HLA-DMA*0103 and HLA-DMB*0104 alleles as novel prognostic factors in rheumatoid arthritis

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Objective: To evaluate HLA-DM alleles as markers for disease severity in rheumatoid arthritis (RA).

Methods: Two distinct cohorts of patients with RA were oligotyped for HLA-DRB1 and HLA-DMA genes using PCR amplified genomic DNA with sequence specific oligonucleotide probes. Cohort 1 comprised 199 unselected patients with RA (mean (SD) age 45.5 (13.5) years; disease duration 11.9 (8.8) years), whose disease severity was assessed using Larsen score on hand and foot radiographs. Cohort 2 comprised 95 patients with severe RA and 70 patients with benign RA according to the Larsen method.

Results: In cohort 1, after stratification according to DRB1 genotypes, patients positive for HLA-DMA*0103 and negative for HLA-DRB1*04 tended to have greater articular damage on hands and wrists (p = 0.07 by Mann-Whitney U test) and reached statistical significance for the Larsen score per year (p = 0.05). This association between HLA-DMA*0103 and articular damage was especially observed in patients with HLA-DRB1*01. Similarly, HLA-DMB*0104 positive patients had higher Larsen score on hands and wrists (p = 0.02). This association was even stronger in DRB1*04 positive patients (p = 0.005). In cohort 2, HLA-DMA*0103 was associated with severe RA in patients negative for HLA-DRB1*04 (OR = 5.4; p = 0.014). HLA-DMB*0104 allele frequency tended to be higher in patients with severe RA but without reaching significance.

Conclusion: This is the first study evaluating the role of HLA-DM genes in the severity of RA. Our results suggest that HLA-DMA*0103 and HLA-DMB*0104 alleles may represent new genetic markers of RA severity. The HLA-DMA*0103 allele tends to be associated with patients with RA negative for DRB1*04 and could predict a more severe form of disease especially in HLA-DRB1*01 positive patients. The HLA-DMB*0104 allele could have an additive effect in HLA-DRB1*04 patients. Combined determination of HLA-DM and HLA-DRB1 alleles could facilitate identification of patients likely to have a poor disease course.
MATERIALS AND METHODS

Patients

HLA-DM genes distribution was examined in two distinct cohorts of patients with RA. Cohort 1 was composed of 199 patients who participated in a previous study to determine whether HLA-DMA and DMB genes contribute to the genetic susceptibility to RA. 

Patient records were analysed for clinical, biological and radiological informations. Cohort 2 was composed of 165 patients, divided according to Larsen’s grade damage into those with severe (n = 95) and those with benign (n = 70) RA. 

Two unrelated control populations were used in this study. Healthy volunteer bone marrow donors served as control groups. One group consisted of 147 individuals who were randomly selected and typed for HLA-DRB1 and HLA-DM. Another group of 218 individuals was closely matched for the HLA-DRB1 genotype with the RA patient population. Patients fulfilled ≥4 of the American College of Rheumatology 1987 revised criteria for the classification of RA. All patients and controls were of white origin and from the Montpellier area.

Methods

Genomic DNA was extracted from peripheral blood mononuclear cells according to the classic salting out procedure. HLA-DRB1 alleles were typed as previously described. Polymorphisms of the HLA-DMA and DMB genes were determined following PCR amplification of genomic DNA using two specific pairs of primers. Hybridisation of the PCR products with the respective DMA and DMB panel of sequence specific oligonucleotide probes was performed using a nonradioactive direct dot blot procedure. HLA-DMA*0101 to 0104 and HLA-DMB*0101 to 0106 were oligotyped. All patients had been HLA-DRB1* and HLA-DM genotyped.

Assessment of radiographic disease progression

All films were evaluated by two different trained rheumatologists who were blinded to clinical data and typing results. The films were assessed according to Larsen method. This method evaluates 32 joints: 10 metacarpophalangeal joints, 8 proximal interphalangeal joints, 2 interphalangeal joints of the thumbs, 2 wrists, 8 metatarsophalangeal joints II-V, and the interphalangeal joints of the great toes. Grading of joints is based on standard radiographic films and varies between 0 and 5. For the wrist, the score was multiplied by 5. Scores were added for the hands and feet separately. The total score per patient can range from 0 to 150 for the hands and 0 to 50 for the feet. To examine a correlation between HLA-DMA alleles and radiological progression, a separate analysis of x rays of hands and feet was made because these were not available for all patients. Because radiological progression varies with disease duration, Larsen score was divided by the number of years of disease duration (Larsen score/year).

For cohort 2, the severity of the disease was essentially evaluated on structural damage assessed by Larsen’s grade of damage classification calculated on hand and foot x rays. A Larsen’s grade ≥4 on hands and feet defined a severe RA whereas a Larsen’s grade <2 defined a benign RA, as previously described.

Statistical analyses

Statistical analyses were performed using the program S.plus (Department of Epidemiology and Statistics of University of Medicine, Montpellier, France). Odds ratios were calculated by the method of Woolf, with Haldane’s modification for small numbers. For quantitative values, the significance of difference was determined by the Mann-Whitney U test, with significance set at 0.05.

RESULTS

Patient characteristics

A total of 199 unselected patients were included in cohort 1 (157F and 42M, mean (SD) age 45.5 (13.5) years (range 2–84), mean (SD) disease duration (8.8) 11.9 years); 40 patients (20.1%) had rheumatoid nodules, 28 patients (14.1%) had Raynaud’s syndrome, and 73 (36.8%) had Sjögren’s syndrome. Other extra-articular features were seen in 19 patients: 10 skin vasculitis, 2 pericarditis, 3 with association of skin vasculitis and pericarditis, 1 amyloidosis, 1 psoriasis, 1 steatohepatitis, and 1 lymphoma.

Table 1

<table>
<thead>
<tr>
<th>Genes</th>
<th>RA cohort 1</th>
<th>RA cohort 2</th>
<th>Random controls</th>
<th>Matched controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>GF (%)</td>
<td>n</td>
<td>GF (%)</td>
</tr>
<tr>
<td>DRB1*041</td>
<td>100</td>
<td>50.3</td>
<td>80</td>
<td>25.6</td>
</tr>
<tr>
<td>*04/01</td>
<td>20</td>
<td>10.1</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>*04/01/X</td>
<td>14</td>
<td>7</td>
<td>12</td>
<td>7.2</td>
</tr>
<tr>
<td>DBR1*011</td>
<td>51</td>
<td>25.6</td>
<td>47</td>
<td>28.5</td>
</tr>
<tr>
<td>*01/01</td>
<td>6</td>
<td>3</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>*01/04</td>
<td>14</td>
<td>7</td>
<td>12</td>
<td>7.2</td>
</tr>
<tr>
<td>*01/X</td>
<td>31</td>
<td>15.9</td>
<td>30</td>
<td>18.2</td>
</tr>
<tr>
<td>DRB1*0101/X</td>
<td>62</td>
<td>31.1</td>
<td>50</td>
<td>30.3</td>
</tr>
</tbody>
</table>

GF, genotype frequency. X corresponds to any HLA-DRB1 gene other than DRB1 alleles.

Table 2

<table>
<thead>
<tr>
<th>Gene</th>
<th>n</th>
<th>GF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRB1*0401/0401</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>DRB1*0401/0404</td>
<td>9</td>
<td>4.5</td>
</tr>
<tr>
<td>DRB1*0401/0405</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>DRB1*0401/0404</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>DRB1*0405/0405</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>DRB1*0408/0408</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>DRB1*0101/0404</td>
<td>10</td>
<td>0.5</td>
</tr>
<tr>
<td>DRB1*0101/0404</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>DRB1*0101/0401</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>DRB1*0102/0401</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>DRB1*0102/0404</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>DRB1*0401/X</td>
<td>41</td>
<td>20.6</td>
</tr>
<tr>
<td>DRB1*0404/X</td>
<td>19</td>
<td>9.5</td>
</tr>
<tr>
<td>DRB1*0405/X</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>DRB1*0101/0101</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>DRB1*0101/X</td>
<td>28</td>
<td>14</td>
</tr>
<tr>
<td>DRB1*0102/X</td>
<td>3</td>
<td>1.5</td>
</tr>
</tbody>
</table>

GF, genotype frequency.
and 1 Felty’s syndrome. There were 115 patients (57.8%) positive for rheumatoid factor and 45 (22.7%) for ANA. Patients with RA had been treated with 3.88 (2.22) disease modifying antirheumatic drugs (DMARDs) and 96 (47.7%) had had surgery. HLA-DRB1 and HLA-DM genotype and allele frequencies are presented in tables 1–4. Of the patients had orthopaedic surgery on 65% of patients with RA, extra-articular features, RF, ANA, and treatment received distributions. Orthopaedic surgery was performed on 65% of patients carrying HLA-DM-A0103. Therefore the presence of HLA-DM-A0103 tended to be associated with greater articular damage to hands and wrists (table 7). This association reached statistical significance for the Larsen score/year (p = 0.05). A similar trend was observed for feet, but was not significant (p = 0.11).

In cohort 2, the relation of HLA-DM-A0103 with articular damage was particularly evident in the groups of patients shown in tables 5 and 6. RA susceptibility allele HLA-DRB1*04 but not DRB1*01 was significantly associated with severe RA (p<0.05), whereas patients without any of the RA susceptibility allele had benign RA (p<0.05).

### HLA-DM-A0103 phenotype correlates with greater radiographic damage

In cohort 1, 198 patients with RA were typed for HLA-DM-A. Distribution of HLA-DM alleles was not different for sex, age at disease onset, extra-articular features, rheumatoid factor (RF), anti-nuclear antibodies (ANA), or medical treatments. Orthopaedic surgery was performed on 65% of patients carrying HLA-DM-A0103. Therefore the presence of HLA-DM-A0103 tended to be associated with greater articular damage to hands and wrists (table 7). This association reached statistical significance for the Larsen score/year (p = 0.05). A similar trend was observed for feet, but was not significant (p = 0.11).

In cohort 2, the relation of HLA-DM-A0103 with articular damage was particularly evident in the groups of patients

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**Table 3** Allele and genotype frequencies of HLA-DMA in patients with RA, and matched and random controls

<table>
<thead>
<tr>
<th>DMA</th>
<th>DRB1*01/01 and 01/X</th>
<th>DRB1*04/04 and 04/X</th>
<th>DRB1*X/X</th>
</tr>
</thead>
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<tr>
<td>n (% AF)</td>
<td>n (% AF)</td>
<td>n (% AF)</td>
<td>n (% AF)</td>
</tr>
<tr>
<td>RA cohort 1</td>
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<td>Random</td>
<td>RA cohort 1</td>
</tr>
<tr>
<td></td>
<td>[n = 44]</td>
<td>[n = 41]</td>
<td>[n = 100]</td>
</tr>
<tr>
<td>*0101</td>
<td>57 (77)</td>
<td>74 (84)</td>
<td>72 (87.8)</td>
</tr>
<tr>
<td>*0102</td>
<td>6 (8.1)</td>
<td>13 (14.8)</td>
<td>10 (11.4)</td>
</tr>
<tr>
<td>*0103</td>
<td>1 (1.4)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>*0101/01</td>
<td>20 (54)</td>
<td>74 (84)</td>
<td>72 (87.8)</td>
</tr>
<tr>
<td>*0103/01</td>
<td>6 (16.2)</td>
<td>13 (14.8)</td>
<td>10 (11.4)</td>
</tr>
<tr>
<td>*0101/02</td>
<td>10 (27)</td>
<td>1 (1.1)</td>
<td>0</td>
</tr>
<tr>
<td>*0102/02</td>
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<td>0</td>
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<td>*0103/03</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>*0103/04</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>*0101/01</td>
<td>1 (2.7)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>*0102/02</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>*0103/03</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>*0103/04</td>
<td>0</td>
<td>0</td>
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</tr>
</tbody>
</table>

**Table 4** Allele and genotype frequencies of HLA-DM-A in patients with RA, and matched controls

<table>
<thead>
<tr>
<th>DMB</th>
<th>RA cohort 1 [n = 198]</th>
<th>Matched [n = 81]</th>
</tr>
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<tr>
<td></td>
<td>n (% GF)</td>
<td>n (% GF)</td>
</tr>
<tr>
<td>*0101/0101</td>
<td>33 (40.7)</td>
<td>45 (55.6)</td>
</tr>
<tr>
<td>*0101/0102</td>
<td>2 (2.5)</td>
<td>5 (6.2)</td>
</tr>
<tr>
<td>*0101/0201</td>
<td>1 (1.4)</td>
<td>0</td>
</tr>
<tr>
<td>*0101/0301</td>
<td>1 (0.8)</td>
<td>0</td>
</tr>
<tr>
<td>*0102/0101</td>
<td>1 (0.8)</td>
<td>0</td>
</tr>
<tr>
<td>*0102/0201</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>*0102/0301</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>*0103/0101</td>
<td>2 (2.2)</td>
<td>1 (1.2)</td>
</tr>
<tr>
<td>*0103/0201</td>
<td>3 (3.3)</td>
<td>0</td>
</tr>
<tr>
<td>*0103/0301</td>
<td>5 (6.2)</td>
<td>0</td>
</tr>
<tr>
<td>*0101</td>
<td>109 (59.9)</td>
<td>120 (74)</td>
</tr>
<tr>
<td>*0102</td>
<td>8 (4.4)</td>
<td>8 (4.9)</td>
</tr>
<tr>
<td>*0103</td>
<td>38 (20.8)</td>
<td>29 (17.9)</td>
</tr>
<tr>
<td>*0104</td>
<td>27 (14.8)</td>
<td>5 (3)</td>
</tr>
</tbody>
</table>

**Table 5** Distribution of HLA-DRB1 genotypes in patients with severe and benign RA (cohort 2)

<table>
<thead>
<tr>
<th>DRB1*</th>
<th>Severe RA [n = 95]</th>
<th>Benign RA [n = 70]</th>
<th>OR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>04/04</td>
<td>10 (10.5)</td>
<td>5</td>
<td>9.1 (1 to 73.7)</td>
<td>0.014</td>
</tr>
<tr>
<td>04/01</td>
<td>11 (11.6)</td>
<td>1</td>
<td>2.1 (1.08 to 4.29)</td>
<td>0.03</td>
</tr>
<tr>
<td>04/X</td>
<td>37 (39.1)</td>
<td>16</td>
<td>2.1 (1.08 to 4.29)</td>
<td>0.03</td>
</tr>
<tr>
<td>01/01</td>
<td>4 (4.2)</td>
<td>1</td>
<td>1</td>
<td>NS</td>
</tr>
<tr>
<td>01/X</td>
<td>20 (21.1)</td>
<td>10</td>
<td>2.1 (1.08 to 4.29)</td>
<td>0.03</td>
</tr>
<tr>
<td>X/X</td>
<td>13 (14)</td>
<td>37</td>
<td>0.14 (0.06 to 0.29)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

X is any HLA-DRB1 gene other than HLA-DRB1*04 and 01 alleles. χ² test was used for analysis.
listed as severe and benign RA. As shown in table 8, HLA-DMA*0103 frequency in non-DRB1*04 patients was much higher in severe RA (p = 0.014). In the benign RA group, HLA-DMA*0103 frequency was similar to that observed in an HLA matched control group. In DRB1*01 positive patients, the presence of HLA-DMA*0103 was associated with severe RA (p = 0.037).

**HLA-DMB*0104 phenotype correlates with greater radiographic damage**

In cohort 2, 160 patients were typed for HLA-DMB. HLA-DMB*0104 positive patients with RA received more DMARDs than DMB*0104 negative patients (4.74 (2.23) vs 3.74 (2.19); p = 0.015). They also had a significantly higher Larsen score on hand and wrist x-rays (p = 0.02). Very similar results were observed for patients with the HLA-DMB*0104/DRB1*04 and HLA-DMB*0104/non-DRB1*04 haplotypes (table 9). These patients were treated with more DMARDs and had more articular damage on hands and wrists. There was the same trend on foot x-rays, but it did not reach statistical significance. In cohort 2, we observed the same tendency; HLA-DMB*0104 allele frequency was higher in severe RA compared with benign RA, but it was not statistically significant (data not shown).

**DISCUSSION**

In this retrospective study, we examined the relation between known markers of severity and HLA-DM alleles in two different cohorts of patients with RA. We particularly focused on HLA-DMA*0103 and HLA-DMB*0104, because we had previously reported that these alleles were associated with RA susceptibility. Patients with RA carrying HLA-DMA*0103 and HLA-DRB1*01 had greater articular damage, and the HLA-DMA*0103 positive patients tended to have more surgical procedures. HLA-DMB*0104 was associated with structural damage in patients positive for HLA-DRB1*04. Sex ratio, age at disease onset, extra-articular features, and biological features in our population of patients with RA were very similar to those observed in other studies, and 70% of our patients carry at least one HLA-DRB1 RA susceptibility allele (HLA-DRB1*0401, *0404, *0405, or *0408, or DRB1*0101 or *0102). The high prevalence of the HLA-DRB1*01 and DRB1*04 genotypes has been extensively reported, and the HLA-DM phenotypic frequencies in our population of patients with RA are similar to those observed in healthy controls except for HLA-DMA*0103 and DMB*0104 as previously reported. Phenotypic frequencies of these alleles in patients with RA were significantly higher. For HLA-DMA*0103, this frequency was 13% in controls and for HLA-DMB*0104, 17% in RA. In the literature, there are conflicting results regarding the association between HLA-DM alleles and RA. Half of these studies reported a difference in the distribution of HLA-DM genes between patients and controls. Toussirot et al found the same association with HLA-DMA*0103, but not with DMB*0104. In two other French studies, authors reported an association with HLA-DMB*0101 in western France and HLA-DMA*0101 in Corsica. Five studies did not find any association. These discrepancies may result from ethnic disparities, as two of these studies concerned Asian patients. The distribution of HLA-DRB1 alleles has also been described according to racial origin. However, ethnic variation is probably not the only explanation, because the three other studies comprised white populations. Geographical factors may account for variations in distribution of HLA-DM alleles, and size samples may also explain the absence of statistical differences between groups. For example, Singal and Ye reported very similar phenotype frequencies for HLA-DMA alleles but in significantly smaller samples: HLA-DMA*0103 frequencies were 14.29% in 54 patients with RA and 3.70% in 36 controls. In the two cohorts

### Table 6 Distribution of the HLA-DMA alleles and genotypes in patients with severe and benign RA (cohort 2) and matched controls, according to the HLA-DRB1 genotypes

<table>
<thead>
<tr>
<th>Allele</th>
<th>n (% AF)</th>
<th>n (% AF)</th>
<th>n (% AF)</th>
<th>n (% AF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe</td>
<td>Benign</td>
<td>Severe</td>
<td>Benign</td>
<td>Severe</td>
</tr>
<tr>
<td>DMA*0101</td>
<td>39 (81.3)</td>
<td>19 (86.4)</td>
<td>85 (90.4)</td>
<td>35 (83.3)</td>
</tr>
<tr>
<td>DMA*0102</td>
<td>1 (2)</td>
<td>2 (9)</td>
<td>5 (5.3)</td>
<td>6 (14.3)</td>
</tr>
<tr>
<td>DMA*0103</td>
<td>8 (16.7)</td>
<td>1 (4.5)</td>
<td>2 (2.1)</td>
<td>1 (2.4)</td>
</tr>
<tr>
<td>DMA*0104</td>
<td>0</td>
<td>0</td>
<td>3 (3.2)</td>
<td>0</td>
</tr>
<tr>
<td>DMA<em>0101/DMA</em>0103</td>
<td>15 (62.5)</td>
<td>8 (72.7)</td>
<td>39 (83)</td>
<td>15 (71.4)</td>
</tr>
<tr>
<td>DMA<em>0101/DMA</em>0102</td>
<td>1 (4.2)</td>
<td>2 (18.2)</td>
<td>3 (6.4)</td>
<td>5 (23.8)</td>
</tr>
<tr>
<td>DMA<em>0101/DMA</em>0103</td>
<td>8 (33.3)</td>
<td>1 (9)</td>
<td>1 (2.1)</td>
<td>0</td>
</tr>
<tr>
<td>DMA<em>0101/DMA</em>0104</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DMA<em>0102/DMA</em>0103</td>
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<td>DMA<em>0103/DMA</em>0103</td>
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</tr>
</tbody>
</table>

AF, allele frequency; OR, odds ratio; CI, confidence interval.

### Table 7 Articular damage on hands and feet in patients with HLA-DMA*0103 phenotype in cohort 1

<table>
<thead>
<tr>
<th>Allele</th>
<th>n</th>
<th>Larsen score: hands and wrists</th>
<th>p</th>
<th>Larsen score: year: hands and wrists</th>
<th>p</th>
<th>Larsen score: feet</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMA*0103</td>
<td>25</td>
<td>62.5 (42.2)</td>
<td>0.27</td>
<td>ND</td>
<td>19.7 (14.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMA*0101</td>
<td>173</td>
<td>53.3 (41.9)</td>
<td>0.27</td>
<td>ND</td>
<td>17.7 (16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DRB1*04 negative</td>
<td>9</td>
<td>63.4 (38.8)</td>
<td>0.07</td>
<td>8.5 (7.5)</td>
<td>19.8 (13.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMA<em>0103 + DMA</em>0101</td>
<td>90</td>
<td>42.8 (40.5)</td>
<td>0.07</td>
<td>5.8 (6.4)</td>
<td>15.9 (14.1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

+, DMA*0103 positive patients; –, DMA*0103 negative patients. ND, not done. Analyses were assessed in all patients and in non-DRB1*04 with a Mann-Whitney U test.
of patients with RA, HLA-DMA*0103 was associated with radiographic severity in non-HLA-DRB1*04 patients. Linkage disequilibrium between HLA-DMA*0103 and HLA-DRB1*01 may explain this association, as Reviron et al., in a population geographically close to ours, found that differences in DMA phenotype frequencies between patients and controls were secondary to linkage disequilibrium. However, we did not find any linkage disequilibrium using the $\Delta$ values for non-random assortment of alleles in control subjects. Moreover, for HLA-DMA*0103, this association could not be explained by a linkage disequilibrium because this allele was also increased in patients negative for the RA susceptibility DRB1*04 alleles. The HLA-DMA*0103 allele was not correlated with known markers of severity in RA including sex, age at disease onset, extrarticular features, erythrocyte sedimentation rate, C reactive protein, and rheumatoid factor (RF).

Patients with RA with HLA-DMA*0103 allele did not require more DMARDs but tended to undergo more surgical procedures. This association between HLA-DMA*0103 and bone destruction was more evident when considering the HLA-DRB1* RA susceptibility allele. Patients with HLA-DMA*0103 and non HLA-DRB1*04 susceptibility alleles had higher Larsen score and Larsen score/year calculated on hand x ray. The role of HLA-DMA*0103 in the severity of RA became statistically significant when we compared allele distribution in groups of patients with severe and benign RA. Indeed, patients with severe RA (Larsen score >4) had a higher frequency of HLA-DMA*0103/non DRB1*04 phenotype compared with those with benign RA (p = 0.014) and in matched controls (p<0.0001). This independence between DMA*0103 and DRB1*04 suggests that HLA-DMA*0103 could be associated with the progression and severity of RA in patients without DRB1*04 alleles. Moreover, in cohort 2, the presence of HLA-DMA*0103 associated with HLA-DRB1*01 was higher in patients with severe RA than in those with benign RA and in matched controls. Thus, HLA-DMA*0103 may represent an additional predictive factor of structural damage in patients with RA and HLA-DRB1*01 alleles.

HLA-DMB*0104 may also be involved in the severity of RA. Patients carrying HLA-DMB*0104 took more DMARDs and had a higher Larsen score on hand x ray. This was also true for HLA-DRB1*04. HLA-DMB*0104 may represent an additional factor of severity in patients with DRB1*04 or DRX alleles.

This is the first study evaluating the role of HLA-DM genes in the severity of RA. These results support the idea that HLA-DMA*0103 and HLA-DMB*0104 are associated with the progression of structural damage in RA. Such correlation between genes and the degree of disease severity has only been described previously for HLA-DRB1* genes. The DRB1*04 alleles correlate with more severe forms of disease, whereas HLA-DRB1*01 associated with milder RA. It may be useful in these HLA-DRB1*01 positive patients to determine the presence or not of HLA-DMA*0103, as a combination of these two alleles is more often distributed in severe RA. The combined presence of these two alleles in addition to other prognostic factors could help to identify patients with poor prognosis. This result has to be confirmed by prospective studies. The clinical benefit of HLA-DMB*0104 typing seems to be less evident because of its reliance on HLA-DR1*04. However, because of this close relation between these two alleles, it might be of interest to study the functional consequences of HLA-DM and HLA-DR interaction on antigen presentation in patients with RA carrying both HLA-DRB1*04 and HLA-DMB*0104.

In conclusion, our study brings evidence that the HLA-DMA*0103 and DMB*0104 alleles could be new genetic markers of RA severity. HLA-DMA*0103 could predict a more severe form of disease in patients with no HLA-DRB1*04 susceptibility alleles, especially in HLA-DRB1*01 positive

### Table 8: Articular damage to hands and feet in patients with RA with HLA-DMA*0103 phenotype in cohort 2

<table>
<thead>
<tr>
<th>Allele</th>
<th>Matched controls (n = 218)</th>
<th>Severe RA (n = 95)</th>
<th>Benign RA (n = 70)</th>
<th>OR (95% CI) p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non DRB1*04</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMA*0103 +</td>
<td>120 28 45</td>
<td>5.4 (1.4 to 21.2)</td>
<td>0.014</td>
<td></td>
</tr>
<tr>
<td>DMA*0103 –</td>
<td>1 8 1</td>
<td>11.8 (0.62 to 225)</td>
<td>0.037</td>
<td></td>
</tr>
</tbody>
</table>

**Table 8** Articular damage to hands and feet in patients with RA with HLA-DMA*0103 phenotype in cohort 2

**Key messages**

- HLA-DMA*0103 and HLA-DMB*0104 may represent new predictive markers of severity in RA.
- Patients carrying the HLA-DRB1*01 gene along with HLA-DMA*0103 or HLA-DRB1*01, and HLA-DMB*0104 have greater structural damage.
patients. HLA-DMB*0104 could have an additive effect in HLA-DRB1*04 patients. Combined determination of HLA-DM and HLA-DRB1 alleles could facilitate identification of patients likely to have a poor disease course, particularly joint destruction. This genetic analysis may help to select patients who could benefit of the more efficacious drug therapies.

ACKNOWLEDGMENTS

This work was supported by grants from Société Française de Rhumatologie.

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REFERENCES

12 Patalk SS, Luch JD, Blum JS. Cutting edge: editing of recycling class II peptide complexes by HLA-D. J Immunol 2001;167:532–5