Distribution of HLA-B27 in Romanian spondyloarthritides patients

O. M. Popa, M. Bojinca, V. Bojinca, C. Ciufu, M. I. Dutescu, A. Bardan, R. Sfrent-Cornateanu, M. Petrek, C. Bara & L. Popa

Summary
The analysis of 310 Romanian spondyloarthritides patients confirmed the association of the HLA-B27 marker with the susceptibility to different diseases of this group. For ankylosing spondylitis, the HLA-B27 frequency in Romanian patients (72.1%) was similar to that found in several regions in the Mediterranean area.

Introduction
The association between HLA-B27 and spondyloarthritides (SpA) is one of the strongest among HLA-associated diseases. However, the association degree varies markedly among different diseases of this group and also between different populations. Ankylosing spondylitis (AS) is the most common subtype of SpA (Zochling et al., 2005) and exhibits the strongest association with HLA-B27, while psoriatic arthritis (PsA) shows the lowest degree of association (Paladini et al., 2005). Genetic association data are still missing for some ethnic groups, especially for SpA other than AS.

Romania is a South-Eastern European country with its genetic background resulting from admixture between Roman and ancient local populations and further influences (to a variable and questionable degree) from subsequent migrating populations (Slavonic, Uralic, Turkish, etc.), as discussed by Cavalli-Sforza et al. (1994) and Stefan et al. (2001). The aim of this study was to characterize for the first time a relatively large group of Romanian spondyloarthritides patients from the point of view of the HLA-B27 status.

Materials and methods
A group of 310 Romanian spondyloarthritides patients – ankylosing spondylitis (n = 158), psoriatic arthritis (n = 60), reactive arthritis, ReA (n = 22) and undifferentiated spondyloarthritis, USpA (n = 70) – were enrolled into the study, after diagnosis at the Division of Rheumatology, Galati County Hospital, and ‘St. Maria’ Hospital and ‘I.C. Cantacuzino’ Hospital, Bucharest, Romania. The European Spondyloarthropathy Study Group (ESSG) diagnostic criteria for SpA (Dougados et al., 1991) and additional specific criteria for the different diseases (Moll & Wright, 1973; Willkens et al., 1981; Van der Linden et al., 1984) were used. AS patients with psoriasis or enteropathies were not included in this study. The age of SpA patients ranged between 16 and 86 years (median 39 years). Among the patients, 50% of the PsA, 55% of the ReA and 60% of the USpA were males. In the AS group, the male/female ratio was 4:1 (125 males/33 females). A group of 211 Romanian non-related potential bone marrow/organ donors served as a control population. Romanian ethnicity was based on surnames and anamnestic (subject history) details. The study was approved by the local ethics committee; the details were explained to both control subjects and patients and afterwards, consent for genetic screening was obtained.

HLA-B*27 was genotyped by PCR-SSP using the B27-SSP low resolution kit (Olerup, Sweden). A group of 55 randomly selected individuals (~10%), from the 521 healthy and diseased subjects investigated, were genotyped twice with the same method and concordant result was obtained in each case. Because we have typed only for HLA-B*27 allelic group, homozygosity status for HLA-B*27 could not be evaluated. For this reason we will hereafter use the term HLA-B27 to name the phenotype characterized by the presence of an HLA-B*27 allele, regardless of its homozygosity/heterozygosity status in a given individual.

The data were analysed using the OpenEpi Collection of Epidemiologic Calculators Version 2.3. The
95% confidence limits (CL) for the HLA-B27 proportions in the analysed populations were estimated using the Mid-P exact test. The significance of the association was determined using the Mid-P exact test. The odds ratios (OR) were calculated as conditional maximum likelihood estimates and the corresponding 95% confidence intervals (CI) were calculated by the Mid-P exact method (Martin & Austin, 1991). The statistical power ($\alpha = 0.05$, two tailed) was calculated based on normal approximation. The comparison between populations was performed with a two tailed t-test.

**Results and discussion**

In the present study we have determined for the first time the distribution of HLA-B27 in SpA patients of Romanian ethnicity. For the whole group of SpA we have found 161 of 310 patients to be B27-positive (51.9%). The highest frequency of HLA-B27 (72.1%) was observed in AS patients, while in the undifferentiated spondyloarthritis group, HLA-B27 was present in 37.1% of cases. Psoriatic arthritis and ReA patients revealed the lowest frequencies of the investigated marker, 25% and 27.2% respectively. Among the psoriatic arthritis group, 18 patients (30%) exhibited the axial pattern of arthritis, but only four of them were B27 positive (22%). In the control group of healthy Romanian subjects, the HLA-B27 marker was detected in 22 of 211 individuals (10.4%).

Statistical analysis confirmed the positive association of HLA-B27 with susceptibility to each of the investigated SpA disease groups and to SpA as a whole (Table 1). The study reports adequate levels of statistical power ranging from 78.7% for PsA to 100% for AS and SpA group as a whole and a lower statistical power for the ReA disease (60.3%).

We compared the frequency of the HLA-B27 marker in our Romanian population samples with the frequency of the same marker in several other European populations. In the general population, the HLA-B27 frequency in Romania was significantly higher than that reported from Italy (5%, $P = 0.03$, Ferri et al., 1982), but not different from those reported from United Kingdom (26%, Woodrow & Ilchysyn, 1985) and Spain (22%, Collantes et al., 2007). In Italy, there are two other studies reporting frequencies of HLA-B27 that are not statistically different from our study (13% in Sardinia, Paladini et al., 2005; 32.6% in Scarpa et al., 1992). In our PsA group, the pattern of arthritis appears to have no influence on the association of this disease with HLA-B27 as reported for some populations (Lopez-Larrea et al., 1990; Queiro et al., 2006).

For ReA, the HLA-B27 frequency in our group was 27.2%, while between 50% and 80% in some studies (Olivieri et al., 2002; Kataria & Brent, 2004), and between 30% and 50% in others (Carter, 2006). However, as the number of our ReA patients was small, the HLA-B27 frequency we found may not reflect the real situation, as implied also by the suboptimal statistical power of this part of the study. Several authors reported that the HLA-B27 association in ReA is influenced by the causative agent of the disease (Kwiatkowska & Filipowicz-Sosnowska, 2009). We were not able to associate the HLA-B27 status with the initial infection event, because of the lack of data regarding the triggering bacteria in our Romanian ReA patients.

For undifferentiated SpA patients, there are few genetic studies and the reported frequency of HLA-B27 varies from 70% to 84% in different populations

<table>
<thead>
<tr>
<th>Disease</th>
<th>HLA-B27 positive % (95% CL)</th>
<th>P-value, Mid-P exact test</th>
<th>OR</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spondyloarthritides (n = 310)</td>
<td>51.9 (46.4–57.5)</td>
<td>&lt;0.0000001</td>
<td>9.24</td>
<td>5.7–15.5</td>
</tr>
<tr>
<td>Ankylosing spondylitis (n = 158)</td>
<td>72.1 (64.8–78.7)</td>
<td>&lt;0.0000001</td>
<td>22.00</td>
<td>12.7–39.3</td>
</tr>
<tr>
<td>Psoriatic arthritis (n = 60)</td>
<td>25 (15.3–37.1)</td>
<td>&lt;0.01</td>
<td>2.85</td>
<td>1.3–5.9</td>
</tr>
<tr>
<td>Reactive arthritis (n = 22)</td>
<td>27.2 (11.9–48.3)</td>
<td>0.02</td>
<td>3.20</td>
<td>1.1–8.9</td>
</tr>
<tr>
<td>Undifferentiated spondyloarthritis (n = 70)</td>
<td>37.1 (26.5–48.9)</td>
<td>&lt;0.000001</td>
<td>5.04</td>
<td>2.6–9.8</td>
</tr>
<tr>
<td>Controls (n = 211)</td>
<td>10.4 (6.8–15.1)</td>
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P-value for the disease association with HLA-B27; OR, odds ratio; CI, confidence interval for OR.
Distribution of HLA-B27 in Romanian spondyloarthritides patients


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### Table 2. Comparison of HLA-B27 distribution in AS patients from Romania versus other European regions

<table>
<thead>
<tr>
<th>Country</th>
<th>HLA-B27 positive (%)</th>
<th>P-value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Romania</td>
<td>72.1</td>
<td></td>
<td>Minev et al. (1979)</td>
</tr>
<tr>
<td>Bulgaria</td>
<td>88</td>
<td>0.0005</td>
<td>Goemoer et al. (1977)</td>
</tr>
<tr>
<td>Hungary</td>
<td>92.7</td>
<td></td>
<td>Collantes et al. (2007)</td>
</tr>
<tr>
<td>Spain</td>
<td>84.14</td>
<td>0.0018</td>
<td>Collantes et al. (2007)</td>
</tr>
<tr>
<td>Spain-Galicia</td>
<td>94.3</td>
<td>&lt;0.0001</td>
<td>Fernandez-Sueiro et al. (2004)</td>
</tr>
<tr>
<td>Germany</td>
<td>82.2</td>
<td>0.029</td>
<td>Rudvalai et al. (2009)</td>
</tr>
<tr>
<td>Norway (North)</td>
<td>93</td>
<td>&lt;0.0001</td>
<td>Bakland et al. (2005)</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>90.2–94</td>
<td>&lt;0.0002</td>
<td>Brown et al. (1996), Freeston et al. (2007)</td>
</tr>
<tr>
<td>Finland</td>
<td>93</td>
<td>&lt;0.0001</td>
<td>Jaakkola et al. (2006)</td>
</tr>
</tbody>
</table>

(Zochling et al., 2005). Considering these as expected values, the HLA-B27 frequency in our Romanian USpA sample is significantly lower (P < 0.0001). The HLA-B27 frequency in USpA is influenced by the prospective course of the disease, because at least some of the patients – 59% according to a German study (Mau et al., 1988) – will develop AS.

HLA-B27 represents a group of over 60 allelic variants and about 50 proteins (http://www.anthonynolan.org.uk/research/hlainformaticsgroup), but only a few are common and have shown association with SpA. Among these, two major alleles, B*2702 and B*2705 show a positive association with the whole group of SpA (Khan, 2002). In this context, the limitation of our study is the inability of determining HLA-B*27 alleles by high resolution typing. In the future, more detailed molecular studies of Romanian spondyloarthritides patients can bring interesting and valuable information about this association.

### Acknowledgements

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### References


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