HLA-B27 and its subtypes in Syrian patients with ankylosing spondylitis

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ABSTRACT

الأهداف: دراسة دور الجين HLA-B*27، ومدى ارتباط أنماطه الفرعية بمرض التهاب الفقار التصلبي لدى المرضى السوريين المصابين به.

الطريقة: أُجريت هذه الدراسة في مختبر الأبحاث والاستشارات الجينية بكلية الطب، جامعة دمشق، دمشق، سوريا وذلك خلال الفترة من ديسمبر 2006م إلى ديسمبر 2007م. شملت الدراسة 50 مريضاً مصاباً بالتهاب الفقار التصلبي ممن تتماشى صفاتهم مع معايير نيويورك لتصنيف وتشخيص هذا المرض (مجموعة المرضى)، بالإضافة إلى 217 من المشاركين الأصحاء (مجموعة الشاهد). لقد تم جمع المشاركين في الدراسة من قسم العيادات الخارجية في مستشفى الأسد، دمشق، سوريا. وتمت الاستعانة بتقنية التفاعل التسلسلي المبلمر من أجل دراسة التعدد الشكلي الحيوي للجين HLA-B*.

النتائج: أشارت نتائج الدراسة إلى ظهور الجين HLA-B*27 في 1.4% من المشاركين في مجموعة الشاهد، و60% من المرضى في مجموعة المرضى المصابين بالتهاب الفقار التصلبي (OR=107, p=0.0001, corrected p=0.003). وكان النمط B*2705 من أكثر أنماط هذا الجين شيوعاً حيث بلغت نسبة ظهوره في المرضى المصابين بالمرض 67%، وتبعه النمط B*2702 الذي وصلت نسبة ظهوره 20%. ولقد تم اكتشاف الجين HLA-B*27 لدى جميع الحالات التي رافقها الإصابة بمرض التهاب العنبية، والتهاب المفاصل المحيطي، ومن لديهم تاريخ أسري بأمراض الفقار.

خاتمة: أظهرت الدراسة بأن نسبة ظهور الجين HLA-B*27 قليلة بين المرضى السوريين المصابين بمرض التهاب الفقار التصلبي مقارنةً بالشعوب الأخرى، وبالرغم من ذلك فإن هناك علاقة قوية بين هذا الجين وزيادة خطر الإصابة بالتهاب الفقار التصلبي. قد يكون لنتائج دراستنا أهمية كبيرة عند دعمها بالدراسات الأخرى الموسعة، وخصوصاً في تشخيص الحالات التي تبدأ أعراضها السريرية بالتهاب العنبية أو التهاب المفاصل المحيطي وقبل اكتمال معايير نيويورك لتشخيص هذا المرض.

Objectives: To assess HLA-B*27 and its subtypes associated with ankylosing spondylitis (AS) in Syrian patients.

Methods: A polymerase chain reaction with specific sequence primer method was used to study the HLA-B* locus polymorphism in 50 Syrian patients fulfilling the modified New York criteria for classification of ankylosing spondylitis and 217 unrelated healthy Syrian controls. Patients were recruited from the Outpatients Department, Alassad University Hospital, Damascus, Syria between December 2006 and December 2007. The study took place at the Laboratory for Research and Genetic Consultations, Faculty of Medicine, Damascus University, Damascus Syria.

Results: HLA-B*27 allele was found in 1.4% healthy Syrians and 60% in patients with AS (OR=107, p=0.0001, corrected p=0.003). The most common HLA-B*27 variants in patients were B*2705, which was found in 67% of patients, followed by B*2702 found in 20% of patients. HLA-B*27 was identified in all cases with uveitis, peripheral arthritis, and positive family history.

Conclusion: Although HLA-B*27 allele frequency in this group of Syrian patients with AS is lower compared to the noted AS patients in many populations, its association with the disease risk in our population seems to be the strongest one. If confirmed by larger study, this finding may be of great interest, particularly for diagnosis at disease onset where some clinical features as uveitis and peripheral arthritis may precede the fulfilling of all standard criteria for AS diagnosis.

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Ankylosing spondylitis (AS) is a chronic inflammatory disease affecting mainly the sacroiliac joints, spine, but also enthesis, peripheral joints, and less commonly extra-articular organs. One of the New York criteria for AS diagnosis is a radiographic changes of the sacroiliac joints, which may take years to develop after the disease onset, this often leads to delayed diagnosis,[1,2] and many patients undergo unnecessary investigations and sometimes undergo unnecessary surgery. Substantial evidence indicates that AS arise as a consequence of abnormal immune response, which may be triggered by a complex of genetic and environmental factors.[3] Analysis of twin data indicates a predominant role of the genetic component in the occurrence of the diseases, while the environmental contributes only in small part and seems to be ubiquitous.[3,4] HLA-B*27 allele has been unequivocally implicated in the genetic susceptibility of AS.[3-8] Its direct role in the disease pathogenesis has been confirmed by transgenic rats model, where animals expressing HLA-B27 and beta-2-microglobulin develop a disease that shares many similarities with human AS.[9] Besides, AS prevalence parallels HLA-B27 frequency in different populations.[6] It has been estimated that it accounts for 20-50% of AS genetic risk.[3,6] Although HLA-B*27 appeared as the strongest risk factor for AS, its contribution to the overall genetic risk varies between different populations. HLA-B*27 frequency in AS patients is over 90% in Caucasians with possible dose effect.[3,6] Studies of AS patients groups from middle east populations showed often lower HLA-B*27 frequency: 80% in Greeks, 70% in Turkish,[10] 73% in Iranians,[11] 87% in Kuwaitis, 75% of South Asians living in Kuwait,[12] 75% in patients from north Jordan,[13] 42.9% in Tunisians,[14] and 25.7% in Lebanese patients with AS.[15] HLA-B*27 allele encompasses a family of subtypes, that encode different products sharing a serologic specificity named B27 antigen. There are at least 38 members of this family designated HLA-B*2701 through HLA-B*2738.[16,17] These variants are thought to have evolved from the most widespread subtype B*2705, their distribution and association to the AS risk differs across populations.[18-20] In Syria, there was no previous study that used molecular methods to define an accurate prevalence of HLA-B*27 in AS patients and healthy individual, or the distribution of HLA-B*27 subtype in patients; hence, we planned this study to unravel the role of HLA-B*27 allele and its subtypes in Syrian patients with AS as well as its contribution to uveitis and peripheral arthritis that may precede the spinal manifestations.

Methods. Fifty consecutive adult patients with AS and 217 unrelated healthy individuals were included in the study. Diagnosis of AS was established according to the modified New York criteria for classification of AS.[21] Patients were recruited in the study between December 2006 and December 2007, from those followed at the outpatient department of Alassad University Hospital, Damascus, Syria. All patients have chronic inflammatory low back pain and x-ray evidence of bilateral sacroiliitis (grade 2 at least), none of the patients had symptoms or signs suggesting psoriasis, inflammatory bowel disease, Behcet disease, Familial Mediterranean Fever, or reactive arthritis. Family history was considered positive for spondylitis when spondyloarthropathy was confirmed in at least one of the relative. Only one patient from each family was included. Healthy individuals were recruited from the medical students, university employees, and blood donors. Their ages were between 20-60 years (65% males and 35% females). The study was approved by the University Research Committees and verbal consent was obtained from all patients and controls.

Patients were excluded from the study if they had rheumatic or other kind of symptoms suggesting presence of a serious disease and familial history of rheumatic or any other disease. HLA typing and allele frequency was carried out at the Laboratory for Research and Genetic Consultations, Faculty of Medicine, Damascus University, Damascus, Syria. Using salting out method, genomic DNA was extracted from 5ml peripheral blood of each one of the studied population. The polymorphic Exon 2 of HLA-B* locus was amplified using polymerase chain reaction with sequence specific primer (PCR-SSP) according to the method of Olerup et al.[22]

Statistical analysis was carried out using Chi square or student T test where appropriate. Using Woolf approximation, the confident interval (CI) of the calculated odds ratio (OR) was estimated by approximate 95%. Results were considered significant when the corrected *p*-value was less than 0.05 (corrected *p*-value was calculated by multiplying the *p*-value by the number of alleles found).

Results. The demographic characteristics of 50 Syrian patients with AS are shown in **Table 1**. Distribution of HLA-B* alleles in both AS patients and healthy control is shown in **Table 2**. We found 28 alleles in all individuals included in the study. The only allele presenting a significant frequency difference between patients and healthy individuals was HLA-B*27, which was found in 30/50 (60%) of patients, and 3/217 (1.4%) in health individuals. Although HLA-B*40 allele was higher in the patients group (10% in patients, 5% in healthy individuals), no significant risk could be attributed to this allele. On the other hand, frequency of HLA-B*18 (4% in patients versus 12% in healthy individuals), HLA-B*35 (18% in patients versus 36% in healthy individuals), and HLA-B*38

Table 1 - Demographic characteristics and clinical features in ankylosing spondylitis (AS) patients (N=50).

Characteristics	n (%)
Female to male ratio	4:1
Age at disease onset (years)	
Mean	22.3
Range	9-44
Disease duration (years)	
Mean	9.6
Range	1-35
Peripheral arthritis frequency	6 (12)
Acute anterior uveitis frequency	5 (10)
Frequency of spondylarthritis family history	8 (16)

Table 2 - Frequency of HLA-B* alleles in AS patients and controls.

Allele	Frequency in controls n=(217)		Frequency in patients n=(50)		Odds ratio	*P*-value	*Corrected *P*-value
	n	(%)	n	(%)			
B*05	0		2	(4)			
B*7	11	(5.0)	3	(6)			
B*8	25	(12.0)	1	(2)			
B*13	17	(8.0)	4	(8)			
B*14	11	(5.0)	2	(4)			
B*15	16	(7.0)	3	(6)			
B*18	25	(12.0)	2	(4)	0.3	NS	
B*27	3	(1.4)	30	(60)	107	0.0001	0.003
B*35	79	(36.0)	9	(18)	0.2	0.004	>0.05
B*37	3	(1.4)					
B*38	29	(13.0)	1	(2)	0.15	0.04	>0.05
B*39	5	(2.0)	0				
B*40	12	(5.5)	5	(10)	2.2	NS	
B*41	16	(7.0)	0				
B*42	3	(1.4)					
B*44	21	(10.0)	6	(12)			
B*45	11	(5.0)	1	(2)			
B*49	18	(8.0)	2	(4)			
B*50	30	(14.0)	4	(8)			
B*51	33	(15.0)	5	(10)			
B*52	16	(7.0)	4	(2)			
B*53	5	(2.0)	0				
B*54	2	(1.0)					
B*55	5	(2.0)	1	(2)			
B*57	6	(3.0)	0				
B*58	6	(3.0)	0				
B*73	2	(1.0)	0				
B*78	0		1	(2)			

Note: Bold type indicates significant association, NS - Not Significant, AS - ankylosing spondylitis. *Corrected p was calculated by multiplying *p* value by number of alleles found (28 alleles)

(2% in patients versus 13% in healthy individuals) alleles in patients was lower than that observed in the healthy individuals, however, no significant protective effect correlated to any of them. In fact, the *p*-value for HLA-B*35 and B*38 alleles, became insignificant when normalized by the number of the found alleles. No clear difference in distribution was observed for other alleles. The comparison between HLA-B*27 positive patients and HLA-B*27 negative patients regarding uveitis, peripheral arthritis, age at disease onset, and presence of family history of sponyloarthropathy, is shown in **Table 3**. HLA-B*27 allele was represented by all patients with anterior uveitis, peripheral arthritis, and family history of sponyloarthropathy, in contrast, these clinical features have not observed in any of HLA-B*27 negative patients. As shown in **Table 3**, a significant HLA-B*27 correlation with the family history and the peripheral arthritis was demonstrated by student t test analysis. Concerning the age of patients at disease onset, no obvious difference was observed between the patients within HLA-B*27 and those without HLA-B*27 (mean age at onset disease was 22 years for HLA-B*27 positive patients, and 27 years for HLA-B*27 negative patients).

Table 3 - Clinical features in ankylosing spondylitis (AS) patients according to presence or absence of HLA-B*27.

Variables	Patiens with B*27 (n=30)	Patients without B*27 (N=20)	*P*-value
Acute anterior uveitis frequency	5 (17)	0	0.07
Peripheral arthritis frequency	6 (20)	0	0.03†
Family history	8 (27)	0	0.03†
Age at onset (years) Mean ± SD	22 ± 5	27 ± 6.2	
Range (years)	12 - 46	9 - 40	

†significant. Student t test was used to analyze frequency difference of each variable between HLA-B*27 positive and HLA-B*207 negative patients

Table 4 - HLA-B*27 subtypes frequency in patients and controls.

HLA-B*27 subtype	Frequency in HA-B*27 positive control n=3	Frequency in HLA-B*27 positive patients n=30
B*2702	0	6 (20.0)
B*2705	3 (100)	20 (67.0)
B*2707	0	2 (6.6)
B*2713	0	2 (6.6)

No statistical analysis was carried out for any of HLA-B*27 subtype distribution in patients and controls because of the very low number of individuals with HLA-B*27 in the healthy group

Distribution of HLA-B*27 variants in patients and healthy control is shown in **Table 4**. HLA-B*2705 variant was the most prevalent in our patients (67%), followed by B*2702 (20%), whereas HLA-B*2707, and B*2713 were each seen in 2 patients (6.6%). Concerning the healthy group, all 3 HLA-B*27 found were represented by HLA-B*2705 variant. Unfortunately, to verify if the HLA-B*27 risk is raised from particular subtypes, no statistical comparison could be realized for B*27 variants distribution between the patients and the healthy group, because of the limited number of individuals within HLA-B*27 in the healthy group (n=3).

Discussion. The allelic variation of HLA-B* locus in 217 healthy individuals and 50 Syrian patients with AS was assessed. HLA-B*27 allele prevalence in the healthy Syrian population in this study was lower than that noted in Caucasians and resembles that reported by some studies in other Arabic countries such as Lebanon, Oman, and Emirates.[15,23,24] Similarly, HLA-B*27 prevalence in our patients was lower than that documented in some areas in the Middle Eastern and European populatios,[3-6] However, it seems to confer a higher AS risk in our population, since the highest reported odd ratio was 100[7] whereas the OR in our study reached up to 107.

Analysis of overall HLA-B* locus polymorphism showed some differences in allelic distribution between our 2 study groups (**Table 2**); HLA-B*40 was increased, in contrast of HLA-B*18, HLA-B*35 and HLA-B*38, which were decreased in patients compared to controls. However, because of the limited size of the studied group, no significance could be attributed to these frequency differences. We should point out, that HLA-B*40 encodes B60 serologic specificity, which was associated with AS risk in HLA-B*27 negative patients and increased the risk in HLA-B*27 positive patients in both Europe and Taiwan, and that HLA-B*35, was noted as other risk allele.[5,6,25]

We identified 4 HLA-B*27 variants in our studied groups. In fact, we could not accurately define the contribution of each one of the identified HLA-B*27 subtype to AS risk, because of the very low B*27 frequency in the healthy group. However, the 2 prevalent variants in our patients HLA–B*2705 and B*2702 are the most common subtypes worldwide and show very strong association with AS.[17-19] HLA-B*2707, which was seen in 2 of our patients is not always associated with AS,[26] and it has been considered as a protective allele in a Greek population.[27] Finally, B*2713 was also observed in 2 of our patients, it has been reported in other studies between other AS associated subtypes.[28] HLA-B*27 allele was represented in all our patients with AS family history, acute anterior uveitis and peripheral arthritis. This may suggest a possible role of this allele in the occurrence of those clinical features. HLA-B*27 Homozygosis was detected only in 3 male patients (data not shown) so no conclusions can be drawn regarding this point. The relatively lower HLA-B*27 prevalence in Syrian patients with AS raises the question about the involvement of other genes and/or a common infectious agent. The role of other genetic factors in AS susceptibility is suggested by the fact that only 5-8% of HLA-B27 positive individuals develop AS.[3,6] Many non-MHC investigated genes demonstrated association with AS such tumor necrosis factor (TNF), IL-1 cluster, aminopeptidase regulator of TNFR1 shedding (ARTS1), and IL-23 receptor.[3,4,29]

Despite the limited size of our study, it provides interesting information on the HLA-B*27 association with AS in a sample of Syrian patients. The low B*27 prevalence in the healthy group, coupled with the remarked strong AS risk demonstrated by our results, if proved by larger studies may have implications on the use of HLA-B*27 in the diagnosis of early and difficult cases, and cases where peripheral joint affection or uveitis may precede the spinal symptoms, since HLA typing in our country is cheaper than MRI of the sacroiliac joints, which was included in the recently validated AS classification criteria for axial arthropathy,[30] and many experts had justified its use.[31]

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Related topics

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