EXTENDED REPORT

Finnish HLA studies confirm the increased risk conferred by HLA-B27 homozygosity in ankylosing spondylitis

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Objective: To determine the influence of *HLA-B27* homozygosity and *HLA-DRB1* alleles in the susceptibility to, and severity of, ankylosing spondylitis in a Finnish population.
Methods: 673 individuals from 261 families with ankylosing spondylitis were genotyped for *HLA-DRB1* alleles and *HLA-B27* heterozygosity/homozygosity. The frequencies of *HLA-B27* homozygotes in probands from these families were compared with the expected number of *HLA-B27* homozygotes in controls under Hardy–Weinberg equilibrium (HWE). The effect of *HLA-DRB1* alleles was assessed using a logistic regression procedure conditioned on *HLA-B27* and case–control analysis.
Results: *HLA-B27* was detected in 93% of cases of ankylosing spondylitis. An overrepresentation of *HLA-B27* homozygotes was noted in ankylosing spondylitis (11%) compared with the expected number of *HLA-B27* homozygotes under HWE (4%) (odds ratio (OR) = 3.3 (95% confidence interval, 1.6 to 6.8), p = 0.002). *HLA-B27* homozygosity was marginally associated with reduced BASDAI (*HLA-B27* homozygotes, 4.5 (1.6); *HLA-B27* heterozygotes, 5.4 (1.8) (mean (SD)), p = 0.05). Acute anterior uveitis (AAU) was present in significantly more *HLA-B27* positive cases (50%) than *HLA-B27* negative cases (16%) (OR = 5.4 (1.7 to 17), p<0.004). *HLA-B27* positive cases had a lower average age of symptom onset (26.7 (8.0) years) compared with *HLA-B27* negative cases (35.7 (11.2) years) (p<0.0001).
Conclusions: *HLA-B27* homozygosity is associated with a moderately increased risk of ankylosing spondylitis compared with *HLA-B27* heterozygosity. *HLA-B27* positive cases had an earlier age of onset of ankylosing spondylitis than *HLA-B27* negative cases and were more likely to develop AAU. *HLA-DRB1* alleles may influence the age of symptom onset of ankylosing spondylitis.

Ankylosing spondylitis is a chronic inflammatory rheumatic disease with a strong genetic component determining both susceptibility to, and severity of, the disease.[1 2] Genes of the human major histocompatibility complex (MHC), in particular *HLA-B27*, are major genetic factors influencing the familial clustering of ankylosing spondylitis, as evidenced by linkage and association studies.[3] The strong association between ankylosing spondylitis and *HLA-B27* has been known since 1973[4 5]; however, the exact genetic mechanism of the pathogenic link remains enigmatic. Whether homozygosity for *HLA-B27* contributes any additional effect on the disease is unclear. Contradictory reports on the effect of homozygosity for *HLA-B27* have been published,[6 7] and there is no consensus as to whether *HLA-B27* homozygosity increases the susceptibility to, or severity of, ankylosing spondylitis.

Several haplotypic and association studies suggest that more than one gene within the human MHC may influence the susceptibility to ankylosing spondylitis. An association between *HLA-DRB1*01* and spondyloarthropathy has been reported in British and Mexican populations,[8 9] while a recent Sardinian study reported an association between *HLA-DRB1*15-B27* haplotype and ankylosing spondylitis.[10 11]

The objective of this study was to clarify the HLA associations in ankylosing spondylitis in a Finnish population. The Finnish population has a restricted gene pool,[12] a property which can be useful in dissecting the relevant disease causing polymorphisms.[13] We have investigated the influence of *HLA-DRB1* alleles and *HLA-B27* homozygosity/heterozygosity in susceptibility to, and clinical manifestations of, ankylosing spondylitis in a Finnish population.

METHODS

Ankylosing spondylitis families and controls

Fifty three cases of sporadic ankylosing spondylitis and 620 persons (237 affected with ankylosing spondylitis and 383 unaffected family members) from 208 families with the disease were recruited to the study from the Rheumatism Foundation Hospital in Heinola, Finland. Of the affected families, 181 (87%) were single case families, 25 (12%) had two affected individuals, and two (1%) had three affected individuals. Ankylosing spondylitis was defined according to the modified New York Criteria for the disease,[14] and all cases were assessed by a qualified rheumatologist. The data for all patients for the present study were evaluated and coded by one physician (KL). A structured questionnaire was used to assess the presence of acute anterior uveitis (AAU), inflammatory bowel disease, psoriasis, and peripheral arthritis, and to define the age of symptom onset, age at diagnosis, diagnostic delay, disease duration, and disease severity scores, including the Bath ankylosing spondylitis disease activity index (BASDAI)[15] and Bath ankylosing spondylitis functional index (BASFI).[16] Of the ankylosing spondylitis cases, 270 (93%) completed the questionnaire, and these cases were used to assess the clinical manifestations of the disease.

Abbreviations: AAU, acute anterior uveitis; BASDAI, Bath ankylosing spondylitis disease activity index; BASFI, Bath ankylosing spondylitis functional index; HWE, Hardy–Weinberg equilibrium; LD, linkage disequilibrium; MHC, major histocompatibility complex; QTDT, quantitative transmission disequilibrium test

Table 1 Clinical description of the ankylosing spondylitis cases stratified according to their HLA-B27 genotype and allele status

Variable	HLA-B27 genotypes			p Value
	B27 +/+ cases (n = 31)	B27 +/− cases (n = 220)	B27−/− cases (n = 19)	
AAU (n (%))	19 (61%)	104 (47%)	3 (16%)	0.004
IBD (n (%))	0	15 (7%)	3 (16%)	NS
Psoriasis (n (%))	2 (6%)	4 (2%)	0	NS
Peripheral arthritis (n (%))	21 (68%)	176 (80%)	13 (68%)	NS
BASDAI (mean (SD))	4.5 (1.6)	5.4 (1.8)	5.1 (2.4)	0.05
BASFI (mean (SD))	3.5 (2.6)	4.0 (2.3)	4.4 (2.3)	NS
Age of symptom onset (mean (SD)) (range)	25.9 (7.3) (15 to 44)	26.8 (8.1) (14 to 55)	35.7 (11.2) (18 to 54)	p<0.0001
Age at diagnosis (mean (SD)) (range)	32.5 (9.1) (21 to 52)	35.1 (9.7) (18 to 73)	43.4 (10.0) (25 to 55)	p<0.0001
Diagnostic delay (mean (SD)) (range)	6.1 (6.6) (0 to 24)	8.4 (10.9) (0 to 53)	6.9 (6.8) (0 to 26)	NS
Disease duration (mean (SD)) (range)	23.8 (10.2) (8 to 48)	23.9 (10.3) (3 to 60)	18.3 (12.0) (3 to 49)	NS

The statistical significances of differences in categorical and continuous variables were calculated using χ^2 analysis (comparing B27 antigen positive and negative cases) and the quantitative transmission disequilibrium test (total association within families), respectively. All the *HLA-B27* positive ankylosing spondylitis cases were used in the data analysis.
AAU, acute anterior uveitis; BASDAI, Bath ankylosing spondylitis disease activity index; BASFI, Bath ankylosing spondylitis functional index; IBD, inflammatory bowel disease.

HLA-B/DRB1 untransmitted parental haplotypes of Finnish parent–case trios with children with type 1 diabetes mellitus were used as a control sample set (n = 1763, of whom 183 were *B27* positive).[17]

Genotyping of HLA-DRB1 and HLA-B

HLA-DRB1 genotyping was carried out using either direct sequencing (see the appendix for details), the primer extension method developed in this laboratory,[18] or the polymerase chain reaction–sequence specific primer (PCR-SSP) approach.[19] All the probands (n = 261) were genotyped using both the primer extension method and either direct sequencing or the PCR-SSP; the consistency between the genotypes was checked. All the family members (n = 412) were genotyped using the primer extension approach; Mendelian segregation of *HLA-DRB1* alleles was confirmed. The control samples were typed for the HLA-DRB1 alleles *01 to *10, using serological methods.[17]

Limited *HLA-B* locus genotyping was undertaken by PCR-SSP to assess *HLA-B27* homozygosity and heterozygosity.[19] One PCR reaction was carried out to determine the *HLA-B27* carrier status, and five group specific PCR reactions were done to determine all the other *HLA-B* alleles. PCR amplification was achieved as described by Bunce.[19] Control primers amplifying a 796 base pair (bp) fragment from the third intron of *HLA-DRB1* were included in the PCR reactions. Positive controls with known *HLA-B* genotypes and negative H_2O controls were used in all the reactions. Mendelian segregation of *HLA-B* alleles was confirmed.

Table 2 The expected and observed number of *HLA-B27* homozygotes of the 240 *HLA-B27* positive probands

	Observed	Expected
HLA-B27 homozygote	27 (11%)	9 (4%)
HLA-B27 heterozygote	213 (89%)	231 (96%)
Total	240	240

The expected number of homozygotes was calculated using Hardy–Weinberg equilibrium. To assess independent effects, only probands were used in the analysis.

Statistical analysis

The expected number of *HLA-B27* homozygotes was calculated assuming Hardy–Weinberg equilibrium (HWE) with regard to *HLA-B27*. The antigen frequency of *HLA-B27* was determined from a previously published source of Finnish Bone Marrow Donor Registry based on 10 000 samples.[12] Of the 10 000 individuals, 1444 were *HLA-B27* positive, corresponding to an antigen frequency of 14.4%. The expected frequency of homozygotes for *HLA-B27* in healthy controls was calculated using HWE, as follows:

Let the *HLA-B27* allele frequency be *a* and non-*HLA-B27 b*, such that $a+b = 1$. The likelihood of being *HLA-B27*-antigen positive is given by:

$P(B27/B27 \text{ or } B27/nonB27) = a^2 + 2ab$
$= a^2 + 2a(1-a) = 0.144.$
$a^2 + 2ab + b^2 = 1;\ b^2 = 1-0.144;\ b = 0.925;$
$a = 1-b = 0.0748.$

The probability of being *HLA-B27* homozygote in individuals known to be *HLA-B27* positive is given by:

$P(B27/B27 \mid B27/B27 \text{ or } B27/nonB27)$
$= a^2/(a^2 + 2ab) = a^2/(a^2 + 2a(1-a)) = 0.0748^2/0.144$
$= 0.039\ (3.9\%)$

The SIMWALK2 program (version 2.83) was used for haplotype reconstruction in all the ankylosing spondylitis families.[20] Only families where at least two individuals were fully genotyped for *HLA-DRB1* and *HLA-B27* heterozygosity/homozygosity were included in the analysis. In order to assess independent effects, only one affected individual per family (proband) was chosen as a case for the case–control analysis. The *HLA-DRB1* results from the sequencing, the primer extension reactions and the PCR-SSP were pooled to correspond to the classic *HLA-DRB1* specificities *01 to *10, as this was the typing resolution in the control population. Haplotype frequencies in ankylosing spondylitis cases and the control population were compared by χ^2 analysis. The relative predispositional effect (RPE) method was used to evaluate the relative effects of *HLA-DRB1* alleles.[21] A stepwise conditional logistic regression procedure was applied to further assess the relative importance of *HLA-DRB1* alleles in disease susceptibility, controlling for the effect of linkage disequilibrium (LD) with *HLA-B27*.[22]

The quantitative transmission disequilibrium test (QTDT)[23] was used to calculate the significance of differences in continuous variables between different HLA groups. QTDT incorporates variance components methodology in the

Table 3 The number of *HLA-DRB1-B27* haplotypes in ankylosing spondylitis cases and controls

	HLA-B27 positive **AS haplotypes** (n (%))	*HLA-B27* positive **control haplotypes** (n (%))	OR	p Value
DRB1*01	57 (26)	45 (25)	0.9	0.82
DRB1*02	10 (5)	12 (7)	0.7	0.38
DRB1*03	5 (2)	4 (2)	0.9	0.91
DRB1*04	50 (23)	37 (20)	1.0	0.98
DRB1*05	9 (4)	11 (6)	0.7	0.41
DRB1*06	15 (7)	11 (6)	1.0	0.98
DRB1*07	6 (3)	0	11.1	0.02
DRB1*08	66 (30)	60 (33)	0.8	0.42
DRB1*09	1 (0.5)	2 (1)	0.5	0.45
DRB1*10	2 (1)	1 (0.5)	1.3	0.73
Total	221	183		

221 independent ankylosing spondylitis haplotypes were reliably obtained using a family based haplotype reconstruction program SIMWALK2 (see Methods for details). The control sample set consists of *HLA-B27* positive untransmitted parental haplotypes of Finnish parent–case trios with children with type 1 diabetes mellitus.
AS, ankylosing spondylitis; OR, odds ratio.

analysis of family data and includes exact estimation of p values for analysis of small samples and non-normal data. It also estimates the magnitude of the reduction or increase in the continuous variables resulting from a particular allelic transmission. Disease duration and sex correlated with BASFI and BASDAI in this dataset and they were treated as covariates in the analysis. A 2×2 contingency table using χ^2 analysis was constructed to analyse the clinical manifestations of the disease. All the p values shown are two tailed and uncorrected for multiple comparisons; p values less than 0.05 were considered significant.

The LD between *HLA-B27* and *HLA-DRB1* alleles was calculated using Lewontin's standardised disequilibrium coefficient D',[24] calculated employing the program 2BY2.[25] The founder haplotypes estimated using the program SIMWALK2 (version 2.83) were used as the input.[20] The statistical significance of the finding was assessed using the χ^2 test.

RESULTS

In this dataset, the male to female ratio was 2.1:1. One hundred and twenty six subjects (47%) also had AAU, 18 (7%) had inflammatory bowel disease, six (2%) had psoriasis, and 210 (78%) had peripheral arthritis. The mean (SD) age at symptom onset was 27 (9) years; age at diagnosis, 35 (10) years; diagnostic delay, 8 (8) years; disease duration, 24 (11) years; age at study, 51 (11) years; BASDAI, 5.2 (1.9); and BASFI, 3.9 (2.3).

HLA class I

Table 1 gives the clinical characteristics of the ankylosing spondylitis cases stratified according to their *HLA-B27* genotype status. *HLA-B27* antigen was detected in 251 of the 270 cases (93%). Of the 126 ankylosing spondylitis cases with AAU, 123 (98%) were *HLA-B27* positive—a significant increase compared with the 122 (88%) of the 138 ankylosing spondylitis cases without AAU ($\chi^2 = 8.4$, $p = 0.004$; odds ratio (OR) = 5.4 (95% confidence interval (CI), 1.7 to 17)). No significant differences were noted between ankylosing spondylitis associated with IBD, psoriasis, or peripheral arthritis and the carriage status of *HLA-B27*.

HLA-B27 positive cases had a significantly younger age of symptom onset (by 5.3 years, $p<0.0001$) and younger age at diagnosis (by 5.5 years, $p<0.0001$). *HLA-B27* was associated with a marginal decrease in BASDAI ($p = 0.05$) by QTDT analysis. This was primarily because *HLA-B27* homozygotes had a lower mean BASDAI, while no difference was apparent comparing *HLA-B27* antigen positive and negative cases (table 1). No significant associations were noted between other clinical characteristics and *HLA-B27*.

Twenty seven probands with ankylosing spondylitis were homozygotes for *HLA-B27*, 213 probands were heterozygotes, and 19 probands were *HLA-B27* negative (table 2). Clear *HLA-B27* homozygote/heterozygote genotyping could not be obtained from two probands, who were therefore not considered in further analysis. Assuming HWE with regard to *HLA-B27*, among the *HLA-B27* positive cases the expected number of *HLA-B27* homozygotes of 259 probands was 9 (0.039×240) and the expected number of heterozygotes, 231 (240−9). There was an overrepresentation of *HLA-B27* homozygotes among the probands ($\chi^2 = 9.7$, $p = 0.002$; OR = 3.3 (95% CI, 1.6 to 6.8)).

HLA class II

The frequencies of *HLA-DRB1-B27* haplotypes between cases and controls are presented in table 3. *HLA-B27* positive case and control haplotypes were compared. A marginal increase in the HLA-DRB1*07-B27 haplotype frequency was observed

Table 4 The associations between the age of symptom onset and *HLA-DRB1* alleles and haplotypes, calculated using the quantitative transmission disequilibrium test (QTDT)

HLA allele or haplotype	Direction of association	Magnitude (years)	p Value
HLA-DRB1*08 allele	Younger age of symptom onset	2	0.05
HLA-DRB1*03 allele	Older age of symptom onset	6	0.001
HLA-DRB1*13 allele	Older age of symptom onset	2	0.05
HLA-DRB1*03-non-B27 haplotype	Older age of symptom onset	6	0.006

The estimated magnitude of the reduction or increase of the age of onset associated with a particular allelic or haplotypic transmission is presented in column 3.

compared with the controls (OR = 11.1, p = 0.02), but the sample size was very small (6 *v* 0). No other statistically significant differences were noted.

Conditioning on *HLA-B27* within-family analysis showed no independent associations between *HLA-DRB1* alleles and ankylosing spondylitis susceptibility (logistic regression transmission disequilibrium test: $\chi^2 = 0.42$, p = 0.52; genotype relative risk analysis: $\chi^2 = 3.9$, p = 0.70).

Several weak associations were noted between quantitative traits and *HLA-DRB1-B27* haplotypes or *HLA-DRB1* alleles. The *HLA-DRB1*09-B27* haplotype was associated with a 2.8 decrease in BASDAI ($\chi^2 = 5$, p = 0.03), *HLA-DRB1*04-nonB27* with a 0.7 increase in BASFI ($\chi^2 = 5$, p = 0.03), *HLA-DRB1*08-nonB27* with a 0.8 decrease in BASFI ($\chi^2 = 5$, p = 0.02), and the *HLA-DRB1*12* haplotype with a 1.5 decrease in BASFI ($\chi^2 = 5$, p = 0.03). Associations noted between *HLA-DRB1* alleles or haplotypes and the age of disease onset are presented in table 4. The disease severity scores did not correlate with the age of symptom onset. No significant associations between *HLA-DRB1* alleles and ankylosing spondylitis complicated by AAU, inflammatory bowel disease, or peripheral arthritis were seen.

LD between HLA-B27 and HLA-DRB1 alleles

In the probands a significantly positive LD was noted between *HLA-B27* and *HLA-DRB1*01* (D′ = 0.19, p = 0.05), and *HLA-DRB1*08* (D′ = 0.53, $p = 2\times10^{-7}$). A negative LD was noted between *HLA-B27* and *HLA-DRB1*02* (D′ = −0.6, $p = 9\times10^{-5}$), *HLA-DRB1*03* (D′ = −0.47, p = 0.03), *HLA-DRB1*11* (D′ = −0.43, p = 0.04), and *HLA-DRB1*13* (D′ = −0.42, p = 0.002).

DISCUSSION

Previous studies on the influence of *HLA-B27* homozygosity in the development of ankylosing spondylitis have been contradictory. A significant excess of HLA homozygotes among cases of ankylosing spondylitis has been reported,[6] but this has not been confirmed by others.[7] The contribution of *HLA-B27* homozygosity is likely to be modest and large samples sizes are required to investigate this issue. In other studies, homozygosity for HLA has been reported to be associated with susceptibility to autoimmune diseases,[26] common variable immunodeficiency,[27] and an increased difficulty in clearing infections.[28]

In our family based cohort, *HLA-B27* homozygosity was significantly increased from that expected under HWE. There are genetic theories that could explain our findings. The increased susceptibility associated with *HLA-B27* homozygosity could be explained by the threshold model of polygenic disease, where the presence of increased number of susceptibility alleles increases the likelihood of developing the disease. Another explanation could be the possibility that some *non-HLA-B27* alleles are relatively protective. There are also theoretical molecular mechanisms that could explain the findings. First, *HLA-B27* homozygotes may be more likely to carry abnormal HLA-B27 molecules such as homodimers or misfolded proteins. Second, *HLA-B27* homozygotes may express an increased level of HLA-B27 molecules. The latter is supported by the observation of greater expression of HLA-B27 molecules in patients with ankylosing spondylitis than in healthy controls.[29] However, it is also possible that the genotyping method employed failed to detect some *HLA-B* alleles, which would subsequently increase the number of apparent homozygotes. This could be overcome by performing full *HLA-B* locus genotyping for all the homozygous cases. It is estimated that the frequency of the alleles that are not detected by this method is very low. This is supported by the fact that no Mendelian inconsistencies were detected in these families.

A previous report has suggested that *HLA-B27* homozygosity may influence disease severity.[7] A greater frequency of involvement of peripheral joints has been reported among the *HLA-B27* homozygotes.[6] Surprisingly, this study noted a significant, albeit marginal, decrease in BASDAI among the *HLA-B27* homozygotes, suggesting that *HLA-B27* homozygote patients may have milder disease than *HLA-B27* heterozygotes. It is of note that BASDAI aims to measure the actual disease activity, which may have been decreased by more aggressive disease modifying drug treatment in patients with most severe disease. None of the other disease severity indices showed significant differences. These findings are consistent with the whole genome disease severity screen, in which no linkage between the disease severity indices and the MHC region was observed.[30]

It has been proposed that *HLA-B27* positive and *HLA-B27* negative ankylosing spondylitis represent a heterogeneous group of phenotypically similar diseases that may have different aetiopathogenic mechanisms. *HLA-B27* negative ankylosing spondylitis is rarely familial and is associated with a later age of disease onset.[31 32] The current study showed a significant association between *HLA-B27* and an earlier age of symptom onset and diagnosis in ankylosing spondylitis patients, confirming the previously reported associations. However, *HLA-B27* positivity may be used as a diagnostic tool and therefore cases will be detected earlier. A recent report suggested that *HLA-B27* is also associated with an earlier onset of psoriatic arthritis.[33] *HLA-B27* appears to alter the threshold for developing the disease at an earlier age, but the pathogenic mechanism of this process remains unclear. The large difference between the average ages of onset suggests that the underlying disease process between the *HLA-B27* positive and negative patients may be different. In sub-Saharan Africa the epidemic of HIV infection has been associated with a dramatic increase in the prevalence of *HLA-B27* negative spondyloarthritis,[34] suggesting that environmental factors may play a crucial role in the development of *HLA-B27* negative spondyloathropathy. *HLA-B27* negative patients could merely represent phenocopies of classical *HLA-B27* associated disease. Certainly, it should not be assumed that *HLA-B27* positive and negative ankylosing spondylitis is homogeneous with regard to their aetiology or pathogenesis.

Previous studies have found several distinct associations between *HLA-DRB1* alleles. Our case–control analysis showed that *HLA-DRB1*07-B27* haplotype frequency was marginally increased compared with the controls, but this may merely be a reflection of small sample size and random statistical fluctuation. The logistic regression analysis on *HLA-DRB1* alleles conditioned on *HLA-B27* noted no statistically significant difference between distinct *HLA-DRB1* alleles and ankylosing spondylitis susceptibility. Because of the significant LD within the MHC, *HLA-DRB1* alleles were conditioned on *HLA-B27*. Owing to the extreme polymorphic nature of the *HLA-DRB1* locus and the expected modest effects of HLA class II genes on susceptibility to ankylosing spondylitis, large scale studies are required to dissect these effects. Because of limited sample size this study was underpowered to detect weak effects.

An association between *HLA-DRB1*08* and juvenile ankylosing spondylitis has been reported in Norwegian and Mexican cases.[35 36] A weak association between *HLA-DRB1*08* and early age at symptom onset is reported here, supporting these previous findings. Such findings strongly suggest that both *HLA-B27* and *HLA-DRB1*08*, either independently or as a haplotype, contribute to the genetic susceptibility of early onset disease. Significant positive LD between *HLA-B27* and *HLA-DRB1*08* was observed, raising the possibility that the observed association is caused by

linkage disequilibrium with *HLA-B27*. In this dataset neither the *HLA-DRB1*08-B27* haplotype or *HLA-DRB1*08-nonB27* haplotypes were associated with an earlier age of symptom onset, and thus it is unlikely that *HLA-B27* alone is responsible for the observed association.

Early onset of symptoms did not correlate with BASDAI or BASFI, suggesting that even if disease occurs earlier in the presence of predisposing genes, it is not more severe. This is consistent with previous studies, indicating that although cases with juvenile onset of ankylosing spondylitis are more likely to develop hip arthritis, their disease is otherwise similar in character to cases with adult onset disease ankylosing spondylitis.[37] *HLA-DRB1*03* allele and *HLA-DRB1*03-nonB27* haplotype were associated with a later age of symptom onset, suggesting *HLA-DRB1*03*—or another gene on the *HLA-B27* negative chromosomal strand—may be involved in determining this. Overall, these findings suggest that *HLA-DRB1* alleles may influence the age of symptom onset of ankylosing spondylitis. However, the reported p values are not corrected for multiple comparisons made, and the associations reported here may merely be attributable to stochastic statistical fluctuations.

AAU is more common in *HLA-B27* positive than *HLA-B27* negative cases with ankylosing spondylitis,[38 39] confirmed by this study. There is no consensus among previous studies concerning the association between *HLA-DRB1*08* and AAU. An association has been reported in a Japanese population,[40] and negative findings have been reported in Norwegian and Mexican studies.[35 36] A possible explanation is that the juvenile ankylosing spondylitis may increase AAU. However, a recent study noted that the prevalence of iritis correlates positively with disease duration, but not with age of symptom onset.[41] The present study noted no significant association between *HLA-DRB1*08* and AAU, and thus does not support the Japanese finding. The discrepant associations may be due to different ethnic background of the populations.

The disease severity was assessed using a structured questionnaire, which imposes limitations on the method. Peripheral arthritis and other extra-articular manifestations of the disease were defined merely using the questionnaire. Systematic radiographic disease severity indices such as BASRI were not available. However, BASDAI and BASRI are closely correlated (Calin A, personal communication). Also, BASDAI and BASFI are highly heritable,[2] suggesting that the random fluctuation does not significantly reduce their accuracy. Future studies using radiographic disease severity indices are warranted for a better assessment of the effect of HLA genes on disease severity.

The complexity of the LD and the density of genes make the detection of the causative variants a challenging task in the HLA region. In the healthy Finnish population *HLA-B27* has previously been reported to be in LD with *HLA-DRB1*01*, **04*, and **08*.[42] Positive LD between *HLA-B27* and *HLA-DRB1*01* and **08*, and negative LD between *HLA-B27* and *HLA-DRB1*02, *03, *11*, and **13* was observed. The British and Finnish populations have a similar overall LD pattern.[8] The finding of this study of a significant overrepresentation of *HLA-B27*-homozygotes—and of previous studies of over-representation of *HLA-B60* in *HLA-B27* positive and negative cases[1 43]—have major implications for studies of MHC genes other than *HLA-B* in ankylosing spondylitis. The past practice of matching cases and controls for *HLA-B27* antigen status is clearly not adequate, and future studies must match at least for *HLA-B27* at the allelic level.

Conclusions

HLA-B27 homozygosity is associated with a moderately increased risk of ankylosing spondylitis compared with *HLA-B27* heterozygosity, but otherwise does not significantly affect clinical manifestations. *HLA-B27* positive cases have an earlier age of onset than *HLA-B27* negative cases and are more likely to develop acute anterior uveitis. *HLA-DRB1* alleles may influence the age of symptom onset of ankylosing spondylitis, but otherwise do not play a major role in susceptibility to the disease in Finns.

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APPENDIX

The sequence based method involved eight group specific PCR amplifications with the primers HLA-DRB1*01 (5'CAGTGTCTTCTCAGGTGGCT), HLA-DRB1*15/16 (5'GGCC GCCTTGTGACCGGATG), HLA-DRB1*03/08/11/13/14 (5'GCC TCAGGAAGACAGAGGAG), HLA-DRB1*04 (5'CTTGGGATC AGAGGTAGATTTT), HLA-DRB1*07 (5'CGGCGTCGCTGTC AGTGTT), HLA-DRB1*09 (5'CAGTTAAGGTTCCAGTGCCA), HLA-DRB1*10 (5'CCCACAGCGTTCTTGGAGG), and HLA-DRB1*12 (5'AGTGTCTTCTCAGGACGCCA) prepared in a 50:50 mix with a generic reverse primer with an M13-21 sequencing tag (5'TGTAAAACGACGGCCAGTGCCGCTGCA CTGTGAAGCTCTC). Amplification was carried out in a 10 μl reaction mixture containing 50 ng DNA, 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$ (for HLA-DRB1*01 and HLA-DRB1*03/08/11/13/14) or 2.5 mM $MgCl_2$, 25 μM of each dNTP, 0.25 U AmpliTaq Gold polymerase (Applied Biosystems, Warrington, UK), and 0.4 μM primers. The cycling conditions were as follows: 94℃ for 14 minutes; 35 cycles of 94℃ for 30 seconds, 55℃ (for HLA-DRB1*15/16, HLA-DRB1*04 and HLA-DRB1*10) or 60℃ for 30 seconds, and 72℃ for 30 seconds. PCR products were separated on a 3% agarose gel stained with ethidium bromide and visualised under ultraviolet light. PCR products were subsequently sequenced using M13-21 Big Dye dye primer sequencing kit (Applied Biosystems) using following conditions: 15 cycles of 96℃ for 10 seconds, 55℃ for 5 seconds, 70℃ for one minute, and 15 cycles of 96℃ for 10 seconds and 70℃ for one minute. The products were separated on 4.75% polyacrylamide gels using ABI 373 semiautomated sequencer (Applied Biosystems) and analysed using Sequencing Analysis version 3.0 and Factura version 2.0.1 (PE Applied Biosystems).

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