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ARTICLE

Autoimmune and immunogenetic profile of patients with optic neuritis in a population-based cohort

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ABSTRACT

Background: Optic neuritis (ON) is an inflammatory optic neuropathy, where the genetic and autoimmune dependency remains poorly characterized.

Objective: To investigate autoimmune and immunogenetic aspects of ON.

Method: In a prospective population-based cohort 51 patients with ON were included. At follow up 20 patients had progressed to multiple sclerosis (MS-ON). All patients were screened for neuronal and systemic autoantibodies. HLA genotypes and allele and genotype frequencies of the PTPN22 C1858T and the PD-1.3 single-nucleotide polymorphisms (SNPs) were determined and compared to a cohort of Danish blood donors, acting as healthy controls.

Results: Median follow-up was 366 days (301-430) for MS-ON patients and 375 (range 50-436) for isolated ON (ION). Autoantibodies against myelin oligodendrocyte glycoprotein (MOG-IgG), were positive in two patients, no patients had anti-aquaporin-4 antibodies. Coexisting neural autoantibodies were detected in two patients and in 12 patients other systemic autoantibodies were found. Four (8%) had other autoimmune disorders. A family history of autoimmunity was observed in 12 (24%) and of demyelinating disease in six patients (12%). In MS-ON patients the frequencies of HLA-DQB1*06:02 and HLA-DRB1*15:01 tended to be higher compared to controls (p=0.08). Stratification of patients with presence of oligoclonal bands (OCB) showed an association to the HLA-DQB1*06:02-HLA-DRB1*15:01 haplotype in ION (HLA-DQB1*06:02 and HLA-DRB1*15:01 (p=0.03)), and in MS-ON patients (HLA-DQB1*06:02 and HLA-
DRB1*15:01 ($p=0.03$)). No significant associations to PTPN22 1858 C/T or PD-1.3 G/A were found in any group comparison.

**Conclusions:** ON patients had a general susceptibility to autoimmunity and two were MOG-IgG positive. HLA-DQB1*06:02 and HLA-DRB1*15:01 were associated with the presence of OCB in ON patients.

**INTRODUCTION**

Optic neuritis (ON) is a focal inflammatory demyelinating event highly associated with multiple sclerosis (MS)(Toosy et al., 2014). ON may also be seen in relation to other autoimmune diseases(Asgari et al., 2011; Frigui et al., 2011), infections(Kallenbach and Frederiksen, 2008) or in a paraneoplastic context(Asproudis et al., 2005; Soelberg et al., 2016). Furthermore, autoantibodies against myelin oligodendrocyte glycoprotein (MOG-IgG) or the astrocyte water channel aquaporin-4 (AQP4) have been identified in ON patients(Asgari et al., 2011; Pache et al., 2016). In general, MS-ON and antibody-mediated ON are different in therapeutic response and prognosis, which likely reflect differences in disease mechanisms(Jarius et al., 2016a). ON is a common first symptom of MS and identification of risk factors for the development of MS has become more important due to the introduction of early disease modifying treatments(Jarius et al., 2016a).

Variation in antigen presentation by the human leukocyte antigen (HLA) molecules plays an important role in autoimmunity(Fernando et al., 2008). A genetic susceptibility for MS is supported by an overrepresentation of certain HLA alleles(Frederiksen et al., 1997). Susceptibility to MS has been linked mainly to the HLA-DRB1 locus, with the HLA-DR15 haplotype (DRB1*15:01, DQA1*01:02-DQB1*06:02, DRB5*01:01) as the predominating risk factor for disease development in Caucasians(Barcellos et al., 2003; Brynedal et al., 2007). In ON and MS-ON patients the frequency of HLA-DR15 haplotype has also been shown to be
higher as compared to healthy controls (Frederiksen et al., 1997). The association with the HLA molecules points to T cell involvement and supports a role for autoimmunity in MS (Compston and Coles, 2002).

Apart from HLA, PTPN22 1858T allele has been linked to several autoimmune diseases (Zheng et al., 2012). However, the PTPN22 1858T allele has been shown to have negligible association with MS and neuromyelitis optica spectrum disorders (NMOSD) (Asgari et al., 2012). Disruption of the PD1 pathway has been linked to breakdown of self-tolerance and development of autoimmune disease (Francisco et al., 2010). In our previous study (Asgari et al., 2012), the presence of the PD-1.3A allele was significantly increased in NMOSD and MS patients compared to healthy controls (Asgari et al., 2012). We aimed to investigate autoimmune traits and immunogenetic aspects of ON in this prospective population-based cohort.
MATERIAL AND METHODS

Patients and controls
Patients (n=51) originated from a population-based, prospective case series with one year of follow-up as reported previously (Soelberg et al., 2017). In brief, patients were included if they fulfilled the diagnosis of ON as described by the Optic Neuritis Study Group (1991), in the present study evaluated independently by a neurologist and an ophthalmologist. Patients were excluded if already diagnosed with MS or NMOSD. MS was diagnosed according to the 2010 (Polman et al., 2011) criteria and, the diagnosis of NMOSD was made according to the 2015 International Panel for NMOSD Diagnosis criteria (Wingerchuk et al., 2015). Randomly selected healthy Danish blood donors living in the Region of Southern Denmark, acted as controls and were genotyped for HLA class I and II (n=1576) and for the SNPs PD-1.3 (n=155) and PTPN22 C1858T (n=354). All assays used have been validated ensuring specificity above > 95% in blood donors. Being blood donors, these controls have no symptoms of autoimmune disease. Their familial history is unknown.

Laboratory methods

*Determinations of AQP4-IgG and MOG-IgG autoantibodies*
Presence of IgG autoantibodies to AQP4 were determined with a recombinant immunofluorescence assay using HEK293 cells transfected with recombinant human full-length
AQP4 gene (Asgari et al., 2012) and re-evaluated by means of an in-house cell based assay at the University of Heidelberg in a blinded fashion (Jarius et al., 2010).

MOG-IgG were determined using two cell-based assays employing fixed and live HEK293 cells, respectively, transfected with full-length human MOG as previously described (Jarius et al., 2016b; Mader et al., 2011).

**Determination of other autoantibodies**

Screening for other neural and systemic autoantibodies in serum was performed using validated standard methods in an accredited laboratory at the Department of Clinical Immunology, Odense University Hospital (Asgari et al., 2017). Briefly, screening for antinuclear antibodies (ANA) was done by indirect immunofluorescence (IIF) using HEp2 cells as substrate at a 1:160 dilution (AESKU Diagnostics, Wendelsheim, Germany); anti-dsDNA antibodies were detected by both enzyme-linked immunosorbent assay (ELISA) (Phadia, Uppsala, Sweden) and IIF using Critidia Luciliae as substrate (INOVA Diagnostics, California, USA); IgG extractable nuclear antigen antibodies (ENA) (anti-centromeres, anti-Jo1, anti-RNP, anti-Ro52, anti-Ro60, anti-Scl-70, anti-Sm and anti-SS-B,) were determined by chemiluminescent immunoassay (CLIA) (INOVA Diagnostics, California, USA) and anti-histones by ELISA (INOVA Diagnostics, California, USA).

Smooth muscle (actin) antibodies, parietal cell (H+/K+ ATPase) antibodies, IgA tissue transglutaminase 2 antibodies and mitochondrial (E2 subunits of pyroate dehydrogenase complex (PDC-E2), branched chain 2-oxo-acid dehydrogenase complex (BCOADC-E2) and 2-oxo glutarate dehydrogenase complex (OGDC-E2)) antibodies were all determined by ELISA (INOVA Diagnostics, California, USA). Acetylcholine receptor antibodies were determined by radioimmunoassay (IBL, Hamburg, Germany).

Screening for IgG antibodies against neuronal antigens (NMDAR, CASPR2, LGI1, AMPAR and GABABR) was performed using transfected HEK293 cells expressing the respective recombinant target antigens at a 1:10 dilution (Euroimmun, Luebeck, Germany).

Screening for IgA, -G, -M antibodies associated with paraneoplastic neurologic syndromes (anti-CV2 (CRMP5), anti-amphiphysin, anti-Hu, anti-Ma2, anti-Ri, anti-Yo and anti-SOX1) was done by IIF using monkey cerebellum as substrate (Euroimmun, Luebeck, Germany). Sera were
screened at 1:10 and 1:100 dilutions. All results were confirmed by line immune assay (LIA) detecting IgG antibodies (Euroimmun, Luebeck, Germany). Positive results were confirmed independently at the Euroimmun Laboratory.

**Human Leucocyte Antigen (HLA) Typing**

High resolution (4 digits) typing of HLA class I and II were performed at HistoGenetics LLC (New York, USA) using Next Generation Sequencing (NGS) technology on the Illumina MiSeq platform (Cereb et al., 2015).

*Genotyping of the programmed death-1.3 (rs11568821) and PTPN22 C1858T (rs2476601) Single Nucleotide Polymorphisms (SNPs)*

DNA extraction and genotyping of PD1.3A and PTPN22 C1858T SNPs were performed as previously described (Asgari et al., 2012).

Standard protocol approvals, registrations, and patient consents:
The Regional Health Research Ethics Committee for the Region of Southern Denmark (ref. no. S-20130137) and the Danish Data Protection Agency (ref. no. 14/26345) approved the study. All patients provided oral as well as written informed consent.

Statistical analysis
Genotype and allelic frequencies were compared using Fisher’s exact test. Bonferroni’s correction was used for multiple comparisons (simultaneous inference), multiplying the value of \( p \) obtained in the statistical test by the total number of alleles tested (corrected \( p , cp \)) (data not shown). In view of the known association between HLA-DQB1*06 and HLA-DRB1*15 alleles and MS, no correction was made for the number of antigens tested (corrected and uncorrected statistics are shown). Odds ratios (ORs) were obtained from Woolf’s method. A \( p \)-level of 0.05 was used as limit of significance.
RESULTS

Clinical characteristics and antibody findings

All patients were of Caucasian origin. Thirty five were women and 16 men (ratio 2.2:1). Median age at diagnosis was 38 (range 16-66) years. Median follow-up time was 366 days (range 301-430) for MS-ON patients, 374.5 (range 372-377) for MOG-IgG patients and 375 (range 50-436) for isolated ON (ION). There was no significant difference between the follow-up period for the patients diagnosed with MS compared to the rest of the group (\(p=0.3798\)). Within the first year of follow-up, 20 (39%) patients converted to MS. Cerebrospinal fluid (CSF) analysis showed oligoclonal bands (OCB) in 22 (47%) patients, 6 with ION and 16 with MS-ON, (\(p<0.001\)). Two (4%) patients had MOG-IgG and 29 (57%) retained the diagnosis of ION. All patients were negative for AQP4-IgG. Autoimmune and clinical characteristics of the patients are depicted in Table 1. A diagnosis of another autoimmune disease was established in 4/51 (7.8%) prior to onset of ON (three with ION (respectively autoimmune thyroiditis, coeliac disease and psoriasis); and one with MS-ON and autoimmune thyroiditis). Familial occurrences of other autoimmune diseases were observed in 12/51 (23.5%) patients (nine with isolated ON, three with MS-ON). A family history of demyelinating disease occurred in six patients (12%), MS in five (three with ION, two with MS-ON) and ION in one. Statistical comparisons did not reach
significance (data not shown), but over all there was a tendency towards a higher frequency of autoimmune associations in patients who retained the diagnosis of ION.

In total, neural or systemic autoantibodies were detected in serum in 16/51 (31%) patients. Neural autoantibodies were positive in four patients and 12 had systemic autoantibodies. As mentioned before, two patients had anti-MOG. Antibodies against collapsin-responsive mediator protein-5 (CRMP-5, also called anti-CV2) were detected in one patient with ION and anti-SOX1 antibodies in one patient with MS-ON. No clinical observations or symptoms compatible with a paraneoplastic neurologic syndrome (PNS) were present in these two patients at follow-up and the patients were referred for further observation. The frequency of autoantibodies in the subgroup of patients who retained the diagnosis of ION was 12/31 (39%), compared to 4/20 (20%) in patients who progressed to MS (Table 1). Anti-nuclear antibodies (ANA) were found in three patients (two with ION and one with MS-ON). Anti-histone antibodies were positive in five (10%) patients (four with ION and one with MS-ON) and weakly positive anti-Sm antibodies were detected in one patient, who had MOG-IgG related ON. This patient did not have symptoms indicating systemic lupus erythematosus or antibodies against double stranded DNA (dsDNA). Anti-smooth muscle antibodies were found in five (10%) patients (three with ION and two with MS-ON). Anti-parietal cell antibodies were found in two patients, both with ION, however vitamin B₁₂ was within normal range in both subjects.

HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQB1, and HLA-DPB1 allele frequencies

Initial analyses comparing frequencies of HLA class I (A, B, C) and class II (DRB1, DQB1, DPB1) in cases with ON and controls did not show any significant differences (data not shown). After stratification no significant differences were observed between, ION, MS-ON, MOG-IgG-ON and controls. HLA-genotyping results of the full cohort including subgroups are available in the Supplementary Material (table 5). The two patients with MOG-IgG were HLA-DQB1*06:02-02:01; HLA-DRB1*15:01-03:01 and HLA-DQB1*03:02-02:01; HLA-DRB1*07:01-04:01, respectively (see Table 5).

HLA-DQB1*06:02 and HLA-DRB1*15:01 allele frequencies
Comparison of HLA-DQB1*06:02 and HLA-DRB1*15:01 allele frequencies in patients with a final diagnosis of ION and patients with MS in relation to healthy controls showed no significant differences (Table 2). However, comparing MS-ON patients and controls revealed near significantly increased frequencies of HLA-DQB1*06:02 (OR 1.97 CI 0.98-3.9) and HLA-DRB1*15:01 (OR 1.94 CI 0.97-3.8, p=0.08) in MS-ON patients (Table 2).

Association between HLA and the presence of OCB in ION and MS-ON

A significantly higher frequency of OCB was observed in MS-ON as compared to ION patients (p<0.001), as previously reported elsewhere (Soelberg et al., 2017). Presence of OCBs in CSF was associated to the HLA-DQB1*06:02 (OR 2.36 CI 1.2-4.9, p=0.03) and HLA-DRB1*15:01 (OR 2.33 CI 1.14-4.9, p=0.03) haplotype in patients with MS-ON, compared to healthy controls. Similar findings were made when comparing ION patients with presence of OCBs in CSF (HLA-DQB1*06:02 (OR 3.7 CI 1.3-10.5, p=0.03) and HLA-DRB1*15:01 (OR 3.7 CI 1.3-10.4, p=0.03)), to healthy controls (Table 2). The one MOG-IgG positive patient with HLA-DQB1*06:02-02:01; HLA-DRB1*15:01-03:01 haplotype did not have OCB.

Frequency of alleles and genotypes of PTPN22 C1858T single-nucleotide polymorphism

Since MOG-IgG-mediated ON is considered a separate disease entity, the two patients with MOG-IgG were excluded when comparing allele and genotype frequencies. Frequency of alleles and genotypes of the PTPN22 C1858T SNP in in the whole population, ION, MS-ON and healthy controls are shown in Table 3. No significant differences were observed between ION, MS-ON and healthy controls for either allele or genotype frequencies.

Frequency of alleles and genotypes of programmed death 1.3 single-nucleotide polymorphism

Frequency of alleles and genotypes of the PD 1.3 SNP in the whole population, ION, MS-ON and healthy controls are shown in Table 4. No significant differences were observed between ON, MS-ON and healthy controls for either allele or genotype frequencies. However, a tendency towards an increased frequency of the PD1.3 AG genotype (p=0.09) and PD-1.3A allele (p=0.11) in patients with MS-ON compared to healthy controls was seen.
DISCUSSION

In this population-based prospective study with a one-year follow-up of patients with acute ON (Soelberg et al., 2017), we found frequent coexistence of autoimmune disease and family history of autoimmune or demyelinating disease. In line with these findings, circulating autoantibodies were detected in a high proportion of patients (31%). In addition, we observed a tendency towards an increased frequency of the HLA-DQB1*06:02-HLA-DRB1*15:01 haplotype in MS-ON patients. Stratification for presence of OCBs showed significant increase of the HLA-DQB1*06:02-HLA-DRB1*15:01 haplotype in both ION and MS-ON patients. Furthermore, two other genetic risk markers of autoimmunity, the PD-1.3A allele and PTPN22 1858T allele, were investigated. We observed a tendency towards increased frequencies of the PD1.3 AG genotype and PD-1.3A allele only in MS-ON compared to healthy controls. However, this did not reach statistically significant levels, perhaps due to small population size. These data suggest clustering of autoimmunity in patients with a focal demyelinating event on the optic nerve, some of whom are at risk for later conversion to MS.

Autoimmune mechanisms are thought to be involved in the pathogenesis of inflammatory demyelinating disease of the central nervous system. In the present study, 8% of the patients with ON had a diagnosis of another autoimmune disorder. Moreover, seropositivity for both neural and systemic autoantibodies could be detected in 31% of ON patients, with a tendency towards higher frequency of autoantibodies in the subgroup of patients who retained the diagnosis of isolated ON (39%) versus patients who had developed MS (MS-ON) at follow-up (20%). Antibodies associated with PNS were detected in two patients, one with ION (anti-CV2/CRMP5) and one who developed MS (anti-SOX1). However, no clinical observations or symptoms compatible with a PNS were present in these two patients at follow-up. Paraneoplastic optic neuritis (PON) associated with anti-CV2 antibodies have previously been described in non-small cell lung cancer (Asproudis et al., 2005), renal cancer (Cross et al., 2003) and thyroid cancer (Cross et al., 2003). Anti-SOX1 antibodies are not well characterized onconeural antibodies, but are instead termed cancer-related (Graus et al., 2010). A recent study (Berger et al., 2016) investigated the prevalence of anti-SOX1 in various neurological diseases and found anti-
SOX1 reactivity in 2/247 (0.8%) of patients with MS. One of these patients developed a thyroid cancer (Berger et al., 2016). These data suggest a general susceptibility to autoimmunity in ON.

In this study, we have shown that patients who developed MS (MS-ON) tended to have increased frequencies of the HLA-DQB1*06:02-DRB1*15:01 haplotype compared to controls. Remarkably, patients with the HLA-DQB1*06:02-DRB1*15:01 haplotype had a significantly higher frequency of OCBs in CSF. HLA-DRB1*15:01 has previously been shown to be a strong risk factor for OCB positivity in MS-patients (Mero et al., 2013). This data corresponds to other studies showing that the HLA-DQB1*06:02-DRB1*15:01 haplotype, dominate the genetic contribution to MS disease-risk. Additionally, studies from different geographical areas have shown that DRB1*15 (part of the HLA-DR15 haplotype) is also a significant predisposing factor for ON (Frederiksen et al., 1997; Soderstrom et al., 1998) compared to healthy controls (Tuwir et al., 2007). In the present study we did not identify specific allelic differences between ION and MS-ON that suggest risk factors for the evolution of ION to MS-ON. This result may partly be a result of the small sample size and the short follow-up period of one year, however, we did show an association between specific haplotypes and the presence of OCB in CSF in ION and MS-ON. All in all, these data suggest that the HLA-DR15 haplotype in with the concurrent presence of OCB is involved in susceptibility to an initial focal demyelinating event. Due to strong linkage disequilibrium in the HLA II region, it has been difficult to establish the isolated effects of each allele predisposing to MS. HLA-DQB1*06:02 and HLA-DRB1*15:01 are commonly placed on the same haplotype HLA-DR15 and therefore it is uncertain whether or not both of them play an important pathogenic role. Interestingly, an experimental study in an MS-disease model has established that DQB1*06:02, but not DRB1*15:01, determines disease-susceptibility in HLA-transgenic mice, suggesting a role for DQB1*06:02 as a predisposing risk allele in MS (Kaushansky et al., 2012).

Identification of the MOG-IgG phenotype of ON creates a differential diagnosis when diagnosing ON. Currently, it is unclear if MOG-IgG are a discrete disease entity different from MS and AQP4-IgG seropositive disease. It seems important to clarify in large cohort studies whether MOG-IgG is associated with certain HLA haplotypes.

Apart from HLA, two molecules of interest for the T cell mediated immune reactivity have been investigated in this study. We have previously observed an increased frequency of the
PD-1.3A allele in NMOSD (Asgari et al., 2012). In addition, we and others have shown significantly increased frequency of the PD-1.3A allele in MS-patients (Asgari et al., 2012; Trabattoni et al., 2009). In the present study, a tendency towards increased frequency of the PD1.3 AG genotype and PD-1.3A allele was observed only in ON patients who progressed to MS (MS-ON). In line with this observation a previous study suggested that the PD-1.3A allele is a contributing genetic modifier of the progression of MS (Trabattoni et al., 2009). Additionally, investigation of experimental autoimmune encephalomyelitis has shown that PD-1 blockade resulted in accelerated and more severe disease (Salama et al., 2003). MS is believed to be a T-cell mediated disease (Lassmann, 2012) and PD-1 interact with PD-L1 and PD-L2 to down regulate T-cell immune responses (Fife and Bluestone, 2008). This supports a role for the PD-1.3A allele as a common susceptibility allele for autoimmune disease.

The PTPN22 gene is a strong non-HLA susceptibility gene shared by many autoimmune diseases with the 1858T allele increasing the risk of disease (Zheng et al., 2012). In accordance with previous studies of MS immunogenetics (Asgari et al., 2012), we did not find a disease association to the PTPN22 1858T allele. It has been suggested, that the PTPN22 1858T allele is involved in the pathogenesis of antibody-mediated diseases, but not in T-cell mediated diseases such as MS (Lassmann, 2012; Zheng et al., 2012).

In conclusion, we describe autoimmune traits in patients with acute ON from a population-based Caucasian cohort and report a high frequency of circulating autoantibodies, coexisting autoimmune disease and family history of autoimmune as well as demyelinating disease. These findings suggest an autoimmune predisposition in patients with ON. We investigated selected immunogenetic aspects of ON and the association between HLA and development of MS. Moreover, we showed a link between HLA distribution and the presence of OCB in CSF in ION and MS-ON. This study adds information to current knowledge on ON.

Disclosures:

**K. Soelberg**: No conflicts of interest.

**AC. Nilsson**: No conflicts of interest.
C. Nielsen: No conflicts of interest.

S. Jarius: No conflicts of interest.

M. Reindl: The Neurological Research Laboratory (Medical University of Innsbruck and Tirol Kliniken, Markus Reindl) receives payments for antibody assays (AQP4- and anti-neuronal antibodies) and for AQP4- and MOG-antibody validation experiments organized by Euroimmun (Germany). Markus Reindl was supported by research grant ‘BIG WIG MS’ from the Austrian Federal Ministry of Science, Research and Economy.

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S.T. Lillevang: No conflicts of interest.

N. Asgari: No conflicts of interest.
References:


Table 1. Optic neuritis and co-existence of other autoimmune diseases. Clinical and serological associations in optic neuritis (ON) versus ON with conversion to multiple sclerosis (MS-ON).

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<th>MS-ON N = 20</th>
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<td>Parietal cell Antibodies</td>
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<td>None</td>
</tr>
<tr>
<td>Acetylcholine receptor Antibodies</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>IgA Tissue transglutaminase 2 Antibodies</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Mitochondrial Antibodies</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Antibodies associated with autoimmune encephalitides</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Anti-AMPAR1/2 (Glutamate receptor 1/2)</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Anti-CASPR2</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Anti-GABAR</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Anti-LGI1</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Anti-NMDAR</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Antibodies associated with paraneoplastic neurologic syndromes</td>
<td>2</td>
<td>None</td>
</tr>
<tr>
<td>Anti-CV2 (CRMP5)</td>
<td>1</td>
<td>None</td>
</tr>
<tr>
<td>Anti-SOX1</td>
<td>None</td>
<td>1</td>
</tr>
<tr>
<td>Anti-Amfiphysin</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Anti-Hu</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Anti-Ma2</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Anti-Ri</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Anti-Yo</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

*Coexisting cytoplasmatic pattern.

**Abbreviations:** ON: Optic neuritis; MS: Multiple sclerosis; MOG: Myelin oligodendrocyte glycoprotein; AQP4: Aquaporin4; RNP: Ribonucleoproteins; dsDNA: double stranded DNA; CASPR2: Contactin-associated protein-like 2; GABAR: Gamma-aminobutyric acid type B receptor subunit 1; LGI1: Leucinerich glioma-inactivated protein 1; NMDAR N-methyl-D-aspartate receptor subunit NR1; CV2 (CRMP5): Dihydropyrimidinase-related protein 5 antibody
Table 2. Distribution of HLA-DQB1*06, and HLA-DRB1*15 allele frequencies in patients with optic neuritis (ON), patients with multiple sclerosis (MS) and healthy controls. Numbers and results in italic are from patients with oligoclonal bands in cerebrospinal fluid.

<table>
<thead>
<tr>
<th>Alleles</th>
<th>ON</th>
<th>MS vs. controls</th>
<th>ON vs. controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-DQB1*06</td>
<td>0 0 0 0</td>
<td>0 0 0 0</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>HLA-DRB1*15</td>
<td>2.36</td>
<td>0 ns</td>
<td>3.7 1.3-10.5 ns</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Alleles</th>
<th>Entire cohort vs. controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-DQB1*06</td>
<td>1.4 0.9 0</td>
</tr>
<tr>
<td>HLA-DRB1*15</td>
<td>2.3 1 7</td>
</tr>
</tbody>
</table>
### Table

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>4.9</th>
<th>0</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
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</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

### Notes

- **OR**, odds ratio; **CI**, confidence interval; **p**, *p*-values from Pearson's chi-square test; **cP**, *P* corrected by Bonferroni's method; **ns**: not significant.

# all other HLA-DQB1*06 subtypes

## all other HLA-DRB1*15 subtypes
Table 3 Frequency of alleles and genotypes of PTPN22 C1858T single-nucleotide polymorphism in patients with *optic neuritis* (ON), patients with multiple sclerosis (MS) and healthy controls.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>ON (n=29)</th>
<th>MS (n=20)</th>
<th>Controls (n=354)</th>
<th>ON vs. MS</th>
<th>ON vs. controls</th>
<th>MS vs. controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>CC</td>
<td>21</td>
<td>72.4</td>
<td>16</td>
<td>80.0</td>
<td>28</td>
<td>81.6</td>
</tr>
<tr>
<td>CT</td>
<td>7</td>
<td>24.1</td>
<td>4</td>
<td>20.0</td>
<td>65</td>
<td>18.3</td>
</tr>
<tr>
<td>TT</td>
<td>1</td>
<td>3.5</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

*CT + TT genotypes against CC genotypes.
Table 4. Frequency of alleles and genotypes of 7146G/A programmed death-1.3A (PD-1.3A) single-nucleotide polymorphisms in patients with optic neuritis (ON), patients with multiple sclerosis (MS) and healthy controls.

<table>
<thead>
<tr>
<th></th>
<th>ON (n=29)</th>
<th>MS (n=20)</th>
<th>Controls (n=155)</th>
<th>ON vs. MS</th>
<th>ON vs. controls</th>
<th>MS vs. controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>OR [CI]</td>
<td>P</td>
</tr>
<tr>
<td>Genotypes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>24</td>
<td>82.8%</td>
<td>14</td>
<td>70.0%</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td></td>
<td>4</td>
<td>5</td>
<td>[0.1]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*0.4</td>
<td>[0.1-1.71]</td>
<td>*0.3</td>
<td>[0.5-2.06]</td>
<td>*0.5</td>
<td>[0.5-3.61]</td>
</tr>
<tr>
<td>AG</td>
<td>5</td>
<td>17.2%</td>
<td>6</td>
<td>30.0%</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
<td>2</td>
<td>0</td>
<td>[1.71]</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>0</td>
<td>0%</td>
<td>0</td>
<td>0%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>53</td>
<td>91.4%</td>
<td>34</td>
<td>85.0%</td>
<td>0.54</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td></td>
<td>9</td>
<td>2</td>
<td>[0.1]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>[0.1-2.06]</td>
<td>[0.1-4.03]</td>
<td>[3.45]</td>
<td>[6.26]</td>
<td>[0.5-6.26]</td>
<td>[0.5-4.03]</td>
</tr>
<tr>
<td>A</td>
<td>5</td>
<td>8.6%</td>
<td>6</td>
<td>15.0%</td>
<td>8</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td></td>
<td>0</td>
<td>0</td>
<td>[2.06]</td>
<td></td>
</tr>
</tbody>
</table>
*AG + AA genotypes against GG genotypes.

**Highlights**

- Co-existence of autoimmunity and family occurrence of autoimmune or demyelinating disease were frequently observed.
- Circulating autoantibodies was detected with a higher frequency of autoantibodies in the subgroup of patients, who retained the diagnosis of isolated optic neuritis (ION) versus patients who developed MS during follow-up.
- HLA-DQB1*0602 and HLA-DRB1*1501 were associated with the presence of OCBs in CSF in both ION and MS-ON.