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Contribution of Rare Genetic Variation to Disease Susceptibility in a Large Scandinavian Myositis Cohort

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Objective. Idiopathic inflammatory myopathies (IIMs) are a heterogeneous group of complex autoimmune conditions characterized by inflammation in skeletal muscle and extramuscular compartments, and interferon (IFN) system activation. We undertook this study to examine the contribution of genetic variation to disease susceptibility and to identify novel avenues for research in IIMs.

Methods. Targeted DNA sequencing was used to mine coding and potentially regulatory single nucleotide variants from ~1,900 immune-related genes in a Scandinavian case–control cohort of 454 IIM patients and 1,024 healthy controls. Gene-based aggregate testing, together with rare variant– and gene-level enrichment analyses, was implemented to explore genotype–phenotype relations.

Results. Gene-based aggregate tests of all variants, including rare variants, identified *IFI35* as a potential genetic risk locus for IIMs, suggesting a genetic signature of type I IFN pathway activation. Functional annotation of the *IFI35* locus highlighted a regulatory network linked to the skeletal muscle–specific gene *PTGES3L*, as a potential candidate for IIM pathogenesis. Aggregate genetic associations with *AGER* and *PSMB8* in the major histocompatibility complex locus were detected in the antisynthetase syndrome subgroup, which also showed a less marked genetic signature of the type I IFN pathway. Enrichment analyses indicated a burden of synonymous and noncoding rare variants in IIM patients, suggesting increased disease predisposition associated with these classes of rare variants.

Conclusion. Our study suggests the contribution of rare genetic variation to disease susceptibility in IIM and specific patient subgroups, and pinpoints genetic associations consistent with previous findings by gene expression profiling. These features highlight genetic profiles that are potentially relevant to disease pathogenesis.

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INTRODUCTION

Idiopathic inflammatory myopathies (IIMs), also collectively known as myositis, are a heterogeneous group of rare chronic inflammatory autoimmune diseases with a multifactorial etiology (1). Inflammation mainly affects the skeletal muscle, but it can also occur in the lungs, skin, joints, heart, and gastrointestinal tract. IIMs can be categorized into major subgroups, including polymyositis (PM) and dermatomyositis (DM), which are defined by distinct clinical and histopathologic features (2–4). Taking serologic profiles into account, 2 additional subgroups have been identified: antisynthetase syndrome (ASyS), of which the anti–Jo-1–positive subgroup is the most prevalent, and immune-mediated necrotizing myopathy (5). IIMs are characterized by an interferon (IFN) signature, with the type I IFN and type II IFN pathways differentially activated in muscle tissues in different disease subgroups (6,7). While transcriptomic studies on muscle tissue have provided substantial evidence of distinctive IFN gene signatures in different types of IIMs, genetic studies have not achieved the same level of resolution mainly due to the small sample size of IIM patient cohorts and of clinically and serologically defined disease subgroups.

In recent years, classical genetic association studies have implicated, in addition to the HLA region, noncoding potentially regulatory common variation at several risk loci in IIM and specific disease subgroups (8,9). For complex disorders such as IIM, rare variation has also been implicated as contributing to the genetic architecture of the disease (1). However, for IIM, comprehensive studies investigating this class of genetic variation are lacking. Large-scale targeted sequencing of patient cohorts now represents a valid and accessible approach to explore genetic variation at the lower range of allele frequency (10). Moreover, the tailored implementation of statistical algorithms based on the aggregation of rare variants into specific analysis units allows for accurate modeling of these variants, thus resulting in increased discovery power (11).

Here, we present a next-generation sequencing-based study of IIM, designed to explore the contribution of rare (minor allele frequency [MAF] <0.01) single nucleotide variants (SNVs) to disease susceptibility and to identify novel candidate loci. We performed targeted DNA sequencing of coding and potentially regulatory regions of a set of ~1,900 immune-related genes in a Scandinavian IIM case–control cohort. We implemented gene-based aggregate testing in the whole patient cohort to maximize the power to explore the genetic underpinnings of the disease. This approach was also extended to examine the genetic background of subgroups of clinically and serologically distinct patients. To further characterize the genetic landscape of IIMs, we performed comprehensive functional annotation of candidate loci, as well as investigating differential allelic burden for specific rare variant categories and the genes potentially driving that burden. In summary, we comprehensively evaluated genetic variation underlying IIMs for the full allele frequency spectrum to gain novel insights into disease susceptibility and biology.

PATIENTS AND METHODS

Study patients. The Scandinavian IIM discovery cohort consisted of patients recruited in Sweden, Denmark, and Norway, as well as Swedish and Norwegian healthy blood donors and population controls. The Bohan and Peter criteria (2,3) were applied for the diagnosis of possible, probable, or definite PM or DM, and the Connors criteria (12) were used to evaluate ASyS. Patients with inclusion body myositis (IBM) were excluded (13). After quality control, the final data set included 454 IIM patients and 1,024 control samples. Patient characteristics and serologic profiles are summarized in Supplementary Tables 1 and 2 (http://onlinelibrary.wiley.com/doi/10.1002/art.41929/abstract). For detailed descriptions of the methods, see Supplementary Methods (available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41929/abstract). See Appendix A for a list of members of the DISSECT Consortium and the ImmunoArray Development Consortium.

Ethics approval. All subjects provided informed consent to participate in the study, and the study was approved by the regional ethics board in Uppsala (Dnr 2015/450 and 2016/155) and by the local research ethics committee for the UK Myositis Network (MREC North West [Haydock Park], 98/8/86).

Targeted DNA sequencing and bioinformatics analysis. Targeted DNA sequencing was performed on the same technology platform on the Scandinavian IIM case–control cohort using an Illumina HiSeq 2500. An average sequencing read depth of >30× per sample was achieved. The targeted array comprised ~1,900 genes involved in immune function and immunologic diseases, for which both coding and potentially regulatory sequences were captured, as outlined elsewhere (14). As detailed in Supplementary Figure 1 (http://onlinelibrary.wiley.com/doi/10.1002/art.41929/abstract), bioinformatics analyses comprised mapping, genotype calling, variant-based and individual-based quality control (including relatedness and ancestry estimation [Supplementary Figure 2]), and generated a high-quality data set containing 264,956 SNVs characterized by high call rates (i.e., ≥98%).

Genetic association analyses. Single-marker association analysis of common SNVs (MAF ≥0.05) was performed with PLINK version 1.9 using a logistic regression model and assuming additive effects. Following classical HLA alleles imputation using SNP2HLA, the same statistical model was applied for conditional analysis on *DRB1*0301*. Aggregate association testing using all SNVs and analysis units defined as RefSeq gene body coordinates (+2 Kb upstream and +2 Kb downstream) was first

performed using SKAT-O (15). For this analysis, we used a weighted kernel with default settings and higher weight for rare variants. Employing the same analysis units, gene-based aggregate testing with all SNVs and with the inclusion of metrics defining variant functional potential was implemented using GenePy, version 1.2 (16). Gene- and region-based annotations were set according to Annovar, and the functional metrics included in the algorithm was based on the Combined Annotation Dependent Depletion (CADD) version 1.3 annotation (17). The gene score distributions between the patient and the control groups, including relevant patient subgroups (Supplementary Tables 1 and 2, http://onlinelibrary.wiley.com/doi/10.1002/art.41929/abstract), were modeled through logistic regression using the glm function with the "binomial" parameter in R.

All implemented association analyses and statistical models incorporated the most significant population principal components (PCs; PC1, PC2, and PC3) (Supplementary Figure 3, http://onlinelibrary.wiley.com/doi/10.1002/art.41929/abstract) and sex as covariates. The population PCs were generated in PLINK version 1.90 (--pca) after excluding long-range linkage disequilibrium (LD) regions (18), variants with MAF <0.05, and variants in LD ($r^2 > 0.2$). The significance of the generated PCs was assessed by evaluating the corresponding overall eigenvalues in a scree plot and identifying where the characteristic plateau occurs. PCs were also projected onto a 2-dimensional scatter plot to assess their convergence to a homogenous structure without any apparent cluster or batch effect (Supplementary Figure 3). The correlation between disease status and sex distribution was evaluated using Fisher's exact test, which identified a statistically significant sex imbalance between IIM patients and control subjects ($P < 1 \times 10^{-16}$). Statistical significance was based on Bonferroni and false discovery rate (FDR) corrections ($\alpha = 0.05$).

Variant annotation and enrichment analyses. Rare SNVs (MAF <0.01) were partitioned into relevant functional categories, for which the allelic burden between IIM patients and controls was evaluated globally using Mann-Whitney U test and at the gene level using GenePy. For the rare variant functional categories showing a statistically significant increased allelic burden in patients, we performed gene set enrichment analyses using the corresponding representative set of variants. An exhaustive variant functional annotation was performed using publicly available database resources and software, such as ENCODE, Roadmap Epigenomics, GTEx, and SnpEff (19–22).

Data generation and gene-based aggregate testing results in the replication cohort. The replication cohort consisted of IIM patients only, recruited through the UK Myositis Network (n = 397). Patients met the Bohan and Peter criteria for probable or definite PM or DM, or the Connors criteria for ASyS, and no patients had IBM. We did not have matched UK controls available for targeted sequencing. The UK IIM patients were subjected to sequence capture, targeted DNA sequencing, and subsequent bioinformatic analysis of the generated reads following the same procedures described for the discovery data set. An average individual sequencing read depth of >30× was achieved. The same criteria were also used for the individual- and variant-based quality controls. The resulting quality-controlled data set composed of patients only was employed as a replication data set (n = 348) (Supplementary Tables 2 and 3, http://onlinelibrary.wiley.com/doi/10.1002/art.41929/abstract).

Importantly, we explored the possibility of including out-of-study matched controls by utilizing the UK10K Avon Longitudinal Study of Parents and Children (ALSPAC) data set (23), which includes low read depth (~7×) whole-genome sequences of 1,927 control individuals from the UK. Despite implementing extensive and rigorous quality control procedures for data set harmonization, the substantially lower read depth of the ALSPAC controls prevented an unbiased and equivalent calling of rare variant alleles compared to the IIM UK patients. This precluded use of the ALSPAC cohort as a control population for the aggregate testing results replication. An alternative out-of-study source of matched controls could be the UK Biobank, for which genotype array-based and recently generated whole exome sequencing data (but no whole genome sequencing) are currently available (24,25). Nevertheless, neither of these technologies can provide full capture of the whole spectrum of rare variation detectable by our targeted array. For these reasons, to replicate the rare variation analysis results, we implemented an alternative method, intended to be fully unbiased and free from any confounders derived from systematic biases or technical artefacts, especially when focusing on rare variation.

First, for all UK patients, we generated CADD-based gene scores using GenePy. For all phenotypic contrasts, we then compared the distribution of the GenePy-derived gene scores for the patients in the discovery and replication cohorts using logistic regression, including the data set–specific most significant population PCs (as previously described) and sex as covariates. Assuming that a difference in the score of the same genes between the 2 groups of patients is likely to reflect ancestry or technical dissimilarities, we excluded genes that showed a significant difference ($P < 0.05$). This is a very conservative and low-sensitivity approach, which may also remove potentially relevant genes whose difference in score underlies a true involvement in disease etiology. However, by excluding these significantly different genes, we ensure harmonization and homogeneity of the data and minimize the risk of reporting erroneous results. Finally, we considered genes to be replicated if they matched the significantly associated genes (5% FDR) resulting from the GenePy aggregate analyses in the discovery data set.

Data availability. The data sets generated and/or analyzed in the present study are not publicly available due to the

inclusion of information that could compromise research participant privacy and consent. However, they are available from the corresponding authors upon reasonable request and on a collaborative basis.

RESULTS

In this study, we performed targeted DNA sequencing of coding and regulatory regions of immunologic genes to collectively explore the contribution of genetic variants to IIM. Outlines of these analyses can be found in Supplementary Figure 4 (http://onlinelibrary.wiley.com/doi/10.1002/art.41929/abstract).

Confirmation of HLA as a major risk locus in IIM by single-variant association analysis. In a single-variant association analysis performed on 69,110 common variants, an experiment-wide statistical threshold of $P < 7.2 \times 10^{-7}$ was exclusively reached by variants located in the major histocompatibility complex (MHC) region. The strongest association was detected for an intronic variant of *HLA–DQA1* (rs9272729-A; raw $P = 3.1 \times 10^{-31}$; odds ratio 4.0 [95% confidence interval 3.2–5.1]). No loci outside the MHC region exceeded the suggestive statistical significance threshold for single marker analysis ($P = 1 \times 10^{-5}$) (Supplementary Figure 5, http://onlinelibrary.wiley.com/doi/10.1002/art.41929/abstract). After analysis conditioning on rs9272729, no additional statistically significant signals

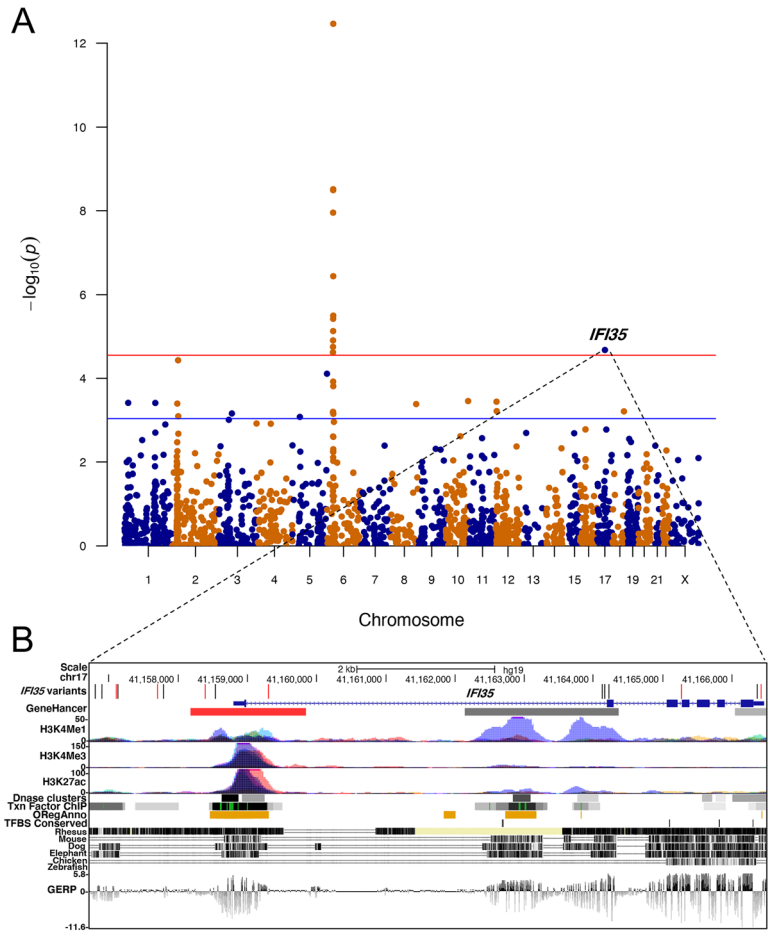

Figure 1. GenePy case–control gene-based association analysis of idiopathic inflammatory myopathies (IIMs) and "zoom in" of the associated locus. **A**, Manhattan plot showing the results of case–control gene-based association analysis (using GenePy) for IIM. Every point represents a gene region, with their associated *P* values plotted against chromosome location. Red line indicates the Bonferroni statistical significance threshold ($P = 2.9 \times 10^{-5}$), and blue line represents the 5% false discovery rate ($P = 8.7 \times 10^{-4}$). **B**, UCSC genome browser view of *IFI35*, i.e., the non–major histocompatibility complex, Bonferroni-corrected associated gene in the GenePy case–control aggregate association test for IIM. *IFI35* variants detected and tested in this study are indicated and are color-coded red if they represent GTEx expression quantitative trait loci. The *IFI35* locus overlaps with strong regulatory marks, including GeneHancer promoter (red bar) and enhancer (grey bars) regions, ENCODE histone modifications (H3K4Me1, H3K4Me3, H3K27ac), DNase I hypersensitivity sites (DNase clusters), transcription factor binding sites (Txn Factor ChIP), open regulatory region associated with active gene expression (ORegAnno), conserved transcription factor binding sites (TFBS conserved), as well as region of evolutionary constraint across 100 vertebrates and defined by Genomic Evolutionary Rate Profiling rejection submission scores.

remained (Supplementary Figure 6, http://onlinelibrary.wiley.com/doi/10.1002/art.41929/abstract). The same effect was obtained using imputed classical HLA alleles and conditioning on the well-established *DRB1*0301* IIM risk allele.

Suggestion of *IFI35* as a novel IIM genetic risk locus via aggregate association testing. To test for association between IIM and variants covering the full spectrum of allele frequency, as well as aggregating in genes, we first performed gene-based analysis using the SKAT-O software (15). In this gene-based analysis between all patients and controls, only 2 MHC genes were significantly associated with IIM after Bonferroni correction: *NOTCH4* (Bonferroni-adjusted $P = 0.022$) and *MICB* (Bonferroni-adjusted $P = 0.035$).

In addition to a classical aggregate test, we performed a gene-based analysis incorporating variant functional potential using GenePy (16). This analysis focused on the 1,737 gene units derived from the integrated Annovar variant annotation and constituted by a minimum of 2 genetic variants for which a CADD score was available. These 1,737 gene units were also used for stringent multiple-testing Bonferroni correction. Consistent with its sample size–independent increased discovery potential, this algorithm detected statistically significant associations with IIM exceeding Bonferroni correction in the MHC region (11 genes, top hit *HLA–DQA1*; Bonferroni-adjusted $P = 5.98 \times 10^{-10}$), and in *IFI35* on chromosome 17 (Bonferroni-adjusted $P = 0.036$) (Supplementary Table 4, http://onlinelibrary.wiley.com/doi/10.1002/art.41929/abstract). The association with *IFI35* derives from 16 variants spanning ~10 Kb (hg19-chr17:41,156,792–41,166,417) (Figure 1 and Supplementary Table 5, http://onlinelibrary.wiley.com/doi/10.1002/art.41929/abstract). A Kruskal-Wallis test for each of the *IFI35* rare variants (MAF <0.01) demonstrated that the difference in *IFI35* gene score distribution was not driven by any country-of-origin–specific subset of patients or controls showing unique population-specific alleles. With a less conservative statistical threshold (i.e., 5% FDR), we detected significant associations with non-MHC genes, including *PRDX3*, *SLAMF1*, *ZFAT*, and *PTPN6*, which further showed evidence of replication in downstream analyses (Supplementary Table 4).

Functional annotation of the *IFI35*-associated locus suggests *PTGES3L* as a potentially novel candidate gene for IIM. To functionally annotate the *IFI35* locus (the only non-MHC gene significant after Bonferroni correction), we used the GTEx Portal to investigate whether any of the variants contributing to the aggregate association overlapped with expression quantitative trait locus (eQTL) markers ($P < 1 \times 10^{-4}$) in relevant tissues. Of the 16 analyzed variants, 7 variants colocalized with eQTLs for *IFI35* and/or for other genes in the vicinity (Supplementary Tables 6 and 7, http://onlinelibrary.wiley.com/doi/10.1002/art.41929/abstract).

Overall, we observed strong pleiotropic effects on nearby genes, indicating the presence of considerable coregulatory mechanisms. In skeletal muscle tissue, *PTGES3L* and *NBR2* were the most significant eQTL gene targets ($P < 1 \times 10^{-6}$). Strikingly, in the 1000 Genomes Project European population, we observed high LD ($r^2 > 0.95$) between the strongest eQTLs for *PTGES3L* (rs34638441-T and rs10840-A, located in the *IFI35* gene) and 1 variant located in *PTGES3L* (rs35444712-A), suggesting their occurrence on a single haplotype. Moreover, these regions show evidence of a long-distance interaction (GeneHancer Regulatory Interactions marks GH17J043006/GH17J042979 and GH17J043014/GH17J042979), confirming a potentially shared regulatory control at this locus (Figure 2 and Supplementary Table 8, http://onlinelibrary.wiley.com/doi/10.1002/art.41929/abstract). Of note, the genes are localized within a common topologically associating domain (TAD) region (hg19-chr17:41,080,000–41,160,000) in the psoas muscle, as shown in the 3DIV web resource. Additionally, the expression of *PTGES3L* appears to be skeletal muscle–specific, with rs35444712 (1.2×10^{-16}) being a strong eQTL in this tissue (Supplementary Figure 7, http://onlinelibrary.wiley.com/doi/10.1002/art.41929/abstract).

Distinct genetic signature in patients with ASyS revealed by aggregate testing. To further dissect the IIM genetic landscape, we extended GenePy case–control aggregate testing to the major clinical subgroups: PM (n = 170) and DM (n = 133). The vast majority of the genes significantly associated with PM and DM were located in the MHC region (top hits *C2* [FDR-corrected $P = 0.0036$] and *HLA–DQA1* [FDR-corrected $P = 0.0035$], respectively).

Additionally, we restricted this analysis to clinically defined and autoantibody-specific patient subgroups, and focused on the most prevalent subgroup, the ASyS patients (n = 142) (Supplementary Tables 1 and 2, http://onlinelibrary.wiley.com/doi/10.1002/art.41929/abstract). Besides the most significant association with *AGER* (FDR-corrected $P = 5.56 \times 10^{-6}$), in these patients we detected, among others, aggregate associations with *PSMB8* (FDR-corrected $P = 0.0018$) and *PSMB9* (FDR-corrected $P = 0.021$). These genes are all located in the MHC region. Supplementary Table 9 summarizes the GenePy aggregate testing results in the patient subgroups (http://onlinelibrary.wiley.com/doi/10.1002/art.41929/abstract). Using Fisher's exact test on allele counts, we observed that the ASyS patients interestingly also showed a marginal underrepresentation ($P = 0.054$) of minor alleles for the 16 collectively associated *IFI35* variants when compared to the rest of the patients (Figure 3).

Replication of the aggregate testing results. Considering that the replication cohort included UK IIM patients only, and matched controls were not available for targeted DNA sequencing or technically amenable for inclusion in the replication stage after retrieval from publicly available resources, we implemented a strategy based on the comparison of the GenePy-derived gene scores between the patients in the

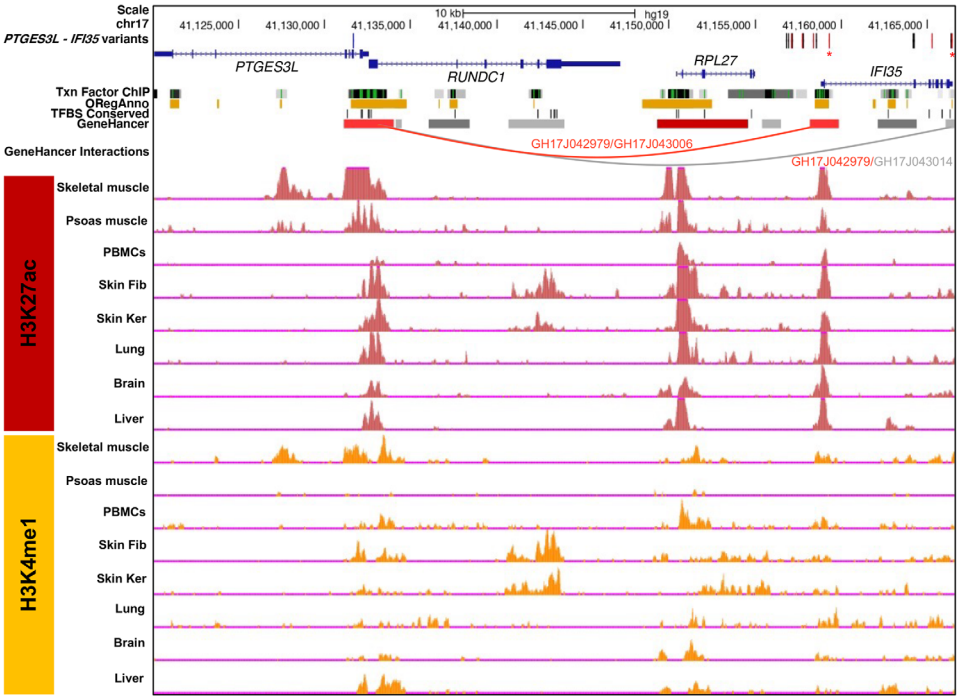

Figure 2. Functional annotation of the extended *IFI35* region. The *PTGES3L/IFI35* variants track shows the *PTGES3L* and *IFI35* variants detected and tested in this study. In this locus, a *PTGES3L* variant (rs35444712, hg19-chr17:41,131,645) (blue) shows high linkage disequilibrium (LD) ($r^2 > 0.95$) with 2 *IFI35* variants (rs34638441, hg19-chr17:41,159,301; rs10840, hg19-chr17:41,166,417) (red asterisks). These *IFI35* variants represent GTEx eQTLs (red) and are located in GeneHancer promoter (GH17J043006) and enhancer (GH17J043014) regions interacting with the *PTGE3L* promoter (GH17J042979). The histone modification marks (H3K27ac, H3K4me1) associated with active enhancers and mapped for different tissues according to Roadmap Epigenomics indicate the presence of skeletal muscle–specific enhancers. PBMCs = peripheral blood mononuclear cells; skin fib = skin fibroblasts; skin ker = skin keratinocytes (see Figure 1 for other definitions).

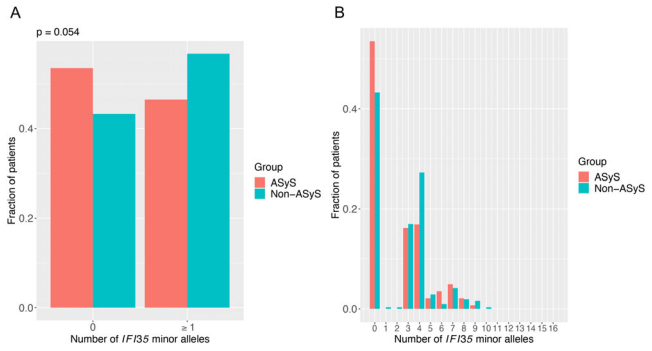

Figure 3. Enrichment of *IFI35* minor alleles in antisynthetase syndrome (ASyS) patients and those without ASyS (non-ASyS). **A**, Summed minor allele distribution for the 16 *IFI35* variants collectively associated with idiopathic inflammatory myopathies via GenePy. The number of patients with 0 alleles versus those with ≥1 allele was compared among the ASyS patients and the group without ASyS. The *P* value was obtained using Fisher's exact test on the parent contingency table composed of ASyS patients with 0 alleles (n = 76), non-ASyS patients with 0 alleles (n = 135), ASyS patients with ≥1 allele (n = 66), and non-ASyS patients with ≥1 allele (n = 177). **B**, Bar graph showing the breakdown of the differential minor allele distribution for all 16 *IFI35* variants total allele count bins in the ASyS and non-ASyS patients.

replication and discovery cohorts. A logistic regression model was implemented to compare the GenePy-derived gene scores between patients in the UK replication cohort (n = 348) and Scandinavian discovery cohort (n = 454) for all of the previously tested phenotypic comparisons. The genes showing evidence of heterogeneity ($P < 0.05$) were conservatively excluded. We considered genes to be replicated if they matched the discovery GenePy-based significantly associated (5% FDR) gene list. Using this approach, among the non-MHC genes significantly associated with IIM, *PRDX3*, *SLAMF1*, *ZFAT*, and *PTPN6* replicated, whereas *IFI35* did not show evidence of replication. Furthermore, the ASyS-associated genes *AGER*, *PSMB8*, and *PSMB9* all showed evidence of replication in the UK ASyS patients. Information on the associated genes in the Scandinavian discovery cohort and whether they showed evidence of aggregate testing replication is provided in Supplementary Table 4 and Supplementary Table 9 (http://onlinelibrary.wiley.com/doi/10.1002/art.41929/abstract).

Evidence of an increased regulatory rare variant allelic burden in IIM patients. Our data set offers the possibility to explore the extent to which rare variation contributes to

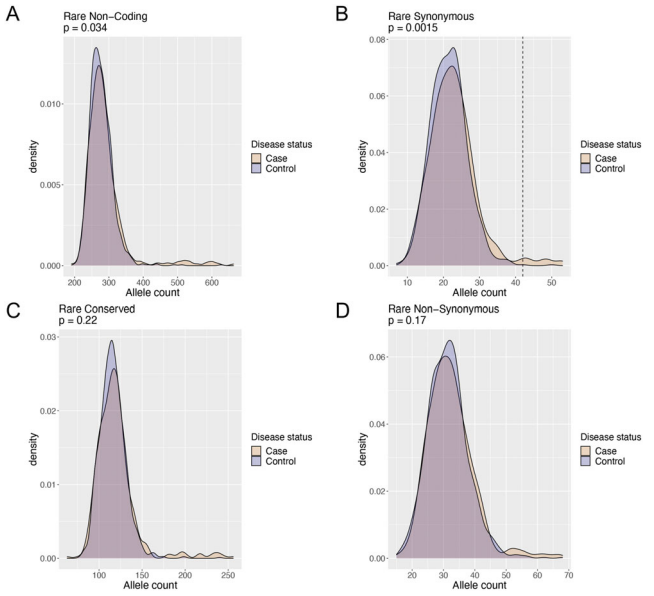

Figure 4. Distribution of single nucleotide variant functional categories in idiopathic inflammatory myopathy (IIM) patients and controls. **A**, Rare noncoding variants. **B**, Rare synonymous variants. Dashed line indicates the 2.5% right tail of the distribution in cases. **C**, Rare conserved variants (Genomic Evolutionary Rate Profiling rejection submission score >2). **D**, Rare nonsynonymous variants. *P* values indicate differences in allele burden between IIM patients and controls. Raw *P* values are shown (Bonferroni-corrected significance threshold of $P = 0.012$).

disease risk. After grouping all rare variants (MAF <0.01) into 4 functional categories, we sought to examine whether there was an increased allelic burden in IIM patients compared to controls, and whether this was driven by enrichment in specific genes or simply reflected a generalized cumulative effect across all the analyzed genomic regions.

For the 101,712 rare noncoding variants detected in the whole cohort, we found statistical evidence of increased allele burden in patients ($P = 0.034$) (Figure 4A), which is consistent with the finding that the majority of autoimmune complex disease–associated variants are located in regulatory regions (26). Although the enrichment was not robust to the conservative correction for multiple testing (i.e., correction for the 4 functional categories tested), 5 genes were significant when considering the allelic burden at the specific gene level: *ITIH4* (FDR-corrected $P = 0.018$), *PXN* (FDR-corrected $P = 0.024$), *TH* (FDR-corrected $P = 0.025$), *IFI35* (FDR-corrected $P = 0.034$), and *VRK1* (FDR-corrected $P = 0.047$).

Next, we detected a significant difference in allele burden in IIM patients when examining synonymous variants (n = 6,898; $P = 0.0015$) (Figure 4B). Although no individual gene was found to drive this enrichment, the gene set harboring these variants in the clinically heterogeneous patients representing the 2.5% right tail of the distribution showed a major enrichment for the JAK/STAT signaling pathway (adjusted $P = 1.4 \times 10^{-5}$). Conversely, the genes from the residual distribution were primarily enriched for the general immune system pathway (Figure 5 and Supplementary Table 10, http://onlinelibrary.wiley.com/doi/10.1002/art.41929/abstract). Interestingly, the predicted likely pathogenic case-only synonymous variants from the right tail of the distribution included a number of candidates in genes with known skeletal muscle–related function (Supplementary Table 11 and Supplementary Methods, http://onlinelibrary.wiley.com/doi/10.1002/art.41929/abstract).

Following the assumption that rare variants located in evolutionary constrained elements (Genomic Evolutionary Rate Profiling [GERP] rejection submission [RS] score >2) (27) have functional potential, we evaluated these variants (n = 42,448) and found no enrichment in cases ($P = 0.22$) (Figure 4C). No

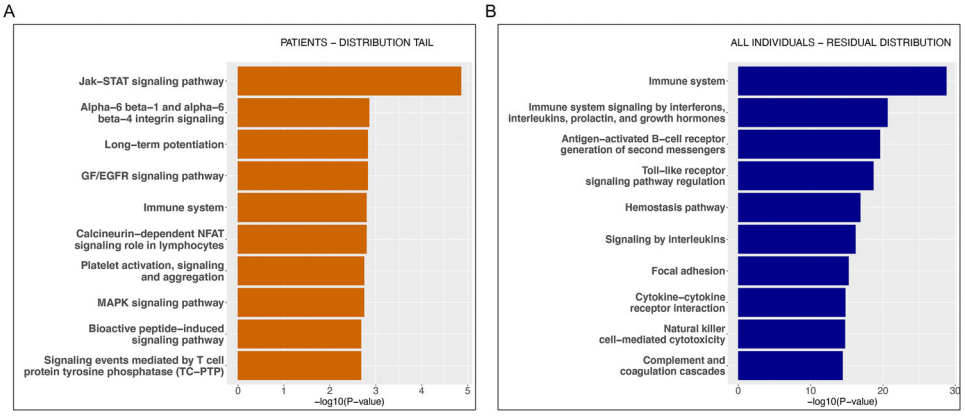

Figure 5. Gene set/pathway enrichment analysis of rare synonymous variants. **A**, Top 10 enriched gene sets/pathways derived from the rare synonymous variants with ≥1 minor allele detected in the patients constituting the 2.5% right tail of the distribution depicted in Figure 4B. The top 3 enriched gene sets/pathways here are respectively ranked 13th, 91st, and 343rd in the enrichment ranking from the analysis in **B**. **B**, Top 10 enriched gene sets/pathways derived from the rare synonymous variants with ≥1 minor allele detected in all individuals from the residual distribution indicated in Figure 4B. The top 3 enriched gene sets/pathways here are respectively ranked 5th, 221st, and 294th in the enrichment ranking from the analysis in **A**. Adjusted *P* values are shown.

evidence of allelic enrichment was detected for rare missense coding variants (n = 12,247) in patients ($P = 0.17$) (Figure 4D).

DISCUSSION

Targeted DNA sequencing, used here for the first time to study the genetic underpinnings of IIM, provides a valuable framework for exploring the genetic contribution to complex diseases characterized by effects from the full spectrum of allele frequencies, including common, low-frequency, and rare variations.

Our study confirmed that the strongest genetic risk for IIM exists within the HLA locus. The single-variant analysis top signal in *HLA–DQA1* presumably reflects the association with the 8.1 ancestral haplotype, as demonstrated by the loss of the whole association signal after conditional analysis on the well-established *DRB1*0301* IIM risk allele.

To effectively investigate rare variation, aggregate association testing algorithms that incorporate variants' functional annotations have recently been proposed to boost discovery power (16,28). Using GenePy, the gene-based aggregate analysis of all variants in the whole patient cohort identified the *IFI35* gene as a potential genetic risk locus for IIM. *IFI35* is preferentially induced by type I IFN and regulates the innate immune response (29). Type I IFN–inducible genes have been found to be overexpressed in the blood, muscle, and skin of DM and/or PM patients (30–32), and blockade of the type I IFN pathway has been investigated as a potential treatment option in such patients (33). Recently, *IFI35* overexpression has been detected and type I IFN pathway activation confirmed in muscle tissue from patients with DM and ASyS (6). Likely due to limited sample size, in the present study, we could only detect a nominal *IFI35* aggregate genetic association with the DM subgroup (raw $P = 0.0024$, FDR-corrected $P = 0.25$), which is also the clinical subgroup with strongest links to type I IFN in blood and tissues. ASyS did not show any association with *IFI35* (raw $P = 0.092$), which could also be due to a less strong association to type I IFN, as discussed below. Nevertheless, consistent with those findings that indicate a key role of type I IFN in the pathogenesis of certain subgroups of IIM, our study corroborates and provides additional evidence of its involvement in IIM at the genetic level.

It is well established that clinical and autoantibody-specific subgroups of IIM have distinct molecular pathway activation profiles. Here, we detected, among others, aggregate genetic associations in the *AGER*, *PSMB8*, and *PSMB9* genes with the ASyS subgroup. *AGER* encodes a multiligand cell surface receptor (receptor for advanced glycation end products [RAGE]) largely expressed in the lung. This gene has previously been associated with lung function and disease (34,35). It is noteworthy that interstitial lung disease is one of the key detrimental phenotypes associated with ASyS (12). *PSMB8* and *PSMB9* are interferonopathy genes involved in the proteasomal degradation pathway and are associated with proteasome-associated autoinflammatory syndromes (36). Interestingly, *PSMB8* has been indicated as the most significantly up-regulated gene in muscle tissue of ASyS patients (6). A reduced activation of the type I IFN pathway compared to the type II IFN pathway has also been described in ASyS patients (6,7). Consistently, our data suggest lower levels of genetic association with the type I IFN pathway in these patients compared to the rest of the IIM patients in our cohort. In fact, compared to all other patients, ASyS patients showed a suggestive depletion of minor alleles for the 16 collectively associated *IFI35* variants.

Autoimmune complex diseases have been largely associated with common noncoding genetic variation, but rare variants may also contribute to disease risk (37). In this study, we present evidence that rare variant alleles located in functionally important regulatory regions are enriched in IIM patients. Interestingly, this disease-driven overrepresentation was more pronounced for rare synonymous variants, which can be generally involved in gene expression, splicing, messenger RNA structure stability, and protein translation and folding. Gene set enrichment analysis indicated that the synonymous variant alleles showing overrepresentation in a clinically heterogeneous subset of patients do cluster in the JAK/STAT signaling pathway. Notably, JAK/STAT pathway inhibition has been proposed as a therapeutic target for DM (38,39). Intriguingly, when focusing on the subset of overrepresented case-only variants, a number of these were located in genes associated with muscular dysfunction. *TTN*, *EXOSC10*, *CDC42BPB*, and *CARM1* have been associated with Mendelian forms of myopathies, muscular atrophies, and motor neurodevelopmental disorders (40). Additionally, *COL1A1* and *COL4A5* could account for impaired collagen metabolism leading to muscle pain. Consistent with the genetic architecture of other polygenic disorders, IIM exhibits rare genetic variants that have the potential to exert monogenic effects in the relevant corresponding organ systems (41).

Despite not surviving multiple test correction, we also observed enrichment of rare noncoding regulatory variant alleles in IIM patients, with *IFI35* among the main gene drivers. It is interesting how 2 aggregate analyses based on different, albeit not fully independent, variant sets (i.e., all variants and all rare noncoding variants) implicate the *IFI35* locus as a potential genetic risk factor for IIM, thus cross-verifying each other.

When examining the potentially regulatory variants in the *IFI35* locus, we found from published chromatin immunoprecipitation sequencing data (20,21) that specific transcription factors active in immune signaling bind to several regions with variants and presumably regulate *IFI35* expression. These transcription factors include Bcl-3, which is crucial in controlling both innate and adaptive immunity (42), and FoxA1, which establishes a T cell regulatory population involved in inflammatory diseases (43).

Nearly half of the variants contributing to the *IFI35* aggregate association represent eQTLs in multiple tissues. Interestingly, these variants not only modulate *IFI35* expression but can also affect the level of several nearby gene transcripts, including

PTGES3L and *NBR2*. Our study suggests that the skeletal muscle–specific gene regulatory network, linking *IFI35* and *PTGES3L*, potentially contributes to the etiology of IIM. We hypothesize that, within this regulatory circuit, which is defined by direct across-gene enhancer– and promoter–promoter interactions, bordered by a distinct TAD in skeletal muscle, and characterized by high levels of LD, cumulative genetic contribution of eQTLs at the *IFI35* locus might also alter *PTGES3L* expression. Intriguingly, *PTGES3L* is predominantly expressed in skeletal muscle (Supplementary Figure 7, http://onlinelibrary.wiley.com/doi/10.1002/art.41929/abstract), which is supported by strong tissue-specific enhancers and the promoter for this gene. Notably, *PTGES3L* is associated with type 2A distal arthrogryposis (40). Arthrogryposis is a rare disorder characterized by systemic muscle weakness at birth and stiffness, thus showing some similarities with the IIM phenotype. Therefore, *PTGES3L* emerges as an attractive target for research in IIM, although further experiments and mechanistic evidence are required to confirm our hypothesis.

The *IFI35* locus eQTLs can also strongly affect *NBR2* expression in skeletal muscle. Interestingly, *NBR2* encodes a long noncoding RNA involved in metabolic and energy stress response (44), a process perturbed in muscle undergoing IIM pathogenic events (45). The non-MHC genes associated with IIM by gene-based aggregate testing, as well as showing evidence of replication (i.e., *PRDX3*, *SLAMF1*, *ZFAT*, and *PTPN6*), represent potentially novel genetic risk factors pointing to immune- and nonimmune-mediated mechanisms implicated in the development of muscle weakness and damage, such as oxidative stress (46), autophagy (47), and Toll-like receptor signaling (48,49). These mechanisms have all been suggested to contribute to the pathophysiology of IIM (1).

This study has the limitation of applying the old classification criteria for IIM, as samples were collected before the approval of the new European Alliance of Associations for Rheumatology/American College of Rheumatology classification criteria (50). Some patients might therefore be reclassified based on these more recent criteria. We also recognize that *IFI35* failed in our attempt to replicate the discovery aggregate testing results in the UK cohort. This could be due to the limited statistical power derived from the replication cohort size, the ancestry-specific aggregate effect of this gene (for which contribution of rare variation is key), or alternatively to the unique and overconservative strategy we used for replication in our study due to the lack of analogously sequenced UK controls. A further limitation is that our array targets mainly immune-related genes and covers only a fraction of the genome, thus preventing the investigation of additional genes and noncoding regions potentially involved in IIM pathogenesis.

In summary, aggregate genetic association suggests a potential role for *IFI35* and *PTGES3L* in the pathogenesis of IIM. We found a genetic signature indicating type I IFN pathway activation in IIM and highlighted specific genetic associations in patients with ASyS that are consistent with previous findings on their gene expression profile in muscle tissue and with lung involvement. Overall, these findings and the indication that genetic perturbations of the JAK/STAT pathway might occur in a subset of patients independently of the myositis subtype suggest that inhibitors of this pathway might be beneficial in a broader spectrum of patients. Our study highlights the contribution of rare genetic variation to disease susceptibility in a Scandinavian myositis cohort and in specific patient subgroups. These findings may collectively inform disease treatment options in the context of future personalized medicine practice.

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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. Bianchi 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. Bianchi, Kozyrev, Notarnicola, Rönnblom, Lindblad-Toh, Lundberg, with contributions from members of the ImmunoArray Development Consortium.

Acquisition of data. Bianchi, Kozyrev, Notarnicola, Karlsson, Andersson, Cooper, Padyukov, Tjärnlund, Dastmalchi, Pyndt Diederichsen, Molberg, Chinoy, with contributions from members of the DISSECT Consortium.

Analysis and interpretation of data. Bianchi, Kozyrev, Notarnicola, Hultin Rosenberg, Pucholt, Rothwell, Alexsson, Sandling, Meadows, Lamb, Rönnblom, Lindblad-Toh, Lundberg, with contributions from members of the DISSECT Consortium.

ROLE OF THE STUDY SPONSOR

AstraZeneca had no role in the study design or in the collection, analysis, or interpretation of the data, the writing of the manuscript, or the decision to submit the manuscript for publication. Publication of this article was not contingent upon approval by AstraZeneca.

ADDITIONAL DISCLOSURES

Authors Hultin Rosenberg and Pucholt are employees of Olink Proteomics. Author Alexsson is an employee of Qiagen.

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APPENDIX A: THE DISSECT CONSORTIUM AND THE IMMUNOARRAY DEVELOPMENT CONSORTIUM COLLABORATORS

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