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Protective role of HLA-DRB1*11 against juvenile idiopathic arthritis living in North Eastern Iran

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ABSTRACT

Objective(s): Juvenile idiopathic arthritis (JIA) is one of the most common chronic rheumatic diseases in children. The complex nature of this immune-mediated disease owes itself to several predisposing genes and environmental factors affecting its pathogenesis. Conducted in Iran, this study was originally intended to investigate every possible association between HLA DRB1 alleles and a susceptibility to JIA.

Materials and Methods: In this case-control study, 45 patients with a definite diagnosis of JIA based on International League against Rheumatism (ILAR) criteria were compared against 46 healthy controls. DNA samples taken from both groups were analyzed using PCR-sequence specific primers (PCR-SSP) method. Data analysis including parametric and nonparametric test and multivariate analysis was undertaken using the SPSS 11.5 software. A *P-value*< 0.05 was regarded as statistically significant.

Results: Mean ages in case group and healthy controls were 14.64±6.21 and 13.73±6.39, respectively with no significant difference between the two groups (*P*=0.515). Sex difference between JIA group and healthy controls was also not significant (*P*=0.068) .The frequency of HLA-DRB1*01 was found the most frequent HLA-RB1 in our patients (33.3%). No significant statistical correlation between various HLA-DRB1 alleles and clinical subtypes of the disease could be established from the data. HLA-DRB1*11 was shown to raise protection to JIA (*P*=0.035, OR=2.755, 95% CI=0.963-8.055) in northeastern Iran. In addition, we found that HLA-RB1*09 is nominally associated with an increased risk of JIA (*P*=0.56, OR=2, 05, 95% CI=0.18-23.63).

Conclusion: HLA-DRB1*11 was shown to raise protection to JIA in northeastern Iran. The disparity of findings in other ethnicities prompts further investigations with larger sample sizes.

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Introduction

Juvenile idiopathic arthritis (JIA) is one of the most common chronic inflammatory disorders in pediatric medicine, which involves the joints (1).The incidence rate of JIA has been estimated as 4 to 14 cases aged 0-17 years old in each 100,000 children per year (2).

Rheumatoid arthritis (RA) associated with HLA-DRB1 alleles encodes DRβ1 chains with conserved amino acid sequence in positions 70-74 (3). More than 50 studies on tissue compatibility antigens in RA or JIA have been performed that clearly refer to the role of HLA in susceptibility to disease. The main difference between these studies and ours is the evidence of JIA and adult states. HLA class 2 genetic is more complex and has greater association with JIA (4-6).

JIA is caused by a combination of multiple genetic susceptibility and environmental factors (7), which promote disease progression. Polymorphism at the DRB1 locus, represented by 58 known alleles in humans, has been existed for at least 30 million years and has been shared by humans (8). Some studies have mentioned that JIA is positively associated with HLA DRB1*01, DRB1*08, DRB1*11, DRB1*13, DPB1*02 and DQB1*04 (6, 9, 10).This allows physicians to target therapies more effectively through the use of genetic analysis (2). HLA DRB1*11 is associated with oligoarticular JIA (10).In addition, an association has been found between HLA-DRB1*04:05 and polyarticular JIA (11).

The aim of the present study was to investigate the relationship between HLA class II alleles and JIA in the north east of Iran.

Materials and Methods

The study population included 45 patients with JIA criteria who attended at Rheumatology section of Ghaem Hospital, Mashhad University of Medical Sciences, Mashhad, Iran. According to the revised ILAR (*International League of Associations for Rheumatology*) classification criteria, the patients were classified into one of the seven ILAR subgroups (12). Forty six ethnically age and sex matched participants without any genetics and autoimmune disorders were enrolled as healthy controls.

The study was conducted in accordance with the principles of Declaration of Helsinki (version 1996). Design and all study related documents were reviewed

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Table 1. Demographic and clinical characteristic of study population

	JIA patients(n=45)	Controls (n=46)	*P*-value
Age, years mean ± SD	14.64±6.21	13.73±6.39	0.515
Sex n (%)			
Male	15(33.3)	24 (52.2)	0.069
Female	30 (66.6)	22 (47.8)	
Disease duration (mean±SD)	7.34±5.09	-	-
Age of disease onset	7.11±4.54	-	-
ESR (mean±SD)	27.76±24.25	3.35±2.5	0.035
HB	11.081±1.57	12.5±1.3	0.038
WBC	8676.92±3552.031	8256.14±2786.01	0.074
PLT ($\times 10^3$)	347.93±109.221	324.45±98.650	0.763
Crp positive n (%)	20(69)		
RF positive n (%)	5(18.5)		
Anti ccp positive n (%)	7(15.5)		
Disease category			
Systemic onset JIA	2(4.4)		
Oligoarticular (persistent)	23(51.1)		
Oligoarticular (extended)	1(2.2)		
Polyarticular RF(-)	8(17.8)		
Polyarticular RF(+)	7(15.6)		
Psoriatic arthritis	1(2.2)		
Enthesitis- related Arthritis	2(4.4)		
Undifferentiated	1(2.2)		

JIA: Juvenile idiopathic arthritis, CRP: C-reactive protein (CRP), ESR: Erythrocyte sedimentation rate, Hb: Hemoglobin, Plt: Platelets, ANA: Antinuclear antibody test

and approved by the Human Research Ethics Committee of Mashhad University of Medical Sciences. All patients or their parents signed the informed consent, allowing us to conduct the study.

Blood samples were obtained in the EDTA tube and stored at -20 ˚C until DNA extraction was performed using the Genetbio DNA extraction kit (Genetbio, South Korea).The extraction was performed by columns provided in the kit according to manufacturer's instruction.

PCR-SSP (sequence specific primers) method was used for determining the HLA DR. For this purpose, Histotype DR Low resolution kit (BAG, Germany) was used. Each kit contains 20 to 24 wells per plate and allele-specific primers with internal control primers for GAPDH (Glyceraldehyde-3-Phosphate Dehydrogenase) gene. The PCR condition of HLA-DRB1 amplification was as follows: initial denaturation: 96 ˚C for 5 min, 5 cycles of 96 ˚C (denaturation), 20 sec and 68 ˚C (annealing) 1 min, 10 cycles of 96 ˚C for 20 sec, 64 ˚C for 50 sec and 72 ˚C for 45 sec, 15 cycles of 96 ˚C for 20 sec, 61 ˚C 50 for sec and 72 ˚C for 45 sec and finally 1 cycle at 72 ˚C for 5 min.

Statistical analyses

The statistical analysis was performed using the SPSS 11.5 program (SPSS Inc., Chicago, IL, USA). Values are reported as mean ±SD for normally distributed variables. According to "Kolmogorov-Smirnov" test, the normal distribution in case and control groups was checked. Baseline demographics and clinical characteristics were compared amongst the groups using the t-student test, one way ANOVA test, chi-square test and/or Fisher exact test as appropriate. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated. A *P*-value < 0.05 was considered statistically significant.

Results

The mean age of participants in the case and control group was 14.64±6.21 and 13.73±6.39 years, respectively. There is no significant difference in age between the two groups (*P*=0.515).In case group, 33.3% of individuals were male and in the control 52.2% were male (*P*=0.069). There were significant difference in erythrocyte sedimentation rate (ESR) and hemoglobin between the groups (*P*<0.05) (Table 1).

Mean disease duration in patients with JIA was 7.34±5.09 years and age of onset was 7.11±4.54 years. The most common disease manifestations in patients were persistent oligoarticular (51.1%), Polyarticular RF (-), Polyarticular RF (+), Systemic Onset JIA, Enthesitis-related arthritis, and Oligoarticular (extended) (Table 1).

The results of HLA-DRB1 typing showed that the frequency of HLA- DRB1*01 was higher in patients with JIA (38.5%) than that of control group (25.6%); however, no statistically significant difference was observed between the two groups (*P*=0.22). The frequency of HLA-DRB1*11 was lower in JIA patients (25.6%) compared to the healthy control group (48.7%) and significant difference was observed between the two groups (*P*=0.037, OR=0.36, 95% CI=0.14-0.94). This result suggests that HLA-DRB1*11 is a protective factor

Table 2. Frequency of HLA-DRB1 types in patients with juvenile idiopathic arthritis in comparison with healthy controls

HLA-DRB1 allele	Patients n=45 N (%)	Controls n= (46) N (%)	Odd Ratio	(95% CI)	*P*-value
HLA-DRB1*01	15 (38.5)	10(25.6)	1.81	(95% CI=0.64-5.1	NS
HLA-DRB1*03	8(20.5)	4(10.3)	2.27	(95% CI=0.54-11)	NS
HLA-DRB1*04	6(15.4)	6(15.4)	1.02	(95% CI=0.25-4.20)	NS
HLA-DRB1*07	4(17.9)	7(17.9%)	0.54	(95% CI=0.10-2.35)	NS
HLA-DRB1*08	2(5.1)	2(5.1)	1	(95% CI=0.71-14.7)	NS
HLA-DRB1*09	2(5.1)	1(2.6%)	2.09	(95% CI=0.1-126.16)	NS
HLA-DRB1*10	0	1(2.6)	-	-	NS
HLA-DRB1*11	10(25.6)	19(48.7)	0.4	(95% CI=0.14-1.10)	0.04$
HLA-DRB1*13	7(17.9)	3(7.7)	1.93	(95% CI=0.44-9.67)	NS
HLA-DRB1*14	4(10.3)	4(10.3)	1	(95% CI=0.17-5.89)	NS
HLA-DRB1*15	6(15.4)	7(17.9)	0.85	(95% CI=0.21-3.28)	NS
HLA-DRB1*16	2(5.1)	2(5.1)	1	(95% CI=0.07-14.70)	NS

NS: nonsignificant. $:significant

against development to JIA.

There were no significant difference between case and control group in any HLA-RB1 (P>0.05) (Table 2).We analyzed the results of HLA-DRB1 typing between sex and age; however, no significant difference was observed. Furthermore, as far as the number of HLA-DRB1 alleles is too small according to clinical and laboratory finding, the results were also not significant.

Discussion

Numerous relations between various HLA polymorphisms and JIA have been reported across different ethnic groups. The present study is one the great association study in north east of Iran for JIA and HLA-RB1. Some scientists believe that an association between JIA and the HLA-DRB1 alleles exists (14). In this study, 45 cases of JIA were evaluated for 12 HLA-DRB1 alleles. Farivar *et al*. worked on 33 cases in 2011 (13) and they found that HLA-DRB1*11 is the most common allele in oligoarthritis and in RF negative polyarthritis JIA.

Hisa *et al*. examined a shared epitope (SE), which is encoded by several HLA-DRB1 alleles and the peptidyl arginine deiminase type 4 (PADI4) in 48 RF-positive polyarthritis patients and 188 healthy control. The results showed that frequencies of the HLA-SE were higher in RF-positive polyarticular JIA compared to the healthy control group (15).

In a meta-analysis in six study populations of Western European by Ombrello *et al*. they reported that *HLA-DRB1*11 has a* strong association with systemic JIA (sJIA) *(16). However, in the present study* we observed that the frequency of HLA-RB1*11 allele is higher in healthy controls in comparison with patients suggesting that this allele has negative association with JIA in our population and therefore differences in genetic background should be taken into account. Others studies demonstrated the association of various HLA-DRB1 epitopes with JIA. Haas *et al*. showed that HLA-DRB1*08 is associated with polyarthritis RF negative JIA (17). In 2010, Ramirez *et al*. demonstrated association of HLA-DRB1*01 and HLA-DRB1*04 with JIA after correction of

P value with Bonferroni's test in the Mexican population (18). Sandoughi and colleagues reported that HLA-DRB1*10 is the most common HLA-BR1 allele in adult patients of RA and they demonstrated that DRB1*0103 is protective for RA (19). We have previously reported that HLA-DRB1*01, DRB1*11 and DRB1*13 alleles are associated with RA patients in north east of Iran. In the normal healthy controls, HLA-DRB1*11 (31.95) and HLA-DRB1*13 (34.7%) were the most frequent alleles (20).

In a group of 65 JRA patients and 65 controls, it has been reported that HLA-DRB1*1104 in comparison with HLA-DRB1*1602 and HLA-DRB1 alleles has a significant association in JRA patients. Furthermore, a protective relation has been demonstrated between HLA-DRB1*1501 and HLA-DRB1* 1402 in patients with JRA (21). In 2010, Wu *et al*. showed that HLA-DRB1*08 allele was significantly higher in JIA patients than in healthy controls; the study was on 94 JIA patients and 226 healthy controls (22). It is worth noting that in the previous studies, HLA-DRB1*15 allele has been considered as a protective allele. Similarly, in the present study we found that HLA-RB1*11 has a similar effect (3, 21). Also, the study by Wu *et al*. showed that HLA-DRB1*12 has also protective role (22). In 2014, Sparchez *et al.* demonstrated, in a cohort of 61 Romanian JIA patients and 30 controls, a protective effect for HLA-DRB1*15 and HLA-DQB1*06 (23). Taken together, the frequencies of HLA-DRB1 alleles amongst different populations are controversial. Furthermore, this controversy is also reported in healthy population. Esmaeili *et al.* performed HLA typing in a large normal population of Mashhad. The data showed that the most common types of HLA-DRB1 alleles were DRB1*15 (20.0%) and DRB1*13 (16.2%) (24). In contrast, in our study the most frequent HLA-DRB1 alleles in control group were HLA-DRB1*011 (48.7%) and HLA-DRB1*01 (25.6%). Another study conducted in Kerman, south eastern Iran demonstrated that HLA-DRB1*13 (23.2%) and HLA-DRB1*15 (18.9%) are the most frequent alleles in the control groups. More interestingly, HLA-DRB1*04 was the only allele shown to be negatively associated with ulcerative colitis (UC) (25). In multiple sclerosis (MS), it has been reported that HLA-DRB1*03 is significantly more frequent in patients compare to controls. Furthermore, the frequencies of HLA-DRB1 14 and 16 were significantly higher in controls than that of MS patients. The HLA-DRB1*15 (20%) was the most frequent allele in healthy controls, although no significant difference was observed between patients and healthy controls (26). Analysis of HLA-DRB1 in 60 Iranian patients with acute myelogenous leukemia (AML) and 180 unrelated normal controls exhibited that HLA-DRB1*11 allele (24.7%) was the most frequent allele in controls group and significant association for this allele was found between patients and controls (27). Sayad *et al*. reported that HLA-DRB1*11 is the most frequent allele in both healthy controls (40%) and patients with Hodgkin's lymphoma (41%) (28). Thus, the ethnic background, sex and age matching of the study population and environmental factors seems to influence both the association between HLA-DRB1 alleles and disease susceptibility and also difference in HLA-DRB1 allele frequency among control group. Further studies with larger sample sizes are needed to clarify the role HLA-DRB1 in JIA. Furthermore, analysis of allelic variants of HLA-DRB3, DRB4 and DRB5, which are inked with HLA-DRB1, would be informative.

Conclusion

The results of the present study showed that HLA-DRB1*11 is a protective factor and it is associated with low risk of JIA development. The disparity of findings in other ethnicities prompts further investigations with a larger sample size.

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Conflict of Interests

There is no conflict for posing the current manuscript.

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