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Association of HLA-DQA1*0101/2 and DQB1*0502 with Myasthenia Gravis in Southern Iranian Patients

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ABSTRACT

Background: Myasthenia gravis is an autoimmune disorder of neuromuscular junction characterized by skeletal muscle weakness and fatigability. Different genes may control the induction and clinical presentation of this disease. Various HLA alleles are reported as predisposing or protective genetic elements in myasthenia gravis. **Objective:** The aim of this study was to investigate the probable association between HLA-DQ alleles and myasthenia gravis in southern Iranian patients. **Methods:** HLA-DQA1 and DQB1 alleles were determined in 104 sporadic patients with myasthenia gravis using polymerase chain reaction - restriction fragment length polymorphism method and the results were compared to 816 healthy controls. **Results:** HLA-DQA1*0101/2 (39.4%) and DQB1*0502 (21.6%) were the most frequent alleles in southern Iranian patients with myasthenia gravis. These alleles revealed positive associations with the disease with relative risks of 1.69 and 2.41, respectively. The most common haplotype was DQA1*0101/2-DQB1*0502 in these patients. **Conclusion:** According to the results of this study, DQA1*0101/2 and DQB1*0502 alleles might be considered as predisposing genetic factors to myasthenia gravis while DQA1*0501, DQB1*0301 and *0602/3 show protective roles against this disease.

Keywords: HLA-DQA, HLA-DQB, Myasthenia gravis

INTRODUCTION

Myasthenia gravis (MG) is an autoimmune neuromuscular disorder leading to skeletal muscle weakness and fatigability caused by autoantibodies against acetylcholine receptors (AchR) or the muscle-specific kinase at the post-synaptic neuromuscular junction (1,2).

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The severity of muscle weakness is highly variable, ranging from a localized form in ocular myasthenia to a generalized form, which affects many muscles or results in respiratory distress. Thymic hyperplasia or thymoma may also be associated with MG in some cases. Tensilon test, electromyography, and detection of high titer of autoantibodies are used for diagnosis. Cholinesterase inhibitors and immunosuppressants are routinely used for its treatment. Plasmapheresis or intravenous IgG are also applied for short-term assistance of acute attacks while thymectomy is a long-term solution for patients with thymic hyperplasia or thymoma (1).

Although most of the MG patients have almost a normal life with treatment, some of them need treatment for their whole life. Since MG is considered an autoimmune disease, immunotherapy might be useful in its treatment. Altered peptide ligands (APLs) which transfer impaired signals to T cells are potential candidates for a novel treatment of patients with MG (3). To design appropriate APLs, determination of HLA in the patients is necessary. Furthermore, HLA genes play a prominent role in predisposition to many autoimmune diseases. Because HLA region on the short arm of chromosome six is a highly dense genetic region, closely linked HLA and/or non-HLA loci might be involved in susceptibility to autoimmune diseases (e.g. MG) (4). Therefore, in this study, the frequencies of HLA-DQA1 and DQB1 alleles were determined in southern Iranian patients with myasthenia gravis.

MATERIALS AND METHODS

In this study, blood samples were collected with informed consent from 104 Iranian patients with sporadic myasthenia gravis whose disease was diagnosed by edrophonium test, electromyogram, and repetitive stimulation test. DNA was extracted by salting out method. HLA DQA1 and DQB1 alleles were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method (5) and the results were compared to the data from 816 unrelated healthy Iranians as the control group (6). Allele and haplotype frequencies in each group were analyzed using Arlequin ver. 2. Significant variation of allele frequencies between patients and controls was calculated by Chi-square test using Epi Info ver. 6.

RESULTS AND DISCUSSION

Out of 104 (61 females and 38 males) MG patients studied here, 86 patients (82.7%) were early-onset and 18 patients (17.3%) were late-onset MG.

HLA-DQA1*0101/2 (39.4%) was the most common allele in the MG patients whereas *0501 (39.2%) revealed the highest frequency among the controls (Table 1). DQA1*0501 allele may be considered a protective HLA allele against MG disease ($p=0.026$, $RR=0.73$). This is in agreement with the data reported from China (7) while in a report from Sweden, DQA1*0103 revealed a negative association to the disease (8). In our study, DQA1*0101/2 was significantly more frequent in MG patients than in normal controls ($p=0.00007$, $RR=1.69$). This allele might be considered a predisposing genetic factor for MG. DQA1*0101/2 and DQB1*0503 showed higher frequencies in male MG patients ($p=0.038$ and $p=0.025$, respectively), while, the frequency of DQB1*0601 was higher in female patients ($p=0.012$).

When we analyzed our data according to the age of disease onset, no significant difference was observed except for DQB1*0301 which was more frequent in the late-onset patients (p=0.031).

In this study, DQB1*0502 (21.6%) and DQB1*0301 (27.1%) alleles revealed the highest frequencies in MG patients and healthy controls, respectively (Table 1). The former allele showed a positive relation to the disease (p=0.000, RR=2.41) while the latter allele revealed a negative association with the disease (p=0.034, RR=0.71). DQB1*0502 was also reported as the most frequent allele in MG patients of Turkey (9). A correlation between DQB1*03 and early-onset MG in females was previously reported in Japan (10) while an association between DQB1*0302 and MG was observed in China (7) and DQB1*0201 was remarkably detected in Swedish females with early-onset MG (8). As shown, variable HLA alleles may protect or predispose people to MG and may also affect the age of disease onset, clinical presentation or severity of the disease. Furthermore, these associations are inconsistent among different populations, which could be explained by HLA gene diversity among races.

Table 1. HLA-DQA1 and DQB1 allele frequencies in southern Iranian patients with myasthenia gravis and normal controls

DQA1 allele	Controls (2n=1632)	MG patients (2n=208)	P.val., RR	Male MG (2n=76)	Female MG (2n=132)	P.val.	Early-onset MG (2n=172)	Late-onset MG (2n=36)	P.val.
0101/2	429 (26.3%)	82 (39.4%)	0.00007, 1.69	37 (48.7%)	45 (34.1%)	0.038	71 (41.3%)	11 (30.6%)	NS
0103	217 (13.3%)	18 (8.7%)	NS	4 (5.3%)	14 (10.6%)	NS	13 (7.6%)	5 (13.9%)	NS
0201	140 (8.6%)	15 (7.2%)	NS	3 (3.9%)	12 (9.1%)	NS	12 (7.0%)	3 (8.3%)	NS
0301	171 (10.5%)	23 (11.1%)	NS	7 (9.2%)	16 (12.1%)	NS	19 (11%)	4 (11.1%)	NS
0401	33 (2.0%)	2 (0.1%)	NS	1 (1.3%)	1 (0.8%)	NS	1 (0.6%)	1 (2.8%)	NS
0501	640(39.2%)	65 (31.1%)	0.026, 0.73	23 (30.3%)	42 (31.8%)	NS	53 (30.8%)	12 (33.3%)	NS
0601	2 (0.1%)	3 (1.4%)	0.0006, 5.37	1 (1.3%)	2 (1.5%)	NS	3 (1.7%)	–	NS
DQB1 allele									
0201	344 (21.1%)	43 (20.7%)	NS	12 (15.8%)	31 (23.5%)	NS	38 (22.1%)	5 (13.9%)	NS
0301	442 (27.1%)	42 (20.2%)	0.034, 0.71	16 (21.1%)	26 (19.7%)	NS	30 (17.4%)	12 (33.3%)	0.031
0302	10 (0.6%)	–	NS	–	–		–	–	
0303	157 (9.6%)	15 (7.2%)	NS	8 (10.5%)	7 (5.3%)	NS	14 (8.1%)	1 (2.8%)	NS
0402	39 (2.4%)	2 (1.0%)	NS	–	2 (1.5%)	NS	2 (1.2%)	–	NS
0501	86 (5.3%)	14 (6.7%)	NS	6 (7.9%)	8 (6.1%)	NS	10 (5.8%)	4 (11.1%)	NS
0502	144 (8.8%)	45 (21.6%)	0.000, 2.41	19 (25%)	26 (19.7%)	NS	39 (22.7%)	6 (16.7%)	NS
0503	90 (5.5%)	16 (7.7%)	NS	10 (13.2%)	6 (4.5%)	0.025	13 (7.6%)	3 (8.3%)	NS
0601	114 (7.0%)	15 (7.2%)	NS	1 (1.3%)	14 (10.6%)	0.012	10 (5.8%)	5 (13.9%)	NS
0602/3	158 (9.7%)	10 (4.8%)	0.022, 0.50	3 (3.9%)	7 (5.3%)	NS	10 (5.8%)	–	NS
0604	47 (2.9%)	6 (2.9%)	NS	1 (1.3%)	5 (3.7%)	NS	6 (3.5%)	–	NS

RR, Relative Risk; NS, non significant

The most common haplotype was DQA1*0101/02-DQB1*0502 (19.2%) in the MG patients while DQA1*0501-DQB1*0301 with a frequency of 25.3% was the predominant haplotype among the controls (Table 2).

Table 2. DQA1-DQB1 haplotype frequencies (HF) in southern Iranian patients with myasthenia gravis and normal controls

Patients (n=104)		Controls (n=816)	
DQA1-DQB1	**HF%**	**DQA1-DQB1**	**HF%**
0101/2-0502	19.2	0501-0301	25.3
0501-0301	18.1	0501-0201	11.9
0501-0201	10.2	0101/2-0502	8.8
0101/2-0503	7.2	0201-0201	7.7
0103-0601	6.3	0301-0303	7.6
0101/2-0501	5.8	0103-0601	6.4
0201-0201	5.2	0303-0602/3	6.2
0301-0303	4.7	0101/2-0503	5.3
0301-0201	3.3	0101/2-0501	5.1
0101/2-0604	2.9		

In summery, the results of this study revealed a positive association between DQA1*0101/2 and DQB1*0502 alleles, and myasthenia gravis while DQA1*0501 showed a protective role against this disease. However, in this study we focused on HLA-DQ loci because of their critical roles in immune responses. Other genes (e.g. the subunits of the AChR and chemokines) are also considered as candidate genes in recent decades. The AChR of muscle is a pentameric glycoprotein of four different subunits ($\alpha 2\beta\gamma\delta$). CHRNA1 gene, which encodes the α-subunit, is the main target of pathogenic autoantibodies in MG (11). Association of a biallelic variant in the CHRNA1 promoter with early-onset of MG was reported in France and United Kingdom (11). Giraud et al. suggested that, both the CHRNA1 promoter variant and autoimmune regulator gene modulate CHRNA1 expression in thymic epithelial cells and adjust the threshold of autoreactive T cells for self-tolerance versus autoimmunity (11). Feferman et al. detected increased expression of CXL10 and CXCR3 in thymus and muscle of MG patients. According to the association of CXCL10/CXCR3 signalling with MG pathogenesis, they proposed these molecules as novel drug targets to treat MG (12). Therefore, finding a gene with strong association to MG not only can shed more light on the pathogenesis of this disease but also would be helpful in therapeutic designations.

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