

The LMP2 polymorphism is associated with susceptibility to acute anterior uveitis in HLA-B27 positive juvenile and adult Mexican subjects with ankylosing spondylitis

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Abstract

Introduction—An association between polymorphism of the HLA linked LMP2 locus and the development of acute anterior uveitis (AAU) has previously been described in B27 positive white subjects with ankylosing spondylitis (AS). This study evaluated LMP2 alleles in two HLA-B27 positive Mexican populations of patients with spondyloarthropathy known to have a different clinical spectrum of disease from white people.

Patients and Methods—The study populations consisted of 90 AS patients from Guadalajara with predominantly adult onset disease and 80 AS patients from Mexico City with predominantly juvenile onset disease. LMP2-CfoI amplified fragment length polymorphisms were determined after polymerase chain reaction amplification and digestion with CfoI restriction enzyme.

Results—There was an increased LMP2A allelic frequency in patients who had had AAU in both Guadalajara (31.8%) and Mexico City (33.3%) when compared with non-AAU patients (15.2% and 17.7% of Guadalajara and Mexico City populations, respectively). The odds ratio relating LMP2A allelic frequency and AAU for the combined population, stratified by age at onset of disease, was 2.51 ($p=0.01$). LMP2 alleles did not influence the age at onset of disease or the development of peripheral arthritis.

Conclusions—These data support the view that polymorphism at the LMP2 locus is associated with the development of AAU in B27 positive subjects with AS. The requirement for both the less common LMP2 allele and HLA-B27 is consistent with the low prevalence of AAU in Mexican patients with spondyloarthritis.

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Numerous findings derived from epidemiological studies and studies of families of patients with ankylosing spondylitis (AS) strongly implicate a role for genes additional to HLA-B27 in the pathogenesis of AS.^{1,2} Subsequent work has implicated a role for the HLA

Bw60 allele³ and polymorphism of the HLA linked proteasomal subunit LMP2 (low molecular weight polypeptide)⁴ gene.^{5,6} The proteasome constitutes a large cytoplasmic multicatalytic proteinase complex implicated in antigen processing for major histocompatibility complex (MHC) class I associated antigen presentation to cytotoxic T cells.⁷ The LMP2 and LMP7 subunits of this complex are HLA encoded, γ interferon inducible proteins,⁸ which influence the proteolytic specificity of the proteasome as demonstrated by transfection data⁹ and studies of LMP2 and LMP7 knockout mice.^{10,11} The LMP2 locus encodes 2 alleles, LMP2A and LMP2B, distinguished by an arginine for histidine amino acid substitution, respectively, at position 60 of the coding sequence.¹²

Evaluation of the LMP2 gene polymorphism in a white population of AS patients from Edmonton, Canada, has previously shown an association between LMP2B and the occurrence of acute anterior uveitis (AAU)^{5,6} while a study of AS patients from England was unable to confirm this finding.¹³ On the other hand, a large study of LMP2 gene polymorphism in patients with predominately juvenile onset AS from Norway has not only shown an association between LMP2B and the occurrence of AAU but also a primary association with disease unrelated to linkage disequilibrium with other HLA class I or class II alleles.¹⁴ In particular, the disease association with HLA-Bw60 was shown to be secondary to linkage disequilibrium with LMP2B. Possible reasons for these discrepancies include clinical ascertainment error in so far as AAU may occur at any time during the course of AS, and the probable presence among different ethnic groups of different aetiological agents whose antigenic processing is variably influenced by the incorporation of LMP2 into the proteasome.

AS in Mexican Mestizo populations differs from AS in a white population in that juvenile onset of disease is far more common as is presentation with a characteristic enthesopathy and severe tarsal involvement.¹⁵⁻¹⁷ AAU, on the other hand, is far less common than AS in a white population.¹⁵ In view of data derived from Norwegian AS patients showing a preferential LMP2 gene disease association with

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Table 1 Spondyloarthropathy patient details of two populations of HLA-B27 positive Mexican Mestizo from Guadalajara and Mexico City

Patients	M:F	Average age (range)	Age at onset (range)
Guadalajara			
Total patients	68:22	34.4 (16–59)	23.4 (6–46)
AAU+	9:2	40.9 (21–54)	24.7 (6–46)
AAU–	59:20	33.5 (16–59)	23.8 (6–46)
PA+	45:13	32.7 (16–56)	20.6 (6–46)
PA–	23:9	37.7 (21–59)	27.4 (12–46)
Mexico City			
Total patients*	70:10	24.1 (11–55)	16.1 (5–52)
AAU+	14:1	23.2 (15–44)	15.6 (8–27)
AAU–	56:9	24.3 (11–55)	16.1 (5–52)
Undifferentiated spondyloarthropathy	17:3	22.9 (14–55)	16.9 (8–52)

AAU = acute anterior uveitis, PA = peripheral arthritis. * 93.8% of Mexico City patients had peripheral arthritis.

juvenile compared with adult onset disease, we have explored LMP2 gene disease associations in both juvenile and adult AS in a Mexican population in the hope that the findings may provide some clarification as to whether the primary influence of the LMP2 gene centres on the development of AAU or predisposes to a juvenile onset of disease. In a preliminary study of one Mexican population with AS, we were unable to discern any effect of LMP2 polymorphism on the phenotype of disease, although this study lacked sufficient numbers of AAU patients to arrive at any definitive conclusions.¹⁸ In this study, we have studied LMP2 gene polymorphism in two geographically distinct populations of Mexican Mestizo people with AS, one with predominantly juvenile onset and the other with predominantly adult onset disease, who have been well characterised clinically.

Methods

PATIENTS

AS was defined according to the modified New York criteria.¹⁹ Table 1 summarises patient details from two populations of Mexican Mestizo selected for HLA-B27 positivity. The Guadalajara study population consisted of 90 HLA-B27 positive Mexican Mestizo subjects with AS. Seventeen (18.9%) of these had juvenile onset disease (that is, under 16 years of age). Eleven (12.2%) patients had had AAU while 58 (64.4%) had peripheral arthritis. The second population consisted of 80 HLA-B27 positive Mexican Mestizo subjects with AS from Mexico City. Fifty two (65.0%) had juvenile onset disease (under 16 years of age). Fifteen (18.8%) had had AAU and 75 (93.8%) had peripheral arthritis. This Mexico City

Table 2 LMP2CfoI genotypes in HLA-B27 positive Mexican Mestizo spondyloarthropathy patients from Guadalajara and Mexico City

	LMP2-CfoI genotype		
	AA (%)	AB (%)	BB (%)
Guadalajara			
Total patients	4 (4.4)	23 (25.6)	63 (70.0)
AS/AAU+	2 (18.2)	3 (27.3)	6 (54.5)
Juvenile onset	0 (0)	4 (23.6)	13 (76.4)
AS/PA+	3 (5.2)	14 (24.1)	41 (70.7)
Mexico City			
Total patients	4 (5.0)	25 (31.2)	51 (63.8)
AS/AAU+	2 (13.3)	6 (40.0)	7 (46.7)
Juvenile onset	2 (3.8)	18 (34.6)	32 (61.6)
Undifferentiated spondyloarthropathy	1 (5.0)	7 (35.0)	12 (60.0)

population also included 13 patients with juvenile onset (under 16 years) undifferentiated spondyloarthropathy (or seronegative enthesopathy and arthropathy (SEA) syndrome)²⁰ and seven patients with adult onset undifferentiated spondyloarthropathy. Two patients with SEA syndrome had had AAU. AAU was confirmed by a complete ophthalmology assessment in both patient groups while peripheral arthritis was defined as arthritis occurring outside the axial skeleton—that is, excluding the shoulders and hips—and confirmed by a rheumatologist. Sacroiliitis was confirmed by the presence of a pelvic x ray or computed tomography, or both. None of the patients had psoriasis or inflammatory bowel disease.

DNA EXTRACTION

Genomic DNA was extracted from sodium EDTA anticoagulated blood using a modified salt precipitation method.²¹

LMP2 GENE POLYMORPHISM

LMP2 genotype assignments were made after polymerase chain reaction amplification of the second exon of LMP2 from genomic DNA followed by restriction enzyme digestion with CfoI as described previously.⁶ LMP2 genotype assignments were made before knowledge of the clinical history of each patient.

HLA TYPING

HLA-B27 typing was performed in the Department of Immunogenetics, INDRE, Mexico City, using standard microcytotoxicity assays and typing serum. Where this was not available (for the Guadalajara population), molecular typing for HLA-B27 was performed as described previously.²²

STATISTICAL ANALYSIS

This was conducted mainly by stratified exact 2 × 2 and 2 × 3 table analysis. The predictor variable, the LMP2 allele, was categorised in two ways: first, the frequency and second, the prevalence of the LMP2A allele. The other predictor variable was population (Mexico City *v* Guadalajara). To examine whether the LMP2 polymorphism primarily influences the development of AAU or predisposes to juvenile onset disease, we first considered age at onset as an outcome variable and tested LMP2A and B alleles, population (Mexico City *v* Guadalajara), and development of AAU as predictor variables. Subsequently, development of AAU was considered as an outcome variable and LMP2A and B alleles, age at onset, and population as predictor variables. The EGRET epidemiological software was used to derive conditional maximum likelihood estimates (MLE) for the variable of interest. The stratified exact odds ratios (OR) are given together with 95% confidence intervals (CI).

Results

Table 2 illustrates the distribution of LMP2 genotypes in B27 positive subjects from Guadalajara and Mexico City subdivided according to presence or absence of AAU, peripheral

Table 3 LMP2CfoI genotypes in Mexican AS patients stratified by age at onset and history of AAU

	LMP2-CfoI genotype		
	AA (%)	AB (%)	BB (%)
Guadalajara*			
Juveniles			
AAU+	0	1 (50)	1 (50)
AAU-	0	3 (20)	12 (80)
Adults			
AAU+	2 (22.2)	2 (22.2)	5 (55.6)
AAU-	2 (3.1)	17 (26.6)	45 (70.3)
Mexico City†			
Juveniles			
AAU+	2 (16.7)	4 (33.3)	6 (50)
AAU-	0	14 (35)	26 (65)
Adults			
AAU+	0	2 (66.7)	1 (33.3)
AAU-	2 (8.0)	5 (20)	18 (72)

* Stratified exact OR = 2.12 (95% CI, 0.46, 9.24; $p = 0.40$).
 † Stratified exact OR = 2.27 (95% CI, 0.62, 8.54; $p = 0.25$).
 Combined population stratified exact OR = 2.30 (95% CI, 0.90, 5.91; $p = 0.087$). OR = The exact conditional maximum likelihood estimates, stratified by age at onset of disease, for the odds ratio relating LMP2A allelic frequency and history of AAU.

Table 4 LMP2CfoI allelic frequencies in Mexican Mestizo with AS stratified by age at onset and history of AAU

	LMP2-CfoI allele	
	A (%)	B (%)
Guadalajara*		
Juveniles		
AAU+	1 (25)	3 (75)
AAU-	3 (10)	27 (90)
Adults		
AAU+	6 (33.3)	12 (66.7)
AAU-	21 (16.4)	107 (83.6)
Mexico City†		
Juveniles		
AAU+	8 (33.3)	16 (66.7)
AAU-	14 (17.5)	66 (82.5)
Adults		
AAU+	2 (33.3)	4 (66.7)
AAU-	9 (18)	41 (82)

* Stratified exact OR = 2.58 (95% CI, 0.80, 7.62; $p = 0.12$).
 † Stratified exact OR = 2.31 (95% CI, 0.84, 6.12; $p = 0.11$).
 Combined population stratified exact OR = 2.51 (95% CI, 1.21, 5.09; $p = 0.013$). OR = The exact conditional maximum likelihood estimates, stratified by age at onset of disease, for the odds ratio relating LMP2A allelic frequency and history of AAU.

arthritis, and juvenile onset disease (under 16 years). The distribution of LMP2 genotypes in both populations of AS patients was similar. The prevalence of the LMP2 BB genotype was (70%) and (63.8%) in the Guadalajara and Mexico City populations, respectively.

Table 3 shows the LMP2 genotypes stratified according to age at onset and history of AAU. An increased prevalence of the LMP2A allele was noted in AAU positive versus AAU negative AS patients in both populations. Five of 11 AAU positive Guadalajara patients carried the LMP2A allele (45.4%) compared with 22 of 79 AAU negative patients (27.8%). Similarly, eight of 15 AAU positive Mexico City patients carried the LMP2A allele (53.3%) compared with 21 of 65 AAU negative patients (32.3%). The exact stratified by age at onset of disease conditional MLE for the odds ratios relating AAU and the prevalence of LMP2 A are provided in table 3. Exact odds ratios, stratified by history of AAU, with juvenile onset disease as the primary outcome showed no significant associations with LMP2A allelic prevalence (OR (Guadalajara)

= 0.67, OR (Mexico City) = 1.19; OR (combined) = 1.07; $p > 0.05$ for all analyses).

We have also analysed the prevalence of the LMP2 AA and LMP2AB genotypes and shown a significantly increased prevalence of the LMP2 AA genotype in AAU patients. The prevalence of LMP2 AA was 15.4% in AAU patients (4 of 26) versus 2.8% in non-AAU patients (4 of 144), while LMP2 AB was present in 34.6% (9 of 26) and 27.1% (39 of 144) of AAU and non-AAU patients, respectively. The exact OR stratified by age at onset relating AAU with the LMP2 AA and LMP2 AB genotypes were 9.03 (95% CI, 1.42, 60.08; $p = 0.02$) and 1.72 (95% CI, 0.6, 4.78; $p = 0.36$) respectively for the combined population. The few patients carrying genotype AA did not permit analysis for each city separately when stratified by age at onset of disease (table 3).

Table 4 shows that there was an increased allelic frequency for the LMP2A allele in AS patients who had had AAU compared with non-AAU patients in both Guadalajara and Mexico City. LMP2A allelic frequency in Guadalajara patients was 31.8% (7 of 22 alleles) in AAU positive subjects compared with 15.2% (24 of 158 alleles) in AAU negative subjects. LMP2A allelic frequency in Mexico City patients was 33.3% (10 of 30 alleles) in AAU positive subjects compared with 17.7% (23 of 130 alleles) in AAU negative subjects. The exact OR stratified by age at onset of disease are shown in table 4. In neither population was LMP2A allelic frequency predictive of the development of juvenile onset disease when stratified by previous history of AAU (OR(Guadalajara) = 0.59; OR (Mexico City) = 0.97; OR (combined population) = 0.91; $p > 0.05$ for all analyses).

The exact OR indicated no significant influence of either age at onset or disease population on the prevalence of AAU (data not shown). Within group comparisons of patients with and without peripheral arthritis showed no significant differences in the distribution of LMP2 genotypes or allelic frequencies ($p > 0.05$).

Discussion

Our study of two Mexican populations with spondyloarthropathy has provided consistent data to implicate polymorphism of the LMP2 locus in predisposition to AAU in HLA-B27 positive AS patients. LMP2 alleles did not seem to influence either age at onset or the development of peripheral arthritis.

At first hand, these findings seem to be at odds with previous studies in white populations of AS patients where the LMP2B rather than the LMP2A allele seems to predispose to AAU or juvenile onset disease, or both.^{5,6,14} One explanation for these differences may reflect the distinct clinical background of spondyloarthritis in the Mexican Mestizo population. In addition to a much lower prevalence of AAU during the course of disease, juvenile onset is more common particularly in the setting of erosive disease, enthesopathy, and tarsal involvement compared with adult

onset disease in white populations. Both our populations exemplified these clinical differences showing a low prevalence of AAU (12.2% and 18.8% in the Guadalajara/Mexico City populations, respectively) and a high prevalence of peripheral arthritis (64.4% and 93.8% of the Guadalajara/Mexico City populations, respectively). Patients with SEA were included with AS patients in the Mexico City cohort as follow up studies have shown that most patients presenting with SEA ultimately develop the more typical features of AS.²³

Although the explanation(s) accounting for this difference in the clinical spectrum of disease remains speculative, one might propose that different environmental agents (for example, bacteria) or immunopathogenetic mechanisms, or both, are responsible. Firstly, one might propose that the LMP2A allele influences the antigenic processing of a protein derived from one or more bacteria endemic to the Mexican population while the LMP2B allele conversely influences the processing of an endemic antigen to which white populations are exposed. Processing of a B27 binding motif that cross reacts with a uveitogenic cryptic self peptide(s) may stimulate autoreactive T cells demonstrating specificity for antigens in the anterior uvea. Secondly, recently described disease associations with other HLA alleles in Mexicans with spondyloarthropathy (for example, HLA-B49 and the FC31 clone-type)²⁴ distinct from those seen in white populations (for example, HLA-Bw60) reinforces the concept of a distinct, multifactorial aetiology for this disease in different ethnic groups. Thus, competition between HLA-B alleles for binding of potential arthritogenic or uveitogenic peptides, or both, might influence the priming of autoreactive CTL.²⁵ It seems unlikely, however, that differences in B27 subtypes from those seen in white patients with AS can account for differences in disease phenotype as B27 subtyping in the Mexico City population (data not shown) as well as previous work²⁶ has shown that the predominant subtypes related to Mexican AS, B2702 and B2705, have the same prevalence as that seen in other AS populations. Further development of this hypothesis will require functional studies of LMP2 polymorphism in the context of potentially disease relevant antigens.

Our data do not preclude the possibility that the association of LMP2 polymorphism with predisposition to AAU simply reflects another disease locus in the HLA region on an extended HLA-B27 haplotype or on the non-B27 chromosome. Previous work in AS families raises the possibility of a second AS susceptibility gene on an extended B27/DR1 haplotype²⁷ while other work has implicated HLA-Bw60.^{3,28} Consequently, the differing LMP2 allelic associations with disease in different populations may reflect differences in population prevalence of distinct disease associated HLA haplotypes.

The association between the presence of an LMP2A allele and predisposition to AAU is

also consistent with the low prevalence of AAU in Mexican populations with spondyloarthritis. Thus, the A allele is much less common than the B allele in Mexican patients with spondyloarthritis (18.8% *v* 81.2%) while homozygosity for the A allele was seen in only 4.4% and 5.0% of the Guadalajara/Mexico City AS populations, respectively. In contrast, 50% of all LMP2 AA homozygotes had had AAU. Consequently, one explanation for the low prevalence of AAU in Mexican AS might be the requirement for both an LMP2A allele and HLA B27 in the processing and presentation of a disease relevant antigen endemic to Mexico that is associated with the development of AAU.

In conclusion, our findings in two Mexican populations of B27 positive patients with juvenile and adult onset AS support the view that polymorphism of the LMP2 gene is associated with the development of AAU rather than age at onset. Our results also suggest potential immunopathological mechanisms that might account for different clinical presentations of spondyloarthropathy in different parts of the world.

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