Susceptibility to and severity of tuberculosis is genetically controlled by human leukocyte antigens

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ABSTRACT

الأهداف: لكشف النقاب عن دور التعدد الشكلي لمنظومة الهلا HLA في الاستعداد الوراثي للإصابة بداء السل في سوريا.

الطريقة: اعتمدنا تقنية تفاعل السلسلة العديد PCR–SSP، باستخدام البادئات النوعية لألائل الموقع در وذلك من أجل تعيين الأليل در DRB1* في هذا الموقع لدى مجموعتي المرضى والأصحاء. تضمنت مجموعة المرضى 147 مريضاً بداء سل الرئوي تم اختيارهم بشكل عشوائي من مراجعي المركز الوطني لمكافحة السل، خلال الفترة مابين 2005م وحتى 2007م، وذلك بناء على إيجابية الفحص المباشر أو إيجابية زرع القشع بحثاً عن عصية السل. أما مجموعة الأصحاء فتضمنت 209 فرداً من الأصحاء الذين تم اختيارهم بناء على سلبية فحص السلين الجلدي لديهم. تمت الدراسة في مختبر البحوث والاستشارات الوراثية – كلية الطب البشري – جامعة دمشق – سوريا.

النتائج: أظهرت نتائج الدراسة انخفاضاً هاماً في نسبة الأليل در DRB1*11 في مجموعة المرضى مقارنة بمجموعة الأصحاء، (34.7% في مجموعة المرضى) (51% في مجموعة الأصحاء)، OR=0.51 p=0.003 القيمة المصححة p=0.04. بينما لوحظت زيادة في نسبة الأليل در DRB1*04 في مجموعة المرضى قياساً بمجموعة الأصحاء (38.8% في مجموعة المرضى) (26.4% في مجموعة الأصحاء)، p=0.01، OR=1.77 القيمة المصححة p>0.05. هذه الزيادة تصبح هامة عندما نستبعد الأشخاص الذين يحملون الأليل در DRB1*11 من مجموعتي المرضى والأصحاء (33% في مجموعة المرضى غير الحاملين للأليل در) مقابل (17% في مجموعة الأصحاء الغير الحاملين للأليل در)، p=0.003، OR=2.5، القيمة المصححة p=0.03. فضلا عن ذلك، لوحظت زيادة هامة في نسبة التكهف الرئوي في مجموعة المرضى الذي يحملون الأليل در DRB1*04 قياساً للمرضى الذين لا يحملون هذا الأليل (33% في المرضى الذين يحملون الأليل در) (16% في المرضى الذين لا يحملون الأليل در) p=0.04، OR=2.7.

خاتمة: يمكننا أن نستنتج مما سبق أن الأليل در(DRB1*04) يؤهل وراثيا للإصابة بداء السل بينما الأليل در(DRB1*11) يساعد على الوقاية من هذا الداء في الشعب السوري. إضافة إلى ذلك، يبدو أن الأليل در(DRB1*04) يساعد على تشكل الكهوف الرئوية في سياق الإصابة بالسل الرئوي.

Objective: To assess the role of HLA polymorphism in the susceptibility to tuberculosis in Syria.

Methods: We used the polymerase chain reaction with sequence specific primer method to study the DRB1* locus in 147 Syrian patients with positive sputum smear or sputum culture for *Mycobacterium Tuberculosis* strains, and 209 Syrian healthy matching individuals with negative tuberculin skin test. Patients were randomly recruited from the Damascus Health Center of Tuberculosis and Pulmonary Diseases during 2005-2007. The study was carried out at the Laboratory for Research and Genetic Consultations, in the Faculty of Medicine of Damascus University, Damascus, Syria.

Results: A significant decrease of the DRB1*11 allele was observed in patients compared to controls (34.7% in patients versus 51% in control, odds ratio [OR]=0.51, p=0.003, corrected p=0.04), whereas the DRB1*04 allele was increased in patients (38.8% in patients versus 26.4% in controls, OR=1.77, p=0.01, corrected p>0.05). This increase became significant when individuals with the DRB1*11 allele were removed from both patients and controls (33% in DRB1*11 negative patients versus 17% in DRB1*11 negative controls, OR=2.5, p=0.003, corrected p=0.03). In addition, pulmonary cavitation was significantly increased in the DRB1*04 positive patients compared to patients without the DRB1*04 allele (33% in DRB1*04 positive patients versus 16% in DRB1*04 negative patients, OR=2.7, p=0.04).

Conclusions: The DRB1*04 allele is associated with susceptibility to pulmonary tuberculosis, whereas DRB1*11 is associated with protection from pulmonary tuberculosis in the Syrian population. In addition, cavity formation in patients with pulmonary tuberculosis seems to be favored by presence of the DRB1*04 allele.

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Tuberculosis (TB) is a serious health problem with an estimated 8-10 million new cases per year worldwide resulting in 1-2 million deaths.[1] In Syria, the incidence of TB is estimated at 17/100,000.[1] In addition to factors such as virulence of TB bacilli, social conditions, HIV, AIDS, diabetes and steroid therapy, genetic background may be involved in the evolution from the primary infection to clinical TB.[2,3] Genetic influence of susceptibility to clinical TB has been supported by racial variation,[4,5] familial clustering of the disease, and a markedly increased concordance of disease in monozygotic twins compared to dizygotic twins both before and after adjustment for effect of other factors.[6] Furthermore, it has been shown that <10% of the infected population progresses to clinical TB,[7] a fact that suggests the genetic component as a determinant of the final or terminal result of challenge between the host and TB bacilli. Also, the role of the genetic factors in the determination of the severity of the clinical manifestation of the TB disease was indicated by occurrence of familial cases of disseminated Bacillus Calmette Guerin (BCG) infection after immunization.[8] Candidate susceptibility genes to TB should be among those controlling the immune response, since protection from TB infection is related to efficacy of both innate and adaptive immunity of the host. Many studies have focused on genes of cytokines and innate immunity.[9-12] In the adaptive immunity field, the HLA system has gained much interest because of the effect of its hyper polymorphism on the immune system behavior toward different challenges. Antigen presenting cells present mycobacterial TB antigen to CD4+ T cells in the context of HLA class II molecules, raising the adaptive Th1 response against *Mycobacterium tuberculosis* (*M. Tuberculosis*) infection. It has been shown that the lymphocyte response to TB antigens is influenced by the type of DR molecules.[13] To date, results obtained by studies performed for this goal varied according to the studied population.[14-22] In Syria, no such studies has yet been carried out despite the worrying incidence of TB. Therefore, exploring the relationship between HLA and TB seems warranted. We analyzed the DRB1* polymorphism in 147 pulmonary TB patients with sputum smear positive for TB bacilli and 209 healthy individuals to explore the relationship between HLA and TB.

Methods. This work was carried out during 2005 and 2007 at the Laboratory for Research and Genetic Consultations, at the Faculty of Medicine, Damascus University, Damascus, Syria. The study comprised 147 unrelated patients and 209 healthy unrelated individuals. Sample size was calculated using Epi info 2002 statistical software for 90% power of study, and α=0.05. Patients were included in the study with a definite diagnosis of TB confirmed by positive sputum smear or sputum culture for *M. Tuberculosis* strains, they were recruited during 2005 and 2007 from the Damascus Health Center of Tuberculosis and Pulmonary Diseases (National Tuberculosis Program). After Ministry of Health approval, patients were recruited and consented for the study and peripheral blood samples were obtained. Cases with any other associated diseases were excluded. The patients age varied from 17-55 years, and we classified them according to the initial chest radiograph (infiltration, cavitations in one or 2 lung zones). Healthy individuals with negative tuberculin skin test (TST) were chosen from students and employees of Damascus University. Their ages were also between 20-55 years. A negative TST was defined as <10 mm induration. All patients and controls had been vaccinated with BCG. Patients and controls were matched for age, gender, ethnicity, and socioeconomic status. Sampling was carried out in fashion to ensure a simple random selection of both patients and healthy individuals. Using the salting out method, genomic DNA was extracted from 5 ml peripheral blood of each of the patients and controls, the polymorphic HLA-DR locus was amplified (exon 2) using polymerase chain reaction with sequence specific primer according to the method described by Olerup et al.[23] This method allows specific DNA amplification of the different DRB1* alleles. Specific primers for DRB* alleles were purchased from BAG (Lich, Germany) in the BAG-DR low-resolution standard kit. To define the DRB1* genotype, numbers of the wells with allelic specific amplification were displayed in the software supplied by BAG. Besides the DRB allele specificities, chest radiology, and clinical data were entered into a database and analyzed after transferring the data to SPSS worksheet where frequencies of different DRB1* allelic specificities were analyzed and Chi square test was performed. The 95% confidence interval (CI) of the calculated odd ratio (OR) was estimated. The corrected *p*-value was calculated by multiplying the *p*-value by number of alleles found. Results were considered significant when corrected the *p*-value was <0.05.

Results. The distribution of DRB1* alleles in TB patients and healthy groups is illustrated in **Table 1**, where a significant decrease of DRB1*11 allele frequency in patients compared to controls was observed. In contrast, the DRB1*04 allele increased in patients compared to controls. The other DRB1* alleles were almost similarly distributed in patients and controls. **Table 2** shows the analysis of the different DRB1*04 combination frequencies (with the second DRB1* locus) in patients and controls. There was an increased

frequency of DRB1*04/DRB1*04 and DRB1*04/DRB1*03 genotypes in patients compared to healthy controls, which was not significant. Since the DRB1*04 allele did not significantly increase in patients, we verified whether a significant positive association of the DRB1*04 allele is masked by a possible dominant effect of the DRB1*11 allele. We calculated DRB1*04/non DRB1*11 genotype frequency in patients and controls. As shown in **Table 2**, when individuals with DRB1*04/DRB1*11 genotype were removed from patients and controls, a significant increase of DRB1*04 haplotypes appeared in patients compared to controls. According to the chest radiology data, 31 patients had cavitations in one or more pulmonary regions. To see if the DRB1* allelic variation correlates with the clinical evolution of the disease, we analyzed the DRB1* alleles and entire genotypes in patients developing cavitation. We found that 58% of patients with cavitation had DRB1*04 allele (18 from 31 patients with cavity), so cavitation increased in the DRB1*04 positive patients compared to the DRB1*04 negative patients (33% in DRB1*04 positive patients versus 16% in DRB1*04 negative patients, OR=2.7, p=0.04).

Discussion. The allelic variation of the HLA-DRB1* locus in 147 Syrian patients with clinical and laboratory evidence of TB, and 209 Syrian healthy individuals were studied to assess the effect of HLA class II polymorphism on the susceptibility to pulmonary

Table 1 - The DRB1 alleles frequency in controls (N=209) and patients (N=147) with pulmonary tuberculosis.

Allele	Frequency in controls		Frequency in patients		Odds ratio	*P*-value	Corrected *p*-value
	n	(%)	n	(%)			
DRB1*01	26	(12.5)	17	(11.6)			
DRB1*03	31	(14.9)	22	(14.9)			
DRB1*04	55	(26.4)	57	(38.8)	1.77	0.01	>0.05
DRB1*07	43	(20.8)	27	(18.4)			
DRB1*08	7	(3.4)	6	(4.0)			
DRB1*09	2	(0.9)	1	(0.7)			
DRB1*10	9	(4.3)	7	(4.8)			
DRB1*11	106	(51.0)	51	(34.7)	0.51	0.003	0.04
DRB1*12	5	(2.4)	4	(2.7)			
DRB1*13	42	(20.2)	32	(21.8)			
DRB1*14	23	(11.0)	10	(6.8)			
DRB1*15	31	(14.9)	31	(21.0)			
DRB1*16	7	(3.4)	2	(1.4)			

Table 2 - The DRB1*04/DRB1*X combinations frequency in control (N=209) and patients (N=147) with pulmonary tuberculosis.

Genotype	Frequency in controls		Frequency in patients		Odds ratio	*P*-value	Corrected *p*-value
	n	(%)	n	(%)			
DRB1*04/01	3	(1.4)	4	(2.7)			
DRB1*04/03	3	(1.4)	9	(6.0)	4.47	0.03	>0.05
DRB1*04/04	3	(1.4)	6	(6.0)	4.47	0.03	>0.05
DRB1*04/07	6	(2.9)	7	(4.7)			
DRB1*04/10	1	(0.5)	1	(0.7)			
DRB1*04/11	20	(9.5)	11	(7.4)			
DRB1*04/13	11	(5.3)	5	(3.4)			
DRB1*04/14	4	(1.9)	4	(2.7)			
DRB1*04/15	4	(1.9)	5	(3.4)			
DRB1*04/nonDRB1*11	35	(17.0)	48	(33.0)	2.5	0.003	p=0.03

TB. A protective effect of DRB1*11 allele was reported. This effect seems to be dominant over a predisposing effect of the DRB1*04 allele, which became significant only after removing the DRB1*11 allele. Nevertheless, a significant increase of pulmonary cavitation frequency was seen in patients within the DRB1*04 allele compared to those without the DRB1*04 allele. This suggests that the DRB1*04 allele may be involved in both susceptibility to TB infection and post infection clinical evolution in the studied population.

Although DRB1*04/DRB1*03 and DRB1*04/DRB1*04 genotypes were increased in patients compared to controls, this increase remained not significant when a corrected *p*-value was considered. A study of a larger number of patients is needed to verify a possible synergy effect between DR3 adn DR4 haplotypes. A predisposing effect of the DRB1*04 allele has also been reported in a polish population,[14] and in an Italian population with historical TB.[15] Some results of other studies were different from ours. A positive TB association with DRB1*15 has been reported in Mexican[16] and Indian populations,[17] DRB1*07 allele in an Iranian population,[18] DRB1*1302 allele in a South African population,[19] and with DRB1*0803 allele in a Korean population.[20] Studies based on serological determination reported an increase in both HLA-DR14 and DR17 in an Iranian study.[21] Finally, the meta analysis of 22 serological studies showed a positive association of DR2 and DR8 to TB disease.[22] Discrepancy in reported HLA- association between various populations can be explained partly by diversity of technique used, small sample size, and other limitations in the study design, also, by diversity of linkage disequilibrium and the frequent HLA haplotypes in populations ethnically different. However, multiplication of studies of HLA-association in different ethnics, with large sample size, remains a powerful tool that enables us to design a consistent model where accumulated data can be harmonized and the effect of different HLA alleles with predisposition or protection can be better understood on a base of clustering of particular residues that are located in critical positions in the HLA pocket, and give a shared peptide binding characteristics for acceptance of a given *M. Tuberculosis* derived epitope. Agreeing with this notion, Nepom et al[24] demonstrated a critical contribution of beta chain residue 57 in the peptide binding ability of both HLA-DR and DQ molecules. This notion may have a potential role in developing a new recombinant peptide based vaccine, since HLA profiles are likely to be related to vaccine efficacy.

We should note that although our study showed a positive correlation between occurrence of TB and HLA DRB1*04 allele, it remains unclear if this allele is responsible for the susceptibility to TB by itself, or if this is related to another one which may be closely linked to it, particularly a DQ allele. Therefore, it will be necessary to study the role of other HLA locus in the susceptibility to TB. A functional study in the presence of selected HLA alleles will be also helpful to clarify the mechanism underlying the role of HLA polymorphism in the susceptibility to or protection from TB infection.

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Related topics

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