Monocyte chemotactic protein-1 single nucleotide polymorphisms do not confer susceptibility for the development of adult onset polymyositis/dermatomyositis in UK Caucasians

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Objectives. Polymyositis (PM) and dermatomyositis (DM) form part of the idiopathic inflammatory myopathies (IIMs). The chemokine monocyte chemotactic protein-1 (MCP-1) is expressed at sites of the T cell inflammatory response in the IIMs. We thus investigate whether genetic markers in the MCP-1 gene confer disease susceptibility for the development of PM and DM.

Methods. DNA samples were analysed from a group of 195 UK Caucasian IIM patients, comprising 103 PM and 92 DM. Their results were compared with those of 162 ethnically matched controls. The polymorphic positions of three single nucleotide polymorphisms (SNPs) and one insertion–deletion sequence within regions coding for MCP-1 were tested. The SNPs examined were located in intron 1 (rs2857657, C/G), exon 2 (rs4586, A/G) and the 3′ untranslated region (rs13900, C/T). The insertion–deletion sequence was located in intron 1 (rs3917887, AGCTCCTCCTTCTC/−). Each SNP was tested for Hardy-Weinberg equilibrium and allelic/genotypic associations. Haplotype frequencies were estimated using the Expectation/Maximization algorithm.

Results. There was strong linkage disequilibrium present between three out of these four markers. The majority of controls were in Hardy Weinberg equilibrium. No allelic, genotypic or haplotypic associations were detected when comparing PM or DM cases to controls, or when PM and DM were compared with each other.

Conclusions. Genetic markers in the MCP-1 gene do not demonstrate significant genetic associations with the IIMs, and do not discriminate PM from DM in a UK Caucasian population.

Key words: Myositis, Polymyositis, Dermatomyositis, Monocyte chemotactic protein-1, Single nucleotide polymorphisms.

Introduction

The idiopathic inflammatory myopathies (IIMs) are a heterogeneous group of diseases defined by the presence of acquired muscle inflammation and weakness, which include polymyositis (PM) and dermatomyositis (DM). In PM and DM, a characteristic histopathological feature in biopsied muscle tissue is that of mononuclear inflammatory cell infiltrates, in differing pattern and location depending on disease subtype. Thus, in PM, a higher proportion of CD8⁺ T cells are present, and localized in the endomysium, while in DM, macrophages and CD4⁺ T cells are more commonly present in perivascular and perimysial sites [1, 2].

Monocyte chemoattractant protein-1 (MCP-1) is an important member of the C-C family of chemokines, and acts as a chemoattractant and activator of T lymphocytes and monocytes in the pathogenesis of diseases characterized by mononuclear infiltrates. Tissue expression studies of MCP-1 in muscle biopsies from IIM patients show that MCP-1 correlates with areas of T cell inflammatory responses [3–5]. MCP-1 expression is also thought to play an important role in tissue inflammation in other autoimmune conditions. For example, high levels have been isolated in plasma/urine of patients with conditions characterized by inflammatory vascular injury, including lupus nephritis [6], Kawasaki disease [7] and giant cell arteritis (GLA) [8]. The MCP-1 gene is located on chromosome 17q11.1-q21.1, and consists of three exonic regions. MCP-1 gene polymorphisms have been demonstrated in a number of autoimmune conditions [9–13], and it is plausible that such polymorphisms may influence susceptibility in these diseases.

Based on this evidence, we hypothesized that mutations in the MCP-1 gene may affect disease susceptibility in inflammatory myositis. Furthermore, as the pattern and type of inflammatory infiltrates in affected muscle differs between PM and DM, it is also possible that MCP-1 genetic markers could differentiate these disease subtypes. We thus investigated large cohorts of PM and DM patients to determine whether mutations in the MCP-1 gene affect susceptibility to the development of IIMs, stratified according to clinical classification and phenotype.
Methods

Study design

A case-control, cross-sectional genetic association study was undertaken to investigate associations between genetic markers in the gene and disease susceptibility in PM and DM.

Study population

Between 1999 and 2004, a UK-wide collaboration comprising 56 rheumatologists and four neurologists [the Adult Onset Myositis Immunogenetic Collaboration (AOMIC)] recruited 195 UK Caucasian patients aged 18 years of age or older at disease onset. Patients with PM or DM had probable or definite PM/DM, based on the Bohan and Peter criteria [14, 15]. A standardized clinical data collection form was used, detailing demographics and individual clinical details. Physicians at each study site assessed for interstitial lung disease, by pulmonary function testing and thoracic imaging, and cancer-associated myositis, by relevant investigations. One hundred and sixty-two healthy unrelated Caucasian control subjects were recruited, representing UK blood donors collected as controls for other disease studies [16]. Collection of data and blood from patients and controls was under regulation of the local research ethics committees.

Serological typing

Sera were obtained from patients for determination of myositis specific/associated antibodies (MSA/MAAs), as described [17].

Genotyping

Three SNPs (rs2857657, rs4586, rs13900) and one insertion–deletion (rs3917887) were selected from the National Center for Biotechnology Information SNP database (www.ncbi.nlm.nih.gov/SNP). Genotyping assays were based on TaqMan (PE Applied Biosystems, Warrington, UK). dNTP primer extension methods were designed for polymorphic sites within MCP-1. SNP location and nucleotide substitutions are shown in Table 1. Additionally, one insertion–deletion site was typed using SNaPshot (PE Applied Biosystems, Warrington, UK). HLA genotyping was performed as previously described [17].

TaqMan

Two MCP-1 SNPs were genotyped using Assay-By-Design (ABI), consisting of unlabelled PCR primers and TaqMan MGB probes (FAM and VIC dye-labelled) in a 40× probe mix. Primers and probes used were as follows: (1) rs2857657: forward primer GTAT AGCCAGAAGCACTGGGATTTA; reverse primer CAGAAA AGAGTCATGGAGAAAAAGCA; VIC ATGAGCTCTTTG TCTTCT (reverse probe) and FAM ATGAGCTCTTTCTCT TCT (reverse probe); (2) rs4586: forward primer TGCAATCA TCTTCT (reverse probe) and FAM ATGAGCTCTTTG TCTTCT (reverse probe) and VIC ATGAGCTCTTTATAGCA (reverse probe). One MCP-1 SNP (rs13900) was genotyped using ABI Assays-On-Demand, assay ID: C___7449810_10.

Templates of 10 ng were amplified with 0.25 μl of 40× probe mix (Applied Biosystems) and 2.5 μl of 2 × PCR mastermix (Eurogentec) in a 5 μl final volume. Assays were incubated at 50°C for 2 min, 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min.

Sizing reaction

The insertion–deletion marker (rs3917887) was genotyped using an ABI 3100 DNA analyser. Primers used were as follows: forward primer 6-FAM - GCTGATCTTCCCTGGTGCTGAT; reverse primer CATTAAATCCTCCAGTGGCTTGCCTA; The forward primer was fluorescently labelled with 6-FAM attached to the 5′ primer end during synthesis. PCRs were performed in a 10μl total reaction volume, containing 10ng of DNA, 10 pmol of each PCR primer, 4 mmol of each of the four deoxynucleotide triphosphates, 0.2 units Taq polymerase (Bioline) in 1.5 mM MgCl₂ buffer. The PCRs were performed in 384-well microtitre plates on Dyad thermal cyclers with 35 denaturation cycles, primer annealing at 40°C and extension at 72°C. PCR products were diluted 1:200, mixed with ROX 400 size standard, and loaded onto the ABI 3100 for capillary electrophoresis.

Statistical analyses

Genotyping frequencies for each marker were tested for Hardy-Weinberg equilibrium (HWE) in cases and controls to ensure that the subjects studied were selected independently and at random. Allelic and genotype frequencies were compared between myositis cases and controls, using Fisher’s exact test. Where significant, data were expressed as odds ratios (OR) with exact 95% confidence intervals (CI). Linkage disequilibrium (LD) was calculated with r values, using HelixTree (version 3.1.2, Golden Helix, Inc., MT, USA). Haplotypes were estimated in individuals where SNP data were complete, and constructed using the Expectation/Maximization (EM) algorithm. A two-marker moving window haplotype analysis was also undertaken. Unless otherwise stated, the statistical package Stata (release 8, Stata Corp., College Station, TX) was used to perform statistical analysis. The study had 80% power to detect the effect of a SNP in the myositis groups combined, with a 10% minor allele frequency conferring an OR of 2.0 at the 1% significance level, assuming a dominant mode of inheritance.

Results

Demography

Of the 195 UK Caucasian myositis patients recruited, 103 had PM (69 females, 70%) and 92 DM (65 females, 70.6%). There was no significant difference in the mean age at the onset of myositis between the disease subgroups (mean 48 years). The median duration of disease at data capture was 5 years. The allelic and genotype frequencies for all four MCP-1 genetic markers did not deviate from HWE in either cases or controls. LD correlation was strong between the MCP-1 genetic markers (r > 0.8), with the exception of SNP-2 (r < 0.4). No between-gender differences were noted within cases or control subjects for any of the MCP-1 markers tested (P > 0.2 for both comparisons).

Genotype and genotype frequencies

Genotype and allelic frequencies were examined for each individual polymorphism and the results summarized in Table 1. No significant differences were observed in the frequencies between cases and controls, even when the myositis subgroups were combined into one overall myositis group. Patients were also stratified according to whether or not they were HLA-DRB1*03 positive (a known myositis genetic risk factor) [17, 18], to see if this unmasked underlying associations. Analysis was also stratified by the presence or absence of circulating MSA/MAAs, interstitial lung disease or associated malignancy, but no differences in the allele or genotype frequencies were observed between cases and controls (data not shown). The PM and DM groups were then directly compared to each other, but no significant differences in allele or genotype frequencies were noted.
This study represents the largest myositis genetic association study undertaken to date examining genetic markers outside of the HLA region, and utilizing large cohorts of UK Caucasian PM and DM patients. For the first time in the IIMs, genetic markers have been studied within the MCP-1 gene to assess their role in conferring susceptibility for the development of myositis, and to determine whether the markers could discriminate PM from DM. Four common genetic markers in the MCP-1 gene, including three SNPs and one insertion–deletion sequence were examined. Unfortunately, no significant allelic, genotypic or haplotypic differences were demonstrated between cases and controls. No associations were demonstrated after stratification for the presence of myositis antibodies, clinical features or after allowing for the presence of HLA-DRB1*03 [17, 18]. Furthermore, no significant differences were noted when a direct comparison between PM and DM was made. The MCP-1 genetic markers tested here are not therefore significant risk factors in the development of myositis, and do not discriminate PM from DM.

The role of MCP-1 in tissue overexpression has been convincingly demonstrated in IIM muscle biopsy studies [3–5]. For example, MCP-1 mRNA is highly expressed in PM, DM and inclusion body myositis (IBM), but detectable at much lower levels in muscle biopsy tissue from normal controls. In DM, immunohistochemistry studies demonstrated that MCP-1 expression was most evident in the perimysium, whereas in PM and IBM, expression was strongest around inflammatory cells invading non-necrotic fibres. MCP-1 has not only been found in T cell-infiltrated muscle cells, but also in neighbouring extracellular matrix, suggesting a role in lymphocyte activation and migration [4]. Additionally, the MCP-1 receptor, CCR2, is highly expressed on inflammatory and endothelial cells in the IIMs [19, 20]. In PM/DM, MCP-1 expression may only be influenced at a local level secondary to other inflammation processes, and this may explain the lack of significant MCP-1 genetic associations found. MCP-1 genetic polymorphisms in the IIMs may only influence quantitative expression of MCP-1, which we have not tested for.

The SNPs examined in our study also appeared to be good candidate markers to examine. All markers had a minor allelic frequency of at least 20%, one marker was present in the 3’UTR regulatory region, one a synonymous coding exonic SNP and one an insertion–deletion sequence. The SNPs also formed significant haplotype associations in a study comparing biopsy-proven GCA patients to controls [13]. MCP-1 gene polymorphisms have been examined in other autoimmune diseases, including systemic lupus erythematosus [9, 10], systemic sclerosis [11], Crohn’s disease [12] and GCA [13]. A number of these studies examined a -2518 polymorphism in the MCP-1 promoter region. This SNP appears to influence transcriptional activity, as in vitro studies have shown that, in response to IL-1β, cells from GG or AG individuals produce more MCP-1 protein compared to AA individuals [21].

The failure to detect differences between IIM patients and controls may be due to a number of factors. First, this may be due to false-negative results. However, genotyping errors are unlikely, as the genetic markers showed no HWE deviation, and the control allele frequencies are very similar to those previously reported (http://www.ncbi.nlm.nih.gov/SNP/) [13]. The power calculation confirmed adequate power to detect potential differences between cases and controls. Subgroup analysis aims to create homogenous patient groups, but case numbers are inevitably reduced, making it less likely to detect small but significant differences. The MCP-1 genetic markers in this study are not significant risk factors in the IIMs, but other MCP-1 SNPs, including those in the promoter region, may show evidence of association. Other candidate C-C chemokine genes on the same chromosome should also be examined to more fully characterize the role of chemokines in the IIMs.

Up-regulation of MCP-1 expression has previously been demonstrated in muscle biopsy tissue from IIM patients [3–5, 19, 20], suggesting a role is played by this chemokine in the inflammatory processes involved in IIMs.
the pathophysiology of the IIMs. However, we have not demonstrated significant associations in relation to SNPs and an insertion–deletion sequence tested within the MCP-1 gene. In conclusion, our findings do not support a role of the studied MCP-1 genetic markers for IIM susceptibility in UK Caucasian population.

### Key messages

- The largest myositis genetic study examining genetic markers outside the HLA region.
- The findings do not support a role of MCP-1 genetic markers for IIM susceptibility in UK Caucasians.

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The authors have declared no conflicts of interest.

## References