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*HLA-DQB1*06* May Predispose People to Multiple Sclerosis (MS)

N. Khalili[a], R. Nakhaei Sistani[b,*] and H. Shirzad[c]

[a]*Department of Cellular and Molecular Biology, Faculty of Chemistry, University of*
[b]*Department of Cellular and Molecular Biology, Faculty of Chemistry, University of Kashan*
[c]*Research Institute of Police Science and Social Studies, Tehran, Iran*

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ABSTRACT

Background: Multiple sclerosis (MS) is a chronic disease of the central nervous system that leads to the disability of the affected people. The etiology of the disease is not clear, but it is believed that it has an autoimmune nature. Several studies suggested that the major histocompatibility complex (MHC) class II loci are the most prominent genetic risk factor for MS susceptibility. The Human leukocyte antigen (HLA) DQB1*06 allele was introduced in numerous studies as the MS genetic predisposition factor, but there is a debate about it in different regions. Objective: This study aims to analyze the association of the HLA-DQB1*06 allele in Tehran. Method: Blood samples were collected from 117 MS and 114 healthy people. DNA was extracted, and genotyping was performed using allele-specific primers. Results: Our results showed that the HLA-DQB1*06 allele is significantly associated with MS (*p-value* < 0.000, odds ratio = 0.059, 95% CI = 0.02 to 0.14). The homozygous genotype was also more prevalent in the MS group compared with the control (*p-value < 0.000*, odds ratio = 0.2, 95% CI = 0.1-0.39). Our results show there is no difference in allelic distribution of this gene between men and women. Conclusion: Our data suggest that the HLA-DQB1*06 could be considered as an important genetic risk factor for MS in Tehran.

Keywords: Multiple sclerosis, HLA-DQB1, Association, Disease susceptibility, Genetic predisposition

INTRODUCTION

Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS) [1]. MS is the most prevalent disease of the CNS causes lifelong debility in young adults. It is an inflammatory disease characterized by the demyelination of neurons and axonal loss [2]. These damages probably result from the activation of autoreactive T-lymphocytes [3], which causes recurrent immune attacks on the CNS [4]. The etiology of MS is not clear, but a plethora of evidence suggests that both genetic and environmental factors underlie MS susceptibility, progression, and symptoms [5].

The mono- and dizygotic twin studies and sibling studies [6], familial aggregation of cases, and the higher prevalence in some ethnics regardless of geographical location [7] indicate the role of the genetic component. Studies concerning the genetic loci involved in MS have identified several MS-associated genes, and most of them are related to the immune system [8]. MHC loci are the most prominent genetic players in MS, and the other loci are less important [9]. Many of the non-MHC genes are also involved in the cell-mediated immune response [10].

The association of MHC loci with MS is consistent with this enduring opinion that this disease is essentially an antigen-specific autoimmune disease, but also implies that the risk assessment is complicated because there are multiple statistically independent alleles and haplotypes [11,12]. The MHCs are glycoproteins, which are responsible for the initiation of the immunological response for an antigen [13]. The MHC antigens are classified as HLA-I, HLA-II, and HLA-III. The Class II genes have been shown to contribute dominantly to the MS genetic risk [11].

The HLA-II genes are located on the HLA-D region of chromosome 6p21 [14]. The HLA-II proteins are mainly present on the surface of the antigen-presenting cells (APCs) and are involved in the presentation of extracellular antigens to T-cells. They are composed of two polypeptide chains α

*Corresponding author. E-mail: r.nakhaei@kashanu.ac.ir

and β and encoded by HLA-DP, -DQ, or DR loci [11,15]. The HLA-DQ sub-region consists of two loci DQA1 and DQB1 for α and β polypeptide chains, respectively.

The HLA-DQB1 gene helps the immune system distinguish the body's proteins from foreign invaders. The HLA-DQB1 gene provides instructions for making a protein that forms a complex with another protein from the HLA-DQA1 gene. This complex displays foreign peptides to the immune system to trigger the body's immune response.

HLA-DQB1*06 alleles may affect the recognition and presentation of certain myelin antigens, such as myelin basic protein (MBP), proteolipid protein (PLP), or myelin oligodendrocyte glycoprotein (MOG), by the immune system. Depending on the allele, the HLA-DQB1*06 molecule may bind and present these antigens more or less efficiently, or with different conformations, leading to different T-cell responses. Some of these responses may be pathogenic, meaning they cause inflammation and damage to the myelin, while others may be tolerogenic, meaning they induce tolerance and prevent autoimmunity [16].

It has been shown that the HLA-DQB1 locus is strongly associated with MS [17]. One of the first studies about the contribution of this locus on MS susceptibility was published in 1989 and reported that in Norwegian MS patients, three variants of the HLA-DQB1 gene were associated with the disease [18]. Since then, there have been numerous studies about the involvement of various alleles of the HLA-DQB1 gene in MS susceptibility with controversial results [17,19]. As several loci participate in MS development, these discrepancies could be attributed to the genetic background of different populations. Therefore, it is important to address the transferability of regional association data to the interested population [19]. For example, there are several reports about the different immunogenetic backgrounds of MS in Asian and Western populations [20,21]. In this study, we investigated the association of HLA-DQB1*06 alleles with MS in Tehran.

MATERIALS/PATIENTS AND METHODS

Patients and Samples

This work was a case-control study, consisting of 117 MS cases and 114 individuals with no diagnosed MS as the control group. The cases were of members of the Iranian MS society from Tehran with a well-documented MS history. The sampling period was between 6/12/2014 to 29/5/2016. The case and control groups were similar regarding age and gender. The participants were between 8 and 46 years old, and 77.3% of them were female. All the people were informed about their participation in the study.

Blood samples were collected in the Yekta laboratory by specialist personnel. From each person, 5 ml of blood was taken in a tube containing EDTA. The samples were stored at -80 ºC until DNA extraction.

This work was performed according to the Iran National Committee for Ethics in Biomedical Research with the IR.UMZ.REC.1399.030 ethical code.

DNA Extraction

DNA was extracted according to a modified salting-out method [22]. In brief, a low salt buffer and Triton-X were used to lyse the red blood cells (RBCs). The pellet of white cells was treated with high salt buffer and SDS, and then with 6M NaCl to precipitate the proteins. DNA precipitation was performed by using isopropanol. The quality of DNA samples was assessed with gel electrophoresis and spectrophotometer.

Primer Design and PCR

To perform the genotyping of the HLA-DQB1 locus, primers capable of discriminating the HLA-DQB1*06 alleles were designed. To do this, the sequence of exons one and two of all HLA-DQB1 alleles were obtained from dbMHC (the data are now available in ftp://ftp.ncbi.nlm.nih.gov/pub/mhc/mhc/Final Archive). The sequences were grouped according to two-digit numbers in their names. Each sequence group was multiply aligned separately, and the consensus sequence for each group was obtained by using Emboss Explorer (http://www.bioinformatics.nl/emboss-explorer/). Next, the consensus sequences were multiply aligned to determine the discriminating position of the HLA-DQB1*06 consensus sequence. Then, the PCR primers were designed (Table 1) according to the simple allele-discriminating PCR (SAP-PCR) [23].

The Ampliqon Taq DNA polymerase 2X master mix red was purchased from Pishgam Biotech Co. The standard polymerase chain reaction (PCR) was performed with a final

Table 1. The Sequences of Primers Used in This Study

Primer name	Primer sequence 5' to 3'	Tm	Amplicon length
Forward	TGCTACTTCAACGGGAC	65.7	
Reverse 06	CTGCTGTTCCAGTACTCGGTA	65.8	153 bp
Reverse others	GCTGTTCCAGTACTCGGTG	64.5	

concentration of 0.2 uM for each primer in the final volume of 25 µl. 40-150 ng of genomic DNA was used in each PCR reaction tube. The same PCR program was used for both primer pairs, beginning at 95 ºC for 5 min, followed by 30 cycles of 95 ºC for 30 s, 62.5 ºC for 30 s, and 72 ºC for 30 s, and ended at 72 ºC for 5 min. The PCR products were run on 1.5% agarose gel.

The Statistical Analysis

The Chi-square test and odds ratio statistics were calculated to analyze the association of HLA-DQB1 with MS. The analysis was performed with R software version 4.2.0. The normal distribution of data was analyzed by the Shapiro-Wilk test and a comparison of means was performed using the Wilcoxon test for non-normal data. The Hardy-Weinberg was tested using HardyWeinberg package version 1.7.5.

Table 2. The Distribution of Samples Regarding Age and Sex in Both Case and Control Groups

	Age < 40		Age >= 40	
	Female	Male	Female	Male
Not MS	51	12	38	12
MS	61	16	30	13

Table 3. The Distribution of HLA-DQB1*06 Allele in Patients and Controls

	Allele 06 exist	Allele 06 not exist
Not MS	60	53
MS	115	5

RESULTS

Samples Demographics

To analyze the association of the HLA-DQB1*06 allele group with MS, a total of 231 individuals (114 healthy and 117 affected people) were enrolled in this study, 22.7% were male, and 77.3% were female (Table 2.). The Shapiro-Wilk test was performed on age data and showed that age data is not normal (*p-value* = 2.047e-06). The Wilcoxon test showed that the case and the control groups are not significantly different regarding age (*p-value* = 0.2089).

The control people were selected so that the control group was not significantly different from the case group regarding age and sex. This was approved by the Chi-square test (*p-value* = 0.8).

Hardy-Weinberg Test

The HardyWeinberg package was used to assess whether the samples were in Hardy-Weinberg equilibrium or not. The results show that the control sample and the whole sample are in Hardy-Weinberg equilibrium, but the case group has deviated from it (*p-value* = 0.005). This indicates an association of the HLA-DQB1*06 with MS.

Genotype Analysis

The genotype of people was determined by SAP-PCR. Our data (Table 3.) showed that in the control group, 64 over 114 people (56.1%) have the HLA-DQB1*06 allele, while in the case group, 112 out of 117 individuals (95.7%) have this allele. The difference between the two groups was highly significant (p-value < 0.000). The odds ratio for the presence of the HLA-DQB1*06 allele was 0.059, with 95% CI = 0.02 to 0.14.

There is also a highly significant difference between the case and control groups regarding the genotypes (p-value < 0.000) (Table 4). The odds ratio for the homozygous

Table 4. The Distribution of HLA-DQB1*06 Among Case and Control Groups

	Homozygote 06	Heterozygote 06	Homozygote other 06
Not MS	17	43	53
MS	98	17	5

genotype for the HLA-DQB1*06 allele was 0.2, 95% CI = 0.1-0.39.

DISCUSSION

It is believed that MS is an autoimmune disease that results from recurrent attacks of auto-reactive T-lymphocytes. The autoreactive T-cells are produced as a result of the failure of the mechanisms that eliminate them. T-cell mediated suppression is one of such mechanisms that make the adult immune system unresponsive to self-antigens. It has been shown that the suppression by CD8+ T-cells is HLA-DQ dependent [24]. It is possible that during T-cell maturation, particular HLA antigens interact with T-cells to elicit positive or negative T-cell selection. Therefore, some DQ antigens may be inefficient in provoking thymic negative selection of auto-reactive T-cells. Alternatively, some DQ antigens may play a role in the peripheral suppression of anti-self T-cells [25]. Another scenario about how the HLA-DQB1 alleles could be involved in MS pathogenesis is the evidenced role of some of its alleles in raising antibodies against interferon-β protein [26].

Since yet, there are numerous reports about the association of HLA-DQB1 alleles in MS. These works indicate a predisposing or protecting role for different HAL-DQB1 alleles in various populations. It is also mentioned that some alleles may be involved in the type, progression, and response to treatment of MS and the patients. Although, there is a debate about the role of any particular allele, and, it seems that this is dependent on the population under study.

In 1989, Vartdal *et al.* reported that alleles of HLA-DQB1, which shared a particular amino acid stretch, are associated with MS [18]. In 1992, it was shown that in Norwegian Caucasians, the allele HLA-DQB1*0602 has a higher frequency in MS patients than in normal samples. The authors also suggested that there may be an association between MS and a particular combination of HLA-DQB1 and -DQA1 alleles [27]. The study conducted on Sardinian Island reported that alleles HLA-DQB1*0201 and *0302 are associated with MS [25]. The authors reported that HLA-DQB1*0502 was diminished in MS patients. Another HLA-DQB1 allele, *0301, is associated with MS in Korean children [28]. It was found in the Slovak population that the HLA-DQB1*06 allele and HLA-DQB1*06/HLA-DQB1*06 genotype were positively associated with MS. A protective effect was also detected for the HLA-DQB1*03 allele and HLA-DQB1*03/HLA-DQB1*05 genotype [17]. On the other hand, the study conducted on African-Americans could not show any association of this locus with MS [19].

In the current study, the association of the HLA-DQB1*06 allele with MS was studied. Our results showed a highly significant higher frequency of the allele in MS patients (p-value < 0.000) with odds ratio = 0.059, 95% CI = 0.02 to 0.14. Interestingly, 84.61% of MS patients were homozygous for the studied allele, compared with only 15% in the control group (p-value < 0.000, odds ratio = 0.2, 95% CI = 0.1-0.39). In Iran, in a sample of MS patients from all around the country, it was shown that the DQ3 allele (DQA1*0501/DQB1*0301 or DQA1*0301/DQB1*0302) is associated with MS. The work studied DQ1, DQ2, and DQ3 alleles and, therefore, there was no data in their work about the HLA-DQB1*06 alleles [29]. Another study conducted by Amirzargar *et al.* showed that a haplotype containing the HLA-DQB1*0602 allele predisposes people to MS, while haplotype containing HLA-DQB1*0601 allele may have a protective effect against MS [30]. Also, Kollaee et al. stated that a haplotype containing the HLA-DQB1*0602 allele is associated with MS in an Iranian sample [31]. The positive association of HLA-DQB1*0602 with MS was also reported in the study performed by Amirzargar and colleagues [32]. In the study on MS patients of Khuzestan province of Iran, Zabihi et al. could not find any significant relationship between the HLA-DQB1*0602 allele and MS. The frequency of the mentioned allele in their samples was also high (more than 60%) [33].

It is noteworthy that in this study, the studied allele is overrepresented in the patient group (allele frequency of 91.88%) compared with other studies. This can be attributed

to the difference between samples. Alternatively, other researchers studied the HLA-DQB1*0602 sub-group and/or haplotypes. However in this study, we investigated the HLA-DQB1*06 totally and independently. Another possibility is that the allele discriminating primer, which is designed based on the comparison of HLA-DQB1 alleles consensus sequences, might amplify some allele sub-groups that do not belong to HLA-DQB1*06 but involved in MS susceptibility. If this is true, the primer binding site or its corresponding position in the protein sequence may have functional relevance.

CONCLUSION

In general, our data are consistent with the results obtained from numerous works in Iran and other countries. This study showed that the HLA-DQB1*06 allele is associated with MS in Tehran, and the individuals homozygous for this allele are more prone to be affected. Therefore, the HLA-DQB1*06 allele could be considered as a strong genetic risk factor in Tehran.

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