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Introduction: The association between susceptibility to MS and class II alleles of the major histocompatibility complex (MHC) is well established in MS patients but not in NMOR. The ethnicity has an important role in MS HLA DQ and DR profile. Brazilian population has ethnic particularities with a high mixed African and Caucasian Mediterranean population. We previously demonstrated the association of HLA-DR2 in white Brazilians MS patients from Rio de Janeiro. Here we analyze the HLA DQ and DR haplotypes in NMOR patients with from the same population.
Methods: 64 NMOR patients (Afro: 41, white:23) attended in Hospital da Lagoa (Rio de Janeiro, Brazil) were selected for the study and genotyped for the DQA1, DQB1 and DRB1 loci. The results were compared with 106 MS patients and 103 healthy controls. Anti-AQP4 positive and negative subgroups were compared.
Results: No association was found between NMOR and the extended haplotype DRB1*1501 DQA1*0102 DQB1*0602 or its alleles, neither in the total group nor in white and Afro Brazilian subgroups. White NMOR and MS patients differ concerning this haplotype ($p = 0.001$). There were no differences in AQP4 positive subgroup compared to the AQP4 negative.
Conclusion: This is the first study that definitely showed the lack of association of the HLA DR2 in NMO. This observation reinforces the distinction between NMO and MS.

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MEFV gene mutations in German multiple sclerosis patients and controls

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Objective: To investigate the MEFV mutation frequency in patients with multiple sclerosis (MS) and in controls from Germany and to define the phenotype of heterozygous mutation carriers.
Background: Familial Mediterranean fever (FMF; MIM 249100) is a hereditary autoinflammatory disorder characterised by recurrent episodes of sterile, painful peritonitis, pleuritis, arthritis, and cutaneous manifestations, accompanied by fever. It is caused by mutations in the MEFV gene on chromosome 16p13.3 encoding the protein pyrin and is frequently observed in individuals from the Middle East and Mediterranean countries. A possible relationship between FMF and MS has been discussed in Turkish patients suffering from both FMF and demyelinating CNS disease.
Methods: Two groups of patients and one control group were investigated. 147 MS patients (group 1) from our outpatient clinic were screened for mutations in exons 2, 3, and 10 of the MEFV gene. 77 MS patients of group 1 had at least one symptom compatible with an autoinflammatory syndrome. In addition, 260 unselected, independent MS patients (group 2) from a nearby hospital specialized on MS and 400 Caucasian controls (group 3) were analyzed for the presence of the low-penetrance pyrin mutations E148Q and K695R encoded by MEFV exons 2 and 10.
Results: Thirteen MS patients (16.8%) of group 1 with symptoms suggestive of FMF tested positive for one mutation in the MEFV gene. FMF symptoms were mild to moderate without classical fever episodes. In four of these 13 individuals, diagnosis of MS was delayed due to an unusual onset of MS and additional rheumatological symptoms. Four patients had ancestors from Mediterranean countries. Three MS patients (4.2%) of group 1 without FMF symptoms were also heterozygous carriers of a MEFV mutation. In group 2, ten (3.8 %) MS patients were heterozygous for one of the two low-penetrance mutations (E148Q: $n = 7$; K695R: $n = 3$). Seventeen German controls (4.2 %) also tested positive for these two amino acid substitutions (E148Q: $n = 10$; K695R: $n = 7$).
Conclusion: We identified a group of MS patients with additional symptoms compatible with FMF who were heterozygous MEFV mutation carriers. As about 4% of unselected German MS patients and controls were E148Q and K695R heterozygotes, FMF should be considered as a differential diagnosis in MS patients as well as in non-MS patients from central Europe with a history suggestive of an autoinflammatory disease.

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LA. Gerdes T. Wacker and P. Lohse have nothing to disclose

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Lack of association of a KIF1B polymorphism with disease progression in Greek patients with multiple sclerosis

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Background: KIF1B is a neuronally expressed gene with a putative role on axonal loss that has been recently found to be associated with susceptibility to multiple sclerosis (MS). A possible effect of KIF1B on disease progression and severity has not been extensively studied to date.
Objective: To investigate a possible association of the KIF1B gene with disease progression and severity in a cohort of Greek patients with MS.
Patients and Methods: We studied a total of 554 Greek patients with MS. The rs10492972 SNP in the KIF1B gene was genotyped in all patients. We used Kaplan-Meier analysis with time to reach EDSS 4.0 or 6.0 as end-points and the MS Severity Score (MSSS) to assess a possible effect of the KIF1B gene on disease progression and severity.
Results: Our cohort included 358 female and 196 male patients with MS. Disease course was relapsing-remitting in 396, secondary progressive in 83 and primary progressive in 75 patients. Time to reach EDSS 4.0 was similar for C-allele carriers (15.0 yrs, 95% CI: 11.1-18.9) and non-carriers (13.0 yrs, 95% CI: 10.2-15.8; p 0.16). Time to reach EDSS 6.0 was also similar for C-allele carriers (22.0 yrs, 95% CI: 19.3–24.7) and non-carriers (29.0 yrs, 95% CI: 23.8–34.2; p 0.50). Finally, the MSSS was not significantly different between C-allele carriers (3.5 ± 2.9) and non-carriers (3.9 ± 2.8; p 0.11).
Conclusions: No association was found between the rs10492972[C] variant of the KIF1B gene and disease progression or severity in Greek patients with MS.

The authors report no conflict of interest.

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Angiotensin II receptor type 2 (AT2R) -1332 A/G gene polymorphism as a risk factor for multiple sclerosis

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Background: Multiple sclerosis (MS) is a complex inflammatory, demyelinating disease of central nervous system (CNS). All the essential components of the renin-angiotensin system (RAS) are presented in the mammalian brain. The angiotensin II (Ang II), biologically active octapeptide is not only a vasoconstrictor, but also a pro-inflammatory factor. Many of the classical and of the hypothetical functions of brain Ang II are mediated by stimulation of AT1 receptors (AT1R). Brain AT2 receptors (AT2R) are highly expressed during development. In the adults, AT2R are restricted to areas predominantly involved in the process of sensory information. The AT2R −1332 A/G polymorphism was proposed to influence AT2R protein expression, and is the most studied polymorphism in this gene, in other diseases. Recently, the striking appearance of the RAS in MS brain was described. However, the role of AT2R remains to be clarified. Thus, the aim of our study was to establish if there is an association between AT2R −1332 A/G gene polymorphism and predisposition of MS.

Methods: Subjected group consisted of 122 female and 70 male patients with MS and 75 female and 50 male controls from population of Serbia. Genotyping was done by PCR and restriction digestion with EcoRI enzyme.
Results: The genotype and allele frequencies for AT2R −1332A/G gene polymorphism are analyzed separately in females and males, since this gene is located on X chromosome. We detected significant overrepresentation of −1332A/G AA genotype (OR 1.6, 95% CI:1.0–2.7, $p < 0.05$) in female patients with MS compared to female controls. In hemizygous males we didn't found any difference between patients and controls.
Conclusion: The role of RAS genes in MS was neglected until recently. Than, it was shown that the role of RAS in the CNS is beyond the regulation of cardiovascular function. Until now AT2R (−1332A/G) gene polymorphism was widely studied and associated with hypertension and other vascular disease. Until now, there were no studies concerning role of Ang II receptor polymorphisms in MS. This study suggest possible role of AT2R in MS. Further studies are needed to elucidate this result.

The authors have nothing to disclose.

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Transcriptional response signature of immune cells to IFN-b reveals distinct patterns in monocytes

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Monocytes, which have been acknowledged as important players in MS pathogenesis, are outnumbered by neutrophils and lymphocytes in peripheral blood mononuclear cells (PBMC). Interferon-b (IFN-b) is a widely used immuno-modulatory drug in MS and much effort has been invested to identify a gene expression signature associated with either good or poor response to the drug. Our research hypothesis was that the monocytes distinct response to IFN-b might be obscured by other PBMC and that differences in the monocytes expression profile may contribute to the drug response variability in MS patients. CD14+ monocytes and CD3+ T lymphocytes were isolated from 3 healthy blood donors using commercially available kits and treated with IFN-b. RNA was harvested and hybridized to gene expression arrays; data was analyzed using JMP Genomics and GOrilla. ANOVA yielded 397 and 147 differentially expressed transcripts in monocytes and in T cells respectively (p-value $\leq 10^{-3}$ and fold change ≥ 2), from which only 6 were common to both. Function enrichment analysis revealed immune functions in both cell types, but these were much more prominent in the T lymphocytes. The results were validated using RNA from 3 additional donors by RT-PCR on 8 genes, seven novel with respect to IFN-b response and one known, and further validation on the protein level is in progress. These results suggest that in monocytes IFN-b activates a signaling pathway that is different from the IFN-b canonical pathway, and includes monocytes-specific genes that might have been overlooked among the general immune cells. Future studies should evaluate the effect of monocyte-related genes on the response profile of MS patients treated with IFN-b.

The authors have nothing to disclose.

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Exploring the CLEC16A gene reveals a MS-associated variant with correlation to the relative expression of CLEC16A isoforms in thymus

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Genomewide association studies have implicated the CLEC16A gene in several autoimmune diseases, including multiple sclerosis (MS) and type 1 diabetes. However the most associated SNP varies, and causal variants are still to be defined. In MS, two SNPs in partial linkage disequilibrium with each other, rs6498169 and rs12708716, have been validated at genomewide significance level. To explore the CLEC16A association in MS in more detail, we genotyped 57 SNPs in 807 Norwegian MS patients and 1027 Norwegian controls. Six highly associated SNPs emerged and were then replicated in two large independent sample sets (Norwegian and British) together including 1153 MS trios, 2308 MS patients and 4044 healthy controls. In combined analyses, SNP rs12708716 gave the strongest association signal in MS ($P = 5.3*10–8$, OR 1.17 95% CI 1.11–1.25), and was found to be superior to the other SNP associations in conditional logistic regression analyses. Expression analysis revealed that rs12708716 genotype was significantly associated with the relative expression levels of two different CLEC16A transcripts in thymus ($P = 0.004$), but not in blood, possibly implying a thymus- or cell-specific splice regulation.

The authors have nothing to disclose.

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Validation of IRF5 as multiple sclerosis risk gene: putative role in interferon beta therapy and human herpesvirus-6 infection

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Background: In a recent paper, two SNPs in the IRF5 (interferon regulatory factor 5) gene (rs4728142 and rs3807306) showed significant association with MS susceptibility in three studied populations. IRF5 is expressed mainly in lymphocytes and dendritic cells, but it is induced in other cells in response to type I interferon. IRF5- deficient mice have been reported to exhibit an increased susceptibility to viral infection, linked to a significant decrease in the induction of serum type I interferon.
Objectives: In the present study, we evaluated the association of two IRF5 polymorphisms with MS predisposition and we also addressed whether these polymorphisms were associated with active replication of HHV-6 observed in a subgroup of MS patients. In addition, we tested the role of the IRF5 polymorphisms as genetic predictors of IFN-beta response.
Methods: A total of 1496 MS patients and 1506 ethnically matched controls were genotyped for rs4728142 and rs3807306 with TaqMan pre-designed assays in three independent Spanish cohorts: Madrid, Bilbao and San Sebastián. Serum samples were collected and analyzed for the presence of HHV−6 at 6, 12, 18 and 24 months of follow-up. One hundred and six patients were classified as responders to IFN-beta therapy (no relapses or increases in EDSS over the 2-year follow-up) and 112 as non-responders (at least two relapses or an increase in EDSS of at least 1 point during the same period).
Results: The rs3807306 T allele was significantly associated with MS in the Madrid cohort ($P = 0.03$). Neither rs4728142 nor rs3807306 reached significance in any of the other individual case-control datasets. The combined analysis of this study in conjunction with the previously published cohorts yielded an effect size on MS susceptibility with ORMantel-Haenszel = 1.14 ($p < 0.002$) for both IRF5 polymorphisms. Additionally, the T allele of rs3807306 unraveled as a promising marker for both, infection with HHV−6 [$p = 0.05$, OR (95% CI) = 1.56 (1.00–2.44)] and response to IFN-beta therapy [$p = 0.09$, OR (95% CI) = 1.39 (0.95–2.05)].
Conclusions: The T allele of rs3807306 emerges from this study as a significant marker for both susceptibility to MS and infection with HHV-6, and seems to be a promising marker for response to IFN-beta therapy.

The authors have nothing to disclose.