Associations of HLA-DRB and -DQB genes with two and five year outcome in rheumatoid arthritis

K Eberhardt, E Fex, U Johnson, F A Wollheim

Abstract

Objective—To evaluate the clinical usefulness of genomic HLA typing during the first five years of established rheumatoid arthritis (RA).

Methods—The HLA-DRB and -DQB alleles were determined by restriction length polymorphisms and polymerase chain reaction amplification with sequence specific primers in 99 Swedish patients with RA. Clinical features after two and five years disease duration were related to the genetic pattern. Seventy four patients were seropositive, 25 had nodules, 90 developed erosions, and 15 required joint replacements. Twelve patients were in remission after five years. Disability was assessed by health assessment questionnaire, and radiographic damage in hands and feet by the Larsen method.

Results—Eighty seven per cent of the patients carried the conserved third hypervariable region sequence (HVR3), 32% had DRB1*04 on one allele, and 26% had DRB1*04 on both alleles (all frequencies significantly greater than in controls). Frequencies of DRB1*04 associated DQB*0301 and *0302 were normal. Patients carrying DRB1*04 on both alleles tended to have more radiographic changes after two years, but this difference had diminished after five years. Disability did not vary with regard to the genotype. Homozygous HVR3 patients had about three times greater risk of undergoing joint replacement. Homozygosity for HVR3 and presence of DQB*0302 both tended to be associated with erosive disease.

Conclusions—We confirmed a strong association of disease with the presence of the shared epitope on one or two alleles. However, genotype was not strongly associated with disease severity after two and five years disease duration, and thus the value of genomic typing to select patients for early aggressive therapy is questionable.

Although it has been claimed that rheumatoid arthritis (RA) is becoming a less destructive disease, it has the potential to cause severe physical disability and shorten the average life expectancy. In addition, it has a considerable socioeconomic impact, even in the early stages of the disease. However, disease severity varies widely, and the disease may remain benign for prolonged time intervals in many patients; it is difficult to predict the course for the individual patient. Availability of better prognostic instruments would make it possible to select predictably severe cases for aggressive therapy at an early stage, at the same time avoiding unnecessary exposure of patients with mild disease. Such knowledge would also facilitate improved designs of drug trials.

The role of genetic factors has been much investigated since the demonstration of an increased prevalence of HLA-DR4, and to a lesser extent HLA-DR1, in patients with RA in many white populations. Further studies revealed 11 different subtypes of DR4, of which only DRB1*0401(Dw4), DRB1*0404/0408(Dw14), and DRB1*0405(Dw15) are associated with RA. These alleles encode a conserved amino acid sequence in the third hypervariable region (HVR3) of the DR β chain. Similar sequences are also shared with other RA associated alleles, for instance HLA-DRB1*0101(DR1) and -DRB1*1001 (Drw10), that are found in white populations. It has been suggested that the structural element encoded by this sequence confers susceptibility to RA—the so called 'shared epitope' hypothesis. This epitope is present in 80–90% of RA patients.

An important practical issue is whether genetic factors that predict disease severity can be identified. Whereas severe RA has been reported to be associated with DR4 in several cross sectional studies of patients with long disease duration, studies of the prognostic value of early serological DR4 typing have given conflicting results. The question arises whether more precise DNA technology could provide better prognostic information. In cross sectional studies of RA patients with long disease duration, Weyand et al observed that patients in whom the susceptibility HVR3 sequence was present on two alleles were likely to suffer from a more severe disease course characterised by a greater frequency of major extra-articular manifestations and joint surgery. They suggested that genomic typing might be useful to identify patients eligible for early aggressive therapy. In an early synovitis clinic, knowledge of DR type allowed prediction of persistent and erosive disease after one year. However, there is little information from prospective studies concerning the influence of the genetic markers on the subsequent course of established disease.

DR region genes exhibit linkage disequilibrium. DR4 positive haplotypes carry preferentially the DQB encoded variants...
*0301(Dqw7) or *0302(Dqw8). Cross sectional studies have indicated that DR4 associated DQB*0301 may be a severity marker both for extra-articular and for articular disease, but other studies could not confirm these results. It has also been suggested that the proposed DQB*0301 association in Felty’s syndrome, at least, is secondary to a primary association with the DRB1 subtype.

The aim of this study was to evaluate the importance of DR and DQ types as markers of disease severity in unequivocal RA during the first five years of disease, by examining the correlation between genotype and both disability and radiological damage at two and five years in a cohort followed prospectively.

**Patients and methods**

**Patients**

We investigated 99 patients who were taking part in a continuing prospective study of RA in Southern Sweden, and enrolled during the years 1985–87. The inclusion criteria were definite RA with a disease duration of less than 24 months, and age greater than 18 years. Disease duration was defined as time from onset of symptoms. The patients were 33 men and 66 women, all white, with a mean (SD) age of 52-1 (12-8) years at disease onset and a mean disease duration at inclusion to the study of 11-4 (6-4) months. An additional six patients were included in the RA cohort during this period, but were lost to follow up and therefore not subjected to genotyping; four had died of causes unrelated to RA, and two had moved away. Most of the patients were referrals from primary care physicians as a result of a special campaign to recruit patients with RA of recent onset. The study design allowed inclusion of all degrees of disease severity.

**Immunoochemical assay**

Rheumatoid factor (RF) of IgM class was analysed with an enzyme linked immunosorbent assay (ELISA).

**DNA analysis**

Genomic DNA was extracted from peripheral blood cells using the salting out procedure. HLA-DR and -DQ alleles were typed by restriction fragment length polymorphism analysis (RFLP). The DNA probes used for typing were a 790 bp HindIII-Sacl fragment from clone pII-B-3 and a 627 bp AvaI-AvaI fragment from clone pIIIB1, respectively. The probes were labelled with α-[32P]dCTP (Amersham, Solna, Sweden) by random hexanucleotide priming. For the RFLP analysis DNA 5–10 μg was cleaved by the restriction enzyme TaqI (3–5 U/μg DNA), according to the manufacturer’s instructions. The cleaved DNA was separated on 0–7–1% agarose gels and blotted onto nitrocellulose membranes. For analysis of DRB and DQB alleles, membranes were prehybridised for one hour at 65°C (0.25 mmol/l Na2HPO4, pH 7.2; 2.7% (w/v) sodium dodecyl sulphate (SDS); 250 mg/l denatured sheared herring sperm DNA) and hybridised with added labelled probe for 16–20 hours at 65°C. The membranes were washed twice at 65°C for 15 minutes with 20 mmol/l Na2PO4 and 1% (w/v) SDS, followed by two final washes at 65°C for 15 minutes with 10 mmol/l Na2PO4 and 0.5% (w/v) SDS. After drying, the membranes were subjected to autoradiography.

The HLA-DR1 and -D4 specificities were subtyped using polymerase chain reaction amplification with sequence specific primers (PCR-SSP) according to Olerup and Zetterquist. Subtyping of HLA-DQ3 was also performed by PCR-SSP. All sequence specific primers used were kindly supplied by Olle Olerup. The distribution of HLA-DR and -DQ alleles in the normal healthy local population was calculated from the blood of 100 healthy blood donors.

**Outcome measures**

Disability and radiographic changes were chosen as the main outcome variables for this study. Disability was assessed with a validated Swedish version of the Stanford Health Assessment Questionnaire (HAQ) Disability Index that assigns scores from 0 to 3.0. Radiographic evaluation of hands, wrists, and feet was performed at the start of the study and annually thereafter, and the findings scored by the Larsen method, modified according to Pettersson, grading changes from 0 (normal) to 5 (maximal damage). Thirty two joints were evaluated. The wrist score was multiplied by 5, then all scores were added to give a joint damage score with a theoretical range of 0–200. All radiographs were assessed by one blinded observer (EF). Radiographic examination of other joints was performed only if indicated clinically.

Destruction of a large joint leading to joint replacement was considered a major morbidity feature and was therefore included in the outcome measures.

Remission was defined according to the American Rheumatism Association criteria.

**Statistical analysis**

Differences between groups were analysed by the Wilcoxon or Mann-Whitney tests for continuous data, and by χ2 test or Fisher’s exact test for discrete data. Evaluation of the predictive value of different immunogenetic markers compared clinical features between patients with and without each marker. The value for significance was set at p = 0.01.

Sensitivity, specificity, and relative risks were calculated according to the following definitions. Sensitivity: number of patients with a certain disease feature and presence of genetic marker divided by total number of patients with that disease feature. Specificity: number of patients lacking both disease feature and marker divided by total number of patients without disease feature. Relative risk estimates: quotient between number of patients with disease feature and marker divided by total
Table 1 Occurrence of rheumatoid arthritis associated HLAB1 antigens in patients and controls

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Patients (n = 99)</th>
<th>Controls (n = 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-DRBI*0101/0101</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>HLA-DRBI*0101/0401/4/8</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>HLA-DRBI*0101/x</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>HLA-DRBI*0401/4/0401/4/5/8</td>
<td>26***</td>
<td>1</td>
</tr>
<tr>
<td>HLA-DRBI*0401/4/8/x</td>
<td>32</td>
<td>28</td>
</tr>
<tr>
<td>HLA-DRBI*0408/1001</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>HLA-DRBI*1001/x</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

x = Non-disease-associated allele.

**p = 0.001 compared with controls (χ² test).

number of patients with marker, and number of patients with disease feature but without marker divided by total number of patients without marker.

Results

DISTRIBUTION OF HLA DR AND DQ ALLELES

Table 1 displays the occurrence of the RA associated DRBI1 antigens in patients and local control subjects. Altogether 87% of the patients carried the conserved HVR3 sequence, compared with 50% of the controls (p < 0.0001). Thirty two patients expressed HLA-DRBI*04 on one allele and 26 expressed DRBI*04 on both alleles. Eleven of these 26 were homozygous for *0401, one for *0404, one for *0408, and the remaining 13 carried *0401/0404, *0401/0405, or *0401/0408. Thirty one individuals in the control group expressed at least one of the relevant HLA-DRBI*04 alleles. Only one control subject was homozygous and was typed as *0401/0401. The excess of HLA-DRBI*04 and DRBI*04 homozygosity among patients was highly significant. Twenty three patients expressed the *0101 allele; nine of them carried *04 on the other allele. This combination *01/04 tended to be more common among patients (p = 0.03): of the 20 normal controls carrying the *0101 allele, only two showed this combination. Six patients carried *0101, one in combination with *0408, and one control carried *1001; thus DRBI*1001 tended to be more common among patients (p = 0.05).

Forty four patients (65%) carried DR4-associated DQB*0301 and 46 (68%) expressed DQB*0302. Four patients were homozygous for DQB*0301; three of them also typed DRBI*04/04 on both alleles, and six patients were homozygous for DQB*0302, all of them also homozygous for DRBI*04/04. Fifteen of 31 DR4 positive controls carried DQB*0301 (48%) and 23 (74%) carried DQB*0302. There was no significant difference in DR4 associated DQB*0301 and *0302 between patients and controls.

CLINICAL FEATURES

Table 2 shows disability index and radiographic score in the hands and feet of the patients after two and five years disease duration. The individual variation was considerable. However, group comparison showed that both scores had increased significantly between the two time points.

Table 3 shows further clinical features. Seventy four patients were seropositive and 25 had nodular disease. There was radiographic evidence of erosions in 57 patients at the start of the study, in 84 after two years, and in 90 after five years disease duration. At five years, 12 patients had been in remission for at least six months; average remission time was 36-4 months (range 7-54 months). Four patients developed severe extra-articular manifestations, one cutaneous vasculitis and mono-neuritis, and three renal amyloidosis verified by biopsy specimen.

The patients were treated according to common principles. A total of 62 patients received slow acting antirheumatic drugs during the first five years—42 receiving one drug and 20 receiving two or more. The drugs used most commonly were chloroquine (39 patients), D-penicillamine (22), gold compounds (14) and sulphasalazine (nine). Six patients were treated with low doses of corticosteroids by mouth. The patients received, on average, two intra-articular injections of steroid per year (range 0–13).

Table 2 Clinical findings in 99 patients with early rheumatoid arthritis after two and five years disease duration

<table>
<thead>
<tr>
<th>After two years</th>
<th>After five years</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAQ disability index (0–3)</td>
<td>0.8 (0.4–1.1)</td>
</tr>
<tr>
<td>Joint damage score (0–200)</td>
<td>17 (8–32)</td>
</tr>
</tbody>
</table>

Values are median (interquartile range).

**p < 0.001 compared with two years (Wilcoxon's test for paired data).

Table 3 Demographic and clinical characteristics of the five immunogenetically defined rheumatoid arthritis patient groups

<table>
<thead>
<tr>
<th>Patients with DRBI*04/04 (group A) (n = 26)</th>
<th>Patients with DRBI*04/01 + *04/1001 (group B) (n = 10)</th>
<th>Patients with DRBI*04/x (group C) (n = 12)</th>
<th>Patients with DRBI*01/x + *1001/x (group D) (n = 19)</th>
<th>Patients with x/x (group E) (n = 12)</th>
<th>Total for all groups (n = 99)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at onset (yr)†</td>
<td>47±4 (14–7)</td>
<td>54±8 (11–16)</td>
<td>52±8 (10–5)</td>
<td>50±3 (11–18)</td>
<td>64±8 (11–4)**</td>
</tr>
<tr>
<td>Male gender</td>
<td>13 (50)</td>
<td>2 (20)</td>
<td>7 (22)</td>
<td>6 (22)</td>
<td>5 (42)</td>
</tr>
<tr>
<td>Seropositivity</td>
<td>22 (85)</td>
<td>6 (60)</td>
<td>24 (75)</td>
<td>16 (64)</td>
<td>6 (50)</td>
</tr>
<tr>
<td>Nodular disease</td>
<td>9 (31)</td>
<td>2 (20)</td>
<td>8 (25)</td>
<td>5 (26)</td>
<td>2 (17)</td>
</tr>
<tr>
<td>Erosive disease 2 yr</td>
<td>24 (92)</td>
<td>9 (90)</td>
<td>27 (84)</td>
<td>15 (79)</td>
<td>9 (75)</td>
</tr>
<tr>
<td>Erosive disease 5 yr</td>
<td>26 (100)</td>
<td>10 (100)</td>
<td>29 (91)</td>
<td>15 (79)</td>
<td>10 (83)</td>
</tr>
<tr>
<td>Joint replacement</td>
<td>7 (27)</td>
<td>2 (20)</td>
<td>4 (13)</td>
<td>2 (11)</td>
<td>9 (8)</td>
</tr>
<tr>
<td>Remission at 5 yr</td>
<td>4 (15)</td>
<td>1 (10)</td>
<td>2 (6)</td>
<td>2 (11)</td>
<td>3 (25)</td>
</tr>
<tr>
<td>HAQ 2 yr</td>
<td>0.7 (0–4.1)</td>
<td>1.0 (0–5–1)</td>
<td>0.8 (0–4.1)</td>
<td>0.7 (0–1.2)</td>
<td>1.0 (0–7–1)</td>
</tr>
<tr>
<td>HAQ 5 yr</td>
<td>1.0 (0–7.4)</td>
<td>1.0 (0–5–1)</td>
<td>1.0 (0–5–1)</td>
<td>1.0 (0–5–1)</td>
<td>1.0 (0–6–1)</td>
</tr>
<tr>
<td>Joint damage score 2 yr</td>
<td>27 (13–54)</td>
<td>17 (8–30)</td>
<td>19 (7–32–5)</td>
<td>14 (6–32)</td>
<td>12 (4–24)</td>
</tr>
<tr>
<td>Joint damage score 5 yr</td>
<td>52 (15–78.5)</td>
<td>31.5 (13–63)</td>
<td>47 (18–59–5)</td>
<td>28.5 (14–57)</td>
<td>10 (5–55)</td>
</tr>
</tbody>
</table>

x = Non-RA-associated allele.

Values are number (%) except for fmean (SD) and fmedian (interquartile range). HAQ = Health assessment questionnaire.

**p < 0.001 compared with other groups (Mann-Whitney test).
Fifteen patients (seven men and eight women) required joint replacement (18 hip joints, two shoulder joints, and one knee joint). Joint surgery was performed after mean 46 (16) months.

**RELATION BETWEEN CLINICAL PICTURE AND GENETIC SUBTYPES**

The patients were divided into five groups according to their DRB1 subtype (table 3). Patients without any disease related allele (group E) were significantly older. Male gender tended to be more frequent in patients with DRB1*0404 (p = 0.04). There was no significant difference in frequency of seropositivity, nodular disease or remission rate between the groups. Patients with *04 on both alleles tended to have a greater joint damage score at two years (p = 0.04), but this difference from the other groups had diminished at five years (p = 0.08). Groups A + B (homozygosity for the conserved HVR3 sequence) tended to contain more patients with joint replacement (p < 0.04) (relative risk (95% confidence interval) 2.7 (1.1 to 7.1)). Homozygosity for the conserved HVR3 sequence tended also to be associated with erosive disease after five years (p = 0.03) (sensitivity 40%; specificity 100%; relative risk 1.2 (1.1 to 1.3)).

Of the four patients with extra-articular disease, one carried DRB1*0401/0408, one had *0401/0101, and the remaining two carried *0401/x (where x is an allele not associated with disease). Two patients carried DQB*0301 and the other two *0302.

Table 4 shows demographic and clinical characteristics according to DR4-associated DQ subtype. There was no difference in disability or amount of radiographic damage at two or five years for either the DQB*0301 or the DQB*0302 group. The DQB*0302 group tended to have a greater frequency of patients developing erosive disease (p = 0.02, sensitivity 49% and 50%; specificity 80% and 89%; relative risks 1.2 (1.0 to 1.4) and 1.1 (1.02 to 1.3) for two and five years, respectively). There was no specific linkage disequilibrium regarding DRB1*04 subtype in these patients.

**Discussion**

Genomic typing of patients attending an early synovitis clinic has been shown to allow distinction of patients who later developed erosive disease. However, the subsequent course in such patients may vary considerably. The prognostic value of genomic typing is therefore also dependent on its relation to disease severity during the following years. We have evaluated this in a well defined and ethnically homogeneous group of definite RA patients who had been followed prospectively during the first five years of their disease. Mean disease duration at inclusion in the study was about one year, but some patients had had symptoms for up to two years. The earliest possible commencement of follow up evaluation was therefore two years after the onset of symptoms. Disability and radiographic damage in hands and feet were chosen as main outcome variables. In addition to its importance for quality of life, functional ability is a predictor of work loss and mortality.

Radiographic changes provide a practical and objective outcome measure, and joint destruction in the hands and feet develop early in the disease course. The amount of radiographic change was the most important variable. The presence of erosions was also included in the analyses, though most patients with RA develop erosions, and this measure is probably more meaningful in the very early phase of synovitis, to distinguish between those patients developing or not developing RA.

The association of disease with presence of the conserved HVR3 sequence was again strongly confirmed in this study. Eighty seven per cent of the patients carried at least one of these alleles. There was a high frequency of HLA-DR4, as predicted from serological typing, and a high frequency of homozygosity for this antigen. As the shared epitope was present in the large majority of the patients, it was not suitable for use as a prognostic factor in predicting disease severity and we therefore focused on the prognostic value of different genetic patterns, dividing the patients into five immunogenetically defined groups as described previously. The patients were reasonably evenly distributed among these five groups, in contrast with the cohort studied by Weyand et al, among whom almost 50% carried the disease associated sequence in both alleles, only very few patients having two non-disease-linked alleles. This probably reflects sampling differences between the studies. Genetic associations also may vary in different patient groups; for example in hospital populations with established disease, a strong association with HLA-DRB1*04 can be shown, whereas in newly diagnosed cases from the community no such association is present.

In our cohort, four patients to date have developed severe extra-articular manifestations; all were DR4 positive, but only one carried DRB1*04 on both alleles. The patients were too few to allow statistical comparison. Severe extra-articular features are a very important outcome measure, but probably more relevant to studies with longer disease duration. Weyand et al found that the majority of patients with major organ involvement were typed as *04/04.
Patients without RA associated alleles were older at disease onset, implying that without the involvement of genetic factors, more time is needed for clinically manifest disease to develop. Male patients tended to be more common in the group carrying DR*04/04, giving some support to previous findings. Patients carrying the conserved HVR3 sequence on both alleles had about a three times greater risk of requiring replacement of a large joint during the first five years of their disease. The frequency of joint replacement in this cohort was as high as 15%, and hip joints were most commonly affected. We have recently reported on this feature in detail. Symptoms characteristically appeared very late, mostly at a stage when the joints were already totally destroyed, therefore, even if the increase of risk is moderate, it may have practical implications by making the physician more alert to symptoms from the hip region. Erosive disease tended to be associated with presence of the shared epitope on two alleles, and with DR4 associated DQB*0302. However, these associations had little clinical relevance. The relative risks were only marginally in excess of one; specificity was good, but sensitivity was unacceptably low. Gough et al reported somewhat stronger associations between presence of the shared epitope and erosive disease after one year, especially when RF status was added. However, the clinical usefulness of predicting erosive disease remains doubtful. Although 90% of our patients developed erosions, many of them had a non-progressing or very slowly progressing joint destruction. Others have shown that the rate of change in radiographic progression is unique to an individual patient and is not constant over time. Brook et al have reported that in as many as 40% of their patients erosions became non-progressing within two years. In recent analyses of five year data on radiographic findings, presence of erosions at the initial examination was not found to be predictive for subsequent joint damage progression (Fex et al, manuscript in preparation).

There was no clinically useful correlation between the HLA markers and disease severity regarding disability and joint damage in the hands and feet after two or five years disease duration. There was a trend for greater radiographic changes after two years in patients carrying DRB1*04 on both alleles, but this relationship was somewhat weaker after five years. Weyand et al have very convincingly shown the strong correlation between the conserved HVR3 and severe disease in cross sectional studies with a highly selected group of patients with longstanding disease. However, our findings show clearly that conclusions drawn from such a study relating to the earlier phase of the disease may not be valid. Similar conclusions were reached in a recent Canadian study; in a community based cohort of 103 RA patients with a disease duration of six to seven years, no correlation was found between HLA-DRB1 subtype and disease severity. The frequency of DR4 related DQB*0301 and *0302 did not differ between patients and controls. Both normal frequencies and increased frequencies of either DQB*0301 or DQB*0302 have been reported, the difference probably depending on patient sampling methods. Patients with erosive disease tended to have a greater frequency of DQB*0302 that seemed independent of the DRB1 subtype. However, this study gave no support to the view that DQB*0302 is a marker of disease severity.

Although DQ related disease expression is intriguing, the addition of DQ typing did not improve the clinical usefulness of genomic typing.

We conclude that, though genomic HLA typing is useful to predict the development of persisting RA, its value in the selection of patients for early aggressive therapy is limited. There was a trend for a more severe disease course during the first five years in patients homozygous for HVR3, but the associations were not strong enough to be of use in clinical practice.

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