Confirmation of association of the REL locus with rheumatoid arthritis susceptibility in the UK population.

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Genome-wide association studies (GWAS) have contributed to the identification of at least 14 rheumatoid arthritis (RA) susceptibility loci (1). One of the first RA GWAS included 1,522 cases and 1,850 controls from the US/Sweden and identified TRAF1/C5 as a novel RA locus (2). Recently this GWAS was repeated after including an additional 1,550 cases and 3,310 controls from the US and restricting analysis to the US subjects (3). In the expanded sample, two novel single nucleotide polymorphisms (SNPs) mapping to the REL locus showed association with RA. REL encodes c-Rel, a member of the NF-κB family of transcription factors and one of the associated SNPs (rs13031237) maps to an intron of this gene. The association was validated in an independent sample of 2,604 RA cases and 2,882 controls from the US/Canada with strong evidence for association in the combined samples (rs13031237 p = 3.1 x 10^{-14}). We aimed to test association of the same variants with RA in a large UK case control sample.

Caucasian patients with RA were recruited from 6 centres across the UK with ethical committee approval (MREC 99/8/84) and after providing informed consent (4;5). Genotyping was performed using Sequenom and only samples and SNPs exceeding 90% success rate were included in subsequent analysis. Genotype frequencies were compared between cases and controls using the trend test implemented in PLINK (6).

DNA samples from 3,962 RA cases and 3,531 controls were available for testing and the clinical characteristics have been described previously (4;5). The two SNPs, rs13031237 and rs13017599, strongly associated with RA in the previous US/Canadian study were genotyped in the UK samples and both SNPs showed strong evidence for association, with no deviation from Hardy-Weinberg expectations (Table 1). In the previous study, the subjects investigated were overwhelmingly positive for autoantibodies (3). We, therefore, undertook subgroup analysis in autoantibody positive groups. The strength of association was stronger in anti-CCP positive, RF positive and autoantibody positive subgroups compared with the overall group. A meta-analysis of data from the previous US/Canadian sample and the current UK group was undertaken and increased the strength of evidence for association to 1.7 x 10^{-17} (Figure 1).

In this large sample, we provide confirmation of association of the REL locus with RA in a UK population. The associated markers map 28.5kb apart on chromosome 2p, are in almost complete linkage disequilibrium (r^2 = 0.97, D' = 1) and, in logistic regression models, it was not possible to determine which was driving the association. Rs13017599 is a synonymous substitution (asparagine) within the ribosomal protein S12 pseudo gene 3 (RPS12P3), which is not an obvious candidate RA gene. Rs13031237 maps to an intron of REL, which is a stronger candidate RA gene because, firstly, it encodes a component of the NF-κB signalling pathway and, secondly, c-Rel deficient mice are resistant to induction of collagen-induced arthritis, suggesting a crucial role for c-Rel in the development of systemic autoimmunity (7). There are no other confirmed genes within the linkage disequilibrium block defined by SNPs with r^2 > 0.5 with either of the SNPs tested.

Interestingly, many of the RA loci identified, like the one confirmed here, show stronger effects in autoantibody-positive subgroups suggesting that autoantibody positive RA may have different underlying pathogenic mechanisms underpinned by different genetic loci compared with autoantibody negative disease.
However, it should be noted that the number of autoantibody negative samples included in studies is often quite small.

In summary, we provide confirmatory support for the association of the REL locus with RA. Fine mapping and functional studies will be required to identify the causal variant(s) and inform our understanding of how these variants influence the pathogenesis of RA.

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References


Table 1. Genotype counts and frequencies for SNPs mapping to chromosome 2p in UK RA cases and controls and association of SNPs in subgroups stratified by auto-antibody status.

<table>
<thead>
<tr>
<th></th>
<th>rs13031237</th>
<th>rs13017599</th>
</tr>
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<tbody>
<tr>
<td>Case: n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 / 2</td>
<td>508 (14.9)</td>
<td>507 (14.8)</td>
</tr>
<tr>
<td>1/ 2</td>
<td>1674 (49.0)</td>
<td>1672 (48.9)</td>
</tr>
<tr>
<td>1 / 1</td>
<td>1234 (36.1)</td>
<td>1239 (36.2)</td>
</tr>
<tr>
<td>Control: n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 / 2</td>
<td>350 (12.7)</td>
<td>352 (12.8)</td>
</tr>
<tr>
<td>1/ 2</td>
<td>1271 (46.2)</td>
<td>1270 (46.1)</td>
</tr>
<tr>
<td>1 / 1</td>
<td>1128 (41.1)</td>
<td>1133 (41.1)</td>
</tr>
</tbody>
</table>

Case control comparison:
- p-trend allelic OR (95% CI):
  - rs13031237: 5.26 x 10^{-05} (1.16 (1.08-1.25))
  - rs13017599: 6.96 x 10^{-05} (1.16 (1.08-1.25))

RF + (n=2370) vs controls (n=2758):
- p-trend allelic OR (95% CI):
  - rs13031237: 2.83E-06 (1.21 (1.12-1.31))
  - rs13017599: 3.91E-06 (1.21 (1.11-1.31))

RF - (n=784) vs controls (n=2758):
- p-trend allelic OR (95% CI):
  - rs13031237: 0.37 (1.06 (0.94-1.19))
  - rs13017599: 0.42 (1.05 (0.93-1.18))

Anti-CCP + (n=1184) vs controls (n=2758):
- p-trend allelic OR (95% CI):
  - rs13031237: 2.12E-05 (1.24 (1.12-1.37))
  - rs13017599: 2.67E-05 (1.23 (1.12-1.36))

Anti-CCP - (n=433) vs controls (n=2758):
- p-trend allelic OR (95% CI):
  - rs13031237: 0.34 (0.93 (0.8-1.08))
  - rs13017599: 0.41 (0.94 (0.81-1.09))

Auto-antibody + (n=2593) vs controls (n=2758):
- p-trend allelic OR (95% CI):
  - rs13031237: 7.71 x 10^{-07} (1.22 (1.12-1.31))
  - rs13017599: 1.01 x 10^{-06} (1.21 (1.12-1.31))

1 = major allele; 2 = minor allele
RF+ = RF positive; RF - = RF negative
Anti-CCP + = anti-CCP positive; Anti-CCP - = anti-CCP negative
Autoantibody positive + = positive for either RF or anti-CCP antibodies.

Figure 1. Meta-analysis of current UK data with previous data.
US and US/Canadian allele counts from ref (3).
Combined p-value = 1.53 x 10^{-17}
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