Interferon-Gamma and Interleukin-4 Gene Polymorphisms in UK Caucasian Idiopathic Inflammatory Myopathy Patients

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ABSTRACT

Objectives: To determine whether interferon-gamma (IFN-γ) and interleukin-4 (IL-4) genes confer susceptibility for the idiopathic inflammatory myopathies (IIMs).

Methods: A large cross-sectional study of UK Caucasian adults with polymyositis (PM, n=101), dermatomyositis (DM, n=94) and myositis overlapping with a connective tissue disease (myositis/CTD-overlap, n=70) was completed. 177 ethnically matched controls were available for comparison. Single nucleotide polymorphisms (SNPs) within intronic regions coding for IL-4, IFN-γ and a microsatellite marker within intron 1 of the IFN-γ gene were typed.

Results: Strong linkage disequilibrium was present between SNPs in each gene. In the IFN-γ gene, a weak allelic association was observed in PM vs. controls at rs1861493 (odds ratio [OR] 1.6, 95% confidence interval [CI] 1.03-2.4). The microsatellite IFN-γ CA(14) allele was associated with risk for IIMs overall (OR 3.3, 95% CI 1.4-7.8), the strongest association being observed within the anti-U1-RNP group (OR 6.0, 95% CI 1.5-23.1), and persisting after adjustment for known myositis HLA class II associations.

Conclusions: Genetic markers in the IFN-γ gene demonstrate significant allelic associations with the IIMs in a UK Caucasian population. The SNPs tested in this study within the region coding for IL-4 fail to show significant associations to IIM disease susceptibility.

Keywords
Myositis, genetic, single nucleotide polymorphisms, interferon-gamma, interleukin-4
INTRODUCTION

The idiopathic inflammatory myopathies (IIMs) are a heterogeneous group of diseases which include polymyositis (PM), dermatomyositis (DM) and myositis overlapping with other connective tissue diseases (myositis/CTD-overlap). There is increasing recognition that genetic factors are involved in the development of IIMs.[1] Most myositis studies to date have only examined the human leucocyte antigen (HLA) region, where in Caucasians, HLA-DRB1*0301 and HLA-DQA1*0501 are significant risk factors.[2;3] Few IIM genetic studies have been conducted outside of HLA.[1]

In multifactorial diseases mediated by inflammatory processes, variations in disease susceptibility/expression may be influenced by functional cytokine polymorphisms, or up/down-regulation of genes encoding for cytokine production.[4] A Th1/Th2 cytokine imbalance is postulated in various autoimmune disorders. Th1 cytokines play a role in cell-mediated immunity, but are detrimental in organ-specific responses; Th2 cytokines induce antibody production and mediate allergic responses. Furthermore, Th1 and Th2 cytokine responses are antagonistic and downregulate each other.

Interferon-gamma (IFN-γ) is a T cell-derived Th1 cytokine and a strong inducer of major histocompatibility complex (MHC) class I expression in IIM inflammatory muscle tissue.[5] The IFN-γ gene on chromosome 12q14 comprises four exons (Figure 1). A variable dinucleotide repeat, (CA)n, has been demonstrated in intron 1, acting as a non-specific tissue enhancer element. (CA) repeat variations are associated with various autoimmune diseases.[4] In contrast, interleukin-4 (IL-4) is a Th2 cytokine produced by activated T cells and functions as a B-cell growth factor. The IL-4 gene is on chromosome 5q31.1, comprising 4 exons (Figure 1). IL-4 SNPs have been associated with severe asthma and multiple sclerosis.[4] Variable expression of IL-4 and IFN-γ has been demonstrated in IIM muscle biopsy specimens.[6-8] Furthermore, an imbalance of Th1/Th2 cytokines in favour of Th1 is postulated in the IIMs.[9]

In view of these findings, we hypothesise that Th1/Th2 cytokine responses are aetiologically involved in genetic susceptibility in myositis, and thus investigate the role of IFN-γ and IL-4 genes in a large cohort of IIM patient subtypes.
MATERIALS AND METHODS

Study design: A case-control, cross-sectional, genetic-association study, comparing IIM cases with normal subjects.

Cases: Between 1999-2004, a UK-wide collaboration of 56 rheumatologists and 4 neurologists comprising the Adult Onset Myositis Immunogenetic Collaboration, (AOMIC, for details see Appendix in [3]) recruited 272 Caucasian patients, ≥18 years at disease onset. The inclusion criteria for PM/DM was probable or definite disease, according to Bohan and Peter.[10] For myositis/CTD-overlap, myositis may be less rigorously diagnosed as part of another CTD. Thus, 17 of the 70 (24%) myositis/CTD-overlap patients were included if they: 1) met published criteria for their primary CTD; 2) possessed at least two of four Bohan/Peter criteria and 3) possessed at least one myositis specific/associated antibody (MSA/MAA). The remaining 53 myositis/CTD-overlap patients fulfilled criteria for their primary disease and satisfied probable/definite myositis criteria. Collaborating physicians confirmed/excluded interstitial lung disease (ILD) and cancer-associated myositis (CAM) by relevant investigations. Subjects’ written consent was obtained according to the Declaration of Helsinki.

Controls: 177 UK Caucasian control subjects were recruited from primary population registers in the UK, as part of previously described epidemiological studies.[3] Control marker frequencies are comparable to those found in the National Center for Biotechnology Information (NCBI) SNP database (www.ncbi.nlm.nih.gov/SNP) and those previously published.[11] Collection of clinical data and blood was under regulation of local research ethics committees.

Serological typing: Sera were obtained from patients for determination of MSAs (anti-tRNA synthetases: -Jo-1, -PL-7, -PL-12, -EJ, -OJ, -KS; -Mi-2, -SRP) and MAAs (anti-PM-Scl, -Ku, -U1-RNP, -U3-RNP), as described.[3]

Genotyping. Genetic markers were selected from the NCBI SNP database (Figure 1). Assays were based on TaqMan or SNaPshot (PE Applied Biosystems [ABI], UK). The IFN-γ microsatellite marker and insertion-deletion were genotyped using an ABI 3100 DNA analyser.[12] Primers and conditions are available on a supplementary file on-line. HLA genotyping was performed as described.[3]

Statistical analyses: Genotyping frequencies for each SNP were tested for Hardy-Weinberg equilibrium (HWE) in controls. Allelic and genotype frequencies were compared between myositis cases and controls. Probabilities were calculated using Fisher’s exact test, and unless otherwise stated, corrected for multiple comparisons using Bonferroni for the number of SNPs tested. Data were expressed as odds ratios (OR) with exact 95% confidence intervals (CI). Linkage disequilibrium (LD) was calculated with r squared (r²) values, using HelixTree (version 3.1.2, Golden Helix, Inc., MT, USA). Haplotypes were estimated and constructed using the Expectation/Maximisation (EM) algorithm. The statistical package Stata (release 8, Stata Corp., TX) was used to perform statistical analysis. The study had 80% power to detect the effect of a SNP, with a range of 17-40% minor allele frequency in the tested SNPs, conferring an OR of 2.0 at the 5% significance level, assuming a dominant mode of inheritance.
RESULTS

Demography: The 265 cases recruited for the study included 101 PM, 94 DM and 70 myositis/CTD-overlap patients. The myositis/CTD-overlap patients had the following primary diagnoses: 45 systemic sclerosis (SSc), 9 mixed CTD, 7 Sjögren’s syndrome, 7 systemic lupus erythematosus (SLE) and 2 rheumatoid arthritis (RA). A detailed summary of further demographics is provided in [3].

Genetic associations: All markers, except rs734244, showed no deviation from HWE. Strong LD ($r^2 > 0.64$) was observed across all markers, except rs1861494 ($r^2 < 0.5$). The LD detected between markers in the IFN-$\gamma$, IL-4 and HLA class II genes was weak ($r^2 < 0.1$).

IFN-$\gamma$ and IL-4 genotype and allele frequencies are summarised in Table 1. In the IFN-$\gamma$ gene, a weak allele association for the A allele in rs1861493 was noted in PM. The frequency of the AA genotype was increased under a recessive model. This allele was also increased in anti-tRNA synthetase positive patients (78% cases vs. 67% controls, OR 1.7, 95% CI 1.0-3.0, uncorrected probability [p uncorr 0.04]). No further significant associations were observed in the other subgroups, groups combined, or in the IL-4 markers.

Sequencing analysis of the IFN-$\gamma$ microsatellite marker revealed six allelic variants, ranging from 11 to 16 CA dinucleotide repeats, and a summary of these frequencies in clinical subgroups is provided in Table 2. The overall microsatellite marker allelic distribution, and specifically, the (CA)14 polymorphism was significantly different in myositis/CTD-overlap patients vs. controls (Table 2). The (CA)14 polymorphism was increased in anti-U1-RNP positive patients vs. controls (10% vs. 2% controls, OR 6.0, 1.5-23.1, $p_{uncorr} = 0.005$), losing significance after correction for tested MSA/MAAs. Weaker associations for the (CA)14 polymorphism were observed in PM, DM and anti-tRNA synthetase positive patients. A multivariate logistic regression model was created, including the microsatellite marker and known myositis HLA class II alleles as predictors (DRB1*03, DQA1*05, DQB1*02 [3]). The (CA)14 polymorphism remained a significant risk factor in overall and myositis/CTD-overlap groups after adjusting for the HLA alleles, but no additive or multiplicative effects were observed. Only one (CA)14/(CA)14 homozygote was observed, in the DM group. The frequency of the heterozygous genotype (CA)12/(CA)14 was significantly increased in myositis/CTD-overlap patients vs. controls (6.8% overlap vs. 1.3% controls, OR 13.4, 95% CI 2.7-128.1, corrected $p=0.002$). No other genotypic associations, including homozygous states, were observed for the microsatellite marker. No significant associations were observed in patients with other MSA/MAAs, overlap subgroups, CAM or ILD.

Haplotypes: Haplotype frequencies were calculated for the IFN-$\gamma$ markers, capturing >93% of the variation. After correction for multiple comparisons, no significant differences between cases and controls were noted.
DISCUSSION

We report evidence for disease-association of the IFN-γ gene in the largest SNP IIM study to date. The IFN-γ associations for disease are found primarily in the microsatellite marker, where the CA(14) repeat represents a risk factor, specifically in myositis-CTD/overlap and anti-U1-RNP positive patients. This association is independent of HLA class II members of the 8.1 ancestral haplotype and shows no interaction with these alleles.[3] However, the strength of the CA(14) repeat association is less than that of HLA class II alleles.[3] These findings are unlikely to be causal, as the tested associations are weak or not present for individual SNPs, and generally no longer present after correction for multiple comparisons. Certainly, testing multiple genetic markers in several subgroups may lead to potential problems in analyses, especially when associations are weak. Myositis/CTD-overlap is a somewhat heterogeneous group, therefore the described genetic associations may refer to general autoimmunity rather than specifically for myositis. IFN-γ microsatellite polymorphism associations have been described in other autoimmune disorders. In American SLE patients,[13] the CA(16) polymorphism is associated with anti-U1-RNP and severe disease markers. In giant cell arteritis, IFN-γ associations correlate with specific clinical severity features.[14] The CA(12) and CA(13) repeats are also associated with increased and reduced serum IFN-γ production respectively.[11;15] We have not examined whether the described genetic polymorphisms correlate with aberrant serum cytokine production.

The major genetic associations so far detected in myositis lie within the HLA region.[1] New technologies, e.g. whole genome association studies, may locate new disease susceptibility genes. We conclude that the tested markers within the IFN-γ, but not the IL-4 gene demonstrates significant genetic associations in UK IIM Caucasians. The IFN-γ gene requires further investigation, including on a functional basis, to elucidate the genetic role of the Th1/Th2 cytokine responses in the aetiopathogenesis of IIMs.
ACKNOWLEDGEMENTS AND FUNDING

Dr Hector Chinoy is an Arthritis Research Campaign Clinical Research Fellow. We are grateful to the Myositis Support Group UK, who funded myositis autoantibody testing. We also wish to thank the 60 participating physicians in the AOMIC (see appendix in [3]), without whom this research would not have been possible.

COMPETING INTERESTS

Nil
Table 1: Genotype and allele frequencies of IFN-γ and IL-4 polymorphisms in myositis patients and controls

<table>
<thead>
<tr>
<th>IL-4 markers</th>
<th>Controls</th>
<th>PM</th>
<th>DM</th>
<th>Overlap</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2070874 n=156</td>
<td>n=156</td>
<td>n=88</td>
<td>n=84</td>
<td>n=65</td>
</tr>
<tr>
<td>CC, CT, TT</td>
<td>75.6, 23.1, 1.3</td>
<td>79.5, 20.5, 0</td>
<td>77.4, 21.4, 1.2</td>
<td>69.2, 29.2, 1.6</td>
</tr>
<tr>
<td>C, T</td>
<td>87.2, 12.8</td>
<td>89.8, 10.2</td>
<td>88.1, 11.9</td>
<td>83.8, 16.2</td>
</tr>
<tr>
<td>rs734244 n=161</td>
<td>n=161</td>
<td>n=97</td>
<td>n=90</td>
<td>n=70</td>
</tr>
<tr>
<td>CC, CT, TT</td>
<td>77.6, 12.4, 9.9</td>
<td>75.3, 10.3, 14.4</td>
<td>77.6, 10.3, 12.1</td>
<td>71.4, 18.6, 10.0</td>
</tr>
<tr>
<td>C, T</td>
<td>83.8, 16.2</td>
<td>80.4, 19.6</td>
<td>80.7, 19.3</td>
<td>81.3, 18.7</td>
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<tr>
<td>rs2227284 n=156</td>
<td>n=156</td>
<td>n=87</td>
<td>n=83</td>
<td>n=64</td>
</tr>
<tr>
<td>GG, GT, TT</td>
<td>59.0, 36.5, 4.5</td>
<td>59.8, 35.6, 4.6</td>
<td>68.7, 60, 25.3</td>
<td>56.3, 35.9, 7.8</td>
</tr>
<tr>
<td>G, T</td>
<td>77.2, 22.8</td>
<td>77.6, 22.4</td>
<td>81.3, 18.7</td>
<td>78.0, 22.0</td>
</tr>
<tr>
<td>rs2227282 n=151</td>
<td>n=151</td>
<td>n=88</td>
<td>n=84</td>
<td>n=67</td>
</tr>
<tr>
<td>GG, GC, CC</td>
<td>58.3, 37.1, 4.6</td>
<td>55.7, 38.6, 5.7</td>
<td>70.2, 23.8, 6.0</td>
<td>58.2, 34.3, 7.5</td>
</tr>
<tr>
<td>G, C</td>
<td>76.8, 23.2</td>
<td>75.0, 25.0</td>
<td>82.1, 17.9</td>
<td>75.4, 24.6</td>
</tr>
<tr>
<td>rs2243266 n=156</td>
<td>n=156</td>
<td>n=87</td>
<td>n=85</td>
<td>n=61</td>
</tr>
<tr>
<td>CC, CT, TT</td>
<td>76.9, 21.8, 1.3</td>
<td>78.2, 21.8, 0</td>
<td>77.6, 21.2, 1.2</td>
<td>70.5, 27.9, 1.6</td>
</tr>
<tr>
<td>C, T</td>
<td>87.8, 12.2</td>
<td>89.1, 10.9</td>
<td>88.2, 11.8</td>
<td>84.4, 15.6</td>
</tr>
<tr>
<td>rs2243267 n=156</td>
<td>n=156</td>
<td>n=90</td>
<td>n=85</td>
<td>n=65</td>
</tr>
<tr>
<td>GG, GC, CC</td>
<td>76.3, 22.4, 1.3</td>
<td>79.0, 21.2, 0.8</td>
<td>77.6, 21.2, 1.2</td>
<td>69.2, 29.2, 1.6</td>
</tr>
<tr>
<td>G, C</td>
<td>87.5, 12.5</td>
<td>89.4, 10.6</td>
<td>88.2, 11.8</td>
<td>83.8, 16.2</td>
</tr>
</tbody>
</table>

IFN-γ markers

| rs1861494 n=151 | n=151 | n=85 | n=72 | n=60 |
| TT, TC, CC | 47.0, 42.2, 10.6 | 56.5, 36.5, 7.0 | 50.0, 37.5, 12.5 | 56.7, 35.0, 8.3 |
| T, C | 68.2, 31.8 | 74.7, 25.3 | 68.8, 31.2 | 74.2, 25.8 |
| rs1861493 1 n=148 | n=148 | n=83 | n=74 | n=63 |
| AA, AG, GG | 44.6, 45.3, 10.1 | 59.0, 35.0, 6.0 | 48.6, 37.8, 13.6 | 58.7, 31.8, 9.5 |
| A, G | 67.2, 32.8 | 76.5, 23.5 | 67.6, 32.4 | 74.6, 25.4 |
| rs2069733 n=159 | n=159 | n=101 | n=90 | n=69 |
| G/G, G/-, -/- | 52.8, 39.0, 8.2 | 60.4, 35.6, 4.0 | 48.9, 43.3, 7.8 | 62.3, 30.4, 7.3 |
| G, - | 72.3, 27.7 | 78.2, 21.8 | 70.6, 29.4 | 77.5, 22.5 |
| rs2069718 n=156 | n=156 | n=87 | n=82 | n=66 |
| GG, GA, AA | 35.9, 51.9, 12.2 | 47.1, 41.4, 11.5 | 37.8, 41.5, 20.7 | 33.3, 57.6, 9.1 |
| G, A | 61.9, 38.1 | 67.8, 32.2 | 58.5, 41.5 | 62.1, 37.9 |

Key: PM=Polymyositis; DM=dermatomyositis. n may vary between markers due to genotyping failures. XX, XY, YY refers to percentage genotype frequencies. X, Y refers to percentage allele frequencies. Allele percentage frequencies are based on 2n.

1rs1861493 A allele, PM vs. controls, puncorr=0.04, pcorr>0.05, odds ratio 1.6, 95% confidence interval 1.0-2.4. rs1861493 AA genotype, PM vs. controls, puncorr=0.04, pcorr>0.05, odds ratio 1.8, 95% confidence interval 1.0-2.3.2.
Table 2: Allele frequencies in the IFN-γ microsatellite marker

<table>
<thead>
<tr>
<th>Amplicon size (bp)</th>
<th>IFN-γ (CA)n</th>
<th>Controls</th>
<th>PM</th>
<th>DM</th>
<th>Overlap</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2n=316</td>
<td>2n=186</td>
<td>2n=178</td>
<td>2n=136</td>
<td>2n=500</td>
<td></td>
</tr>
<tr>
<td>122</td>
<td>11</td>
<td>0</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
<td>0.2</td>
</tr>
<tr>
<td>124</td>
<td>12</td>
<td>49.7</td>
<td>49.5</td>
<td>47.2</td>
<td>43.4</td>
<td>47.0</td>
</tr>
<tr>
<td>126</td>
<td>13</td>
<td>45.2</td>
<td>40.3</td>
<td>44.4</td>
<td>44.8</td>
<td>43.0</td>
</tr>
<tr>
<td>128</td>
<td>14</td>
<td>1.9</td>
<td>5.4</td>
<td>5.0</td>
<td>8.1</td>
<td>6.0</td>
</tr>
<tr>
<td>130</td>
<td>15</td>
<td>2.9</td>
<td>3.8</td>
<td>3.4</td>
<td>3.7</td>
<td>3.6</td>
</tr>
<tr>
<td>132</td>
<td>16</td>
<td>0.3</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
<td>0.2</td>
</tr>
<tr>
<td>p</td>
<td>0.1</td>
<td>0.3</td>
<td>0.02</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Key as per table 1. Percentage frequencies presented. *n represents the (CA) dinucleotide repeat number. ¹Overlap vs. controls, OR 4.5 (1.7-12.1), p uncorr=0.005, p_corr=0.03. ²Comparison of distribution of alleles between cases and controls using Fisher’s exact test.
FIGURE

Figure 1: Schematic representations of genetic markers examined as they appear in the genome. Data obtained from the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov). Legend: UTR=untranslated region; MS=microsatellite; arrows indicate direction of transcription
REFERENCES


IFN-γ gene, chromosome 12q14

IL-4 gene, chromosome 5q31.1
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