The Protein Tyrosine Phosphatase N22 Gene Is Associated With Juvenile and Adult Idiopathic Inflammatory Myopathy Independent of the HLA 8.1 Haplotyp in British Caucasian Patients

H. Chinoy,1 H. Platt,2 J. A. Lamb,2 Z. Betteridge,3 H. Gunawardena,3 N. Fertig,4 H. Varsani,5 J. Davidson,6 C. V. Oddis,4 N. J. McHugh,5 L. R. Wedderburn,3 W. E. R. Ollier,2 and R. G. Cooper,7 on behalf of the UK Adult Onset Myositis Immunogenetic Collaboration and the Juvenile Dermatomyositis Research Group

Objective. To examine single-nucleotide polymorphisms (SNPs) of the protein tyrosine phosphatase N22 gene (PTPN22) and to study the relationship between PTPN22 and the HLA region in patients with idiopathic inflammatory myopathies (IIMs).

Methods. PTPN22 SNPs were assessed in a large, cross-sectional, case–control study from the UK involving patients with adult or juvenile IIM, comprising patients with polymyositis (PM) (n = 114), dermatomyositis (DM) (n = 102), myositis associated with another connective tissue disease (myositis–CTD overlap syndrome) (n = 64), or juvenile DM (n = 101), in comparison with 748 control subjects. Seventeen PTPN22 SNPs were genotyped using the Sequenom MassArray iPLEX platform. Serotyping for myositis-specific/myositis-associated autoantibodies (MSAs/MAAs) was performed by radioimmunoprecipitation.

Results. A significant association was noted between the R620W variant (rs2476601) and IIM (corrected P < 0.0009 versus controls), and specifically with the clinical subgroup of PM (P < 0.003 versus controls). A weaker association was noted with juvenile DM (P < 0.009 versus controls). No significant associations were noted after stratification by serologic subgroups. The association with the R620W variant was independent of alleles forming the HLA 8.1 haplotype. No other PTPN22 SNPs were associated with IIM. The PTPN22 haplotype containing the R620W T allele was the only haplotype significantly associated with IIM.

Conclusion. The R620W variant is a significant risk factor for IIM, independent of the HLA 8.1 haplotype. Unlike that in the HLA region, risk is not increased in individuals possessing MSAs/MAAs. These results are further evidence that the PTPN22 gene confers autoimmune susceptibility.

The idiopathic inflammatory myopathies (IIMs) are a heterogeneous group of rare autoimmune diseases characterized by acquired proximal muscle weakness, inflammatory cell infiltrates in muscle biopsy tissue, and the presence of circulating myositis-specific/myositis-associated autoantibodies (MSAs/MAAs). The most common myositis subgroups are polymyositis (PM), dermatomyositis (DM), and myositis overlapping with...
another connective tissue disease (myositis–CTD overlap syndrome). IIMs may also be present in children, with the most common subgroup being juvenile DM. The etiopathogenesis of IIM is likely to be multifactorial, i.e., arising from both genetic and environmental factors and their interactions (1).

It is well recognized that a major genetic contribution conferring susceptibility in a variety of autoimmune diseases arises from the major histocompatibility complex (MHC). However, recent genetic research has demonstrated that genes outside of the MHC may also be important for conferring susceptibility to autoimmunity. For instance, large-scale genetic association studies have confirmed a missense single-nucleotide polymorphism (SNP) in the gene coding for protein tyrosine phosphatase N22 (PTPN22), on chromosome 1. The SNP in question is a C-to-T change at position 1858, which causes an amino acid substitution at residue 620 from arginine to tryptophan (R620W) in the lymphocyte phosphatase (LYP) protein. This SNP represents a disease susceptibility gene in several autoimmune diseases (2), especially in diseases in which HLA markers are associated with disease-specific autoantibodies.

In adult IIM and juvenile IIM, many characteristic MSAs/MAAs are strongly associated with the HLA region, notably with components of the 8.1 common ancestral haplotype (HLA–B*08/DRB1*03/DQB1*02/DQA1*05) (3–7). We therefore examined SNPs from the PTPN22 gene as part of a large, ongoing case–control association study of adult and juvenile IIMs in patients from the UK, and investigated the relationship of the PTPN22 gene with genetic markers from the HLA region.

PATIENTS AND METHODS

Patients. DNA was available from 381 UK Caucasian patients with IIM. Patients with adult IIM (n = 280), who were ages ≥18 years at disease onset, were recruited through the UK Adult Onset Myositis Immunogenetic Collaboration (AOMIC) (7). Patients with juvenile DM (n = 101) were recruited via the UK Juvenile DM National Registry and Repository (8,9). Patients with PM, DM, or juvenile DM had probable or definite myositis, based on the Bohan and Peter criteria (10,11). For patients with myositis–CTD overlap syndrome, use of these criteria is problematic, since myositis is often diagnosed less rigorously in the context of another CTD (likely reflecting the lack of expertise in electromyography [EMG] and muscle histology among physicians in nonteaching centers in the UK). Thus, 15 (23%) of 64 patients with myositis–CTD overlap syndrome were included if they fulfilled all of the following requirements: 1) met the published criteria for their primary CTD (12–16) or for mixed CTD (MCTD) (17); 2) possessed at least 2 of 4 Bohan and Peter criteria (proximal muscle weakness, elevated muscle enzyme levels, characteristic myopathic changes on EMG, diagnostic confirmation on muscle biopsy); and 3) possessed at least 1 MSA/MAA. The remaining 49 patients with myositis–CTD overlap syndrome fulfilled the criteria for their primary disease or MCTD and had probable/definite myositis according to the Bohan and Peter criteria.

A standardized clinical data collection form, detailing demographics and individual clinical characteristics, was used. For adult patients, the collaborating physicians at each AOMIC study site confirmed/excluded the presence of interstitial lung disease (ILD) by pulmonary function tests and thoracic imaging, and confirmed/excluded the presence of cancer-associated myositis (CAM) by relevant investigations. CAM was defined as cancer occurring in patients with probable/definite PM/DM (18) that was diagnosed within 3 years of the myositis diagnosis according to the modified Bohan and Peter classification.

Controls. Seven hundred forty-eight UK Caucasian control subjects were recruited from among blood donors and general practitioner registers as described previously (7). Collection of data and blood from patients and controls was undertaken in accordance with the regulations of the local research ethics committees, and informed consent was obtained from all subjects according to the Declaration of Helsinki.

Autoantibody typing. Serum was obtained from all patients for the determination of MSAs, comprising antisynthetases (anti–Jo-1, anti–PL-7, anti–PL-12, anti–EJ, anti–OJ, and anti–KS), anti–Mi-2, anti–signal recognition particle, and anti–155/140, and MAAs, comprising anti–PM-Scl, anti–Ki, anti–U1 RNP, and anti–U3 RNP. The autoantibodies were determined using radioimmunoprecipitation, as previously described in studies of adult IIM (7,19) and juvenile IIM (9). The presence of the newly described anti–small ubiquitin-like modifier 1 activating enzyme was also determined, as previously described (20).

Genotyping. DNA samples were extracted from peripheral blood obtained from all patients and control subjects using a standard phenol-chloroform method. SNPs were genotyped using the Sequenom MassArray iPLEX platform, in accordance with the manufacturer’s instructions (online at http://www.sequenom.com/seq-genotyping.html). Cases were typed for the HLA–DRB1, HLA–DQB1, and HLA–B loci with broad specificity, using a commercially available polymerase chain reaction–sequence-specific oligonucleotide probe typing system (Dynal Biotech, Hamburg, Germany). Data for the HLA–DQA1 locus were derived from the results of testing for DRB1 and DQB1, as previously outlined (9). The HLA class II typing has been described previously (7).

PTPN22 SNPs. Seventeen SNPs within the PTPN22 gene were selected. Of these, 9 haplotype-tagging SNPs (tag-SNPs) were selected for genotyping by pairwise tagging, using the HapMap Center d’Etude du Polymorphisme Humain population (release 20, National Center for Biotechnology Information Build 35 assembly, online at www.ncbi.nlm.nih.gov), with an r² cutoff value of ≥0.8 and a minor allele frequency (MAF) of 10%. The other 8 SNPs were selected on the basis of having a putative functional role. Of these 17 SNPs, 10 were previously reported to have a role in patients with rheumatoid arthritis (RA) (2). Three SNPs (rs1217412, rs1775759, and
were removed from further analysis because of an assay success rate of 90%. For this analysis, the cutoff value for successful genotyping per sample was set at 80%.

Statistical analysis. Genotype frequencies for each PTPN22 SNP were tested for Hardy-Weinberg equilibrium (HWE) in each group. Allele and genotype frequencies of these PTPN22 SNPs were compared between myositis cases and controls, using Fisher’s exact test or chi-square test, as appropriate. In cases of significant differences between groups, data were expressed as the odds ratio (OR) with exact 95% confidence interval (95% CI). Pointwise P values were corrected using permutation testing (10,000 permutations), as implemented in the PLINK program (21). Linkage disequilibrium (LD) was calculated using both D’ and pairwise r² values, and PTPN22 haplotypes were estimated and constructed using the expectation-maximization algorithm, using HelixTree (version 3.1.2; Golden Helix, Bozeman, MT). The analyses were also repeated after stratification for serologic subgroups and the presence of CAM/ILD in myositis patients. Unless otherwise stated, the statistical package Stata (release 9.2; StataCorp, College Station, TX) was used to perform statistical analyses.

A power calculation was applied to the SNP with the lowest MAF (rs2476601), using allele data deposited by Dr. Anne Barton (UK Arthritis Research Campaign Epidemiology Unit) and published online as part of the British 1958 Birth Cohort DNA data collection (22). For 80% power to detect an effect size of 1.65 at a 95% significance level (based on data from an RA discovery study [2]), a sample size of 390 cases and 390 controls would be required. Significant differences in allele frequencies were also verified using the 1958 Birth Cohort DNA data.

RESULTS

Clinical details. The 381 patients with IIM recruited for the study included 114 with PM, 102 with DM, 64 with myositis–CTD overlap syndrome, and 101 with juvenile DM. The patients with myositis–CTD overlap syndrome had the following primary diagnoses: 41 with systemic sclerosis, 9 with MCTD, 6 with Sjögren’s syndrome, 7 with systemic lupus erythematosus, and 1 with RA. The median age at myositis onset was 49 years (SD 14.1 years) for patients with adult-onset IIM, and 6 years (SEM 3.6 years) for patients with juvenile DM. The overall proportion of female patients was 73%.

Genotype and allele associations. All of the analyzed PTPN22 SNPs conformed to HWE, in both the total IIM patient group (combined cases) and controls. Table 1 summarizes the genotype and allele frequencies for the tested PTPN22 SNPs. The minor T allele (allele 2) of the rs2476601 SNP (R620W) was a significant risk factor in the combined cases compared with controls (Table 2). This result remained significant when the British 1958 Birth Cohort DNA data collection was used as an additional control data set (Table 2). A significant genotype association was also present for R620W in both recessive (TT versus CT + CC) and dominant (CT + TT versus CC) models of inheritance (Table 3).

The genotype data were also analyzed according to heterozygosity or homozygosity of the T allele. The association was significant for TT homozygotes (TT versus CC). A weaker association was noted for TC heterozygotes (TC versus CC). No genotype or allele associations were observed for any of the other tested SNPs.

Table 1. PTPN22 SNP allele and genotype frequencies in combined idiopathic inflammatory myopathy (IIM) cases compared with controls*

<table>
<thead>
<tr>
<th>SNP/rs number</th>
<th>No. of cases</th>
<th>No. of controls</th>
<th>Minor allele frequency</th>
<th>Genotype frequency of 1/1, 1/2, 2/2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/rs1217385</td>
<td>360</td>
<td>286</td>
<td>0.44</td>
<td>0.42</td>
</tr>
<tr>
<td>2/rs1235005</td>
<td>365</td>
<td>289</td>
<td>0.44</td>
<td>0.42</td>
</tr>
<tr>
<td>3/rs2488457</td>
<td>362</td>
<td>287</td>
<td>0.23</td>
<td>0.21</td>
</tr>
<tr>
<td>4/rs17510162</td>
<td>364</td>
<td>289</td>
<td>0.18</td>
<td>0.20</td>
</tr>
<tr>
<td>5/rs1217414</td>
<td>364</td>
<td>708</td>
<td>0.27</td>
<td>0.25</td>
</tr>
<tr>
<td>6/rs2488458</td>
<td>361</td>
<td>680</td>
<td>0.27</td>
<td>0.25</td>
</tr>
<tr>
<td>7/rs1217418</td>
<td>364</td>
<td>289</td>
<td>0.46</td>
<td>0.43</td>
</tr>
<tr>
<td>8/rs12760457</td>
<td>361</td>
<td>678</td>
<td>0.28</td>
<td>0.31</td>
</tr>
<tr>
<td>9/rs2730735</td>
<td>350</td>
<td>645</td>
<td>0.28</td>
<td>0.31</td>
</tr>
<tr>
<td>10/rs2476601†</td>
<td>363</td>
<td>735</td>
<td>0.14</td>
<td>0.08</td>
</tr>
<tr>
<td>11/rs1310182</td>
<td>361</td>
<td>587</td>
<td>0.45</td>
<td>0.45</td>
</tr>
<tr>
<td>12/rs1217389</td>
<td>356</td>
<td>279</td>
<td>0.27</td>
<td>0.23</td>
</tr>
<tr>
<td>13/rs1217388</td>
<td>357</td>
<td>693</td>
<td>0.26</td>
<td>0.25</td>
</tr>
<tr>
<td>14/rs3811021</td>
<td>344</td>
<td>678</td>
<td>0.19</td>
<td>0.19</td>
</tr>
</tbody>
</table>

* For frequencies of each single-nucleotide polymorphism (SNP) genotype, 1 refers to the major allele (1/1 being homozygous, 1/2 being heterozygous), and 2 refers to the minor allele.
† See Tables 2 and 3 for detailed analyses of this association.
Clinical and MSA/MAA associations. To examine the R620W association in more detail, data were stratified by clinical subgroups and by subtypes of MSAs/MAAs. The associations with clinical subgroups and significant associations with MSAs/MAAs are summarized in Table 2. Significant associations with the R620W allele were observed in the PM subgroup as compared with both sets of controls. A weaker association was observed in the juvenile DM subgroup, and possible associations (which were not significant after correction in probability analyses) were observed in the anti–Jo-1 antibody– and anti-155/140 antibody–positive subgroups. No significant associations were detected in any of the other clinical or MSA/MAA subgroups, or in those patients with no demonstrable antibodies.

An additional association with the R620W allele was noted in IIM patients with ILD (uncorrected $P = 0.0007$, OR 1.7, 95% CI 1.2–2.4; patients with polymyositis (PM) $P = 0.0006$, OR 1.9, 95% CI 1.3–2.8; patients with juvenile dermatomyositis (DM) $P = 0.003$, OR 1.8, 95% CI 1.2–2.8. NS = not significant; CTD = connective tissue disease.

Table 2. Allele associations of rs2476601 in patients with idiopathic inflammatory myopathies (IIMs) and in IIM subgroup analyses stratified by clinical and antibody specificities*

<table>
<thead>
<tr>
<th>No. of subjects</th>
<th>Allele frequency, %</th>
<th>OR (95% CI)</th>
<th>$P$</th>
<th>$P_{cor}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>735</td>
<td>91.8</td>
<td>8.2</td>
<td>–</td>
</tr>
<tr>
<td>Combined cases</td>
<td>363</td>
<td>86.4</td>
<td>13.6</td>
<td>1.8 (1.3–2.4)</td>
</tr>
<tr>
<td>Clinical subgroup</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM</td>
<td>110</td>
<td>83.6</td>
<td>16.4</td>
<td>2.2 (1.4–3.3)</td>
</tr>
<tr>
<td>DM</td>
<td>98</td>
<td>90.3</td>
<td>9.7</td>
<td>1.2 (0.7–2.0)</td>
</tr>
<tr>
<td>Myositis–CTD overlap</td>
<td>63</td>
<td>88.9</td>
<td>11.1</td>
<td>1.4 (0.7–2.5)</td>
</tr>
<tr>
<td>Juvenile DM</td>
<td>91</td>
<td>84.1</td>
<td>15.9</td>
<td>2.1 (1.3–3.3)</td>
</tr>
<tr>
<td>Antibody subgroup†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jo-1</td>
<td>50</td>
<td>86.0</td>
<td>14.0</td>
<td>1.8 (0.9–3.3)</td>
</tr>
<tr>
<td>155/140</td>
<td>32</td>
<td>82.8</td>
<td>17.2</td>
<td>2.3 (1.4–2.6)</td>
</tr>
</tbody>
</table>

* $P$ values (uncorrected and corrected [$P_{cor}$]) are for comparisons with controls. In comparisons with control data from the British 1958 Birth Cohort DNA data set, associations were as follows: combined cases $P = 0.0004$, odds ratio (OR) 1.5, 95% confidence interval (95% CI) 1.2–2.0; patients with polymyositis (PM) $P = 0.0006$, OR 1.9, 95% CI 1.3–2.8; patients with juvenile dermatomyositis (DM) $P = 0.003$, OR 1.8, 95% CI 1.2–2.8. NS = not significant; CTD = connective tissue disease.
† Only significant associations are shown.

Interaction with the HLA 8.1 haplotype. Alleles forming part of the 8.1 haplotype are known to confer risk of disease in adult and juvenile IIM (3–7). Therefore, it was of interest to investigate the relationship between the HLA region and the PTPN22 gene in IIM. Associations of the R620W SNP with each of HLA–B*08, HLA–DRB1*03, HLA–DQA1*05, or HLA–DQB1*02 were examined in a multivariate logistic regression model. The association of R620W with the 8.1 haplotype remained significant. However, no multiplicative interaction with any of the tested 8.1 alleles was noted.

Since anti–Jo-1 and anti–PM-Scl antibodies are known to be strongly associated with the 8.1 haplotype (7), data were also stratified for the presence or absence of these antibodies. The R620W association remained significant in the subgroup of patients lacking the anti–Jo-1 or anti–PM-Scl antibodies, as compared with controls (uncorrected $P = 0.0007$, OR 1.7, 95% CI 1.3–2.4).

Linkage disequilibrium. The tested PTPN22 SNPs were assessed for LD with each other, using data from the control group. Strong pairwise LD between SNPs was noted, using the $D'$ measure. When the more stringent measure of pairwise $r^2$ was used, rs1217388 showed the greatest LD with rs2476601 (pairwise $r^2 = 0.24$). Minimal LD was noted between the tested PTPN22 SNPs and the tested HLA alleles ($D' ≤ 0.27$).

Table 3. Genotype associations of rs2476601 in combined idiopathic inflammatory myopathy cases compared with controls*

<table>
<thead>
<tr>
<th>Genotype test</th>
<th>$P$</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT vs. CT + CC</td>
<td>0.005</td>
<td>4.1 (1.3–15.5)</td>
</tr>
<tr>
<td>CT + TT vs. CC</td>
<td>0.0005</td>
<td>1.7 (1.3–2.4)</td>
</tr>
<tr>
<td>TT vs. CC</td>
<td>0.003</td>
<td>4.5 (1.4–17.0)</td>
</tr>
<tr>
<td>TC vs. CC</td>
<td>0.004</td>
<td>1.6 (1.1–2.2)</td>
</tr>
</tbody>
</table>

* $P$ values are uncorrected. OR = odds ratio; 95% CI = 95% confidence interval.
To enable comparison of haplotype results with previously published data, haplotypes were constructed from tagSNPs previously identified in a study by Carlton et al in patients with RA (2). Thus, 5 haplotypes were identified at a frequency of 5% in the control population (Table 4). The most frequent haplotype corresponded to the haplotype most frequently observed in the study by Carlton et al (2). Only 1 haplotype (C-A-G-A-T-T-C-T; underline indicates the substitution) carried the R620W T allele. This haplotype showed a significant association in the combined IIM cases compared with controls. When stratified by disease subtypes, significant associations of this haplotype were also seen in patients with PM and in those with juvenile DM (Table 4).

Other haplotype associations were also observed in groups stratified by serologic subtypes. In the subgroup with anti–Mi-2 antibodies, an association was observed with C-G-A-G-C-C-T-T (frequency of 11%; \( P = 0.01 \) versus controls), while anti–Jo-1 antibody positivity was associated with C-A-G-A-C-T-T-C-T (frequency of 6%; \( P = 0.007 \) versus controls), and the subgroup with anti-155/140 antibodies had an association with C-A-G-A-T-T-C-T (19%; \( P = 0.009 \) versus controls), although it is likely that the low numbers obtained after stratification by serologic subtype could affect the validity of these results. Haplotype frequencies were reanalyzed in the absence of the T (risk) allele (forming the R620W variant). Other than the haplotype associations of the C allele already described, no further associations were observed.

### DISCUSSION

Herein we describe an association of the R620W variant of the PTPN22 gene in both adult and juvenile IIM, representing the first description of a major IIM disease susceptibility gene outside of the HLA region. No significant associations were present for the remainder of the tested PTPN22 SNPs. The R620W association appears to be strongest in individuals homozygous for the T allele. The R620W association was also observed after stratification by clinical disease subgroups; the association was present in patients with PM and in those with juvenile DM, but not in patients with DM or those with myositis–CTD overlap syndrome. It is interesting that even after stratification into traditional IIM clinical subgroups, the association with PM remained strong. The major PTPN22 haplotype that showed an association was that containing the R620W variant, in keeping with published data on patients with RA (2,23).

Until now, the major IIM disease susceptibility gene has been thought to reside in the HLA region, as part of the 8.1 haplotype (6,7,9). We demonstrated that the R620W–IIM association is independent of the 8.1 haplotype (albeit with a weaker strength of association). After stratification for the presence of antisynthetase or anti–PM-Scl antibodies, the association with the 8.1 haplotype becomes stronger (7,24). In contrast, after stratification for MSAs/MAAs, we found that the R620W association became far weaker, or lost significance altogether. Patients with PM and those with juvenile DM, the IIM subgroups associated with the strongest risk for the R620W variant, had the lowest overall MSA/MAA frequencies when compared with patients with DM or those with myositis–CTD overlap syndrome (7,19). We observed a weak association between the anti-155/140 antibody and the R620W variant; notably, this antibody is associated with an HLA allele outside of the 8.1 haplotype (HLA–DQA1*0301) (25).

### Table 4. PTPN22 haplotype frequencies in combined idiopathic inflammatory myopathy (IIM) cases and IIM clinical subgroups compared with controls*

<table>
<thead>
<tr>
<th>TagSNP number</th>
<th>Controls (2n = 1,124)</th>
<th>Combined IIM cases (2n = 622)</th>
<th>PM (2n = 216)</th>
<th>DM (2n = 192)</th>
<th>Myositis–CTD overlap (2n = 122)</th>
<th>Juvenile DM (2n = 114)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 C G A G C C T T</td>
<td>0.31</td>
<td>0.27</td>
<td>0.26</td>
<td>0.30</td>
<td>0.29</td>
<td>0.20†</td>
</tr>
<tr>
<td>6 T G G A C C T T</td>
<td>0.24</td>
<td>0.28</td>
<td>0.29</td>
<td>0.29</td>
<td>0.25</td>
<td>0.29</td>
</tr>
<tr>
<td>8 C G G A C T C T</td>
<td>0.19</td>
<td>0.19</td>
<td>0.16</td>
<td>0.20</td>
<td>0.20</td>
<td>0.21</td>
</tr>
<tr>
<td>9 C A G A C T C T</td>
<td>0.16</td>
<td>0.12</td>
<td>0.11</td>
<td>0.10</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>10 C A G A T T C T</td>
<td>0.08</td>
<td>0.13§</td>
<td>0.17§</td>
<td>0.10</td>
<td>0.10</td>
<td>0.15¶</td>
</tr>
</tbody>
</table>

* The 8 haplotype-tagging single-nucleotide polymorphisms (tagSNPs) correspond to SNPs 1, 2, 18, 21, 22, 27, 32, and 36 from the study by Carlton et al in patients with rheumatoid arthritis (2).

† \( P = 0.01 \) versus controls, odds ratio (OR) 0.5, 95% confidence interval (95% CI) 0.3–0.9.
‡ \( P = 0.0007 \) versus controls, OR 1.7, 95% CI 1.2–2.4.
§ \( P = 0.00003 \) versus controls, OR 2.4, 95% CI 1.5–3.6.
¶ \( P = 0.01 \) versus controls, OR 2.1, 95% CI 1.1–3.6.
The R620W association was also observed in IIM patients with ILD, although this may represent a general association with the IIMs. No effect of sex or age at disease onset was observed, in contrast to the findings in patients with RA (26,27).

Association with the R620W SNP has already been described in other autoimmune diseases, including RA, systemic lupus erythematosus, type 1 diabetes mellitus, and autoimmune thyroid disease (28), with effect sizes similar to those seen in the present study in patients with IIM. Generally, PTPN22 associations have been found in diseases in which autoantibodies potentially play a prominent role, and not in those diseases in which antibodies are generally not detectable (e.g., psoriasis, psoriatic arthritis, multiple sclerosis) (23).

In one study of patients with RA, the R620W variant showed no interaction with the HLA shared epitope (29), although the pooled data from another study of patients with RA suggested a gene–gene interaction (30). A strong interaction between the R620W variant and anti–cyclic citrullinated peptide antibodies has also been demonstrated in the development of RA (26,27). A further study failed to demonstrate any association in rheumatoid factor (RF)–negative patients with RA (31). However, another study of patients with RA demonstrated an association of the R620W variant in patients with antinuclear antibody (ANA)–positive or ANA-negative juvenile idiopathic arthritis and in patients with RF-positive or RF-negative RA (23).

The lack of association between PTPN22 and anti–PM-Scl–positive and anti–PM-Scl–positive IIM cases may reflect the strong relationship that these antibodies have with the 8.1 haplotype. The R620W variant and other PTPN22 SNPs may exert a smaller effect in other IIM antibody systems or in patients who lack detectable antibodies, but the present study lacked the power to detect such associations. Thus, in the IIMs, the R620W variant appears to generate susceptibility to disease regardless of MSA/MAA status.

A previous, small case–control study from the US examined the R620W variant in Caucasian patients with IIM, but no significant associations were observed (32). However, the frequency of the T allele in the control group was higher than that seen in the present study (12% in the US study versus 8% in our study; among the patients, 10% in the US study versus 14% in our study). Since the frequency of homozygosity for the T allele is low in Caucasians, use of a small sample size may lead to overestimation of the relative frequency of the C allele. It is therefore possible that an association does exist in US Caucasian patients with IIM, but the cited study was insufficiently powered to detect this.

The amino acid at residue 620 is located in a proline-rich motif of the LYP protein. R620 (arginine) enables binding of LYP to the Src homology domain of C-terminal Src kinase (Csk) (28,33). The LYP–Csk complex inhibits the T cell receptor (TCR) signaling pathway, but W620 (tryptophan) disrupts the complex, rendering LYP*W620 unable to bind Csk (33). Initially, LYP*W620 was thought to be a loss-of-function variant, due to a reduced ability to down-regulate T cell activation, thus leading to increased autoimmune reactivity. Recently, data have suggested that W620 is a gain-of-function mutation, which is able to dephosphorylate signaling proteins more efficiently than LYP*R620, leading to increased inhibition of T and B lymphocytes, thymic hyporesponsiveness, and increased circulating autoreactive T cells (34,35). Furthermore, LYP*W620 / LYP*W620 homozygote T cells suppress TCR signaling to a greater extent than that exhibited by LYP*R620 / LYP*W620 heterozygote T cells (34). The findings of the present study are consistent with this hypothesis of a R620W gene-dosage effect, since we were able to demonstrate a greater risk of IIM in individuals homozygous for the T allele.

Due to the rarity of adult and juvenile IIMs, it is challenging to recruit a sufficient number of cases for analysis in genetic association studies that examine SNPs with a modest effect size. The present study was not powered to detect associations after stratification by disease subgroups (although a strong association was still observed within the PM subgroup). This may also explain why no significant associations were observed for the other PTPN22 SNPs, since these may have a more modest effect size, and why no interaction with the 8.1 haplotype was noted. Although, to date, there are no replication data from other IIM cohorts to support our findings, the demonstrated association with IIM is consistent with findings from studies of other autoimmune diseases.

The findings from the present study demonstrate that the R620W variant is a significant risk factor in IIM and is independent of the 8.1 haplotype. Thus, the genetic risk of developing an IIM resides not only within the HLA, but also possibly within multiple genetic regions, all of which may contribute to disease susceptibility. The polygenic nature of susceptibility risk is already becoming apparent in RA and other common autoimmune diseases, in which novel associations with modest effect sizes are coming to light as a result of such investigations as the recent Wellcome Trust Case Con-
control Consortium whole-genome scan (36). To further our understanding of the genetics of IIM, large-scale collaborative genetic studies now appear necessary to identify further susceptibility genes.

ACKNOWLEDGMENTS

We thank the UK Arthritis Research Campaign for providing the infrastructure that made this collection of DNA samples from adult myositis patients possible, and the UK Myositis Support Group, which provided the funds necessary to undertake the genetic analysis. We thank the patients and their families for agreeing to contribute to the National Juvenile DM Registry and Repository (UK and Ireland) (JDRG) and all of the JDRG contributors (see ref. 9). We also thank Dr. Mark Lunt for assisting with the statistical analyses, and the UK physicians who contributed to the AOMIC (see ref. 7).

AUTHOR CONTRIBUTIONS

Dr. Cooper 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 design. Chinoy, Ollier, Cooper.

Acquisition of data. Chinoy, Platt, Betteridge, Gunawardena, Fertig, Varssani, Davidson, Oddin, McHugh, Wedderburn, Cooper.

Analysis and interpretation of data. Chinoy, Lamb, Betteridge, Gunawardena, McHugh, Ollier, Cooper.

Manuscript preparation. Chinoy, Gunawardena, Davidson, McHugh, Wedderburn, Ollier, Cooper.

Statistical analysis. Chinoy.

REFERENCES

22. Genetic information from the British 1958 Birth Cohort. URL: http://www.b58cgen.ssgl.ac.uk.
24. O’Hanlon TP, Carrick DM, Targoff IN, Arnett FC, Reveille JD, Carrington M, et al. Immunogenetic risk and protective factors for the idiopathic inflammatory myopathies: distinct HLA-A,-B,-Cw,
-DRB1, and -DQA1 allelic profiles distinguish European American patients with different myositis autoantibodies. Medicine (Baltimore) 2006;85:111–27.


36. The Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007;447:661–78.