM *et al.* Medium- and long-term functional outcomes in a multicenter cohort of children with juvenile dermatomyositis. Arthritis Rheum 2000;43:541–9.
5 Ramanan AV, Feldman BM. Clinical features and outcomes of juvenile dermatomyositis and other childhood onset myositis syndromes. Rheum Dis Clin North Am 2002;28:833–57.
6 Pilkington CA, Wedderburn LR. Paediatric idiopathic inflammatory muscle disease: recognition and management. Drugs 2005;65:1355–65.
7 Rider LG, Miller FW. Classification and treatment of the juvenile idiopathic inflammatory myopathies. Rheum Dis Clin North Am 1997;23:619–55.
8 Buchbinder R, Forbes A, Hall S, Dennett X, Giles G. Incidence of malignant disease in biopsy-proven inflammatory myopathy. A population-based cohort study. Ann Intern Med 2001;134:1087–95.
9 Hill CL, Zhang Y, Sigurgeirsson B *et al.* Frequency of specific cancer types in dermatomyositis and polymyositis: a population-based study. Lancet 2001;357:96–100.
10 Love LA, Leff RL, Fraser DD *et al.* A new approach to the classification of idiopathic inflammatory myopathy: myositis-specific autoantibodies define useful homogeneous patient groups. Medicine 1991;70:360–74.

11 Targoff IN. Myositis specific autoantibodies. Current Rheumatol Rep 2006;8:196–203.
12 Feldman BM, Reichlin M, Laxer RM, Targoff IN, Stein LD, Silverman ED. Clinical significance of specific autoantibodies in juvenile dermatomyositis. J Rheumatol 1996;23:1794–7.
13 Rider LG, Miller FW, Targoff IN *et al.* A broadened spectrum of juvenile myosistis. Arthritis Rheum 1994;37:1534–8.
14 Oddis CV, Fertig N, Goel A *et al.* Clinical and serological characterization of the anti-MJ antibody in childhood myositis. Arthritis Rheum 1997;40(Suppl):S139.
15 Mamyrova G, Targoff IN, Lindsley C *et al.* Distinguishing clinical features of JDM associated with a myositis-associated autoantibody (MAA) directed against a 155 kd protein [abstract]. Arthritis Rheum 2003;48(Suppl 9).
16 Sato S, Hirakata M, Kuwana M *et al.* Autoantibodies to a 140-kd polypeptide, CADM-140, in Japanese patients with clinically amyopathic dermatomyositis. Arthritis Rheum 2005;52:1571–6.
17 Kaji K, Fujimoto M, Hasegawa M *et al.* Identification of a novel autoantibody reactive with 155 and 140 kDa nuclear proteins in patients with dermatomyositis: an association with malignancy. Rheumatology 2007;46:25–8.
18 Targoff IN, Mamyrova G, Trieu EP *et al.* A novel autoantibody to a 155-kd protein is associated with dermatomyositis. Arthritis Rheum 2006;54:3682–9.
19 Chinoy H, Fertig N, Oddis CV, Ollier WE, Cooper RG. The diagnostic utility of myositis autoantibody testing for predicting the risk of cancer-associated myositis. Ann Rheum Dis 2007;66:1345–9.
20 McCann LJ, Juggins AD, Maillard SM *et al.* The Juvenile Dermatomyositis National Registry and Repository (UK and Ireland)–clinical characteristics of children recruited within the first 5 yr. Rheumatology 2006;45:1255–60.
21 Bohan A, Peter JB. Polymyositis and dermatomyositis (first of two parts). N Engl J Med 1975;292:344–7.
22 Bohan A, Peter JB. Polymyositis and dermatomyositis (second of two parts). N Engl J Med 1975;292:403–7.
23 Lovell DJ, Lindsley CB, Rennebohm RM *et al.* Development of validated disease activity and damage indices for the juvenile idiopathic inflammatory myopathies. II. The Childhood Myositis Assessment Scale (CMAS): a quantitative tool for the evaluation of muscle function. The Juvenile Dermatomyositis Disease Activity Collaborative Study Group. Arthritis Rheum 1999;42:2213–9.
24 Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee.Preliminary criteria for the classification of systemic sclerosis (scleroderma). Arthritis Rheum 1980;23:581–90.
25 Tan EM, Cohen AS, Fries JF *et al.* The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271–7.
26 Betteridge ZE, Gunawardena H, North J, Slinn J, McHugh NJ. Anti-synthetase syndrome: a new autoantibody to phenylalanyl transfer RNA synthetase (anti-Zo) associated with polymyositis and interstitial pneumonia. Rheumatology 2007;46:1005–8.
27 Wedderburn LR, McHugh NJ, Chinoy H *et al.* HLA haplotype and autoantibody associations in children with juvenile dermatomyositis, and scleroderma overlap. Rheumatology 2007, (in press).
28 Targoff IN, Trieu EP, Levy-Neto M, Prasertsuntarasai T, Miller FW. Autoantibodies to Transcriptional Intermediary Factor 1-gamma (TIF1-g) in dermatomyositis. Arthritis Rheum 2006;54(Suppl):S518.
29 Casciola-Rosen L, Nagaraju K, Plotz P *et al.* Enhanced autoantigen expression in regenerating muscle cells in idiopathic inflammatory myopathy. J Exp Med 2005; 201:591–601.