Psoriasis vulgaris and psoriatic arthritis share a 100 kb susceptibility region telomeric to HLA-C

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Objective. To map the locus for susceptibility to psoriasis in patients with psoriatic arthritis.

Methods. Seventy-four patients with psoriatic arthritis and 95 patients with psoriasis vulgaris were included in this study. Polymorphic genes and microsatellite markers centromeric (C1_2_5) and telomeric (C1_4_4, OTF3, HCR and the corneodesmosin gene) to HLA-C were studied in an association analysis. Typing was also performed on a control population of 104 matched donors.

Results. The allele Cw*0602 was associated both with psoriasis (49 vs 21%, Pc < 0.0003; odds ratio (OR) = 3.6, aetiological factor (AF) = 0.72) and with psoriatic arthritis (62 vs 21%, Pc < 0.000001; OR = 6.1, AF = 0.83). In psoriatic patients a susceptibility region telomeric to HLA-C that includes C1_4_4 (56 vs 22%, Pc < 0.0002; OR = 4.39, AF = 0.77), OTF3 (85 vs 60%, Pc < 0.0002; OR = 3.7, AF = 0.73) and HCR (63 vs 26%, Pc < 0.00001; OR = 3.8, AF = 0.74) was observed. In psoriatic arthritis patients the susceptibility region was delimited by HLA-C and C1_4_4 (384 allele, 56 vs 22%, Pc < 0.0002; OR = 4.37, AF = 0.77).

Conclusions. Comparing the susceptibility regions associated with the two diseases, an overlapping interval of 100 kb between HLA-C and OTF3, which might contain the psoriasis gene, can be defined.

Key words: Psoriatic arthritis, Psoriasis vulgaris, HLA-C, C1_4_4.

Psoriasis vulgaris (PV) is a common inflammatory chronic disease characterized by keratinocyte hyperproliferation and the recruitment of T lymphocytes and mononuclear cells in the affected skin. The causes of psoriasis are still unknown, but there is clear evidence, from association, twin and family studies, of a strong heritable component. Linkage and association analyses have shown that the major histocompatibility complex (MHC) is the major genetic determinant related to psoriasis susceptibility. Within the MHC, HLA-Cw*0602 (Cw6) is the allele that shows the strongest association with psoriasis and with psoriatic arthritis. Other HLA alleles (B13, B37 and B57) have also been associated with PV. Nevertheless, it has been established that the association between these alleles and Cw6 is secondary to linkage disequilibrium.

A significant proportion of psoriatic patients (10–40%) develop chronic arthritis. Psoriatic arthritis (PsA) is an inflammatory arthritis associated with psoriasis. PsA is also associated with certain HLA alleles. Thus, B13, B16 (B38/B39), B17 and Cw6 have been related to PsA [8–11]. The common association of Cw6 with both diseases suggests that patients with psoriasis and those with PsA are likely to share a similar locus for susceptibility to psoriasis in the HLA region.

Genetic studies in PsA are complicated by the clinical heterogeneity of the disease. Consequently, stratification by clinical subset is useful in reducing the heterogeneity of the disease in MHC association studies. The age of the patients at onset of psoriasis has also been suggested...
as a stratification variable. Thus, PsA patients with early-onset psoriasis show HLA associations similar to those of patients with type I psoriasis [12].

The region telomeric to HLA-C has been studied widely in patients with psoriasis in order to find PSORS1 (the psoriasis gene in the MHC) (reviewed in [13]). However, this region has not been studied in PsA patients. The aim of this study was to map the locus for susceptibility to psoriasis in PsA patients by comparing the associations found in these patients with those detected in matched patients with psoriasis alone. Thus, we analysed several polymorphic markers and genes spanning a region from the microsatellite C1_2_5, located 19 kilobases (kb) centromeric to HLA-C, to the corneodesmosin gene (CDSN), located 147 kb telomeric to HLA-C.

Methods

Patients and controls

Seventy-four patients with PsA, diagnosed in accordance with the criteria established by Moll and Wright [7], at the Rheumatology Unit of Hospital Central de Asturias, were examined in this study; these patients were selected randomly from those who had early onset of psoriasis (onset of psoriasis before the age 40 yr) from a larger population of 180 patients. All PsA patients had classic psoriasis patterns of skin lesions. The mean age at presentation of psoriasis and arthritis in PsA patients was 23 ± 9 and 36 ± 12 yr respectively. Several clinical and radiological features were evaluated, and the patients were divided accordingly into disease subsets [14]: 29 with oligoarthritis, 18 with polyarthritis and 27 with spondylarthritis. Ninety-five unrelated patients with early-onset chronic plaque psoriasis (type I) without any other extra-epithelial manifestations were also included in the study. The average age at onset of psoriasis in these patients was 26.9 ± 15.1 yr. Typing was also performed on a control population of 104 matched donors from the Spanish population. All patients gave written informed consent before enrolling in the study. The study was carried out with the approval of the ethics committee of our hospital.

The differences among the frequencies in the populations were assessed using the $\chi^2$ test with Yates’ correction. The odds ratio (OR) was calculated as the cross-product ratio. The $P$ values were corrected ($P_c$) by multiplying them by the number of comparisons at each locus. The potential impact for each marker was estimated as the aetiological fraction (AF), which indicates the proportion of disease cases among the total population that are attributable to one allele when $OR > 1$.

The extent of linkage disequilibrium between two loci is expressed as the observed disequilibrium value ($D$), i.e. a proportion of the theoretical maximum disequilibrium value ($D_{max}$) achievable for the combination of alleles. The standardized value of $D$ ($\Delta$) was calculated using the formula: $\Delta = \frac{Pa - (Pa \times Pb)}{P_a(1 - Pb)} = A \cdot D_{max}$, where $Pa$ is the frequency of individuals with allele a, $Pb$ the frequency of individuals with allele b and $Pab$ the frequency of individuals with both alleles.

Genotyping of the polymorphic markers

HLA-C was typed using the polymerase chain reaction with sequence-specific primers (PCR-SSP) [15]. The polymorphisms of the octamer transcription factor 3 gene (OTF3) and the corneodesmosin gene (CDSN) were analysed as described previously [16]. The reported polymorphism at position +251 (C/T) in the HCR gene [17] was analysed by the amplification refractory mutation system (ARMS) [18] using the sense PCR primer 5'-GCCAAAGCAGCAAGGCAGG-3' and the antisense primer 5'-GCTGCCAGAGCTGGCGGC-3' for +256C and the antisense primer 5'-GCTGCAGACGGTCGGCT-3' for +256T.

The microsatellites C1_2_5 and C1_4_4 were amplified using the PCR primers described by Tamiya et al. [19]. Twenty-four and 14 alleles respectively were detected in these microsatellites. Alleles of each microsatellite marker were named on the basis of the length of the amplified fragment. After amplification, the number of repeats was determined using an automated DNA sequencer (ALF Express II, Amersham Biosciences, Uppsala, Sweden).

Results

A total of 95 Spanish patients with PV, 74 patients with PsA and 104 controls were studied for association analysis using two microsatellite markers and three polymorphic genes located around the HLA-C gene (Table 1 and Fig. 1). In PV patients an association was found over a region flanked by HLA-Cw*0602 and HCR (Table 1). Strong linkage disequilibrium between Cw*0602 and 384 (C1_4_4) was found in PV ($\Delta = 0.75$); consequently it was not possible to determine which marker is primarily associated with psoriasis in these patients (data not shown). An association with the CDSN gene was not detected. The results define a critical region of 147 kb associated with

<table>
<thead>
<tr>
<th>Marker</th>
<th>Increased allele</th>
<th>Controls (n=104)</th>
<th>PsA (n=74)</th>
<th>PV (n=95)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1_2_5</td>
<td>218</td>
<td>15 (15%)</td>
<td>14 (19%)</td>
<td>30 (32%)</td>
</tr>
<tr>
<td>HLA-C</td>
<td>Cw*0602</td>
<td>22 (21.1%)</td>
<td>46 (62%)</td>
<td>47 (49.4%)</td>
</tr>
<tr>
<td>C1_4_4</td>
<td>384</td>
<td>24 (22.6%)</td>
<td>42 (56.7%)</td>
<td>54 (56.8%)</td>
</tr>
<tr>
<td>OTF3 HidIII</td>
<td>OTF3-B</td>
<td>63 (60.5%)</td>
<td>50 (67.5%)</td>
<td>81 (85.2%)</td>
</tr>
<tr>
<td>HCR (Pg8)</td>
<td>256T</td>
<td>27 (26%)</td>
<td>26 (35%)</td>
<td>60 (63.1%)</td>
</tr>
<tr>
<td>CDSN</td>
<td>186Phe</td>
<td>101 (97.1%)</td>
<td>70 (94.5%)</td>
<td>93 (97.8%)</td>
</tr>
<tr>
<td></td>
<td>393Gly</td>
<td>102 (98%)</td>
<td>72 (97.3%)</td>
<td>95 (97.8%)</td>
</tr>
<tr>
<td></td>
<td>394Ser</td>
<td>90 (86.5%)</td>
<td>72 (97.3%)</td>
<td>92 (96.8%)</td>
</tr>
</tbody>
</table>

PsA, $^1P_c < 0.000001, OR = 6.1, AF = 0.83; $^2P_c < 0.0002, OR = 4.37, AF = 0.77 (patients vs controls).

PV, $^3P_c < 0.0003, OR = 3.6, AF = 0.72; $^4P_c < 0.0002, OR = 4.39, AF = 0.77; $^5P_c < 0.0002, OR = 3.7, AF = 0.73; $^6P_c < 0.00001, OR = 3.8, AF = 0.74 (patients vs controls).
PV susceptibility, telomeric to HLA-C and centromeric to CDSN.

Cw*0602 (62 vs 21%, $P_c < 0.000001$; OR = 0.61, AF = 0.83) and allele 384 of microsatellite C1_4_4 (56.7 vs 22.6%, $P_c < 0.00002$; OR = 4.37, AF = 0.77) were found to be increased in PsA. Linkage disequilibrium between HLA-C and C1_4_4 occurs to a lesser extent in PsA ($D_e = 0.42$). It was not possible to determine which of these are strongly associated with PsA. Telomeric markers such as OTF3 (100 kb), HCR (113 kb) and CDSN (147 kb) were not significantly associated. No differences were found either in the allele distribution of these or in the onset of psoriasis among the different groups of patients according to the clinical pattern described above (see Patients and controls). These results define a region associated with PsA between HLA-C and C1_4_4. The susceptibility interval of 100 kb between HLA-C and OTF3 shared by PsA and PV might contain the PSORS1 gene (Fig. 1).

Discussion

The aim of this study was to map the locus for susceptibility to psoriasis, comparing PsA patients and patients with psoriasis alone. Most patients with PsA have the classic PV pattern of skin lesions [20]. However, there are certain genetic differences between the two forms of the disease, as the putative psoriasis gene is in linkage disequilibrium with different HLA haplotypes in each form of the disease. PV is mainly associated with the ancestral haplotypes AH57.1 and AH13.1 in most populations that have been studied. However, PsA is not associated with AH13.1, but is associated with the AH38.1 and AH39.1 haplotypes. Analysis of those polymorphic markers, associated with both forms of the disease, might be useful in delimiting the segment associated with disease susceptibility.

In the present study, several polymorphic markers spanning this region were studied and the overlap between PsA and psoriasis was analysed. The results obtained strongly suggest that PsA and psoriasis are associated with the same locus, located in a region telomeric to HLA-C.

Our findings limit the psoriasis susceptibility locus to a region telomeric to HLA-C and excluding OTF3, SC1, HCR and CDSN. An association has been reported between psoriasis and several genes located telomeric to OTF3 [17, 21, 22], some of which are expressed in keratinocytes. However, none of these studies has reported an independent association of any of these markers with the disease.

Polymorphism 384 of the microsatellite marker C1_4_4 was associated with both PsA and PV (Table 1). However, no other telomeric marker was associated with PsA. Our results allow us to define a critical region for psoriasis susceptibility between HLA-C and the OTF3 gene. This conclusion, however, needs to be confirmed in further studies in other populations. This segment is included in the MHC region that we have previously reported to contain the PSORS1 gene in psoriasis [16], and it further delimits this region to a segment of 100 kb. Several association and linkage analyses have studied markers located telomeric to HLA-C. Recently, a linkage disequilibrium analysis of 339 families with psoriasis has identified a fragment of 60 kb of the EH57.1 fragment, which is present in all risk haplotypes [23]. This fragment, which partially overlaps with the region delimited by us, excludes the HCR and CDSN genes.

An important question is why, if PV and PsA are associated with the same susceptible locus, the risk haplotypes differ between the two forms of disease. A plausible answer has been suggested by us recently. We have reported that MICA-A9 (MICA gene transmembrane polymorphism A9) is associated with arthritis in psoriatic patients, but not with psoriasis itself [24–26]. Those psoriatic patients who present haplotypes carrying MICA-A9 would be more susceptible to the development of arthritis. Thus, it is to be expected that the haplotypes associated with PsA contain both a susceptibility locus for arthritis and a susceptibility locus for psoriasis.

The MHC class I region has been completely sequenced [27]. No functional gene has been described within the 100 kb critical region located telomeric to HLA-C, although this region contains several transcription units. Nevertheless, because of the high degree of polymorphism of the MHC, the sequence and the presence of genes may vary between haplotypes. Therefore, it will be necessary to isolate these potential genes in the risk haplotypes in order to study their disease-associated polymorphism in any attempt to determine the exact position of the causative gene.

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References