HLA class I associations of ankylosing spondylitis in the white population in the United Kingdom

M A Brown, K D Pile, L G Kennedy, A Calin, C Darke, J Bell, B P Wordsworth, F Cornèlís

Abstract

Objective—To investigate the HLA class I associations of ankylosing spondylitis (AS) in the white population, with particular reference to HLA-B27 subtypes. Methods—HLA-B27 and -B60 typing was performed in 284 white patients with AS. Allele frequencies of HLA-B27 and HLA-B60 from 5926 white bone marrow donors were used for comparison. HLA-B27 subtyping was performed by single strand conformation polymorphism (SSCP) in all HLA-B27 positive AS patients, and 154 HLA-B27 positive ethnically matched blood donors. Results—The strong association of HLA-B27 and AS was confirmed (odds ratio (OR) 171, 95% confidence interval (CI) 135 to 218; p < 10⁻⁹). The association of HLA-B60 with AS was confirmed in HLA-B27 positive cases (OR 3·6, 95% CI 2·1 to 6·3; p < 5 × 10⁻³), and a similar association was demonstrated in HLA-B27 negative AS (OR 3·5, 95% CI 1·1 to 11·4; p < 0·05). No significant difference was observed in the frequencies of HLA-B27 allelic subtypes in patients and controls (HLA-B*2702, three of 172 patients v five of 154 controls; HLA-B*2705, 169 of 172 patients v 147 of 154 controls; HLA-B*2708, none of 172 patients v two of 154 controls), and no novel HLA-B27 alleles were detected. Conclusion—HLA-B27 and -B60 are associated with susceptibility to AS, but differences in HLA-B27 subtype do not affect susceptibility to AS in this white population.


The strong association between HLA-B27 and ankylosing spondylitis (AS) has been known since 1973. However, despite many advances in our understanding of the molecular biology of HLA class I molecules in general, and of HLA-B27 in particular, a comprehensive explanation for this association remains elusive. Molecular mimicry between bacterial antigens and HLA-B27 has been invoked by some as the means by which pathological autoreactive immune responses might be initiated, while others favour the presentation of 'arthritogenic peptides' specifically bound by HLA-B27. A series of amino acid substitutions clustered around the antigen binding site generate at least nine HLA-B27 allelic variants. These could influence susceptibility to AS either by altering antigenic epitopes on the HLA-B27 molecule itself, or by affecting the range of peptides that these HLA-B27 variants bind. It is therefore of particular interest that two alleles, HLA-B*2706 in Thailand and HLA-B*2703 in the Gambia, appear not to be associated with AS. In contrast, the commonest alleles in white populations (HLA-B*2705 and -B*2702) and the oriental variant (HLA-B*2704) are all associated with AS, though it has not been formally established whether the attributable risk is identical for each of these alleles. In recent years, it has been suggested that HLA-B60 also is associated with AS, increasing the risk of disease threefold, but only in individuals who are also HLA-B27 positive; however, this has not been a universal finding.

We have investigated a large population of white patients with AS from the United Kingdom to confirm the role of HLA-B60. In addition, we have used the technique of single strand conformation polymorphism (SSCP) to define HLA-B27 alleles in the population, looking for evidence of differential association of AS with the various HLA-B27 alleles. This technique has the added potential for defining novel alleles, and has not previously been applied to typing large populations of HLA-B27 positive individuals.

Subjects and methods

Patients and controls

We recruited 284 white patients fulfilling the New York criteria for the diagnosis of AS. The HLA-B60 and -B27 phenotype and inferred genotype frequencies among 5926 white bone marrow donors (typed by the National Institutes of Health microlymphocytotoxicity assay) were used in the comparisons. In addition, 154 HLA-B27 positive, ethnically matched blood donors were identified as controls for the HLA-B27 subtype analysis. They were not known to have AS, but did not undergo specific radiological screening.

HLA typing for HLA-B27 and -B60

DNA was extracted from frozen whole blood by the standard phenol-chloroform-proteinase K method. The presence of HLA-B27 was detected by polymerase chain reaction sequence specific primers (PCR/SSP) using the primers HLA-B27L (5'-CTCCATGAGGTATTTCCAC-3') and HLA-B27R (5'-CTGTCCTTGCCCTTGCA-3') (fig 1). HLA-B60 typing was also performed using...
PCR/SSP, using the primers HLA-B60L (5'-AGATCCTCCAGCGCAAGTT-3') and HLA-B60R (5'-TCAGGGCGCTCCAGC-TTG-3'). These amplify a 127 base pair segment of exon 3 of the HLA-B60 gene. A 101 base pair fragment of the α 1 antitrypsin gene was amplified as a positive control in each sample for both HLA-B27 and HLA-B60 typing, using primers PIL (5'-GCTGTTAGA-TTGAGGTGTC-3') and PIR (5'-TAGATCAGCAAGGGCATGG-3'). The reaction mix consisted of potassium chloride 50 mmol/l, gelatin 0 01%, TRIS-hydrochloric acid (1 mol/l, pH 8.3) 0 5 μl, magnesium chloride 1 2 mmol/l, formamide 2-5%, 200 μmol/l each of guanine, adenine, cytosine, and thymine triphosphates, 1 μmol/l of each primer, 200 ng of DNA template, AmpliTag 2 U (Perkin-Elmer Cetus), and distilled water to a volume of 50 μl. The reaction mix was then overlaid with mineral oil. A hot start procedure was used, followed by 28 cycles of denaturation (94°C for one minute), annealing (52°C for one minute), and extension (72°C for 30 seconds).

STATISTICAL ANALYSIS
The significance of differences in individual genotype or phenotype frequencies between patients and controls was assessed by the uncorrected χ² test. The cross product ratio was used to calculate odds ratios (OR), and 95% confidence intervals (CI) were computed by standard methods.

Results
HLA-B27 was present in 268 of 284 patients (94%), compared with 528 of 5926 controls (9.5%), giving an OR of 171 (95% CI 135 to 218; p < 10⁻⁹⁹). The phenotype frequency of HLA-B60 in the HLA-B27 positive AS patients (32 of 268, 11.9%) was significantly different from that in HLA-B27 positive controls (19 of 528, 3.6%) (OR 3-6, 95% CI 2-1 to 6-3; p < 5 x 10⁻⁴). The HLA-B60 phenotype frequency was significantly increased in HLA-B27 negative AS patients (three of 16, 19%) compared with HLA-B27 negative controls (33 of 5398, 6.2%) (OR = 3-5, 95% CI 1-1 to 11; p < 0 05).

No significant difference was observed between the frequencies of HLA-B27 allelic subtypes in patients and controls (HLA-B*2702, three of 172 patients v five of 154 controls; HLA-B*2705, 169 of 172 patients v 147 of 154 controls; HLA-B*2708, none of 172 patients v two of 154 controls), and no novel HLA-B27 alleles were detected.

Discussion
This study has confirmed the strength of the association of HLA-B27 and AS, and also the association of HLA-B60 and AS, though this was relatively a much weaker effect. We have also demonstrated a significant association of HLA-B60 with AS in HLA-B27 negative AS, though the number of patients studied was...
small. Thus HLA-B60 may function as a disease susceptibility gene for AS independent of HLA-B27. The association of HLA-B60 and AS could be attributable to a direct property of HLA-B60, or to a further nearby linked disease susceptibility gene. HLA-B60 might function as a restriction element for presentation of an arthritogenic peptide, or might itself provide an antigenic peptide. Similar questions remain as to how HLA-B27 itself functions as an AS susceptibility gene.

Polymerisms in exon 2 of HLA-B27 are known to affect the in vitro function of HLA-B27 as a restriction element. 12 Whether these in vitro effects have any clinical relevance is uncertain, but could account for the apparent rarity of AS in carriers of HLA-B*2703 and HLA-B*2706. 13 Our study had > 90% power to detect a 7% difference in the prevalence of HLA-B*2702 compared with other subtypes amongst AS patients, at a significance level of p < 0.05. In common with previous studies of white populations, we found no differential strength of association of the HLA-B27 subtypes between AS patients and controls. 8 13-16

The study demonstrated the usefulness of single strand conformation polymorphism in analysing allelic variants in large populations. HLA-B27 subtyping by SSCP differentiates all the known subtypes with sequence variation within exon 2 (fig 2). The technique makes it possible to divide the nine published subtypes into the groups HLA-B*2701, -B*2702, -B*2703, -B*2704/B*2706, -B*2705/B*2707/ B*2709, and -B*2708. As the sequence of exon 2 is shared in HLA-B*2704 and -B*2706, and in HLA-B*2705, -B*2707, and -B*2709, this technique is unable to differentiate these subtypes. Differentiation of HLA-B*2708 from HLA-B*2705/B*2707/B*2709 is relatively difficult using this technique, and we may have underestimated the frequency of HLA-B*2708. Two of our control HLA-B*2708 subjects were initially typed as HLA-B*2705. However, previous data, based on serology, have shown that HLA-B*2708 is a comparatively rare allele, with a frequency of 0.045% in Wales, 17 so any mistyping is unlikely to have affected our results significantly.

SSCP has the theoretical advantage that previously unreported subtypes that vary within the amplified region may be detected. In the PCR products of 200 or fewer base pairs, 70–95% of mutations are detected. 18 19 Our technique is thus suitable for population screening for novel HLA-B27 alleles, in addition to typing the major HLA-B27 alleles. In our study, which is the largest survey of HLA-B27 subtypes published to date, no new HLA-B27 subtypes were detected, and no differential susceptibility to AS was evident between different HLA-B27 subtypes.

We are grateful to the National Ankylosing Spondylitis Society and the many patients who cooperated in this study. The work was funded by the Arthritis Foundation of Australia, and the work was supported by the Arthritis and Rheumatism Council. AC and LGK were supported by the Cold W W Pilkington Charitable Trust and the John Coates Charitable Trust.
HLA class I associations of ankylosing spondylitis in the white population in the United Kingdom.
M A Brown, K D Pile, L G Kennedy, A Calin, C Darke, J Bell, B P Wordsworth and F Cornélis

doi: 10.1136/ard.55.4.268

Updated information and services can be found at:
http://ard.bmj.com/content/55/4/268

These include:

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/