EXTENDED REPORT

The role of HLA genes in familial spondyloarthropathy: a comprehensive study of 70 multiplex families

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Ann Rheum Dis 2002;**61**:201–206

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Accepted 2 July 2001

Objectives: To investigate whether HLA alleles, other than HLA-B27, influence predisposition to spondyloarthropathy (SpA) in multiplex families.

Methods: Seventy French families with at least two affected SpA members were recruited. Patients, and their first degree relatives were typed for HLA-A, B, C, and DR, and extended HLA haplotypes were determined. The distribution of HLA-A, C, and DR alleles carried on HLA-B27+ haplotypes in SpA families was compared with the distribution of these alleles among HLA-B27+ haplotypes in the French general population. Contribution to SpA susceptibility of HLA-A, B, C, and DR alleles, other than HLA-B27, was tested by transmission disequilibrium test. The contribution of HLA alleles to specific presentation features of SpA was examined.

Results: Frequencies of HLA-A, C, and DR alleles carried on HLA-B27+ haplotypes from SpA families were comparable with those seen in the French population, except for DR13 which was overrepresented among patients (pcorr<0.001). Most interestingly, the HLA-DR4 allele was transmitted in excess to patients with SpA, independently of linkage to HLA-B27 (pcorr=0.05), and in a direction opposite to that for HLA-B27+ unaffected siblings (pcorr=0.01). Finally, the distribution of HLA alleles was not related to the presentation feature of SpA.

Conclusion: HLA predisposition to familial SpA appears not to be limited to HLA-B27, but some HLA-DR alleles also have a significant influence. In particular, HLA-DR4 contributes significantly to a genetic predisposition to SpA, which may have implications in our understanding of SpA pathogenesis.

Ankylosing spondylitis (AS), reactive arthritis, a subset of psoriatic arthritis (PsA), arthritis associated with idiopathic inflammatory bowel diseases (IBD), and undifferentiated spondyloarthropathy (uSpA) form a group of inflammatory rheumatic diseases identified as the spondyloarthropathies (SpA).[1] It has long been recognised that different presentation features of SpA tend to combine within the same families.[2 3] We previously conducted a systematic analysis of SpA phenotypic expression in a large number of multiplex families.[4 5] Results of this study support the hypothesis that although familial SpA shows several distinct phenotypes, these all have predisposing genetic factors in common.[4 5] This indicates that, at least in this particular context, genetic investigations should be conducted on the whole group of patients with SpA, rather than restricted to specific subsets.[6]

One genetic factor shared by all the varieties of SpA has already been identified—that is, HLA-B27.[7] Studies on AS suggest that additional genetic factors, distinct from the HLA region, are involved.[8 9] However, it has also been proposed that HLA-B27 itself may not explain all the predisposition to SpA borne within the major histocompatibility complex (MHC) region.[10 11] In this study we took advantage of having recruited a large number of SpA multiplex families to investigate whether HLA genes, other than HLA-B27, might also contribute to SpA predisposition.

SUBJECTS AND METHODS

Subjects

Seventy multiplex families with SpA who have been recruited throughout France by the Groupe Français d'Etude Génétique des Spondylarthropathies, as previously described,[4 5] were included in this study. Of the 188 patients identified, 187 met the criteria of Amor *et al*,[12] and 182 met the European Spondylarthropathy Study Group (ESSG) criteria.[1] Table 1 shows characteristics of the patients. One hundred and twenty (64%) of the patients had AS according to the modified New York criteria.[13] Among the 67 (36%) patients with SpA without definite sacroiliitis, PsA was diagnosed in 12, arthritis associated with IBD in 4, Reiter's syndrome in 1,[14] and uSpA in the remaining 51. An age matched group of 71 HLA-B27+ siblings belonging to the same families, for which a diagnosis of SpA could not be made, was used as a control group (table 1).

HLA typing

Standard serological methods were routinely used for HLA-A, B, and C typing.[15] In several cases, HLA-A and B generic typing was performed on DNA extracted from peripheral venous blood leucocytes by a polymerase chain reaction-based sequence-specific primers method, using a commercial kit designed to recognise all the known broad specificities (Dynal France SA, Compiègne, France). HLA-DRB1 typing was performed by polymerase chain reaction-sequence-specific oligonucleotide probing in all cases, and frequently also by a standard serological method. HLA-A, B, and DRB1 typing was performed on all 188 patients with SpA, and their first degree healthy relatives. HLA-C typing was only performed on a first set of 38 families. MHC haplotypes were deduced by segregation analysis of HLA-A, B, C, and DRB1 genotypes within pedigrees.

Abbreviations: AS, ankylosing spondylitis; CI, confidence interval; IBD, inflammatory bowel disease; MHC, major histocompatibility complex; PsA, psoriatic arthritis; RA, rheumatoid arthritis; RR, relative risk; SpA, spondyloarthropathy; TDT, transmission disequilibrium test; uSpA, undifferentiated spondyloarthropathy

Table 1 Characteristics of the study group*

Characteristic	HLA-B27+ healthy siblings (n=71)	Patients		
		All patients (n=188)	Patients with AS (n=120)	Non-AS patients (n=67)
Sex ratio, (male/female)	0.65	1.14	1.5	0.4
Age, mean (95% CI)	42 (38.3 to 44.9)	42 (40.4 to 44.4)	44 (41.5 to 46.5)	40 (36.1 to 42.9)
Duration of disease, mean (95% CI)	NA	18 (16.4 to 20)	22 (19.6 to 24.2)	12 (9.3 to 14.1)
HLA-B27 positivity, No (%)	71 (100)	185 (98)	120 (100)	65 (96)
Axial symptoms, No (%)				
All	0 (0)	180 (96)	120 (100)	60 (88)
Back or buttock inflammatory pain	0 (0)	179 (95)	119 (99)	60 (88)
Sacroiliitis (pelvic *x* ray)*	0/69 (0)	120/187 (64)	120 (100)	0 (0)
Peripheral enthesitis, No (%)	3 (4)	109 (58)	61 (51)	50 (74)
Peripheral arthritis, No (%)	1 (1)	90 (48)	59 (49)	22 (32)
Uveitis, No (%)	0 (0)	57 (30)	51 (42)	6 (9)
Psoriasis, No (%)	3 (4)	35 (19)	22 (18)	13 (19)
Crohn/ulcerative colitis, No (%)†	0/1 (1)	3/5 (4)	2/3 (4)	1/2 (4)

*AS, ankylosing spondylitis; non-AS, undifferentiated spondyloarthropathy (n=51), psoriatic arthritis (n=12), inflammatory bowel disease associated arthritis (n=4), and Reiter's syndrome (n=1); 95% CI, 95% confidence interval; NA, not applicable.
*Radiographic sacroiliitis of at least bilateral grade II or unilateral grade III. The number of patients evaluated in each group was 69, 187, 120, and 67 respectively; †Percentages are the sum of the percentages for Crohn's disease and ulcerative colitis.

Study design and statistical analysis

Influence of HLA alleles, other than HLA-B27, on predisposition to SpA was investigated differently for alleles encoded by an HLA-B27+ haplotype and for alleles encoded by an HLA-B27– haplotype.

The frequency of alleles encoded by an HLA-A, C, or DRB1 locus carried on an HLA-B27+ haplotype was compared with the distribution of those alleles in a reference panel of 198 HLA-B27+ haplotypes representing the French general population. This control panel was retrieved from a collection of 3424 haplotypes (Borot N, unpublished results), which have been previously established on a sample of 1356 French families collected equally from the different French regions (to constitute a representative sample), and fully typed across the MHC by serological methods.[16] For this part of the analysis, each individual haplotype identified in an SpA family was counted only once, even if present in several patients. Allelic frequencies were compared between familial patients with SpA and the control French population, using Fisher's exact test. Odds ratio and its 95% confidence interval (CI) were also determined.

The contribution of individual alleles to SpA predisposition was also tested by transmission disequilibrium test (TDT), as previously described.[17] Alleles were tested independently of each other, using binomial probability to determine whether transmission of a given allele by heterozygous parents to affected offsprings was significantly different than the expected frequency of 50%. In addition to patients, HLA-B27+ healthy siblings were also included in the analysis, to test for transmission distortion in the direction opposite to that for SpA siblings, using the unmatched 2×2 χ^2 test.[18] Alleles encoded by an HLA-B27– haplotype, could specifically be tested by this method to discriminate effects independent of linkage with HLA-B27.

Comparison of allele frequency between patients and unaffected HLA-B27+ siblings, and between different subgroups of patients with SpA, was performed, using Fisher's exact test. The relative risk (RR), and its 95% CI were also determined.

In all cases, pcorr refers to corrected p values which were obtained by multiplying crude p values by the number of comparisons. Definite statistical significance required a pcorr<0.05.

RESULTS

Frequency of HLA-A, C, and DR alleles carried on HLA-B27+ haplotypes

Typing of HLA-A, B, and DRB1 in 70 French families with multiple cases of SpA, and of HLA-C in a subset of 38 families

Table 2 Comparison of the frequency of HLA-A alleles carried on HLA-B27+ haplotype between patients with familial spondyloarthropathy and the French control population*

HLA-A allele	Patients haplotypes (n=81)	Control haplotypes (n=196)	p Value†	OR (95% CI)
A1	1(1.2)	11(5.6)	0.19	0.2 (0.03 to 1.7)
A2	31(38.3)	61(31.3)	0.26	1.4 (0.8 to 2.4)
A3	8(9.9)	20(10)	1	1 (0.4 to 2.29)
A11	12(14.8)	18(9.4)	0.2	1.7 (0.8 to 3.8)
A23	1(1.2)	2(1.2)	1	1.2 (0.1 to 13.6)
A24	4(4.9)	24(12.1)	0.08	0.4 (0.1 to 1.1)
A25	3(3.7)	1(0.6)	0.08	7.5 (0.8 to 73.2)
A26	1(1.2)	13(6.5)	0.07	0.2 (0.02 to 1.4)
A28	7(8.6)	11(5.6)	0.42	1.6 (0.6 to 4.3)
A29	2(2.5)	4(2.1)	1	1.2 (0.2 to 6.8)
A30	0(0)	8(3.8)	0.11	0.1 (0.01 to 2.4)
A31	2(2.5)	6(2.9)	1	0.8 (0.2 to 4.1)
A32	7(8.6)	16(8.3)	1	1.1 (0.4 to 2.7)
A33	1(1.2)	1(0.6)	0.5	2.4 (0.2 to 39.5)
A43	1(1.2)	0(0)	0.29	7.3 (0.3 to 182)

OR, odds ratio; CI, confidence interval.
*Except where otherwise indicated, values are the number (%) of haplotypes; †statistical comparisons were performed, using Fisher's exact test.

Table 3 Comparison of the frequency of HLA-C alleles carried on HLA-B27 positive haplotype between patients with familial spondyloarthropathy and the French control population*

HLA-C allele	Patients haplotypes (n=48)	Control haplotypes (n=169)	p/pcorr†	OR (95% CI)
C1	20(41.7)	69(40.8)	1	1 (0.5 to 2)
C2	21(43.8)	90(53.3)	0.26	0.7 (0.4 to 1.3)
C3	1(2.1)	2(1.2)	0.53	1.8 (0.2 to 20)
C4	0(0)	1(0.6)	1	1.2 (0.05 to 29)
C5	1(2.1)	2(1.2)	0.53	1.8 (0.2 to 20)
C6	0(0)	1(0.6)	1	1.2 (0.05 to 29)
C7	4(8.3)	3(1.8)	0.04/0.35	5 (1.1 to 23.3)
C8	1(2.1)	0(0)	0.22	10.7 (0.4 to 267)

OR, odds ratio; CI, confidence interval.
*Except where otherwise indicated, values are the number (%) of haplotypes;†statistical comparisons were performed, using Fisher's exact test. pcorr=p value corrected for the number of comparisons.

Table 4 Comparison of the frequency of HLA-DR alleles carried on HLA-B27 positive haplotype between patients with familial spondyloarthropathy and the French control population*

HLA-DR allele	Patients haplotypes (n=81)	Control haplotypes (n=184)	p/pcorr†	OR (95% CI)
DR1	17(21)	47(25.5)	0.53	0.8 (0.4 to 1.5)
DR2	8(9.9)	20(10.9)	1	0.9 (0.4 to 2.1)
DR3	2(2.5)	16(8.7)	0.07	0.3 (0.06 to 1.2)
DR4	19(23.5)	36(19.6)	0.51	1.3 (0.7 to 2.4)
DR7	3(3.7)	8(4.3)	1	0.9 (0.2 to 3.3)
DR8	4(4.9)	11(6)	1	0.8 (0.3 to 2.7)
DR9	0(0)	5(2.7)	0.33	0.2 (0.01 to 3.7)
DR10	0(0)	1(0.5)	1	0.8 (0.03 to 18.6)
DR11	13(16)	27(14.7)	0.85	1.1 (0.5 to 2.3)
DR12	1(1.2)	3(1.6)	1	0.8 (0.08 to 7.4)
DR13	8(9.9)	0(0)	<0.0001/<0.001	42.7 (2.4 to 749)
DR14	6(7.4)	10(5.4)	0.58	1.4 (0.5 to 4)

OR, odds ratio; CI, confidence interval.
*Except where otherwise indicated, values are the number (%) of haplotypes;†statistical comparisons were performed, using Fisher's exact test. pcorr=p value corrected for the number of comparisons.

resulted in the identification of 81 distinct HLA-B27+ extended haplotypes in patients. Noteworthy, is that in 10 families, two B27+ haplotypes were found in B27 homozygous patients. Of those haplotypes, 48 included HLA-C (A;C;B27;DR), whereas 33 did not (A;B27;DR). Allelic distribution at locus A (table 2) and C (table 3) in familial SpA did not differ from the distribution seen in a panel of HLA-B27+ haplotypes from the French general population. Distribution of HLA-DR alleles was also similar between familial SpA and the control group for most alleles, except for DR13, which was significantly overrepresented among SpA HLA-B27+ haplotypes (table 4).

Transmission of HLA-A, B, and C alleles to patients

TDT was used to determine if class I MHC alleles, other than HLA-B27, were randomly transmitted to patients with SpA. For each HLA-A and C allele tested we performed a global TDT which included all haplotypes, and also a TDT restricted to HLA-B27– haplotypes, to search for transmission disequilibrium independent of linkage between A or C alleles and HLA-B27. For HLA-B alleles, only TDT applied to HLA-B27– haplotypes is shown.

Transmission of most HLA-A, B, and C alleles to patients was not significantly different from random. A limited number of alleles encoded by those class I MHC loci displayed some degree of unbalanced transmission, which failed to reach statistical significance after correction for the number of comparisons (table 5). Such weak transmission disequilibrium, was rarely observed in TDT restricted to HLA-B27– haplotypes. Notably, a non-significant trend towards an excess of transmission to patients was seen for the HLA-B60 allele (table 5).

Transmission of HLA-DR alleles to patients and healthy HLA-B27+ siblings

TDT of HLA-DR alleles to patients with SpA was first performed with alleles carried on all haplotypes. Results of this TDT displayed unbalanced transmission of several alleles, reaching statistical significance after correction for the number of comparisons (table 6). Hence, an excess of transmission to patients with SpA was observed for DR1 (pcorr=0.02) and DR4 (pcorr=0.002), whereas a reduced transmission was noticed for DR2 (pcorr=0.05). In addition, trends towards reduced transmission were found for DR3 and DR13, but failed to reach statistical significance after correction (table 6). The transmission of HLA-DR alleles to unaffected HLA-B27+ siblings of patients with SpA was analysed in parallel using TDT, and compared with the pattern of transmission to patients with SpA, using an unmatched 2×2 χ^2 test. We found a significant distortion of transmission of DR4 allele to HLA-B27+ unaffected siblings, in the direction opposite to that for SpA siblings (table 6; pcorr= 0.006). No such distortion was seen with any other HLA-DR allele. These results suggest that an excess of transmission of DR4 to a patient with SpA might exist independently of linkage disequilibrium between DR4 and HLA-B27. A TDT restricted to HLA-DR alleles carried on HLA-B27– haplotypes was next performed to examine this possibility. This TDT showed an excess of transmission of the DR4 allele to patients with SpA (table 7; pcorr=0.05). Furthermore, a distortion of transmission of DR4 allele to HLA-B27+ unaffected siblings in the direction opposite to that for SpA siblings was also confirmed with HLA-B27– haplotypes (table 7; pcorr= 0.01).

Table 5 Results of the transmission disequilibrium test performed for HLA-A, B, and C alleles in patients with familial spondyloarthropathy*

	All haplotypes			HLA-B27 negative haplotypes		
HLA allele	Transmitted	Not transmitted	p/pcorr†	Transmitted	Not transmitted	p/pcorr†
A1‡	20	26	0.38	18	8	0.05/0.45
A28	19	8	0.04/0.32	6	6	1
B5§				9	18	0.09
B18				15	6	0.05/0.6
B60				12	5	0.09
C1¶	27	11	0.01/0.08	2	1	NA
C2	34	19	0.04/0.32	3	9	0.09
C3	10	25	0.02/0.16	8	14	0.21

*NA, statistical analysis not applicable because of small sample size; †p values were obtained using binomial probability. pcorr=p value corrected for the number of comparisons; ‡balanced transmission was observed for all the following HLA-A alleles tested: A2, A3, A9 (A23 + A24), A10 (A25 + A26), A11, A19 (A29 + A30 + A31 + A32 + A33 + A34), and A29; §balanced transmission was observed for all the following HLA-B alleles tested: B7, B8, B12 (B44 + B45), B14 (B64 + B65), B15, B16 (B38 + B39), B17 (B57 + B58), B21, and B35; ¶balanced transmission was observed for the following HLA-C alleles tested: C4, C5, C6, C7, and C8.

Contribution of HLA alleles to specific presentation features of SpA

The foregoing results were obtained by examining all patients with SpA from mutiplex families. We also considered whether specific features of the SpA spectrum were preferentially associated with HLA alleles. No statistically significant association of HLA-A, B, and C alleles with specific manifestations was seen after correcting for the number of comparisons (data not shown). Likewise, most HLA-DR alleles were uniformly distributed among patients with SpA, whatever the manifestation examined (table 8). Only the DR13 allele appeared to be less frequent among patients with sacroiliitis, and was conversely overrepresented among patients with IBD, but the differences failed to reach statistical significance (table 8). Notably, the DR13 allele which was present in 50% of patients with IBD was carried on a B27– haplotype. Finally, the

Table 6 Results of the transmission disequilibrium test performed for HLA-DR alleles carried on all haplotypes*

	Patients (n=169)			Unaffected HLA-B27+ siblings (n=71)				
HLA-DR allele†	Transmitted	Not transmitted	p/pcorr‡	Transmitted	Not transmitted	p‡	Unmatched χ^2	p/pcorr§
DR1	46	21	0.002/0.02	22	11	0.11	0.04	0.84
DR2	24	48	0.005/0.05	21	18	0.64	4.42	0.036/0.32
DR3	20	38	0.02/0.18	11	18	0.2	0.1	0.75
DR4	52	20	0.0002/0.002	10	18	0.13	11.4	0.0007/0.006
DR7	26	32	0.43	8	14	0.2	0.47	0.49
DR8	13	17	0.47	3	7	0.21	0.56	0.46
DR11	44	36	0.38	25	16	0.16	0.4	0.53
DR13	22	40	0.03/0.3	7	4	0.49	3.09	0.08
DR14	22	13	0.13	10	6	0.37	0.0006	0.98

*pcorr, p value corrected for the number of comparisons; †number of alleles DR9, DR10, and DR12 available for transmission disequilibrium test were too small to give valid results; ‡p values were obtained using binomial probability; §p values were obtained using χ^2 test to compare the balance of transmission between patients and HLA-B27+ siblings.

Table 7 Results of the transmission disequilibrium test performed for HLA-DR alleles carried on HLA-B27 negative haplotypes*

	Patients (n=156)			Unaffected HLA-B27+ siblings (n=70)				
HLA-DR allele†	Transmitted	Not transmitted	p/pcorr‡	Transmitted	Not transmitted	p‡	Unmatched χ^2	p/pcorr§
DR1	13	10	0.53	8	8	1	0.16	0.69
DR2	16	26	0.13	12	11	0.84	1.2	0.27
DR3	14	18	0.48	8	4	0.25	1.83	0.18
DR4	20	6	0.006/0.05	2	8	0.06	9.85	0.002/0.012
DR7	20	20	1	9	8	0.81	0.04	0.84
DR8	10	11	0.83	3	4	NA	NA	NA
DR11	20	16	0.51	7	9	0.62	0.62	0.43
DR13	13	18	0.37	8	5	0.41	2.2	0.14
DR14	8	7	0.8	1	3	NA	NA	NA

*NA, statistical analysis not applicable because of small sample size. pcorr=p value corrected for the number of comparisons; †number of alleles DR9, DR10, and DR12 available for transmission disequilibrium test were too small to give valid results; ‡p values were obtained using binomial probability; §p values were obtained using χ^2 test to compare the balance of transmission between patients and HLA-B27+ siblings.

Table 8 Frequency of HLA-DR alleles in familial spondyloarthropathy (SpA) according to specific manifestations*

HLA-DR allele	Number (%) of healthy B27+ siblings (n=71)	Number (%) of patients with SpA: All/B27+ patients (n=188/185)	Radiographic sacroiliitis† (n=120)	Arthritis (n=89)	Uveitis (n=57)	Psoriasis (n=35)	IBD (n=8)
DR1	24(34)	59(31/32)	40(33)	31(35)	19(33)	10(29)	2(25)
DR2	20(28)	35/34(19/18)	22(18)	19(21)	11(19)	6(17)	1(13)
DR3	10(14)	22/21(12/11)	15(12)	11(12)	9(16)	4(11)	1(13)
DR4	11(15)	63(34)‡	42(35)	26(29)	15(26)	13(37)	1(13)
DR7	9(13)	32(17)	22(18)	17(19)	11(19)	7(20)	1(13)
DR8	4(6)	18(10)	15(12)	6(7)	7(12)	3(9)	1(13)
DR9	1(1)	2(1)	2(2)	2(2)	1(2)	1(3)	0(0)
DR10	1(1)	2(1)	1(0.8)	1(1)	0(0)	1(3)	0(0)
DR11	27(38)	54/53(29)	31(26)	22(25)	16(28)	8(23)	3(38)
DR12	0(0)	4(2)	2(2)	1(1)	0(0)	2(6)	0(0)
DR13	15(21)	35/32(19/17)	15(12)§	18(20)	12(21)	3(9)	4(50)¶
DR14	7(10)	25(13/14)	19(16)	12(13)	9(16)	8(23)	0(0)

*IBD, inflammatory bowel disease; †radiographic sacroiliitis of at least bilateral grade II or unilateral grade III; ‡B27+ patients *v* B27+ healthy siblings: p=0.003/pcorr=0.04, using Fisher's exact test; relative risk (95% CI)1 .3 (1.1 to 1.5); §radiographic *v* no radiographic sacroiliitis: p=0.006/pcorr=0.07, using Fisher's exact test; relative risk (95% CI)=0.6 (0.4 to 0.9). ¶IBD *v* no IBD: p=0.06/pcorr=0.72, using Fisher's exact test; relative risk (95% CI)=3.8 (1 to 14.4).

HLA-DR4 allele appeared to be uniquely overrepresented among HLA-B27+ patients, as compared with unaffected HLA-B27+ siblings (table 8; pcorr=0.04).

DISCUSSION

Genetic linkage between AS, the prototypical form of SpA, and the MHC region has been previously established.[9 10 19] The strongest association between the MHC antigen and AS is unequivocally attributable to HLA-B27.[11 20] However, there are some suggestions that other MHC alleles besides HLA-B27 may also contribute towards increasing susceptibility for AS.[10 11] Numerous studies have attempted in the past to identify such non-B27 genes within the MHC which would directly contribute to AS susceptibility.[11 21] However, such studies have commonly been hampered by the strong linkage disequilibrium between alleles encoded in separate loci within the MHC region, by the overwhelming presence of HLA-B27 in AS, and also by a lack of power with regard to highly polymorphic HLA loci. In this study we took advantage of having recruited a large number of mutiplex SpA families to examine separately the contribution of MHC alleles encoded by an HLA-B27+ haplotype and the contribution of alleles encoded by B27– haplotypes.

To analyse the genetic contribution of HLA alleles encoded by an HLA-B27+ haplotype, we compared the frequency of alleles encoded by SpA haplotypes to alleles encoded by HLA-B27+ haplotypes from the French general population. The distribution of alleles was remarkably similar between both populations, even though the control haplotypes were obtained from a historical study.[16] This result confirms, on a larger scale, previous reports concerning HLA-A and C loci.[22] It is also consistent with a priority role for HLA-B27 over putative secondary predisposing gene(s) located within the MHC region.[20] The only remarkable difference concerned the HLA-DR13 allele, which was significantly represented among SpA B27+ haplotypes, whereas it was not detected among HLA-B27+ control haplotypes. This absence of DR13 in controls is unlikely to reflect a technical bias, because the DR13 allele was commonly detected among B27– haplotypes in the French population study (Borot N, unpublished results). Rather, it is consistent with a previously reported negative linkage disequilibrium between DR13 and HLA-B27 in the general population.[23] Nevertheless, TDT failed to support linkage between DR13 and SpA within families. Hence, this increased prevalence of B27;DR13 haplotypes in SpA families may reflect population stratification (as a consequence of some putative degree of common origin, which would make patients more likely to share the DR13 allele with each other than with a control population), rather than a direct role for the DR13 allele in genetic predisposition to SpA. Alternatively, it might be secondary to the presence of another as yet unidentified predisposing gene carried on the B27;DR13 haplotype.

The most striking findings in this study were obtained with TDT, which allows one to determine both linkage and association between disease and given alleles. When all haplotypes were included in the TDT, unbalanced transmission of several HLA alleles to patients was found. In most cases this result was not confirmed by TDT restricted to HLA-B27– haplotypes, suggesting that it was accounted for by linkage disequilibrium between such alleles and HLA-B27. Notably, the excess transmission of HLA-DR1 to patients could entirely be attributed to linkage disequilibrium with B27 in this study, albeit DR1 has been suspected to predispose to AS independently of HLA-B27.[23] In contrast, HLA-DR4 was transmitted in excess to patients independently of its positive linkage disequilibrium with HLA-B27. Furthermore, an opposite trend towards a lack of transmission of DR4 to unaffected HLA-B27+ siblings was seen. Together, these data show that the presence of DR4 contributed to SpA susceptibility, in addition to the HLA-B27 effect. For some of the other alleles, the small amount of meiosis available for TDT in this study might have been associated with a lack of power in demonstrating a statistically significant effect. This particularly might be the case for HLA-B60, which has been reported in several studies to increase the risk of AS.[11]

In this study we analysed the whole spectrum of SpA together, because we had previously shown that in familial SpA different subsets of SpA probably share major predisposition factors.[4 5] Nevertheless, genes located within the MHC region may contribute to phenotypic diversity. In particular, HLA-DR4 might have preferentially contributed to peripheral arthritis, as previously suggested.[24] Such an assumption was not confirmed by our data, which showed equivalent association between DR4 and SpA, whatever the presentation feature. Notably, this study had a statistical power >95% to detect a twofold increase in peripheral arthritis among patients with DR4. Furthermore, this study failed to repeat any of the previously reported preferential associations between specific features of SpA and HLA alleles.[21] Several explanations might account for such discrepancies, among which are ethnic variations between different studies and the familial context, which was specific to this study. Another study in AS, which was largely based on familial cases, also failed to identify a significant influence of HLA-DR alleles on

phenotypic presentation, including peripheral arthritis, uveitis, psoriasis, or IBD.[23] Results of this study are suggestive of a negative association between HLA-DR13 and sacroiliitis and, conversely, of a positive association between DR13 and IBD, which was not statistically significant. However, this study had only a power of 12% to demonstrate an RR of IBD among DR13 patients, of a magnitude of two with 95% CI (that is, without correcting for the number of comparisons), because of the low proportion of IBD cases. Hence, we would have needed to include 4213 subjects to reach an 80% power of demonstrating an RR of such magnitude, or 1583 subjects to reach an 80% power of demonstrating an RR of three, after correcting for the number of comparisons. Nevertheless, these data highlight again HLA-DR13 as a candidate marker to be further investigated in a genetic study of SpA.

To our knowledge, this study is the first to show that HLA-DR4, another HLA allele within the MHC region besides HLA-B27, is associated with predisposition to SpA. The possibility that preferential association between SpA and a more important MHC gene in tight linkage disequilibrium with DR4 accounted for this result cannot entirely be excluded. Nevertheless, it is a further step towards improving our understanding of the role of the MHC in SpA. It has been proposed that CD4+ T cells might have a more important role in SpA pathogenesis than CD8+ T cells.[25] In line with this hypothesis, HLA-DR4, which is a class II allele, might preferentially affect CD4+ T cell biology. Most interestingly, HLA-DR4 is also known as a major genetic factor predisposing to rheumatoid arthritis (RA).[26] It has been reported that HLA-B27, in addition to HLA-DR4, may contribute to RA susceptibility.[27 28] Such observations support the hypothesis that both alleles might contribute additively to chronic inflammatory joint disease, and that RA and SpA may share some pathogenic mechanism. Further work will be required to identify whether DR4 subtypes associated with RA are those preferentially transmitted to patients with SpA, which would strengthen this latter hypothesis.

ACKNOWLEDGMENTS

We gratefully acknowledge the contribution of Dr Bernard Amor, Dr Francis Berenbaum, Dr Jean-Marie Berthelot, Dr Jean-Paul Blanquet, Dr Bernard Combe, Dr Emmanuelle Dernis-Labous, Dr Agnès Duché, Dr Jacques Fechtembaum, Dr Sandrine Guis, Dr Pierre Miossec, Dr Aleth Perdriger, Dr Anne-Marie Prieur, and Dr Alain Saraux, from Groupe Français d'Etude Génétique des Spondylarthropathies to the selection of patients, and the excellent technical assistance of Maryse Mezières, Anne-Marie Poussy, and Gilles Michard.

This work was supported by grants from Programme Hospitalier de Recherche Clinique (PHRC; 1995), Société Française de Rhumatologie (SFR; 1997 and 1999), Association de Recherche Clinique en Rhumatologie (ARCR; 1996 and 1997), and Searle-Monsanto (1998 and 1999). Corinne Miceli-Richard was supported by a grant from Association de Recherche sur la Polyarthrite (ARP; 1999 and 2000).

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REFERENCES

1 **Dougados M**, Van der Linden S, Juhlin R, Huitfeldt B, Amor B, Calin A, *et al*. The European Spondylarthropathy Study Group preliminary criteria for the classification of spondylarthropathy. Arthritis Rheum 1991;34:1218–27.
2 **Moll JM**, Haslock I, Macrae IF, Wright V. Associations between ankylosing spondylitis, psoriatic arthritis, Reiter's disease, the intestinal arthropathies, and Behçet's syndrome. Medicine (Baltimore) 1974;53:343–64.
3 **Hochberg MC**, Bias WB, Arnett FC. Family studies in HLA-B27 associated arthritis. Medicine (Baltimore) 1978;57:463–75.
4 **Said-Nahal R**, Miceli-Richard C, Berthelot JM, Duché A, Dernis-Labous E, Le Blévec G, *et al*. The familial form of spondylarthropathy: a clinical study of 115 multiplex families. Arthritis Rheum 2000;43:1356–65.
5 **Said-Nahal R**, Miceli-Richard C, D'Agostino MA, Dernis-Labous E, Berthelot JM, Duché A, *et al*. Phenotypic diversity is not determined by independent genetic factors in familial spondylarthropathy. Arthritis Care Res 2001;45:478–84.
6 **Miceli-Richard C**, Said-Nahal R, Breban M. Impact of sex on inheritance of ankylosing spondylitis [letter]. Lancet 2000;355:1097–8.
7 **Amor B**, Feldmann JL, Delbarre F, Hors J, Beaujan MM, Dausset J. HL-A antigen W27 - a genetic link between ankylosing spondylitis and Reiter's syndrome? [letter]. N Engl J Med 1974;290:572.
8 **Brown MA**, Kennedy LG, MacGregor AJ, Darke C, Duncan E, Shatford JL, *et al*. Susceptibility to ankylosing spondylitis in twins. The role of genes, HLA, and the environment. Arthritis Rheum 1997;40:1823–8.
9 **Laval SH**, Timms A, Edwards S, Bradbury L, Brophy S, Milicic A, *et al*. Whole-genome screening in ankylosing spondylitis: evidence of non-MHC genetic-susceptibility loci. Am J Hum Genet 2001;68:918–26.
10 **Rubin LA**, Amos CI, Wade JA, Marti JR, Bale SJ, Little AH, *et al*. Investigating the genetic basis for ankylosing spondylitis. Linkage studies with the major histocompatibility complex region. Arthritis Rheum 1994;37:1212–20.
11 **Wordsworth P**. Genes in the spondyloarthropathies. Rheum Dis Clin North Am 1998;24:845–62.
12 **Amor B**, Dougados M, Mijiyawa M. Critère diagnostique des spondylarthropathies. Rev Rhum Mal Osteoartic 1990;57:85–9.
13 **Van der Linden S**, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. Arthritis Rheum 1984;27:361–8.
14 **Willkens RF**, Arnett FC, Bitter T, Calin A, Fisher L, Ford DK, *et al*. Reiter's syndrome. Evaluation of preliminary criteria for definite disease. Arthritis Rheum 1981;24:844–9.
15 **Terasaki PI**, McClelland JD. Microdoplet assay of human serum cytotoxine. Nature 1964;204:998–1000.
16 **Cambon-Thomsen A**, Ohayon E. Analyse des données génétiques sur l'échantillon global des provinces Françaises. In: Ohayon E, Cambon-Thomsen A, eds. *Human population genetics*. Colloque INSERM 1986:297–318.
17 **Spielman RS**, McGinnis RE, Ewens WJ. Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). Am J Hum Genet 1993;52:506–16.
18 **Lie BA**, Rønningen KS, Akselsen HE, Thorsby E, Undlien DE. Application and interpretation of transmission/disequilibrium tests: transmission of HLA-DQ haplotypes to unaffected siblings in 526 families with type 1 diabetes. Am J Hum Genet 2000;66:740–3.
19 **Reveille JD**, Suarez Almazor ME, Russell AS, Go RC, Appleyard J, Barger BO, *et al*. HLA in ankylosing spondylitis: is HLA-B27 the only MHC gene involved in disease pathogenesis. Semin Arthritis Rheum 1994;23:295–309.
20 **Martinez-Borra J**, Gonzalez S, Lopez-Vazquez A, Gelaz MA, Armas JB, Kanga U, *et al*. HLA-B27 alone rather than B27-related class I haplotypes contributes to ankylosing spondylitis suceptibility. Hum Immunol 2000;61:131–9.
21 **al-Khonizy W**, Reveille JD. The immunogenetics of the seronegative spondyloarthropathies. Baillieres Clin Rheumatol 1998;12:567–88.
22 **Lochead JA**, Chalmers IM, Marshall WH, Larsen B, Skanes VM, Payne RH, *et al*. HLA-B27 haplotypes in family studies of ankylosing spondylitis. Arthritis Rheum 1983;26:1011–16.
23 **Brown MA**, Kennedy LG, Darke C, Gibson K, Pile KD, Shatford JL, *et al*. The effect of HLA-DR genes on susceptibility to and severity of ankylosing spondylitis. Arthritis Rheum 1998;41:460–5.
24 **Miehle W**, Schattenkirchner M, Albert D, Bunge M. HLA-DR4 in ankylosing spondylitis with different patterns of joint involvement. Ann Rheum Dis 1985;44:39–44.
25 **Breban M**, Falgarone G, Blanchard H, Dernis-Labous E, Lamarque D. Animal models of the spondyloarthropathies. Curr Rheumatol Rep 2000;2:282–7.
26 **Reveille J**. The genetic contribution to the pathogenesis of rheumatoid arthritis. Curr Opin Rheumatol 1998;10:187–200.
27 **Rantapaa Dahlqvist S**, Strom H, Bjelle A, Moller E. HLA antigens in rheumatoid arthritis patients with and without a family history of polyarthritis. Scand J Rheumatol 1985;14:375–80.
28 **Khan MA**, Wolfe F, Kleinheksel SM, Molta C. HLA DR4 and B27 antigens in familial and sporadic rheumatoid arthritis. Scand J Rheumatol 1987;16:433–6.