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Dense Genotyping of Immune-Related Loci in the Idiopathic Inflammatory Myopathies Confirms HLA alleles as Strongest Genetic Risk Factor and Suggests Different Genetic Background for Major Clinical Subgroups

Simon Rothwell[1,*], **Robert G. Cooper**[2], **Ingrid E. Lundberg**[3], **Frederick W. Miller**[4], **Peter K. Gregersen**[5], **John Bowes**[1], **Jiri Vencovsky**[6], **Katalin Danko**[7], **Vidya Limaye**[8], **Albert Selva-O'Callaghan**[9], **Michael G. Hanna**[10], **Pedro M. Machado**[10], **Lauren M. Pachman**[11], **Ann M. Reed**[12], **Lisa G. Rider**[4], **Joanna Cobb**[13], **Hazel Platt**[14], **Øyvind Molberg**[15], **Olivier Benveniste**[16], **Pernille Mathiesen**[17], **Timothy Radstake**[18], **Andrea Doria**[19], **Jan De Bleecker**[20], **Boel De Paepe**[20], **Britta Maurer**[21], **William E. Ollier**[14], **Leonid Padyukov**[3], **Terrance P. O'Hanlon**[4], **Annette Lee**[5], **Christopher I. Amos**[22], **Christian Gieger**[23], **Thomas Meitinger**[24,25], **Juliane Winkelmann**[26,27], **Lucy R Wedderburn**[28], **Hector Chinoy**[29], **Janine A Lamb**[14], and **Myositis Genetics Consortium**

[1]Centre for Genetics and Genomics, Arthritis Research UK, University of Manchester, Manchester, M13 9PT, UK [2]Department of Musculoskeletal Biology, Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, L69 3GA, UK [3]Rheumatology Unit, Department of Medicine, Karolinska University Hospital, Karolinska Institutet, Stockholm, 171 76, Sweden [4]Environmental Autoimmunity Group, Clinical Research Branch, National Institute of Environmental Health Science, National Institutes of Health, Bethesda, MD 20892-1301, USA [5]The Robert S Boas Center for Genomics and Human Genetics, Feinstein Institute for Medical Research, Manhasset, NY 11030, USA [6]Institute of Rheumatology and Department of Rheumatology, 1st Faculty of Medicine, Charles University, Prague, 12850, Czech Republic [7]Division of Clinical Immunology, Department of Internal Medicine, University of Debrecen, Hungary [8]Royal Adelaide Hospital and University of Adelaide, North Tce, Adelaide, Australia [9]Department of Internal Medicine, Vall d'Hebron Hospital, Passeig Vall d'Hebron, Barcelona 08035, Spain [10]MRC Centre for Neuromuscular Diseases, UCL Institute of Neurology, London, WC1N 3BG, UK [11]Northwestern University Feinberg School of Medicine and Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL 60611, USA [12]Department of Pediatrics, Duke University, Durham, NC 27710, USA [13]Arthritis Research UK, NIHR Manchester Musculoskeletal Biomedical Research Unit, Central Manchester NHS Foundation Trust, Manchester Academic Health Science Centre, University of Manchester, Manchester, M13 9PT, UK [14]Centre for Integrated Genomic Medical Research, University of Manchester, Manchester, M13 9PT, UK [15]Department of Rheumatology, Oslo University Hospital, Oslo 0424, Norway [16]Pitié-Salpêtrière Hospital, UPMC, APHP, Paris 75013, France [17]Paediatric Department, Naestved Hospital,

Corresponding Author: Simon Rothwell, Centre for Genetics and Genomics, Arthritis Research UK, University of Manchester, Manchester, M13 9PT, UK, s.rothwell@manchester.ac.uk, Phone: 07979501439.

Supplementary Materials

Supplemental materials include extended methods, five figures and 15 tables.

Næstved 4700, Denmark [18]Department of Rheumatology and Clinical Immunology, University Medical Center Utrecht, 3584 CX Utrecht, the Netherlands [19]Department of Medicine, University of Padova, 35128 Padova, Italy [20]Department of Neurology and Neuromuscular Reference Centre, Ghent University Hospital, 9000 Ghent, Belgium [21]Department of Rheumatology and Center of Experimental Rheumatology, University Hospital Zurich, Zurich CH-8091, Switzerland [22]Geisel School of Medicine, Dartmouth College, Hanover, NH 03750, USA [23]Helmholtz Zentrum München, Deutsches Forschungszentrum für Gesundheit und Umwelt (GmbH), D-85764 Neuherberg, Germany [24]Institute of Human Genetics, Technische Universität München, 81675, Munich, Germany [25]Institute of Human Genetics, Helmholtz Zentrum München, German Research Center for Environmental Health, 85764 Neuherberg, Germany [26]Neurologische Klinik und Poliklinik, Klinikum rechts der Isar, Technische Universität München, 81675 Munich, Germany [27]Institute of Neurogenomics, Helmholtz Zentrum München, German Research Center for Environmental Health, 85764 Neuherberg, Germany [28]Arthritis Research UK Centre for Adolescent Rheumatology, and Institute of Child Health, University College London, London, WC1N 1EH, UK [29]National Institute of Health Research Manchester Musculoskeletal Biomedical Research Unit, Centre for Musculoskeletal Research, University of Manchester, Manchester, M13 9PT, UK

Abstract

The idiopathic inflammatory myopathies (IIM) are a heterogeneous group of rare autoimmune diseases characterized by muscle weakness and extramuscular manifestations such as skin rashes and interstitial lung disease. We genotyped 2,566 IIM cases of Caucasian descent using the Immunochip; a custom array covering 186 established autoimmune susceptibility loci. The cohort was predominantly comprised of dermatomyositis (DM, n=879), juvenile dermatomyositis (JDM, n=481), polymyositis (PM, n=931) and inclusion body myositis (IBM, n=252) patients collected from 14 countries through the Myositis Genetics Consortium. The human leukocyte antigen (*HLA*) and *PTPN22* regions reached genome-wide significance ($p<5\times10^{-8}$). Nine regions were associated at a significance level of $p<2.25\times10^{-5}$, including *UBE2L3*, *CD28* and *TRAF6,* with evidence of independent effects within *STAT4.* Analysis of clinical subgroups revealed distinct differences between PM, and DM and JDM. *PTPN22* was associated at genome-wide significance with PM, but not DM and JDM, suggesting this effect is driven by PM. Additional suggestive associations including *IL18R1* and *RGS1* in PM and *GSDMB* in DM were identified. HLA imputation confirmed that alleles *HLA-DRB1*03:01* and *HLA-B*08:01* of the 8.1 ancestral haplotype (8.1AH) are most strongly associated with IIM, and provides evidence that amino acids within the HLA, such as *HLA-DQB1* position 57 in DM, may explain part of the risk in this locus. Associations with alleles outside the 8.1AH reveal differences between PM, DM, and JDM. This work represents the largest IIM genetic study to date, reveals new insights into the genetic architecture of these rare diseases and suggests different predominating pathophysiology in different clinical subgroups.

Keywords

Genetics; idiopathic inflammatory myopathy; myositis; HLA; association

Introduction

The idiopathic inflammatory myopathies (IIM) are a heterogeneous group of rare autoimmune diseases, the major phenotypes of which are dermatomyositis (DM), polymyositis (PM), inclusion body myositis (IBM) and DM/PM overlapping with other connective tissue diseases.[1] IIM are primarily characterised by the presence of proximal muscle weakness, elevated levels of skeletal muscle enzymes and inflammatory infiltrates in skeletal muscle, but may also present with extramuscular manifestations including skin rashes, interstitial lung disease and malignancy that often correlate with serum antibody status.[2]

IIM are thought to be complex genetic diseases, initiated by immune activation following specific environmental events in genetically predisposed individuals. The strongest genetic association in the IIM has been consistently within the major histocompatibility complex (MHC),[3] specifically with the 8.1 ancestral haplotype (8.1 AH). A recent genome-wide association study (GWAS) in DM, and a candidate gene study, also indicate overlap of genes implicated in other autoimmune diseases.[4, 5] The Immunochip is a custom-designed array containing coverage of 186 established autoimmune susceptibility loci and extended coverage across the MHC.[6] In this study, we report an Immunochip analysis of 2,566 global IIM cases and 15,651 controls, representing the largest genetic association study to date in IIM.

Methods

Samples

2,954 samples from 14 countries were collected through the Myositis Genetics Consortium (MYOGEN), and written informed consent was obtained from all cases with approval from research ethics boards at each participating centre. There is overlap between these samples and previous IIM genetic studies.[3–5] IIM cases were included if they fulfilled probable or definite Bohan and Peter classification criteria for PM, juvenile PM (JPM), DM or Juvenile DM (JDM),[7, 8] and Griggs or European Neuromuscular Centre (ENMC) or Medical Research Council (MRC) criteria for IBM.[9–11] Eleven samples met the criteria for anti-synthetase syndrome,[12] however available clinical data was not able to differentiate between PM or DM. These were included in the combined IIM analysis, but removed from the clinical subgroup analysis.

Immunochip control data from 12 countries was provided by four disease consortia (online supplementary methods).

Genotyping and quality control

Genotyping was performed in accordance with Illumina's protocols in the UK (Centre for Genetics and Genomics Arthritis Research UK, University of Manchester, UK) and the US (Feinstein Institute, New York, USA). Standard sample and SNP QC was performed in PLINK v1.07 (online supplementary methods).

Statistical Analysis

Statistical analysis was performed in PLINK v1.07 using a logistic regression applying an additive model, including the top ten principal components as covariates to control for population stratification. Evidence for additional independent effects was investigated using a stepwise logistic regression including the most associated variants as covariates in subsequent conditional analyses.

Functional annotation

Associated loci were interrogated for potentially causative variants using eQTL databases, and the functional prediction tools PolyPhen-2,[13] SIFT,[14] and phastCons17-way[15] (online supplementary methods).

MHC imputation and association analysis

Classical human leukocyte antigen (*HLA*) alleles and corresponding amino acid sequences were imputed using SNP2HLA. A logistic regression assuming an additive model was used to test for association, and forward stepwise logistic regression was used to test for independent effects (online supplementary methods). Classical 4-digit HLA alleles were preferentially reported, unless an amino acid association explained more risk than HLA alleles alone.

Results

Genotyping quality control

After stringent SNP and sample quality control we analysed 90,536 genetic variants in 2,566 IIM cases and 15,651 controls of Caucasian descent (Table 1). A breakdown of this cohort by clinical subgroup is reported in Table S1. Australia, Denmark and Switzerland did not have an ethnically matched control group; however, these were adequately matched by existing cohorts (UK, Sweden, and Germany respectively). By including the top ten principal components as covariates and calculating the genomic inflation on a set of null SNPs (from a study investigating the genetic basis for reading and writing ability)[16] on the Immunochip gave a $\lambda GC_{1000} = 1.05$, indicating that cases and controls are well matched for ethnicity (online supplementary Figure S1).

HLA and *PTPN22* are the most strongly associated regions in IIM

Two regions in this study reached genome-wide significance ($p<5\times10^{-8}$) (Figure 1A and Table 2). As expected, the most strongly associated region was within the MHC ($p=9\times10^{-133}$) (online supplementary Figure S2). HLA-imputation was performed separately on this locus.

The other region reaching genome-wide significance was within the *PTPN22* locus (rs2476601; $p=7.22\times10^{-9}$), an established autoimmune risk locus. This SNP/locus has been previously associated in an IIM candidate gene study,[3] but was not associated in a GWAS in DM.[4]

A further nine regions were associated at a suggestive level of significance

We next investigated associations reaching our suggestive tier of association ($p=2.25\times10^{-5}$) calculated using the genetic Type 1 Error Calculator.[31] This estimates the effective number of independent tests based on the LD between SNPs contained on the genotyping array. Here, we found evidence of a further nine associated loci (Table 2).

The third most strongly associated SNP in this analysis was in the *YDJC* gene (rs5754467) on chromosome 22 (4.67×10^{-7}). This SNP tags a large haplotype block containing *UBE2L3*, an established autoimmune risk locus.

STAT4 is a susceptibility locus for many autoimmune diseases. The lead SNP in this region was protective, which is a novel finding in IIM (rs4853540, $p=1.57\times10^{-6}$). Stepwise logistic regression analysis in this region suggested an independent risk effect of rs10174238 ($p=1.08\times10^{-5}$, OR=1.17, 95% CI=1.09–1.26) (online supplementary Figure S3) and a further potential independent effect was seen at rs932169 ($p=2.88\times10^{-5}$, OR=1.25, 95% CI=1.13–1.39).

Further variants reaching our suggestive significance threshold reveal loci of interest that have been previously associated with autoimmune disease, including DGKQ, EOMES, CD28 and PRR5L/TRAF6. Associated SNPs that tag risk haplotypes (r2 >0.7) in other autoimmune diseases are reported in Table 2, and the direction of effect is reported in online supplementary Table S2.

Subgroup analysis reveals unique associations within PM and DM

We stratified our cohort by the two largest subgroups within IIM, consisting of 931 adult PM cases (Figure 1B, Table 2) and 1,360 DM cases (Figure 1C, Table 2). JDM cases were included in the DM analysis both to increase power, and on previous evidence that there is not extensive genetic heterogeneity between the subgroups.[4] The only non-HLA region to reach genome-wide significance in either subgroup was *PTPN22* in PM (rs2476601, $p=7.9\times10^{-11}$). Interestingly, with a smaller sample size, the association in PM with *PTPN22* was stronger than in the combined IIM analysis. There was no evidence of association in DM (p=0.19), therefore the stronger effect in PM appears to be driving the association in the combined IIM analysis. Other interesting regions reaching a suggestive level of significance were *SL26A1/IDUA* and *RGS1* in PM, and *GSDMB* in DM (online supplementary Table S3).

Exonic and eQTL SNPs suggest potential causal variants

Potential functionally relevant variants were investigated for non-synonymous SNPs (Table 3) or eQTLs (Table 4) that are tagged by the lead SNP ($r^2 > 0.9$). Two variants within the *GSDMB* gene, suggestively associated in DM, are ‘potentially damaging’ as predicted by PolyPhen-2. The *PTPN22* variant is confirmed to be conserved across vertebrates, as well as a SNP in *UBE3B*. Evidence for eQTLs in cells with immune function (lymphoblastoid cell lines and monocytes) was found in six loci and may help annotate our associations, for example, the association with *NAB1* in PM may be due to an eQTL affecting the expression of *STAT1,* 275Kb upstream.

HLA Imputation confirms alleles of the 8.1 ancestral haplotype as the strongest association in IIM

Due to the complex linkage disequilibrium/haplotype structure in the MHC, interpretation of causal associations and independent effects using SNPs may be inadequate. We used SNP2HLA to impute classical HLA alleles and amino acids from SNP genotyping information. For each analysis, all variants reaching statistical significance ($p<6.8\times10^{-6}$) after each round of conditioning are included in online supplementary Table S4–S15. For many associations, amino acids unique to classical HLA risk alleles were associated at similar levels of significance to the HLA allele. For consistency, 4-digit HLA alleles are reported, unless an amino acid is significantly more associated than individual HLA alleles. In the combined IIM analysis (n=2,566), the most associated variants were classical HLA alleles, with *HLA-DRB1**03:01 being the most significant 4-digit allele ($p=2.58\times10^{-135}$, OR=1.88, 95% CI=1.68–2.11). *HLA-DRB1**03:01 forms part of the 8.1 AH which has been consistently associated with IIM. After conditioning on the effects of *HLA-DRB1**03:01, a strong association was found with *HLA-B**08:01 ($p=3.23\times10^{-14}$, OR=1.58, 95% CI=1.41–1.78) suggesting that there is an independent effect within this locus. Further residual associations highlight the heterogeneity within this cohort, so analysis was then conducted on clinical subgroups. Similar associations were found with PM (n=931), *HLA-DRB1**03:01 being the most significant 4-digit allele ($p=6.11\times10^{-80}$, OR=1.99, 95% CI=1.67–2.36) and an independent effect with HLA-B*08:01 ($p=4.17\times10^{-9}$, OR=1.71, 95% CI=1.43–2.05). As the effect size of the HLA is strong in IIM, we hypothesised that we may be able to detect any potential differences between adult and juvenile DM, even with a reduced sample size. In adult DM (n=879), *HLA-B**08:01 was the most significant allele ($p=2.46\times10^{-42}$, OR=1.90, 95% CI=1.66–2.17). Conditioning on *HLA-B**08:01, there was evidence of multiple independent effects within the *HLA-DQB1* locus, therefore we analysed imputed amino acid residues. Amino acid position 57 of *HLA-DQB1* was more significantly associated with DM than individual *HLA-DQB1* alleles ($p=8.95\times10^{-14}$), with alanine ($p=1.29\times10^{-12}$ OR=1.62, 95% CI=1.44–1.83) and serine ($p=9.28\times10^{-7}$, OR=2.15, 95% CI=1.60–2.84) conferring the greatest risk. Further association with *HLA-DQB1* remains after conditioning, notably an independent effect of *HLA-DQB1**04:02 ($p=2.01\times10^{-6}$, OR=1.99, 95% CI=1.52–2.58). In the JDM subgroup (n=481), *HLA-DRB1**03:01 was the most associated allele ($p=7.91\times10^{14}$, OR=1.90, 95% CI=1.61–2.22) and an independent association was observed with *HLA-C**02:02 ($p=3.28\times10^{-7}$, OR=1.99, 95% CI=1.55–2.52) which is not a part of the 8.1 ancestral haplotype.

Discussion

This is the largest genetic study to date in IIM, and has revealed several novel suggestive associations in adult and juvenile IIM emphasising the autoimmune architecture of these diseases. We have confirmed *HLA* and *PTPN22* as the most strongly associated regions in IIM, and identified nine additional associations at a suggestive level of significance. Subgroup analysis has identified distinct differences between PM and DM. Identification of exonic and eQTL SNPs has localised association signals to several potential causal variants.

It is reassuring that associations such as *PTPN22*, *STAT4* and *UBE2L3* follow a similar genetic profile as reported in other autoimmune diseases. The most significantly associated SNP in the *PTPN22* region is the rs2476601 variant, a C>T polymorphism that results in a non-synonymous arginine (R) to tryptophan (W) amino acid change at position 620. Although this SNP has been extensively studied in the context of autoimmunity, there is no consensus regarding the functional consequences of this SNP. Some studies report a gain of function mutation by enhancing the inhibitory effect on TCR signalling,[39] while others report a loss of function by increased degradation of the protein and a diminished inhibitory effect on T-cell activation.[40]

STAT4 is an important transcription factor for many genes involved in T-cell differentiation and has previously been associated with DM in the Japanese population.[41] Stepwise logistic regression analysis was conducted on all regions in this study; however *STAT4* is the only locus with evidence of independent associations. The three independent SNPs are in LD with associations in different diseases. The lead SNP in *STAT4* is protective, and in moderate LD with protective SNPs in *STAT4* reported in inflammatory bowel disease (IBD), Crohn's and ulcerative colitis.[16] The independent risk effect of rs10174238 is the same SNP reported in juvenile idiopathic arthritis,[18] and is in strong LD with disease-associated SNPs in RA and SLE.[19, 42] A SNP in high LD with rs932169 has been reported to be associated with primary biliary cirrhosis.[25]

The most significantly associated SNP in the *YDJC* gene tags an established autoimmune susceptibility locus where *UBE2L3* is thought to be the causal gene.[43] This risk haplotype is thought to increase *UBE2L3* expression in B cells and monocytes and amplify NF-κB activation.[43]

Stratification by clinical subgroup revealed further novel suggestive associations. These distinct differences between PM and DM suggest different autoimmune pathways in these subsets of IIM. For example, when splitting the total IIM cohort into PM and DM, we have shown that the association with *PTPN22* is predominantly driven by a strong association with PM. For all associations, we have stratified by clinical subgroup and reported the summary statistics in online supplementary Table S3. IBM patients were included in the combined IIM analysis on the basis of their diagnosis as an inflammatory autoimmune myopathy, however we did not analyse this subgroup separately due to a lack of power (n=252). Removing this group from our analyses did not make any substantial difference in associated regions, however the strength of the signals were attenuated in most instances. With eight non-HLA loci reaching our level of suggestive significance in PM, and only 3 in DM, it may be that the Immunochip is explaining less of the genetic risk to DM. This may be due to lack of power, the selected content of the Immunochip, heterogeneity of phenotypes within DM, or a weaker genetic influence compared to other autoimmune diseases. Some previous reported associations with DM failed to replicate in this study. We looked for evidence of association with loci that have previously been associated with IIM that did not reach our suggestive level of significance. For example, in the DM and JDM subgroup analysis, an association was found with rs2618476 ($p=3.2\times10^{-5}$, OR=1.2, 95% CI=1.1–1.32), a SNP in B Lymphoid Tyrosine Kinase (*BLK*). rs2618476 is a proxy for a SNP that was associated with DM in the Japanese population,[44] and is also highly

correlated (r^2>0.8) with associations found in SLE and RA.[20, 29] With this knowledge, this association becomes more convincing, whereas the single association in the *FAM167A-BLK* region in the PM subgroup (rs17799348) that is independent of the established risk haplotype, is less so.

It is important to note that this study was conducted on Caucasian IIM individuals. While there is evidence that risk loci may be shared across populations, such as *STAT4* and *BLK* in the Japanese population, the association between *PTPN22* and autoimmune disease is unique to Caucasians as the R620W variant is rarely seen in Asian populations. There is therefore a need to conduct further genetic studies on different IIM populations.

The Immunochip contains a dense set of SNPs covering 186 loci based on evidence of association with 12 different autoimmune and inflammatory diseases.[18] IIM was not one of these diseases, so this study can be seen as an exploratory investigation to assess genetic overlap with other autoimmune diseases, rather than the identification of genes novel to IIM. With 2,566 samples, Immunochip studies of similar size have revealed multiple non-HLA associations reaching genome-wide significance.[18, 45] The fact that only a single locus reached this threshold may due to low statistical power owing to phenotypic heterogeneity within IIM. A more conservative level of significance ($p<2.25\times10^{-5}$) revealed suggestive associations of interest. SNPs that are the same, or in high LD with established autoimmune variants, along with biological knowledge and/or evidence of functionality may lead us to pursue these associations with more confidence. Indeed, a recent Immunochip study in T1D calculated a Bayesian posterior probability of disease association >0.9 of SNPs reaching a suggestive level of significance ($p<1\times10^{-5}$) when there is evidence of genome-wide significance in other Immunochip studies.[30]

Due to the extended haplotypes that are present in the HLA region, for many associations, alleles carried on the same haplotype reached an equivalent significance level. For consistency, the most associated allele was used in the stepwise conditional analysis; however, this is not to say that the allele is causative. Interestingly in PM, two alleles frequently inherited together on the 8.1 AH (*HLA-DRB1**03:01 and *HLA-B**08:01) show evidence of having independent effects. This may also be the case in DM, however, after conditioning on *HLA-B**08:01, the association with other alleles of the 8.1 AH did not reach genome-wide significance. In DM, the independent association with amino acid position 57 in *HLA-DQB1* may explain part of the risk within this gene. Indeed, this position is an established risk factor for T1D.[46] In this study, classical 4-digit HLA alleles were preferentially reported, unless an amino acid association explained more risk than HLA alleles alone. However looking at amino acids may give insight into functionality. For example, the association with *HLA-DRB1*03:01* may be explained by the presence of amino acids that are unique to that allele. An asparagine at position 77, and an arginine at position 74 also were highly associated with IIM (online supplementary Table S4), and these residues are predominantly found on *DRB1*03* alleles. As there are multiple residues unique to this allele, it is hard to tease out which positions may be functionally important; however the location of these amino acids in the *HLA-DRB1* molecule may give insight (online supplementary Figure S4). Amino acid position 74 of *HLA-DRB1* lies within the peptide binding groove, and almost all of the risk at this position can be explained by the presence of

an arginine ($p=3.1\times10^{-72}$, OR=2.83, 95% CI=2.53–3.17). The location of Arg-74 may change the structure of the peptide binding groove in such a way as to accommodate autoantigenic peptides. Indeed Arg-74 is an established risk factor for the development of autoimmune diseases.[47] A similar phenomenon is seen with *HLA-B*08:01* and the occurrence of Phe-67 and Asp-9 (online supplementary Figure S5).

Alleles of the 8.1 AH have frequently been associated with the presence of myositis autoantibodies such as anti-Jo-1 and anti-PM-Scl. It may be that the association with the 8.1 AH and IIM is due to the prevalence of these antibodies, and weak associations with other *HLA*-alleles may be due to associations with autoantibodies that are less frequent in the disease subgroup. Further work is planned to stratify patients by serotype to clarify these differences.

This study has revealed new suggestive associations with IIM in the Caucasian population, and independent associations with PM and DM. and has shown that subgrouping patients into clinical subgroups is important to expand our knowledge of IIM. This international collaboration has made it possible to perform the largest study to date in IIM and it has considerably expanded our knowledge about the genetic architecture of this rare disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Further MYOGEN Investigators

Study investigators of the Myositis Genetics Consortium, in addition to the authors of this article, are as follows: Drs. Christopher Denton (Royal Free Hospital, London, UK), Herman Mann (Institute of Rheumatology, Prague), David Hilton-Jones (John Radcliffe Hospital, Oxford, UK), Patrick Kiely (St. George's Hospital, London, UK), Paul H. Plotz, Mark Gourley (National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, MD), Kelly Rouster-Stevens (Emory University School of Medicine, Atlanta, GA), Adam M Huber (Dalhousie University, Halifax, Nova Scotia, Canada), Galina Marder (North Shore Univeristy Hospital, Great Neck, NY) and Mazen Dimachkie (University of Kansas Medical Center, Kansas City, KS).

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Maria Lidén (Uppsala University), Awat Jalal (Örebro University), Helena Hellström (Rheumatology Clinic Falun), Thomas Skogh (Linköping University), Aladdin Mohammad (Lund).

UK Adult Onset Myositis Immunogenetic Collaboration (UKMYONET)

Members of the UK Adult Onset Myositis Immunogenetic Collaboration who recruited and enrolled subjects are as follows: Drs. Yasmeen Ahmed (Llandudno General Hospital), Raymond Armstrong (Southampton General Hospital), Robert Bernstein (Manchester Royal Infirmary), Carol Black (Royal Free Hospital, London), Simon Bowman (University Hospital, Birmingham), Ian Bruce (Manchester Royal Infirmary), Robin Butler (Robert Jones & Agnes Hunt Orthopaedic Hospital, Oswestry), John Carty (Lincoln County Hospital), Chandra Chattopadhyay (Wrightington Hospital), Easwaradhas Chelliah (Wrightington Hospital), Fiona Clarke (James Cook University Hospital, Middlesborough), Peter Dawes (Staffordshire Rheumatology Centre, Stoke on Trent), Joseph Devlin (Pinderfields General Hospital, Wakefield), Christopher Edwards (Southampton General Hospital), Paul Emery (Academic Unit of Musculoskeletal Disease, Leeds), John Fordham (South Cleveland Hospital, Middlesborough), Alexander Fraser (Academic Unit of Musculoskeletal Disease, Leeds), Hill Gaston (Addenbrooke's Hospital, Cambridge), Patrick Gordon (King's College Hospital, London), Bridget Griffiths (Freeman Hospital, Newcastle), Harsha Gunawardena (Frenchay Hospital, Bristol), Frances Hall (Addenbrooke's Hospital, Cambridge), Beverley Harrison (North Manchester General Hospital), Elaine Hay (Staffordshire Rheumatology Centre, Stoke on Trent), Lesley Horden (Dewsbury District General Hospital), John Isaacs (Freeman Hospital, Newcastle), Adrian Jones (Nottingham University Hospital), Sanjeet Kamath (Staffordshire Rheumatology Centre, Stoke on Trent), Thomas Kennedy (Royal Liverpool Hospital), George Kitas (Dudley Group Hospitals Trust, Birmingham), Peter Klimiuk (Royal Oldham Hospital), Sally Knights (Yeovil District Hospital, Somerset), John Lambert (Doncaster Royal Infirmary), Peter Lanyon (Queen's Medical Centre, Nottingham), Ramasharan Laxminarayan (Queen's Hospital, Burton Upon Trent), Bryan Lecky (Walton Neuroscience Centre, Liverpool), Raashid Luqmani (Nuffield Orthopaedic Centre, Oxford), Jeffrey Marks (Steeping Hill Hospital, Stockport), Michael Martin (St. James University Hospital, Leeds), Dennis McGonagle (Academic Unit of Musculoskeletal Disease, Leeds), Neil McHugh (Royal National Hospital for Rheumatic Diseases, Bath), Francis McKenna (Trafford General Hospital, Manchester), John McLaren (Cameron Hospital, Fife), Michael McMahon (Dumfries & Galloway Royal Infirmary, Dumfries), Euan McRorie (Western General Hospital, Edinburgh), Peter Merry (Norfolk & Norwich University Hospital, Norwich), Sarah Miles (Dewsbury & District General Hospital, Dewsbury), James Miller (Royal Victoria Hospital, Newcastle), Anne Nicholls (West Suffolk Hospital, Bury St. Edmunds), Jennifer Nixon (Countess of

Chester Hospital, Chester), Voon Ong (Royal Free Hospital, London), Katherine Over (Countess of Chester Hospital, Chester), John Packham (Staffordshire Rheumatology Centre, Stoke on Trent), Nicolo Pipitone (King's College Hospital, London), Michael Plant (South Cleveland Hospital, Middlesborough), Gillian Pountain (Hinchingbrooke Hospital, Huntington), Thomas Pullar (Ninewells Hospital, Dundee), Mark Roberts (Salford Royal Foundation Trust), Paul Sanders (Wythenshawe Hospital, Manchester), David Scott (King's College Hospital, London), David Scott (Norfolk & Norwich University Hospital, Norwich), Michael Shadforth (Staffordshire Rheumatology Centre, Stoke on Trent), Thomas Sheeran (Cannock Chase Hospital, Cannock, Staffordshire), Arul Srinivasan (Broomfield Hospital, Chelmsford), David Swinson (Wrightington Hospital), Lee-Suan Teh (Royal Blackburn Hospital, Blackburn), Michael Webley (Stoke Manderville Hospital, Aylesbury), Brian Williams (University Hospital of Wales, Cardiff), and Jonathan Winer (Queen Elizabeth Hospital, Birmingham).

UK Juvenile Dermatomyositis Research Group

Dr. Kate Armon, Mr. Joe Ellis-Gage, Ms. Holly Roper, Ms. Vanja Briggs, and Ms. Joanna Watts (Norfolk and Norwich University Hospitals); Dr. Liza McCann, Mr. Ian Roberts, Dr. Eileen Baildam, Ms. Louise Hanna, Ms. Olivia Lloyd and Ms Susan Wadeson (The Royal Liverpool Children's Hospital, Alder Hey, Liverpool); Dr. Phil Riley and Ms. Ann McGovern (Royal Manchester Children's Hospital, Manchester); Dr. Clive Ryder, Mrs. Janis Scott, Mrs. Beverley Thomas, Professor Taunton Southwood, Dr. Eslam Al-Abadi and Lisa Charles (Birmingham Children's Hospital, Birmingham); Dr. Sue Wyatt, Mrs. Gillian Jackson, Dr. Tania Amin, Dr. Mark Wood, Dr. Vanessa Van Rooyen, and Ms. Deborah Burton (Leeds General Infirmary, Leeds); Dr. Joyce Davidson, Dr. Janet Gardner-Medwin, Dr. Neil Martin, Ms. Sue Ferguson, Ms. Liz Waxman, and Mr. Michael Browne (The Royal Hospital for Sick Children, Yorkhill, Glasgow); Dr. Mark Friswell, Professor Helen Foster, Mrs. Alison Swift, Dr. Sharmila Jandial, Ms. Vicky Stevenson, Ms. Debbie Wade, Dr. Ethan Sen, Dr. Eve Smith, Ms. Lisa Qiao, Mr. Stuart Watson and Claire Duong (Great North Children's Hospital, Newcastle); Dr. Helen Venning, Dr. Rangaraj Satyapal, Mrs. Elizabeth Stretton, Ms. Mary Jordan, Dr. Ellen Mosley, Ms. Anna Frost, Ms. Lindsay Crate, Dr. Kishore Warrier and Ms Stefanie Stafford (Queens Medical Centre, Nottingham); Professor Lucy Wedderburn, Dr. Clarissa Pilkington, Dr. N. Hasson, Mrs. Sue Maillard, Ms. Elizabeth Halkon, Ms. Virginia Brown, Ms. Audrey Juggins, Dr. Sally Smith, Mrs. Sian Lunt, Ms. Elli Enayat, Mrs. Hemlata Varsani, Miss Laura Kassoumeri, Miss Laura Beard, Miss Katie Arnold, Mrs. Yvonne Glackin, Miss Stephanie Simou, Dr. Beverley Almeida, Dr Raquel Marques, Dr Claire Deakin and Ms Stefanie Dowle (Great Ormond Street Hospital, London); Dr. Kevin Murray (Princess Margaret Hospital, Perth, Western Australia); Dr. John Ioannou and Ms. Linda Suffield (University College London Hospital); Dr. Muthana Al-Obaidi, Ms. Helen Lee, Ms. Sam Leach, Ms. Helen Smith, Dr Anne-Marie McMahon, Ms Heather Chisem and Ruth Kingshott (Sheffield's Children's Hospital); Dr. Nick Wilkinson, Ms. Emma Inness, Ms. Eunice Kendall, Mr. David Mayers, Ruth Etherton and Dr Kathryn Bailey (Oxford University Hospitals); Dr Jacqui Clinch, Ms Natalie Fineman and Ms Helen Pluess-Hall (Bristol Royal Hospital for Children, Bristol); Ms Lindsay Vallance (Royal Aberdeen Children's Hospital); Ms Louise Akeroyd (Bradford Teaching Hospitals).

US Childhood Myositis Heterogeneity Study Group

The following members of the US Childhood Myositis Heterogeneity Study Group contributed to this study: Drs. Barbara S. Adams (University of Michigan, Ann Arbor, MI), Catherine A. Bingham (Hershey Medical Center, Hershey, PA), Gail D. Cawkwell (All Children's Hospital, St. Petersburg, FL), Terri H. Finkel (Children's Hospital of Philadelphia, Philadelphia, PA), Steven W. George (Ellicott City, MD), Harry L. Gewanter (Richmond, VA), Ellen A. Goldmuntz (Children's National Medical Center, Washington, DC), Donald P. Goldsmith (St. Christopher's Hospital for Children, Philadelphia, PA), Michael Henrickson (Children's Hospital, Madera, CA), Lisa Imundo (Columbia University, New York, NY), Ildy M. Katona (Uniformed Services University, Bethesda, MD), Carol B. Lindsley (University of Kansas, Kansas City), Chester P. Oddis (University of Pittsburgh, Pittsburgh, PA), Judyann C. Olson (Medical College of Wisconsin, Milwaukee), David Sherry (Children's Hospital of Philadelphia, Philadelphia, PA), Scott A. Vogelgesang (Walter Reed Army Medical Center, Washington, DC), Carol A. Wallace (Children's Medical Center, Seattle, WA), Patience H. White (George Washington University, Washington, DC), and Lawrence S. Zemel (Connecticut Children's Hospital, Hartford).

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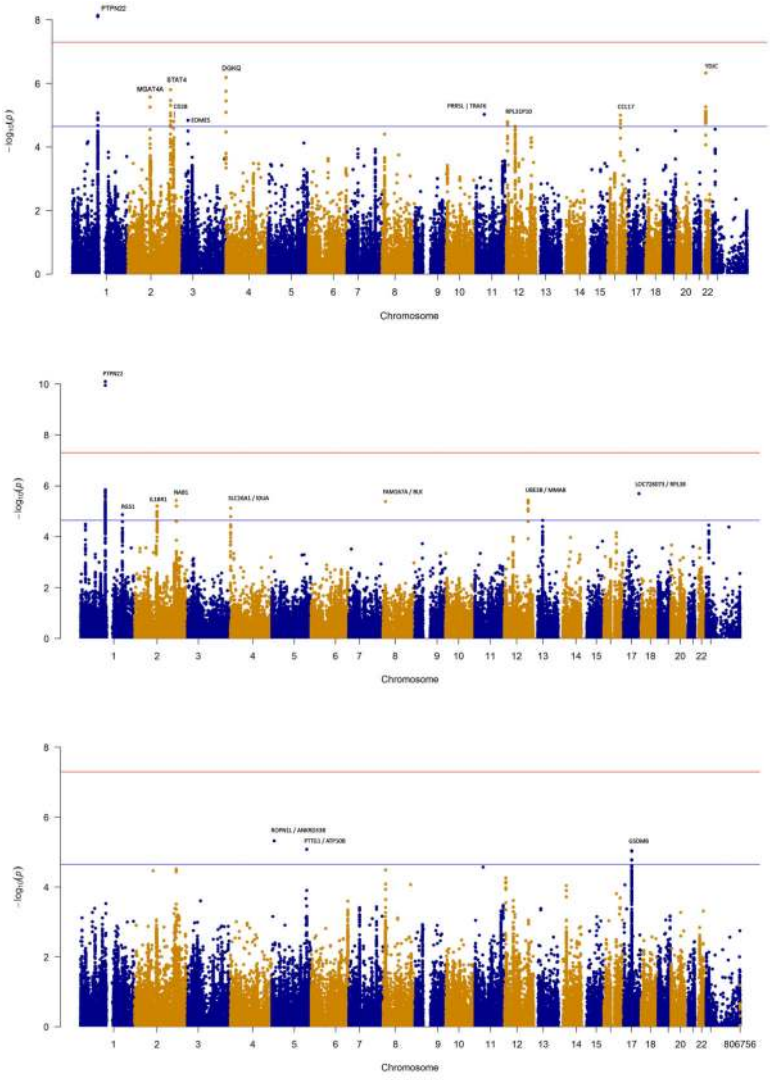

Figure 1. Manhattan plots of the IIM, PM and DM+JDM analyses, with the MHC region removed

The red and blue lines represent genome-wide level of significance ($p=5\times10^{-8}$) and suggestive significance ($p=2.25\times10^{-5}$) respectively. A) Analysis of 2,566 IIM cases and 15,651 controls. B) Analysis of 931PM cases and 15,651 controls. C) Analysis of 1,360 DM +JDM cases and 15,651 controls.

Table 1

Number of IIM cases included in the analysis.

Number of Cases		Number of Controls	
Australia	120	-	
Belgium	12	Belgium	351
Czech Republic	236	Poland	526
Denmark	53	-	
France	37	France	497
Hungary	209	Hungary Germany	257 1029
Italy	37	Italy Italy (RAF cohort)	969 813
Netherlands	38	Netherlands	2020
Norway	63	Norway	730
Sweden	269	Sweden	1938
Spain	73	Spain	409
Switzerland	3	-	
United Kingdom	993	United Kingdom	4332
United States	423	United States	1780
Total	***2,566***	***Total***	***15,651***

Number of IIM cases after QC, by country of origin. Control samples shared from Immunochip consortia matched by closest ethnicity.

Table 2

Loci associated with IIM, PM, DM and JDM cases

Subgroup	Gene Region	Chr	Position	Most Significant SNP	Minor Allele	MAF cases	MAF controls	P-value	OR (95% CI)	Localisation of LD to nearest genes (r2 ≥0.9)	Overlap with other autoimmune diseases
IIM	**HLA**	**6**	**32395726**	**rs3129843**	**G**	**0.24**	**0.11**	**9.14×10^{-133}**	**2.74 (2.53–2.97)**	**MHC**	**Multiple**
IIM	**PTPN22**	**1**	**114377568**	**rs2476601**	**A**	**0.12**	**0.09**	**7.22×10^{-9}**	**1.32 (1.20–1.45)**	**Exon 12 of PTPN22**	**ATD,**[17] **IBD,**[16] **JIA,**[18] **RA,**[19] **SLE,**[20] **SSC,**[21] **T1D,**[22] **VIT,**[23]
IIM	YDJC	22	21985094	rs5754467	G	0.23	0.20	4.67×10^{-7}	1.21 (1.12–1.30)	Intron 1 of UBE2L3 | complete YDJC	CEL,[6] IBD,[16] JIA,[18] PSO,[24] RA,[19] SLE,[20]
IIM	DGKQ	4	956047	rs6599390	A	0.30	0.34	6.48×10^{-7}	0.85 (0.79–0.90)	Intron 21 of DGKQ to intron 2 of SLC26A1 and intron 2 of IDUA	
IIM	STAT4	2	191917317	rs4853540	T	0.19	0.22	1.57×10^{-6}	0.83 (0.77–0.89)	Intron 3 to intron 14 of STAT4	JIA,[18] PBC,[25] RA,[19] SLE,[20]
IIM	MGAT4A	2	99389870	rs10189330	T	0.50	0.46	2.68×10^{-6}	1.16 (1.09–1.23)	Intergenic of C2orf55 (KIAA1211L) and MGAT4A incorporating uncharacterized LOC101927070	
IIM	PRR5L | TRAF6	11	36492191	rs570676	A	0.37	0.40	9.42×10^{-6}	0.87 (0.82–0.92)	Intergenic PRR5L (also known as FLJ14213) | complete TRAF6	
IIM	CCL17	16	57445376	rs223900	T	0.28	0.25	9.97×10^{-6}	1.17 (1.09–1.25)	Intron 1 of CCL17	
IIM	EOMES	3	28076283	rs376072	T	0.26	0.29	1.45×10^{-5}	0.86 (0.80–0.92)	310.68kb upstream of EOMES	
IIM	CD28	2	204592021	rs3116494	G	0.28	0.26	1.54×10^{-5}	1.16 (1.09–1.24)	Intron 1 to 8.5kb downstream of CD28	PSC,[26] RA,[19]

Subgroup	Gene Region	Chr	Position	Most Significant SNP	Minor Allele	MAF cases	MAF controls	P-value	OR (95% CI)	Localisation of LD to nearest genes (r2 ≥0.9)	Overlap with other autoimmune diseases
IIM	RPL31P10	12	6523249	rs11064180	T	0.38	0.41	1.61×10^{-5}	0.87 (0.82–0.93)	Intergenic of LTBR and CD27	
PM	**HLA**	**6**	**31434366**	**rs3094013**	**T**	**0.25**	**0.12**	**6.36×10^{-76}**	**2.97 (2.64–3.33)**	**MHC**	**Multiple**
PM	**PTPN22**	**1**	**114377568**	**rs2476601**	**A**	**0.12**	**0.09**	**7.90×10^{-11}**	**1.58 (1.38–1.81)**	**Exon 12 of PTPN22**	**ATD,**[17] **IBD,**[16] **JIA**[18], **RA,**[19] **SLE,** [20] **SSC,**[21] **T1D,**[22] **VIT,**[23]
PM	LOC728073 | RPL38	17	71527243	rs9905921	C	0.48	0.43	2.01×10^{-6}	1.26 (1.14–1.39)	Intronic (provisional in refseq) of SDK2	
PM	UBE3B | MMAB	12	109980516	rs7956536	C	0.44	0.46	3.66×10^{-6}	0.80 (0.72–0.88)	Intron 19 of MYO1H, complete KCTD10; UBE3B; MMAB; MVK	
PM	NAB1	2	191535576	rs2286896	G	0.15	0.13	3.76×10^{-6}	1.35 (1.19–1.53)	Complete NAB1	
PM	FAM167A | BLK	8	11333521	rs17799348	T	0.36	0.39	4.13×10^{-6}	0.80 (0.71–0.87)	Intergenic FAM167A | BLK	
PM	IL18R1	2	103012902	rs1420095	G	0.07	0.09	6.16×10^{-6}	0.63 (0.52–0.78)	Intron 3 of IL18R1 : 1L18RAP	
PM	SLC26A1 | IDUA	4	980464	rs4690220	G	0.49	0.45	7.47×10^{-6}	1.25 (1.13–1.37)	2.14 Kb 3' to intron 2 of SLC26A1	SJO,[27]
PM	RGS1	1	192545099	rs7535818	G	0.17	0.18	1.37×10^{-5}	0.74 (0.65–0.85)	25kb upstream to intron 1 of RGS1	CEL,[6] MS,[28] T1D,[22]
DM+JDM	**HLA**	**6**	**32395726**	**rs3129843**	**G**	**0.21**	**0.11**	**1.72×10^{-48}**	**2.18 (1.97–2.42)**	**MHC**	**Multiple**
DM+JDM	ROPN1L | ANKRD33B	5	10517908	rs4702698	G	0.34	0.30	4.77×10^{-6}	1.22 (1.12–1.33)	Intergenic of ROPN1L and ANKRD33B	
DM+JDM	PTTG1 | ATP10B	5	159928876	rs4921293	G	0.39	0.35	8.27×10^{-6}	1.21 (1.11–1.31)	Intergenic of PTTG1 and ATP10B	
DM+JDM	GSDMB	17	38066267	rs1008723	T	0.47	0.49	9.05×10^{-6}	1.20 (1.11–1.30)	Complete IKZF3, ZPBP2 GSDMB	IBD,[16] MS,[28] PBC, [25] RA,[29] T1D,[30]

Analysis of 2,566 IIM cases, 931 PM cases, and 1,360 DM and JDM cases, compared to 15,651 controls. Loci reported at genome wide significance p $<5\times10^{-8}$ (bold) or at suggestive significance p $<2.25\times10^{-5}$. Coordinates based on GRCh37 assembly. Overlap defined as high LD (r^2 >0.7) between the associations in IIM (including independent effects) and the lead association in another autoimmune disease (as reported on www.immunobase.org). ATD – Autoimmune Thyroid Disease, CEL – Celiac Disease, Chr – chromosome, IBD – Inflammatory Bowel Disease, JIA – Juvenile Idiopathic Arthritis, MAF – minor allele frequency, MS – Multiple Sclerosis, OR – Odds ratio, PBC – Primary Biliary Cirrhosis, PSC - Primary Sclerosing Cholangitis, PSO – Psoriasis, RA – Rheumatoid Arthritis, SJO - Sjogren Syndrome, SLE – Systemic Lupus Erythematosus, SSC - Systemic Scleroderma, T1D – Type 1 Diabetes, VIT – Vitiligo, 95% CI – 95% confidence interval

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Table 3

Potentially causal exonic SNPs.

Subgroup	Mapped Genes	Most Significant SNP	SNP in LD	SNP Location	Amino acid change	PolyPhen-2/SIFT	phastCons17-way
IIM + PM	PTPN22	rs2476601	rs2476601	Non-synonymous coding in PTPN22	R620W	Benign/tolerated	0.99
IIM	DGKQ	rs6599390	rs3796622	Non-synonymous coding SLC26A1	Q556R	Benign/tolerated	0.35
PM	UBE3B | MMAB	rs7956536	rs7298565	Non-synonymous coding UBE3B	R346Q	Benign/tolerated	1
			rs9593	Non-synonymous coding in MMAB	M239K	Benign/tolerated	0.17
DM+JDM	GSDMB	rs1008723	rs11078928	Splice acceptor variant in GSDMB			0
			rs2305480	Non-synonymous coding GSDMB	P298S	Possibly damaging/Tolerated	0
			rs2305479	Non-synonymous coding GSDMB	G291R	Probably damaging/Tolerated	0

SNPs investigated for functionality may be the lead association or any SNP with $r^2 \geq 0.9$. PolyPhen-2,[13] and SIFT,[14] was used to predict the possible impact of an amino acid substitution. phastCons17-way,[15] gives a score of evolutionary conservation in 17 vertebrates, with 1 being the most conserved at that base position.

Table 4

Evidence of expression quantitative trait loci (eQTL)

Subgroup	Mapped Genes	Immunochip lead SNP	eQTL within r^2 >0.9	References
IIM	*UBE2L3* | *YDJC*	rs11089637	*UBE2L3*, *RIMBP3*	[32] [33]
PM	*UBE3B* | *MMAB*	rs7956536	*KCTD10*, *MMAB*	[32] [33] [34]
PM	*RGS1*	rs7535818	*RGS1*	[32]
PM	*NAB1*	rs2286896	*STAT1*	[35]
DM+JDM	*GSDMB*	rs1008723	*ORMDL3, MED24, KRT222, NR1D1, GSDMB*	[34] [35] [36] [33]

eQTLs for the expression of a gene in cells with an immune function (Lymphoblastoid and Monocytes). RegulomeDB,[37] Genevar,[38] and the Pritchard lab eQTL browser (http://eqtl.uchicago.edu/cgi-bin/gbrowse/eqtl/) were interrogated. The most significant SNP or any SNP with r^2 ≥0.9 with most significant SNP was used. Genes are indicated along with studies in which the eQTLs were reported.