

# HLA-DRB1 ALLELE FREQUENCY IN MYASTHENIA GRAVIS PATIENTS FROM SOUTHERN IRAN

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## ABSTRACT

HLA-DRB1 allele frequencies of 28 myasthenia gravis (MG) patients, residents of southern Iranian provinces, were studied using PCR-SSP method. The control group consisted of 100 healthy individuals of the same ethnic group. The allele HLA-DRB1\*16 (RR= 2.04,  $p= 0.028$ ) was found to be significantly increased in MG patients compared to the control group, whereas HLA-DRB1\*15 (RR=0.17  $p= 0.043$ ) and HLA-DRB1\*07 ( $p= 0.036$ ) were found to be significantly increased in the control group compared to the patients, and seem to be negatively associated with the disease.

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**Keywords:** Myasthenia gravis, HLA-DRB1, PCR-SSP.

## INTRODUCTION

MG is an autoimmune disease in which production of autoantibody against the acetylcholine receptor in patients causes fatigability and muscle weakness. The association of MG with products of major histocompatibility complex genes has been studied in various races and ethnic groups worldwide. Of particular interest is the association of HLA-B8 and MG in Caucasians.<sup>1</sup>

Further study indicated that in Caucasian MG patients there is a linkage between HLA-B8 and DR3.<sup>2</sup> However, in the Swedish population, polymorphism in HLA-DQ alleles has been shown to determine susceptibility.<sup>3</sup> A recent report from Italian MG patients indicated that DQB10\*502 allele is significantly associated with this patient group.<sup>4</sup> In MG patients from a North Indian population the highest frequency has been reported for Bw21 and Bw35.<sup>5</sup> But as expected from the polymorphism of HLA genes in different populations, other alleles have been found to be associated in non-Caucasian patients. In Japanese, it has been reported that a combination of HLA-DRB1/DQB1 is responsible for susceptibility.<sup>6</sup> In Chinese, HLAB46/DR9 has been reported to

be associated with MG patients.<sup>7</sup> We reported recently on the frequency of HLA- class I alleles in Iranian patients with MG, and it was shown that HLA-B8 and HLA-B51 are associated with the disease in southern Iranians patients.<sup>8</sup> Here, we report on the frequency of HLA-DRB1 alleles in patients from southern Iran using PCR-SSP method.

## MATERIALS AND METHODS

### Patients

The patient group consisted of 28 individuals (14 men, 14 women) all from southern Iranian provinces. Mean age of onset was calculated to be 37 years (women 27, men 45). The diagnosis of MG was clinically based on fatigability and muscle weakness with diurnal variation which dramatically responded to IV edrophonium HCl (Tensilon test) and were alleviated by maintenance of anti-cholinesterase medications. 26 patients had generalized and 2 patients had ocular forms of the disease.

### Controls

The control group consisted of 100 healthy individuals which were residents of southern Iranian provinces.

### Genomic DNA extraction

Genomic DNA was extracted according to a modified

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method of Miller.<sup>9</sup> Blood was transferred to a 50 mL tube and cold red cell lysis buffer I, containing 0.144 M NH<sub>4</sub>Cl and 1mM NaHCO<sub>3</sub>, was added to a final volume of 50 mL. The mixture was centrifuged for 5 min at 2400 g. Then the supernatant was discarded and the pellet was homogenized by vortexing. This step was repeated until almost all red cells were lysed. The pellet was homogenized and 10 mL of red cell lysis buffer II, containing 0.3 M sucrose, 10 mM Tris-HCl (pH 7.5), 5 mM MgCl<sub>2</sub> and 1% Triton-X100, was added. The pellet was treated with 4.5 mL of WBC lysis buffer containing 75 μM NaCl and 24 μM Na-EDTA. Subsequently, 125 μL of 10% SDS and 1mL of 5 M NaClO<sub>4</sub> were added. The mixture was vortexed for 10 seconds and 2 mL of 6 M NaCl was added and vortexed again. The mixture was centrifuged for 5 min at 1500 g. The supernatant was carefully transferred into a clean 50 mL tube and 7 mL absolute isopropanol was added and mixed gently. The precipitated DNA was removed. The excess isopropanol was squeezed out. The DNA was washed twice in 1.5 mL of 70% ethanol. The pellet was dried at room temperature and resuspended in 100-500 μL of double distilled water (ddH<sub>2</sub>O) depending on the yield of the extracted DNA.

#### HLA-DRB1 typing

HLA-DRB1 typing was performed by PCR-SSP as previously described by Olerup.<sup>10</sup> This method was achieved by 19 reactions, eighteen for assigning DRB1 alleles and one as a negative contamination control. All primer mixes were obtained from the Department of Transplantation Immunology, University of Heidelberg, Germany. In each PCR reaction, in addition to alleles or group specific primers, there were a pair of primers for non-allelic sequences of DRB1 gene as an internal positive control. The PCR mix-

ture (17 μL PCR mixture, 1 μL template DNA and 2 μL Taq DNA polymerase) was covered with 30 μL of mineral oil and subjected to 30 cycles amplification under the following PCR conditions: denaturation at 94°C for 30 seconds, annealing at 55°C for one minute and extension at 72°C for one minute using an Applegene thermal cycler (Applegene, France). The extension was continued for a further 5 min at 72°C. PCR products were electrophoresed on 1.5% agarose gel and the presence of the specific DNA bands were analyzed under UV light.

#### Statistical analysis

Statistical analysis was performed by EPI-5 software. The frequencies of DRB1 alleles were compared between the patients and control group using chi-square and Fisher's exact tests.

### RESULTS

The HLA-DRB1 allele frequencies of control and patient groups are presented in Table I. According to the results, HLA-DRB1\*16 allele (RR= 2.04, *p*= 0.028) was found to be significantly increased in the patient group in comparison to the control group, and show association with the disease. The HLA-DRB1\*7 allele (*p*= 0.036) and HLA-DRB1\*15 allele (RR= 0.17, *p*= 0.043) were found to be significantly increased in the control group compared to the patients and be negatively associated with the disease. The HLA-DRB1\*16 allele was mostly found (although not significantly) in women under 40 years of age.

### DISCUSSION

Being an autoimmune disease, myasthenia gravis, like other autoimmune diseases, is a T cell and finally an MHC dependent disease. Since MHC class II molecules act as antigen presenting molecules for T cells, it is usually a beneficial task to detect what type of MHC class II molecules (alleles) are associated with the disease. Serological analysis of MHC-II molecules has several disadvantages because of the poor specificity of antibodies used for this purpose and the cross reactivity that may occur. Therefore, new methods have been recently developed. PCR-SSP is one of these methods, which recently has been given a great deal of importance. This method detects the MHC at the gene level and refutes the disadvantage adherent to the serological methods. With this impetus, we undertook this study which is the first report on HLA allele distribution in the southern Iranian population of MG patients. The results show that the southern Iranian population of MG patients is associated with HLA-DRB1\*16 allele. It is a well established fact that HLA-A1, B8, DR3 haplotype is associated with MG patients of Caucasian origin, especially female patients under 40 years of age.<sup>1</sup> In our patients, although the HLA-

**Table I.** Allele frequency of HLA-DRB1\* in Iranian patients with MG and normal controls.

DRB1 Alleles	Cont.(%) N= 100	MG (%) n= 28	<i>p</i> value	OR	RR
1	11(5.5)	2 (3.6)	NS		
3	20 (10)	10 (17.9)	NS		
15	24 (12)	1 (1.8)	0.043	0.13	0.17
16	13 (6.5)	9 (16.1)	0.028	2.75	2.47
4	21 (10.5)	3 (5.4)	NS		
7	13 (6.5)	0	0.036		
8	3 (1.5)	2 (3.6)	NS		
9	6 (3)	1 (1.8)	NS		
10	8 (4)	2 (3.6)	NS		
11	50 (25)	13 (23.2)	NS		
12	3 (1.5)	3 (5.4)	NS		
13	14 (7)	7 (12.5)	NS		
14	14 (17)	3 (5.4)	NS		

RR= Relative Risk, OR= Odd's Ratio, NS= Non-significance.

DRB1\*03 allele was found to be increasingly (but not significantly) occurring in this group of patients, the patients group typed for HLA-DRB1 was too small to reach a decisive conclusion. As exemplified in the introduction, the association of HLA alleles in MG patients represents a broad heterogeneity among races and populations worldwide. This heterogeneity seems to be more consistent with the frequency of HLA class II alleles. This view is the case even for Japanese and Chinese MG patients which one would expect more similarity within their HLA alleles.<sup>2,6</sup> Whether the heterogeneity of HLA molecules in MG patients is a reflection of disease heterogeneity or disease subgroups need larger size samples to be analyzed remains to be determined.

Two recent reports on the role of HLA-DQ alleles and MG patients are of particular interest. Horiki et al. described the association of an HLA-DR/DQ combination which is responsible for susceptibility in Japanese MG patients.<sup>6</sup> More importantly, the report by Raju et al. using transgenic mice provided evidence that polymorphism at the HLA-DQ locus contributes to the incidence and the severity of experimental MG.<sup>2</sup> By considering the ambiguity which largely exists in the serological analysis of HLA-DQ allele, it is expected that the possible association of HLA-DQ alleles in MG patients will be re-investigated by more reliable and molecular methods in the future.

In conclusion, the present work points to a possible association of HLA-DRB1\*16 alleles with MG in southern Iranian province population. To our knowledge this is the first report on the association of this allele with MG patients.

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