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ORIGINAL PAPER

Analysis of HLA DR2&DQ6 (DRB1*1501, DQA1*0102, DQB1*0602) Haplotypes in Iranian Patients with Multiple Sclerosis

Mojdeh Ghabaee · Asghar Bayati · Shahla Amri Saroukolaei · Mohamad Ali Sahraian · Mohammad Hosein Sanaati · Parisa Karimi · Massoud Houshmand · Homa Sadeghian · Leila Hashemi Chelavi

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Abstract Multiple sclerosis (MS) is prototype of inflammatory demyelinating disease of the central nervous system .The etiology of MS remains unclear, but according to current data the disease develops in genetically susceptible individuals and may require additional environmental triggers. The human leukocyte antigen (HLA) class II alleles (DRB1*1501, DQA1*0102, DQB1*0602) may have the strongest genetic effect in MS. In this study, the role of these alleles were investigated in 183 Iranian patients with multiple sclerosis and compared with 100 healthy individuals. HLA typing for DRB1*1501, DQA1*0102, DQB1*0602 was performed by polymerase chain reaction (PCR) amplification with sequence-specific primers (PCR-SSP) method. The results show that, HLA DR B1*1501 was significantly more frequent among MS patients (46% vs. 20%, PV = 0.0006) but DQA1*0102 haplotype was negatively associated with MS (30% vs. 50%, PV = 0.0049) and no significant association was found with DQB1*0602 and MS patients in comparison with control group (24% and 30%, PV = 0.43). No significant correlation was observed among these alleles with sex, type of disease; initial symptoms, expanded disability status scale (EDSS), as well as age at onset and familial MS. This study therefore indicates that there is no association of above HLA haplotypes with clinical presentation, disease duration, and disability in Iranian patients with MS which is in line with other previous studies in different ethnic groups.

Keywords Multiple sclerosis · HLA DRB1*1501 · HLA DQA1*0102 · HLA DQB1*0602 · Haplogroup

M. Ghabaee (✉) · A. Bayati · H. Sadeghian · L. Hashemi Chelavi
Department of Neurology, Iranian Center of Neurological Research, Imam Khomeini Hospital, Tehran University of Medical Sciences, Keshavarz Blvd, Tehran, Iran
e-mail: ghabaeem@tums.ac.ir

S. Amri Saroukolaei · M. H. Sanaati · M. Houshmand
National Institute on Genetic Engineering and Biotechnology, Tehran, Iran

M. A. Sahraian
Sina Hospital, Tehran University of Medical Sciences, Tehran, Iran

P. Karimi
Immunology, Asthma and Allergy Research Institute, Tehran University of Medical Sciences, Tehran, Iran

Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disorder of the central nervous system characterized by the destruction of myelin (Poser 2007).The etiology and pathogenesis of MS have not been fully defined (Laplaud and Confavreux 2006). It is widely believed that MS is the result of an autoimmune process in which activation of CD4+ autoactive T cell and their activation into Th1-phenotype are a crucial

event in the initial steps and also in long-term evolution of disease (Agrawal and Yong 2007; Mireia and Rolando 2005).

Linkage and association studies have established that the strongest genetic determinant of susceptibility to MS maps to the major histocompatibility complex (MHC) class II region in chromosome 6p21.3 (Lincoln et al. 2005). This association varies in different parts of the world probably due to varying racial susceptibility to MS (Alves-Leon et al. 2007). Association studies in Northern European, American, and American-black MS patients indicated a significant positive association of MS with the HLA-DR2, DQ6 (short for DRB1*1501, DQA1*0102, DQB1*0602) haplotypes (Dunne et al. 2006; Haegert and Francis 1993). The mechanism underlying the genetic association of these alleles with MS are not yet fully understood but one possibility is that these MHC molecules fail to negatively select (delete) auto reactive T cells within the embryonic thymic microenvironments (Prat et al. 2005). In this study, we attempted to find out the frequency of HLA DRB1*1501, DQA1*0102, DQB1*0602 alleles in Iranian patients with MS and examined probable relationship among these alleles with gender, sex, age at onset, initial symptoms, EDSS, course of disease, and positive family history (Kurtzke 1983).

Materials and Methods

Patients and Control Group

Hundred and eighty-three Iranian MS patients according to McDonald criteria were studied in department of neurology of Iranian center of neurological research, during 2004–2007. HLA data were compared with clinical categories including age at onset, gender, disease course, initial symptoms, EDSS and positive familial history.

Age at onset was defined as the first episode of neurological dysfunction suggestive of demyelinating disease. Disease course, defined as relapsing–remitting (RR) with at least two relapses with partial or full recovery, secondary progressive (SP) with initial relapsing–remitting course but the disability increases over time with or without overt relapses, primary progressive (PP) having had a steady progressive course or progressive-relapsing (PR) with continuous progression from the beginning, and several relapses over during the time (Iuliano et al. 2007).Positive family history defined as occurrence of MS in first or second-degree relatives of patients. EDSS of the patients was assumed from 0 to 10 based on Kurtzke expanded disability status scale (Kurtzke 1983). Peripheral blood samples were collected after obtaining informed consents from the patient and getting approval from the local ethical committee.

HLA Typing

HLA DR2, DQ6 haplotypes (DRB1*1501, DQA1*0102, DQB1*0602) in MS patients were investigated by polymerase chain reaction (PCR) amplification with sequence-specific primers (PCR-SSP) method from peripheral blood samples. Each sample was typed twice and typing was repeated if discordant results were obtained. The results were compared with those of 100 healthy blood donors of random Iranian individuals provided by the blood transfusion center (Amirzargar et al. 2001).The protocol of primers are listed below:

0102:ex2-F 5′-CT GAC CAC GTT GCC TCT TGT-3′
0102:ex2-R 5′-ATT GGT AGC AGC GGT AGA GTT-3′
0602:ex2-F 5′-TC CCC GCA GAG GAT TTC GTG T-3′
0602:ex2-R 5′-TCC TGC AGG GCG ACG ACG CTC ACC TCT CC-3′
1501: 5′-CCG CGC CTG CTC CAG GAT-3′
1501: 5′-TCC TGT GGC AGC CTA AGA G-3′

Beta Actin

Beta actin is used as a positive control for primers which means in ideal PCR condition, 600 bp bond must appear. Beta actin are as follows:

(1) CCA AGG CCA ACC GCG AGA AGA TGA C
(2) AGG GTA CAT GGT GGT GCC GCC AGA C

Statistical Analysis

The frequencies of the HLA DRB1*1501, DQA1*0102, DQB1*0602 alleles were compared

between the patients and control group using SPSS 14 statistical software and the chi square test.

Results

Clinical Features

The clinical features of the patients MS are summarized in Table 1.

One hundred and eighty three patients with MS (133 females and 50 males) were investigated in this study.

The mean age of patients was 28.01 ± 9.19 (range of 11–57 years), mean disease duration was 4.66 ± 4.46 (range of lower 1–25 years), and mean EDSS was 3.6 ± 1.98 (range of 0–8). Most of them had EDSS less than 3. Initial presenting symptoms were motor in 73 (39.9%) followed by sensory 56 (30.6%), brainstem–cerebellar 52 (28.5%), visual disturbances 41 (22.4%), and urinary symptoms 11 (6%), respectively.

Table 1 Clinical summary of Iranian MS patients

Clinical information	MS patients
Total individuals with MS (*n*)	183
Female/male ratio	3:1
Mean age at onset in years (S.D)	28 ± 9.2
Mean disease duration in years (S.D)	4.6 ± 4
Initial symptom (*n*, %)	
Long tract motor	73 (39.9)
Long tract sensory	56 (30.6)
Visual (optic) disturbance	41 (22.4)
Brainstem–cerebellum	52 (28.5)
Urinary symptoms	11 (6)
Seizure	1 (0.5)
Disease course (*n*, %)	
RR	129 (70)
PP	22 (12.6)
SP	21 (11.5)
PR	11 (6)
EDSS	
≤3	90 (49.2)
3.5–6	63 (34.4)
>6	30 (16.4)
Familial history	
No	171 (93.4)
Yes	12 (6.6)

Table 2 Frequency of selected HLA antigens in MS patients and controls

HLA allele	MS patients ($n = 183$)		Control subjects ($n = 100$)		*P* value
	Number	%	Number	%	
DRB1*1501	82	46	12	20	0.0006
DQA1*0102	53	30	29	50	0.0049
DQB1*0602	43	24	17	30	0.43*
Total	178	100	58	100	–

* Not significant

One hundred and twenty-nine (70%) patients were classified as relapsing-remitting (RR) MS, 22 (12.6%) as primary-progressive (PP), 21 (11.5%) as secondary progressive (SP), and 11 (6%) patients as progressive-relapsing MS.

Twelve (6.6%) patients had positive family history of MS.

Genetic Analysis of HLA Loci and Clinical Expression

HLA DRB1*1501, DQA1*0102, DQB1*0602 alleles were investigated in 183 MS patients and compared with 100 unrelated healthy individuals (Table 2); results show that the frequency of HLA DRB1*1501 had increased significantly among MS patients compared with control group (46% vs. 20%, $P = 0.0006$), but DQA1*0102 haplotype was negatively associated with MS (30% vs. 50%, $P = 0.0049$) and no significant association was found with DQB1*0602 and MS patients in comparison with control groups (24% and 30%, $P = 0.43$). The effect of HLA loci on clinical variables was evaluated; no significant correlation was observed between DRB1*1501, DQA1*0102, DQB1*0602 alleles with sex, type of disease, initial symptoms, EDSS, as well as age at onset disease and familial MS.

There is no significant relationship between HLA typing, disease duration, and EDSS at time of disease presentation.

Discussion

An association between MS and HLA class II was first noted by Jersild et al in 1973 (Jerslid et al. 1973).

The HLA class II DR2 haplotypes (DRB1*1501, DQA1*0102, DQB1*0602) have been associated with MS in all ethnic groups especially Caucasians (Fernández et al. 2004).

HLA DRB1*1501 is the major variant conferring disease risk (Hooper-van Veen et al. 2006). In nearly all of the studies that analyzed DRB1*1501 allele, researchers found the frequency of this allele to be considerably higher in patients than in controls (Schmidt et al. 2007).

In few studies in non-European populations such as Chinese, African Americans, and Afro-Brazilians investigators reported slightly lower prevalence of DRB1*1501 in MS cases than in controls, and/or low frequencies of this allele in both cases and controls less than 10% (Kelly et al. 1995; Caballero et al. 1999; Karni et al. 1999; Oksenberg et al. 2004).

Our study shows that there is a positive association with HLA DRB1*1501 among MS patients compared with control group (46% vs. 20%, PV = 0.0006). This association is in line with studies done in many parts of the world and pervious study in Iran that was done by Kalanie in Caucasoid Iranian RR & PP MS patients (Kalanie et al. 2000) but in contrast to Amirzargar study in Iran that was done exclusively on chronic progressive MS patients (Amirzargar et al. 1998).

In most of the studies higher prevalence of DQA1*0102 allele in MS cases was reported (Marrosu et al. 2006). Two studies from Mediterranean island of Sardinia, showed lower frequencies of DQA1*0102 in MS cases than in controls (Marrosu et al. 1993; Haegert et al. 1993).

In a recent study HLA DQA1*0102 was negatively associated with MS (30% vs. 50%, PV = 0.0049). These data are similar to results in Mediterranean island of Sardinia and pervious study in Iran (Kalanie et al. 2000).

DQB1*0602 was consistently found to be more prevalent in cases than in controls in European (Dunne et al. 2006) and Caucasian (Fernández et al. 2004) populations and also in certain non-Caucasian populations, including African Americans (Oksenberg et al. 2004) and Martinicans (Quelvennec et al. 2003), although some studies failed to detect a significant association, particularly those conducted in Southern European regions such as Sardinia (Marrosu et al. 1993) and northeastern Italy (Zivadinov et al. 2003).

None of the Asian or Middle Eastern populations studied had a significantly greater frequency of the DQB1*0602 allele in cases than in controls, with the exception of Ashkenazi Jews living in Israel (Kwon et al. 1999) and Turks (Saruhan-Direskeneli et al. 1997). Our data about HLA DQB1*0602 in MS patients (24% vs. 30%, PV = 0.4) are similar to pervious study in Iran (Amirzargar et al. 1998), Sardinia and Northeastern Italy (Marrosu et al. 1993; Haegert et al. 1993).

Inconsistent results were obtained between HLA class II alleles and clinical characteristics of MS (Schmidt et al. 2007); some studies found DRB1*1501 to be associated with a younger age at onset (Hensiek et al. 2002; Smestad et al. 2007) and others did not (Amirzargar et al. 1998; Ballerini et al. 2004).

Overrepresentation of DRB1*1501 in females is seen in some studies. Others reported no gender difference (Hensiek et al. 2002; Zivadinov et al. 2007). Several studies examined the genetic association with disease severity (Hensiek et al. 2002; Perini et al. 2001).

In studies performed in the United States and United Kingdom, no association between DRB1*1501 and clinical course was found. (Hensiek et al. 2002; Weinshenker et al. 1998).

In this study no significant correlation was observed between DRB1*1501, DQA1*0102, DQB1*0602 haplotype with sex, initial symptoms, type of disease, EDSS, as well as age at onset, and positive family history of MS.

How an autoimmunity works with in HLA effect comes from the results that copolymer 1 antigen of myelin contains class II binding motifs, and once bound to groove of HLA-DR molecules, COP1 may act as either a blocking peptide, or as antagonist, or partial agonist, resulting in suppression of autoimmune T cell responses, or anergy or both (Hareli et al. 1999).

Our study was limited by examining the patients from different parts of the country that may have variable ethnic backgrounds. Future larger studies in different geographic parts and subgroup analysis may shed more light on the association of HLA with multiple sclerosis.

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