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Associations of HLA DRB1 alleles with IgG oligoclonal bands and their influence on multiple sclerosis course and disability status

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ABSTRACT

Background and aim: Oligoclonal bands (OCB) may be associated with the genes of HLA complex, which allows to consider the possible interaction of genetic and immunological factors and its importance in the development and progression of multiple sclerosis (MS). The aim of this study was to evaluate the associations between HLA DRB1 alleles and oligoclonal bands (OCBs) in the disease course and disability of multiple sclerosis (MS) patients.

Materials and methods: This was a prospective study of 120 patients with MS. HLA DRB1 alleles were genotyped using the polymerase chain reaction. Matched cerebrospinal fluid (CSF) and plasma samples were analyzed using isoelectric focusing and IgG specific immunofixation to test for the presence of intrathecal specific OCB.

Results: HLA DRB1*08 allele was related to a lower degree of disability. Oligoclonal bands were an independent and significant factor that influenced disability status irrespective of HLA DRB1* 04, *07, *08, *13, *15 and *16 alleles. Age at the onset and duration of the disease were independent and significant factors for MS progression in all logistic regression models with each newly added HLA DRB1 allele. HLA DRB1*08 allele was related to 75% lower odds that relapsing remitting (RR) MS will change to a progressive course MS irrespective of the other factors investigated. Detection of OCBs in the CSF was associated with the higher possibility of RR MS progression in all cases, except when the *08 allele was present.

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Conclusions: OCBs had an influence on disability status, while HLA DRB1*08 allele was significantly associated with lower possibility that RR MS will change to progressive course MS.

1. Introduction

Multiple sclerosis (MS) is known to be the most common and usually severe disease among many idiopathic demyelinating conditions that affect the central nervous system, believed to involve complex etiopathogenetic interactions between a variety of genetic and environmental factors [1–3]. These beliefs are strongly supported by data from epidemiological and immunological research. As epidemiological studies have shown, MS can be triggered by environmental factors in a genetically susceptible individuals [4]. On the other hand, in patients with MS the development of the disease and certain clinical and immunological features are strongly influenced by the HLA complex [3,4]. The mechanism through which such influence might occur is believed to be the interdependence between the HLA-DRB1 genotype and the phenotypic status of the oligoclonal immunoglobulin G (IgG) bands (OCBs) [5] that are present in the cerebrospinal fluid (CSF) in the majority (85–95%) of clinically confirmed MS patients [6,7] and are known to reflect the intrathecal synthesis of IgG antibodies [8,9], but depending on the geographical position, OCBs can be detected in 76–97% of Western Europeans with MS [10–12] and in 55–56% of MS patients in Asian countries [13–16]. Although opinions about OCB impact on the course of MS course are somewhat controversial – some authors state that detection of OCBs could be associated with more aggressive course of the disease [13,18–21], while the others believe that OCBs are related to slower MS progression, better course, lower disability and female sex [14] – it is clear that OBCs foment a variety of the manifestations of MS in a major way. At the same time, higher prevalence of HLA-DRB1 *1501 allele may be linked to an earlier onset of the disease, less favorable course, and a female gender – all factors which are believed to be particularly important in the prognosis and treatment of MS [6,7]. Such differences indicate that geographic location and ethnic features might be very important confounding variables that affect expression of immunogenetic factors in MS [11–17].

Hence, we conducted the prospective cohort observational study with an explicit aim of determining the relationship between the immunogenetic risk factors, clinical features, and level of the disability in MS patients among Lithuanian population.

2. Materials and methods

2.1. Enrolment of the patients

The study was approved by the Kaunas Regional Bioethics Committee [No. 2R-493]. The study group was comprised of 120 MS patients, all older than 18 years, who were referred to the Department of Neurology at the Hospital of Lithuanian University of Health Sciences in Kaunas during 2009–2010 and were willing to participate in the study. The study included patients with confirmed MS diagnosis only. MS diagnosis was established according to widely accepted and revised McDonald criteria (2005) [22]. An informed consent form was signed by each patient before entering the study. Lumbar puncture and cerebrospinal fluid (CSF) examination was performed at the time of the diagnosis. Matched CSF and plasma samples were analyzed using isoelectric focusing and IgG specific immunofixation with the purpose of testing for the presence of intrathecal specific OCB and comparing directly with the serum samples [18]. Positive OCBs were defined when more than 2 bands were present in the CSF, while absent in the corresponding blood serum [8]. Demographic (age at onset of the first symptoms, gender) and clinical data (disease course and duration of the symptoms, disability status), 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.

2.2. HLA genotyping

Blood samples were obtained from patients with MS and stored at −20 °C. DNA was extracted from blood leukocytes by standard phenol-chloroform method. DNA was dissolved in the sterile double distilled water. HLA DRB1 alleles for MS patients were genotyped using a 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 and 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, *03, *04, *07, *08, *09, *11, *12, *13, *14, *15, and *16. Laboratory analyses were carried out in the Laboratory of Clinical Chemistry and Genetics, Hospital of Lithuanian University of Health Sciences.

2.3. Statistical analysis

Analysis of the collected data was performed using the statistical package SPSS version 13.0. Comparisons of mean age at onset of MS across groups were carried out using the Student *t* test. Parametric statistical criteria were used for the

normally distributed quantitative variables (estimated with Kolmogorov–Smirnov and Shapiro–Wilk tests) and the mean and 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 when searching for the associations. For the control of type I error, the level of significance was preselected to be $\alpha = 0.05$. Values of p lower than 0.05 ($P < \alpha$) were considered to indicate statistical significance. Logistic regression models were constructed for the estimation of the impact of immunological, genetic and clinical variables on the progression of disease (disability and disease course). For the investigation of the effect of two interdependent factors (genetic and immunological) on the outcome variable (disability) we used two-factor analysis of variance.

3. Results

The MS group consisted of 120 subjects: 44 (36.7%) men and 76 (63.3%) women. The mean age of the MS patients was 43.75 ± 10.1 years (men, 41.79 ± 9.97 years; women, 44.8 ± 10.05 years). Of the 120 MS patients, 50.0% had the relapsing-remitting (RR) course of the disease; 40.0%, the secondary-progressive (SP) course; and 10.0%, the primary progressive (PP) course. The mean duration of the first symptoms was 11.93 ± 8.0 years (Table 1).

The HLA DRB1 *08 allele was more frequently documented among the RR MS patients than among the patients with the progressive forms of MS (25% vs. 8.3%, respectively; $P = 0.014$), while the HLA DRB1*15 allele was more prevalent among the patients with the progressive forms of MS (35% vs. 20.8%, respectively; $P < 0.001$). During the last visit, the lowest EDSS score (3.15 ± 1.95) was found among the patients with the HLA DRB1 *08 allele ($P = 0.006$) and the highest (4.60 ± 2.10), among those with HLA DRB1 *15 allele ($P = 0.047$). However, there were no significant associations between these alleles and the duration of the disease and disability. Positive OCBs were more prevalent among MS patients with higher degree of disability (4.61 ± 1.96 vs. 3.31 ± 1.89, respectively; $P = 0.002$) and more frequent conversion to the progressive course observed during the last visit (56.6% vs. 43.2%, respectively; $P = 0.036$). The HLA DRB1*15 allele was more common among the MS patients with OCBs in the CSF than among those without (80.6% vs. 64.2%; OR = 2.3, 95% CI 1.017–5.301; $P = 0.043$) (Table 2).

The HLA DRB1 *08 allele demonstrated a relation of borderline significance to lower degree of disability (according to the EDSS score: 3.69 ± 0.3 vs. 4.30 ± 0.1, $P = 0.062$), while no relation was found among other HLA DRB1 alleles and disability status, irrespective of the presence or absence of OCBs. Oligoclonal bands were an independent and significant factor that was related to disability status irrespectively of the HLA DRB1 * 04,*07,*08,*13,*15 and *16 alleles. Disability status (according to the EDSS score) was also dependent on the interaction between HLA DRB1 *07 allele and OCBs ($P = 0.049$). No statistically significant associations were found between the other alleles and OCBs (Table 3).

Table 1 – The main demographical and clinical data of patients with multiple sclerosis.

Characteristic	MS patients N = 120
Gender ratio (M:F)	1:1.72 (44:76)
Age at onset, mean ± SD (range), years	30.86 ± 7.92 (16–55)
Disease course, *n* (%)	
Relapsing-remitting	60 (50.0)
Secondary-progressive	48 (40.0)
Primary-progressive	12 (10.0)
Duration of the first symptoms, years, mean ± SD	11.93 ± 8.0
Duration of the disease (time of diagnosis), years, mean± SD	6.84 ± 3.54
Relapse rate per year, mean ± SD	1.36 ± 0.88
EDSS[A] score, mean ± SD	4.26 ± 2.01
EDSS[B] score, mean ± SD	3.8 ± 1.0
OCB, *n* (%)	88 (73.3)

EDSS, Expanded Disability Status Scale; EDSS[A], score during last visit; EDSS[B], score at time of diagnosis; OCB, oligoclonal bands.

Table 2 – Frequency of HLA DRB1 gene alleles and oligoclonical bands in patients with multiple sclerosis.

HLA DRB1 alleles	MS group (N = 120), *n* (%)	OCB		*P*
		OCB (+) N = 88, *n* (%)	OCB (–) N = 32, *n* (%)	
*01	9 (7.5)	5 (55.6)	4 (44.4)	NS
*03	10 (8.3)	6 (60)	4 (40)	NS
*04	18 (15.0)	10 (55.6)	8 (44.4)	NS
*07	30 (25.0)	21 (70)	9 (30)	NS
*08	20 (16.7)	12 (80)	8 (40)	NS
*09	0 (0.0)	–	–	–
*11	27 (22.5)	16(59.2)	11 (40.8)	NS
*12	9 (7.5)	5 (55.6)	4 (44.4)	NS
*13	19 (15.8)	11(57.9)	8 (42.1)	NS
*14	3 (2.5)	2 (66.7)	1 (33.3)	NS
*15	67 (55.8)	54 (80.6)	13(19.4)	0.043
*16	4 (3.3)	2 (50)	2 (50)	NS

OCB, oligoclonical bands; NS, not significant.

Table 3 – HLA DRB1 allele, oligoclonal bands and their interaction to disability (EDSS score) of multiple sclerosis patients (two-factorial analysis).

HLA DRB1 alleles value to disability			OCB value to disability		HLA DRB1 alleles and OCB interaction value to disability
Allele	EDSS score	*P*	EDSS score	*P*	*P*
*01	3.34 ± 0.12 vs. 3.70 ± 0.1	0.2	3.35 ± 1.2 vs. 3.78 ± 1.1	0.7	0.38
*03	3.49 ± 0.18 vs. 3.98 ± 0.2	0.19	4.05 ± 0.2 vs. 4.30 ± 0.1	0.11	0.69
*04	4.58 ± 0.2 vs. 5.20 ± 0.1	0.087	2.7 ± 0.3 vs. 3.9 ± 0.12	0.008	0.92
*07	3.19 ± 0.2 vs. 3.35 ± 0.2	0.93	3.00 ± 1.3 vs. 4.30 ± 1.1	0.0001	0.049
*08	3.69 ± 0.3 vs. 4.30 ± 0.1	0.062	4.22 ± 0.4 vs. 5.2 ± 0.1	0.018	0.87
*11	4.4 ± 0.24 vs. 4.70 ± 0.1	0.14	3.78 ± 1.2 vs. 4.80 ± 1.1	0.005	0.41
*12	4.69 ± 1.3 vs. 5.0 ± 1.4	0.11	2.78 ± 2.3 vs. 3.30 ± 1.8	0.14	0.51
*13	2.54 ± 0.3 vs. 3.03 ± 0.3	0.18	3.9 ± 1.1 vs. 5.0 ± 1.21	0.005	0.83
*14	3.09 ± 1.3 vs. 3.60 ± 1.2	0.32	3.19 ± 0.2 vs. 3.43 ± 0.4	0.7	0.13
*15	4.11 ± 0.4 vs. 4.30 ± 0.1	0.4	4.01 ± 0.3 vs. 5.12 ± 0.2	0.003	0.73
*16	3.09 ± 0.23 vs. 3.5 ± 0.12	0.23	4.19 ± 1.2 vs. 5.4 ± 1.1	0.024	0.30

EDSS, Expanded Disability Status Scale; OCB, oligoclonical bands.

Logistic regression models were constructed and revealed an impact of the patient's age at onset of the disease, duration of MS, gender, HLA DRB1 alleles, and oligoclonal bands on MS progression. Patient's age at the onset of the disease and duration of MS were independent and significant factors for MS progression in all logistic regression models with each newly added HLA DRB1 allele (excluding models with *07,*13, and *14 alleles where age at onset had only borderline significance). The HLA DRB1*08 allele was related to 75% lower odds that RR MS will convert to progressive course MS irrespectively of other factors investigated (Table 4). In the model with *08 allele, immunological factors had no impact on MS progression. Detection of oligoclonal bands in the CSF was associated with the higher possibility of RR MS conversion to a progressive course in all cases except in the model with *08 allele.

4. Discussion

The results of our study have shown that OCB and HLA DRB1 alleles could influence both, the disease course of MS and disability level. The HLA DRB1 *08 allele may be associated with a lower possibility that RR MS would convert to the progressive course of the disease regardless of other analyzed factors. Meanwhile, the immunological factors (especially oligoclonal bands) were associated with the higher probability of transition to the progressive course of the disease. They also influenced the progression of the disability among MS patients in Lithuania.

Table 4 – HLA DRB1*08 allele, oligoclonal bands, disease duration, gender and age at onset value of the risk of relapsing remitting to switch from progressive form of multiple sclerosis (logistic regression model).

Factor	β	OR (95% CI)	*P*
Age at onset	0.072	1.07 (1.00–1.15)	0.046
Gender	0.127	1.13 (0.47–2.70)	NS
HLA DRB1*08	1.214	0.25 (0.06–0.92)	0.03
OCB	−1.649	1.37 (0.61–3.08)	NS
IgG index	0.893	2.41 (0.31–18.9)	NS
Disease duration	0.076	1.08 (1.01–1.13)	0.005

β (beta), regression coefficient; OR, odds ratio; CI, confidence interval; OCB, oligoclonical bands; IgG index, immunoglobulin G index; NS, not significant.

We found that the HLA DRB1 *08 allele tended to be associated with lower disability and was more frequent among the RR MS, while the HLA DRB1 *15 allele was more prevalent among the patients with the progressive forms of MS. Other HLA DRB1 alleles had no impact on the disability and the disease course. Presence of OCBs in CSF in MS patients correlated with higher degree of disability and progressive course of the disease. Oligoclonal bands were associated with disability status independently of HLA DRB1 * 04, *07, *08, *13, *15 and *16 alleles. Interaction between HLA DRB1 *07 allele and OCBs had an impact on disability status. Age at the onset and disease duration were independent and significant factors for the course of the disease to become progressive. Independently of the other factors analyzed, the HLA DRB1*08 allele was associated with the 75% lower odds that RR MS would change to progressive disease form. Furthermore, when this allele was detected the immunogenetic factors did not influence changes of the disease course. Oligoclonal bands detected in the CSF increased the possibility for RR MS form to become progressive in all the analyzed cases except the cases when the HLA DRB1 *08 allele was detected. On the basis of this finding it might be presumed that OCBs could be considered as a marker that is related to the conversion of MS course [18–20]. Moreover, independently of the HLADRB1 allele type (an exception being HLA DRB1 *08 allele which was associated with a better prognosis) our findings indicated that immunogenetic factors, especially OCBs, were related to the higher disability level and more progressive course of the disease. According to the controversial and quite sparse data from the literature, OCBs can be related to higher disability level, especially when the HLA DRB1 *15 allele is detected; conversely the HLA DRB1*04 allele can be associated with higher disability level independently of the OCBs presence [23]. Other authors claim that OCBs can indicate tendency to improved disability [14]. However, Koch et al. have found no relation between the OCBs and disability and no tendency of RR MS change to SP

form [18]. On the other hand, Romero-Pinel et al. suggested that HLA DRB1 alleles had no impact on MS course when comparing MS relapsing with progressive onset [24]. All of that controversies point to the lack of data that estimate effect of oligoclonal bands and HLA DRB1 gene alleles on clinical manifestation of the MS. Therefore, much larger studies are needed to detect the most significant factors influencing the MS course. Our results indicate that oligoclonal bands could be used to evaluate the clinical activity of the disease and to forecast its course. It can be accomplished by detecting HLA DRB1 gene alleles in the early stage of MS. The results of such test might be useful in forecasting the clinical course of MS and for choosing the most appropriate prevention and management strategy. However, importance of the detection of HLA DRB1 gene alleles in the early stage of the disease for the purposes of reducing progression of the disease and disability should be studied more extensively in a much larger cohort. Our study was limited to the relatively small patient cohort and therefore should be considered as a pilot study, therefore results awaiting to be reconfirmed on the larger set of Lithuanian MS patients in the near future.

5. Conclusions

This study points to the both distinct and shared genetic risk factors in Lithuanian MS patients that differ by the presence or absence of the OCBs in CSF. OCBs influenced level of disability and the disease course irrespective of the HLA DRB1 alleles and increasing the probability of the disease progression. HLA DRB1 *08 allele can be associated with the lower probability of RR MS transformation into the progressive course MS. The HLA DRB1 *15 allele was found to be associated to the progressive course of the disease, and a higher level of disability, while the HLA DRB1*08 allele was more common in the patients with relapsing-remitting course of the MS and a lower level of disability. In summary, our results suggest that OCBs and HLA DRB1 alleles may have an impact on the disability, natural course of the disease and thus might supply additional information on the disease prognosis of a patient; however, further and large-scale studies are still needed.

Conflict of interest

The authors declare that they have no competing interests.

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