Concise Report

**MHC2TA** promoter polymorphism (−168*G/A, rs3087456) is not associated with susceptibility to rheumatoid arthritis in British Caucasian rheumatoid arthritis patients

P. Harrison, J. J. Pointon, C. Farrar¹, A. Harin¹ and B. P. Wordsworth

**Objective.** To investigate the association of a single-nucleotide polymorphism (SNP) in the promoter region of **MHC2TA** gene (−168*G/A, rs3087456), which has previously been described in a Swedish rheumatoid arthritis (RA) cohort, in British Caucasian RA patients.

**Methods.** We genotyped 733 RA patients and 613 healthy controls for **MHC2TA** −168*G/A SNP by amplification-refractory mutation system (ARMS). Data were analysed using SPSS version 13.0 software and the chi-square test was applied where appropriate.

**Results.** The **MHC2TA** −168*G/A SNP was not associated with increased susceptibility to RA in our patients. Stratifying the patients according to the presence or absence of rheumatoid factor (RF) showed the SNP to be more common in RF negative patients, but this did not reach statistical significance.

**Conclusion.** We did not confirm the previously reported association of this **MHC2TA** polymorphism with RA in our UK population despite its ethnic similarities with the Swedish population in which it was first described.

**Key words:** **MHC2TA**, Rheumatoid arthritis, HLA, Rheumatoid factor negative.

**Introduction**

Rheumatoid arthritis (RA) is a classic example of a common complex disease with a multifactorial aetiology. It is a clinically heterogeneous condition, the seropositive erosive form of which has a substantially higher sibling recurrence risk than forms of the mild non-erosive disease [1]. The strongest association is with **HLA-DRB1** alleles, estimated at around 30% of the total genetic effect [2]. It is likely that several other genes are also involved, but only **PTPN22**, encoding a protein tyrosine phosphatase important in regulating T-cell activation, has been reliably and reproducibly implicated to date [3, 4]. **HLA-DRB1** is one of a number of genes within the class II region of the major histocompatibility complex (MHC), expression of which is predominantly regulated by the MHC class II transactivator (CIITA). CIITA production is dependent on the transcription of its gene **MHC2TA**, which is further controlled by four functional promoters [5]. Recently a Swedish group described a single-nucleotide polymorphism (SNP) (−168*A/G, rs3087456) in the type III promoter of **MHC2TA** associated with susceptibility to RA, multiple sclerosis and myocardial infarction [6]. This result was not replicated in a smaller German population [7]. Since the power to replicate positive findings is heavily influenced by the sample size, particularly where the genetic effect is weak. Our aim was to study the rs3087456 polymorphism in a large sample of British Caucasian RA patients.

**Method**

Patients with RA (n = 733) were recruited from the Nuffield Orthopaedic Centre in Oxford. All RA patients were Caucasians living in the UK and satisfied the 1987 American College of Rheumatology criteria. The controls (n = 613) were healthy British Caucasian blood donors from the same geographical area. All subjects gave informed, written consents, and approval was granted by the Oxford Research Ethics Committee. Medical records were examined to determine the rheumatoid factor (RF) status of each patient. Patients who were positive at two different time points were considered to have seropositive disease. Where the data was not available, RF was measured using nephelometric technique and a titre over 40 IU/ml was considered positive.

Genomic DNA was extracted from whole blood. Cases and controls were genotyped for **MHC2TA** −168*G/A polymorphism using amplification-refractory mutation system (ARMS) with additional mismatch (underlined). The primer sequences used were: for allele A, forward 5'-GTGAAATTAATTTACAG AGGTTAGA and reverse 5'-AGAAGCAGACACAGCCTCATCA; for allele G, forward 5'-ATGACTGTGCCCCCATCTGG and reverse 5'-CCTCCTTAAGCCCTCCAC. The PCR conditions were as following: 95°C for 10 min, 35 cycles of 95°C for 30 s, 58°C for 30 s for allele A or 60°C for allele G, 72°C for 30 s, followed by 72°C for 10 min. PCR products were resolved on 3% agarose gel stained with ethidium bromide and visualized under ultraviolet light. All samples were genotyped in duplicates and
positive and negative controls were included. Statistical analysis for association and Hardy–Weinberg equilibrium were evaluated using chi-square test. P-values <0.05 were considered significant. For continuous variables statistical software package SPSS version 13.0 for Windows was used.

Results

All the genotypes were in Hardy–Weinberg equilibrium, and there was no association of the MHC2TA –168*G/A with susceptibility to RA (Table 1). When we stratified our RA population for the presence of RF, the minor allele frequency for RF positive patients was 0.25 (similar to healthy controls) compared with 0.30 for RF negative patients, but this did not reach statistical significance.

No significant associations were observed when the patients were stratified for gender, age of onset or HLA-DRB1 genotype.

Discussion

It has become evident that distinct autoimmune diseases can share common genetic polymorphisms, a classic example for which is in PTPN22. This gene encodes a protein tyrosine phosphatase essential for T-cell signalling. The R620W variant has now been associated with several autoimmune diseases, including type I diabetes, RA and systemic lupus erythematosus [8–10]. Another possible example of a genetic variant common to several diseases was recently suggested: a promoter SNP in MHC2TA was associated with RA, multiple sclerosis and myocardial infarction in Swedes. Including our study, there have been two subsequent independent studies assessing the MHC2TA –168*G/G polymorphism in German and British RA patient populations. The Swedish, German and British study groups were all reasonably powerful statistically, containing 1288, 319 and 733 RA patients, compared with 709, 463 and 613 healthy controls, respectively. In contrast to the initial association of MHC2TA –168*G/G SNP with RA in the Swedish RA population, we could not confirm this in our British RA population, and this is consistent with the recent German report [7]. The likely explanation for this apparent difference between the three populations may lie in the minor allele frequencies found in different control populations. It is striking that the minor allele frequencies are very similar in the RA patients from each population (24.4% in Swedes, 25% in German and 26% in British) but in contrast, it is only 20.5% in the Swedish controls compared with 26% in the Germans and 25% in the British. Such a difference in the control samples could have occurred by chance, generating a spurious association with RA in the Swedes. It seems relatively unlikely, given the very similar allele frequencies in all but the Swedish control group, that this is a true ethnic-related genetic effect. Furthermore, if the minor allele frequency observed in the Swedish controls were spuriously low, it might also account for the association seen with the other diseases. In particular, it could account for the surprising association between MHC2TA and myocardial infarction, which defies an obvious biological explanation. In the original Swedish study, a surprisingly low proportion (62.5%) of the RA group were RF positive compared with our sample. MHC2TA may, therefore, be more relevant to the aetiology of seronegative RA, and we did find a small (non-significant) difference between our seropositive and negative patients. Replication of the initial results in a further independent Swedish population is, therefore, desirable.

In their analysis of the Swedish population, Swanberg et al. [6] indicated a significant effect for the GG genotype in a codominant model of susceptibility. This equates with an excess of 21 patients with the GG genotype in the RA group compared with predictions (n=62) based on the genotype frequency in controls (4.8%). This would be a tiny patient subgroup (constituting only 1.6% of their study population) compared with other known RA associations, such as HLA-DRB1.

Swanberg et al. [6] indicated that the putative disease-associated polymorphism caused a modest (≤50%) reduction in IFN-γ-induced MHC2TA and HLA-DR mRNA expression in peripheral blood cells. Since HLA-DR molecules are believed to be important in the pathogenesis of RA, it is tempting to conclude that factors involved in regulating their expression might also be involved in the aetiology of the disease. CIITA expression in lymphoid cells is almost exclusively regulated by type III promoter in contrast to cells of myeloid origin, where CIITA expression is regulated by promoter I or induced by INF-γ. Promoter IV is responsible for CIITA expression in non-professional antigen-presenting cells and thymic epithelial cells and is strongly influenced by INF-γ [11]. It is also interesting that statins (HMG-CoA reductase inhibitors), by inhibiting MHC2TA gene activation, also repress INF-γ induced MHC class-II expression [12]. It remains to be seen whether there may be potential future benefit from suppressing such MHC class II expression that can be harnessed in the treatment of diseases where MHC class II-dependent T-cell activation is required. Thus, MHC2TA and its regulation are attractive candidates for therapeutic manipulation in RA, but we suggest that genetic variation at this locus is unlikely to play a significant role in its genetic aetiology.

### Table 1. Genotyping data and allele frequencies

<table>
<thead>
<tr>
<th>Allele frequency</th>
<th>Genotyping frequency Codominant model</th>
<th>Genotyping frequency G-dominant model</th>
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<tr>
<td></td>
<td>A (%) G (%) OR, 95% CI</td>
<td>AA (%) AG (%) GG (%) P-value</td>
</tr>
<tr>
<td>RA cases n = 733</td>
<td>1082 (74) 384 (26) 1.0 (0.9–1.1) P = 0.7</td>
<td>404 (55) 274 (37) 55 (8) 0.4</td>
</tr>
<tr>
<td>Controls n = 613</td>
<td>914 (75) 312 (25) 0.8 (0.6–1.0) P = 0.08</td>
<td>336 (55) 242 (39) 35 (6) 0.2</td>
</tr>
<tr>
<td>RF pos n = 559</td>
<td>834 (75) 284 (25) 1.0 (0.8–1.2) P = 1.0</td>
<td>315 (56) 204 (37) 40 (7) 0.4</td>
</tr>
<tr>
<td>RF pos vs RF neg</td>
<td>1.3 (1.0–1.7) P = 0.08</td>
<td>0.2</td>
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* vs healthy controls.

Key message

- Polymorphism in the third promoter of MHC2TA gene is not associated with increased susceptibility to RA in British Caucasian patients.
Acknowledgements

The authors are grateful to the patients for their cooperation. The authors specifically want to thank Dr J. David and Dr P. Bowness for the access to the patients, and nurses V. Ziel and C. Jess for their assistance in sample collections.

The authors have declared no conflicts of interest.

References