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Identification of distinctive interferon gene signatures in different types of myositis

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Abstract

Objective

Activation of the type 1 interferon (IFN1) pathway is a prominent feature of dermatomyositis (DM) muscle and may play a role in the pathogenesis of this disease. However, the relevance of the IFN1 pathway in patients with other types of myositis such as the antisynthetase syndrome (AS), immune-mediated necrotizing myopathy (IMNM), and inclusion body myositis (IBM) is largely unknown. Moreover, the activation of the type 2 interferon (IFN2) pathway has not been comprehensively explored in myositis. In this cross-sectional study, our objective was to determine whether IFN1 and IFN2 pathways are differentially activated in different types of myositis by performing RNA sequencing on muscle biopsy samples from 119 patients with DM, IMNM, AS, or IBM and on 20 normal muscle biopsies.

Methods

The expression of IFN1- and IFN2-inducible genes was compared between the different groups.

Results

The expression of IFN1-inducible genes was high in DM, moderate in AS, and low in IMNM and IBM. In contrast, the expression of IFN2-inducible genes was high in DM, IBM, and AS but low in IMNM. The expression of IFN-inducible genes correlated with the expression of genes associated with inflammation and muscle regeneration. Of note, *ISG15* expression levels alone performed as well as composite scores relying on multiple genes to monitor activation of the IFN1 pathway in myositis muscle biopsies.

Conclusions

IFN1 and IFN2 pathways are differentially activated in different forms of myositis. This observation may have therapeutic implications because immunosuppressive medications may preferentially target each of these pathways.

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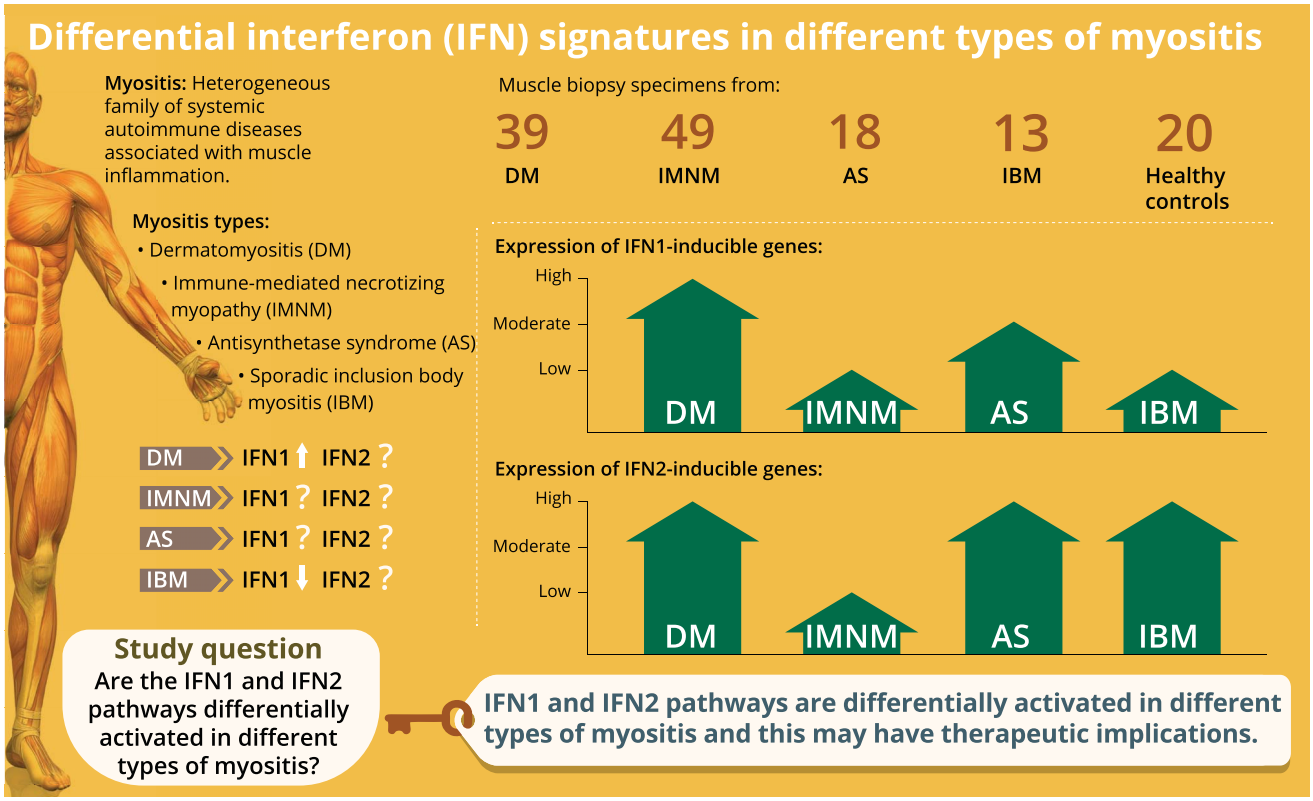
Glossary

AS = antisynthetase syndrome; **CK** = creatine kinase; **DM** = dermatomyositis; **FPKM** = fragments per kilobase of transcript per million mapped reads; **HMGCR** = 3-hydroxy-3-methylglutaryl-CoA reductase; **IBM** = inclusion body myositis; **IFN** = interferon; **IMNM** = immune-mediated necrotizing myopathy; **JAK** = Janus kinase; **MDA** = melanoma differentiation-associated protein; **MSA** = myositis-specific autoantibodies; **NXP** = nuclear matrix protein; **SRP** = signal recognition particle; **STAT** = signal transducer and activator of transcription; **TIF** = transcriptional intermediary factor.



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Myositis is a heterogeneous family of systemic autoimmune diseases that includes the following groups: dermatomyositis (DM), immune-mediated necrotizing myopathy (IMNM), the antisynthetase syndrome (AS), and sporadic inclusion body myositis (IBM).[1,2] Myositis-specific autoantibodies (MSAs) help define additional myositis subgroups with unique clinical phenotypes.[1] For example, anti–transcriptional intermediary factor (TIF) 1γ and anti-melanoma differentiation-associated protein (MDA) 5 autoantibodies are each found in patients with DM who have myositis and rash. However, whereas anti-TIF1γ –positive patients have a high risk of cancer and a low risk of lung involvement, anti-MDA5–positive patients have a relatively low risk of cancer and a high risk of lung involvement. Additional MSAs associated with distinct clinical phenotypes include those found in patients with DM (anti-Mi2 and anti–nuclear matrix protein [NXP] 2), IMNM (anti–signal recognition particle [SRP] and anti–3-hydroxy-3-methylglutaryl-CoA reductase [HMGCR]), and AS (anti-Jo1, anti-PL7, and anti-PL12).

The pathogenic mechanisms underlying the different types and subtypes of myositis are incompletely understood. However, the type 1 interferon (IFN) pathway has emerged as potentially relevant to DM pathogenesis.[3] Specifically, a marked overexpression of IFN1-inducible genes has been demonstrated in the muscle,[3] peripheral blood,[4,5] and skin[6] of patients with DM. Moreover, the expression levels of IFN1-inducible genes correlate with indicators of DM disease activity.[4,5]

Three different families of ligands may activate the IFN pathway by binding to cell surface receptors: type 1 IFNs (IFN1; including IFN-α and IFN-β), type 2 IFNs (IFN2; i.e., IFN-γ), and type 3 IFNs (IFN3; i.e., IFN-λ).[7] These proteins bind to their corresponding surface receptors, which, via the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathway, stimulate the expression of IFN-inducible genes.[8] Although there is considerable overlap between the sets of genes induced by the different types of IFN,[9,10] a handful of genes are specifically stimulated by either IFN1 (e.g., *ISG15*,[11,12]

IFI6,[13] and *MX1*[14]) or IFN2 (e.g., *GBP1*, *GBP2*,[10,15] and *PSMB8*[16]).

Prior studies have established the preferential activation of the IFN1 pathway in DM muscle.[3] However, activation of the IFN1 pathway has not been compared between patients with DM with different DM subtypes defined by the presence of different DM autoantibodies. Furthermore, the IFN1 pathway activation was found to be relatively low in IBM but has not been systematically explored in AS or IMNM.[3,17,18] Similarly, although IFN2 pathway activation has been implicated in IBM muscle,[19,20] activation of IFN2 pathways in muscle biopsies from patients with IMNM, AS, and IBM has not been systematically analyzed. In this study, we assessed activation of both IFN1 and IFN2 pathways by analyzing gene expression data from RNA sequencing performed on a large number of muscle biopsies from patients with DM, IMNM, AS, and IBM, as well as normal comparator tissue.

Methods

Patients, samples, and autoantibody testing

All the available muscle biopsies from patients enrolled in investigational review board–approved longitudinal cohorts of the NIH (Bethesda, MD), the Johns Hopkins Myositis Center (Baltimore, MD), the Clinic Hospital (Barcelona), and the Vall d'Hebron Hospital (Barcelona) were included in the study if the patients fulfilled IBM criteria according to Lloyd et al.[21] or had one of the following MSAs: anti-NXP2, -Mi2, -TIF1γ, -MDA5, -HMGCR, -SRP, or -Jo1. Autoantibody testing was performed as previously described for anti-HMGCR[22] and by line blot for the others (EUROLINE Myositis Profile 4). Patients were classified as having AS if they had autoantibodies against Jo-1; as being in the DM group if they had autoantibodies recognizing Mi2, NXP2, TIF1γ, or MDA5; and as being in the IMNM group if they tested positive for anti-SRP or anti-HMGCR autoantibodies. Creatine kinase (CK) levels and strength assessments obtained closest to the time of muscle biopsy were used to assess the clinical activity of the disease. Muscle strength was evaluated by the examining physician using the Medical Research Council scale. This scale was transformed to the Kendall 0-to-10 scale; the right- and left-side measurements for arm abduction and hip flexion strength were combined, and the average was used for calculations (possible range 0–10) as previously described.[23] Normal muscle biopsies were obtained from the Johns Hopkins Neuromuscular Pathology Laboratory (n = 10) and the Skeletal Muscle Biobank of the University of Kentucky (n = 10).

Standard protocol approvals and patient consents

This study was approved by the Institutional Review Boards at participating institutions, and written informed consent was obtained from each participant.

RNA sequencing

RNA sequencing was performed as previously described.[24] Briefly, RNA was prepared with TRIzol (Thermo Fisher Scientific). Libraries were prepared with the NeoPrep system according to the TruSeqM Stranded mRNA Library Prep protocol (Illumina, San Diego, CA) and sequenced with the Illumina HiSeq 2500 or 3000. Reads were aligned with STAR version 2.5[25]; the abundance of each gene was quantified with StringTie version 1.3.3[26]; and the differential gene expression was performed with DESeq2 version 1.20.0.[27] The Benjamini-Hochberg correction was used to adjust for multiple comparisons, and a corrected value of p (q value) ≤0.05 was considered statistically significant.

IFN genes and pathways

IFN pathway genes were collected from the Reactome biorepository (reactome.org/). General IFN-related genes and genes from the IFN1 and IFN2 pathways were merged into a single list. The 13 genes included in the previously proposed IFN score in myositis were also added to the list.[3] The expression of the genes of this list was analyzed in the different autoantibody and clinical myositis subsets.

Data analysis

Gene expression (fragments per kilobase of transcript per million mapped reads [FPKM]) values were log-transformed (logFPKM: log2[FPKM + 1]) for visualization purposes with the Python programming language and the Numpy, Pandas, and Seaborn packages. Correlation among continuous variables was measured with the Spearman ρ.

Data availability

Any anonymized data not published within the article will be shared by request from any qualified investigator.

Results

Ranking IFN-inducible gene expression in myositis muscle biopsies

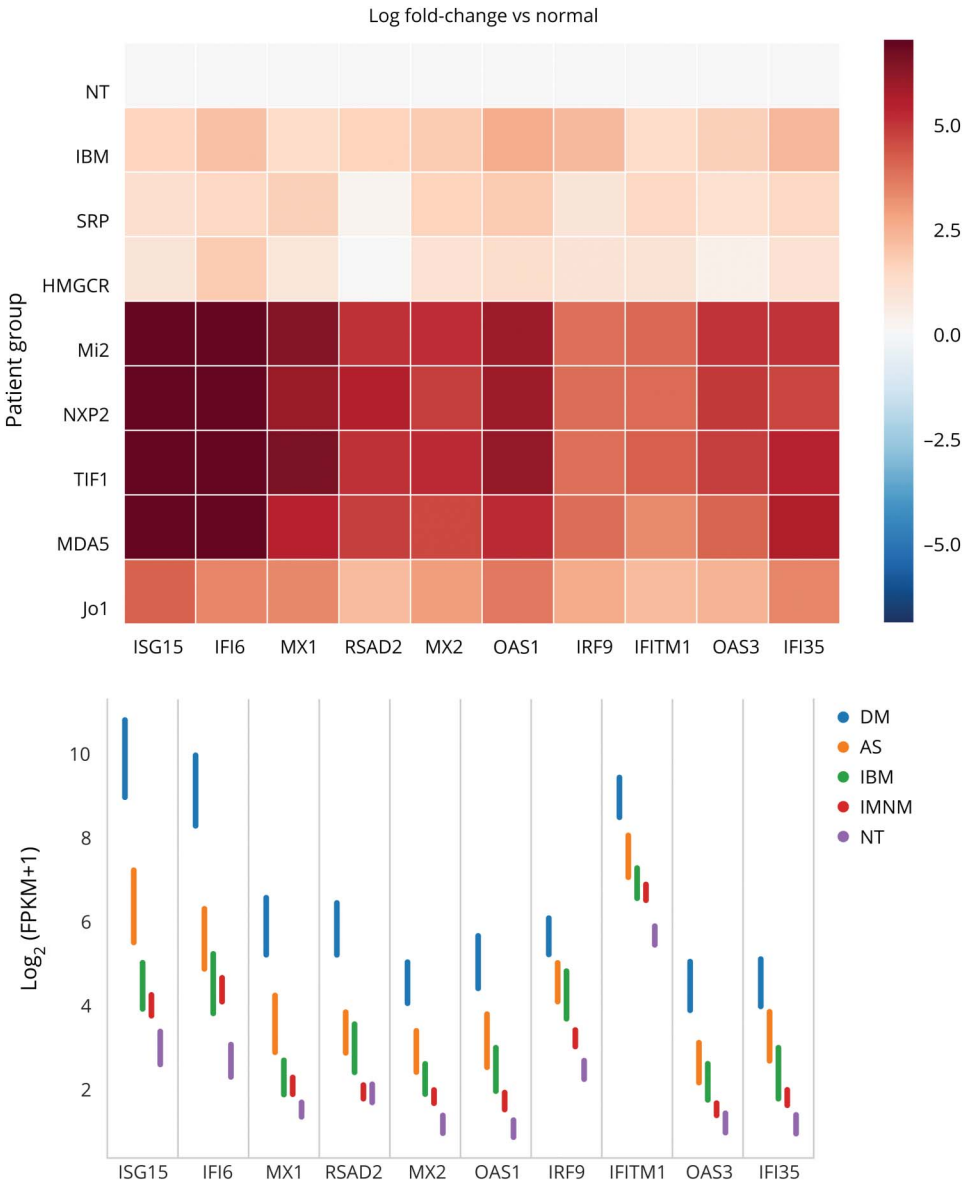
Muscle biopsy specimens were available from 119 myositis patients, including 39 with DM (11 anti-Mi2–, 12 anti-NXP2–, 11 anti-TIF1γ–, and 5 anti-MDA5–positive), 49 with IMNM (9 anti-SRP– and 40 anti-HMGCR–positive), 18 with anti-Jo1–positive AS, and 13 with IBM. Twenty normal muscle biopsy specimens were used as comparators. Expression levels of all genes were determined by RNA sequencing. The expression level of each gene from each major type of myositis (i.e., DM, IMNM, AS, and IBM) and each autoantibody group (i.e., anti-Mi2, -NXP2, -TIF1γ, -MDA5, -SRP, and -HMGCR) was compared to the expression level of the same gene in the comparator group. Differentially expressed genes were rank ordered by the degree of significance according to the adjusted p value. From among the complete list of differentially expressed genes, IFN-inducible genes were identified; the top 10 upregulated IFN-inducible genes for each group are listed in the table.

Table Expression levels of the top 10 most significantly expressed genes of the IFN pathway in the different clinical and serologic myositis subgroups

DM (All)				Mi2				NXP2				TIF1				MDA5			
Gene	**Order**	**FC**	**q Value**	**Gene**	**Order**	**FC**	**q Value**	**Gene**	**Order**	**FC**	**q Value**	**Gene**	**Order**	**FC**	**q Value**	**Gene**	**Order**	**FC**	**q Value**
ISG15	1	101	1.1E-91	*IFI6*	1	62	2.6E-43	*ISG15*	1	110	1.5E-55	*ISG15*	1	84	1.5E-47	*ISG15*	1	163	1.2E-51
IFI6	2	67	2.7E-80	*ISG15*	2	67	5.9E-43	*IFI6*	2	70	7.8E-48	*IFI6*	2	62	1.7E-43	*IFI6*	2	72	2.6E-37
MX1	3	29	2.6E-56	*MX1*	3	32	1.9E-33	*RSAD2*	3	23	2.1E-33	*MX1*	3	41	9.0E-39	*IFI35*	4	30	2.3E-24
RSAD2	4	18	1.2E-49	*OAS1*	4	25	9.7E-28	*MX1*	4	29	8.6E-33	*MX2*	4	22	6.9E-32	*PSMB8*	9	19	3.3E-20
MX2	5	17	2.5E-49	*MX2*	5	18	1.2E-27	*IFIT2*	5	22	5.6E-31	*OAS1*	5	29	3.8E-31	*IFI27*	10	18	2.2E-19
OAS1	6	23	4.0E-48	*RSAD2*	7	17	8.1E-26	*OAS1*	7	26	4.7E-30	*IFITM1*	6	12	2.7E-28	*MX2*	14	16	1.6E-18
IRF9	7	10	2.5E-43	*OAS3*	9	16	3.6E-25	*OAS3*	8	16	1.3E-26	*RSAD2*	7	18	2.0E-27	*IRF7*	15	15	1.7E-18
IFITM1	8	10	8.6E-43	*IFITM1*	11	9	1.5E-22	*HERC5*	9	25	3.4E-26	*IFI44*	9	18	4.1E-27	*RSAD2*	16	16	1.7E-18
OAS3	10	14	2.3E-41	*UBE2L6*	12	11	3.7E-22	*MX2*	10	15	4.8E-26	*ISG20*	11	27	2.2E-26	*MX1*	20	18	8.6E-18
IFI35	11	18	2.3E-41	*IRF9*	13	9	3.8E-22	*IRF9*	11	10	7.7E-26	*UBE2L6*	13	13	6.4E-26	*IRF9*	25	10	4.3E-17
AS (Jo1)				**IBM**				**IMNM (All)**				**SRP**				**HMGCR**			
Gene	**Order**	**FC**	**q Value**	**Gene**	**Order**	**FC**	**q Value**	**Gene**	**Order**	**FC**	**q Value**	**Gene**	**Order**	**FC**	**q Value**	**Gene**	**Order**	**FC**	**q Value**
PSMB8	1	13	2.6E-25	*GBP2*	2	7	1.3E-18	*IFI30*	40	7	4.0E-13	*IFI30*	52	7	1.9E-07	*NCAM1*	31	4	9.6E-14
GBP2	3	7	1.1E-22	*PSMB8*	4	9	1.7E-16	*NCAM1*	42	4	5.9E-13	*NCAM1*	67	4	1.1E-06	*IFI30*	39	7	1.3E-12
GBP1	9	12	9.8E-21	*GBP1*	5	11	3.5E-16	*SOCS3*	89	6	3.1E-10	*VCAM1*	159	4	3.1E-05	*SOCS3*	50	8	8.6E-12
IFI30	10	16	1.0E-20	*GBP5*	8	17	4.0E-15	*TRIM38*	148	3	7.4E-09	*ICAM1*	212	3	7.6E-05	*TRIM38*	67	3	7.5E-11
IRF1	14	8	5.0E-20	*GBP4*	17	6	3.4E-13	*VCAM1*	158	4	1.1E-08	*SOCS3*	220	5	8.2E-05	*GBP2*	143	3	6.8E-09
ISG20	35	11	2.1E-16	*STAT1*	24	6	1.5E-12	*GBP2*	191	3	2.4E-08	*GBP2*	289	3	2.2E-04	*VCAM1*	203	4	5.6E-08
ICAM1	36	7	2.2E-16	*B2M*	33	5	6.2E-12	*ICAM1*	197	3	3.0E-08	*TRIM38*	385	2	4.3E-04	*ICAM1*	227	3	8.3E-08
UBE2L6	41	6	7.9E-16	*CIITA*	47	6	1.7E-11	*MT2A*	312	3	4.6E-07	*MT2A*	407	3	5.8E-04	*MT2A*	301	4	4.2E-07
TRIM38	46	4	1.3E-15	*TRIM38*	52	4	2.3E-11	*TRIM62*	418	2	1.8E-06	*CD44*	425	3	6.3E-04	*TRIM8*	391	1	1.5E-06
GBP5	63	13	1.7E-14	*GBP6*	56	15	5.1E-11	*IRF5*	464	3	2.7E-06	*IRF5*	428	3	6.4E-04	*TRIM62*	450	2	2.8E-06

Abbreviations: AS = antisynthetase; [Mi2, NXP2, TIF1, MDA5] = DM autoantibody groups; DM = dermatomyositis; FC = fold-change; HMGCR = 3-hydroxy-3-methylglutaryl-CoA reductase; [Jo1] = AS autoantibody group; IBM = inclusion body myositis; IFN = interferon; IMNM = immune-mediated necrotizing myositis; [SRP, HMGCR] = IMNM autoantibody groups.
In each panel, the first column shows the gene name, the second column shows the rank of the gene relative to the whole list of differentially expressed genes (including non-IFN genes), the third column shows the FC, and the fourth column shows the Benjamini-Hochberg–adjusted *p* value (q value).

Figure 1 Expression of IFN1-inducible genes in myositis muscle biopsies



(A) Relative and (B) raw (95% confidence interval) expression levels of the type 1 interferon (IFN1)–inducible genes among the different clinical and serologic groups. AS = antisynthetase syndrome; DM = dermatomyositis; FPKM = fragments per kilobase of transcript per million mapped reads; IBM = inclusion body myositis; IMNM = immune-mediated necrotizing myositis; [Jo1] = AS autoantibody group; [Mi2, NXP2, TIF1, MDA5] = DM autoantibody groups; NT = normal biopsies; [SRP, HMGCR] = IMNM autoantibody groups.

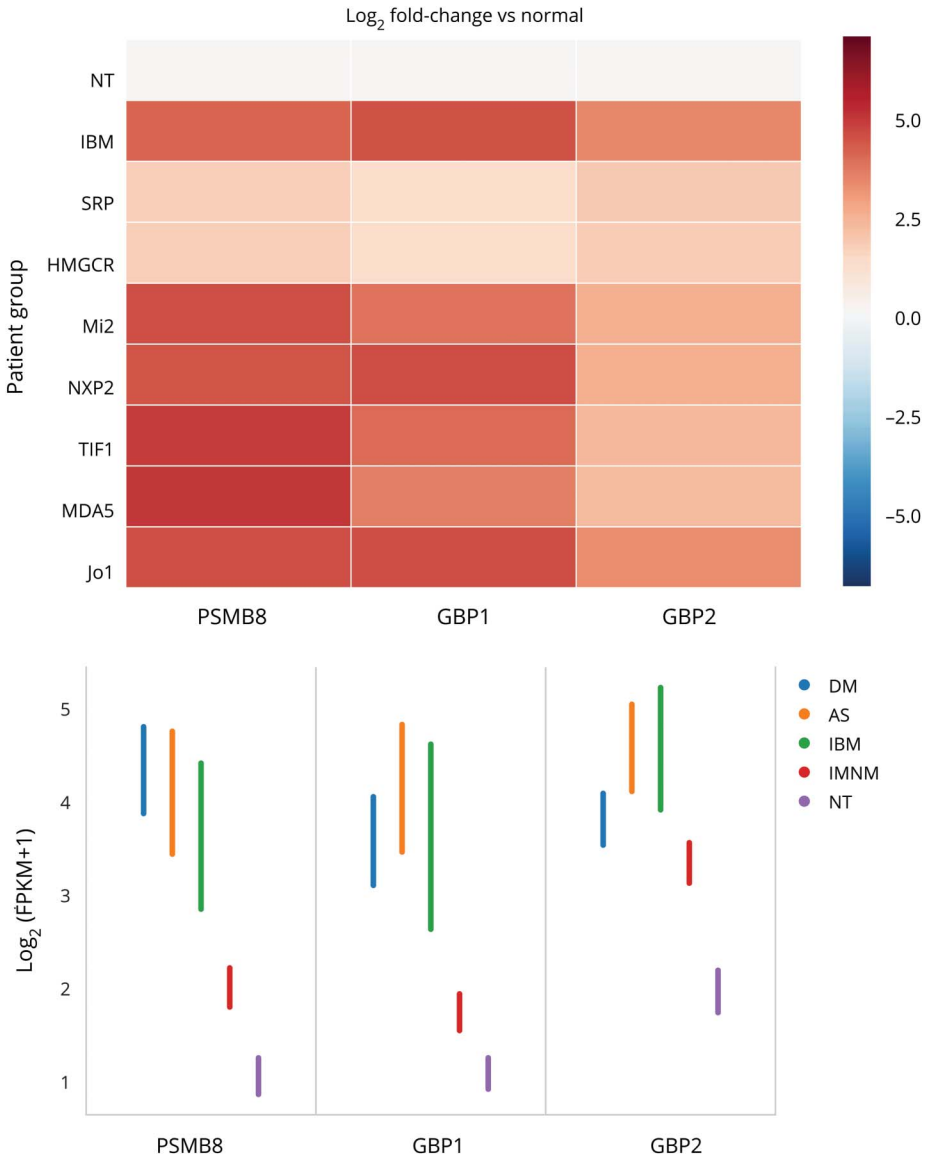
Expression levels of IFN1-inducible genes

The most significantly upregulated IFN-inducible genes in DM muscle biopsies were *ISG15*, *IFI6*, *MX1*, *RSAD2*, *MX2*, *OAS1*, *IRF9*, *IFITM1*, *OAS3*, and *IFI35* (table), all of which are preferentially induced by IFN1 (IFN-α/β signaling of reactome.org/).[11–14] Among all differentially expressed genes in DM (not just IFN-induced genes), these 10 IFN1-inducible genes were also among the most significantly upregulated (with all of them in the top 12 overall differentially expressed genes) (table).

The overexpression of IFN1-inducible genes was not restricted to DM muscle biopsies (figure 1). However, the magnitude of this increase was markedly different among the different myositis types. Specifically, IFN1-inducible genes were expressed at markedly elevated levels in DM, at moderately increased levels in AS, and at minimally increased levels in IBM and IMNM (figure 1). Using *ISG15* expression as an illustrative example, we found a 101-fold increase in DM (q value 1.1×10^{-91}), an 8.7-fold increase in AS (q value 1.8×10^{-13}), a 2.4-fold increase in IBM (q value 0.01), and a 1.8-fold increase in IMNM (q value 0.05) compared to comparator muscle biopsies (figure 1). In DM, *ISG15* expression was 11 times higher than in AS (q value 5.3×10^{-27}), 42 times higher than in IBM (q value 1.6×10^{-48}), and 56 times higher than in IMNM (q value 9.8×10^{-109}). Likewise, *ISG15* expression in AS was higher than in IBM and IMNM by 4 and 4.8 times, respectively (q values 0.001 and 3.8×10^{-11}).

We next analyzed the expression levels of IFN1-inducible genes among autoantibody subgroups. Interestingly, *ISG15*

Figure 2 Expression of IFN2-inducible genes in myositis muscle biopsies



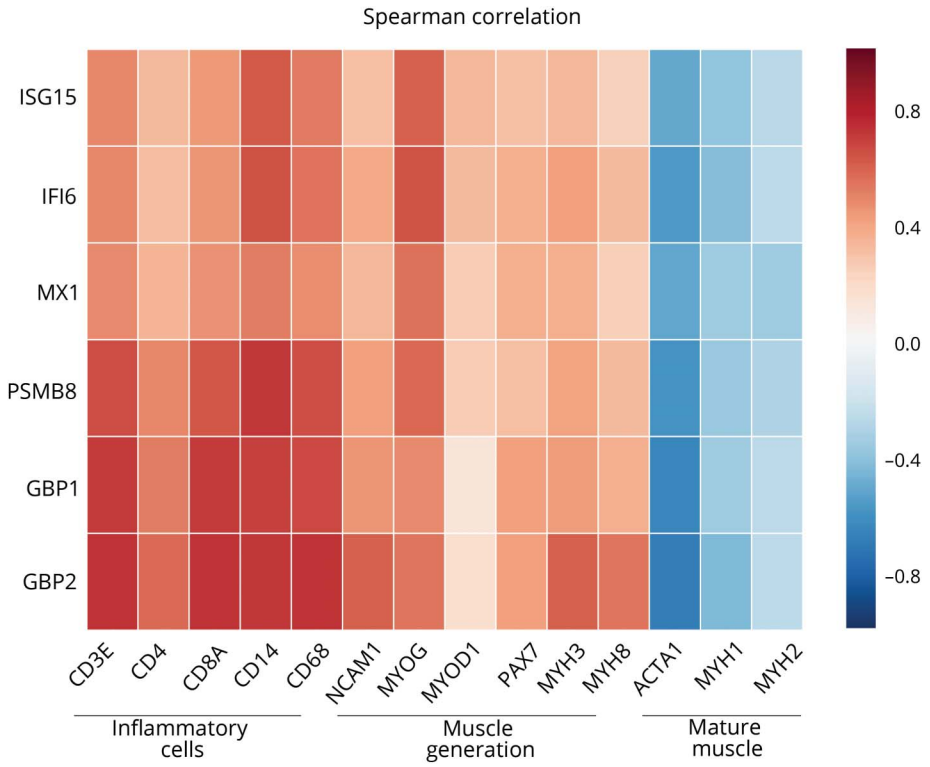
(A) Relative and (B) raw (95% confidence interval) expression levels of type 2 interferon (IFN2)–inducible genes among the different clinical and serologic groups. AS = antisynthetase syndrome; DM = dermatomyositis; FPKM = fragments per kilobase of transcript per million mapped reads; IBM = inclusion body myositis; IMNM = immune-mediated necrotizing myositis; [Jo1] = AS autoantibody group; [Mi2, NXP2, TIF1, MDA5] = DM autoantibody groups; NT = normal biopsies; [SRP, HMGCR] = IMNM autoantibody groups.

and *IFI6* were the most significantly upregulated genes in all DM autoantibody groups (i.e., anti-Mi2, anti-NXP2, anti-TIF1γ, and anti-MDA5) (table). In each DM autoantibody subgroup, these 2 genes were upregulated by at least 60-fold compared to healthy comparators (all q values $<1 \times 10^{-44}$) with no significant differences between the DM subgroups. Within IMNM, the expression of IFN1-inducible genes in those with anti-SRP autoantibodies was not significantly different compared to those with anti-HMGCR autoantibodies.

Expression levels of IFN2-inducible genes

The IFN2-specific genes *GBP1*, *GBP2*, and *PSMB8* were the 3 most significantly upregulated IFN-inducible genes in both AS and IBM. In addition, in muscle biopsies from both patients with AS and those with IBM, these 3 IFN2-inducible genes were within the top 10 most upregulated genes overall (table). Compared to comparators, the expression of IFN2-inducible genes was increased by 7- to 14-fold in IBM, AS, and DM biopsies (all q values $<1 \times 10^{-15}$) (table and figure 2). There were no significant differences between AS or IBM and DM except that *GBP2* had slightly higher expression levels in IBM (fold-change 1.7, q value 0.01) and AS (fold-change 1.8, q value 0.02) compared to DM. In contrast, the magnitude of IFN2-inducible gene overexpression in IMNM compared to comparators was much lower (*PSMB8* fold-change 2.5, q value 7.6×10^{-5}). Compared to IMNM, IFN2-inducible genes were expressed at higher levels in DM (fold-change 5.6, q value 9.5×10^{-25}), AS (fold-change 5.2, q value 2.1×10^{-12}), and IBM (fold-change 3.7, q value 9.2×10^{-7}). There were no significant differences in the expression of IFN2-inducible genes between the different autoantibody subgroups within IMNM or DM.

Figure 3 Correlation of IFN-inducible gene expression with expression of inflammatory cell and muscle regeneration genes



Correlation of type 1 and type 2 interferon (IFN)–inducible genes with the expression of genes related to T cells (CD3E, CD4, and CD8A), macrophages (CD14 and CD68), muscle regeneration (NCAM1, MYOG, MYOD1, PAX7), and adult muscle structural proteins (ACTA1, MYH1, MYH2).

Interestingly, the IFN2-inducible gene *IFI30* was 1 of the 2 most significantly upregulated IFN genes in both anti-SRP– and anti-HMGCR–positive patients with IMNM. Compared to normal biopsies, this gene showed a 7-fold increase in IMNM (q value 4×10^{-13}), a 16-fold-increase in DM (q value 5.7×10^{-32}), a 15.8-fold-increase in AS (q value 1×10^{-20}), and a 7-fold-increase in IBM (q value 2.1×10^{-9}) (table). Apart from *IFI30* gene expression, the relative magnitude of IFN-related genes among all differentially expressed genes in IMNM was modest compared to other types of myositis. In fact, the first-ranked IFN-inducible gene in IMNM was ranked 40th in the list of all differentially expressed genes. In contrast, the first-ranked IFN-inducible gene was also first among all differentially expressed genes in DM and AS and the second among all differentially expressed genes in IBM (table).

Expression levels of genes associated with inflammation and muscle regeneration

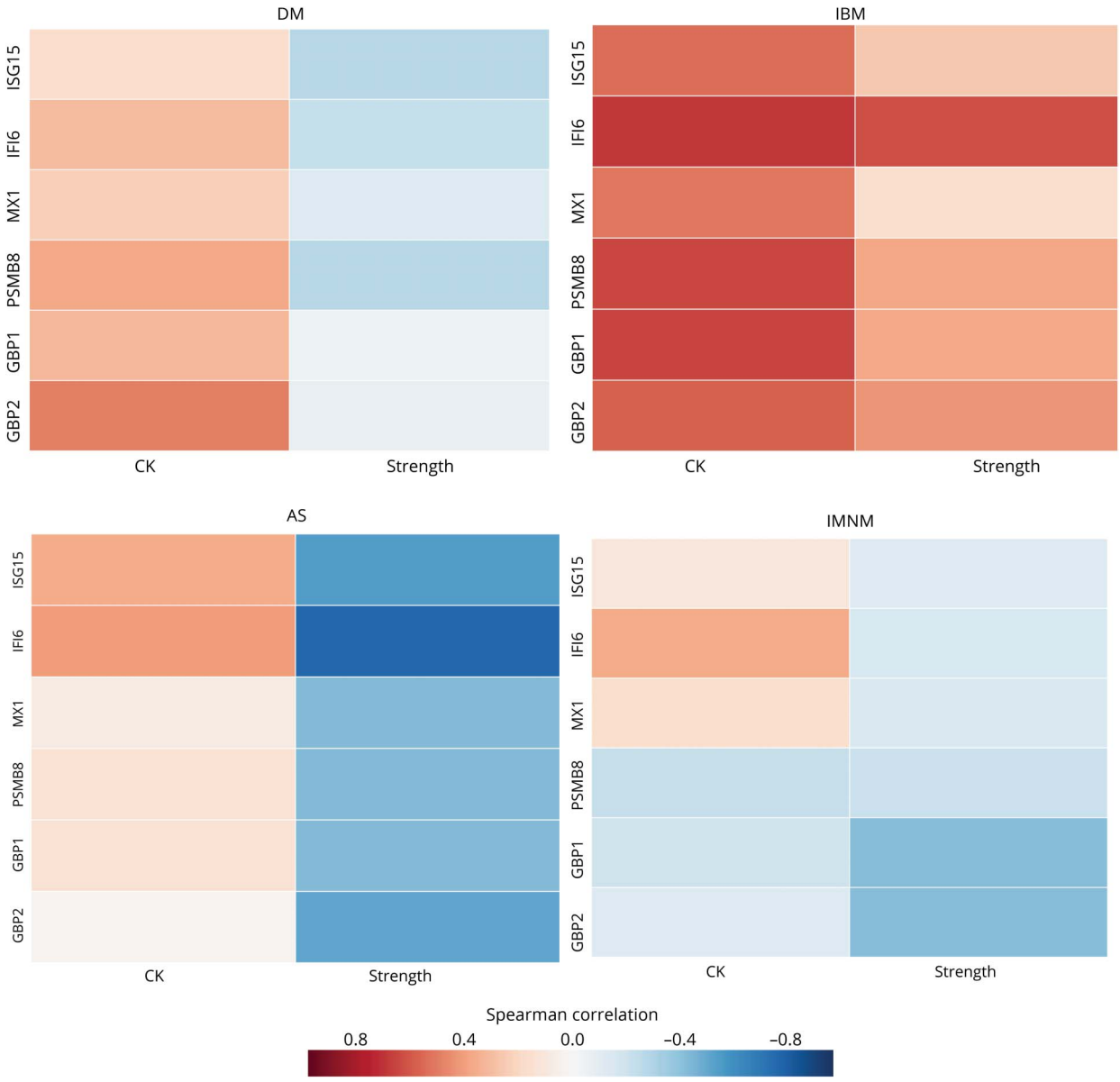
In each of the clinical and autoantibody subgroups studied, the expression of both IFN1- and IFN2-inducible genes was positively correlated with the expression of genes associated with inflammatory cells (T-cells [*CD3E*, *CD4*, *CD8A*] and macrophages [*CD14*, *CD68*]) and genes associated with muscle regeneration (*NCAM1*, *MYOG*, *MYOD1*, *PAX7*, *MYH3*, and *MYH8*) (all q values <0.05) (figure 3). Conversely, IFN-inducible genes were inversely correlated with mature-muscle structural proteins (*ACTA1, MYH1, and MYH2*) (all q values <0.05).

Strength measurements and CK levels obtained near the time of the muscle biopsy were available from 62 of the patients from Johns Hopkins (17 with DM, with 6 AS, 12 with IBM, and 27 with IMNM). Although there was a trend for patients with DM, AS, and IMNM with higher levels of IFN-inducible genes to have higher CK levels and decreased strength, this was not statistically significant (figure 4). However, patients with IBM with higher levels of IFN-inducible genes had significantly higher CK levels (all $p \leq 0.05$) and a nonsignificant trend toward being stronger than those with lower levels of IFN-inducible genes. Because patients with IBM often have relatively preserved muscle strength early in the course of the disease, we hypothesized that IFN-inducible gene expression might also be highest early during the course of the disease. Indeed, we found that patients with IBM with a shorter interval between onset of symptoms and muscle biopsy had higher expression levels of IFN-inducible genes (data not shown).

ISG15 gene expression compared to composite IFN scores to quantify the IFN signature

Several gene scoring systems have been proposed to measure the activation of the IFN pathway in myositis[28] and other autoimmune diseases.[29] Particularly, a score combining 13 IFN1-inducible genes has been used to study the relationship between IFN1-inducible gene expression and disease activity in blood of patients with DM and

Figure 4 Correlation of type 1 and type 2 interferon-inducible genes with the CK and strength in different types of myositis



AS = antisynthetase syndrome; CK = creatine kinase; DM = dermatomyositis; IBM = inclusion body myositis; IMNM = immune-mediated necrotizing myositis.

polymyositis.[28] We tried to test the utility of this score compared with simpler alternatives in myositis muscle biopsies.

First, we analyzed the correlations between the expression levels of the different IFN-inducible genes in all of the muscle biopsies included in the study. This revealed a high correlation between expression levels of each IFN-inducible gene with all the others (figure 5). Second, because *ISG15* was, overall, the most highly expressed IFN1-inducible gene, we correlated the raw expression levels of *ISG15* with the previously proposed IFN1 score. This analysis revealed an almost perfect correlation between *ISG15* expression levels alone and the 13-gene composite IFN1 score (Spearman $\rho = 0.94$, $p = 1.5 \times 10^{-64}$, figure 6A), suggesting that it may be unnecessary to use a more complex scoring system to measure IFN1 pathway activation levels in myositis muscle.

The expression levels of the IFN2-inducible genes *PSMB8*, *GBP1*, and *GBP2* were highly correlated with each other (figure 5). However, the association of these IFN2-inducible genes with the 13-gene IFN1 gene score was restricted to DM. For example, *PSMB8* expression levels correlated well with the composite IFN1 gene score in patients with DM but not in patients with AS or IBM (figure 6B). This suggests that IFN1-inducible gene activation may correlate with IFN2 activation in DM but not in AS or IBM.

Figure 5 Spearman correlation of the different type 1 and type 2 interferon-inducible genes in all the biopsies included in the study

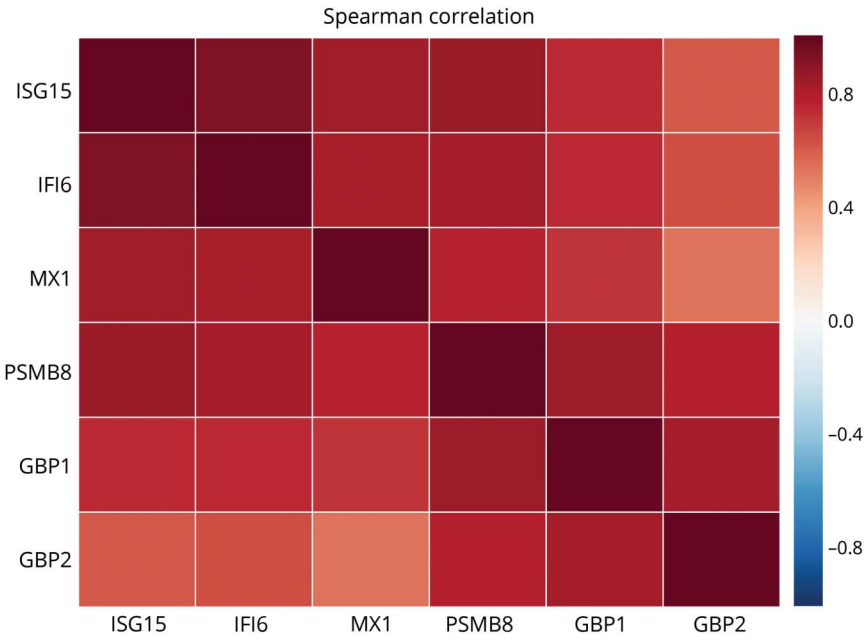
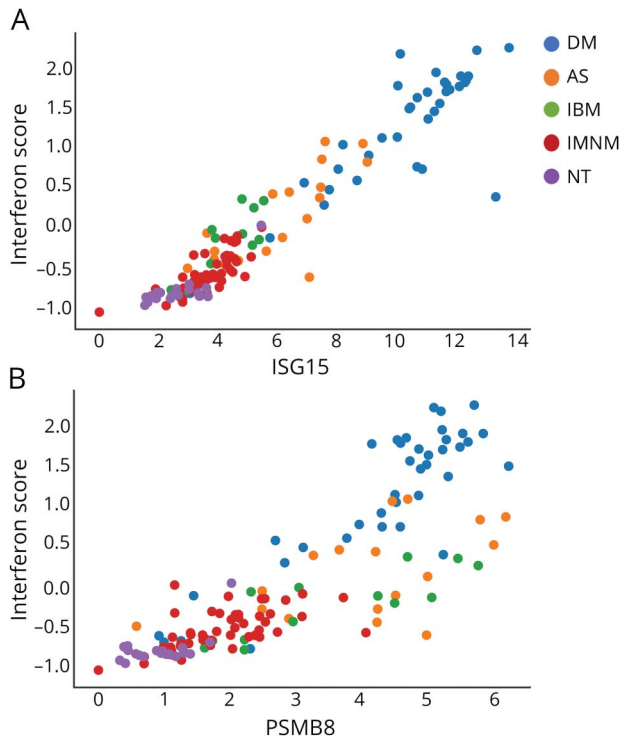


Figure 6 *ISG15* or PSMB8 expression vs composite IFN1-inducible gene scores



Correlation of the expression level (log_2[FKPM+1]) of (A) *ISG15* and (B) PSMB8 with the previously proposed 13-gene type 1 interferon (IFN1) score. AS = antisynthetase syndrome; DM = dermatomyositis; FPKM = fragments per kilobase of transcript per million mapped reads; IBM = inclusion body myositis; IMNM = immune-mediated necrotizing myositis; NT = normal biopsies.

Discussion

In this study, using RNA sequencing data from a large number of myositis and comparator muscle biopsies, we have established that the IFN1 pathway is activated not only in patients with DM, as previously described,[3–6] but also in patients with AS, IMNM, and IBM. Quantitatively, the IFN1 pathway was most upregulated in DM, with intermediate activation of the pathway in AS and lower levels of activation in IBM and IMNM. We also used RNA sequencing data to study activation of the IFN2 pathway, demonstrating robust activation in AS, IBM, and DM but not in IMNM. We were also able to show that activation of the IFN pathway was associated with increased expression of inflammatory cell and muscle regeneration genes. The correlation between this activation and muscle weakness and CK levels, however, did not reach statistical significance.

Interestingly, different collections of IFN-inducible genes were most prominently upregulated in the different groups. For example, the IFN1 genes *ISG15*, *IFI6*, and *MX1* were the most upregulated IFN-inducible genes in DM. In contrast, *IFI30*, *NCAM1*, and *SOCS3* were the most upregulated IFN1-inducible genes in patients with IMNM. Of note, the IFN2 genes *PSMB8*, *GBP2*, and *GBP1* were the most upregulated IFN-inducible genes in both patients with AS and patients with IBM, underscoring the prominence of the IFN2 pathway in these 2 diseases.

It is well established that patients with DM with different myositis autoantibodies have unique clinical manifestations. In

fact, there are differences in muscle biopsy features between patients with DM with different autoantibodies.[30] For example, half of the muscle biopsies from anti-Mi2–positive patients with DM include examples of lymphocytes surrounding and invading healthy muscle fibers; this histopathologic feature was never seen in patients with DM with anti-NXP2 autoantibodies. Despite these histopathologic differences, the IFN gene signature was remarkably similar among patients with DM with different myositis autoantibodies. Indeed, *ISG15* and *IFI6* were the top 2 IFN-inducible genes in each of the serologically defined DM subgroups, and *MX1* and *MX2* were present among the top 10 IFN-inducible genes in each DM subgroup. These findings suggest that, at least with regard to activation of IFN pathways in the muscle, the different autoantibody subgroups of DM are more alike than different. Similarly, in patients with IMNM with either anti-SRP or -HMGCR autoantibodies, *IFI30*, *NCAM1*, *VCAM1*, *ICAM1*, *SOC3*, *GBP2*, and *MT2A* were among the top 10 IFN-inducible genes. We did not have a sufficient number of biopsies from patients with anti-PL7, anti-PL12, or other non-Jo1 antisynthetase autoantibodies to determine whether these serologic subgroups of the AS share a similar IFN gene signature pattern.

Some investigators have shown that immunostaining muscle biopsies for specific IFN-inducible proteins can be used to distinguish between different types of myositis. For example, DM but not AS muscle biopsies stain positive for MxA (*MX1*)[31] or RIG-I (*DDX58*),[32] both IFN1-inducible genes. Our RNA sequencing data, which show higher expression levels of these genes in DM than in AS (MX1 fold-change 4.7 and RIG-1 fold-change 3.3, both q values $<5 \times 10^{-9}$), are consistent with this observation. In addition, *ISG15* overexpression is an established feature in muscle biopsies from patients with DM and perifascicular atrophy.[17] Accordingly, we found a marked preferential overexpression of *ISG15* in patients with DM (*ISG15* fold-change compared to comparator biopsies 101, q value 1.1×10^{-91}).

We also found that *ISG15* expression levels alone can be used to reliably quantify the activation of the IFN1 pathway in myositis muscle biopsies. In fact, measuring *ISG15* levels was equivalent to a composite score derived from measuring expression levels of 13 different IFN1-inducible genes, which is concordant with previous data showing the marked specificity of *ISG15* muscle transcript measurements for DM with perifascicular atrophy.[17] We also noted that both *ISG15* expression levels and the previously proposed composite IFN1 scores were associated with activation of the IFN2 pathway in DM but not in IBM or AS.

This study has several limitations. For example, some less common autoantibody groups (e.g., non–anti-Jo1 AS) could not be included due to an insufficient number of biopsies. In addition, we had relevant CK and strength information only for muscle biopsies obtained at Johns Hopkins, which may have limited our ability to show significant associations between IFN pathway activation and markers of clinical disease activity such as strength and CK levels.

This study demonstrates that DM muscle biopsies are characterized by high levels of both IFN1- and IFN2-inducible genes. In contrast, biopsies from patients with AS and IBM reveal gene expression patterns consistent with prominent IFN2 activation. Finally, RNA sequencing analysis reveals that IMNM biopsies show relatively low activation of the IFN pathway. These findings are consistent with recent case series suggesting the efficacy of JAK/STAT inhibitors in patients with DM.[33–37] They also suggest that these agents may be effective in patients with AS or IBM. However, the relatively modest activation of IFN pathways in IMNM does not provide compelling evidence to support the use of JAK/STAT inhibitors in this patient population.

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Disclosure

The authors report no disclosures relevant to the manuscript. Go to Neurology.org/N for full disclosures.

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Maria Casal-Dominguez, MD, PhD	NIH, Bethesda, MD	Author	Major role in the acquisition of data; revised the manuscript for intellectual content

Appendix *(continued)*

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