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

K. Soelberg[a,b,c,d,e,1], A.C. Nilsson[f,1], C. Nielsen[f], S. Jarius[g], M. Reindl[h], B. Wildemann[g], S.T. Lillevang[f], N. Asgari[a,b,c,*]

[a] Institutes of Regional Health Research and Molecular Medicine, University of Southern Denmark, Odense, Denmark
[b] Patient data Explorative Network (OPEN), Odense University Hospital, Odense, Denmark
[c] Departments of Neurology, Slagelse Hospital, Denmark
[d] Departments of Neurology, Lillebaelt Hospital, Denmark
[e] Department of Ophthalmology, Odense University Hospital, Denmark
[f] Department of Clinical Immunology, Odense University Hospital, Denmark
[g] Molecular Neuroimmunology Group, Department of Neurology, University Hospital Heidelberg, Germany
[h] Clinical Department of Neurology, Medical University Innsbruck, Innsbruck, Austria

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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 1858C/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.

1. 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

* Corresponding author at: Neurobiology, Institutes of Molecular Medicine & Regional Health Research, University of Southern Denmark, J.B. Winsloewsvej 25.2, 5000 Odense C, Denmark
E-mail address: nasgari@health.sdu.dk (N. Asgari).
[1] Sharing first authorship.

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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 over-representation 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.

2. Material and methods

2.1. 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.

2.2. Laboratory methods

2.2.1. Determination 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).

2.2.2. 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 pyrovate 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.

2.2.3. Human Leukocyte 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).

2.2.4. 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).

2.3. 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.

2.4. 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.

3. Results

3.1. Clinical characteristics and antibody findings

All patients were of Caucasian origin. Thirty five were women and