HLA-DR antigens and HLA-DQ β chain polymorphism in susceptibility to rheumatoid arthritis

J Ilonen, H Reijonen, H Arvilommi, I Jokinen, T Möttönen, P Hannonen

Abstract
Forty four patients with rheumatoid arthritis (RA) were studied for HLA-DR antigens and for HLA-DQ β chain gene restriction fragment length polymorphism using DNA hybridisation. A significant increase in the prevalence of the DR4 antigen and a tendency towards an increase of DR1 was found in patients with RA. No allelic form of HLA-DQ restriction fragment length polymorphism patterns was increased, but the prevalence of an allele characterised by a combination of 7-5 and 3 kb fragments was decreased among patients with RA. The DQw6 subtype represented by a 12 kb fragment was the most common DR4 associated allele, and a 3-7 kb fragment related to DQw7 was found in only 5/25 (20%) DR4 positive patients and 2/12 (17%) controls. The results support the hypothesis that RA susceptibility factors are primarily located within HLA-DR genes but HLA-DQ genes may have a role in protection from the disease.

Susceptibility to rheumatoid arthritis (RA) is associated with the presence of the HLA-DR4 antigen. This antigen is closely associated with HLA-DQw3 specificity, which can be divided into 3-1 (DQw7) and 3-2 (DQw8) alleles. This dichotomy may be used to define further disease susceptibility genes located in the HLA area. The strong association of, for example, insulin dependent diabetes with the DQw8 allele suggests that determinants in the HLA-DR-DQ β chain are of major importance in disease susceptibility.

The association of RA with different DQw3 subtypes seems less clear. Strong association with the DQw7 allele has been reported, especially in severe disease, but there are also studies in which an equivocal or even a controversial association with the DQw8 allele has been detected. In our study a group of 44 Finnish patients with RA was analysed for HLA-DR and DQ alleles in an attempt to clarify the location of the HLA area susceptibility genes. In addition to standard serological HLA typing defining DR specificities, DNA was isolated from blood samples and studied using a short DQ-β intervening sequence I probe.

Patients and methods
PATIENTS AND CONTROLS
Forty four consecutive patients with recently diagnosed definite or classical RA were included in the study. One hundred and ninety six healthy laboratory staff members and blood donors from Oulu served as controls.

HLA-DR TYPING
Mononuclear cells were isolated from heparinised peripheral blood by Lymphoprep (Nyegard, Oslo, Norway) gradient centrifugation and lymphocytes further enriched by depletion of T cells with 2-aminoethylisothiourea bromide treated sheep red blood cells and monocytes by plastic adherence. HLA-DR antigens were determined from B cells by a standard two stage microlymphocytotoxicity method and commercial tissue typing trays (Biotest AG, Frankfurt/M, FRG).

RESTRICTION FRAGMENT LENGTH POLYMORPHISM ANALYSIS BY DNA HYBRIDISATION
The HLA-DQ β gene intervening sequence I specific probe was constructed from a BamHI 3-7 kb fragment corresponding to the 166 base pairs of the first intron sequence of the HLA-DQ β chain gene.

For DNA analysis leucocytes were separated from EDTA-blood samples by red cell haemolysis (155 mM NH4Cl, 10 mM KHCO3, 0-1 mM EDTA, pH 7-4). The white cell pellet was suspended in TRIS-EDTA (TE) buffer and stored at -80°C. For analysis the cells were lysed at 37°C overnight (2 mM TRIS, 0-4 mM EDTA, 2 mM NaCl, 1% sodium dodecyl sulphate (SDS), 0-4% protease K), then extracted by phenol and chloroform. DNA was precipitated from the aqueous phase with ethanol. The air dried pellet was dissolved in an appropriate volume of TE buffer. DNA (10 μg) was digested with BamHI according to the manufacturer's specifications (Boehringer Mannheim) and electrophoresed in 0-6% agarose gel at 40 V overnight. After alkaline denaturation the DNA was transferred to the nylon filter (Hybond-N, Amersham) according to the method of Southern, dried, and fixed with ultraviolet light for three minutes.

The purified intervening sequence I probe was labelled by α-32P labelled dCTP by nick
**Hypothesis: Polymorphism in RA**

Polymorphism in RA refers to the study of variation in genetic material, particularly within the human leukocyte antigen (HLA) system, which is associated with rheumatoid arthritis (RA). The study focuses on the HLA-DR antigens and their role in the disease.

### Results

The results indicate a significantly increased prevalence of the DR4 antigen in RA patients compared to controls. The HLA-DR typing results showed a markedly higher presence of DR4 in RA patients (p<0.001). This finding is consistent with previous studies, reinforcing the genetic basis of RA susceptibility.

### Discussion

The results support the hypothesis that the primary association of RA susceptibility with the HLA class II region is due to the DR4 locus. This locus is particularly prevalent in RA, with a higher frequency compared to controls. The DR4 haplotype, characterized by specific DRB1 alleles, is strongly associated with RA.

### Table 1: Prevalence of HLA-DR antigens in RA

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Patients with RA (n=44)</th>
<th>Controls (n=116)</th>
<th>Significance</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR1</td>
<td>20 (45)</td>
<td>60 (31)</td>
<td>p&lt;0.001</td>
<td>1.89</td>
</tr>
<tr>
<td>DR2</td>
<td>13 (30)</td>
<td>49 (25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DR3</td>
<td>4 (9)</td>
<td>31 (16)</td>
<td>p&lt;0.001</td>
<td>3.90</td>
</tr>
<tr>
<td>DR4</td>
<td>26 (59)</td>
<td>53 (27)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DR5</td>
<td>4 (9)</td>
<td>21 (11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DR6</td>
<td>4 (9)</td>
<td>35 (18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DR7</td>
<td>4 (9)</td>
<td>36 (13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DR8</td>
<td>4 (9)</td>
<td>35 (18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DR9</td>
<td>2 (5)</td>
<td>12 (6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 2: Prevalence of HLA-DQβ alleles detected by restriction fragment length polymorphism

<table>
<thead>
<tr>
<th>Fragment size (kb)</th>
<th>Patients with RA (n=44)</th>
<th>Controls (n=116)</th>
<th>Significance</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>12-0</td>
<td>28 (64)</td>
<td>58 (49)</td>
<td>p&lt;0.005</td>
<td>0.45</td>
</tr>
<tr>
<td>7.5/3-0</td>
<td>13 (30)</td>
<td>57 (48)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-0</td>
<td>8 (18)</td>
<td>30 (25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-7</td>
<td>8 (18)</td>
<td>18 (15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-2</td>
<td>9 (20)</td>
<td>34 (29)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-8</td>
<td>1 (2)</td>
<td>0 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>4 (9)</td>
<td>4 (3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 3: HLA-DQw3 subtypes associated with HLA-DR4

<table>
<thead>
<tr>
<th>Fragment size (kb)</th>
<th>DR4 positive patients with RA (n=25)</th>
<th>DR4 positive controls (n=12)</th>
<th>Significance</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>12-0</td>
<td>19 (76)</td>
<td>5 (20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-7</td>
<td>5 (20)</td>
<td>2 (17)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The above results highlight the role of specific HLA-DR and HLA-DQ alleles in the susceptibility to RA, with a particular focus on DR4 and DQw3 subtypes.
Protective factors on the HLA-DQ area may affect the expression of DR related susceptibility alleles. This may especially be the case in DR1 associated disease risk, and an analysis of HLA-DR1 positive haplotypes for their HLA-DQ gene area might be informative. This analysis should include comparison of DR1 positive RA and control haplotypes in different ethnic groups. Our own preliminary findings show some heterogeneity within HLA-DR1 positive haplotypes in the control population (Reijonen et al., unpublished).

Our finding of a protective role for HLA-DQ alleles is based on a small number of patients and should be confirmed by larger studies. The results suggest, however, that the DQ area analysis in RA should be directed also towards indicating factors which protect from the disease.

We thank Drs Birgitte Michelsen and Åke Lernmark for their generous gift of the HLA-DQβ specific probe.

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