American College of Rheumatology
Empowering Rheumatology Professionals

Arthritis & Rheumatology
Vol. 73, No. 6, June 2021, pp 1062–1072
DOI 10.1002/art.41615

Comparison of Lesional Juvenile Myositis and Lupus Skin Reveals Overlapping Yet Unique Disease Pathophysiology

Jessica L. Turnier,[1] Lauren M. Pachman,[2] Lori Lowe,[1] Lam C. Tsoi,[1] Sultan Elhaj,[1] Rajasree Menon,[1] Maria C. Amoruso,[2] Gabrielle A. Morgan,[2] Johann E. Gudjonsson,[1] Celine C. Berthier,[1] and J. Michelle Kahlenberg[1]

Objective. Skin inflammation heralds systemic disease in juvenile myositis, yet we lack an understanding of pathogenic mechanisms driving skin inflammation in this disease. We undertook this study to define cutaneous gene expression signatures in juvenile myositis and identify key genes and pathways that differentiate skin disease in juvenile myositis from childhood-onset systemic lupus erythematosus (SLE).

Methods. We used formalin-fixed paraffin-embedded skin biopsy samples from 15 patients with juvenile myositis (9 lesional, 6 nonlesional), 5 patients with childhood-onset SLE, and 8 controls to perform transcriptomic analysis and identify significantly differentially expressed genes (DEGs; $q \leq 5\%$) between patient groups. We used Ingenuity Pathway Analysis (IPA) to highlight enriched biologic pathways and validated DEGs by immunohistochemistry and quantitative real-time polymerase chain reaction.

Results. Comparison of lesional juvenile myositis to control samples revealed 221 DEGs, with the majority of up-regulated genes representing interferon (IFN)–stimulated genes. *CXCL10*, *CXCL9*, and *IFI44L* represented the top 3 DEGs (fold change 23.2, 13.3, and 13.0, respectively; $q < 0.0001$). IPA revealed IFN signaling as the top canonical pathway. When compared to childhood-onset SLE, lesional juvenile myositis skin shared a similar gene expression pattern, with only 28 unique DEGs, including *FBLN2*, *CHKA*, and *SLURP1*. Notably, patients with juvenile myositis who were positive for nuclear matrix protein 2 (NXP-2) autoantibodies exhibited the strongest IFN signature and also demonstrated the most extensive Mx-1 immunostaining, both in keratinocytes and perivascular regions.

Conclusion. Lesional juvenile myositis skin demonstrates a striking IFN signature similar to that previously reported in juvenile myositis muscle and peripheral blood. Further investigation into the association of a higher IFN score with NXP-2 autoantibodies may provide insight into disease endotypes and pathogenesis.

INTRODUCTION

Juvenile myositis is a potentially life-threatening idiopathic inflammatory myopathy of childhood, often presenting with skin inflammation and following a highly heterogeneous disease course. Skin inflammation frequently persists in the absence of active muscle disease and prevents complete disease remission (1), and there is also uncertainty as to the role of skin disease in directing a change in systemic therapy. Multiple studies have highlighted the importance of skin inflammation

Presented in part at the 83rd Annual Scientific Meeting of the American College of Rheumatology, Atlanta, GA, October 2019 and at Convergence, the 84th Annual Scientific meeting of the American College of Rheumatology, November 2020.

Supported by a Child Health Research Center Career Development Award K12 Program grant to the University of Michigan Department of Pediatrics (grant K12-HD-028820-28). Dr. Turnier's work was supported by a grant from the Cure JM Foundation, by an Investigator award from the Rheumatology Research Foundation, and by a Michigan Institute for Clinical and Health Research Pathway to First award (grant UL1-TR-002240). Drs. Tsoi, Gudjonsson, and Kahlenberg's work was supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH (grant K01-AR-072129 to Dr. Tsoi, grant P30-AR075043 to Dr. Gudjonsson, and grants R01-AR-071384 and K24-AR-076975 to Dr. Kahlenberg).

[1]Jessica L. Turnier, MD, Lori Lowe, MD, Lam C. Tsoi, PhD, Sultan Elhaj, BS, Rajasree Menon, PhD, Johann E. Gudjonsson, MD, PhD, Celine C. Berthier, PhD, J. Michelle Kahlenberg, MD, PhD: University of Michigan, Ann Arbor; [2]Lauren M. Pachman, MD, Maria C. Amoruso, MPH, Gabrielle A. Morgan, MA: Northwestern University Feinberg School of Medicine, Chicago, Illinois.

Drs. Berthier and Kahlenberg contributed equally to this work.

Dr. Gudjonsson has received consulting fees and/or honoraria from Almirall, Novartis, Eli Lilly, and AnaptysBio (less than $10,000 each) and research grants from Almirall, Novartis, Pfizer, Eli Lilly, Celgene, and Bristol Myers Squibb. Dr. Kahlenberg has received consulting fees, speaking fees, and/or honoraria from AstraZeneca, Aurinia, Boehringer Ingelheim, Bristol Myers Squibb, and Eli Lilly (less than $10,000 each) and research grants from Bristol Myers Squibb, Celgene, and Q32 Bio. No other disclosures relevant to this article were reported.

Address correspondence to Jessica Turnier, MD, C. S. Mott Children's Hospital, Department of Pediatrics, Medical Professional Building, Floor 2, 1500 East Medical Center Drive, SPC 5718, Ann Arbor, MI 48109. Email: turnierj@med.umich.edu.

Submitted for publication May 11, 2020; accepted in revised form December 3, 2020.

as an indicator of ongoing disease activity, leading to disease chronicity and damage over time (2,3); however, skin disease in juvenile myositis has not been sufficiently studied.

Interferons (IFNs) are likely important in juvenile myositis pathophysiology. A striking up-regulation of IFN-stimulated genes (ISGs) has been noted in adult dermatomyositis (DM) skin, similar to that seen in systemic lupus erythematosus (SLE) (4). IFN signaling is also known to be up-regulated in juvenile myositis muscle and peripheral blood (5,6). However, the role of IFNs in disease pathogenesis is less clear. While the peripheral blood IFN signature in both juvenile myositis and DM has been shown to correlate with disease activity (7,8), it is not associated with disease duration in juvenile myositis (9). Higher IFN scores in muscle are associated with increased disease severity based on muscle biopsy histopathology, and type II IFN scores may predict a longer time to clinically inactive disease (10). In DM skin disease, type I IFNs have been purported to lead to recruitment of lymphocytes, macrophages, and plasmacytoid dendritic cells (pDCs). Another similarity to lupus (11,12) is that nonlesional juvenile myositis skin may also be abnormal, with increased numbers of pDCs and mast cells (13).

Here, we investigated the transcriptional changes in lesional and nonlesional juvenile myositis skin and compared these with those in childhood-onset SLE skin disease. This was examined in the context of patient data, including myositis-specific autoantibodies (MSAs). This work thus lays the foundation for understanding juvenile myositis skin lesions and identifies IFN-targeting therapies as appropriate for trials in juvenile myositis.

PATIENTS AND METHODS

Sample acquisition and clinical data collection. Biopsy specimens from patients with juvenile myositis and those with childhood-onset SLE were identified at either the University of Michigan or Ann & Robert H. Lurie Children's Hospital of Chicago, with approval from the University of Michigan Institutional Review Board (IRBMED) and a waiver of consent. Diagnosis was verified by chart review of current and historical clinical findings, laboratory data, imaging, histopathology, and specified diagnosis of juvenile DM or childhood-onset SLE by a pediatric rheumatologist. All but 4 of the patients with juvenile DM met the Bohan and Peter criteria for definite or probable juvenile DM (14). Of the 4 patients not meeting these criteria, 2 had amyopathic disease and 2 lacked typical juvenile DM rash at diagnosis. We therefore chose to use the more general term juvenile myositis for our patient cohort. All patients with childhood-onset SLE met the 1997 American College of Rheumatology classification criteria for SLE (15) at time of skin biopsy, with the exception of 1 patient with isolated cutaneous lupus at diagnosis who later developed systemic disease features.

Overall, we identified a total of 25 formalin-fixed paraffin-embedded (FFPE) skin biopsy specimens, including 17 juvenile myositis samples (9 lesional, 8 nonlesional) and 8 childhood-onset SLE samples (all lesional, 2 patients with 2 separate biopsies from different sites at individual time points). Lesional juvenile myositis skin was obtained from varied locations, including the elbow (n = 3), finger (n = 2), arm, knee, leg, and thigh (all n = 1). Lesional childhood-onset SLE skin was also obtained from multiple locations, including the upper arm, toe, cheek, palm, scalp, finger, anterior lateral proximal thigh, and elbow. All nonlesional juvenile myositis skin was obtained from the thigh or lower back. We also obtained FFPE skin from 8 pediatric controls (uninvolved skin removed with nevi excision). Summary reports from patient biopsies performed for clinical care are listed in Supplementary Table 1 (on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41615/abstract).

Clinical data were collected retrospectively by chart review from patients with juvenile myositis and those with childhood-onset SLE both at time of diagnosis and within 1 month of skin biopsy (Table 1). MSAs were measured using either the myositis autoantibody profile from the Oklahoma Medical Research Foundation (OMRF) Clinical Immunology Laboratory (all of the nonlesional samples and 1 lesional sample) or the Myomarker Panel 3 at Mayo Clinic Laboratories (5 lesional samples). These clinically available MSA testing methodologies utilize differing techniques for MSA detection, with the OMRF profile determined predominantly by immunoprecipitation and immunoblotting and the Myomarker Panel 3 by enzyme immunoassay (EIA). While a direct comparison of test performance characteristics between these 2 methodologies has not been published for reference, it has been noted that EIA methodology has a lower sensitivity for detection of some autoantibodies, such as anti–transcription intermediary factor 1γ (anti-TIF1γ), while also potentially leading to more false-positive results (16–19).

RNA isolation and microarray procedures. We obtained ten 10-μm sections per FFPE skin block and isolated RNA using an Omega Bio-Tek FFPE RNA kit. Library preparation and microarray were completed by the Advanced Genomics Core at the University of Michigan. Complementary DNA (cDNA) was prepared with the Ovation PicoSL WTA System V2 (part no. M01226, version 4; NuGEN) from ~30 ng of total RNA. Using the NuGEN Encore Biotin Module (part no. M01111, version 6), 2.5 μg cDNA was biotinylated. A Poly-A RNA Control Kit was used. Affymetrix Human Gene ST 2.1 array plates were run using the Affymetrix GeneTitan system (version 3.2.4.1515). Quality control and robust multiarray average normalization of CEL files were performed in R software (version 3.5.1) using custom CDF (version 23) and the associated modified Affymetrix package from BrainArray (http://brainarray.mbni.med.umich.edu/Brainarray/Database/CustomCDF/CDF_download.asp) (20).

All samples underwent normalized unscaled standard error, relative log expression, and principal components analysis (PCA) quality controls. Two nonlesional juvenile myositis samples and 1 childhood-onset SLE sample were excluded from further

Table 1. Characteristics of the controls, patients with juvenile myositis, and patients with childhood-onset systemic lupus erythematosus*

	Controls (n = 8)	Nonlesional juvenile myositis (n = 6)	Lesional juvenile myositis (n = 9)	Lesional childhood-onset SLE (n = 5)†	P‡	P§
Age at diagnosis, mean ± SEM years	–	7.2 ± 1.6	9.1 ± 1.7	12.8 ± 0.8	0.4560	0.1598
Age at time of biopsy, mean ± SEM years	12.3 ± 1.7	13.0 ± 2.0	10.9 ± 1.7	13.2 ± 0.5	0.4428	0.3561
Female sex	6 (75)	6 (100)	8 (88.9)	5 (83)	0.4346	0.6785
Race						
White	5 (62.5)	6 (100)	7 (77.8)	3 (60)	0.2445	0.5185
African American	0 (0)	0 (0)	2 (22.2)	1 (20)	0.2445	0.9298
Other	2 (25)	0 (0)	0 (0)	1 (20)	–	0.1902
Unknown	1 (12.5)	0 (0)	0 (0)	0 (0)	–	–
Ethnicity						
Non-Hispanic	7 (87.5)	4 (66.7)	8 (88.9)	5 (100)	0.0888	–
Hispanic	0 (0)	2 (33.3)	0 (0)	0 (0)	0.0888	–
Unknown	1 (12.5)	0 (0)	1 (11.1)	0 (0)	0.4346	–
Disease duration at biopsy, mean ± SEM years	–	5.8 ± 1.4	1.8 ± 0.9	0.4 ± 0.2	0.0213	0.4783
Duration of untreated disease prior to diagnosis, mean ± SEM months	–	8.8 ± 2.9	5.9 ± 1.6	–	0.3687	–
Dysphagia at time of biopsy	–	0 (0)	3 (33.3)	–	0.1309	–
CMAS score at time of biopsy, mean ± SEM (range 0–52)	–	44 ± 4.8	39 ± 11.5	–	0.6416	–
Amyopathic disease	–	0 (0)	2 (22.2)	–	0.2445	–
Lupus nephritis	–	–	–	2 (40)	–	–
Skin manifestations						
CLE only	–	–	–	1 (20)	–	–
Discoid lupus	–	–	–	1 (14.2)	–	–
Heliotrope rash	–	2 (33.3)	4 (44.4)	–	0.6934	–
Gottron's sign/papules	–	4 (66.7)	7 (77.8)	–	0.6621	–
Nailfold capillary changes	–	2 (33.3)	7 (77.8)	–	0.0970	–
Calcinosis	–	2 (33.3)	0 (0)	–	0.0699	–
Skin ulceration	–	0 (0)	0 (0)	–	NA	–
Laboratory tests						
Anti-dsDNA positive	–	–	–	2 (40.0)	–	–
C3, mean ± SEM mg/dl	–	–	–	115.8 ± 10.9	–	–
C4, mean ± SEM mg/dl	–	–	–	16.6 ± 2.1	–	–
MAA positive	–	3 (50.0)	1 (12.5)	–	0.0476	–
MSA positive	–	4 (66.7)	3 (60.0)	–	0.8402	–
NXP-2 positive	–	1 (16.7)	2 (40.0)	–	0.4385	–
TIF1γ positive	–	3 (50.0)	1 (20.0)	–	0.3527	–
MSA negative	–	2 (33.3)	2 (40.0)	–	0.8402	–
MSA unknown	–	0 (0)	4 (44.4)	–	0.0623	–
Serum muscle enzymes at time of biopsy, mean ± SEM						
CK, units/liter	–	48.2 ± 8.5	1,474.4 ± 1,341.0	–	0.4514	–
Aldolase, units/dl	–	4.4 ± 1.0	12.8 ± 5.5	–	0.2856	–
LDH, units/liter	–	189.0 ± 13.0	386.9 ± 109.4	–	0.1885	–
AST, units/liter	–	31.6 ± 2.7	68.8 ± 32.0	–	0.4126	–
ALT, units/liter	–	35.0 ± 6.0	41.3 ± 17.7	–	0.8009	–
Medication(s) at time of biopsy						
None	–	0 (0)	4 (44.4)	1 (20)	0.0623	0.3997
Oral steroids	–	6 (100)	3 (33.3)	4 (80)	0.0066	0.1089
IV steroids	–	4 (66.7)	2 (22.2)	1 (20)	0.0970	0.9298
MMF	–	5 (83.3)	1 (11.0)	3 (60)	0.0024	0.0575
Cyclosporine	–	3 (50.0)	1 (11.0)	0 (0)	0.1089	0.4783
HCQ	–	5 (83.3)	0 (0)	2 (40)	<0.0001	0.0426
MTX	–	2 (33.3)	4 (44.4)	0 (0)	0.6934	0.0888
IVIG	–	3 (50.0)	0 (0)	0 (0)	0.0152	–

* Comparisons between patients with lesional and nonlesional skin were made using Student's unpaired 2-tailed *t*-test. Nailfold capillary changes include nailfold capillary dilatation or dropout. Myositis-specific autoantibodies (MSAs) were measured using either the myositis autoantibody profile at the Oklahoma Medical Research Foundation Clinical Immunology Laboratory (all of the nonlesional samples and 1 lesional sample) or the Myomarker Panel 3 at Mayo Clinic Laboratories (5 lesional samples). Data on the Childhood Myositis Assessment Score (CMAS) were missing for 6 patients with lesional skin, data on myositis-associated antibodies (MAAs) were missing for 1 patient with lesional skin, and data on MSAs were missing for 4 patients with lesional skin. Except where indicated otherwise, values are the number (%) of patients. CLE = cutaneous lupus erythematosus; NA = not applicable; anti-dsDNA = anti–double-stranded DNA; NXP-2 = nuclear matrix protein 2; TIF1γ = transcription intermediary factor 1γ; CK = creatine kinase; LDH = lactate dehydrogenase; AST = aspartate aminotransferase; ALT = alanine aminotransferase; MMF = mycophenolate mofetil; HCQ = hydroxychloroquine; MTX = methotrexate; IVIG = intravenous immunoglobulin.
† Two patients with childhood-onset systemic lupus erythematosus (SLE) had 2 separate biopsies from different sites at individual time points.
‡ Lesional juvenile myositis versus nonlesional juvenile myositis.
§ Lesional juvenile myositis versus childhood-onset SLE.

analysis, as 1 patient was an extreme outlier according to PCA and 2 had atypical histopathology. The final cohort consisted of 15 juvenile myositis biopsy samples (9 lesional, 6 nonlesional), 7 childhood-onset SLE biopsy samples (from 5 patients), and 8 control biopsy samples. The baseline $\log_2$ expression value was defined as the minimum +1 SD of the median of all genes. A variance filter of 80% was then applied. Of the 29,635 unique genes represented on the Human ST2.1 chip, a total of 23,698 genes met the defined criteria. Data from the microarrays are available through Gene Expression Omnibus (GEO), accession no. GSE148810.

Canonical pathways and literature-based network analyses, hierarchical clustering, and heatmap generation. Canonical pathways (well-established signaling and metabolic pathways) were identified using Ingenuity Pathway Analysis software (www.ingenuity.com). Biologic literature–based networks were built using Genomatix Pathway System software (www.genomatix.de), with the function-word level as the minimum evidence level parameter. Heatmaps were generated using the gene expression values as input for the HeatmapViewer module in GenePattern (https://cloud.genepattern.org).

Cell-type enrichment analysis. Cell-type enrichment analysis was performed on the normalized data set of 23,698 genes using the xCell webtool (http://xcell.ucsf.edu/) (21).

Calculation of IFN- and juvenile myositis–specific disease signature scores. IFN scores were calculated using 6 IFN-stimulated genes (*IFIT1, IRF7, MX1, EIF2AK2, OASL, IFI44*) with the algorithm described by Feng et al (22), and as previously published (23). With the exception of *EIF2AK2*, these genes were used by Feng et al and include 2 of the 5 recommended ISGs (*OASL* and *MX1*). *EIF2AK2* has been shown to be an ISG in lupus (24) and is also specifically up-regulated in keratinocytes upon IFNα stimulation (25). Our 6-gene IFN score strongly correlated with the IFN score calculated using the 5 ISGs from Feng et al (*LY6E*, *OAS1*, *OASL*, *MX1*, *ISG15*) ($r = 0.9828$, $P < 0.0001$). A skin-directed IFN score was also calculated based on this algorithm, with 18 genes specifically up-regulated in keratinocytes upon IFNα stimulation: *EIF2AK2*, *IFI16*, *IFI27*, *IFI44*, *IFIH1*, *IFIT5*, *IRF9*, *ISG15*, *NMI*, *OAS3*, *PARP12*, *PARP14*, *PARP9*, *PLSCR1*, *SP100*, *STAT1*, *TNFSF10*, and *ZNFX1* (25). Finally, the same algorithm was applied to a juvenile myositis–specific signature consisting of 23 genes (28 genes minus 2 microRNAs, 2 LOCs, and 1 C-orf gene) derived from comparison of childhood-onset SLE and lesional juvenile myositis. The 3-gene juvenile myositis–specific score was calculated using the 3 most regulated genes (smallest q value) with a fold change of ≥2 (*FBNL2* [fold change 2.15; q = 0.0137], *CHKA* [fold change 2.14; q = 0.0137], *SLURP1* [fold change 2.13; q = 0.0239]) in our study samples, as well as skin disease array data sets available from GEO (25). Association between the juvenile myositis–specific signature and the skin-directed IFN score was assessed using Pearson's correlation coefficient with GraphPad Prism, version 8.0.0.

Immunohistochemistry. Four-micron sections were cut from FFPE skin blocks. Skin tissue was deparaffinized in Histo-Clear and rehydrated in graded ethanol. Heat-mediated antigen retrieval was performed in sodium citrate buffer, followed by incubation steps with Bloxall and 1.5% goat serum (Vector). Slides were incubated overnight at 4°C with anti–Mx-1 (1:500 dilution, ab97921; Abcam), rabbit IgG isotype control, or phosphate buffered saline. Slides were developed with biotinylated secondary antibody and HRP/Vectastain Elite ABC Reagent, followed by ImmPACT DAB Peroxidase (HRP) Substrate (all from Vector) for 90 seconds before quenching in water and counterstaining with hematoxylin.

Real-time quantitative polymerase chain reaction (qPCR). Complementary DNA was prepared from FFPE-isolated RNA. Expression of *MX1, IFI44*, *CXCL10*, and *SLURP1* was measured by real-time PCR on an ABI Prism 7900HT (Applied Biosystems) using SYBR Green (Life Technologies). Fold change expression was calculated relative to GAPDH using $2^{-\Delta\Delta C_t}$. Primers were as follows (5′ to 3′): for *MX1,* TACCAGGACTACGAGATTG (forward), TGCCAGGAAGGTCTATTAG (reverse); for *IFI44,* GGTGGGCACTAATACAACTGG (forward), CACACAGAATAAACGGCAGGTA (reverse); for *CXCL10*, GTGGCATTCAAGGAGTACCTC (forward), TGATGGCCTTCGATTCTGGATT (reverse); for *SLURP1,* CTGCAAGCCAGAGGACACA (forward), CACACAGGAGCTGGAGCAG (reverse); and for *GAPDH*, CTGGGCTACACTGAGCACC (forward), AAGTGGTCGTTGAGGGCAATG (reverse).

Statistical analysis. Differentially expressed genes (DEGs) were compared between lesional juvenile myositis, nonlesional juvenile myositis, childhood-onset SLE, and control biopsy specimens with the Significance Analysis of Microarrays method implemented in the Institute for Genomic Research MultiExperiment Viewer application, version 4.9.0 (unpaired analysis) (26). Genes regulated with a false discovery rate q value of less than 0.05 were considered significant and used for further transcriptional and pathway analyses. Statistical analysis of clinical data and gene scores was performed using an unpaired parametric *t*-test with GraphPad Prism, version 8.0.0. *P* values less than 0.05 were considered significant. Comparisons across all groups were performed, and, for clarity, only the most relevant were reported if significant.

RESULTS

Clinical cohort characteristics. In the juvenile myositis cohort, patients with lesional juvenile myositis had shorter disease duration at the time of skin biopsy compared to those with nonlesional juvenile myositis (Table 1). Overall skin manifestations and serum muscle enzyme levels were similar in lesional and nonlesional juvenile myositis. Two patients with lesional juvenile myositis

had amyopathic disease. Among patients with lesional and nonlesional juvenile myositis who were tested for the presence of MSAs, the majority were MSA-positive. In our cohort, patients with juvenile myositis exclusively demonstrated nuclear matrix protein 2 (NXP-2) and TIF1γ MSAs. Only 4 patients with lesional juvenile myositis were treatment-naive at the time of skin biopsy. Patients with nonlesional juvenile myositis were more likely to be receiving oral steroids, mycophenolate mofetil, hydroxychloroquine (HCQ), and intravenous immunoglobulin. In the childhood-onset SLE cohort, only 1 patient had isolated cutaneous lupus at the time of skin biopsy. The majority of biopsy samples showed subacute cutaneous lupus, with only 1 showing discoid lupus (Table 1 and Supplementary Table 1, on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41615/abstract). Overall treatment was similar among patients with lesional juvenile myositis and those with childhood-onset SLE, with the only difference being that more patients with childhood-onset SLE were receiving HCQ at the time of skin biopsy.

Comparison of lesional juvenile myositis skin to control skin. A total of 221 genes were differentially regulated in lesional juvenile myositis compared to controls, with all but 1 up-regulated in lesional juvenile myositis ($q < 0.05$) (Supplementary Table 2, http://onlinelibrary.wiley.com/doi/10.1002/art.41615/abstract). The majority of up-regulated genes in lesional juvenile myositis were IFN-sensitive, with *CXCL10*, *CXCL9*, and *IFI44L* representing the top 3 up-regulated genes (fold change 23.2, 13.3, and 13.0, respectively; $q < 0.0001$). Figure 1 highlights the most up-regulated canonical pathways of the DEGs in lesional juvenile myositis relative to controls (Supplementary Table 3, http://onlinelibrary.wiley.com/doi/10.1002/art.41615/abstract) and provides a heatmap of the genes regulated in each pathway. Canonical pathway analysis revealed IFN signaling as the top up-regulated pathway and showed activation of pathways involving antigen presentation, pattern recognition receptors, communication between innate and adaptive immune cells, T cell signaling, complement system, and DC maturation (Figure 1 and Supplementary Table 3). Literature-based network analysis of all 221 DEGs identified up-regulation of *STAT1* as a central node linking dysregulated genes (fold change 5.16; $q < 0.0001$). The top predicted upstream regulator was IFNα ($P = 7.91 \times 10^{-88}$) (Supplementary Table 4, http://onlinelibrary.wiley.com/doi/10.1002/art.41615/abstract).

Comparison of lesional juvenile myositis skin to nonlesional juvenile myositis skin. Nonlesional juvenile myositis skin had a strikingly different gene expression signature compared to lesional juvenile myositis biopsy specimens, most notably the lack of a prominent IFN signature (Figure 1). Multiple genes and pathways were down-regulated in nonlesional juvenile myositis compared to lesional juvenile myositis, including pathways in protein ubiquitination, glucocorticoid receptor signaling, IFN signaling, and oxidative phosphorylation (Supplementary Tables 5 and

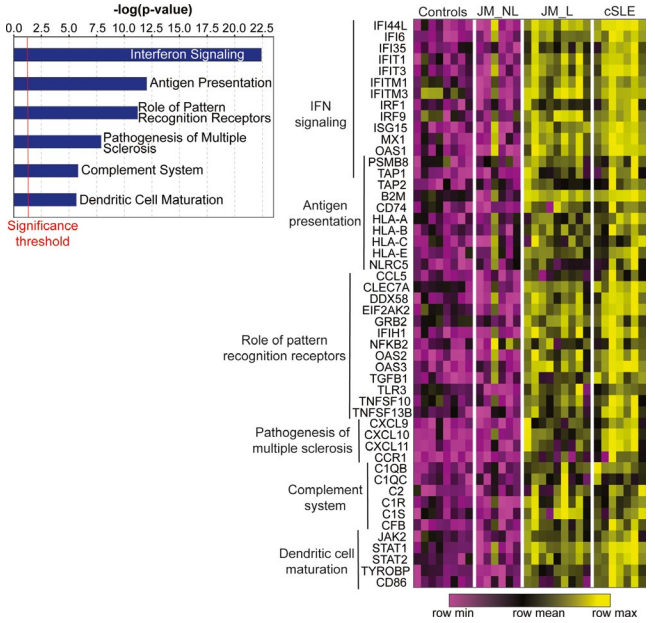
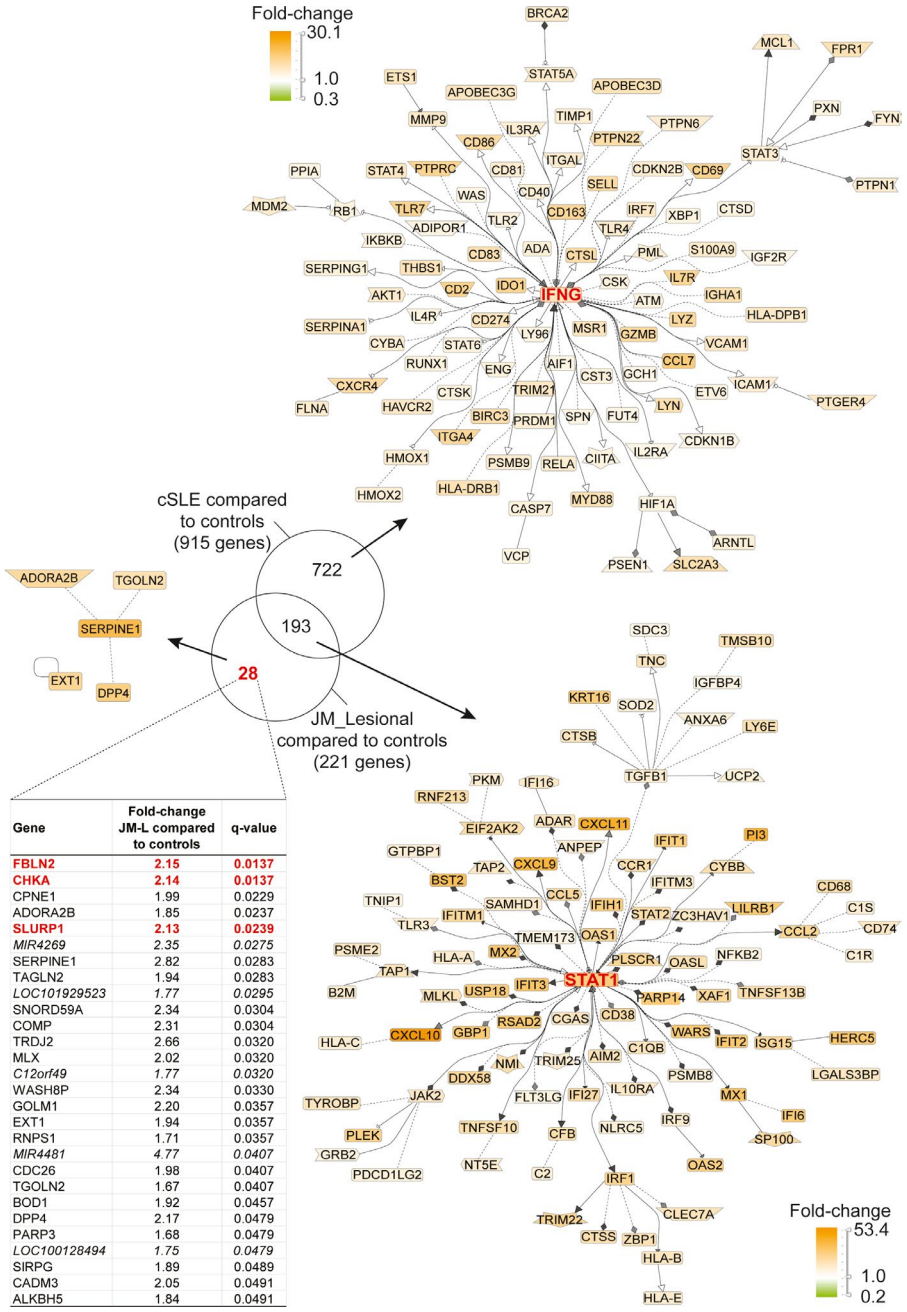


Figure 1. Genome-wide expression analysis of skin biopsy samples from controls, patients with juvenile myositis, and patients with childhood-onset systemic lupus erythematosus (cSLE). Left panel, Selected top canonical pathways ($P < 0.05$) from the 221 genes that were up- or down-regulated in lesional juvenile myositis (JM_L) skin biopsy samples compared to controls ($q < 0.05$). Right panel, Heatmap of selected genes from those top pathways in skin biopsy samples from controls, patients with nonlesional juvenile myositis (JM_NL), patients with lesional juvenile myositis, and patients with childhood-onset SLE. Each column represents an individual patient sample, while each row represents a differentially expressed gene in lesional juvenile myositis relative to controls. Gene expression values are depicted using the color scale shown, with purple to yellow indicating increasing expression. Genes overlapping between pathways are represented only once. IFN = interferon.

6, http://onlinelibrary.wiley.com/doi/10.1002/art.41615/abstract). We applied a cell-type enrichment webtool to the gene expression data (xCell) in order to characterize potential immune cell types in lesional and nonlesional juvenile myositis skin, identifying increased macrophages and CD4+ memory T cells in lesional juvenile myositis skin (Supplementary Figure 1, http://onlinelibrary.wiley.com/doi/10.1002/art.41615/abstract).

Comparison of lesional juvenile myositis skin to lesional childhood-onset SLE skin. Lesional juvenile myositis skin shared a highly similar gene expression pattern with childhood-onset SLE (Figures 1 and 2). Notably, lesional skin both from patients with juvenile myositis and from patients with childhood-onset SLE demonstrated a prominent type I IFN signature. There were only 28 unique DEGs in lesional juvenile myositis skin compared to childhood-onset SLE skin (Figure 2). The most significant unique DEGs in lesional juvenile myositis included *FBLN2*, *CHKA*, and *SLURP1* genes, with diverse roles in extracellular matrix structure, keratinocyte proliferation and differentiation, calcium signaling, and phospholipid metabolism. In contrast,



Gene	Fold-change JM-L compared to controls	q-value
FBLN2	**2.15**	**0.0137**
CHKA	**2.14**	**0.0137**
CPNE1	1.99	0.0229
ADORA2B	1.85	0.0237
SLURP1	**2.13**	**0.0239**
MIR4269	*2.35*	*0.0275*
SERPINE1	2.82	0.0283
TAGLN2	1.94	0.0283
LOC101929523	*1.77*	*0.0295*
SNORD59A	2.34	0.0304
COMP	2.31	0.0304
TRDJ2	2.66	0.0320
MLX	2.02	0.0320
C12orf49	*1.77*	*0.0320*
WASH8P	2.34	0.0330
GOLM1	2.20	0.0357
EXT1	1.94	0.0357
RNPS1	1.71	0.0357
MIR4481	*4.77*	*0.0407*
CDC26	1.98	0.0407
TGOLN2	1.67	0.0407
BOD1	1.92	0.0457
DPP4	2.17	0.0479
PARP3	1.68	0.0479
LOC100128494	*1.75*	*0.0479*
SIRPG	1.89	0.0489
CADM3	2.05	0.0491
ALKBH5	1.84	0.0491

Figure 2. Transcriptomic comparison of childhood-onset SLE skin to lesional juvenile myositis skin ($q < 0.05$). Literature-based networks (Genomatix Pathway System) were obtained from the genes that were up- or down-regulated in childhood-onset SLE and juvenile myositis versus control biopsy samples. In these networks, the 100 best connected genes co-cited in PubMed abstracts in the same sentence linked to a function word (most relevant genes/interactions) are shown. Red type indicates the top 3 differentially expressed genes unique to juvenile myositis compared to childhood-onset lupus. In lesional skin compared to control skin, up-regulated genes are shown in orange, and down-regulated genes are shown in green. See Figure 1 for definitions.

childhood-onset SLE skin had 722 unique DEGs compared to lesional juvenile myositis skin (Supplementary Table 7, http://onlinelibrary.wiley.com/doi/10.1002/art.41615/abstract).

Figure 2 shows that childhood-onset SLE skin uniquely exhibits increased expression of IFNγ relative to control skin, illustrating a more pronounced type II IFN signature in addition to the type I IFN signature it has in common with lesional juvenile myositis. We confirmed a predominant type I IFN signature in lesional juvenile myositis skin by using RNA sequencing data from control keratinocytes treated with either IFNα or IFNγ (Supplementary Figure 2, http://onlinelibrary.wiley.com/doi/10.1002/art.41615/abstract). While both childhood-onset SLE skin and lesional juvenile myositis skin showed up-regulation of genes stimulated by IFNα, childhood-onset SLE demonstrated a more robust

up-regulation of genes regulated by IFNγ stimulation. According to the xCell cell-type enrichment analysis, childhood-onset SLE skin exhibited an overall higher inflammatory cell signature compared to lesional juvenile myositis skin, with increased T cells, B cells, macrophages, and pDCs (Supplementary Figure 1, http://onlinelibrary.wiley.com/doi/10.1002/art.41615/abstract).

Similar skin-directed IFN scores in juvenile myositis and childhood-onset SLE. Patients with lesional juvenile myositis had higher skin-directed IFN scores than those with nonlesional juvenile myositis (P = 0.0001) (Supplementary Figure 3, http://onlinelibrary.wiley.com/doi/10.1002/art.41615). Patients with lesional juvenile myositis and those with childhood-onset SLE had similar skin-directed IFN scores. The findings from our skin-directed IFN scoring of patient samples were validated with a more standard 6-gene IFN score (Supplementary Figure 3). We also evaluated expression levels of 3 candidate ISGs (*MX1*, *IFI44*, and *CXCL10*) using real-time PCR, confirming higher expression levels of *MX1*, *IFI44*, and *CXCL10* in lesional juvenile myositis compared to controls (Supplementary Figure 4, http://onlinelibrary.wiley.com/doi/10.1002/art.41615/abstract). *MX1* and *CXCL10* expression levels were similar in lesional juvenile myositis and childhood-onset SLE, while *IFI44* expression was slightly higher in childhood-onset SLE.

Derivation and evaluation of juvenile myositis disease signature. Using the top 3 unique DEGs in lesional juvenile myositis skin relative to childhood-onset SLE (*FBLN2*, *CHKA*, and *SLURP1*), a juvenile myositis–specific skin score was developed (see Patients and Methods) and evaluated relative to expression data from independent adult skin microarray data sets, which included patients with DM, cutaneous lupus, psoriasis, lichen planus, or graft-versus-host disease. Figure 3 illustrates that the juvenile myositis disease signature was much higher in DM (both pediatric and adult) compared to other skin diseases, including pediatric and adult lupus. We confirmed that *SLURP1* expression was higher in lesional juvenile myositis compared to childhood-onset SLE in our study samples (Supplementary Figure 5, http://onlinelibrary.wiley.com/doi/10.1002/art.41615/abstract). We also evaluated a 23-gene juvenile myositis–specific skin score, obtaining similar results (Supplementary Figure 6, http://onlinelibrary.wiley.com/doi/10.1002/art.41615/abstract). This score was strongly associated with the skin-directed IFN score in lesional juvenile myositis samples (r = 0.8713, P = 0.0022) (Supplementary Figure 6).

Higher IFN scores and increased Mx-1 immunostaining in skin from patients with juvenile myositis who were positive for NXP-2. Upon evaluation of skin-directed IFN scores in patients with juvenile myositis based on clinical features, we found that skin-directed IFN scores did not differ according to individual skin disease manifestations (Figure 4A and Supplementry Figure 7A, http://onlinelibrary.wiley.com/doi/10.1002/art.41615/abstract). Specifically, skin-directed IFN scores did not differentiate between patients with amyopathic disease, nailfold capillary changes, or calcinosis. There was also no difference in skin-directed IFN scores based on treatment status (Figure 4B and

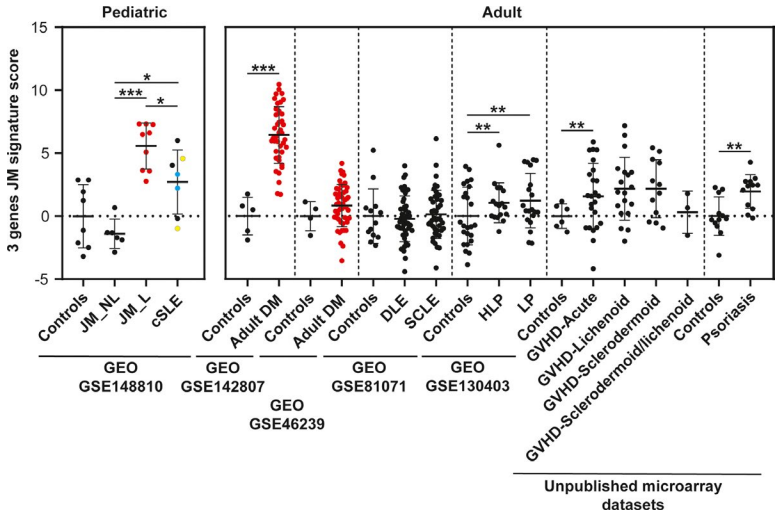


Figure 3. Juvenile myositis disease signature score comparison using transcriptomic data sets of skin lesions from patients with adult dermatomyositis (DM) and other inflammatory skin diseases. The 3-gene juvenile myositis transcriptomic signature identified was the highest in juvenile and adult DM compared to other skin disease lesions (Gene Expression Omnibus [GEO] accession nos. GSE142807, GSE46239, GSE81071, GSE130403, and unpublished microarray data sets, courtesy of Dr. Johann Gudjonsson, Department of Dermatology, University of Michigan) (23,25,41). DM lesional samples are shown in red. Vertical dashed lines separate the studied data sets. Each data set had a control sample set. Patients with childhood-onset SLE who had 2 biopsies are shown in blue and yellow. Each symbol represents an individual sample; bars show the median ± SEM. * = P < 0.05; ** = P < 0.01; *** = P < 0.0001. DLE = discoid lupus erythematosus; SCLE = subacute cutaneous lupus erythematosus; HLP = hypertrophic lichen planus; GVHD = graft-versus-host disease (see Figure 1 for other definitions).

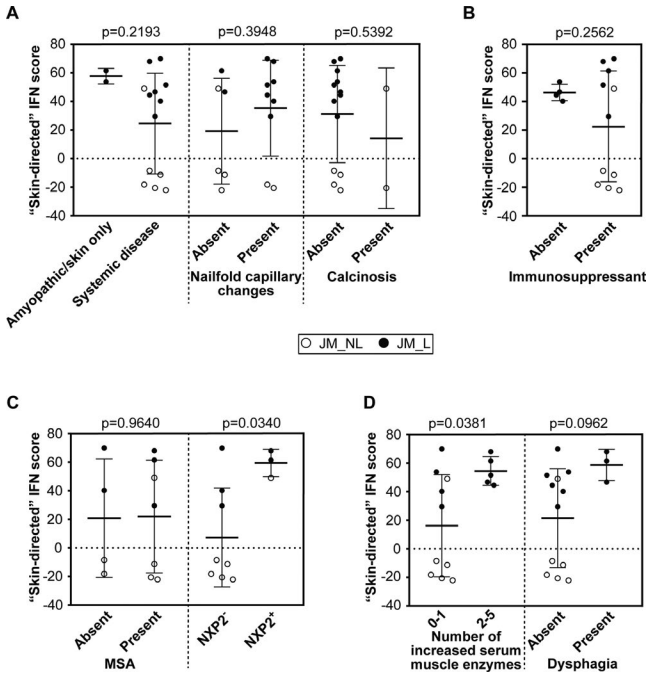


Figure 4. Juvenile myositis skin–directed IFN score in relation to clinical variables. **A**, The skin-directed IFN score was not significantly modified by the presence of systemic disease, nailfold capillary changes, or calcinosis. **B**, The skin-directed IFN score was not significantly changed by treatment status. **C**, The presence alone of any myositis-specific autoantibody (MSA) did not significantly alter the skin-directed IFN score. However, patients with juvenile myositis who were positive for nuclear matrix protein 2 (NXP-2) showed a significantly higher skin-directed IFN score when lesional skin and nonlesional skin were analyzed together. **D**, An increased overall number of serum muscle enzymes was associated with a higher skin-directed IFN score when lesional skin and nonlesional skin were analyzed together. There was a trend toward higher skin-directed IFN scores in the presence of dysphagia. Each symbol represents an individual sample; bars show the median ± SEM. See Figure 1 for other definitions.

Supplementary Figure 7B). While the skin-directed IFN scores did not differ between patients positive for MSAs versus those negative for MSAs, the scores did differ by MSA subtype when lesional skin and nonlesional skin were analyzed together (Figure 4C and Supplementary Figure 7C). Of note, we only had 3 NXP-2–positive patients for this comparison. Anti–NXP-2–positive patients demonstrated higher skin-directed IFN scores than NXP-2–negative patients (fold change 8; $P = 0.034$). Juvenile myositis patients with elevated levels of multiple serum muscle enzymes (e.g., creatine kinase, aldolase, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase) also had higher skin-directed IFN scores when lesional and nonlesional skin were analyzed together (Figure 4D and Supplementary Figure 7D).

Additionally, we evaluated expression of individual ISGs in patients with juvenile myositis. NXP-2–positive juvenile myositis patients, compared to those who were NXP-2 negative, had higher cutaneous expression levels of *MX1*, *IFI44*, and *USP18* when lesional and nonlesional skin were analyzed together (Supplementary Figure 8, http://onlinelibrary.wiley.com/doi/10.1002/art.41615/abstract). In lesional juvenile myositis skin biopsy samples, Mx-1 immunostaining localized to keratinocytes, inflammatory cells, and the perivascular region (Figure 5). Mx-1 staining was more pronounced in NXP-2–positive patients compared to TIF1γ-positive and MSA-negative patients (Figure 5), in accordance with gene expression data (Supplementary Figure 9, http://onlinelibrary.wiley.com/doi/10.1002/art.41615/abstract).

DISCUSSION

In this report, we provide the first characterization of cutaneous gene expression signatures in a juvenile myositis cohort. Similar to previous gene expression studies in juvenile myositis muscle and peripheral blood (5,6,9,27), we identified a striking type I IFN signature in lesional juvenile myositis skin. Interestingly, lesional juvenile myositis skin was found to have a predominant type I IFN signature, whereas childhood-onset SLE exhibited up-regulation of both type I and type II IFNs. A candidate juvenile myositis–specific skin signature was derived using *FBLN2*, *CHKA*, and *SLURP1*, which are all genes not typically considered to have immunomodulatory roles but instead function in cellular structure and metabolism. While a skin-directed IFN score did not distinguish patients with juvenile myositis by cutaneous features or treatment status, the 3 patients with juvenile myositis who were positive for NXP-2 had higher IFN scores and stronger Mx-1 immunostaining in lesional skin.

Our findings suggest that IFNs play a role in juvenile myositis skin disease pathogenesis, consistent with what has previously been described through gene expression and immunohistochemistry studies of adult and juvenile DM skin (4,13,28). The specific mechanisms by which IFNs contribute to juvenile myositis skin disease pathophysiology are not well understood. In DM skin lesions, the number of CXCR3+ lymphocytes correlates with strength of Mx-1 immunostaining (28,29), suggesting a role for IFN-inducible chemokines that are also CXCR3 ligands in recruitment of inflammatory cells. Indeed, we identified *CXCL9* and *CXCL10* within the top 3 DEGs in lesional juvenile myositis skin, suggesting that these chemokines may play a role in cutaneous disease pathogenesis. CXCL10 has also been evaluated as a serum biomarker in juvenile myositis and was demonstrated to outperform creatine kinase as a disease activity marker (30). Using cell-type enrichment analysis, we determined that CD4+ memory T cells and macrophages were increased in lesional juvenile myositis skin compared to skin from patients with nonlesional juvenile myositis and controls, consistent with findings from prior immunohistochemistry studies of DM skin (29,31).

In this study, we also demonstrated that NXP-2–positive patients exhibit a stronger IFN signature in skin, suggesting a potential role for NXP-2 in contributing to the IFN signature. Given that we identified an elevated IFN signature even in a nonlesional skin sample from an NXP-2–positive patient, it is possible that the

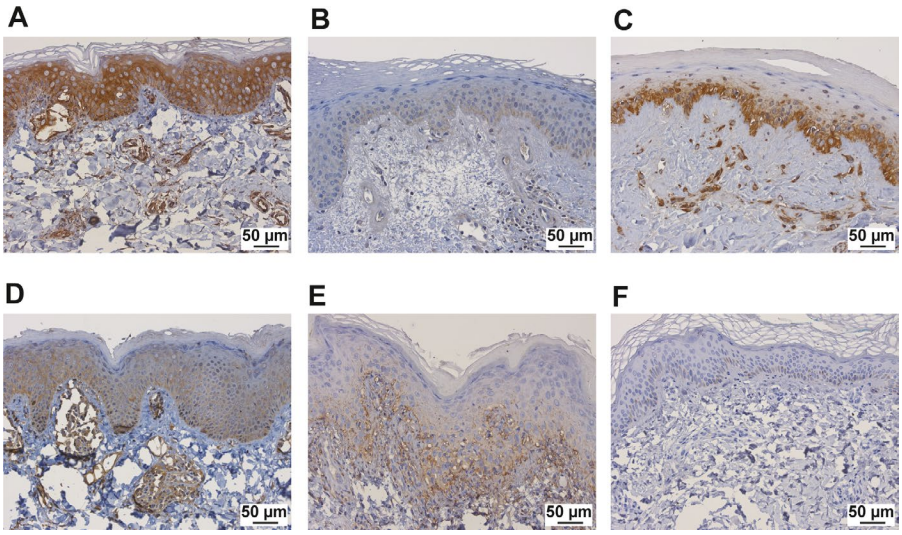


Figure 5. Mx-1 immunostaining in representative study samples, including skin biopsy samples from juvenile myositis patients with nuclear matrix protein 2 positivity (**A**), transcription intermediary factor 1γ positivity (**B**), Ku positivity (**C**), or myositis-specific antibody negativity (**D**), a subject with childhood-onset systemic lupus erythematosus (**E**), and an unaffected pediatric control subject (**F**).

IFN signature is reflective of overall higher levels of systemic inflammation versus skin-specific inflammation. NXP-2, also known as MORC3, has not been widely studied but has been reported to be a protein with RNA-binding activity that functions as an epigenetic regulator (32) and has also been described as both an antiviral factor (33) and a positive regulator of influenza virus transcription (34). Further studies are needed to understand the relationship between IFNs, the autoantigen NXP-2, and how NXP-2 autoantibodies influence disease phenotype.

When comparing lesional juvenile myositis and childhood-onset SLE skin, we noted an overwhelming similarity between gene expression profiles. In particular, juvenile myositis and childhood-onset SLE shared a common type I IFN signature, with a major difference being that childhood-onset SLE lesions had a central IFNγ node on network analysis. While this finding might be reflective of the specific childhood-onset SLE samples in our study, influenced in part by presence of a discoid lesion (23), it is also possible that lack of a strong type II IFN signature distinguishes juvenile myositis from childhood-onset SLE skin, with implications for disease pathogenesis and treatment. We found that a molecular score incorporating expression of the top 3 DEGs, *FBLN2*, *CHKA*, and *SLURP1*, was higher in both juvenile myositis and DM, even when considering other autoimmune skin diseases. Secreted Ly-6/uPAR-related protein 1 is expressed in differentiated keratinocytes (35), whereas fibulin 2 is an extracellular matrix protein involved in basement membrane stability (36), and choline kinase alpha serves as a catalyst in phospholipid biosynthesis. Future validation of this juvenile myositis–specific skin score and how these genes contribute to pathogenesis will be needed.

A major limitation of our study was its small sample size, including only 3 patients with juvenile myositis who were positive for NXP-2, as well as its retrospective nature. Patients with juvenile myositis who were included in our study had variable disease duration at the time of biopsy, which may have limited our ability to detect evolving clinical features, such as calcinosis in the lesional juvenile myositis cohort, as well as influenced gene expression profiles, with nonlesional juvenile myositis samples more skewed toward chronic versus acute inflammatory changes. Given that we had no treatment-naive patients with nonlesional samples and that more patients with nonlesional juvenile myositis were receiving immunosuppressive treatment, inflammatory pathway signatures that might otherwise have been represented in our gene expression data may have been dampened. Lesional juvenile myositis skin samples were also all predominantly from sun-exposed areas, whereas nonlesional juvenile myositis samples were from non–sun-exposed skin, which may have contributed to the difference in gene expression signatures between these 2 types of skin.

Typically, the difference in gene expression identified with FFPE tissue tends to be the more pronounced changes, as sensitivity of detection is diminished. This likely explains why we may not have seen as many DEGs as might be anticipated. However, it has been shown that gene expression from fresh versus frozen versus FFPE tissue can yield comparable findings (37–39), and the genes that we did identify are more likely to be DEGs since differences were likely underdetected. Juvenile myositis clinical phenotypes were also quite heterogeneous, as we did have 2 patients with clinically amyopathic juvenile myositis enrolled in our study. Not all described MSAs were represented in our cohort and MSA data on 4 patients were unavailable; therefore, we could only compare TIF1γ-positive and MSA-negative patients to NXP-2–positive patients. Notably, the study cohort included no patients who were positive for melanoma differentiation–associated gene–like protein (MDA), and higher type I IFN signatures have been reported in the

skin and peripheral blood of MDA-5–positive adult patients with DM (40). MSAs were also tested using 2 methodologies based on the center of enrollment, which may have influenced testing results and patient categorization. It is also not clear which cell types contributed to the IFN signature and how large a role skin-resident immune cells versus infiltrating immune cells might play in disease pathogenesis, given the analysis of bulk tissue. While we did attempt to characterize potential immune cell types present in juvenile myositis skin using xCell, we did not directly quantify cell types and lacked histopathology reports for nonlesional skin biopsy samples. Further work is ongoing to characterize the cellular origin of the type I IFN signature in juvenile myositis skin and to demonstrate how this relates to MSA subtype.

In conclusion, the present study is the largest genome-wide expression analysis of juvenile myositis and childhood-onset SLE skin disease to date, serving to begin characterization of dysregulated genes and pathways specific to skin inflammation in these multisystem disorders. We have identified a link between NXP-2 autoantibodies and strength of the IFN signature in juvenile myositis skin, which may lead to a better understanding of disease heterogeneity and pave the way for individualized treatment in juvenile myositis.

ACKNOWLEDGMENTS

We would like to thank the University of Michigan Advanced Genomics and Research Histology Core for their support and equipment for processing and sequencing of skin samples. We express our many thanks to the myositis and lupus patients for generously sharing their samples for our work. We also thank Matthew Manninen for his expertise in R programming and assistance with calculation of interferon scores, and Tammi Reed for assisting with the FFPE skin biopsy processing protocol. This work was presented in part at both the 2019 and 2020 American College of Rheumatology Annual Meetings.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Turnier had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Turnier, Pachman, Gudjonsson, Berthier, Kahlenberg.

Acquisition of data. Turnier, Lowe, Elhaj, Amoruso, Morgan.

Analysis and interpretation of data. Turnier, Tsoi, Menon, Gudjonsson, Berthier, Kahlenberg.

REFERENCES

1. Almeida B, Campanilho-Marques R, Arnold K, Pilkington CA, Wedderburn LR, Nistala K, et al. Analysis of published criteria for clinically inactive disease in a large juvenile dermatomyositis cohort shows that skin disease is underestimated. Arthritis Rheumatol 2015;67:2495–502.
2. Christen-Zaech S, Seshadri R, Sundberg J, Paller AS, Pachman LM. Persistent association of nailfold capillaroscopy changes and skin involvement over thirty-six months with duration of untreated disease in patients with juvenile dermatomyositis. Arthritis Rheum 2008;58:571–6.
3. Stringer E, Singh-Grewal D, Feldman BM. Predicting the course of juvenile dermatomyositis: significance of early clinical and laboratory features. Arthritis Rheum 2008;58:3585–92.
4. Wong D, Kea B, Pesich R, Higgs BW, Zhu W, Brown P, et al. Interferon and biologic signatures in dermatomyositis skin: specificity and heterogeneity across diseases. PLoS One 2012;7:e29161.
5. Tezak Z, Hoffman EP, Lutz JL, Fedczyna TO, Stephan D, Bremer EG, et al. Gene expression profiling in DQA1*0501+ children with untreated dermatomyositis: a novel model of pathogenesis. J Immunol 2002;168:4154–63.
6. O'Connor KA, Abbott KA, Sabin B, Kuroda M, Pachman LM. MxA gene expression in juvenile dermatomyositis peripheral blood mononuclear cells: association with muscle involvement. Clin Immunol 2006;120:319–25.
7. Greenberg SA, Higgs BW, Morehouse C, Walsh RJ, Kong SW, Brohawn P, et al. Relationship between disease activity and type 1 interferon- and other cytokine-inducible gene expression in blood in dermatomyositis and polymyositis. Genes Immun 2012;13:207–13.
8. Bilgic H, Ytterberg SR, Amin S, McNallan KT, Wilson JC, Koeuth T, et al. Interleukin-6 and type I interferon–regulated genes and chemokines mark disease activity in dermatomyositis. Arthritis Rheum 2009;60:3436–46.
9. Chen YW, Shi R, Geraci N, Shrestha S, Gordish-Dressman H, Pachman LM. Duration of chronic inflammation alters gene expression in muscle from untreated girls with juvenile dermatomyositis. BMC Immunol 2008;9:43.
10. Moneta GM, Marafon DP, Marasco E, Rosina S, Verardo M, Fiorillo C, et al. Muscle expression of type I and type II interferons is increased in juvenile dermatomyositis and related to clinical and histologic features. Arthritis Rheumatol 2019;71:1011–21.
11. Sarkar MK, Hile GA, Tsoi LC, Xing X, Liu J, Liang Y, et al. Photosensitivity and type I IFN responses in cutaneous lupus are driven by epidermal-derived interferon κ. Ann Rheum Dis 2018;77:1653–64.
12. Der E, Ranabothu S, Suryawanshi H, Akat KM, Clancy R, Morozov P, et al. Single cell RNA sequencing to dissect the molecular heterogeneity in lupus nephritis. JCI Insight 2017;2:e93009.
13. Shrestha S, Wershil B, Sarwark JF, Niewold TB, Philipp T, Pachman LM. Lesional and nonlesional skin from patients with untreated juvenile dermatomyositis displays increased numbers of mast cells and mature plasmacytoid dendritic cells. Arthritis Rheum 2010;62:2813–22.
14. Bohan A, Peter JB. Polymyositis and dermatomyositis (first of two parts). N Engl J Med 1975;292:344–7.
15. Hochberg MC, for the Diagnostic and Therapeutic Criteria Committee of the American College of Rheumatology. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus [letter]. Arthritis Rheum 1997;40:1725.
16. Tansley SL, Li D, Betteridge ZE, McHugh NJ. The reliability of immunoassays to detect autoantibodies in patients with myositis is dependent on autoantibody specificity. Rheumatology (Oxford) 2020;59:2109–14.
17. Tansley SL, Snowball J, Pauling JD, Lissina A, Kuwana M, Rider LG, et al. The promise, perceptions, and pitfalls of immunoassays for autoantibody testing in myositis. Arthritis Res Ther 2020;22:117.
18. Mecoli CA, Albayda J, Tiniakou E, Paik JJ, Zahid U, Danoff SK, et al. Myositis autoantibodies: a comparison of results from the Oklahoma Medical Research Foundation myositis panel to the Euroimmun research line blot. Arthritis Rheumatol 2020;72:192–4.
19. Espinosa-Ortega F, Holmqvist M, Alexanderson H, Storfors H, Mimori T, Lundberg IE, et al. Comparison of autoantibody specificities tested

by a line blot assay and immunoprecipitation-based algorithm in patients with idiopathic inflammatory myopathies [letter]. Ann Rheum Dis 2019;78:858–60.
20. Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, et al. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics 2003; 4:249–64.
21. Aran D, Hu Z, Butte AJ. xCell: digitally portraying the tissue cellular heterogeneity landscape. Genome Biol 2017;18:220.
22. Feng X, Wu H, Grossman JM, Hanvivadhanakul P, FitzGerald JD, Park GS, et al. Association of increased interferon-inducible gene expression with disease activity and lupus nephritis in patients with systemic lupus erythematosus. Arthritis Rheum 2006;54:2951–62.
23. Berthier CC, Tsoi LC, Reed TJ, Stannard JN, Myers EM, Namas R, et al. Molecular profiling of cutaneous lupus lesions identifies subgroups distinct from clinical phenotypes. J Clin Med 2019;8:1244.
24. Chiche L, Jourde-Chiche N, Whalen E, Presnell S, Gersuk V, Dang K, et al. Modular transcriptional repertoire analyses of adults with systemic lupus erythematosus reveal distinct type I and type II interferon signatures. Arthritis Rheumatol 2014;66:1583–95.
25. Tsoi L, Gharaee-Kermani M, Berthier CC, Nault T, Hile G, Estadt SN, et al. IL-18-containing five-gene signature distinguishes histologically identical dermatomyositis and lupus erythematosus skin lesions. JCI Insight 2020;5:e139558.
26. Saeed AI, Bhagabati NK, Braisted JC, Liang W, Sharov V, Howe EA, et al. TM4 microarray software suite. Methods Enzymol 2006; 411:134–93.
27. Baechler EC, Bauer JW, Slattery CA, Ortmann WA, Espe KJ, Novitzke J, et al. An interferon signature in the peripheral blood of dermatomyositis patients is associated with disease activity. Mol Med 2007; 13:59–68.
28. Wenzel J, Schmidt R, Proelss J, Zahn S, Bieber T, Tuting T. Type I interferon-associated skin recruitment of CXCR3+ lymphocytes in dermatomyositis. Clin Exp Dermatol 2006;31:576–82.
29. Caproni M, Torchia D, Cardinali C, Volpi W, del Bianco E, D'Agata A, et al. Infiltrating cells, related cytokines and chemokine receptors in lesional skin of patients with dermatomyositis. Br J Dermatol 2004; 151:784–91.
30. Wienke J, Enders FB, Lim J, Mertens JS, van den Hoogen LL, Wijngaarde CA, et al. Galectin-9 and CXCL10 as biomarkers for disease activity in juvenile dermatomyositis: a longitudinal cohort study and multicohort validation. Arthritis Rheumatol 2019; 71:1377–90.
31. Hausmann G, Herrero C, Cid MC, Casademont J, Lecha M, Mascaro JM. Immunopathologic study of skin lesions in dermatomyositis. J Am Acad Dermatol 1991;25:225–30.
32. Li DQ, Nair SS, Kumar R. The MORC family: new epigenetic regulators of transcription and DNA damage response. Epigenetics 2013; 8:685–93.
33. Sloan E, Orr A, Everett RD. MORC3, a component of PML nuclear bodies, has a role in restricting herpes simplex virus 1 and human cytomegalovirus. J Virol 2016;90:8621–33.
34. Ver LS, Marcos-Villar L, Landeras-Bueno S, Nieto A, Ortin J. The cellular factor NXP2/MORC3 is a positive regulator of influenza virus multiplication. J Virol 2015;89:10023–30.
35. Favre B, Plantard L, Aeschbach L, Brakch N, Christen-Zaech S, de Viragh PA, et al. SLURP1 is a late marker of epidermal differentiation and is absent in Mal de Meleda. J Invest Dermatol 2007;127:301–8.
36. Ibrahim AM, Sabet S, El-Ghor AA, Kamel N, Anis SE, Morris JS, et al. Fibulin-2 is required for basement membrane integrity of mammary epithelium. Sci Rep 2018;8:14139.
37. Hodgin JB, Borczuk AC, Nasr SH, Markowitz GS, Nair V, Martini S, et al. A molecular profile of focal segmental glomerulosclerosis from formalin-fixed, paraffin-embedded tissue. Am J Pathol 2010; 177:1674–86.
38. Ben-Moshe NB, Gilad S, Perry G, Benjamin S, Balint-Lahat N, Pavlovsky A, et al. mRNA-seq whole transcriptome profiling of fresh frozen versus archived fixed tissues. BMC Genomics 2018; 19:419.
39. Wimmer I, Troscher AR, Brunner F, Rubino SJ, Bien CG, Weiner HL, et al. Systematic evaluation of RNA quality, microarray data reliability and pathway analysis in fresh, fresh frozen and formalin-fixed paraffin-embedded tissue samples. Sci Rep 2018;8:6351.
40. Ono N, Kai K, Maruyama A, Sakai M, Sadanaga Y, Koarada S, et al. The relationship between type 1 IFN and vasculopathy in anti-MDA5 antibody-positive dermatomyositis patients. Rheumatology (Oxford) 2020;59:918.
41. Shao S, Tsoi LC, Sarkar MK, Xing X, Xue K, Uppala R, et al. IFN-γ enhances cell-mediated cytotoxicity against keratinocytes via JAK2/STAT1 in lichen planus. Sci Transl Med 2019;11:eaav7561.