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Frequency of HLA-DRB1 Gene Alleles in Patients With Multiple Sclerosis in a Lithuanian Population

Renata Balnytė[1], Daiva Rastenytė[1], Dalia Mickevičienė[1], Antanas Vaitkus[1], Erika Skrodenienė[2], Astra Vitkauskienė[2]

[1]Department of Neurology, Medical Academy, Lithuanian University of Health Sciences, Lithuania,
[2]Department of Laboratory Medicine, Medical Academy, Lithuanian University of Health Sciences, Lithuania

***Key words**: multiple sclerosis; human leukocyte antigen; case-control study; heterozygous inheritance; homozygous inheritance.*

***Summary**. The aim of the present study was to investigate the influence of HLA-DRB1 alleles on the genetic susceptibility to multiple sclerosis in the Lithuanian population.*

Material and Methods. A total of 120 patients with multiple sclerosis and 120 unrelated healthy controls were enrolled in this case-control study. Allelic frequencies were compared between the groups. HLA-DRB1 alleles were genotyped using the polymerase chain reaction.

*Results. HLA-DRB1*15 was present in 55.8% of the patients with multiple sclerosis and 10.0% of the controls (OR, 5.58; 95% CI, 3.19–9.77; P<0.0001). The protective alleles that were found to be more prevalent among the controls compared with the patients with multiple sclerosis were HLA-DRB1*01 (26.7% vs. 7.5%, P<0.0001), *03 (17.5% vs. 8.3%, P=0.034), and *16 (11.7% vs. 3.3%, P=0.014). HLA-DRB1*15 was more common among the female patients with multiple sclerosis than among the male patients (68.4% vs. 34.1%; OR, 4.18; 95%, CI 1.90–9.22; P=0.001). The heterozygous inheritance of HLA-DRB1*15 allele was more common in the patients with a history of maternal multiple sclerosis than in those with a history of paternal multiple sclerosis (29.4% vs. 9.8%; P=0.045).*

*Conclusions. HLA-DRB1*15 was found to be associated with multiple sclerosis in the Lithuanian population. This allele was more prevalent among the female patients with multiple sclerosis. Maternal multiple sclerosis was more common than paternal multiple sclerosis, but the relationship with HLA-DRB1*15 allele was not established. HLA-DRB1*01, *03, and *16 appeared to be the protective alleles in this series.*

Introduction

Multiple sclerosis (MS) is a multifactorial disease whose etiology and pathogenesis, despite the abundance of studies, are not completely understood yet. Certain genetic, immunologic, and environmental factors are believed to be involved in the MS development although there is a nonuniformity of the results provided by different studies. Genetic variation is an important determinant of susceptibility to and progression of MS (1). The human leukocyte antigen (HLA) class II region, which is located on the short arm of chromosome 6 at p21.3 part, has been identified as the genetic factor with the strongest influence on the susceptibility to MS (1, 2). The main genetic susceptibility locus for MS was thought to reside specifically at the HLA-DRB1*15 allele within the major histocompatibility complex (MHC) class II region (3, 4). Class II molecule expression occurs on antigen-presenting cells: macrophages, dendritic cells, B lymphocytes, epithelial cells of thymus, activated T lymphocytes, and vascular endothelial cells (2). The expression of these molecules is modulated by cytokines, which are involved in the inflammatory process as well as antigen binding and presentation, and determination of T-cell activity (2, 3, 5). HLA-DRB1*15 allele was investigated as a genetic risk factor for MS in many case-control and family studies around the world. The largest number of studies (n=34, 57%) was performed in populations of Europe, North and South America, and Australia (5–8). It was demonstrated that HLA-DRB1*15 allele was the most prevalent in North European and European Caucasian patients (4), while in other European regions, such as the Mediterranean basin (e.g., Sardinia), association between HLA-DRB1*0301, HLA-DRB1*0405, and HLA-DRB1*1303 and MS was predominantly observed (9). A higher prevalence of HLA-DRB1*07 was observed in Italy (10); meanwhile, HLA-DRB1*13 was more frequently associated with MS in Israel (11). This indicates that the HLA-DRB1 locus is highly polymorphic with different allele distributions among different ethnic groups.

Correspondence to R. Balnytė, Department of Neurology, Medical Academy, Lithuanian University of Health Sciences, Eivenių 2, 50028 Kaunas, Lithuania. E-mail: rbalnyte@yahoo.com

Based on genetic epidemiological studies, the first, second, and third generations of people with a family history of MS have a higher risk of developing the disease compared with the general population. The risk of developing MS is 15–35 times greater among the first-degree relatives than in the rest of population (1, 2, 12, 13). According to the established age-adjusted lifetime risk, the risk of MS is 30.0%±5.0% for monozygotic twins, 6.0%±4.0% for dizygotic twins, 14.0%±4.0% for children who have one parent with MS and 4.0%±0.5% for those who have a sibling with MS, and 2% if there is a second-generation relative with MS (12–14). Although the risk is higher in those with a family history of MS, the inheritance of the disease mechanisms is still not clear.

In Lithuania, the prevalence of HLA-DRB1 alleles as well as the importance of family history among patients with MS has not been studied; thus, the aim of the present study was to analyze the frequency of HLA-DRB1 allelic groups and the relevance of family history in the Lithuanian MS population.

Material and Methods

Subjects. The study group comprised 120 MS patients who were referred to the Department of Neurology at the Hospital of Lithuanian University of Health Sciences in Kaunas during 2009–2010, agreed to participate in the study, and were older than 18 years. The study included patients with a confirmed MS diagnosis only. MS diagnosis was established according to the widely accepted and revised McDonald criteria (2005) (15). All the laboratory data (oligoclonal band [OCB] status), findings of magnetic resonance (MR) imaging, and data of visual evoked potentials (VEPs) were retrospectively reviewed from the medical records of the patients. Lumbar puncture and cerebrospinal fluid examination were performed at the time of diagnosis. All imaging studies were conducted with a 1.5-T MR scanner (MAGNETOM Avanto, Siemens, Erlangen, Germany) with a standard head coil. The standard pattern-shift VEPs were recorded for all 120 patients. Registration of VEPs was done by the Evoked Potential Navigating System (Bio-Logic System Corp., USA). The responses were considered abnormal if the P100 latency was longer than 114 ms (i.e., 2 SD above the mean) (16). Matched cerebrospinal fluid (CSF) and plasma samples were analyzed using isoelectric focusing and IgG specific immunofixation to test the presence of intrathecal specific OCBs and compared directly with the serum samples (17). Positive OCBs were defined if more than 2 bands were present in the CSF, but absent in the corresponding blood serum (18). The IgG index was calculated from the serum and CSF albumin and IgG concentrations using the following formula:

$$\text{IgG index} = (\text{CSF IgG} / \text{serum IgG}) / (\text{CSF albumin} / \text{serum albumin}).$$

The IgG index was considered as high if it was $\geqslant 0.70$ (17). Demographic (age at onset of the first symptoms, gender) and clinical data (disease course, duration of the symptoms, disability status) and the results of all paraclinical tests were collected for all patients. Disability was measured using the Kurtzke Expanded Disability Status Scale (EDSS). The patients were followed up prospectively, and their clinical status was checked every 3 months.

The control group consisted of 120 healthy subjects matched to the patients with MS for age and sex.

HLA Genotyping. Blood samples were obtained from patients with MS and stored at –20°C. DNA was extracted from blood leukocytes by the standard phenol-chloroform method. DNA was dissolved in the sterile double distilled water. HLA-DRB1 alleles for patients with MS were genotyped using the polymerase chain reaction (PCR) with amplification of the second exon of the genes. An amplified product was manually dot blotted onto nylon membranes. Synthetic sequence-specific oligonucleotide probes were 3′-end-labeled with αP32-dCTP and used for hybridization followed by stringency washes and autoradiography. HLA-DRB1 alleles were genotyped using the PCR with sequence-specific primers (HLA-DRB1*-PCR) supplied by Protrans following the manufacturer's recommendations (PROTRANS Medicinische Diagnostische Produkte GmbH, Germany). Each sample was genotyped by a set of 24 PCRs, which resolved HLA-DRB1*01, HLA-DRB1*03, HLA-DRB1*04, HLA-DRB1*07, HLA-DRB1*08, HLA-DRB1*09, HLA-DRB1*11, HLA-DRB1*12, HLA-DRB1*13, HLA-DRB1*14, HLA-DRB1*15, and HLA-DRB1*16. The amplified products were determined by means of agarose gel electrophoresis. Laboratory analyses were carried out at the Laboratory of Clinical Chemistry and Genetics, Hospital of Lithuanian University of Health Sciences.

The study was approved by the Kaunas Regional Bioethics Committee. Informed consent form was signed by each patient before entering the study.

Statistical Analysis. Analysis of the collected data was performed using the statistical package SPSS version 13.0. Comparisons of mean ages at onset across the groups were carried out using the Student *t* test. Parametric statistical criteria were used for the normally distributed quantitative variables (estimated with the Kolmogorov-Smirnov and Shapiro-Wilk tests), and means with standard deviations (SD) were calculated. The χ^2 test was used to compare the qualitative variables and to estimate

possible correlations. Odds ratios (OR) with 95% confidence interval (CI) were calculated to estimate associations. The determined level of significance α=0.05 and P values lower than 0.05 ($P<\alpha$) were considered as statistically significant.

Results

The MS group comprised 120 subjects: 44 men (36.7%) and 76 women (63.3%). The mean age of the patients with MS was 43.75 years (SD, 10.1): 41.79 years (SD, 9.97) for men and 44.8 years (SD, 10.05) for women. Half (50.0%) of the 120 MS patients had the relapsing-remitting disease course. The mean duration of the symptoms was 11.93 years (SD, 8.0). The disease most frequently manifested by visual symptoms (61.7%), followed by the brainstem (47.5%) and pyramidal symptoms (45.0%) (Table 1).

The control group consisted of 53 men (44.2%) and 67 women (55.8%), whose mean age was 41.99 years (SD, 10.9): 41.32 years (SD, 11.1) for men and 42.52 years (SD, 10.9) for women.

The frequencies of the HLA-DRB1 alleles in the patients with sporadic MS and healthy controls are shown in Table 2. A significantly higher frequency of HLA-DRB1*15 as well as HLA-DRB1*08 alleles was found in the patients with MS than in the controls (55.8% vs. 10.0%; OR, 5.58; 95% CI, 3.19–9.77; $P<0.0001$, and 16.7% vs. 6.7%, OR 1.84, 95% CI 1.01–3.36; $P=0.016$, respectively). Some protective alleles, which were negatively associated with MS, were more common in the control group, i.e., HLA-DRB1*01 (26.7% in the control group vs. 7.5% in the study group, $P<0.0001$), HLA-DRB1*03 (17.5% vs. 8.3%, $P=0.034$), and HLA-DRB1*16 (11.7% vs. 3.3%, $P=0.014$). The frequencies of other alleles did not significantly differ between the patients with MS and the controls.

The analysis of the HLA-DRB1 allele frequencies according to sex revealed that HLA-DRB1*15 allele was more common in both sexes in the MS group when compared with the control group (34.1% in men and 68.4% in women in the MS group vs. 11.3% in men and 9.0% in women in the control group,

Table 1. The Main Demographic and Clinical Data of the Patients with Multiple Sclerosis

Characteristic	Patients With Multiple Sclerosis N=120
Male-to-female ratio	1:1.72
Age at onset, mean (SD) [range], years	30.86 (7.92) [16–55]
Disease course	
Relapsing-remitting	60 (50.0)
Secondary progressive	48 (40.0)
Primary progressive	12 (10.0)
Duration of the symptoms, mean (SD), years	11.93 (8.0)
Duration of the disease (from diagnosis to study), mean (SD), years	6.84 (3.54)
Relapse rate per patient per year, mean (SD)	1.36 (0.88)
First symptoms	
Pyramidal	54 (45.0)
Sensory	42 (35.0)
Visual	74 (61.7)
Gastrointestinal/bladder	20 (16.7)
Cerebellar	43 (35.8)
Brainstem	57 (47.5)
Mental	40 (33.3)
EDSS score during the last visit, mean (SD)	4.26 (2.01)
EDSS score at the time of diagnosis, mean (SD)	3.8 (1.0)
Lesions on MRI	
Periventricular	116 (96.7)
Corpus callosum	104 (86.7)
Brainstem	77 (64.2)
Cerebellum	59 (49.2)
Juxtacortical	63 (52.5)
Spinal cord	25 (20.8)
VEP abnormalities, OS/OD	85 (70.8)/83 (69.2)
IgG index, mean (SD)	0.70 (0.29)
Oligoclonal bands	88 (73.3)

Values are number (percentage) unless otherwise indicated. EDSS, Expanded Disability Status Scale; MRI, magnetic resonance imaging; VEP, visual evoked potential; OD, oculi dextri; OS, oculi sinistri; IgG, immunoglobulin G.

Table 2. Frequency of HLA-DRB1 Alleles in Patients With Multiple Sclerosis and Control Patients

HLA-DRB1 allele	Group With Multiple Sclerosis N=120	Control Group N=120	*P*	OR (95% CI)
*01	9 (7.5)	32 (26.7)	0.0001	0.56 (0.45–0.71)
*03	10 (8.3)	21 (17.5)	0.034	0.69 (0.52–0.92)
*04	18 (15.0)	24 (20.0)	0.39	1.41 (0.72–2.77)
*07	30 (25.0)	29 (24.2)	0.9	1.0 (0.55–1.79)
*08	20 (16.7)	8 (6.7)	0.016	1.84 (1.01–3.36)
*09	0 (0.0)	1 (0.8)	0.5	–
*11	27 (22.5)	40 (33.3)	0.084	1.72 (0.97–3.05)
*12	9 (7.5)	7 (5.8)	0.8	1.28 (0.49–3.34)
*13	19 (15.8)	29 (24.2)	0.14	0.65 (0.38–1.12)
*14	3 (2.5)	3 (2.5)	–	1.0 (0.19–5.05)
*15	67 (55.8)	12 (10.0)	0.0001	5.58 (3.19–9.77)
*16	4 (3.3)	14 (11.7)	0.014	0.61 (0.46–0.81)

Values are number (percentage). OR, odds ratio; CI, confidence interval.

P<0.0001). In the MS group, HLA-DRB1*15 was more common in the female patients (68.4% vs. 34.1%; OR, 4.18; 95% CI, 1.90–9.22; *P*=0.001). Among the male patients, HLA-DRB1*08 allele was more frequent in the patients with MS than in the controls (18.2% vs. 5.7%; OR, 2.13; 95% CI, 0.79–5.68; *P*=0.053). HLA-DRB1*01, as a protective allele, was more common in the male patients of the control group (22.6% vs. 6.8%; OR, 0.62; 95% CI, 0.45–0.87; *P*=0.032) (Table 3).

As mentioned above, HLA-DRB1*15 allele was more common among the female patients with MS when compared with the control group (*P*<0.0001). On the contrary, other alleles were more frequently found in the female patients of the control group than in those with MS. These alleles – HLA-DRB1*03 (20.9% vs. 2.6%; OR, 0.47; 95% CI, 0.36–0.63; *P*=0.001) and HLA-DRB1*01 (29.9% vs. 7.9%; OR, 0.52; 95% CI, 0.38–0.71; *P*=0.001) – were found to be protective alleles against the disease (Table 4).

Regarding the inheritance of HLA-DRB1*15 allele, 74.6% of patients were heterozygous and 25.4% of patients were homozygous (*P*<0.0001). No significant associations were found while analyzing the inheritance of other alleles (data not shown).

Of all the MS patients, 24.2% had mothers, 5.0% had fathers, and 2.5% had a sibling with MS. More female MS patients had mothers with MS than male patients (26.3% vs. 20.4%), and no other associations between MS in family members and patient sex were observed (*P*>0.05) (Table 5). The analysis of the association between HLA-DRB1 alleles and MS in family members of the MS patients demonstrated that HLA-DRB1*15 allele was present in 28.4% of the patients who had a mother, in 7.5% of the patients who had a father, and in 1.5% of those who had a sibling with MS; however, a difference between groups was not significant (*P*=0.23) (Fig.). No association between other alleles and family history of MS was observed.

The comparison of heterozygous and homozygous inheritance of HLA-DRB1*15 allele and MS in family members showed that the heterozygous inheritance of this allele was more common in subjects with a family history of maternal MS than in those with a history of paternal MS (29.4% vs. 9.8%, *P*=0.045).

Table 3. Frequency of HLA-DRB1 Alleles in Men With Multiple Sclerosis and Control Patients

HLA-DRB1 allele	Group With Multiple Sclerosis N=44	Control Group N=52	*P*
*01	3 (6.8)	12 (22.6)	0.032
*03	8 (18.2)	7 (13.2)	0.5
*04	6 (13.6)	11 (20.8)	0.3
*07	10 (22.7)	16 (30.2)	0.4
*08	8 (18.2)	3 (5.7)	0.053
*09	0 (0.0)	1 (1.9)	0.3
*11	10 (22.7)	17 (32.1)	0.3
*12	4 (9.1)	4 (7.5)	0.7
*13	9 (20.5)	14 (26.4)	0.5
*14	1 (2.3)	1 (1.9)	0.9
*15	15 (34.1)	6 (11.3)	0.0001
*16	1 (2.3)	4 (7.5)	0.24

Values are number (percentage).

Table 4. Frequency of HLA-DRB1 Alleles in Women With Multiple Sclerosis and Control Patients

HLA-DRB1 allele	Group With Multiple Sclerosis N=76	Control Group N=67	*P* value
*01	6 (7.9)	20 (29.9)	0.001
*03	2 (2.6)	14 (20.9)	0.001
*04	12 (15.8)	13 (19.4)	0.6
*07	20 (26.3)	14 (20.9)	0.5
*08	12 (15.8)	5 (7.5)	0.12
*09	0 (0.0)	0 (0.0)	–
*11	17 (22.4)	23 (34.3)	0.1
*12	5 (6.6)	3 (4.5)	0.5
*13	10 (13.2)	15 (22.4)	0.15
*14	2 (2.6)	2 (3.0)	0.9
*15	52 (68.4)	6 (9.0)	0.001
*16	3 (3.9)	10 (14.9)	0.23

Values are number (percentage).

Table 5. Multiple Sclerosis in Family Members of the Studied Patients With Multiple Sclerosis

Family Members With Multiple Sclerosis	Women N=76	Men N=44
Mother	20 (26.3)	9 (20.4)
Father	4 (4.0)	3 (6.8)
Sibling	2 (2.6)	1 (2.27)

Values are number (percentage).

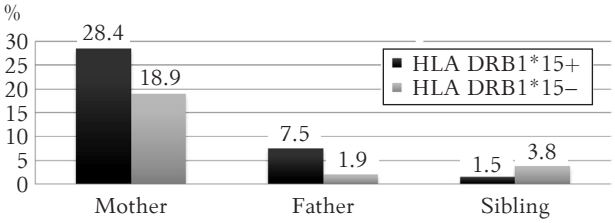

Fig. HLA-DRB1*15 and multiple sclerosis in a family

Discussion

In the present study, 55.8% of all the MS patients were positive for HLA-DRB1*15. HLA-DRB1*08 was also found to be more common in the MS patients than in the healthy controls (16.7% vs. 5.7%). HLA-DRB1*01, *03, and *16 were the protective alleles that were found to be more prevalent among the control patients. HLA-DRB1*15 allele was more frequently found in the MS patients of both sexes (34.1% of men and 68.4% of women) when compared with the control group (11.3% of men and 9.0% of women). Among the MS patients, this allele was more common in women than men. HLA-DRB1*08 allele was more frequent in the male

patients with MS than in the control male patients (18.2% vs. 5.7%). On the contrary, HLA-DRB1*01 allele was found to be more common in the healthy control men than in the men with MS (22.6% vs. 6.8%), while HLA-DRB1*03 and *01 alleles were more frequent in the female patients of the control group than in the MS group (20.9% vs. 2.6% and 29.9% vs. 7.9%, respectively).

The present study has demonstrated for the first time that HLA-DRB1*15 allele is associated with susceptibility to MS in the Lithuanian population as it was found previously in many studies, especially in Caucasian populations (5). The high frequencies of HLA-DRB1*15 have been reported in Northern European series (19, 20); there are also reports on significant associations in Mediterranean populations (21–24) as well as in North and South America (6, 7, 25, 26). Recently, a large study of Australian patients with MS has shown a strong association (27); there is also some evidence from the Far East and India (28, 29).

HLA-DRB1*08 was the second most frequent allele that was found to be associated with MS in the Lithuanian population. Similar findings were reported by Dyment et al. (25). Some other studies have reported HLA-DRB1*03 as the second most frequent allele associated with MS (21, 22, 27). In contrast to these reports, this allele, as well as HLA-DRB1*01 and *16 alleles, was found to be protective in our study. Similarly, Fernandez et al. reported HLA-DRB1*01 allele being protective in the Biscay province population (30). According to the results of other studies, the protective alleles for MS might be HLA-DRB1*11 (6, 24, 31), HLA-DRB1*04 (27), and HLA-DRB1*09 (22). The results of the present study show that HLA-DRB1*15 is more frequent among female patients with MS, and this is consistent with the data of other authors (22, 32–34). No data on HLA and MS associations from our neighboring countries (i.e., Poland, Latvia, or Estonia) were found.

The mothers of our MS patients had MS more often than other family members; moreover, the HLA-DRB1*15 allele was more frequent in the patients who had a mother with MS. Data on the direct transmission from an affected parent to an affected child are controversial. A study from the United States reported that men more often than women transmitted the disease to children, but in a similar study carried out in Canada, no differences were found between paternal and maternal transmission of this disease to children (35, 36). Ramagopalan et al. indicated that disease transmission was more related to mothers (37). In addition, a higher number of affected mother-daughter pairs than father-son pairs were detected by many studies (34, 35). HLA-DRB1*15 allele may be associated with MS in a family to indicate a possible genetic predisposition (38). One of the limitations of our study is that the parents of the patients with MS were not examined. This could have helped to understand the genetic predisposition better.

This is the first report of the association between HLA and MS in the Lithuanian population. This allows a comparison with data of other countries and contribution of new data to the global genomic map of MS.

Conclusions

HLA-DRB1*15 was found to be associated with multiple sclerosis when comparing patients with unrelated healthy controls in the Lithuanian population. This allele was more prevalent among female patients with multiple sclerosis. More multiple sclerosis patients had mothers with multiple sclerosis, but the relationship with HLA-DRB1*15 allele was not established. HLA-DRB1*01, *03, and *16 appeared to be the protective alleles in this series. Maternal multiple sclerosis was more common than paternal multiple sclerosis in a family history of the patients with multiple sclerosis. The heterozygous inheritance of HLA-DRB1*15 allele was more common in the patients with a history of maternal multiple sclerosis than in those with a history of paternal multiple sclerosis.

Statement of Conflict of Interest

The authors state no conflict of interest.

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