Figure 1. Soluble IL-2R levels in serum at the first sampling occasion of individual patients with M-protein associated with polyneuropathy, M-protein without polyneuropathy and in controls without M-protein. Horizontal bars denote mean values and the vertical line denotes mean value ±SD of 50 blood donors.

Table 1. HLA-DR and DQ allele and haplotype frequencies (%) in patients with multiple sclerosis and controls from Asturias (Northern Spain), by DNA-RFLP genotyping

<table>
<thead>
<tr>
<th>Haplotypes</th>
<th>All MS patients (n = 96)</th>
<th>Remittent MS (n = 85)</th>
<th>Progressive MS (n = 11)</th>
<th>Controls (n = 123)</th>
<th>p</th>
<th>p*</th>
<th>p†</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR15(DR2)</td>
<td>39</td>
<td>35</td>
<td>39</td>
<td>19</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>DR16(DR2)</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>DQw5(DQw1)</td>
<td>31</td>
<td>30</td>
<td>31</td>
<td>30</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>DQw6(DQw2)</td>
<td>49</td>
<td>42</td>
<td>49</td>
<td>42</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>DR13(DR6w)</td>
<td>19</td>
<td>19</td>
<td>19</td>
<td>10</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>DR4</td>
<td>28</td>
<td>24</td>
<td>28</td>
<td>24</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>DQw6(DQw3)</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>DR15(DQw6)</td>
<td>36</td>
<td>33</td>
<td>36</td>
<td>33</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS not significant; p All MS patients vs controls; p* Remittent MS vs controls; p† Progressive MS vs controls.

The association between susceptibility to multiple sclerosis (MS) and the HLA system has been shown in previous population studies, but the associated HLA antigens have varied in different ethnic groups.

Southern Europe is an area of interest for the HLA-MS association, since the associations observed in many Northern European populations are usually absent. Previous studies have reached conflicting conclusions on whether particular HLA types influence the clinical course of the disease.

HLA typing has traditionally been accomplished serologically or immunologically, but some subtypes have so far evaded serological recognition. An alternative method is typing at the DNA level using restriction fragment length polymorphism (RFLP). This HLA genotyping detects further subtypes of serologically defined -DR and -DQ specificities, which will permit a better understanding of the HLA-MS association.

We examined MS associated HLA-DR and DQ alleles, characterised by RFLP, at the genomic level in 96 MS patients (63 women and 33 men) from Asturias, a region in Northern Spain with a medium MS prevalence of 24/100,000. MS was defined clinically or by laboratory support using the Poser et al criteria. Eleven had primary chronic progressive MS and 85 had relapsing-remitting MS. The latter group also included patients with a secondary progressive evolution of symptoms. A total of 123 healthy unrelated Spanish individuals were used as controls.

To carry out the HLA typing by DNA-RFLP analysis, the DNA from peripheral blood leucocytes was digested with the restriction endonuclease DNA, and hybridised with probes to DR beta, DQ beta and DQ alpha genes, using standard methodological and analytical procedures. Haplotypes DR-DQ were assigned according to the pattern of bands following the Bidwell method. The results of HLA-class II frequencies in MS patients and controls were compared by using the Chi-square test with Yates's correction and p values were multiplied by the number of allotypes tested.

DR15 (p < 0.05) and DR15/DQw6 (p < 0.05) were significantly increased in the whole MS group compared with the controls (table). DRw13 was significantly decreased (p < 0.05). DQw5 and DRw13/DQw5 were also decreased but the differences were not significant when corrected for the number of allotypes tested.

The frequency of MS patients that were positive for DR15 and/or DR16 (splits of DR2) appears increased compared with the...
controls, but was not significant with the corrected p values. The frequency of DQw5 and/or DQw6 (splits of DQw1) was similar in patients with remittent or primary progressive MS, except for DR4 and DQw8, which were increased in the progressive but not in the remittent form, although the differences were not significant.

The associations most frequently found in white populations have been with the antigens HLA-class II DR2 and DQw1, although the latter has a secondary association because of its linkage disequilibrium with DR2. This association has not been confirmed in all population studies, and its strength decreases from Northern to Southern Europe. Only a small proportion of the total DR2 positive population develops MS.

The area in which we carried out the study (Asturias, Northern Spain) has a medium MS prevalence and here the frequency of DR2 in the general population is less than in populations of Northern Europe with greater prevalences (20–25% vs 30–40%).

Our data show a positive association of the remittent and primary progressive disease with the allotype DR15 and with the haplotype DR15/DQw6 and our results agree with other recent reports. DQw6 does not appear with significantly increased frequency in the patients compared with the controls, thus its association appears secondary to its linkage disequilibrium with DR15. MS is not significantly associated with DR2 in our population, but is associated with one of its splits, the DR15. This shows that this split is a better genetic marker of the association HLA-MS and the advantage of carrying out the typing by DNA-RFLP analysis. DRw13 (split of DRw6) was significantly decreased among the MS patients and for this reason it could be considered as a disease resistance gene.

The DR4 and DQw8 appear with increased frequency in patients with the primary progressive form, but do not occur in those of remittent form. The non significance of these differences may be due to the small number of cases with the progressive form in our study. In Sweden, Olerup et al have also reported a similar association. No DR4 beta 1 subtypes were found to be increased in our MS population.

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