HLA-B27 homozygosity has no influence on clinical manifestations and functional disability in ankylosing spondylitis

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Abstract

Objective

There is an established association between the development of ankylosing spondylitis (AS) and the HLA-B27 allele, but whether or not homozygosity for HLA B27 has any additional effects on the clinical manifestations of AS is unclear. The aim of this study was to determine the influence of HLA–B27 homozygosity on the clinical manifestations of AS in Korea.

Methods

A total of 490 patients were enrolled in this study. Genotyping was carried out using polymerase chain reaction-sequence specific primers (PCR-SSP) to assess HLA-B27 homozygosity or heterozygosity. One PCR reaction was undertaken to determine the HLA-B27 carrier status, and 5 group-specific PCR reactions were carried out to determine all of the other HLA-B alleles. Clinical manifestations of AS and BASFI were also evaluated according to homozygosity or heterozygosity for HLA-B27.

Results

HLA-B27 positive patients had a significantly younger age at symptom onset, more uveitis, and a higher frequency of hip joint involvement than HLA-B27 negative patients. One hundred and forty-six (29.8%) patients were homozygous for HLA-B27. No significant association between HLA-B27 homozygosity or heterozygosity and a history of peripheral arthritis, acute anterior uveitis, age at onset, or the Bath Ankylosing Spondylitis Functional Index (BASFI) was found.

Conclusion

Homozygosity for HLA-B27 does not affect the clinical manifestations of AS in Korean patients.

Key words

Ankylosing spondylitis, HLA-B27, homozygosity.

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Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory disease with a strong genetic component determining both susceptibility to and the severity of the disease (1, 2). The pathogenesis of AS is unknown, but it is well established that genetic factors play a major role in determining a patient's susceptibility to the disease. There is an established association between the development of AS and the presence of the HLA-B27 allele (3). However, the exact genetic mechanism of the pathogenic link remains unknown. Moreover, whether or not homozygosity for the HLA-B27 allele affects the manifestations of the disease is unclear.

Contradictory reports concerning the effects of homozygosity for HLA-B27 have been published (4-9). A recent paper reported a link between HLA-B27 homozygosity and a moderately increased risk of AS in a Finnish population (10). However, there is no consensus as to whether HLA-B27 homozygosity influences the clinical features of AS.

The aim of this study was to evaluate the influence of HLA-B27 homozygosity/heterozygosity on the clinical manifestations of AS patients in a Korean population.

Materials and methods

Patient selection and study design

A total of 490 AS patients (432 males, 58 females) were recruited for this study. All patients were native Koreans with a diagnosis of AS that met the modified New York criteria (11). First, we determined the positive or negative HLA-B27 status of all AS patients in order to compare the clinical features between the two groups. Second, the HLA-B27 positive patients were assessed to determine whether they were homozygous or heterozygous for HLA-B27. Informed consent was obtained from all patients. Clinical data was collected retrospectively from medical records and interviews.

Genotype analysis

All AS patients were genotyped to establish the presence or absence of the HLA-B27 gene using the polymerase chain reaction-sequence specific primer (PCR-SSP) procedure.

In the HLA-B27 positive patients, limited HLA-B locus genotyping by PCR-SSP was performed to assess HLA-B27 homozygosity and heterozygosity. One PCR reaction was carried out to confirm the HLA-B27 carrier status, and 5 group-specific PCR reactions were conducted to determine all the other HLA-B alleles (see Appendix 1a, 1b) (10). Amplification was carried out in a final volume 13μl reaction mixture containing 0.1 and 0.01 μg DNA, 1.3 μl of 10×PCR reaction buffer, 2 mM $MgCl_2$, 200 μM of each dNTP, 0.1875 unit Tag polymerase, and 1~4 μM of specific primers and 0.1μM of control primer.

The cycling conditions were as follows: 1 cycle at 96°C for 1 minute, 5 cycles at 96°C for 25 seconds, 1 cycle at 70°C for 45 seconds, 1 cycle at 72°C for 45 seconds, 21 cycles at 96°C for 25 seconds, 1 cycle at 65°C for 50 seconds, 1 cycle at 72°C for 45 seconds and 4 cycles of 96°C for 25 seconds, 1 cycle at 55°C for 1 minute, and 1 cycle at 72°C for 2 minutes. 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.

Statistical analysis

Differences in 2x2 frequencies were analyzed using chi-square or Fisher's exact tests. Differences between the two variables were compared using two-tailed *t*-tests. A *p*-value less than 0.05 was regarded as statistically significant.

Results

The clinical characteristics of the patients are presented in Table I (patients with missing clinical data were not included). The mean age (SD) of the AS patients was 33.4 (9.9) years, and the mean onset age (SD) was 20.6 (7.9) years. Of these patients, 31.1% had juvenile onset AS (JoAS), and 214 patients (45.1%) had peripheral enthesitis. Two hundred and forty-six patients (50.3%) had a history of peripheral arthritis; 151 patients (30.8%) had a history of uveitis; and 454 patients

Table I. Demographic features of the Korean ankylosing spondylitis (AS) patients.

Clinical features	Cases
Age, mean ± SD (years)	33.4 ± 9.9
Age at onset, mean ± SD (years)	20.6 ± 7.9
Disease duration, mean ± SD (years)	12.7 ± 8.4
Male, no. (total, %)	432 (490, 88.2)
JoAS, no. (total, %)	151 (485. 31.1)
Peripheral enthesitis, no. (total, %)	214 (475, 45.1)
Peripheral arthritis, no. (total, %)	246 (489, 50.3)
Uveitis, no. (total, %)	151 (490, 30.8)
Hip involvement, no. (total, %)	352 (473, 74.4)
Shoulder involvement, no. (total, %)	272 (451, 60.3)
HLA-B27, no. (total, %)	454 (490, 92.7)
BASFI, mean ± SD	1.8 ± 2.0
Initial symptom	
LBP or buttock pain, no. (total, %)	234 (486, 48.1)
Hip joint pain, no. (total, %)	76 (486, 15.6)
Knee arthritis, no. (total, %)	73 (486, 15.0)
Achilles tendonitis, no. (total, %)	40 (486, 8.2)
Ankle arthritis, no. (total, %)	24 (486, 4.9)
Plantar fasciitis, no. (total, %)	13 (486, 2.7)

JoAS: juvenile onset ankylosing spondylitis, BASFI: Bath Ankylosing Spondylitis Functional Index, LBP: low back pain.

Table II. Comparison of clinical features between HLA-B27 positive and HLA-B27 negative groups of Korean AS patients.

Clinical features	HLA-B27 pos. (n=454)	HLA-B27 neg. (n=36)	*p*-value
Age, mean ± SD (years)	33.1 ± 9.5	37.3 ± 13.2	NS
Age at onset, mean ± SD (years)	20.2 ± 7.6	26.2 ± 10.4	0.02
Disease duration, mean ± SD (years)	12.8 ± 8.2	11.2 ± 10.7	NS
Male, no. (total, %)	402 (454, 88.5)	30 (36, 83.3)	NS
JoAS, no. (total, %)	146 (449. 32.5)	5 (36, 13.9)	0.02
Peripheral enthesitis, no. (total, %)	199 (441, 45.1)	15 (34, 44.1)	NS
Peripheral arthritis, no. (total, %)	229 (453, 50.6)	17 (36, 47.2)	NS
Uveitis, no. (total, %)	148 (454, 32.6)	3 (36, 8.3)	0.001
Hip involvement, no. (total, %)	334 (437, 76.4)	18 (36, 50.0)	0.001
Shoulder involvement, no. (total, %)	255 (418, 61.0)	17 (33, 51.5)	NS
BASFI, mean ± SD	1.9 ± 2.0	1.6 ± 1.7	NS
Initial symptom			
LBP or buttock pain, no. (total, %)	216 (454, 47.6)	19 (36, 52.8)	NS
Hip joint pain, no. (total, %)	74 (454, 16.3)	2 (36, 5.6)	NS
Knee arthritis, no. (total, %)	66 (454, 14.5)	7 (36, 19.4)	NS
Achilles tendonitis, no. (total, %)	38 (454, 8.4)	2 (36, 5.6)	NS
Ankle arthritis, no. (total, %)	23 (454, 5.1)	1 (36, 2.8)	NS
Plantar fasciitis, no. (total, %)	12 (454, 2.6)	1 (36, 2.8)	NS

NS: not significant; JoAS: juvenile onset ankylosing spondylitis; BASFI: Bath Ankylosing Spondylitis Functional Index; LBP: low back pain.

(92.7%) had the HLA-B27 allele. Low back pain or buttock pain was the most frequent onset symptom. In cases of peripheral joint involvement, the knee joint was the most commonly affected joint, followed by the ankle joint.

The clinical characteristics of the HLA-B27 positive and HLA-B27 negative AS patients are compared in Table II. HLA-B27 positive patients had a significantly younger age at symptom onset (by 6.0 years, p=0.02), more uveitis (p=0.001), and a higher frequency of hip joint involvement (p=0.001) than HLA-B27 negative patients. However, there were no differences in the frequency of peripheral enthesitis, peripheral arthritis, the Bath Ankylosing Spondylitis Functional Index (BASFI), or symptom onset between the two groups.

One hundred and forty-six (29.8%) AS patients were homozygous for HLA-B27 (Table III).

No significant association between HLA-B27 homozygosity or heterozygosity and a history of peripheral arthritis, acute anterior uveitis, age at onset, or BASFI was found.

Discussion

Some studies have demonstrated that HLA-B27 positive AS patients have an earlier disease onset in terms of age (10, 12) and are more likely to develop acute anterior uveitis (13, 14) than HLA-B27 negative patients. Consistent with these studies, we found a significant association between the presence of the HLA-B27 allele and an earlier age at symptom onset and higher frequency of uveitis. We also found that hip involvement is more frequent in HLA-B27 positive patients. Thus, the presence of the HLA-B27 antigen does affect the clinical manifestations of AS.

The authors of a study performed in Finland demonstrated that increased susceptibility to AS is associated with HLA-B27 homozygosity (10) and suggested several explanations, including: 1) the existence of an increased number of susceptibility alleles could increase the likelihood of developing the disease, 2) HLA-B27 homozygotes may be more likely to carry abnormal HLA-B27 molecules such as homodimers or misfolded proteins, and 3) HLA-B27 molecules might be expressed at higher levels in patients with AS than in healthy controls (15).

However, an alternative viewpoint is that homozygosity for the HLA-B27 allele and, consequently, the limited number of distinct HLA-B27 molecules present on the cell surface of antigen-presenting cells may hinder the likelihood of generating an immune response to antigens (16). Nevertheless, it has been suggested that HLA-B27 homozygosity results in increased susceptibility to AS due to the higher than expected frequencies (11.0-15.5%) of HLA-B27 homozygotes among patients with AS

Table III. Comparison of the clinical features in Korean AS patients heterozygous or homozygous for HLA-B27.

Clinical features	HLA-B27 +/+	HLA-B27 +/-
No.*, total (%)	146 (29.8)	308 (62.8)
Age*, mean ± SD (yrs.)	33.3 ± 9.4	33.0 ± 9.6
Disease duration*, mean ± SD (yrs.)	12.6 ± 8.1	12.9 ± 8.3
Male*, no. (%)	130 (89)	272 (83.3)
JoAS*, no. (%)	35 (24.1)	111 (36.5)
Peripheral enthesitis*, no. (%)	63 (44.7)	136 (45.3)
Peripheral arthritis*, no. (%)	69 (47.3)	160 (52.1)
Uveitis*, no. (%)	49 (33.6)	99 (32.1)
Hip involvement*, no. (%)	113 (80.7)	221 (74.4)
Shoulder involvement*, no. (%)	87 (62.1)	168 (60.4)
BASFI*, mean ± SD	1.86 ± 2.0	1.87 ± 2.1
Initial symptom		
LBP or buttock pain*, no. (%)	77 (52.7)	139 (45.1)
Hip joint pain*, no. (%)	24 (16.4)	50 (16.2)
Knee arthritis*, no. (%)	16 (11.0)	50 (16.2)
Achilles tendonitis*, no. (%)	9 (6.2)	27 (8.8)
Ankle arthritis*, no. (%)	8 (5.5)	15 (4.9)
Plantar fasciitis*, no. (%)	7 (4.8)	6 (1.9)

*not significant between the two groups; JoAS: juvenile onset ankylosing spondylitis; LBP: low back pain.

(4.0-4.2%) found in two studies (5, 10). In addition, 71 of 2,169 healthy Korean bone marrow donors (about 3% of the total control group) were found to carry the HLA-B27 allele (17). The expected frequency of HLA-B27 homozygosity in this study was only 0.87% based on the Hardy-Weinberg equation. The data presented in this study supports the conclusion that HLA-B27 homozygosity is associated with an increased risk of AS, as a high frequency of HLA-B27 homozygotes was detected among patients with AS (29.8%).

A greater frequency of peripheral arthritis was reported among homozygous patients (5), but this result has been contradicted by another study (4). Kvien *et al*. (18) suggested that HLA-B27 homozygosity is probably not responsible for disease onset in childhood. To investigate the clinical contribution of HLA-B27 homozygosity to AS, however, large sample sizes are required. We overcame this limitation in our study, and concluded that HLA-B27 homozygosity has no influence on the clinical manifestations of and functional disability in AS.

All the same, several points should be kept in mind when interpreting these results. First, wide linkage disequilibrium has been observed between HLA-B27 and many other HLA-DR alleles in HLA-B27 positive patients (19-21). Harjacek *et al*. reported that HLA-B*27/HLA-B*07 in combination with the D6S273-134 allele is associated with increased susceptibility to juvenile spondyloarthropathies (22). This was not examined in our study. Thus, additional investigation of the association between AS and other genetic markers would be helpful. Second, among the B27 alleles, B*2706 in southern Asia (23) and B*2709 in Sardinia (24) have been reported not to be associated with AS. Owing to the polymorphic nature of the HLA-B27 subtypes, it would have been desirable to analyze the clinical features according to the HLA-B27 subtype. However, all surveys of the HLA-B27 subtype in Korean AS patients have revealed the presence of only two types: B2705 and B2704 (25, 26). Third, the characteristic features of AS are the growth of new bone and the formation of syndesmophytes (27), possibly leading to ankylosis and spinal fusion. Because we did not assess spinal ankylosis, further radiographic studies are needed to evaluate the influence of HLA-B27 on bony ankylosis.

Nonetheless, in this large-scale study we compared a number of clinical variables in detail as a function of the presence and number of HLA-B27 alleles, and found a clear association between the development of AS and HLA-B27. However, the number of alleles does not appear to affect the clinical manifestations in Korean AS patients.

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Appendix 1

(**a**) Sequences of the HLA-B locus primers used in this study.

Primer mix no.	Orientation	Primer sequences 5' - 3'	Location
1	Sense	GCTACGTGGACGACACGCT	HLA-B exon 2
	Antisense	TCTCGGTAAGTCTGTGCCTT	HLA-B exon 2
2	Sense	ACACAGATCTGCAAGACCAAC	HLA-B exon 2
	Antisense	CCCCAGGTCGCAGCCG	HLA-B exon 3
3	Sense	ACACAGATCTGCAAGACCAAC	HLA-B exon 2
	Antisense	CCTTGCCGTCGTAGGCGTA	HLA-B exon 3
4	Sense	ACACAGATCTGCAAGACCAAC	HLA-B exon 2
	Antisense	TGTCCGCCGCGGTCCAG	HLA-B exon 3
5	Sense	GCGAGGGGACCGCAGGC	HLA-B intron1
	Antisense	GCGCAGGTTCCGCAGGC	HLA-B exon 2
6	Sense	CGCGAGTCCGAGGATGGC	HLA-B exon 2
	Antisense	CAGGTATCTGCGGAGCCA	HLA-B exon 3

(**b**) Alleles recognised by the primers.

Primer mix no.	Alleles recognized	PCR fragment length
1	B*2701-9	150
2	B*0703/8/16/27, *0801-08/10-16, *1310, *1405/6, *1501/3/5/7-12/14/15/18/19/23/24/28-31/33-35/37-40/43/45-58/60/61/63-66/68-73, *1801/3-15/17, *3505/12/16/17/22/30-32/39/43/44, *3801-9, *3901-8/10-20/22-27, *4001-3/5/7-16/18-27/29/31-33/35-40/42/43, *4102-4, *4402-6/8/9/11-14/16/17/19/21-27/29-32, *4801/3-7, *5004, *5106	369
3	B*0703/8/16/27, *0801-13/15/16, *1509/10/30/37/45/48/63, *3502/4/9/12/18/22/31/34/39/44, *3914, *4001/2/4-16/18/19/21-26/28-35/37/39/40/42-44, *4101-6, *4405/25/31, *4801/3-6, *5101-12/14/16-24/26-32, *5201-5, *7801-5	421
4	B*0801-5/7/9-16, *1301-4/6-10, *1401-6, *1501-15/18-21/23-40/42-58/60-66/68-73, *1801-15/17/18, *3501-17/19-30/32-44, *3701/3-5, *3801-9, *3901-20/22-27, *4014/26/28, *4101-6, *4402-30/32, *4501-5, *4802, *4901-3, *5001/2/4, *5101-9/11-24/26-32, *5201-5, *5301-9, *5901, *7801-5	469
5	B*0702-10/12-26/28-31, *0801/4/5/7-16, *1309, *1401-6, *1501-12/14/15/18-21/25-35/37-40/42/44-56/58/60-66/68-73, *1801-8/10-15/17/18, *3501-26/28-44, *3901-19/22-27, *4001-12/14-16/18/20-36/38-40/42-44, *4101-4/6, *4201/2/4, *4409, *4501-6, *4601/2, *4702/3, *4801-7, *5001/2/4, *5401/2, *5501-5/7-11, *5601-6/8-10, *6701/2, *7301, *7801-5, *8101, *8201/2, *8301	376
6	B*1301-4/6-10, *1501/2/4-8/11/13/15-17/20/21/24-28/30-36/39/40/42-45/48/50/55-58/60/63/65-67/70/71/73, *4021, *4601/2, *5701-6/8/9	643