Original Article

Analysis of HLA-DQB1*0602 in Multiple Sclerosis Patients in Khuzestan Province, Iran

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Abstract

Background: Multiple sclerosis (MS) is a chronic, demyelinating, autoimmune and also complex disease of the central nervous system the etiology of which is not completely defined; but both genetic and environmental factors are regarded as main factors in its susceptibility. HLA-DQB1*0602 is considered as one of the most important genetic factors in MS predisposition but contradictory results have been reported in different populations worldwide. Since there are no data with respect to the correlation of HLA-DQB1*0602 and multiple sclerosis in Khuzestan province, and because of ethnic diversity in Khuzestan province, the aim was to examine the association of this allele with multiple sclerosis in Khuzestan.

Methods: This is a case-control study that evaluated 200 MS patients from Khuzestan and 200 healthy individuals from the same geographical region. DNA extraction was performed by salting out method; in addition, HLA typing was carried out by polymerase chain reaction amplification with sequence-specific primers (PCR-SSP) method. The present study also considered probable association among HLA-DQB1*0602 with sex, ethnicity, and type of disease.

Results: Results revealed that distribution of mentioned allele was not statistically different among cases and controls (61.5% vs. 64%, *P* = 0.605); furthermore, no association was shown between this allele and gender, ethnicity or type of disease.

Conclusion: On the whole, our result is consistent with most of the other studies in Iran; but contrasts with most of the studies in European populations.

Keywords: HLA-DQB1*0602, Multiple Sclerosis, PCR-SSP

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Introduction

Multiple sclerosis (MS) is a chronic, demyelinating and inflammatory disease of the central nervous system (CNS) in which both innate and adoptive immune systems contribute to its pathogenesis cascade.[1] It is one of the neurological diseases that endanger young adults more than the others.[2] The prevalence of MS is between 2 and 160 per 100,000 in different populations and more than 2 million individuals suffer from this disease worldwide.[3] It is also worth mentioning that the highest prevalence is in North America and Europe (140 and 108 per 100,000, respectively) and the lowest is in Sub-Saharan Africa and East Asia (2.1 and 2.2 per 100,000, respectively) according to Atlas of MS 2013. About ten years ago, Kalanie, *et al*.[4] reported 200 definite MS patients in Iran but the prevalence and incidence of this disease have increased in Iran, especially during the last decade. There is a wide variation in the prevalence of MS in Iran from 5.3 to 74.28 per 100,000 in different regions. Khuzestan Province, located in south-west of Iran whose population is divided into two major ethnicities, Arab and Persian, is not an exception. The data suggest that the total prevalence and incidence of MS in Khuzestan are 16.28 and 2.20, respectively.[5] In accordance with Atlas of MS 2013, MS is more common in women (M: F ratio 1:2) with the average age of onset at 30 years. MS manifests with various neurological dysfunctions, such as visual and sensory problems, limb weakness or gait disturbance.[6] It is divided into four classical subtypes including: relapsing-remitting MS (RRMS), secondary progressive MS (SPMS), primary progressive MS (PPMS) and primary relapsing MS (PRMS). However, in 85% of cases, there is a clinically isolated syndrome (CIS) that later converts to RRMS.[7,8] Although the precise cause of MS is still unknown, both genetic and environmental factors are involved in its pathogenesis. On the other hand, studies have shown that not only an autoimmune mechanism mediated by auto reactive T cells plays an important role in pathogenesis of MS, but also B cells and innate immune system are involved in its pathway.[1,9] Twin studies have confirmed the contribution of genetic factors to the pathogenesis of MS by comparing monozygotic and dizygotic twins. The concordance rate of monozygotic and dizygotic twins is (~25%–30%) and (~5%), respectively.[3,10] The interleukin 7 receptor (*IL7RA*), the interleukin 2 receptor (*IL2RA*), the CD58 and the c-type lectin domain family 16 member A (*CLEC16A*) genes are some of the most important genetic factors in MS susceptibility,[3] but linkage analysis has confirmed the prominent role of HLA locus that is according to human version of MHC, located in 6p21.3 in predisposition of MS[11]; furthermore, different population studies also highlight the proportion of HLA locus in susceptibility to MS among other genes the same as other autoimmune

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disease. HLA-A*03 and HLA-B*07 were the first alleles whose associations were demonstrated with MS.[12] HLA is divided into three classes: class I, II and III. The class II region contains the classical alpha and beta chain genes including DP, DQ and DR.[13] A prominent role of HLA class II region in the association with MS has been confirmed[14] and it seems that HLA-DRB1*1501, HLA-DQB1*0602 and HLA-DQA1*0102 are considered as the most important factors particularly in association with multiple sclerosis.[15] Each HLA allele name has a unique number that is dependent on the DNA sequence of the allele. HLA-DQB1*0602 is an allele and its sequence is different from others in several nucleotides. Individuals whose DNA sequence is similar to this allele are positive with this allele**.** Although several studies have been carried out on the association of HLA-DQB1*0602 with MS in Iran,[16–18] there are no data so far about this issue in Khuzestan Province. Considering the ethnicity diversion in Khuzestan Province and also due to the fact that unlike Persians, Arabs in Iran are mostly located in Khuzestan and since there are a few data in this regard in Arabic countries, this population study can be of great importance. From this point of view, we decided to consider the association between HLA-DQB1*0602 and MS in Khuzestan Province. In addition, in this study we examined the probable relationship between this allele with gender, ethnicity and subtype of MS.

Materials and Methods

Peripheral blood was collected from 200 MS patients from Khuzestan Province registered in the Khuzestan MS Community. The McDonald criteria were used for MS diagnosis.[19] We supplied a questionnaire containing questions about parameters such as age, gender, ethnicity, positive family history as well as subtype of MS; however, clinical parameters estimation was carried out by experts in the field of neurology.

Two hundred healthy individuals, without any autoimmune disease and familial history of MS, were selected as control group that came to the Shafa Hospital for routine laboratory analysis. Controls were originally from the same geographical region and were matched with cases in ethnicity. Peripheral blood samples were collected from patients and controls in an EDTA tube. The participants were informed about our study and completed a consent form.

DNA extraction was performed by salting out method. Quality and quantity of several random extracted genomes were examined by electrophoresis and nanodrop methods. Typing of HLA-DQB1*0602 was achieved by polymerase chain reaction amplification with sequence-specific primers (PCR-SSP) method and was repeated if discordant results were obtained. Primers were designed using the IMGT/HLA database (http://www.ebi.ac.uk/) and checked out in ncbi/blast. (www.ncbi.nlm.nih.gov). Primers sequence was as follow: forward: 5′- TCCCCGCAGAGGATTTCGTGTT -3′; reverse: 5′- CACCTCGTAGTTGTGTCTGCA -3′. The PCR amplification program was 4 min of initial denaturation at 94ºC followed by 30 cycles of melting at 94ºC for 30 s, annealing at 59ºC for 30 s, and elongation at 72ºC for 30 s followed by 7 min of final elongation at 72ºC. Finally, the results were validated by sequencing several samples randomly.

The frequency of the mentioned HLA was determined as percentage. The significance of association between frequencies in the MS population compared with the control populations was evaluated using SPSS 16 statistical software and chi square test. Statistical significance was defined by a *P*-value of less than 0.05.

Results

As mentioned above, HLA-DQB1*0602 allele was evaluated among 200 MS patients and 200 healthy age- and sex-matched individuals. Summarized characteristics of patients and controls are shown in Table 1. The results showed that the frequency of this allele was almost similar among cases and controls and no association was found among HLA-DQB1*0602 and multiple sclerosis statistically (61.5% vs. 64%, PV = 0.605, OR = 0.899 [95% CI = 0.599–1.348]). Gel electrophoresis is shown in Figure 1.

We also analyzed the correlation of HLA-DQB1*0602 allele with multiple sclerosis in females, males, Arabs and Persians separately, but as shown in Table 2, there was no association between this allele with any of the ethnicities or gender. Also, no significant correlation was demonstrated between the mentioned allele with type of disease.

Table 1. General information of patients and controls.

General information	MS patients	Controls
Total number of individuals	200	200
Female	159(79.5%)	144(72%)
Male	41(20.5%)	56(28%)
Arabs	88(44%)	84(42%)
Persians	112(56%)	116(58%)
Mean age	31.16 ±7.9	57.9 ±6.7
Disease course		
RRMS	175(87.5%)	—
SPMS	5(2.5%)	—
PPMS	5(2.5%)	—
PRMS	1(0.5%)	—
CIS	14(7%)	—
Familial history		
Yes	14(7%)	—
No	186(93%)	—

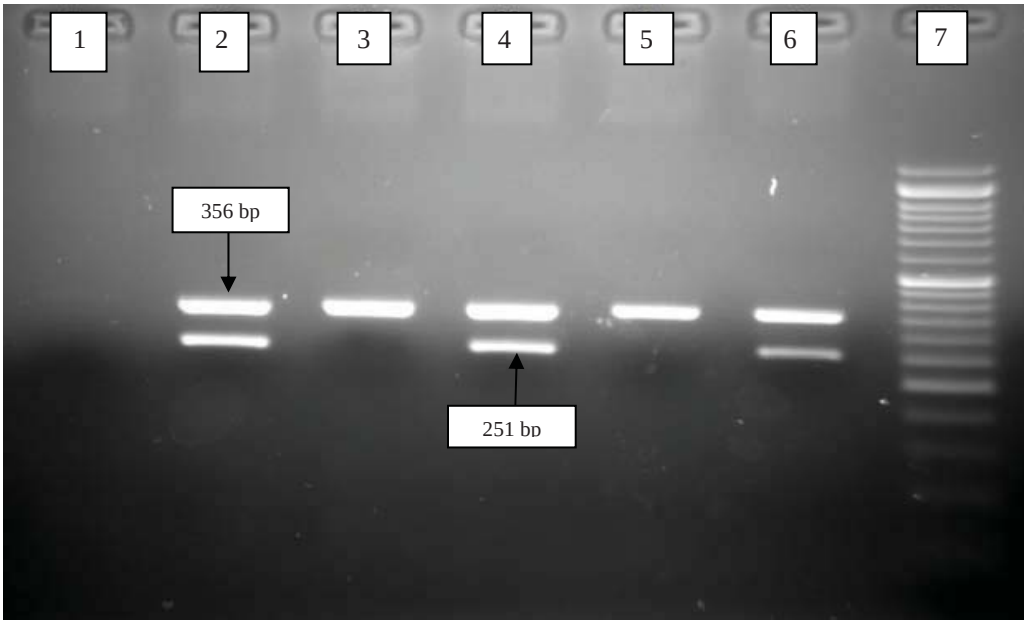

Figure 1. Gel electrophoresis. Column 1 is negative control, columns 2, 4 and 6 are positive samples, columns 3 and 5 are negative samples and column 7 is 50bp marker. Control gene PCR product was 356 bp and HLA-DQB1*0602 PCR product was 251 bp.

Table 2. Analysis of association between HLA-DQB1*0602 with sex and ethnicity separately.

Group	Case (HLA DQB1*0602 positive) (N/%)	Control(HLA DQB1*0602 positive) (N/%)	*P* value	OR	95% CI
Female	92(57.86)	94(65.27)	0.185	0.730	0.459–1.163
Male	26(63.41)	32(57.14)	0.534	1.300	0.569–2.972
Arab	44(50)	48(57.14)	0.348	0.750	0.411–1.368
Persian	74(66.07)	76(65.5)	0.930	1.025	0.593–1.772

Discussion

Multiple sclerosis is a complex and multifactorial disease whose precise cause is unknown but results from the interaction of genetic and environmental factors as well as intrinsic factors and epistatic effects (e.g., gene-gene interactions).[3]

Linkage and association studies have confirmed the role of HLA class II especially HLA-DQB1*1501, DQB1*0602 and DQA1*0102 in multiple sclerosis susceptibility. In most studies that have been performed in European populations, the frequency of HLA-DQB1*0602 in patients have been more than that of the controls[9, 20–22]; except for studies conducted in southern European regions such as Sardinia and northeastern Italy.[23,24] In non-European populations, such as African-Americans and Martinicans, also the association of HLA-DQB1*0602 and MS has been shown.[25,26] In Afro and White Brazilians, HLA-DQB1*0602 was shown to confer susceptibility to MS regardless of ethnicity.[27] In some Asian populations such as Turks, Ashkenazi Jews and Japanese, also the association of HLA-DQB1*0602 and MS has been observed[28–30]; moreover, in Iran, as an Asian country, four studies have been carried out in this regard until now.[16–18,31] Our results are similar to three of them,[16–18] but differ from one of them.[31] However, it should be noted that our allelic frequency was much more than these studies in Iran and this may result from the different genetic pools and ethnic diversity in Khuzestan Province.

We also carried out a PUBMED database survey and reviewed the most important studies ever carried out on the frequency of HLA-DQB1*0602, in Table 3. As shown in this table, the maximum and minimum allelic frequencies have been observed in Norway and Italy, respectively until now. The smallest *P* value and the greatest association have been found in Australia.[32] The frequency of HLA-DQB1*0602 allele was 61.5% in our population that is almost similar to that of Greece with allelic frequency of 69% in MS population.

According to a recent study that was performed by Sharafaddinzadeh, *et al.*, it was confirmed that the prevalence and incidence of MS are higher among Persians in comparison with Arabs in Khuzestan Province,[33] that is why we examined the likely association between HLA-DQB1*0602 with Arabs and Persians separately. In spite of the higher frequency of the mentioned allele in Persians in proportion to Arabs, no significant association was found in either Persians or Arabs compared with the control group. It seems that the only study ever carried out in this regard in Arab population is a study performed in Israel that surveyed different HLA alleles in multiple sclerosis patients and compared those frequencies in Muslim and Christian Arabs separately; no significant association was found between HLA-DQB1*0602 and multiple sclerosis in any of them.[34] Our result about Arabs is also in line with the mentioned study.

Statistical analysis also failed to confirm the association of this allele with sex and type of disease. These findings are also consistent with some other studies.[9,17,18,21]

Table 3. HLA-DQB1*0602 frequency in MS patients and control group in different populations.

Country (Population)	HLA-DQB1*0602 frequency in patients (%)	HLA-DQB1*0602 frequency in controls (%)	*P*-value	OR (95% CI)	Reference
African American	23.5	17.9	0.01	1.4 (1.1–1.9)	25
Australian	54	18	1.1×10^{-7}	—	32
Afro Brazilian	45	17	0.003	—	35
White Brazilians	40	13	<0.001	—	27
Greece	69	51	0.01	—	9
Iranians	11.6	8.5	NS	—	16
	29.2	12.8	0.044	2.80 (0.92–8.36)	31
	24	30	0.43		17
	14.2	7.9	0.041*	1.920 (1.061–3.472)	18
Ireland, County Donegal	53.4	32.5	<0.01	2.382 (1.379–4.116)	20
Ireland, County Wexford	53.3	26.5	<0.001	3.170 (1.631–6.161)	
Israel, Ashkenazi Jews	19.2	8.3	0.014	—	30
Israel, non-Ashkenazi Jews	13.8	5	0.08	—	
Israel, Christians	12.8	4.8	0.10	—	34
Israel, Muslims	6.8	2.2	0.08	—	
Italian	9.6	3.1	—	—	36
Japanese	17	9	—	—	37
Japanese	22.5	6.8	0.04	—	28
Norwegians	94	98	—	—	38
Spain, Gypsies	35.7	2.5	0.0002	—	22
Spain, Caucasians	42.9	20.6	—	—	
Spanish	45	20.6	0.001	3.1 (1.9–5.2)	21
Turks	27	10	0.005	3.2	29

NS = not significant; * = in this study although *P* value is less than 0.05, not significant association was shown.

In general, our result is consistent with most of other studies in Iran but contrasts with most of the studies in European populations; besides, we can conclude that HLA-DQB1*0602 cannot be considered as a genetic risk factor for MS in our population. It is recommended to evaluate this allele in MS population in the other provinces of Iran and Arabic countries for more documented results.

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