HLA-DRB1 alleles associated with polymyalgia rheumatica in northern Italy: correlation with disease severity

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Abstract

Objective—To examine the association of HLA-DRB1 alleles with polymyalgia rheumatica (PMR) in a Mediterranean country and to explore the role of HLA-DRB1 genes in determining disease severity.

Methods—A five year prospective follow up study of 92 consecutive PMR patients was conducted. HLA-DRB1 alleles were determined in the 92 patients, in 29 DR4 positive rheumatoid arthritis (RA) patients, and in 148 controls from the same geographical area by polymerase chain reaction amplification and oligonucleotide hybridisation.

Results—No significant differences were observed in the frequencies of HLA-DRB1 types and in the expression of HLA-DRB1 *0401/4 shared motif between PMR and controls. The frequency of the patients with double dose of epitope was low and not significantly different in PMR and in controls. No significant differences were observed between the frequencies of HLA-DRB1 alleles with polymyalgia rheumatica (PMR) and rheumatoid arthritis (RA). A key role of HLA-DRB1*04 alleles, similar to that seen in rheumatoid arthritis (RA), has been recently demonstrated by Weyand et al, while few studies have examined the relation between HLA-DR4 and PMR severity.

Conclusion—These data from a Mediterranean country showed no association of rheumatoid epitope with PMR in northern Italian patients. A high ESR at diagnosis > 72 mm 1st h in patients with double dose of epitope was low and not significantly different in PMR and controls. The frequency of the patients with double dose of epitope was low and not significantly different in PMR and in controls. No significant differences were observed in the frequencies of HLA-DRB1 types and in the expression of HLA-DRB1 *0401/4 shared motif between PMR and controls. The frequency of the patients with double dose of epitope was low and not significantly different in PMR and in controls. No significant differences were observed in the distribution of HLA-DR4 subtypes were observed between DR4+ PMR, DR+ RA, and DR4+ controls. Results of the univariate analysis indicated that an erythrocyte sedimentation rate (ESR) at diagnosis > 72 mm 1st h, the presence of HLA-DRB1, DR10, rheumatoid epitope, and the type of rheumatoid epitope were significant risk factors associated with relapse/recurrence. Cox proportional hazards modelling identified two variables that independently increased the risk of relapse/recurrence: ESR at diagnosis > 72 mm 1st h (RR=1.5) and type 2 (encoded by a non-DR4 allele) rheumatoid epitope (RR=2.7).

An association between DR4 and polymyalgia rheumatica (PMR), particularly in patients with giant cell arteritis (GCA), has been observed. Recently, some studies have evaluated the HLA-DRB1 alleles associated with PMR. Studies done on white PMR patients originating from the United Kingdom (Manchester area) and from Minnesota (Mayo Clinic) have found an association with HLA-DRB1*04 alleles, similar to that seen in rheumatoid arthritis (RA). A key role of HLA-DRB1*04 subtypes (DRB1*0401, *0404/ *0408, *0405) in RA severity has been clearly demonstrated by Weyand et al, while few studies have analysed the relation between HLA-DR4 and PMR severity.

Conclusion—These data from a Mediterranean country showed no association of rheumatoid epitope with PMR in northern Italian patients. A high ESR at diagnosis > 72 mm 1st h in patients with double dose of epitope was low and not significantly different in PMR and in controls. The frequency of the patients with double dose of epitope was low and not significantly different in PMR and in controls. No significant differences were observed in the distribution of HLA-DR4 subtypes were observed between DR4+ PMR, DR+ RA, and DR4+ controls. Results of the univariate analysis indicated that an erythrocyte sedimentation rate (ESR) at diagnosis > 72 mm 1st h, the presence of HLA-DRB1, DR10, rheumatoid epitope, and the type of rheumatoid epitope were significant risk factors associated with relapse/recurrence. Cox proportional hazards modelling identified two variables that independently increased the risk of relapse/recurrence: ESR at diagnosis > 72 mm 1st h (RR=1.5) and type 2 (encoded by a non-DR4 allele) rheumatoid epitope (RR=2.7).

Methods

PMR PATIENTS

Ninety two consecutive new PMR patients were identified in the Reggio Emilia metropolitan area over a five year period (1992–96). Table 1 shows the clinical and demographic characteristics of the patients. PMR was diagnosed when all the following were present:

1. Persistent pain (for at least one month) involving two of the following areas: neck, shoulders, and/or pelvic girdle;
2. Morning stiffness lasting more than one hour;
3. Rapid response to prednisone (≤ 20 mg/day), and
4. Absence of other diseases capable of causing the musculoskeletal symptoms. Only patients over the age of 50 were included. All PMR patients were rheumatoid factor negative. Eighty two patients had an erythrocyte sedimentation rate (ESR) greater than 40 mm 1st h at diagnosis. Ten patients with typical clinical symptoms, ESR < 40 mm 1st h (median 28 mm 1st h; range: 14–38 mm 1st h) and rapid and complete response to cortico-
Table 1  Demographic and clinical characteristics at diagnosis of the 92 PMR patients studied

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female/male (%)</td>
<td>75/25</td>
</tr>
<tr>
<td>Age at onset of disease (y)</td>
<td>72 (7)</td>
</tr>
<tr>
<td>Duration of disease before diagnosis (months)</td>
<td>2.9 (1.5)</td>
</tr>
<tr>
<td>Duration of treatment (months)</td>
<td>31 (23)</td>
</tr>
<tr>
<td>Duration of follow up (months)</td>
<td>44 (27)</td>
</tr>
<tr>
<td>Systemic symptoms and signs (fever, anorexia, weight loss)</td>
<td>49% (45)</td>
</tr>
<tr>
<td>Morning stiffness (min)</td>
<td>160 (60)</td>
</tr>
<tr>
<td>Peripheral synovitis</td>
<td>23.9% (22)</td>
</tr>
<tr>
<td>Distal swelling with pitting oedema</td>
<td>8.7% (8)</td>
</tr>
<tr>
<td>Biopsy confirmed GCA</td>
<td>6.5% (6)</td>
</tr>
<tr>
<td>Initial prednisone dose (mg/day)</td>
<td>19.5 (11.0)</td>
</tr>
<tr>
<td>Cumulative prednisone dose (g)</td>
<td>6.5 (4.8)</td>
</tr>
<tr>
<td>Cumulative prednisone dose before the first relapse/recurrence (g)*</td>
<td>3.6 (3.1)</td>
</tr>
<tr>
<td>ESR at diagnosis (mm/1st h)</td>
<td>77 (29)</td>
</tr>
<tr>
<td>CRP at diagnosis (mg/dl)</td>
<td>6.1 (4.1)</td>
</tr>
<tr>
<td>ESR &lt;30 mm/1st h at diagnosis (%)</td>
<td>7.6% (7)</td>
</tr>
<tr>
<td>Relapse/recurrence (%)</td>
<td>44% (40)</td>
</tr>
</tbody>
</table>

Data are expressed as percentage or mean (SD). *The dose was computed only for the 40 patients with at least one relapse/recurrence.

Steroids were also included. All these 10 patients had increased C reactive protein (CRP) values at diagnosis (median: 2.1 mg/dl; range: 1.2–5.0 mg/dl).

Temporal artery biopsy specimens were obtained only in patients with cranial signs or symptoms and the diagnosis of GCA was based on a positive temporal artery biopsy. No patient satisfied, at diagnosis, the American Rheumatism Association (ARA) 1987 revised criteria for RA.12

RA PATIENTS

Twenty nine HLA-DR4 positive RA patients fulfilling the ARA 1987 revised criteria for RA were investigated. These patients represented all the DR4 positive patients resident in Reggio Emilia seen as out patients during a one year period (1992) in the Reggio Emilia Rheumatology Unit (all of these patients were also included in a immunogenetic study on RA).13 Seventy six per cent of the patients were seropositive and 72% had erosive disease.

CONTROL GROUP

The healthy control group consisted of a pool of 148 unrelated blood donor volunteers from the same geographical area. HLA-DRB1 alleles were also determined in 41 DR4 positive healthy controls belonging to a larger control group constituted by 351 blood donors from the same geographical area.

FOLLOW UP STUDY

All the 92 patients with PMR were clinically assessed by the same physician at presentation, monthly for the first six months, then every three months during the follow up period. A standardised data collection form was used at every visit to record medical informations. Age, sex, location of aching and morning stiffness, the presence of systemic manifestations and biopsy confirmed GCA, the dose and duration of corticosteroids, and the occurrence of relapses and recurrences were registered. The cumulative prednisone dose was computed. The presence of swelling and tenderness of the joints and periarticular structures with and without pitting oedema, tenosynovitis (defined by the presence of swelling and tenderness along a well defined tenosynovial structure) and the clinical symptoms and the physical findings specific for carpal tunnel syndrome were carefully assessed at each visit. Electromyography (EMG) was performed when considered diagnostically useful. Joint radiography was performed in all patients with joint swelling at some time point during the course of the illness.

At diagnosis and during the follow up ESR was determined by Westergren method and CRP was measured by nephelometry (NA latex CRP kit, Behringwerke, Marburg, Germany) (upper limit of the normal reference range 0.5 mg/dl) in all patients.

Relapse and/or recurrence were considered present if articular symptoms or signs occurred (usually with an ESR greater than 30 mm 1st h) in a patient receiving corticosteroids or after withdrawal of treatment, respectively. The symptoms were suppressed by resumption of, or increase in corticosteroid dose.

The end of the disease was the date of permanent discontinuation of treatment without relapse or recurrence. The end point of patient follow up was the date of the last clinic visit or the date of death.

Only one patient died during the follow up. The cause of death was stroke and at the time of death the disease was in remission and the patient receiving treatment with prednisone 2.5 mg/day.

At the end point of follow up 39 patients (42.4%) were still being treated with corticosteroids, while 53 patients (57.6%) had suspended treatment. Twenty patients had taken corticosteroids for less than two years and at least one year of follow up without treatment after the suspension of corticosteroids. The mean (SD) duration of corticosteroid treatment was 15.9 (5.0) months in these 20 patients. Twenty three patients were receiving corticosteroid treatment for more than four years. The mean (SD) duration of corticosteroid treatment was 67.2 (18.7) months. The cumulative prednisone dose was significantly higher in the patients with more than four years of corticosteroid treatment than in those with less than two years (12.5 g versus 3.8 g, p = 0.0001).

Throughout the follow up period, no PMR patients fulfilled the 1987 ARA revised criteria for RA12 and no clinical evidence of joint deformity or radiological evidence of erosions were observed.

HLA-DNA TYPING

Genomic DNA was extracted from whole blood of patients and controls by using a rapid salting out method.14 A low resolution HLA-DRB1 molecular typing was performed by polymerase chain reaction amplification with sequence specific primers (PCR-SSP), as previously described.15 This method allowed us to type 18 DRB1 alleles, including DRB1*0101/2 and *0103. HLA-DRB1*04 subtypes were distinguished by PCR amplification of DRB1 genes and sequence specific oligotyping using 32P-end labelled probes, according to the 11th Histocompatibility Workshop protocol.16
Table 3 Frequency of associated DRB1 alleles in polymyalgia rheumatica and controls

<table>
<thead>
<tr>
<th>HLA-DRB1 alleles</th>
<th>PMR</th>
<th>Controls</th>
<th>p</th>
<th>RR (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=92)</td>
<td>(n=148)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DRB1*0101, 02</td>
<td>19.6</td>
<td>16.2</td>
<td>NS</td>
<td>1.3 (0.6, 2.5)</td>
</tr>
<tr>
<td>DRB1*0103</td>
<td>0.0</td>
<td>1.4</td>
<td>NS</td>
<td>—</td>
</tr>
<tr>
<td>DRB1*0401</td>
<td>7.6</td>
<td>4.7</td>
<td>NS</td>
<td>1.7 (0.6, 4.9)</td>
</tr>
<tr>
<td>DRB1*0402</td>
<td>1.1</td>
<td>3.4</td>
<td>NS</td>
<td>0.3 (0.04, 2.7)</td>
</tr>
<tr>
<td>DRB1*0403</td>
<td>4.3</td>
<td>3.4</td>
<td>NS</td>
<td>1.3 (0.3, 5.0)</td>
</tr>
<tr>
<td>DRB1*0404</td>
<td>3.3</td>
<td>1.4</td>
<td>NS</td>
<td>2.5 (0.4, 15.0)</td>
</tr>
<tr>
<td>DRB1*0405</td>
<td>0.0</td>
<td>2.0</td>
<td>NS</td>
<td>—</td>
</tr>
<tr>
<td>DRB1*0406</td>
<td>1.1</td>
<td>0.7</td>
<td>NS</td>
<td>1.6 (0.1, 26.1)</td>
</tr>
<tr>
<td>DRB1*0407</td>
<td>3.3</td>
<td>0.7</td>
<td>NS</td>
<td>5.0 (0.5, 48.4)</td>
</tr>
<tr>
<td>DRB1*0408</td>
<td>0.0</td>
<td>0.7</td>
<td>NS</td>
<td>—</td>
</tr>
<tr>
<td>DRB1*0101</td>
<td>3.3</td>
<td>1.4</td>
<td>NS</td>
<td>2.5 (0.4, 15.0)</td>
</tr>
<tr>
<td>Epitope 70-74</td>
<td>30.4</td>
<td>24.3</td>
<td>NS</td>
<td>1.4 (0.8, 2.4)</td>
</tr>
<tr>
<td>DRB1*0404, X+</td>
<td>9.9</td>
<td>6.7</td>
<td>NS</td>
<td>1.5 (0.5, 4.4)</td>
</tr>
<tr>
<td>DRB1*0401, Y+</td>
<td>20.0</td>
<td>17.6</td>
<td>NS</td>
<td>1.2 (0.6, 2.3)</td>
</tr>
</tbody>
</table>

*Y denotes any DRB1 allele other than *01. +X denotes any DRB1 allele other than *04.

Table 4 Frequency of DRB1 *04 subtypes in DR4+ PMR patients compared with DR4+ RA patients and with DR4+ healthy controls

<table>
<thead>
<tr>
<th>DRB4 subtypes</th>
<th>Controls (n=41)</th>
<th>PMR (n=19)</th>
<th>p</th>
<th>RR (CI)</th>
<th>RA (n=29)</th>
<th>p</th>
<th>RR (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRB1*0401</td>
<td>22</td>
<td>37</td>
<td>NS</td>
<td>2.1 (0.7, 7.0)</td>
<td>34</td>
<td>NS</td>
<td>2.0 (0.7, 5.5)</td>
</tr>
<tr>
<td>DRB1*0402</td>
<td>15</td>
<td>5</td>
<td>NS</td>
<td>0.3 (0.04, 3.0)</td>
<td>7</td>
<td>NS</td>
<td>0.5 (0.1, 2.7)</td>
</tr>
<tr>
<td>DRB1*0403</td>
<td>10</td>
<td>21</td>
<td>NS</td>
<td>2.5 (0.6, 11.5)</td>
<td>10</td>
<td>NS</td>
<td>1.1 (0.2, 5.5)</td>
</tr>
<tr>
<td>DRB1*0404</td>
<td>24</td>
<td>16</td>
<td>NS</td>
<td>0.6 (0.2, 2.5)</td>
<td>21</td>
<td>NS</td>
<td>0.9 (0.3, 3.0)</td>
</tr>
<tr>
<td>DRB1*0405</td>
<td>20</td>
<td>0</td>
<td>0.05</td>
<td>—</td>
<td>21</td>
<td>NS</td>
<td>1.2 (0.4, 4.1)</td>
</tr>
<tr>
<td>DRB1*0406</td>
<td>2</td>
<td>5</td>
<td>NS</td>
<td>2.3 (0.1, 38.5)</td>
<td>0</td>
<td>NS</td>
<td>—</td>
</tr>
<tr>
<td>DRB1*0407</td>
<td>10</td>
<td>16</td>
<td>NS</td>
<td>1.8 (0.4, 8.9)</td>
<td>7</td>
<td>NS</td>
<td>0.7 (0.1, 4.1)</td>
</tr>
<tr>
<td>DRB1*0408</td>
<td>5</td>
<td>0</td>
<td>NS</td>
<td>—</td>
<td>0</td>
<td>NS</td>
<td>—</td>
</tr>
</tbody>
</table>

Data shown as percentages.

STATISTICAL ANALYSIS

Statistical analysis was done using SPSS statistical package (SPSS Inc, Chicago, Illinois). The $\chi^2$ test with Yates’ correction and Fisher’s exact test were used to compare the frequencies of HLA antigens. The t test for independent values was used when necessary. Relative risks (RR) were calculated using the Woolf method.

The end point in the survival analysis was the occurrence of at least one relapse/recurrence (the first in the case of more than one) during the follow up. Univariate analysis (Kaplan-Meier method) was used to estimate the cumulative probability of not having relapse/recurrence in relation to the following variables: age (five year interval periods), sex, presence or absence of GCA, systemic signs/symptoms, peripheral synovitis, distal extremity swelling with pitting oedema. ESR at diagnosis (> 72 mm first h, < 72 mm 1st h), CRP at diagnosis (> 5.8 mg/dl, < 5.8 mg/dl), the presence or absence of HLA-DRB1 alleles, rheumatoid epitope and the type of rheumatoid epitope (1= epitope encoded by an HLA-DR4 allele, 2=epitope encoded by a non-DR4 allele and 3=no dose of epitope) were also considered. Youden’s index was used to calculate the pre-assigned cut off value for ESR and CRP (this index identifies the value that best maximises both sensitivity and specificity using ROC curve).

The difference between curves was assessed using the log rank test. Cox proportional hazards models were used to evaluate the relation between the occurrence of at least one relapse/recurrence and the previously defined variables. Only the variables significant at the 0.05 level were chosen for the multivariate analysis.

Results

Analysis of HLA-DR frequencies in PMR showed a significant increase of DR3 in PMR patients compared with controls (table 2). DR4 was only slightly more frequent in PMR patients, while no differences were observed for DR1 antigen. The frequency of DR8 was significantly lower in PMR patients. However, the significance of DR3 positive and DR8 negative associations was lost when the p value was corrected for the number of antigens tested.

Table 3 shows the frequencies of HLA-DRB1 types in PMR and controls. No significant differences were observed between PMR and controls. We did not observe any significant association of PMR with the HLA-DRB 70–74 shared motif. The frequency of double dose of epitope was very low both in PMR patients (3 of 92, 3.3%) and in controls (3 of 148, 2.0%), and the difference was not significant. No significant associations were observed comparing the frequencies of the epitope encoded by an HLA-DR4 allele and a non-DR4 allele in PMR and controls.

Table 4 represents the distribution of HLA-DR frequencies in total PMR, PMR with and without distal manifestations, and healthy controls.
Table 5 Univariate analysis of seven factors significantly related to the risk of relapse/recurrence of polymyalgia rheumatica based on the Kaplan-Meier method

<table>
<thead>
<tr>
<th>Variables</th>
<th>Number</th>
<th>ESR (mm 1st h)</th>
<th>Type of rheumatoid epitope</th>
<th>HLA-DR1</th>
<th>Presence</th>
<th>Absence</th>
<th>Rheumatoid epitope</th>
<th>Presence</th>
<th>Absence</th>
<th>Type of rheumatoid epitope</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>ESR (mm 1st h)</th>
<th>&lt;72</th>
<th>&gt;72</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral arthritis</td>
<td>50</td>
<td>41</td>
<td>12</td>
<td>79</td>
<td>3</td>
<td>38</td>
<td>27</td>
<td>4</td>
<td>7</td>
<td>15</td>
<td>7</td>
<td>9</td>
<td>15</td>
<td>0.42</td>
<td>0.04</td>
<td>0.05</td>
</tr>
<tr>
<td>Absence</td>
<td>41</td>
<td>50</td>
<td>17</td>
<td>74</td>
<td>3</td>
<td>88</td>
<td>27</td>
<td>15</td>
<td>7</td>
<td>15</td>
<td>0.6</td>
<td>0.99</td>
<td>3</td>
<td>-</td>
<td>0.02</td>
<td>0.001</td>
</tr>
<tr>
<td>Distal extremity swelling with pitting oedema</td>
<td>41</td>
<td>50</td>
<td>17</td>
<td>74</td>
<td>3</td>
<td>88</td>
<td>27</td>
<td>15</td>
<td>7</td>
<td>15</td>
<td>-</td>
<td>0.6</td>
<td>0.99</td>
<td>3</td>
<td>-</td>
<td>0.02</td>
</tr>
<tr>
<td>ESR (mm 1st h)</td>
<td>&lt;72</td>
<td>&lt;72</td>
<td>&gt;72</td>
<td>HLA-DR1</td>
<td>Presence</td>
<td>Absence</td>
<td>HLA-DR10</td>
<td>Presence</td>
<td>Absence</td>
<td>ESR (mm 1st h)</td>
<td>0.42</td>
<td>0.02</td>
<td>1.0 (reference)</td>
<td>0.04</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;72</td>
<td>&gt;72</td>
<td>&gt;72</td>
<td>HLA-DR1</td>
<td>Presence</td>
<td>Absence</td>
<td>HLA-DR10</td>
<td>Presence</td>
<td>Absence</td>
<td>ESR (mm 1st h)</td>
<td>0.42</td>
<td>0.02</td>
<td>1.0 (reference)</td>
<td>0.04</td>
<td>0.05</td>
<td></td>
</tr>
</tbody>
</table>

Table 6 Significant variables (at 0.05 level) identified by Cox proportional hazards model

<table>
<thead>
<tr>
<th>Variables</th>
<th>Regression coefficient</th>
<th>p value</th>
<th>Relative risk (95% confidence intervals)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of rheumatoid epitope</td>
<td>1</td>
<td>-0.60</td>
<td>0.11 (0.26, 1.14)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.99</td>
<td>2.68 (1.45, 4.94)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.0 (reference)</td>
<td>1.05 (1.16, 2.19)</td>
</tr>
<tr>
<td>ESR (mm 1st h)</td>
<td>&lt;72</td>
<td>0.42</td>
<td>1.52 (1.06, 2.19)</td>
</tr>
<tr>
<td></td>
<td>&gt;72</td>
<td>0.25</td>
<td>1.32 (0.81, 2.14)</td>
</tr>
</tbody>
</table>

Discussion

We did not observe any significant association between HLA-DRB1*04 and 01 alleles and PMR in our Italian population. The frequency of HLA-DRB 70–74 shared epitope was similar in PMR patients and in healthy controls. At double dose, the frequency of this epitope was very low in PMR patients, as it was in controls. The distribution of HLA-DR4 subtypes was similar in PMR, RA and normal controls.

Studies on British or American white PMR patients attending respectively Manchester and Mayo Clinic rheumatology centres observed a significant association with HLA-DRB1*04 allele. In the British study HLA-DRB1*04 subtyping showed an increase in the frequencies of both DRB1*0401 and DRB1*0404 antigens, similar to RA immunogenetic profile. In the Mayo study the PMR patients were associated to all HLA-DRB1*04 alleles, unlike RA, where the expression of allelic variants of the HLA-DR4 family was restricted to HLA-DRB1*0401 and *0404. Furthermore, Weyand et al showed, in HLA-DRB1*04 negative PMR patients, an under-representation of HLA-DRB1*01 alleles; this haplotype was, instead, frequently seen in DRB1*04 negative RA patients. Unlike the results of the Mayo study, Haworth et al showed a significantly higher frequency of DRB1*0101 in PMR patients compared with controls.

Furthermore, successive European studies have reported conflicting results on the association of PMR with HLA-DRB1*04 or 01 alleles. The first French study from Lille showed in PMR patients a higher frequency of DRB1*0101, but no association with DR4. However, a study from Mediterranean France (Montpellier) showed that phenotype DRB1*04 was significantly increased in PMR patients compared with normal controls, but the frequency of HLA-DRB1*01 was not significantly different from that of controls. In a recent study from Switzerland no significant association of DR4 and DR1 with PMR was observed. Only a significant weak association with the HLA-DRB1 70–74 shared motif (OR=1.8) was demonstrated, and this association was lost when the p value was corrected. Three studies considered, among others, a group of patients with RA. The association of DR4 with RA was much stronger than that observed in PMR. Similarly, we observed in Italian RA patients an association, even if weak, with DR4 (RR=2.4), but no association with PMR. No association with HLA-DR4 and DR1 was also observed in the subgroup of PMR patients with distal musculoskeletal manifestations, who had a disease presentation closer to RA.

HLA-DR4 association in Italian RA patients was stronger in seropositive disease (RR=3.8) and in patients with extra-articular features (RR=4.0) and erosions (RR=3.0). Seronegative RA, like PMR, showed a frequency of DR4 associations with DR4, DR1, and rheumatoid epitope were observed when comparing patients with distal manifestations at diagnosis with controls (table 2). During the follow up period 14 other patients developed at least one episode of peripheral arthritis and/or distal extremity swelling with pitting oedema. Globally, 44 patients (47.8%) developed peripheral arthritis and/or distal swelling with pitting oedema. No significant associations with DR4, DR1, and rheumatoid epitope were observed in these 44 patients (data not shown).

The frequencies of HLA-DR4 and HLA-DR10 were not significantly different between the 20 patients with a corticosteroid treatment duration of less than two years and at least one year of follow up without treatment after corticosteroid suspension and the 23 patients with a treatment duration of more than four years (30.0% versus 21.7% and 0% versus 4.3%, respectively), while the frequency of HLA-DR1 was significantly higher in the latter group (21.7% versus 0%, p=0.03, RR=2.1, 95%CI: 1.5, 2.9). The frequency of rheumatoid epitope was higher in the patients with more than four years of corticosteroid treatment (34.8% versus 0% and 0% versus 4.3%, respectively), while the frequency of HLA-DRB1*01 was not significantly different between HLA-DRB1*04 and 01 alleles. In the Mayo study Haworth et al showed that phenotype DRB1*04 was significantly increased in PMR patients compared with normal controls, but the frequency of HLA-DRB1*01 was not significantly different from that of controls. In a recent study from Switzerland no significant association of DR4 and DR1 with PMR was observed. Only a significant weak association with the HLA-DRB1 70–74 shared motif (OR=1.8) was demonstrated, and this association was lost when the p value was corrected. Three studies considered, among others, a group of patients with RA. The association of DR4 with RA was much stronger than that observed in PMR. Similarly, we observed in Italian RA patients an association, even if weak, with DR4 (RR=2.4), but no association with PMR. No association with HLA-DR4 and DR1 was also observed in the subgroup of PMR patients with distal musculoskeletal manifestations, who had a disease presentation closer to RA.

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HLA-DRB1 alleles in PMR Italian patients

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followed up 47 patients for three years. No
with PMR was observed. 17
observed (16%), no association of this allele
DR4 in general population was the lowest
Emilia and Lille areas, where the frequency of
30% of the patients in the Reggio Emilia
relapse/recurrence and they constituted about
during the follow up, one or more episodes of
relapse/recurrence and they constituted about
50% of the patients in the Reggio Emilia
series. 6 The long term use of corticosteroids in
PMR causes important morbidity. 27 Few stud-
ies have tried to identify risk factors associated
with PMR severity. No reliable predictors of
duration of corticosteroid treatment have been
found. The longer duration of corticosteroid
treatment observed in women by Chuang et al 28
has not been confirmed by other studies. 10 A
reduced percentage of CD8 cells after six
months of treatment has also been proposed as a
useful outcome parameter. 24 However, Corri-
gall et al. found that the %CD8 T cell was not a
good indicator of disease activity. 25
Few PMR studies have examined the
association between rheumatoid epitope and
disease severity. DR4 was found to be increased
in patients with GCA and disabling PMR at
diagnosis. 6 Other studies have not found any
association between peripheral synovitis and rheumatoid epitope. 6, 24 Uddhammar et al
followed up 47 patients for three years. 28 No
difference in the percentages of DR4 positive
and DR4 negative PMR patients still receiving
corticosteroid treatment at the end of follow up
was observed.

Recently, Combe et al did not observe in
PMR and GCA any association between HLA-
DRB1* genes and markers of disease activity
including number of relapses and disease
duration. 4 However, the follow up design of this
study and definition of relapses were not clearly
defined. Furthermore, the authors mixed
together patients with PMR alone, GCA alone
and patients with both the conditions.

We observed a significantly higher frequency of
HLA-DR1 in the patients with more than
four years of corticosteroid treatment than in
those with a corticosteroid treatment duration of
less than two years and one year follow up
period without treatment. No significant differ-ences in DR4 frequencies were observed
between these two groups.

To include all the patients we evaluate in a
multivariate analysis the risk factors associated
with relapse/recurrence, considering clinical
and laboratory parameters and HLA-DRB1* antigens. This study provides evidence that
high ESR at diagnosis (> 72 mm 1st h) and the
presence of rheumatoid epitope encoded by a
non-HLA-DR4 allele are independent risk fac-
tors of relapse/recurrence. These data confirm
that DR1 may have a prognostic value in iden-
tifying the patients with more severe disease. A
high ESR at diagnosis (> 72 mm 1st h) is an
independent risk factor of relapse/recurrence.

In conclusion, our data from a Mediterran-
ean country show no association between
HLA-DRB1* alleles and rheumatoid epitope
and PMR. No differences in the distribution of
HLA-DR4 subtypes were observed between
PMR, RA, and normal controls.

However, the presence of rheumatoid epitope
encoded by a non-DR4 allele (particu-
larly DR1) and a high ESR at diagnosis are
independent valuable markers of disease sever-
ity.

1 Richardson JE, Gladman DD, Fann A, Keystone EC. HLA-
DR4 in giant cell arteritis: association with polymyalgia
associated with HLA-DR4 antigen. Arthritis Rheum 1988;
31:678–82.
3 Weyand CM, Hunder NNN, Hicok KC, Hunder GG, Goronzy J. HLA-DRB1 alleles in polymyalgia rheumatica,
giant cell arteritis, and rheumatoid arthritis. Arthritis Rheum
4 Haworth S, Ridgeway J, Stewart I, Dryer PA, Pepper L, Ollier W. Polymylagia rheumatica is associated with both
5 Weyand CM, McCarthy TG, Goronzy JJ. Correlation
between disease phenotype and genetic heterogeneity in
6 Uddhammar A, Nilsson Soja JK, Rantapää-Dahlqvist S:
HLA antigens in polymyalgia rheumatica in Northern Sweden.
7 Combe B, Sany J, Le Quellec A, Clot J, Eilaou J-F. Distribu-
tion of HLA-DRB1 alleles of patients with polymyalgia rheumatica
and giant cell arteritis in a Mediterranean population. J Rheumatol
8 Salvatani C, Macchioni PL, Mantovani W, Rossi F, Veneziani M, Bosardi L, et al. Extrarheumatic manifestations of
rheumatoid arthritis and HLA antigens in Northern Italy. J Rheumatol
9 Weyand CM, Hunder NNN, Hicok KC, Hunder GG, Goronzy JJ. HLA-DRB1 alleles in polymyalgia rheumatica, and
giant cell arteritis in Mediterranean populations. J Rheumatol
10 Richardson JE, Gladman DD, Fann A, Keystone EC. HLA-
DR4 in giant cell arteritis: association with polymyalgia
11 Healey LA. Long-term follow-up of polymyalgia rheumatica:
12 Arnett FC, Edworthy SM, Bloch DA, MacRae WJ, Fries JF, Cooper NS, et al. The American Rheumatism Associa-
tion 1987 revised criteria for the classification of rheuma-
13 Salvatani C, Macchioni P, Mantovani W, Bragilani M, Collina E, Cremonesi T, et al. HLA-DRB1 alleles associ-
ated with rheumatoid arthritis in Northern Italy: correlation
14 Miller SA, Drydus DD, Poley DF. A simple salting out
procedure for extracting DNA from human nucleated cell.
15 Mantovani V, Martinelli S, Bragilani M, Buzzi M, Selva P,
Collina E, et al. Molecular analysis of HLA genes for the
selection of unrelated bone marrow donor. Bone Marrow
16 Kimura A, Sasazuki T. Eleventh International Histocompat-
itability Workshop reference protocol for the HLA DNA-
typing technique. In Fuij K, Aitawa M, Sasazuki I, eds. HLA
1991 Proceedings of the Eleventh International Histocom-
patibility Workshop and Conference. Oxford: Oxford Science,
1992:397–419.
17 Labbe P, Filpo RM, Fajury J, Hachulla E, Houvenagel E,
Hatren PV, et al. Etude du polymorphisme HLA-DRB1 au
cours de la pseudopolyarthrite rhizomélique et de la ma-
18 Guerne P-A, Salvi M, Setz M, Bruhlmann P, Rivier G, Frey
D, et al. Molecular analysis of HLA-DR polymorphism in
19 Salvarani C, Zizzi F, Macchioni PL, Rossi F, Georgountzos
A, Valentini M, et al. Uno studio clinico dell’artrite reuma-
toido dell’anziano. Confronto con un gruppo di pazienti
con malattia inserta prima dei 60 anni. Il Reumatologo
20 Thomson W, Pepper L, Payton A, Carthy D, Scott D, Ollier
W, et al. Absence of an association between HLA-
DRB1*04 and rheumatoid arthritis in newly diagnosed
cases from the community. Ann Rheum Dis 1993;52:539–
41.
21 Salvarani C, Macchioni PL, Boiardi L. Polymyalgia
22 Gabriel SE, Sunku J, Salvarani C, O’Fallon M, Hunder GG:
Adverse outcomes of antinflammatory therapy among
patients with polymyalgia rheumatica. Arthritis Rheum
23 Chuang T-Y, Hunder GG, Istrup DM, Kurland LT.
Polyarthritis rheumatica: a 10-year epidemiologic and clini-
24 Salvarani C, Boiardi L, Macchioni PL, Rossi F, Tartoni P,
Casadei Maldini M, et al. The role of peripheral CD8 lym-
phocytes and soluble IL-2 receptor in predicting the dura-
tion of corticosteroid treatment in polymyalgia rheumatica
25 Corrigall VM, Dolan AL, Dasgupta B, Panayi GS. The
sequential analysis of T lymphocyte subsets and
interleukin-6 in polymyalgia rheumatica patients as predic-
tors of disease remission and steroid withdrawal. Br J
26 Salvarani C, Rossi F, Macchioni PL, Mantovani W,
Veniezani M, Boiardi L, et al. Synovitis in polymyalgia
rheumatica: an immunogenetic study. [Letter]. Br J Rheu-
matol 1992;31:720.