Immune-Array Analysis in Sporadic Inclusion Body Myositis Reveals HLA–DRB1 Amino Acid Heterogeneity Across the Myositis Spectrum


Objective. Inclusion body myositis (IBM) is characterized by a combination of inflammatory and degenerative changes affecting muscle. While the primary cause of IBM is unknown, genetic factors may influence disease susceptibility. To determine genetic factors contributing to the etiology of IBM, we conducted the largest genetic association study of the disease to date, investigating immune-related genes using the Immunochip.

The views expressed herein are those of the authors and not necessarily those of the (UK) National Health Service (NHS), the NIHR, or the (UK) Department of Health.

Supported by the NIHR (Biomedical Research Unit Funding Scheme) and the University College London Hospitals Biomedical Research Centre. Supported in part by the Association Française Contre Les Myopathies, the European Science Foundation (Research Networking Programme European Myositis Network), the Swedish Research Council, the regional agreement on medical training and clinical research between Stockholm County Council and Karolinska Institutet, the Swedish Research Council, Myositis UK, Arthritis Research UK (grant 18474), and Primes Beatrix Sfierpions (project W.0.R12-15). Dr. Rothwell’s work was supported by the Wellcome Trust (grant 105610/Z/14/Z). Dr. Machado’s work was supported by the NIHR (Rare Diseases Translational Research Collaboration Post-Doctoral Fellowship). The Czech cohort was supported by the Ministry of Health, Czech Republic (Project for Conceptual Development of Research Organization grant 00023728).

1 Simon Rothwell, PhD, John Bowes, PhD, Hazel Platt, BSc, William E. Ollier, PhD, Janine A. Lamb, PhD: University of Manchester, Manchester, UK; 2 Robert G. Cooper, MD: University of Liverpool, Liverpool, UK; 3 Ingrid E. Lundberg, MD: Karolinska University Hospital, Karolinska Institutet, Stockholm, Sweden; 4 Peter K. Gregersen, MD, Annette T. Lee, PhD: Feinstein Institute for Medical Research, Manhasset, New York; 5 Michael G. Hanna, MD, Pedro M. Machado, MD, PhD: University College London, London, UK; 6 Megan K. Herbert, PhD: Beth Israel Deaconess Medical Center, Boston, Massachusetts, and Radboud University Nijmegen, Nijmegen, The Netherlands; 7 Ger J. M. Prujin, PhD: Radboud University Nijmegen, Nijmegen, The Netherlands; 8 James B. Lilleker, MD: University of Manchester, Manchester, UK, and Salford Royal NHS Foundation Trust, Salford, UK; 9 Mark Roberts, MD: Salford Royal NHS Foundation Trust, Salford, UK; 10 Michael F. Seldin, MD: University of California, Davis; 11 Jiri Vencovsky, MD: Charles University, Prague, Czech Republic; 12 Katalin Danko, MD: University of Debrecen, Debrecen, Hungary; 13 Vidya Limaye, PhD, FRACP: Royal Adelaide Hospital, Adelaide, South Australia, Australia; 14 Albert Selva-O’Callaghan, MD: Vall d’Hebron General Hospital, Barcelona, Spain; 15 Øyvind Molberg, MD: University of Oslo, Oslo, Norway; 16 Olivier Benveniste, MD: Hôpital Pitie-Salpêtrière, UPMC, Paris, France; 17 Timothy R. D. J. Radstake, MD: University Medical Center Utrecht, Utrecht, The Netherlands; 18 Andrea Doria, MD: University of Padova, Padua, Italy; 19 Jan De Bleecker, MD, Boel De Paepe, PhD: Ghent University Hospital, Ghent, Belgium; 20 Christian Gieger, PhD: Helmholtz Zentrum München, Neuherberg, Germany; 21 Thomas Meitinger, MD, Juliane Winkelmann, MD: Technische Universität München, Munich, Germany, and Helmholtz Zentrum München, Neuherberg, Germany; 22 Christopher I. Amos, PhD: Dartmouth College, Hanover, New Hampshire; 23 Leonid Padyukov, MD: Karolinska Institutet, Stockholm, Sweden; 24 Hector Chinoy, MD: Central Manchester University Hospitals NHS Foundation Trust, University of Manchester, Manchester, UK.

Drs. Lamb and Chinoy contributed equally to this work. Address correspondence to Simon Rothwell, PhD, Centre for Musculoskeletal Research, University of Manchester, 2.808 Stopford Building, Oxford Road, Manchester M13 9PT, UK. E-mail: s.rothwell@manchester.ac.uk.

Submitted for publication September 12, 2016; accepted in revised form January 10, 2017.
Methods. A total of 252 Caucasian patients with IBM were recruited from 11 countries through the Myositis Genetics Consortium and compared with 1,008 ethically matched controls. Classic HLA alleles and amino acids were imputed using SNP2HLA.

Results. The HLA region was confirmed as the most strongly associated region in IBM ($P = 3.58 \times 10^{-33}$). HLA imputation identified 3 independent associations (with HLA–DRB1*03:01, DRB1*01:01, and DRB1*13:01), although the strongest association was with amino acid positions 26 and 11 of the HLA–DRB1 molecule. No association with anti-cytosolic 5'-nucleotidase 1A–positive status was found independent of HLA–DRB1*03:01. There was no association of HLA genotypes with age at onset of IBM. Three non-HLA regions reached suggestive significance, including the chromosome 3 p21.31 region, an established risk locus for autoimmune disease, where a frameshift mutation in CCR5 is thought to be the causal variant.

Conclusion. This is the largest, most comprehensive genetic association study to date in IBM. The data confirm that HLA is the most strongly associated region and identifies novel amino acid associations that may explain the risk in this locus. These amino acid associations differentiate IBM from polymyositis and dermatomyositis and may determine properties of the peptide-binding groove, allowing it to preferentially bind autoantigenic peptides. A novel suggestive association within the chromosome 3 p21.31 region suggests a role for CCR5.

Sporadic inclusion body myositis (IBM) is an acquired muscle disease characterized clinically by weakness and muscle wasting, predominantly of the quadriceps and long finger flexor muscles. While degenerative changes are recognized, there are also immune-mediated mechanisms at play, characterized by inflammatory features in muscle biopsy specimens and the presence of circulating autoantibodies. These autoantibodies include anti-Ro and a recently identified autoantibody directed against cytosolic 5'-nucleotidase 1A (anti-cN1A), which is present in approximately one-third of patients (1,2). While the primary cause of the disease remains unknown, genetic factors may influence disease susceptibility. A group of hereditary diseases that includes the hereditary inclusion body myopathies and other muscular dystrophies such as the myofibribillary myopathies may mimic clinical features of IBM (3). These diseases may also exhibit similar pathologic features, such as rimmed vacuoles and protein accumulations; clinical and histopathologic suspicion of these diseases should prompt appropriate genetic testing.

To date, the strongest genetic risk identified for IBM lies within the major histocompatibility complex (MHC), in particular with HLA–DRB1*03:01, an allele present on the 8.1 ancestral haplotype that is a risk factor for many autoimmune diseases, including the idiopathic inflammatory myopathies (IIMs) (4). Other HLA–DRB1 alleles such as HLA–DRB1*01:01 and HLA–DRB1*13:01 have also been implicated in IBM, and genotypic combinations of these alleles have been reported to correlate with clinical phenotype (5).

Candidate gene studies in IBM have focused mainly on the MHC, and there are few validated associations outside of this region. Genes associated with neurodegenerative diseases such as Alzheimer’s Disease have been examined in IBM, for example, the genes for β/4-amylolytic precursor protein and apolipoprotein E, although these studies frequently have shown negative or conflicting results (6–8). Other candidate gene approaches have focused on autoantibody targets (1) and genes previously implicated in hereditary inclusion body myopathies (9,10). However, these studies also have failed to find significant common associations.

We recently reported a genetic association study in polymyositis (PM) and dermatomyositis (DM) using the Immunochip array, a custom-designed, high-density genotyping chip that covers genes known to be associated with a variety of autoimmune diseases (11). Samples from patients with IBM were genotyped concurrently and were analyzed separately in this analysis using a previously described method of case–control matching to control for population differences. Using the Immunochip, we have conducted the largest genetic study to date in IBM to investigate potential associations with immune-related genes, and we have used imputation to refine associations within the MHC.

Patients and Methods

Study populations. A total of 252 patients with IBM from 11 countries were recruited through the Myositis Genetics Consortium (MYOGEN). A list of MYOGEN study investigators in addition to the authors of this article is provided in Appendix A. Written informed consent was obtained from all patients with approval from research ethics boards at each participating center. Patients with IBM were included if they fulfilled the following criteria: Griggs (“definite” or “possible”) (12), Medical Research Council (“pathologically defined,” “clinically defined,” or “possible”) (13), or European Neuromuscular Centre (“clínico-pathologically defined,” “clinically defined,” or “probable”) (14). Age at onset for UK patients with IBM was the age at onset of first symptoms as recorded in the clinical record.

Shared control samples from Sweden (the Epidemiological Investigation of Rheumatoid Arthritis study), Spain, and The Netherlands were provided by the Rheumatoid Arthritis Consortium International (15), with control samples from the UK provided by the Wellcome Trust Case Control Consortium (16). Control samples from Italy, Norway, Belgium, and France were provided by the International Multiple Sclerosis Genetics Consortium (17). Polish and Hungarian control samples were provided by the Celiac Consortium (18), and German control samples were provided by the KORAgen consortium (19).
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, Manchester, UK) and the US (Feinstein Institute for Medical Research, Manhasset, NY). Standard quality control was performed as described previously (11). Four controls for each case were matched for ethnicity using principal components analysis coordinates using a method described previously (20).

Statistical analysis. Statistical analyses were performed in Plink version 1.7 (http://zzz.bwh.harvard.edu/plink/index.shtml) using a logistic regression applying an additive model. Sex and population differences were controlled for by including sex and the top 10 principal components as covariates. Significance was defined as $P < 5 \times 10^{-8}$. We also reported variants reaching a second tier of significance of $P < 2.25 \times 10^{-5}$, calculated using the genetic Type I Error Calculator (21). Odds ratios (ORs) are provided with 95% confidence intervals (95% CIs).

To investigate associations with HLA and age at onset, linear regressions were used, with $P$ values less than 0.05 considered significant. Analyses were carried out using Stata statistical software version 13.1 (StataCorp).

Table 1. Numbers of samples from patients with inclusion body myositis and ethnically matched controls included in the analysis after quality control, by country of origin*

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td>44</td>
<td>–</td>
</tr>
<tr>
<td>Belgium</td>
<td>6</td>
<td>23</td>
</tr>
<tr>
<td>Czech Republic</td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td>France</td>
<td>19</td>
<td>35</td>
</tr>
<tr>
<td>Germany</td>
<td>–</td>
<td>29</td>
</tr>
<tr>
<td>Hungary</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Italy</td>
<td>2</td>
<td>44</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>9</td>
<td>69</td>
</tr>
<tr>
<td>Norway</td>
<td>1</td>
<td>30</td>
</tr>
<tr>
<td>Poland</td>
<td>–</td>
<td>8</td>
</tr>
<tr>
<td>Sweden</td>
<td>31</td>
<td>97</td>
</tr>
<tr>
<td>Spain</td>
<td>8</td>
<td>28</td>
</tr>
<tr>
<td>UK</td>
<td>128</td>
<td>485</td>
</tr>
<tr>
<td>US</td>
<td>–</td>
<td>153</td>
</tr>
<tr>
<td>Total</td>
<td>252</td>
<td>1,008</td>
</tr>
</tbody>
</table>

* Control samples were shared from Immunochip consortia. Four controls for each patient were matched based on nearest neighbor by principal components analysis coordinates.

Figure 1. Manhattan plots of the inclusion body myositis (IBM) analysis. Red line represents genome-wide level of significance ($P < 5 \times 10^{-8}$); blue line represents suggestive significance ($P < 2.25 \times 10^{-5}$). Shown is the analysis of 252 patients with IBM and 1,008 matched controls. A, Manhattan plot of the total Immunochip analysis. B, Manhattan plot of the IBM analysis with the major histocompatibility complex (MHC) region (chromosome 6 25–35) removed for visualization purposes.
Functional annotation. Evidence for functional effects and expression quantitative trait loci (eQTLs) were investigated for the lead single-nucleotide polymorphisms (SNPs) in each region, and SNPs in high linkage disequilibrium (LD) ($r^2 \geq 0.8$) were obtained from Phase 3 1000 Genomes data using LDlink (22).

MHC imputation and association analysis. Classic HLA alleles and corresponding amino acid sequences were imputed from ImmuneChip SNP data using the SNP2HLA program as described previously (11). Significance was defined as $P < 6.8 \times 10^{-6}$ based on a Bonferroni correction of the 7,323 markers imputed by SNP2HLA (23). For consistency, the most associated variant was used in the stepwise conditional analysis. Molecular graphics were generated and analyses were performed with the University of California, San Francisco (UCSF) Chimera package version 1.10.2 (Resource for Biocomputing, Visualization, and Informatics at UCSF).

Anti-cN1A detection. Enzyme-linked immunosorbent assay detection of anti-cN1A antibodies was performed using the optimized protocol as described previously (24). Briefly, biotinylated peptides were incubated on Streptawell High Bind microplates (Roche) for 1 hour at 37°C to immobilize the peptides. Unbound peptides were removed by washing the microplates 3 times. Diluted patient serum was then added to the microplate followed by incubation at 37°C for 1 hour. Unbound antigen was removed by further washing the microplate 5 times. Diluted rabbit anti-human Ig was then added, and the plate was incubated for 1 hour at 37°C followed by a further 5 washes. Finally, the bound antibodies were visualized by adding substrate solution, and the reaction was stopped after 5 minutes by adding a stop solution. Signals were quantified by determining optical densities at 450 nm (OD$_{450}$). The OD$_{450}$ value corresponding to the highest Youden Index [calculated as: $\text{[specificity/100]} + \text{[sensitivity/100]} - 1$] (25) at which $\geq 98\%$ specificity was achieved was chosen for each peptide. Sera were assessed as reactive if they were above the established cutoff value for at least one of the peptide antigens (24).

RESULTS

Genotyping quality control. After stringent SNP and sample quality control, we analyzed 104,636 genetic variants in 252 patients with IBM and 1,008 ethnically matched controls (Table 1). Including the top 10 principal components as covariates and calculating the genomic inflation on a set of null SNPs gave a $\lambda_{GC}$ of 1.04, indicating that patients and controls were well matched for ethnicity (see Supplementary Figure 1, available on the Arthritis & Rheumatology web site at http://onlinelibrary.wiley.com/doi/10.1002/art.40045/abstract).

HLA is the most strongly associated region in IBM. SNPs within the MHC region were the only variants reaching genome-wide significance of $P < 5 \times 10^{-8}$ (Figure 1A). The strongest association was with rs3129950 ($P = 3.58 \times 10^{-33}$), a SNP intronic of LOC101929163 and 3’ of BTNL2 (Table 2). As the Immunochip contains high-density SNP coverage across the MHC, this region was subsequently analyzed separately using HLA imputation in an attempt to refine this association to a functional gene. Initially, genes independent of the MHC were investigated that reached a suggestive level of significance.

Suggestive significance of 3 non-HLA associations. Three non-HLA regions reached our second tier of significance (defined as $P < 2.25 \times 10^{-5}$) calculated using the genetic Type I Error Calculator (21). Figure 1B shows the Manhattan plot with the MHC region removed for visualization purposes. The most convincing associations were in the chromosome 3 p21.31 region, where 49 SNPs reached the suggestive level of significance (Figure 2). The strongest association was upstream of CCR2 (rs112088397, $P = 1.93 \times 10^{-6}$; OR 0.42 [95% CI 0.29–0.60]); however, this haplotype contains many genes including CCR3, CCR2, CCR5, and CCRL2.

Potentially functional variants were investigated for all non-HLA SNPs reaching suggestive significance and those tagged by the lead SNP ($r^2 \geq 0.8$) (see Supplementary Table 2. Analysis of the 252 patients with inclusion body myositis compared to 1,008 ethnically matched controls*

<table>
<thead>
<tr>
<th>Gene region</th>
<th>Chr.</th>
<th>Position</th>
<th>SNP</th>
<th>Minor allele</th>
<th>MAF in patients</th>
<th>MAF in controls</th>
<th>$P$ (95% CI)</th>
<th>OR (95% CI)</th>
<th>Localization of LD to nearest genes ($r^2 \geq 0.9$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHC</td>
<td>6</td>
<td>32358201</td>
<td>rs3129950 T 0.08 0.16 1.93</td>
<td>5.69 (4.28–7.55)</td>
<td>0.42 (0.29–0.60)</td>
<td>Downtstream of CCR3 to intron 2 of LTF; incorporating CCR2, CCR5, and CCRL2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCR3/CCR2</td>
<td>3</td>
<td>46389462</td>
<td>rs112088397 C 0.34 0.11 4.03</td>
<td>1.60 (1.31–1.96)</td>
<td>2.23 (1.57–3.17)</td>
<td>Intergenic of FLJ14816 and GLI2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FLJ14816/GLI2</td>
<td>2</td>
<td>12133858</td>
<td>rs1880542 T 0.08 0.16 1.93</td>
<td>5.69 (4.28–7.55)</td>
<td>0.42 (0.29–0.60)</td>
<td>Downtstream of CCR3 to intron 2 of LTF; incorporating CCR2, CCR5, and CCRL2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOC100128517/LOC728961</td>
<td>6</td>
<td>14560180</td>
<td>rs9396510 T 0.11 0.05 7.52</td>
<td>1.60 (1.31–1.96)</td>
<td>2.23 (1.57–3.17)</td>
<td>Intergenic of LOC100128517 and LOC728961</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Coordinates are based on the human assembly GRCh37. Chr. = chromosome; SNP = single-nucleotide polymorphism; MAF = minor allele frequency; OR = odds ratio; 95% CI = 95% confidence interval; LD = linkage disequilibrium.
† Reported at genome-wide significance ($P \leq 5 \times 10^{-8}$).
‡ Reported at second tier of significance ($P \leq 2.25 \times 10^{-5}$).
Tables 1–3, http://onlinelibrary.wiley.com/doi/10.1002/art.40045/abstract). Multiple SNPs within the chromosome 3 p21.31 region had evidence for eQTLs for the expression of CCR5 in monocytes (26). There was 1 missense SNP in the chromosome 3 p21.31 region (rs6441977) that was predicted to be “benign” by PolyPhen-2 (27); however, a frameshift mutation (rs333) is a known variant that results in a 32-bp deletion and a nonfunctional receptor. Conditional analysis on this locus did not identify additional independent variants.

HLA imputation reveals association with HLA–DRB1. To refine associations within the MHC region, HLA alleles were imputed from SNP genotyping information using SNP2HLA (23). Variants reaching statistical significance \((P < 6.8 \times 10^{-6})\) after each round of conditioning are included in Supplementary Tables 4–6, http://onlinelibrary.wiley.com/doi/10.1002/art.40045/abstract. The strongest associations were with alleles that are part of the 8.1 ancestral haplotype, with HLA–DRB1*03:01 reaching \(P = 5.77 \times 10^{-34}\) (OR 7.97 [95% CI 5.88–10.95]). Due to strong LD within the MHC, other alleles within this haplotype such as HLA–DQB1*02:02 and HLA–B*08:01 also had strong associations in this analysis; however, these lost significance after conditioning on the effect of HLA–DRB1*03:01. Conducting stepwise conditional analysis on significant HLA–DRB1 alleles, an independent effect was seen with HLA–DRB1*01:01 \((P = 1.56 \times 10^{-16}, \text{OR} 4.64 [95\% \text{ CI} 3.33–6.49])\). A further independent effect was seen with HLA–DRB1*13:01 \((P = 3.28 \times 10^{-13}, \text{OR} 3.19 [95\% \text{ CI} 2.14–4.72])\).

As risk may lie within multiple HLA alleles, we imputed amino acids to investigate whether shared positions within risk alleles might explain the risk at this locus (see Supplementary Tables 7 and 8, http://onlinelibrary.wiley.com/doi/10.1002/art.40045/abstract). Amino acid position 26 of HLA–DRB1 was associated more significantly than a single classic HLA allele \((P = 5.22 \times 10^{-33})\), with risk attributable to a tyrosine at this position (OR 3.83 [95% CI 2.80–5.29]) (Table 3). This contrasts with PM and DM, where HLA–DRB1 amino acid position 77 was the most strongly associated position (Table 3). Conditioning on the effects of position 26 in IBM revealed an independent effect of position 11 of HLA–DRB1 \((P = 3.80 \times 10^{-13})\). At this position, serine is the most common amino acid in the population and was therefore used as the reference. As many other amino acids are protective at this position, we can infer that serine confers the greatest risk. No further amino acid positions were statistically significant after conditioning on positions 26 and 11 of HLA–DRB1.
patients with 68 anti-cN1A–negative patients, and we found no significant differences in HLA associations between these groups (data not shown).

No distinct HLA association with anti-cN1A positivity. Anti-cN1A antibodies were detected in 36 of the 104 patients serologically tested (35%). After quality control, HLA imputation was conducted on 35 anti-cN1A–positive patients and 140 healthy controls, and the most significant 4-digit classic HLA association was found with HLA–DRB1*03:01 (P = 5.62 × 10^{-8}, OR 11.52 [95% CI 4.95–29.29]) (see Supplementary Table 9, http://onlinelibrary.wiley.com/doi/10.1002/art.40045/abstract). After stepwise conditional analysis, we did not find any additional independent associations of age at onset with HLA–DRB1*01:01 and HLA–DRB1*13:01, suggesting that the HLA–DRB1 gene is important in susceptibility to IBM. In contrast to previous studies, there were no significant associations of age at onset with HLA–DRB1 alleles. Other reported disease-modifying effects of HLA alleles in IBM, such as with disease severity and lower quadriiceps muscle strength, were not investigated due to a lack of consistent clinical data across this multinational, multicenter study. Previous studies have also investigated additional risk factors present on the MHC, such as polymorphisms in the gene for Notch-4 (29) or carriage of secondary HLA–DRB loci such as HLA–DRB3 (30). Although not explicitly investigated in the present study, these associations are in strong linkage with the 8.1 ancestral haplotype, and we do not expect our data to differentiate between these risk factors. Conditioning on the presence of HLA–DRB1, no additional genetic variants within the MHC region were associated with IBM. The frequency of genotypes among patients with IBM (see Supplementary Table 10, http://onlinelibrary.wiley.com/doi/10.1002/art.40045/abstract) suggests that patients homozygous for HLA–DRB1*03:01 and DRB1*01:01 are at lower risk of disease. The contribution of nonadditive effects across HLA alleles has been reported in several autoimmune diseases and may explain higher risk for heterozygote individuals (31). The small numbers in this cohort mean that the study is underpowered to statistically test this in IBM.

As multiple HLA–DRB1 alleles were associated with IBM, we investigated whether there were shared amino acid positions within HLA–DRB1 risk alleles that might explain the risk at this locus. Position 26 of HLA–DRB1 was more strongly associated than a classic HLA allele alone (P = 5.22 × 10^{-43} versus P = 5.77 × 10^{-34}). An additional independent effect was found.

### Table 3. Independent associations of HLA–DRB1 amino acids in clinical subgroups of idiopathic inflammatory myopathy

<table>
<thead>
<tr>
<th>Association, marker</th>
<th>Position 26</th>
<th>Position 11</th>
<th>Most associated in polymyositis‡</th>
<th>Most associated in dermatomyositis‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phenylalanine</td>
<td>Serine</td>
<td>Thrione</td>
<td>Threonine</td>
</tr>
<tr>
<td></td>
<td>Tyrosine</td>
<td>Proline</td>
<td>Asparagine</td>
<td>Asparagine</td>
</tr>
<tr>
<td></td>
<td>Leucine</td>
<td>Valine</td>
<td>Glycine</td>
<td>Leucine</td>
</tr>
<tr>
<td></td>
<td>Position</td>
<td>Omnibus</td>
<td>Reference†</td>
<td>Reference†</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>5.22 × 10^{-43}</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>3.80 × 10^{-13}</td>
<td>1</td>
<td>2.93 (2.53–3.17)</td>
</tr>
<tr>
<td></td>
<td>77</td>
<td>1.65 × 10^{-80}</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>77</td>
<td>1.37 × 10^{-36}</td>
<td>1</td>
<td>2.14 (1.90–2.41)</td>
</tr>
</tbody>
</table>

* P values and odds ratios (ORs) with 95% confidence intervals (95% CIs) were calculated in a logistic regression.
† Reference amino acid is taken as the most frequent in the population.
‡ For comparative purposes, HLA–DRB1 amino acid association statistics for polymyositis and dermatomyositis are shown (from ref. 11).

DISCUSSION

This is the largest genetic association study to date in Caucasian patients with IBM. The results confirm that HLA is the most strongly associated region, identify multiple HLA–DRB1 alleles conferring risk, and suggest amino acid positions that may explain the risk in this locus. A novel suggestive association within the chromosome 3 p21.31 locus indicates genetic overlap with other autoimmune diseases and identifies a potentially functional variant that may contribute to the pathogenesis of IBM.

HLA imputation confirmed that the strongest risk within this region lies with HLA–DRB1*03:01. Stepwise conditional analyses revealed additional independent associations with HLA–DRB1*01:01 and HLA–DRB1*13:01, suggesting that the HLA–DRB1 gene is important in susceptibility to IBM. In contrast to previous studies, there were no significant associations of age at onset with HLA–DRB1 alleles. Other reported disease-modifying effects of HLA alleles in IBM, such as with disease severity and lower quadriiceps muscle strength, were not investigated due to a lack of consistent clinical data across this multinational, multicenter study. Previous studies have also investigated additional risk factors present on the MHC, such as polymorphisms in the gene for Notch-4 (29) or carriage of secondary HLA–DRB loci such as HLA–DRB3 (30). Although not explicitly investigated in the present study, these associations are in strong linkage with the 8.1 ancestral haplotype, and we do not expect our data to differentiate between these risk factors. Conditioning on the presence of HLA–DRB1, no additional genetic variants within the MHC region were associated with IBM. The frequency of genotypes among patients with IBM (see Supplementary Table 10, http://onlinelibrary.wiley.com/doi/10.1002/art.40045/abstract) suggests that patients homozygous for HLA–DRB1*03:01 and DRB1*01:01 are at lower risk of disease. The contribution of nonadditive effects across HLA alleles has been reported in several autoimmune diseases and may explain higher risk for heterozygote individuals (31). The small numbers in this cohort mean that the study is underpowered to statistically test this in IBM.

As multiple HLA–DRB1 alleles were associated with IBM, we investigated whether there were shared amino acid positions within HLA–DRB1 risk alleles that might explain the risk at this locus. Position 26 of HLA–DRB1 was more strongly associated than a classic HLA allele alone (P = 5.22 × 10^{-43} versus P = 5.77 × 10^{-34}). An additional independent effect was found.
with position 11 of HLA–DRB1. Positions 11 and 26 have been associated previously with seropositive autoimmune disease, such as systemic lupus erythematosus (32), which suggests that these positions may determine properties of the HLA–DRB1 peptide-binding groove, allowing it to preferentially bind autoantigenic peptides (Figure 3). Certain amino acids, such as a tyrosine at position 26 and serine at position 11, were associated with risk in this analysis; however, a lack of statistical power means that we were unable to completely characterize the effects of certain amino acids in this molecule.

It is interesting to note that while IBM shares HLA–DRB1*03:01 as a significant risk factor with other inflammatory myopathies, such as PM and DM, the amino acid associations differ between these subtypes. In PM and DM, amino acid position 74 of HLA–DRB1 explains almost all of the risk within this gene (11). These differences in amino acid associations may be explained by the additional independent effects of HLA–DRB1*01:01 and HLA–DRB1*13:01 in IBM that are not associated with PM or DM. Understanding the peptide-binding specificities of these risk alleles may inform future research along with the potential identification of unique autoantigens presented to the immune system in IBM.

We investigated potential associations between specific HLA alleles and the newly described anti-cN1A antibody. The significant association observed with HLA–DRB1*03:01 and the anti-cN1A antibody may be due to an increased association with HLA–DRB1*03:01 as a whole in IBM. No significant differences in HLA associations were observed between anti-cN1A–positive and anti-cN1A–negative patients. A recent study in an Australian cohort of patients also failed to show any association with anti-cN1A antibodies and MHC class II alleles other than HLA–DR3 (33). Furthermore, we do not have complete data on co-occurrence of anti-cN1A and other antibodies, such as anti-Ro, which also has a strong HLA–DR3 association (34). It may be that a significantly larger sample size is needed to detect novel HLA associations in patients with anti-cN1A antibodies.

The Immunochip is a custom-designed chip that contains a dense set of SNPs covering 186 loci based on evidence of association with 12 different autoimmune and inflammatory diseases (18). Therefore, the current study tests a specific hypothesis that IBM shares genetic overlap with other autoimmune diseases. The current study has not comprehensively tested other loci that have been purported to be associated with IBM, such as those predisposing to hereditary inclusion body myopathies or loci associated with other degenerative diseases. The observation that the MHC region is strongly associated, and evidence of association with other genes on

Figure 3. Locations of positions 26 and 11 of HLA–DRB1 within DR β-chain 1. Positions 26 and 11 are independently associated with inclusion body myositis. Arrows indicate the locations of the risk-conferring amino acids Tyr at position 26 and Ser at position 11 within the β-sheet floor of DR β-chain 1. Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/journal/doi/10.1002/art.40045/abstract.
this array, suggests an immune-mediated component to IBM. It is not clear whether this represents a primary or secondary involvement.

No non-HLA loci investigated reached genome-wide significance, although this is to be expected in a study of this size. While comparatively large for IBM, this study is underpowered to detect associations of small effect sizes that are expected in genetic studies of conditions with complex etiologies. Three loci did reach a suggestive level of significance ($P < 2.25 \times 10^{-5}$). Of particular interest is the chromosome 3 p21.31 region, which is known to be associated with multiple autoimmune diseases such as celiac disease, type 1 diabetes mellitus, and Behçet’s disease and is suggestively associated in juvenile idiopathic arthritis (JIA) (35–38). The strongest association in this region was with rs112088397, which tags a large haplotype block where many additional SNPs reached a suggestive level of significance and is the same risk haplotype as that reported in JIA ($r^2 = 0.87$) (35,39). The variant rs112088397 in our study is found at a higher frequency in controls (minor allele frequency [MAF] of 0.08 for patients versus MAF of 0.16 for controls) and is therefore protective against IBM (OR 0.42 [95% CI 0.29–0.60]). Proxies for rs112088397 fall within multiple candidate genes including CCR1, CCR3, CCR2, CCR5, and CCRL2, and therefore it is difficult to identify the causal variant in this region. Interestingly, this haplotype contains a frameshift mutation (rs333) that results in a 32-bp deletion variant (CCR5Δ32) and a non-functional receptor. The most strongly associated non-HLA SNP in IBM is in high LD with this frameshift mutation ($r^2 = 0.86$); furthermore, a number of SNPs in this region are eQTLs for the expression of CCR5 in monocytes (26).

CCR5 binds a number of proinflammatory chemokines that are up-regulated in IIMs and IBM, such as CCL3 (macrophage inflammatory protein 1α [MIP-1α]), CCL4 (MIP-1β), and CCL5 (RANTES). CCR5 has been shown to be predominantly expressed on monocytes, macrophages, and T cells, up-regulated in IBM muscle tissue, and localized on inflammatory cells invading nonnecrotic muscle fibers (40,41). Interestingly, in rheumatoid arthritis (RA) the density of CCR5 molecules on the T cell surface determines efficiency of its function as a chemokine receptor and intensity of T cell migration toward RA synoviocytes (42). We hypothesize that CCR5 is important in the pathogenesis of IBM, consistent with studies showing an up-regulation of CCR5 in muscle tissue of patients. Individuals with the protective rs333 frameshift mutation described above will carry a non-functional variant and/or decreased expression of CCR5, resulting in reduced migration of T cells into muscle fiber.

It is interesting that the suggestive association with the chromosome 3 p21.31 region in this study was found with only 252 individuals. This may be explained by the stronger effect size in IBM compared to JIA (0.42 versus 0.78, respectively) (35), and although IBM is a rare disease, it may mean that replication of this association is possible with ongoing sample collection. Due to the rarity of IBM, it is difficult to ascertain the sample sizes needed for genome-wide association studies; therefore, next-generation sequencing could be an approach to detect rare, potentially functional variants of large effect size. Sequencing studies are currently underway taking either a candidate gene approach (10) or a hypothesis-free approach sequencing exomes of a large number of patients with IBM (9). The present study has not comprehensively tested other loci that have been purported to be associated with IBM, such as those predisposing to hereditary inclusion body myopathies or loci associated with other degenerative diseases. In a disease in which the etiology is unknown, sequencing could be successful in identifying novel variants and/or pathways involved in disease pathogenesis.

In summary, we have conducted the largest genetic association study to date in Caucasian patients with IBM, confirming the involvement of an immune-mediated genetic component of this understudied disease. Studies in the genetics of IBM are hampered by small sample sizes due to the rarity of this disease. Ongoing sample collection, as well as further international collaborative studies, will allow us to further characterize genetic influences on susceptibility to IBM.

ACKNOWLEDGMENTS

We thank all of the patients and their families who contributed to this study. We thank Mr. Paul New (Salford Royal Foundation Trust) for ethical and technical support. The authors would like to acknowledge the assistance given by IT Services and the use of the Computational Shared Facility at The University of Manchester. A list of other acknowledgments is included in Supplementary Materials, available on the Arthritis & Rheumatology web site at http://onlinelibrary.wiley.com/doi/10.1002/art.40045/abstract.

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. Rothwell 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. Rothwell, Cooper, Lundberg, Gregersen, Ollier, Lamb, Chinoy.


Analysis and interpretation of data. Rothwell, Cooper, Lundberg, Lilleker, Bowes, Seldin, Amos, Ollier, Padyukov, Lamb, Chinoy.
REFERENCES


APPENDIX A: 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), Karina Gheorghe (Karolinska Institutet, Stockholm, Sweden), David Hilton-Jones (John Radcliffe Hospital, Oxford, UK), Patrick Kiely (St. George's Hospital, London, UK), and Herman Mann (Institute of Rheumatology, Prague, Czech Republic).