Combination of Myelin Basic Protein Gene Polymorphisms with HLA-DRB1*1501 in Iranian Patients with Multiple Sclerosis

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ABSTRACT

Background: Multiple sclerosis (MS), as a multifactorial autoimmune disease with complex genetic basis, causes demyelination in the central nervous system via cytokine responses to myelin antigens. Myelin basic protein (MBP) is the main protein component of the myelin sheath. HLA-DRB (human leukocyte antigen-DR beta) alleles, particularly HLA-DRB1*1501, may be of significance in the pathogenesis of MS. **Objective**: To examine the association of HLA-DRB1*1501 alleles and MBP VNTR (variable number tandem repeat) polymorphism with the MS susceptibility in Iranian population. **Methods**: Genomic DNA was extracted from peripheral blood. The alleles were determined by the Polymerase Chain Reaction (PCR) method in 259 MS patients and 312 healthy control individuals and analyses were carried out using Fisher's exact test. **Results**: The frequencies of MBP VNTR genotypes (AA, AB and BB) were 47%, 42% and 11% among patients, and 45%, 43% and 12% in control subjects, respectively. HLA-DRB1*1501 allele was more frequent among patients than healthy individuals (OR=1.65, P=0.0045). The frequency of allele A and genotype A/A was significantly higher among HLA-DRB1*1501 positive patients (61% and 32%) than controls (46% and 19%) (OR=1.88, P=0.0013; A/A vs. B/B: OR=5.09, P=0.0004). The two-locus analysis of the interaction between the MBP VNTR polymorphism and the HLA-DRB1 allele showed that the HLADRB1* 1501/A haplotype was more frequent among MS patients than the healthy controls. **Conclusion**: The interaction between the HLA-DRB1*1501 allele and MBP gene may be considered as a predisposing factor in the development and pathogenesis of MS in the case of gene-gene interaction.

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INTRODUCTION

Multiple sclerosis (MS) is a complex autoimmune disease (1) inducing demyelination in the central nervous system (CNS) via T-cell and cytokine responses to myelin antigens (1-6). MS disables patients aged 30 to 40, especially young women (2). This multifactorial disease is affected by the complex interaction between environmental and genetic factors. Based on previous studies, the concordance rates of monozygotic and dizygotic twins are 25-30% and 3-5%, respectively. Positive genetic backgrounds could also increase the risk of developing MS through environmental factors (1,7). Genome-wide association studies have conduced to identifying a myriad region containing genes deemed to be associated with this disease. Major histocompatibility complex (MHC) gene on chromosome 6p21 (8) has also received remarkable attention. Fernández *et al.* (9) proved that there exists an association between HLA-DRB1*1501 allele and MS. In our previous studies, we verified the impacts of the epistatic interaction of HLA-DRB1*1501 on the function of other genes like IL-2 -330 (10), IL-6 -174 (11) and TNF-alpha -308 (12) polymorphisms as far as MS susceptibility in Iranian population is concerned. Other important genes are those responsible for the regulation of immune responses (13) and those involved in myelin formation. Myelin basic protein (MBP) gene is one of the major genes involved in myelin formation, encoding the main protein of the myelin shell of axons in the central nervous system (CNS) (4,14). The MBP gene is located on the long arm of chromosome 18 and it consists of seven exons (15) containing tetranucleotide repeats (TGGA)n region at the 5′side. Repetitive DNA sequences within the human genome are thought to be the main cause of DNA polymorphisms (16,17). MBP is, more often than not, regarded as an effective autoantigen involved in MS, and it is a key structural element that significantly increases the conduction velocity of nerve impulses. This association varies in different parts of the world probably due to various racial susceptibility to MS (18).

The incidence and prevalence of MS is growing in Iran and there are not enough data available on the genetic backgrounds of the Iranian population. In addition, there exist certain controversial findings as to the impact of variable number tandem repeat (VNTR) polymorphism (in the 5′flanking region of the MBP gene) on MS susceptibility; in this regard, this cross-sectional case-control study aimed to investigate the influence of VNTR polymorphism in the 5′flanking region of the MBP gene, HLA-DRB1*1501 and its interaction with HLA-DRB1*1501 allele on a population of Iranian patients with MS in Golestan province.

MATERIALS AND METHODS

Patients. In order to analyze the genetic variations in the MBP gene, we enrolled 259 unrelated MS patients belonging to one center and 312 healthy control individuals. The patients were diagnosed by adept neurologists according to McDonald's criteria and the clinical and para-clinical investigations (MRI, oligo-clonal bands in CSF, and evoked potentials) (19). In order to eliminate the effects of environmental factors, healthy control subjects (matched in terms of age, gender and ethnicity) with no history of autoimmune or inflammatory disorders were selected from the same region. Demographic characteristics (such as age and gender) of the subjects in both groups were specified via a questionnaire. Moreover, the type of MS, age of onset, and

expanded disability status scale (EDSS) were determined for patients with MS. The local ethics committee approved the study and informed consent was further obtained from all study subjects. None of the subjects refused to participate. The mean age of the patients and controls was 31 ± 9 years (ranging from 19 to 57 years) and 39 ± 7 years (ranging from 35 to 64 years), respectively. The mean age of onset and EDSS were 26 ± 6 years (ranging from 10 to 46 years) and 3.5 ± 2 (ranging from 1 to 8), respectively. The female/male ratio was 5:1 in the patients (Table 1).

Table 1. Demographic data of MS patients (n=259).

Mean age	31 ± 9 years (ranging from19 to 57 years)
Female/ Male	5:1
EDSS	3.5±2 (ranging from 1 to 8)
Age at onset	26±6 (ranging from 10 to 46 years)
Clinical subtype	
Relapsing-remitting (RR)	215 (83%)
Primary-progressive (PP)	38 (14.7%)
Secondary- progressive (SP)	3 (1.15%)
Progressive- relapsing (PR)	3(1.15%)

EDSS: expanded disability status scale.

DNA extraction and genotyping. Genomic DNA was extracted from peripheral blood leukocytes (20) using a standard protocol with certain modifications. After aliquoting DNA samples in graded distilled water, DNA concentrations were determined by a UV spectrophotometer at 260 nm (Techne, UK). All samples were diluted and stored at -80°C for future analysis. Polymerase chain reaction (PCR) reagents for MBP genotyping consisted of 20 pmol of each primer, 100 ng of the extracted DNA, 200 μ MdNTPs, 1.5 mM MgCl2, 1 U Taq polymerase and 2.5 μl of 10X PCR buffer with a final volume of 25 μl. PCR was used for genotyping and its conditions were set as follows: 35 cycles of 5 min at 95°C, 45 s at 95°C, 45 s at 56°C, 90 s at 72°C, and a final extension of 7 min at 72°C. Primer pairs were designed and ordered (BIOTEST, Germany) , consisted of MBP-primer-F: 5´- GGA TGA CGA ATG GAT GAA TTG -3´ and MBP-primer-R: 5´- TCA CAT ATT CCT GTA ATA CCA GTC A -3´ and produced two sets of fragments that can be analyzed as two allele groups: Large (allele A: 400 bp) and small (allele B: 330 bp) (Figure 1). DNA genotyping of HLA-DRB1* 1501 allele was carried out by PCR method, using primers and methods previously described by Ghabaee and colleagues (21). The PCR products of HLA-DRB1*1501 and MBP were separated on a 2% agarose gel (Merck, Germany), and bands were visualized by a gel documentation system (UVITEC, UK).

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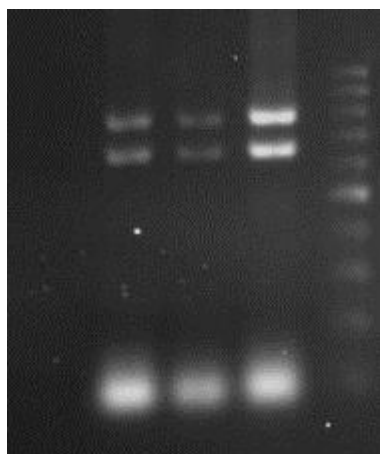
Figure 1. PCR electrophoresis of MBP alleles (330 bp and 400 bp)

Statistical analysis. Data were entered into SPSS software version 16 and the means of parametric variables were computed. Data are presented as mean ± standard deviation (SD) and percentages for parametric and non-parametric values, respectively. Non-parametric tests followed by Fisher's exact analysis using STATA v-8 (CA, US) were employed to calculate and compare allele and genotype frequencies between the groups. P-values less than 0.05 were considered as statistically significant.

RESULTS

Two distinct bands including a 400 bp (allele A) and a 330 bp (allele B) fragment were observed following the amplification of the genomic DNA. The genotype distributions in both study groups were compatible with the Hardy-Weinberg equilibrium. No significant differences were observed between the case and control as regards genotype and allele frequencies of the MBP VNTR polymorphism (Table 2). Moreover, gender and EDSS of patients did not have any major effects on the frequency of alleles and genotypes.

Table 2. Frequency of MBP VNTR allele and genotypes among patients and control subjects.

Alleles an genotypes of MBP gene	MS No (%)	Control No (%)	OR	95% CI	[a]P-Value
AA	123(47)	140(45)	1.19	0.66- 2.40	0.58
AB	108(42)	134(43)	1.09	0.60-1.27	0.78
BB	28(11)	38(12)	1.00	-	-
A	354(68)	414(66)	-	0.87-1.40	0.48
B	164(32)	210(34)	1.00	-	-

As demonstrated in Table 4, the frequency of HLA positive cases is higher in patients (119 out of 259 (46%)) compared to healthy individuals. There were no significant differences between HLA-DRB1*1501-positive and HLA-DRB1*1501-negative patients in terms of onset age and EDSS. The frequency of allele A and genotype A/A was higher among HLA-DRB1*1501 positive individuals (Table 3).

Table 3. Frequency of MBP VNTR allele and genotypes among HLA-DRB1*1501 positive patients and control subjects.

Alleles an genotypes of MBP gene	MS No (%)	Control No (%)	OR	95% CI	[a]P-Value
AA	38(32)	20(19)	5.09	1.90-13.39	0.0004
AB	70(59)	57(54)	3.23	1.41-7.78	0.0034
BB	11(9)	29(27)	1.00	-	-
A	146(61)	97(46)	1.88	1.26-2.78	0.0013
B	92(39)	115(54)	1.00	-	-

The co-existence of HLA-DRB1*1501 and MBP VNTR A alleles was more frequent among MS patients than the healthy controls. Table 4 provides ancillary data on the frequency of haplotypes.

Table 4. Frequency of risk alleles (MBP VNTR A and HLA-DRB1*1501) among patients and control subjects.

Comparison	MS No (%)		Control No (%)		OR	95% CI	[a]P-Value
A+ vs. A	231(89)	28(11)	274(88)	38(12)	1.14	0.66 - 1.99	0.6930
DR15+ vs. DR15-	119(46)	140(54)	106(34)	206(66)	1.65	1.16 - 2.35	0.0045
A+/DR15 vs. A+/DR15-	109(42)	122(47)	77(25)	197(63)	2.28	1.55 – 3.36	0.0001
A+/DR15 vs. A-/DR15+	109(42)	11(4)	77(25)	29(9)	3.73	1.67 – 8.75	0.0004
A+/DR15 vs. A-/DR15-	109(42)	17(7)	77(25)	9(3)	0.74	0.27 – 1.89	NS
A+/DR15 vs. A-/DR15+	122(47)	11(4)	197(63)	29(9)	1.63	0.75 – 3.75	NS
A+/DR15 vs. A-/DR15-	122(47)	17(7)	197(63)	9(3)	0.32	0.12 – 0.80	0.0110
A-/DR15 vs. A-/DR15-	11(4)	17(7)	29(9)	9(3)	0.20	0.06 - 0.65	0.0046

MS: multiple sclerosis, OR: odds ratio, CI: confidence interval. a P values were determined by fisher's exact test for allele frequencies, and by logistic regression test followed by Bonferroni's correction for genotype frequencies.

DISCUSSION

Although it is now well-demonstrated that MS is a multifactorial disease influenced by genetic and environmental factors, the role of these factors has not been entirely elucidated. It has been proven that the dysregulation of inflammatory cytokines has a crucial role in autoimmune diseases like MS; therefore, specific cytokine gene polymorphisms are suspected to be associated with MS susceptibility and its clinical conditions. The MBP gene is capable of inducing the susceptibility to MS because the product of this gene is the main protein component of myelin and it (MBP protein) is a potential autoimmune antigen in the disease (22). MBP gene comprises a larger transcription unit called Golli-MBP which produces a range of mRNA via alternative splicing (23). The expression of MBP gene is regulated at the 5' side region of the first exon of this gene which contains a repetitive sequence (TGGA)n located at the upstream of the MBP initiator methionine. However, this region is of functional importance and needs to be clarified (24) and can be employed as a genetic marker for mapping disease genes (25). On the other hand, the MBP gene involvement in MS development may be a result of MBP-HLA-DRB1 interaction. It has been shown that MBP-specific T cells are clonally expanded in MS patients by the recognition of HLA molecules bound to MBP (16,26-28). In the current case-control study, we investigated polymorphisms at the 5'flanking region of the MBP gene and HLA-DRB1*1501 allele and their interactions. Based on the results, there were no significant differences regarding the allele and genotype frequencies of MBP VNTR polymorphism between patients and healthy individuals. Although several studies have been carried out on the 5' flanking region of the MBP's first exon, most have not found any significant association with MS susceptibility (29-32) C.vandevyver *et al.* observed no significant differences in allele frequencies and found no association between MS and Polymorphism of Tetranucleotide Repeat at the 5' Side of MBP gene (32). Yet another study reported that MBP gene did not have a major effect on MS genetic susceptibility in an Italian population, suggesting that such susceptibility may be a heterogeneous phenomenon possibly affected by the ethnicity of the examined population (30). On the contrary, certain studies have revealed a significant association between the polymorphism of 5' end of the MBP gene and MS. In the present research, no significant association was seen between MBP VNTR polymorphism and MS. Guerini *et al.* reported noticeable differences between their Italian case and control groups concerning genotype frequencies at 5'-flanking region of the MBP first exon. According to their findings, the short fragment (A allele) had a higher frequency in the relapsing remitting MS (RRMS) patients comparisons to healthy individuals. Similarly, the short homozygous pattern (A/A) had a remarkably higher frequency in the RRMS patients (33).

In a study done in the United Kingdom, Tienari *et al.* observed substantial differences in the allele frequencies between the patients and healthy individuals and posited that the 5'-flanking region of the MBP first exon highly influences the susceptibility to MS (34). In another case-control study on a Danish population, there existed a significant association between the 5'-flanking region of the MBP first exon and susceptibility to MS (16).

HLA molecules and cytokine networks are considered as the most important mechanisms responsible for susceptibility to MS (11). The connection between HLA class II and susceptibility to MS was first demonstrated by Jersild *et al.* in 1973 (35).

Marrosu *et al.* also suggested the involvement of MHC gene in genetic susceptibility to MS (36). Furthermore, certain studies have shown the important role of HLA-DRB1*1501 allele as a prognostic factor in the development of MS (37-39). In our pervious study, we demonstrated that the prevalence of HLA-DRB1*1501 allele was 46% in Iranian MS population which is higher than that of the healthy individuals. This percentage was 38.6 in a certain Spanish sample (10). The probable influences of gene–gene interactions on the pathogenesis of MS have been well evidenced (40). The combined association between the alleles of MBP and HLA-DRB1 results in the contribution of the MBP gene to the etiology of MS. This can be explained by the function of the protein products of these genes in the immune system. In MS patients, it has been found, HLA molecules, on the surface of antigen-presenting cells, bind to immunedominant peptides of MBP, presenting them to the antigen-specific T cells. HLA-DR2 mainly restricts the recognition of MBP peptides by the bulk of T cell clones in any particular patient (26); however, E. Cocco *et al.* observed no epistatic interaction between the MBP gene on the HLA-class II and MS in Sardinians (41).

In conclusion, in the present study we evaluated the effect of HLA-DRB1*1501 allele, MBP VNTR polymorphism and their interactions concerning MS susceptibility in 259 MS patients and 312 healthy control individuals. After analyzing the interaction of HLADRB1*1501 and the MBP VNTR polymorphism, an association was observed between A/A genotype and the risk of developing MS among HLA-DRB1*1501-positive individuals. This indicates that MBP VNTR polymorphism increases susceptibility to MS in response to gene-gene interaction.

ACKNOWLEDGEMENTS

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