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Association of HLA-DRB1 Alleles with Ulcerative Colitis in the City of Kerman, South Eastern Iran

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ABSTRACT

The association of HLA class II genes with ulcerative colitis (UC) as an autoimmune disease has been investigated for several years. However, factors responsible for genetic predisposition of this disease have so far not been clearly understood. In this study, for the first time, we aimed to investigate the association between HLA-DRB1 types and UC in the population of Kerman, a city southeast Iran.

HLA typing was performed among 85 UC patients and 95 healthy controls using PCR amplification, employing sequence specific primers (PCR-SSP). The DRB1 frequencies were determined in the patients and controls.

HLA-DRB1*04 was negatively associated with UC. Furthermore, HLA-DRB1*13 was significantly associated with severity of the disease (p=0.01) among UC patients.

This is the novel result that describes an association of HLA-DRB1*13 with UC and also shows the protective role of HLA-DRB1*04 against the disease in people of Kerman.

Keywords: HLA-DRB1; IBD; Iran; PCR-SSP; Ulcerative colitis

INTRODUCTION

Inflammatory bowel disease (IBD), an autoimmune disease of the gastrointestinal tract, is manifested in two clinical forms: Crohn's disease (CD) and ulcerative colitis (UC). The aetiology of IBD involves interaction of genetic and environmental factors.[1] Numerous genome-wide linkage studies in IBD have been performed and these studies recognized at least nine susceptible loci, IBD1–IBD9.[2]Although contradictory

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results exist, it is clear that genetic factors play a major role towards the development of UC.[3-7] Furthermore, several genome-wide studies have confirmed that UC and CD are connected to IBD[3], which includes major histocompatibility complex genes on chromosome 6.[8-11] HLA class II antigens, HLA-DR, DQ and DP are glycoproteins expressed on the antigen presenting cells such as macrophages, dendritic cells and neutrophils. Processing peptide antigens are loaded in the groove of HLA class II antigens and presented to T cell receptors. HLA-DRB1 is the most widely studied gene in IBD and the association between several DRB1 alleles and UC has been reported in different populations.[2] A study published in 1998, in Spanish population, observed a significant association between HLA-DR15 and UC and this allele was reported to be associated with severity of the disease.[12] In another study in Turkey, in 1998, the HLA-DRB1*1502 allele was shown to be significantly associated with UC.[13] In 2000, a study on 114 Ashkenazi Jews with UC reported a significant association between HLA- DRB1*0103 and the disease.[14] Another study conducted in 2002 in Korean population demonstrated a significant association between UC and HLA-DRB1*1502.[15] Two independent studies in 2009 in Japanese population and in 2010 in Mexican population reported a significant association between HLA-DRB1*01 and UC.[16,17] A meta-analysis was conducted in 2006 and a significant association was reported between UC and HLA-DRB1*0103 in populations from Spain, Mexico and North America. Additionally the same study also reported a significant association between HLA-DRB1*1502 allele and UC in the population from Japan, UK, Korea and North America. Results of this meta-analysis study showed a significant association between HLA-DR2, DR9, and DRB1*0301 and UC among the English population.[2] A recent study from Canada has reported an association between HLA-DRB1*01 and UC.[18] While all the above-mentioned studies showed a positive correlation between UC and HLA types and alleles, two reports from Tehran, Iran in 1996 and 2001 showed lack of association between UC and MHC-class I and II antigens, respectively .It should be noted that these two studies used serology HLA-typing method, and the number of UC patients in 1996 and 2001 studies was 30 and 42, respectively.[19,20] Many studies have highlighted the importance of HLA-DRB1 associations in UC; therefore, it is important to study distinct populations, as the association may be influenced by ethnicity. Based on this, we aimed to investigate the association between HLA-DRB1 types and UC in a population from Kerman, a city southeast Iran.

MATERIALS AND METHODS

Patients and Controls

A total of 180 subjects, including 95 healthy controls from Kerman blood centre and 85 patients with UC disease were enrolled in our study. Patients and controls were unrelated, living in Kerman, judged by place of birth. From each subject, 5 mL of blood was collected in tubes containing EDTA. Patients were diagnosed with UC based on the protocol from the American Gastroenterology Association.[21] For all the patients, clinical history and status of the disease were completed by a gastroenterologist. Demographic and clinical data are summarized in Table 1. Demographic data for controls is as follows: males

Table 1. Demographic and clinical characteristics of UC patients

Variables	Patients with ulcerative colitis # (%)
Gender	
Male	38 (44.7%)
Felame	47 (55.3%)
Age (yr)	37.79±15.79
Range	(14-84)
Disease duration (yr)	3.44±3.07
Bowel movements*	
Mild	45 (52.9%)
Moderate	28 (32.9%)
Severe	12 (14.1%)
Blood in stool*	
Mild	39 (45.9%)
Moderate	26 (30.6%)
Severe	20 (23.5%)
Family history of disease	8 (9.4%)
Endoscopic criteria*	
Mild	44 (51.8%)
Moderate	25 (29.4%)
Severe	16 (18.8%)
Total	**85**

*****Patients were diagnosed with ulcerative colitis based on the protocol from the American Gastroenterology Association.[21]

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(frequency=52.4%, age=35.37±1.57 years), females (frequency=46.9%, age=38.57±1.77 years).The healthy controls were selected with no history of systemic or organ specific autoimmune diseases.

Genomic DNA Extraction and PCR-SSP Technique

Genomic DNA was extracted using salting out method.[22] DNA concentration was measured by NanoDrop 100™ and adjusted to 25–40 ng/µL. HLA-typing for DRB1 alleles was performed by employing BAG HEALTH CARE based on PCR-SSP molecular technique according to manufacturer's instructions. Briefly, DNA was amplified in a 11.66 µL volume reaction containing 8.08 µL dH2O, 1.16 µL 10× PCR buffer, 0.09 µL Taq DNA polymerase and 2.33 µL DNA. Lyophilized primers and nucleotides were pre-coated on to PCR wells by Histo Type Kit DR Low (BAG Health Care, Germany). The reaction mixture was covered with strip-caps and PCR amplifications were performed using the following conditions: one cycle of initial denaturation at 96°C for 5 min followed by 5 cycles of denaturation at 96°C for 20 s and annealing and extension at 68°C for 1 min. Subsequently 10 cycles were performed using the following conditions: denaturation at 96°C for 20 s, annealing at 64°C for 50 s and extension at 72°C for 45 s. The PCR-SSP program was performed for additional 15 cycles with the following conditions: denaturation at 96°C for 20 s**,** annealing at 61°C for 50 s and extension at 72°C for 45 s. Final extension was continued for an additional 5 min at 72°C. The PCR products were separated by electrophoresis on 2% agarose gel containing ethidium bromide and visualized by UV light.

Statistical Analysis

Statistical analyses such as logistic regression, independent t-test, Chi-square, Fisher's exact test and descriptive statistics were performed by SPSS software version 17.0. *P* values less than 0.05 were considered as statistically significant.

RESULTS

HLA-DRB1 frequencies in patients with UC were investigated using PCR-SSP and compared with healthy controls. The HLA-DRB1*11, HLA-DRB1*13 and HLA-DRB1*15 were the most frequent alleles in the healthy controls and also in the patient group. Additionally frequency of HLA-DRB1*04 was the same as HLA-DRB1*15, in the healthy control group.As indicated, frequency of HLA-DRB1*04 in the patient group was significantly less than controls (p = 0.03) (Table 2).

Table 2. Frequency of HLA-DRB1types in patients with ulcerative colitis in comparison with healthy controls

HLA DRB1	Patients with ulcerative colitis (n=85)		Healthy controls (n=95)		OddsRatio CI *95%*	*p.* value
	N	Freq. %	N	Freq. %		
DRB1*01	15	17.6	10	10.5	1.82 (0.77-4.3)	0.168
DRB1*03	14	16.5	14	14.7	1.14 (0.50-2.55)	0.75
DRB1*04	7	8.2	18	18.9	0.38 (0.15-0.97)	**0.038**
DRB1*07	17	20.0	17	17.9	1.14 (0.54-2.47)	0.71
DRB1*08	3	3.5	5	5.3	0.66 (0.15-2.84)	0.72 #
DRB1*09	1	1.2	1	1.1	1.11 (0.06-18.17)	0.93 #
DRB1*10	3	3.5	5	5.3	0.66 (0.15-2.84)	0.72 #
DRB1*11	21	24.7	33	34.7	0.61 (0.32-1.18)	2.14
DRB1*12	1	1.2	1	1.1	1.11 (0.06-18.17)	0.93 #
DRB1*13	22	25.9	22	23.2	1.16 (0.58-2.28)	0.67
DRB1*14	9	10.6	12	12.6	0.81 (0.33-2.05)	0.67
DRB1*15	23	27.1	18	18.9	1.58 (0.78-3.20)	0.19
DRB1*16	16	18.8	14	14.7	1.34 (0.61-2.9)	0.46

: with fisher's exact test , other with χ^2 test

Table 3. Impact of HLA-DRB1 types on severity of ulcerative colitis

HLA DRB1	Severity of disease						Total		*p. value*
	Mild		Moderate		Severe		freq.	N	
	N	freq.	N	freq.	N	freq.			
DRB1*01	7	15.9%	2	8.0%	6	37.5%	15	17.6%	0.05
DRB1*03	7	15.9%	5	20.0%	2	12.5%	14	16.5%	0.81
DRB1*04	3	6.8%	3	12.0%	1	6.2%	7	8.2%	0.72
DRB1*07	9	20%	5	20.0%	3	18.8%	17	20.0%	0.98
DRB1*08	2	4.5%	1	4.0%	0	0.0 %	3	3.5%	0.70#
DRB1*09	1	2.3%	0	0.0%	0	0%	1	1.2%	0.62#
DRB1*10	2	4.5%	1	4.0%	0	0%	3	3.5%	0.69#
DRB1*11	13	29.5%	5	20.0%	3	18.8%	21	24.7%	0.56
DRB1*12	1	2.3%	0	0%	0	0%	1	1.2%	0.62#
DRB1*13	7	15.9%	12	48.0%	3	18.8%	22	25.9%	**0.01**
DRB1*14	4	9.1%	1	4.0%	4	25.0%	9	10.6%	0.09#
DRB1*15	14	31.8%	7	28.0%	2	12.5%	23	27.1%	0.33
DRB1*16	7	15.9%	4	16.0%	5	31.2%	16	18.8%	0.37

: with fisher's exact test , other with χ^2 test

Statistical analysis was performed for UC patients to analyse the association between HLA-DRB*1 types and variables such as C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) CRP, ESR, haemoglobin, blood in stool, bowel movements and severity of the disease. According to endoscopic criteria for UC patients, a significant association was detected for HLA-DRB1*13 and severity of the disease (p = 0.01) (Table 3).

DISCUSSION

The frequency of HLA-DRB1 types was investigated in patients with UC and the results were compared with controls. Our population was selected from Kerman, a city southeast in Iran. To our knowledge, this is the first study to report an association between HLA-DRB1*13 and severity of UC. Additionally, HLA-DRB1*04 in patients with UC showed a protective effect against the disease. The frequency of HLA-DRB1 types, DRB1*11, DRB1*13 and DRB1*15, as the most frequent alleles in healthy controls of the present study was 34.7%, 23.2% and 18.9%, respectively. Moreover frequency of HLA-DRB1*04 was the same as HLA-DRB1*15, in the healthy control group. Previously, one study reported the HLA-DRB1*11 (25.0%), HLA-DRB1*15 (14.5%) and HLA-DRB1*04 (10.5%) as the most frequent types in 100 unrelated Iranian individuals from Fars province in southern Iran.[23] Farjadian et al.[24] compared HLA allele frequencies in the Baloch ethnic group of Iran with Baloch and other ethnic groups in Pakistan and reported HLA-DRB1*0301 and *0101 as the most frequent alleles in Baloches of Iran. Ghaderiet al.[25] reported the frequency of HLA-DRB1 alleles in 100 healthy individuals who were residents of southern Iranian province and indicated HLA-DRB1*11 (25%), *14 (17%) and *15 (12%) as the most frequent alleles. Kalaniehet al.[26] showed HLA-DRB1* 11 (46%), *04 (28%) and *07 (27%) as the most frequent alleles in 100 healthy controls who were selected from Tehran, capital of Iran. Abolfazli et al.[27] observed HLA-DRB1*04 (17.5%), *11 (15%) and *13 and *01 (12.5%) as the most frequent alleles in 80 healthy controls who again were selected from Tehran, capital of Iran. Forootan et al.[20] reported the highest frequency of HLA DR-B1 alleles as 45.23%, 28.57% and 27.38% for HLA-DRB1*11, *2 and *4, respectively in 84 healthy individuals who again were selected from Tehran. They also showed lack of association between UC and MHC-class II antigens by employing serology HLA-typing method. The number of patients with UC in their study was less than twice compared to the sample size of patients in our study. Difference in sample size and HLA typing technique, heterogeneity of population in different parts of Iran and lack of

proper judgement about ethnicity of individuals enrolled in studies might be taken into consideration to explain the discrepancies between the results.

The mechanism by which MHC class II genes influence IBD is so far not clearly understood. MHC class II gene polymorphisms are located in the MHC binding groove and interact with anchor residues of the peptide. Therefore, different HLA molecules may bind with various affinities to the same peptide or may bind preferentially to different peptides. Some studies showed that anchor residues from HLA-DRB1*04 are located in DRβ67-71 and contain arginine, which selectively binds to peptides in autoimmune diseases.[28] Results of several studies from autoimmune diseases suggested that DRB1*04 may cause susceptibility or resistance to some of the autoimmune diseases and UC. HLA-DRB1*04 was reported to be negatively associated with UC in a meta-analysis of 15 studies on Japanese and Northern Europeans populations, whereas a strong association of this type with CD was observed.[29] Additionally, the protective effect of HLA-DRB1*04 against UC was associated with the most prevalent subtype DRB1*0401 in the British population and only when inherited as DRB1*0401-DQB1*0301 haplotype.[30] In other autoimmune diseases in Korean population, DRB1*0401 and DRB1*0405 alleles were shown to be significantly associated with rheumatoid arthritis and HLA- DRB1*0405-DQB1*0302 and DRB1*0401-DQB*0302 haplotypes were notably associated with type one diabetes.[31] A similar association was observed between HLA-DRB1*04 and type one diabetes in African population.[32] On the other hand, the protective effect of HLA-DRB1*04 on nephropathy was reported in type one diabetes patients.[33] Results of a study on Swedish population showed an association between HLA-DRB1*04 and the autoimmune disease, systemic lupus erythematosus.[34] Because of these discrepancies between results, the role of HLA-DRB1*04 alleles as a factor for susceptibility or resistance to autoimmune diseases remains unclear and warrants further investigation. Our results are in concordance with meta-analysis data on Japanese, Northern Europeans and British population, which suggested HLA-DRB1*04 to be protective against UC. One of the interesting results of our study was the association between the DRB1*13 and severity of the disease. Inheritance of this type of HLA-DRB1 in our UC patients resulted in decreased severity of the disease. To the best of our knowledge, such a relationship has not been reported so far. Larger sample size and analysing different ethnic groups of patients may further confirm the effect of this allele on the severity of UC in future studies. To our knowledge, the present study is the first HLA-typing molecular based technique performed in Iran that investigated the association between HLA-DRB1 gene polymorphisms and UC. One of limitations in our study was lack of findings with regard to HLA-typing of individuals in high resolution levels specifically for HLA-DRB1*04 and *13.

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