RESEARCH ARTICLE OPEN ACCESS

Conformational Antibodies to Proteolipid Protein-1 and Its Peripheral Isoform DM20 in Patients With CNS Autoimmune Demyelinating Disorders

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Neurol Neuroimmunol Neuroinflamm 2025;12:e200359. doi:10.1212/NXI.0000000000200359

Abstract

Background and Objectives

Antibodies to proteolipid protein-1 (PLP1-IgG), a major central myelin protein also expressed in the peripheral nervous system (PNS) as the isoform DM20, have been previously identified mostly in patients with multiple sclerosis (MS), with unclear clinical implications. However, most studies relied on nonconformational immunoassays and included few patients with non-MS CNS autoimmune demyelinating disorders (ADDs). We aimed to investigate conformational PLP1-IgG in the whole ADD spectrum.

Methods

We devised a new live cell-based assay (CBA) for PLP1-IgG and used it to test 2 cohorts (retrospective exploratory, n = 284; prospective validation, n = 824) of patients with ADDs and controls (n = 177). Patients were classified as MS, neuromyelitis optica spectrum disorders (NMOSDs), myelin oligodendrocyte glycoprotein antibody–associated disease (MOGAD), and other ADDs. PLP1-IgG–positive samples were tested for IgG subclasses, DM20-IgG, and on rat brain tissue-based assay (TBA). Complement-dependent cytotoxicity (CDC) was assessed on a live CBA and antigen specificity and conformational binding through immunoadsorption/colocalization/fixation experiments.

Results

PLP1-IgG were found in 0 of 177 controls and 42 of 1104 patients with ADDs mainly diagnosed as other ADDs (19/42) with frequent myelitis/encephalomyelitis (14/19) and coexisting PNS involvement (13/19). Four of 19 patients with other ADDs fulfilled the

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The Article Processing Charge was funded by the authors.

Coinvestigators are listed in the appendix at the end of the article.

Glossary

ADDs = autoimmune demyelinating disorders; **AQP4** = aquaporin 4; **CBA** = cell-based assay; **CCPD** = combined central and peripheral demyelination; **CDC** = complement-dependent cytotoxicity; **EAE** = experimental autoimmune encephalomyelitis; **EDSS** = Expanded Disability Status Scale; **LETM** = longitudinally extensive transverse myelitis; **MOG** = myelin oligodendrocyte glycoprotein; **MOGAD** = myelin oligodendrocyte glycoprotein antibody–associated disease; **MS** = multiple sclerosis; **ON** = optic neuritis; **PLP1** = proteolipid protein-1; **PNS** = peripheral nervous system; **TBA** = tissue-based assay; **TDL** = tumefactive demyelinating lesion; **TM** = transverse myelitis.

seronegative NMOSD criteria. PLP1-IgG were also found in patients with MOGAD (11/42), more frequently with PNS involvement (p = 0.01), and in patients with MS (12/42), more frequently with atypical features (p < 0.001). PLP1-IgG–positive MOGAD had higher EDSS scores (p < 0.001) and PLP1-IgG–positive MS had higher severity scores (MSSS, p < 0.001) compared with those PLP1-IgG–negative. Overall, PLP1-IgG were found in 24.1% of patients with CNS+PNS-ADD, 21.2% with atypical MS, 8.3% with MOGAD, 12.0% with seronegative NMOSD, and 1.4% with typical MS. Their frequency within each diagnostic subgroup was consistent between the exploratory and validation cohorts. PLP1-IgG a) colocalized with their target on CBA-TBA, where their binding was abolished after immunoadsorption and fixation-induced conformational epitope alteration; b) mostly pertained to the IgG1/IgG3 subclass (68.3%) and were able to induce CDC; and c) coreacted with DM20 in all 12 patients with PNS involvement tested.

Discussion

Conformational PLP1-IgG predominantly identify patients with non-MS ADDs. They should be tested mainly in those with CNS + PNS ADD, coherently with DM20-IgG coreactivity. PLP1-IgG could also be investigated as disease modifiers and prognostic markers in MS and MOGAD. Preliminary evidence supports their pathogenic potential.

Introduction

Autoimmune demyelinating disorders (ADDs) of the CNS are heterogeneous conditions affecting both children and adults, the most common of which is multiple sclerosis (MS).[1]

Within ADDs, the discovery of autoantibodies directed against surface glial targets has allowed the identification of disorders with rather homogeneous clinical features, such as neuromyelitis optica spectrum disorders (NMOSDs) associated with aquaporin-4 (AQP4) antibodies[2] and myelin oligodendrocyte glycoprotein (MOG)–associated disease (MOGAD).[3] Common characteristics of these antibodies are recognition of protein targets when the tertiary structures are preserved (conformational binding) and detectability with conformational methods such as cell-based assays (CBAs). Indeed, MOG-IgG were initially identified with nonconformational techniques in patients with MS and controls but were eventually associated with MOGAD only by using CBAs.[3-8]

Another potential autoantigen in ADDs is the proteolipid protein-1 (PLP1), the most abundant protein of CNS myelin.[9] PLP1 can be used to induce an animal model of ADDs (i.e., experimental autoimmune encephalomyelitis, EAE),[9] and patients with MS harboring particular disease-associated HLA haplotypes show circulating T lymphocytes reactive to PLP1 peptides.[10] In line with this, PLP1 antibodies (PLP1-IgG) were found in a variable proportion of patients with MS (1%–70%), but also in other noninflammatory neurologic disorders, with unclear clinical implications.[11-14] Differences in PLP1-IgG detection strategies, mostly relying on nonconformational assays, likely account for this heterogeneity.[12-15] In addition, PLP1-IgG were not thoroughly investigated in non-MS ADD.

In this study, by devising and validating a new conformational CBA for the detection of PLP1-IgG, we explored their clinical relevance in 2 wide retrospective and prospective cohorts of patients with ADDs.

Methods

Patients and Samples

We included patients from a retrospective exploratory cohort and, subsequently, a prospective validation cohort. The exploratory cohort was used to assess the presence of PLP1-IgG in predefined groups of patients with ADDs[11-15] and controls. We selected paired serum and/or CSF samples of patients with ADDs previously admitted to the Mondino Neurological Institute (Pavia) classified as MS, AQP4-IgG NMOSD, and MOGAD according to disease-specific diagnostic criteria.[2,3,16] MS was divided into typical and "atypical" MS, the latter requiring the presence of at least one of the following features: (1) longitudinally extensive transverse myelitis (LETM); (2) bilateral optic neuritis (ON); (3) chiasm involvement; (4) tumefactive demyelinating lesion (TDL).[17] Patients with ADDs not falling under any of the conditions mentioned above were classified as having other ADDs and further subgrouped into isolated ON, transverse myelitis (TM), seronegative NMOSD (SN-NMOSD),[2] and CNS plus peripheral nervous system (PNS) involvement ADD (CNS + PNS

ADD). In the latter group, PNS involvement was supported by spinal roots' gadolinium contrast enhancement and/or nerve conduction study (NCS) abnormalities with demyelinating features.[18,19] Control serum and, when available, CSF samples were collected from patients with non-inflammatory neurologic disorders (NINDs), including Alzheimer disease (AD), and normal pressure hydrocephalus (NPH), patients with other inflammatory neurologic disorders (OINDs), and healthy controls (HCs). Clinical and paraclinical information was gathered from medical charts. Aliquots of the samples were stored in the Mondino Neuroimmunology Biorepository at −80°C until antibody testing.

A validation cohort was used to confirm our results in a real-life diagnostic setting. We included consecutive serum and/or CSF samples sent for AQP4/MOG antibody testing to our Neuroimmunology Laboratory from April 2020 to April 2021. A diagnostic suspicion following the same disease classification described above was attributed by 3 researchers (S.M., P.B., and G.G.) according to the clinical data provided along with the samples and to the results of AQP4/MOG-IgG testing. Further details were collected for PLP1-IgG–positive patients.

Antibody Testing

Live cell-based assays were implemented using the following plasmids: EGFP-tagged full-length human MOG and untagged rat full-length MOG (kind gift of Prof M. Reindl); GFP-tagged human PLP1 (code: RG218616, Origene); untagged human PLP1 (code: SC119823, Origene); untagged human DM20 (a splicing variant of PLP1, code: SC121107, Origene).

CBA for MOG was performed according to a published protocol.[20] AQP4-IgG were detected using a commercial AQP4 fixed CBA according to the manufacturer's instruction (Euroimmun, Lubeck). PLP1 CBA was performed on transfected live HEK293T cells adapting the protocol for MOG-IgG and initially validated using monoclonal antibodies (mAbs) against the extracellular epitopes $PLP1^{50\text{-}69}$, $PLP1^{178\text{-}191}$, and $PLP1^{200\text{-}219}$ (kind gift of Prof. E.A. Greenfield and Prof. V.J. Kuchroo).[21]

Tissue-based assay (TBA) was performed using immunohistochemistry on lightly fixed rat brain slices according to a published protocol.[22] For colocalization experiments, an indirect immunofluorescence protocol was performed on the same brain slices. CBA and TBA received a semiquantitative score (0–4) based on visual inspection, where 0 corresponds to negative, 1–2 to low positive, and 3–4 to strong positive.[23]

Additional details regarding CBAs and TBAs are reported in eMethods.

All samples included in the study were tested for antibodies against AQP4, MOG, and PLP1 using CBA while only PLP1-IgG–positive samples were tested using TBA. Patients with the PNS involvement were further tested for nodal/paranodal antibodies to neurofascin 155 (NF155), NF140/186, contactin-1 (CNTN1), and contactin-associated protein-1 (CASPR1), using ELISA and/or CBA as previously reported.[24]

Immunoadsorption, Disruption of Conformational Epitopes, and Complement-Dependent Cytotoxicity Assessment

Immunoadsorption experiments were performed by incubating samples with either PLP1-transfected or MOG-transfected HEK293T cells and assessing them afterward using a TBA. To test the effect of fixation on altering conformational epitope recognition, PLP1 CBA and TBA were performed following the same protocols described above after preincubation with either 4% paraformaldehyde or methanol for 10 minutes before the incubation with the serum samples. Complement-dependent cytotoxicity (CDC) of PLP1-IgG was assessed on an in vitro model relying on HEK293 cells. PLP1-transfected cells were incubated with PLP1-IgG–positive serum samples in the presence or absence of normal human serum as a source of complement. Afterward, the proportion of viable cells was measured with the CellTiter-Glo Luminescent Cell Viability Assay kit (Promega, Madison, WI) and their reduction used as an indicator of CDC. Details on the protocols used can be found in eMethods.

Statistical Analysis

Qualitative variables were given as percentages and quantitative variables as medians with ranges. Differences in the proportion of qualitative variables were measured using the chi-square or Fisher exact test. The differences in quantitative variables were tested using the *t* test or Mann-Whitney non-parametric test, depending on whether data were normally distributed. p values ≤0.05 were considered significant (two-sided). All analyses were performed with Stata/SE for Windows V.14. Figures were produced using Prism GraphPad (Version 10.1.2) and BioRender.com.

Data Availability

Raw data are available at the Zenodo repository (10.5281/zenodo.10721216).

Standard Protocol Approvals, Registrations, and Patient Consents

The project was reviewed by the Institutional Review Board of the IRCCS Policlinico San Matteo, Pavia (project codes: 20200026380 approved on 9/3/2020 and 0020308/23 approved on 14/4/23). All patients included in the study provided their informed consent.

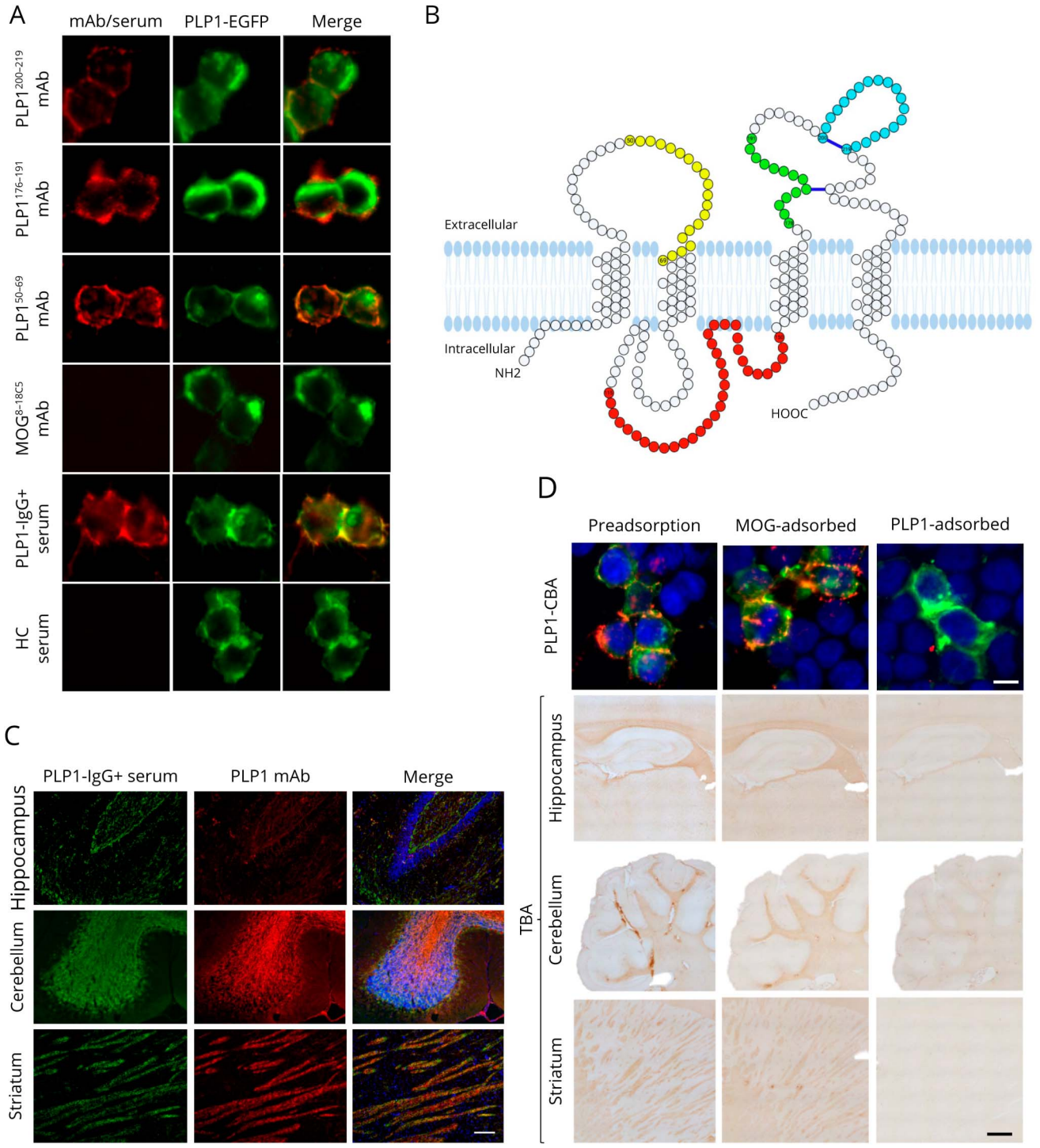
Results

Identification and Validation of PLP1 as an Antibody Target in ADDs

PLP1 Is the Target of Conformational Antibodies

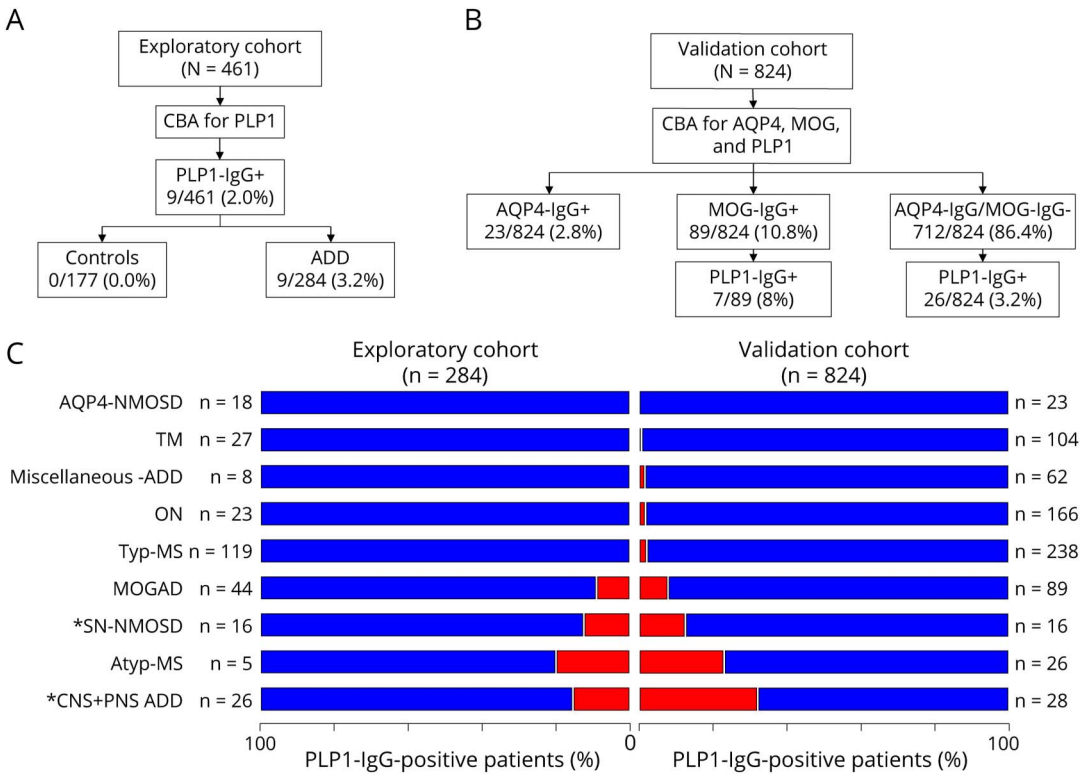
To investigate PLP1 as a potential target of conformational autoantibodies in ADDs, we established a live CBA expressing the full-length protein in HEK293T cells. mAbs specific for the extracellular loops $PLP1^{50\text{-}69}$, $PLP1^{178\text{-}191}$, and $PLP1^{200\text{-}219}$

Figure 1 Conformation-Dependent PLP1 Epitopes Are the Target Antigen of Myelin Antibodies



(A) Monoclonal antibodies against different epitopes of PLP1 and serum from a patient with ADD label the surface of live HEK293T cells transfected with PLP1-EGFP-tag (green). Merged images show colocalization of the staining (orange). No binding is observed with the MOG$^{8\text{-}18C5}$ monoclonal antibody or with serum from a healthy control. (B) Structure of PLP1. Colored aminoacidic sequences identify the different epitopes recognized by the monoclonal antibodies (yellow = PLP1$^{50\text{-}69}$; green = PLP1$^{178\text{-}191}$, and blue = PLP1$^{200\text{-}219}$) or their intracellular portion, which is absent in the DM20 protein isoform (red). (C) Serum of a PLP1-IgG positive patient (green) binds to the myelinated areas (cerebellum, striatum and hippocampus) of lightly fixed rat brain sagittal slices (indirect immunofluorescence). PLP1 monoclonal antibody (red) binds to the same regions, colocalizing with patients' serum (yellow). DAPI stains cell nuclei. Scale bar: 50 µm. (D) Serum from the same patient stains both PLP1-CBA and TBA on lightly fixed rat brain (left column); unmodified staining after preadsorption on MOG-transfected cells (central column); abolition of the binding after preadsorption on PLP1-transfected cells on both CBA and TBA (right column). Created in BioRender. Gastaldi, M. (2024) BioRender.com/v24o621. ADD = autoimmune demyelinating disorder; CBA = cell-based assay; MOG = myelin oligodendrocyte glycoprotein; PLP1 = proteolipid protein-1; TBA = tissue-based assay.

Figure 2 Algorithm of the Study and PLP1-IgG Distribution in Disease Subgroups



Patients from (A) a retrospective exploratory cohort and (B) a prospective validation cohort were tested. (C) Proportions of PLP1-IgG–positive patients within the diagnostic subgroups of both the exploratory and validation cohorts. *Seventy-three of 824 patients without sufficient clinical information were excluded from the figure, all negative for MOG/AQP4/PLP1-IgG. **The SN-NMOSD group includes 3 PLP1-IgG–positive patients (2 from the exploratory and 1 from the validation cohort) also classified as CNS+PNS-ADD. ADD = autoimmune demyelinating disorder; AQP4 = aquaporin 4; CBA = cell-based assay; CNS+PNS ADD = ADD with peripheral and CNS involvement; MOG = myelin oligodendrocyte glycoprotein; MOGAD = myelin oligodendrocyte glycoprotein antibody–associated disease; MS = multiple sclerosis; n = number; NMOSD = neuromyelitis optica spectrum disorder; ON = optic neuritis; PLP1 = proteolipid protein-1; SN = seronegative; TM = transverse myelitis.

confirmed membrane expression of the extracellular epitopes on the live cells (Figure 1, A and B). This PLP1 CBA was used to test a large exploratory cohort of 461 patients, including 124 with MS, 160 with non-MS ADD, and 177 controls (Figure 2A and eTable 1).

Overall, PLP1-IgG were found in 9 of 284 patients with ADDs including 4 of 44 patients with MOGAD (9%), 1 of 5 patients with atypical MS (20%), and 4 of 26 patients with CNS + PNS ADD (15%) (Figure 2B). None of the patients with AQP4-IgG NMOSD and of the controls were positive (Figure 2B). Positive samples bound to the surface of PLP1-transfected cells and provided a strong myelin staining on TBA (Figure 1A and eFigure 1, A and B).

The specificity of the binding was demonstrated by colocalization studies. On TBA, PLP1-IgG in patients' serum colocalized with PLP1 mAbs in myelinated regions (Figure 1C). In addition, preadsorption of 2 PLP1-IgG–positive serum samples with PLP1-transfected HEK293T cells, but not with MOG-transfected cells, completely abolished the binding on PLP1 CBA and the myelin staining on TBA (Figure 1D). Immunoadsorption of a serum sample with coexisting MOG-IgG and PLP1-IgG on either MOG-transfected or PLP1-transfected cells only reduced the TBA reactivity, suggesting a contribution of both antibodies to the staining (eFigure 2).

We also showed that using serum samples of 5 patients with high-titer PLP1-IgG, the PLP1 staining was partially (n = 1) or fully (n = 4) abolished on both CBA and TBA after paraformaldehyde fixation, whereas none of the samples provided any reactivity after methanol fixation (eFigure 3).

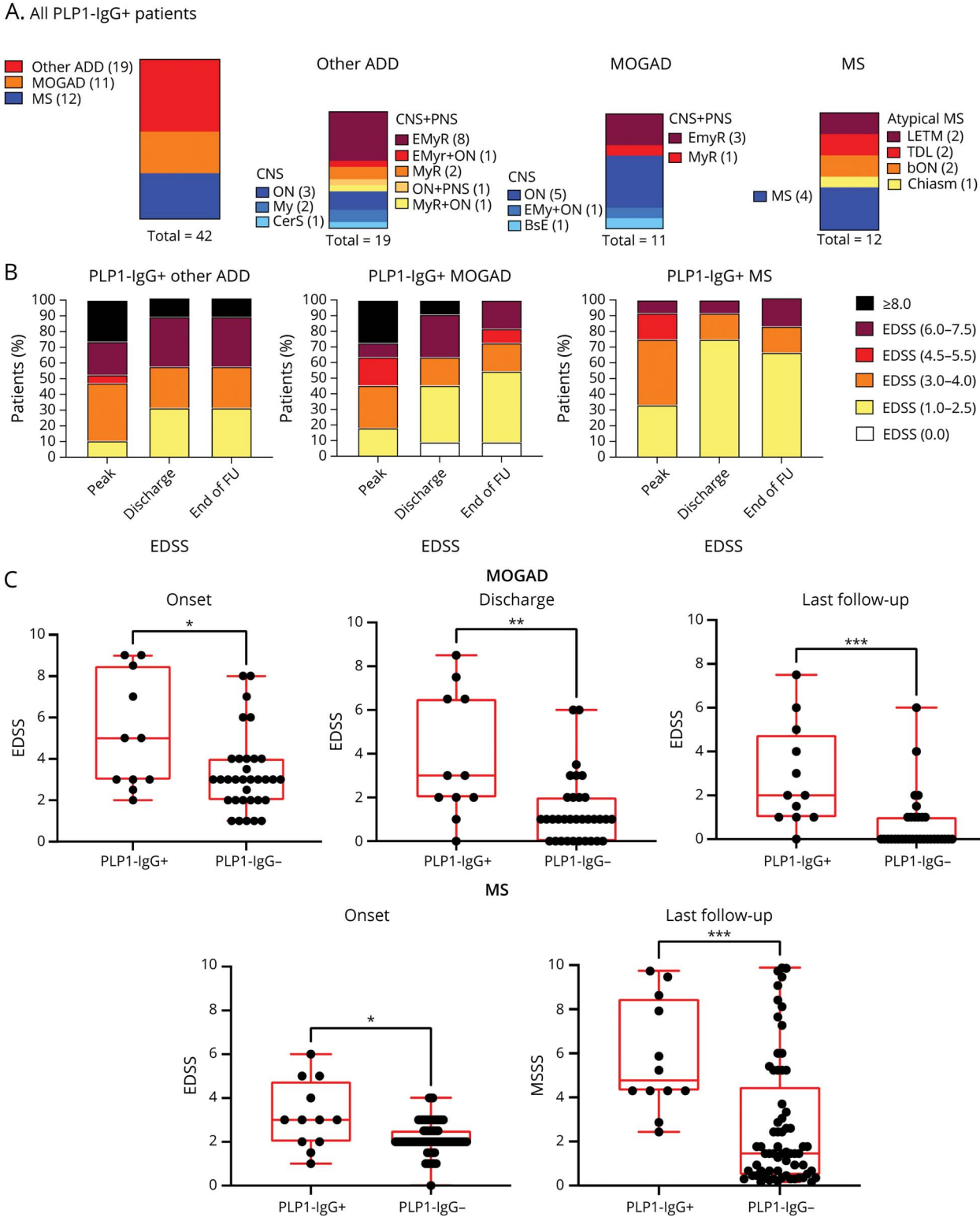
Validation of PLP1 Antibodies in a Prospective Cohort

To investigate the clinical meaning of PLP1-IgG further, we prospectively enrolled 824 consecutive patients and tested them for MOG, AQP4, and PLP1 antibodies (Figure 2B and eTable 1). Of 824 patients, AQP4-IgG were detected in 23 (2.8%), isolated MOG-IgG in 82 (9.9%), and isolated PLP1-IgG in 26 (3.2%). PLP-IgG were found in 15 of 360 with other ADDs (4.2%) and in 11 of 264 with MS (4.2%). In addition, 7 patients had coexisting MOG-IgG and PLP1-IgG and were ultimately diagnosed as MOGAD (Figure 2C).

Clinical and Paraclinical Characterization

After combining results from both the exploratory and validation cohorts, we identified 42 patients positive for PLP1-

Figure 3 Diagnostic Groups and Clinical Features of PLP1-IgG–Positive Patients With Autoimmune Demyelinating Disorders



(A) Clinical phenotypes of patients with ADDs split into 3 groups: PLP1-IgG–positive other ADDs, PLP1-IgG–positive MOGAD, and PLP1-IgG–positive MS; (B) EDSS-measured disability over the disease course; (C) median EDSS scores at the last follow-up (or MSSS for patients with MS) in PLP1-IgG–positive patients with MOGAD or with MS, compared with those measured, over the disease course, in the corresponding PLP1-IgG–negative groups. ADD = autoimmune demyelinating disorder; b = bilateral; BsE = brainstem encephalitis; CerS = cerebellar syndrome; CNS+PNS ADD = ADD with peripheral and CNS involvement; EDSS = Expanded Disability Status Scale; FU = follow-up; EMyR = encephalomyeloradiculitis; LETM = longitudinally extensive transverse myelitis; My = myelitis; MyR = myeloradiculitis; MOGAD = myelin oligodendrocyte glycoprotein antibody–associated disease; MS = multiple sclerosis; MSSS = MS severity score; ON = optic neuritis; PLP1 = proteolipid protein-1; TDL = tumefactive demyelinating lesions.

Table 1 Clinical, Laboratory, and Radiologic Features of PLP1-IgG–Positive Patients

	All patients (42)	MOGAD (11)	MS (12)	Other ADDs (19)	*p* Value
Age, median (range)	51 (8–77)	37 (8–66)	36 (20–70)	55 (17–77)	0.074
Female sex, n (%)	18 (43)	5 (45)	6 (50)	7 (37)	0.755
Prodromal event, n (%)	18 (43)	6 (55)	1 (8)	11 (58)	0.017
Months of FU, median (range)	11 (4–54)	11 (6–52)	12 (5–51)	16 (4–54)	0.505
Syndrome at presentation, n (%)					
Encephalitis	9 (21)	3 (27)	0 (0)	6 (32)	0.248
Meningitis	7 (17)	1 (9)	0 (0)	6 (32)	0.052
Myelitis	25 (60)	5 (45)	6 (50)	14 (74)	0.230
ON	18 (43)	7 (78)	4 (33)	7 (37)	0.264
Bilateral ON	7 (17)	3 (27)	2 (17)	2 (11)	0.495
Brainstem/cerebellar syndrome	15 (36)	4 (36)	3 (25)	8 (42)	0.625
Supratentorial syndrome	10 (24)	4 (36)	1 (8)	5 (26)	0.272
PNS involvement	17 (40)	4 (36)	0 (0)	13 (68)	0.001
Cranial neuropathy	6 (14)	2 (18)	0 (0)	4 (21)	0.241
Brain MRI altered, n (%)	35 (83)	7 (78)	12 (100)	16 (84)	0.064
White matter	34 (81)	7 (78)	12 (100)	15 (79)	0.543
Gray matter	13 (31)	3 (27)	0 (0)	10 (53)	0.003
TDL	4 (9)	2 (18)	2 (17)	0 (0)	0.110
Gd enhancement	23 (55)	5 (45)	7 (58)	11 (58)	0.796
Optic nerve MRI altered, n (%)	19 (45)	7 (78)	5 (42)	7 (37)	0.218
Gd enhancement	10 (24)	6 (55)	1 (8)	3 (16)	0.011
Spinal cord MRI altered, n (%)	32 (76)	4 (36)	11 (92)	15 (79)	0.330
LETM	11 (26)	3 (27)	2 (17)	6 (32)	0.343
Conus medullaris	15 (36)	3 (27)	2 (17)	10 (53)	0.049
Gd enhancement	21 (50)	6 (55)	4 (33)	11 (58)	0.021
Spinal root enhancement	15 (36)	4 (36)	0 (0)	11 (58)	0.001
CSF, n (%)					
Cell >5/mm^3	19 (45)	5 (45)	3 (25)	11 (58)	0.268
B-CSF-B damage	21 (50)	5 (45)	3 (25)	13 (68)	0.124
Unique-to-CSF OCBs	23 (55)	5 (45)	8 (67)	10 (53)	0.527
First-line treatment, n (%)	39 (93)	11 (100)	12 (100)	16 (84)	0.141
Steroids	38 (90)	11 (100)	12 (100)	15 (79)	0.478
PLEX	6 (14)	2 (18)	0 (0)	4 (21)	0.184
IVIg	7 (17)	1 (9)	0 (0)	6 (32)	0.025
Second-line/maintenance treatment, n (%)	22 (52)	5 (45)	12 (100)	5 (19)	0.001
DMT for MS	13 (31)	0 (0)	12 (100)	1 (6)	<0.001
Other immunosuppressants	9 (21)	5 (45)	0 (0)	4 (21)	<0.001
Peak EDSS score, median(range)	4 (1–9)	5 (2–9)	3 (1–6)	5 (1.5–9)	0.044

Continued

Table 1 Clinical, Laboratory, and Radiologic Features of PLP1-IgG–Positive Patients *(continued)*

	All patients (42)	MOGAD (11)	MS (12)	Other ADDs (19)	*p* Value
EDSS score at FU, median(range)	2 (0–9)	2 (0–7.5)	2 (1–6.5)	3 (1–9)	0.471
Relapses, n (%)	17 (40)	6 (55)	6 (50)	5 (19)	0.230
NMOSD criteria fulfilled, n (%)	7 (17)	3 (27)	0 (0)	4 (21)	0.169
ADEM criteria fulfilled, n (%)	9 (21)	3 (27)	0 (0)	6 (32)	0.097

Abbreviations: ADDs = autoimmune demyelinating disorders; ADEM = acute disseminated encephalomyelitis; B-CSF-B = blood-CSF barrier; DMT = disease-modifying therapy; EDSS = Expanded Disability Status Scale; FU = follow-up; Gd = gadolinium; IVIg = IV immunoglobulin; LETM = longitudinally extensive transverse myelitis; MOGAD = myelin oligodendrocyte glycoprotein antibody–associated disease; MS = multiple sclerosis; NMOSD = neuromyelitis optica spectrum disorder; OCBs = oligoclonal IgG bands; ON = optic neuritis; PLEX = plasma exchange; PNS = peripheral nervous system; TDL = tumefactive demyelinating lesions.
Statistical analysis: Kruskal-Wallis for continuous and chi-square for nominal variables; *p* value was set at 0.05.

IgG, who were diagnosed as other ADDs (19/42, 45.2%), MOGAD (11/42, 26.2%), and MS (12/42, 28.6%) (Figure 3A, Table 1). Overall, PLP1-IgG were found in 24.1% of patients with CNS+PNS-ADD, 21.2% of patients with atypical MS, 8.3% of patients with MOGAD, 12% of patients with SN-NMOSD, and 1.4% of patients with typical MS tested, and their frequency within diagnostic subgroups was consistent between the exploratory and validation cohorts (Figure 2C).

Overall, 18 of 42 patients (42.9%) were women. The median age was 51 years (range, 8–77); 4 patients were children, all with MOGAD ($p = 0.002$).

Samples were collected at disease onset in 28 patients, during a relapse in 8, and during remission in 6.

PLP1-IgG–Positive Patients With Other ADDs

Of the 19 patients with other ADDs, most presented with myelitis (n = 5, one with additional ON) or encephalomyelitis (n = 9, one with additional ON); 11 of 19 (57.9%) had a triggering infection (n = 9) or vaccination (n = 2, SARS-CoV-2) (Tables 1 and 2).

PNS involvement was found in 13 of 19 patients (68.4%, 9 with encephalomyelitis, 3 with myelitis, and 1 with ON), assessed with MRI evidence of spinal root contrast enhancement (n = 11), demyelinating or mixed NCS alterations (n = 2), or both (n = 6). In all patients except one, PNS involvement was simultaneous with the CNS syndrome. Seven patients had ON, bilateral in 2. ON was the only manifestation in 2 patients, one with severe form that led to complete blindness. Four of 19 patients (21.1%) fulfilled the criteria for SN-NMOSD, 3 with PNS involvement.

Multifocal demyelinating lesions involving both the brain and spinal cord (15/19 patients, 78.9%) were the most common MRI findings (eFigure 4). Supratentorial lesions were often multiple (n = 13), periventricular (n = 10), and further involving the gray matter (n = 10). Brainstem lesions were adjacent to the root-entry zone of cranial nerves in 6 of 11 patients, and those of the optic nerve involved the chiasm in 4 of 7 patients. Eleven of 16 patients had gadolinium-enhanced brain lesions, involving periependymal and/or leptomeningeal sites in 7. Spinal cord lesions were mostly multiple (n = 13, 10 with conus medullaris involvement and contrast enhancement of spinal roots) and spanned >2 myelomers in 6 cases (LETM, eFigure 4).

CSF analysis revealed inflammatory signs in 17 of 19 patients (89.5%), including high cell count (11/19 patients; 57.9%; median, 32 lymphomonocytes/mm^3; range, 5–580 cells/mm^3), unique-to-CSF oligoclonal IgG bands (10/19 patients; 52.6%), and altered blood-CSF barrier (13/19 patients; 68.4%), in various combinations (Table 2).

The median EDSS score at onset (nadir) was 5 (range 1.5–9.0), with EDSS scores ≥6.0 in 9 of 19 patients (47.3%) (Figure 3, panel B). Sixteen of 19 patients (84.2%) were treated with first-line drugs including IV steroids (n = 16), followed by IV immunoglobulins (n = 5), plasma exchange (n = 2), or both (n = 2), and 13 improved. Relapses occurred in 5 of 19 patients (26.3%), involving the same CNS sites of the index events in 4 of 5 patients. Five patients received second-line treatments (4 anti-CD20 mAbs and one cyclophosphamide), in 4 patients given during the index attack due to poor response to first-line drugs and in one patient after the first relapse. At the end of follow-up (median, 16 months; range, 4–54), no patients reached full recovery and 6 of 19 (31.6%) still had EDSS scores ≥6.0.

PLP1-IgG–Positive Patients With MOGAD

Eleven patients with MOGAD were positive for both PLP1-IgG and MOG-IgG.[3] Clinical syndromes at presentation included encephalomyelitis (n = 4: one with ON, 2 with PNS involvement, and one with ON+PNS), myeloradiculitis (n = 1), isolated ON (n = 5, bilateral in one patient), and brainstem encephalitis (n = 1) (Figure 3A, eTable 2). We compared these 11 PLP1-IgG–positive patients with MOGAD with a group of 33 PLP1-IgG–negative patients with MOGAD of the exploratory cohort for whom detailed clinical information was available

Table 2 Clinical, Laboratory, and Radiologic Features of PLP1-IgG–Positive Patients Classified as Other Demyelinating Disorders of the CNS

Pt no.	Sex, age at onset (y)	Prodromal event, clinical syndrome	Sample timing, FU (mo)	PLP1 CBA	Altered CSF analysis	Brain MRI	Spinal cord MRI	IST	Relapse	EDSS score, acme → FU
1	M, senior adult	None, MyR+men	Relapse, 53	S+(1:80); CSF-IgGs: IgG3	5 LMN/μL, moderate B-CSF-B damage, OCB+	T2/FLAIR multiple WM: juxtacortical, PV, cranial nerves (Gd+); multifocal leptomeningeal (Gd+)	LETM, conus, spinal roots (Gd+)	IV steroids, pr > 3 mo	2, My	8 → 7
2	F, adult	None, EMyR+ON + men, SN-NMOSD	Onset, 32	S+ (1:320); CSF+ (1:40) IgGs: IgG1,2,3	134 LMN/μL, OCB+	T2/FLAIR multiple GM: hippocampus, insular cortex (Gd−); multifocal leptomeningeal (Gd+); optic nerve (Gd−)	Multiple: cervico-thoracic, conus, spinal roots (Gd+)	IV steroids + IVIg and PLEX	1, My	7 → 3.5
3	M, adolescent	None, ON	Onset, 8	S+(1:160); CSF N/A IgGs: none	9 LMN/μL; OCB+	T2/FLAIR left ON and chiasm (Gd+)	N/A	IV steroids	None	3 → 2
4	M, senior adult	Infl. A (H1N1), EMyR	Onset, 11	S+(1:640); CSF+(1:20) IgGs: IgG1	17 LMN/μL, severe B-CSF-B damage; OCB+	T2/FLAIR multiple WM and GM: subcortical, thalamic (Gd+, nodular patchy)	LETM, conus, spinal roots (Gd+)	IV steroids	None	4 → 1
5	F, adult	Cholecystitis, My, SN-NMOSD	Onset, 11	S+(1:320); CSF+(1:5) IgGs: IgG1	Normal	T2/FLAIR multiple WM: subcortical, PV, cer. peduncles (all Gd−)	Multiple: cervico-thoracic, conus (Gd−)	Rtx	None	6.5 → 6.5
6	M, adult	None, bON	Onset, 8	S+(1:160); CSF N/A IgGs: none	Normal	T2/FLAIR bilateral ON and chiasm (Gd+)	Normal	IV steroid + PLEX	None	4 → 4
7	M, senior adult	URTI, My	Onset, 19	S+(1: 2,560); CSF+(1:5) IgGs: IgG1,2,3	30 LMN/μL, severe B-CSF-B damage; OCB+	T2/FLAIR multiple WM: juxtacortical (Gd+), brainstem (Gd−)	Multiple: C3-C4, D3-D5, D8, D11 (Gd−)	None	None	3.5 → 1.5
8	M, adult	URTI, EMyR+men	Remission, 30	S+(1:80); CSF N/A IgGs: none	109 LMN/μL, moderate B-CSF-B damage;	T2/FLAIR multiple WM and GM: PV, subcortical, bulbar (all Gd+); leptomeningeal bulbar (Gd+); TREZ+	Multiple: C3, C4, C6, C7, conus, spinal roots (Gd+)	IV steroids	None	5 → 1
9	M, adult	URTI, EMyR+men	Remission, 52	S-; CSF+(1: 10) IgGs: IgG1	590 LMN/μL; moderate B-CSF-B damage; OCB+	T2/FLAIR multiple WM and GM: PV, bulbar, cer. peduncle, amygdala, IV ventricle (Gd−); multifocal leptomeningeal (Gd+); TREZ+	Multiple: C2, C5-6, D6-7, conus, spinal roots (Gd+)	IV steroids	None	7 → 4.5
10	M, adult	SARS-CoV2 vaccine, MyR	Onset, 27	S+(1: 1,280); CSF N/A IgGs: IgG 1,2,3	Severe B-CSF-B damage	T2/FLAIR single WM: cer. peduncle (Gd−)	Multiple: cervico-thoracic, conus, spinal roots (Gd+)	IV steroid + IVIg	1, My	4 → 1
11	M, senior adult	None, EMy+PNS	Relapse, 54	S+(1:80); CSF N/A IgGs: none	Normal	T2/FLAIR multiple WM: subcortical; PV; cer. peduncle (Gd+)	Single: C5-C6 (Gd+)	IV steroid	1 cer. lesion	3 → 2
12	F, adult	SARS-CoV2 vaccine, ON+PNS	Relapse, 28	S+(1: 1,280); CSF N/A IgGs: IgG1,3	Severe B-CSF-B damage	T2/FLAIR optic nerve and chiasm (Gd−)	N/A	IV steroid + IVIg	1 mON	3 → 4
13	F, adult	URTI, EMyR, SN-NMOSD	Relapse, 54	S+(1:320); CSF-IgGs: IgG3	8 LMN/μL, mild B-CSF-B damage, OCB+	T2/FLAIR multiple WM and GM: PV, centrum semiovale, bulbar, cer. peduncle, optic nerve and chiasm (all Gd−); TREZ+	LETM, conus, spinal roots (Gd−)	IV steroid, Rtx	None	8 → 7
14	F, adult	URTI, EMyR	Onset, 9	S+(1:320); CSF+(1:80) IgGs: IgG1,2,3	290 LMN/μL, moderate B-CSF-B damage, OCB+	T2/FLAIR multiple WM and GM: subcortical, PV, centrum semiovale (all Gd−); bulbar (Gd+); TREZ+	Multiple: cervico-thoracic, conus, spinal roots (Gd+)	IV steroid + IVIg	None	8 → 1

Continued

Table 2 Clinical, Laboratory, and Radiologic Features of PLP1-IgG–Positive Patients Classified as Other Demyelinating Disorders of the CNS *(continued)*

Pt no.	Sex, age at onset (y)	Prodromal event, clinical syndrome	Sample timing, FU (mo)	PLP1 CBA	Altered CSF analysis	Brain MRI	Spinal cord MRI	IST	Relapse	EDSS score, acme → FU
15	M, adult	None, ON	Onset, 10	S+(1:160); CSF N/A IgGs: none	Mild B-CSF-B damage, OCB+	T2/FLAIR multiple WM and GM: subcortical, thalamus (all Gd−); right ON (Gd−)	Single: C3-C4 (Gd−)	IV steroid	None	1.5 → 1.5
16	M, adult	URTI, EMyR+men	Onset, 7	S+(1:80); CSF-IgGs: IgG3	33 LMN/μL, moderate B-CSF-B damage	T2/FLAIR multiple WM and GM: PV, centrum semiovale, bulbar, cer. peduncle, thalamus (Gd+); leptomeningeal (Gd+); TREZ+	LETM, conus, spinal roots (Gd+)	IV steroid + IVIg, CyP	None	7 → 6
17	F, senior adult	None, cerebellar syndrome	Onset, 16	S+(1: 2,560); CSF-IgGs: IgG3	OCB+	T2/FLAIR multiple WM and GM: PV, centrum semiovale (all Gd−); bulbar (Gd+)	N/A	None	None	2.5 → 2
18	M, adult	None, MyR + men + bON + PNS	Onset, 4	S+ (1: 20480), CSF (1: 1,280); IgGs: IgG1,3	77 LMN/μL, severe B-CSF-B damage	T2/FLAIR bilateral optic nerve; leptomeningeal bulbar (Gd+); TREZ+	LETM, multiple cervico-thoracic, conus, spinal roots (Gd+)	IV steroid, PLEX	None	8.5 → 8
19	F, adult	URTI, EMyR; SN-NMOSD	Onset, 8	S N/A, CSF+ (1: 40); IgG1	22 LMN/μL, moderate B-CSF-B damage	T2/FLAIR multiple WM and GM: periventricular, brainstem, thalamus (all Gd−); subcortical (Gd+)	LETM: cervico-thoracic, conus, spinal roots (Gd+)	PLEX, IVIg, Rtx	None	9.5 → 9.5

Abbreviations: B-CSF-B = blood-CSF barrier; bON = bilateral ON; CBA = cell-based assay; cer. = cerebellar; CyP = cyclophosphamide; EDSS = Expanded Disability Status Scale; EMyR = encephalomyeloradiculitis; F = female; FU = follow-up; Gd = gadolinium; GM = gray matter; IgGs = IgG subclass; IST = immunosuppressive treatment; LETM = longitudinally extensive transverse myelitis; LMN = lymphomonocytes; M = male; men = meningitis; mON = monolateral ON; MP = methylprednisone; My = myelitis; MyR = myeloradiculitis; N/A = not available; OCB = oligoclonal IgG band; ON = optic neuritis; PLEX = plasma exchange; PLP1 = proteolipid protein-1; PMN = polymorphonucleate cells; PNS = peripheral nervous system involvement; pr = prednisolone; Pt = patient; PV = periventricular; Rtx = rituximab; S = serum; TREZ = trigeminal root-entry zone T2/FLAIR hyperintensity; URTI = upper respiratory tract infection; WM = white matter.
Age groups were defined as child (0–12 y), adolescent (13–18 y), adult (19–59 y), and senior adult (60 y and older).

(eTable 3). PLP1-IgG–positive patients with MOGAD were older (median age, 37 years; range, 8–66 vs 13 years; 2–50; $p = 0.007$), had more frequent PNS involvement (4/11, 36.4% vs 1/33, 3.0%; $p = 0.01$), and had higher EDSS scores at disease peak (median 5, range 2–9 vs median 3, range 1–8; $p = 0.04$) and at the end of follow-up (median 2, range 0–7.5 vs median 0, range 0–6; $p = 0.001$) (Figure 3, B and C).

PLP1-IgG–Positive Patients With MS

All the PLP1-IgG–positive patients with MS[16] had MS-typical lesions on the brain and/or spinal cord MRI, with high lesion burden (>9) at onset in 9 of 12 (75%) (eTable 4). However, 7 of 12 patients (58.3%) had atypical MS features: 2 with TDL, 2 with LETM, 2 with bilateral ON, and one with optic chiasm involvement.[17] All these manifestations, except for one TDL, occurred at disease presentation.

We then compared the features of the 12 PLP1-IgG–positive patients with MS vs 81 consecutive PLP1-IgG–negative patients with MS of the exploratory cohort with detailed clinical information available. The overall presence of atypical features was more frequent in PLP1-IgG–positive patients with MS vs those PLP1-IgG–negative ($p < 0.001$), in particular for the presence of LETM ($p = 0.02$) and of bilateral ON ($p = 0.04$) (eTable 5).

PLP1-IgG–positive patients with MS, compared with those PLP1-IgG–negative, showed higher median values of EDSS scores at onset (3 vs 2, $p = 0.01$) and of the MS severity score (MSSS) at follow-up (4.7 vs 1.5, $p < 0.001$) (Figure 3, panels B and C).

Serological and Functional Characterization of PLP1-IgG

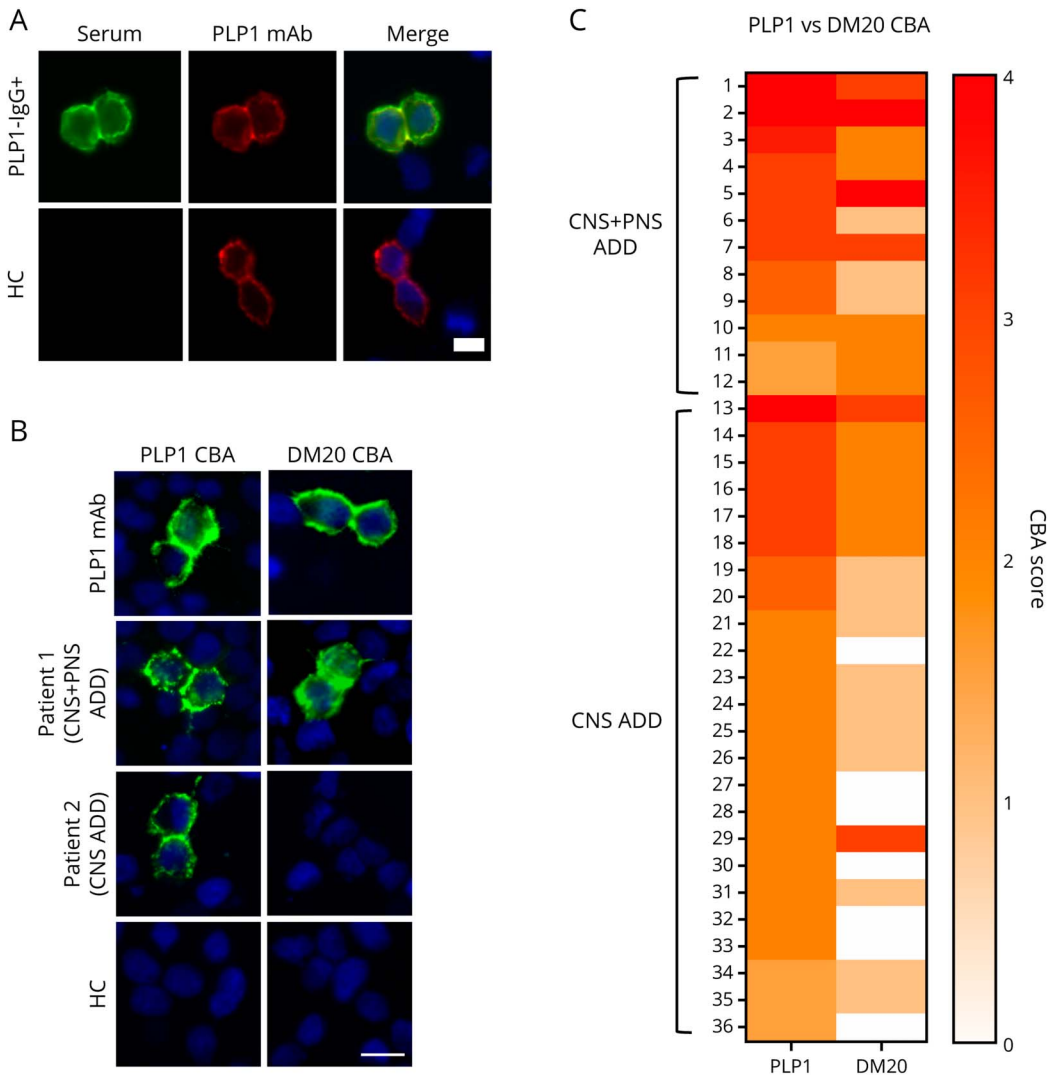
PLP1-IgG CBA and TBA Concordance in Serum and CSF

Among the 42 PLP1-IgG–positive patients on CBA, paired serum and CSF samples were available for 23, serum only for 18, and CSF only for one (total samples, 65). In the former group, 9 patients were positive in serum only (39.1%), 11 in both serum and CSF (47.8%), and 3 in CSF only (13.0%). Overall, 25 of 41 serum samples (60.9%) and 16 of 24 CSF samples (66.7%) were positive for white matter staining on TBA, and 44 of 65 samples (67.8%) yielded concordant results between CBA and TBA (eFigure 5).

PLP1-IgG Can Coreact With the PLP1 Isoform DM20

PLP1 shares all the extracellular domains with its isoform DM20, which is preferentially expressed in the PNS (Figure 1B).[25] We hypothesized that serum samples of PLP1-IgG–positive patients with PNS involvement might coreact with DM20. Using a live CBA expressing DM20 (Figure 4A),

Figure 4 PLP1 Antibodies Can Bind to the Isoform DM20



(A) Serum of a patient with PLP1-IgG binds to DM20-transfected CBA (green) and colocalizes with a PLP1 mAb (red) directed against an extracellular loop of the protein (top row). No binding is observed with serum from a HC (bottom row). (B) Binding to both PLP1- and DM20-transfected cells (green) is observed with both PLP1mAb (first row) and serum from a patient with CNS+PNS ADD (pt#1; second row). Conversely, serum from a patient with CNS ADD binds exclusively to PLP1-transfected cells and not to DM20-transfected cells (third row). No binding is observed with serum from a HC (bottom row). DAPI stains the nuclei. (C) Heatmap representing fluorescence intensity of the available samples (n = 36) in either PLP1 or DM20 CBA measured with a semiquantitative score. All PLP1-IgG–positive patients with CNS + PNS ADD, unlike patients with other disease groups, were positive on both PLP1 and DM20 CBA. Scale bars: 10 μm. ADD = autoimmune demyelinating disorder; CBA = cell-based assay; Ig = immunoglobulin; HC = healthy control; mAbs = monoclonal antibodies; PNS = peripheral nervous system; PLP1 = proteolipid protein-1; Pt = patient.

29 of 36 PLP1-IgG–positive patients with available samples (28 serum samples and 1 CSF sample) resulted positive for DM20-IgG (Figure 4, B and C). All the 12 patients with coexisting CNS + PNS ADD tested (10 with other ADDs and 2 with MOGAD) were positive on both PLP1 and DM20 CBA, compared with 17 of 24 with only CNS ADD (70.8%) (Figure 4C). None of the patients with PLP1-IgG and PNS involvement had antibodies against the nodal/paranodal antigens NF155, NF140/186, CNTN1, or CASPR1.

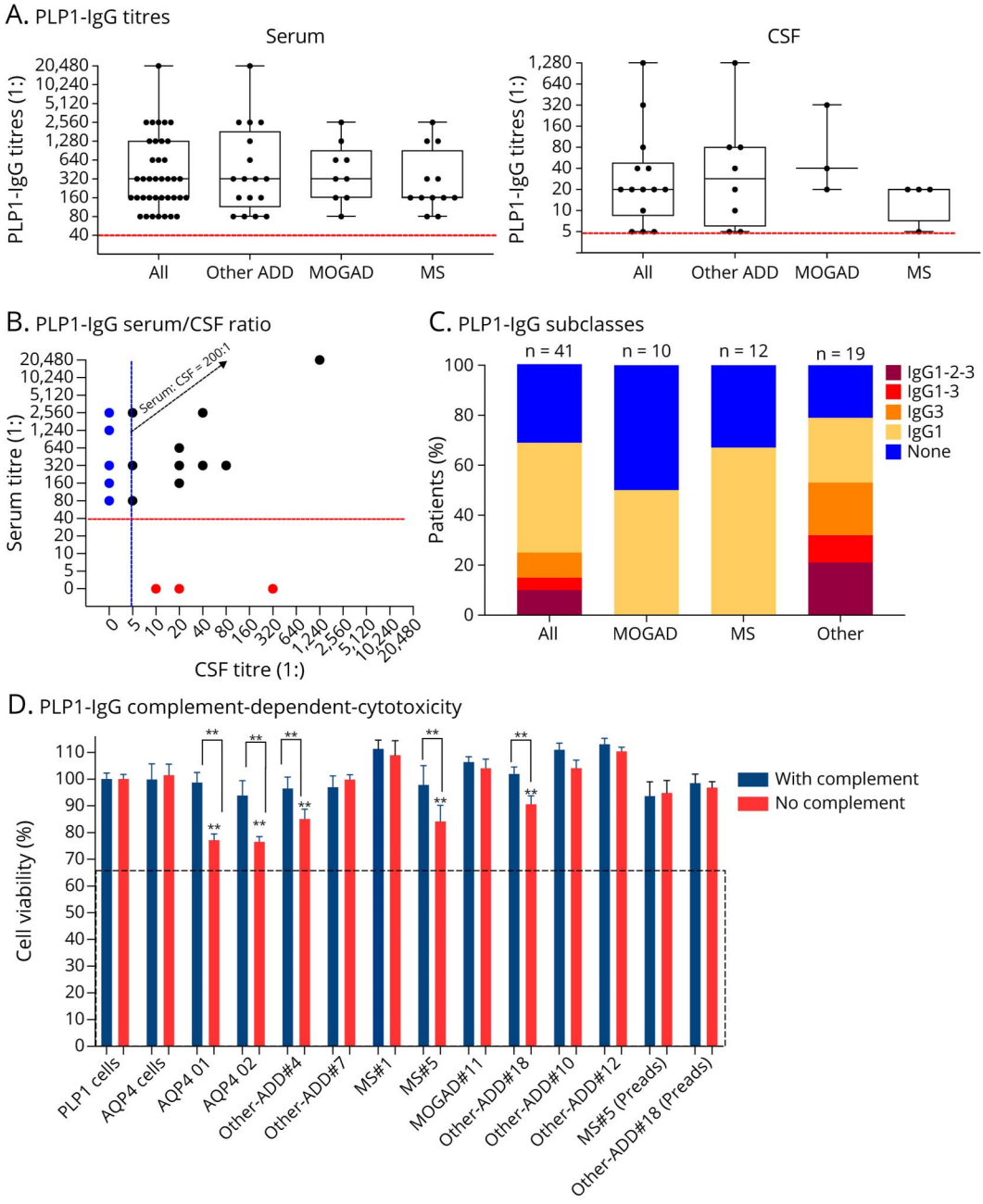
PLP1-IgG Titers and Intrathecal Synthesis

PLP1-IgG median titers were higher in serum (1:320; range, 1:80–1:20,480) than in CSF (1:20; range, 1:5–1:1,280; $p < 0.001$) (Figure 5A, eFigure 6, A and B) and did not differ among the diagnostic groups (Figure 5A). When considering the serum/CSF titer ratio calculated on the available paired samples, 12 of 23 values (37.5%) were less than the 200:1 ratio typical of IgG when B-CSF-B permeability is normal. This, together with the finding of 3 patients positive for PLP1-IgG in CSF samples only, suggests that these antibodies can be intrathecally produced (Figure 5B). In all the 6 patients with follow-up samples, titers were higher at onset and decreased over time ($p = 0.03$), turning negative in 3 (eFigure 6C).

PLP1-IgG Subclasses and Complement-Dependent Cytotoxicity

PLP1-IgG subclasses were tested in 38 serum samples and in 3 CSF samples. Overall, a specific subclass was identified in 28 of 41 samples (68.3%), mostly IgG1 (24/41, 58.5%; co-existing with IgG2/IgG3 in 4, and with IgG3 in 2 patients); 4

Figure 5 PLP1-IgG Titers in Serum and CSF, IgG Subclasses, and Complement-Dependent Cytotoxicity



(A) Serum and CSF PLP1-IgG titers tested in 41 serum and 24 CSF samples. The red dotted line indicates the cutoff for serum (1:40, left graph) and for CSF (1:5, right graph). (B) Serum:CSF PLP1-IgG titer ratio. The dotted arrow marks the 200:1 ratio expected in physiologic conditions. Values below the dotted line might associate with intrathecal synthesis. Blue dots represent patients positive in serum only, red dots patients positive in CSF only, and black dots patients positive in both serum and CSF. (C) PLP1-IgG subclasses in the whole cohort and in the 3 diagnostic groups of PLP1-IgG–positive patients. (D) PLP-IgG1 complement-dependent cytotoxicity. Data are normalized according to the amount of viable cells found in untreated cells (PLP1 cells, first 2 columns), which is considered 100%. The graph represents the finding after incubation with patient's sera. A reduction of viable cells is detected for 2 AQP4-IgG–positive serum samples (AQP4-01 and AQP4-02), which were used as positive controls, only when incubated with AQP4-transfected cells and in the presence of complement. Similarly, 3 of 8 PLP1-IgG–positive serum samples (other ADD#4, MS#5, and other ADD#18) showed reduced cell viability only when incubated with PLP1-transfected cells in the presence of complement. No effect is observed for the same samples in the absence of complement or after PLP1-IgG preadsorption performed on samples MS#5 and other ADD#18. The black dotted line represents the mean transfection rate for AQP4 and PLP1 (35%), which should theoretically set the limit for the maximum CDC effect in this experimental setting. CDC, which should involve only transfected cells, is unlikely below the cutoff. The numeric codes attributed to PLP1-IgG–positive patients correspond to those of Table 2, eTable 2, and eTable 3. ADD = autoimmune demyelinating disorder; AQP4 = aquaporin 4; Ig = immunoglobulin; MOGAD = myelin oligodendrocyte glycoprotein antibody–associated disease; MS = multiple sclerosis; PLP1 = proteolipid protein-1.

patients with other ADDs had isolated IgG3 (Figure 5C). None of the samples was positive for the IgG4 subclass. PLP1-IgG subclasses were not identified in all the tested samples, likely because of the low titers of PLP1-specific total IgG and, possibly, insufficient sensitivity and consistency of the subclass-specific secondary antibodies. Indeed, median serum titers in patients with an identified subclass were higher than in those without a subclass detected ($p = 0.003$; eFigure 6D).

We then assessed the ability of PLP1-IgG to induce CDC in an in vitro model. We selected 8 PLP1-IgG–positive patients with high titers and available material. It is important to note that all samples were negative for AQP4-IgG. In 3 of 8 (2 with CNS + PNS ADD and one with MS), a significant reduction of PLP1-transfected vital cells could be observed exclusively when samples were incubated with normal human serum as a source of complement (Figure 5D). Notably, such reduction was abolished after PLP1-IgG immunoadsorption (performed on 2 samples).

Discussion

PLP1 is one of the major components of the CNS myelin and a likely target for autoimmunity in ADD. In this study, using a candidate antigen approach, we identified conformational antibodies against PLP1 in a retrospective cohort of patients with ADDs, which were confirmed on a larger prospective validation cohort.[26] As a novelty, we found that the most frequent diagnosis in PLP1-IgG–positive patients was other ADD, and the most consistent clinical features were myelitis/encephalomyelitis and coexisting PNS involvement. We further found PLP1-IgG in patients with MOGAD and MS, where they associated with severe disease course and atypical clinical features. We demonstrated that the antibodies targeted surface epitopes of both PLP1 and DM20, likely accounting for the high frequency of PNS involvement in our ADD cohort. PLP1-IgG mostly belong to the IgG1/IgG3 subclasses and can induce CDC, suggesting their pathogenic potential. It is important to note that isolated PLP1-IgG were found in 3.2% of suspect consecutive patients with ADD, which was higher than the proportion of AQP4 antibodies in the same cohort (2.8%).

We developed a conformational live CBA similar to those used for other targets, such as MOG-IgG, to test for PLP1-IgG and thoroughly validated it in a routine diagnostic setting, where it showed high specificity for ADDs (none of the controls was positive). It is important to note that we identified PLP1-IgG in most samples tested with 2 distinct methods, CBA and TBA, owing to the high homology between the human and murine PLP1 isoforms. Differing from other studies,[13,15] PLP1-IgG were not exclusively present in patients with MS, but also, and more frequently, in those with non-MS ADD, either alone or in combination with MOG-IgG. This discrepancy could reflect differences in the cohort selection, as our study included a large proportion of non-MS ADD published so far. In addition, technical variations in the immunoassay used, regarding methods that preserve antigen conformation or different cell substrates for CBA, might account for differences in our results.[11-15]

In a recent study, recombinant mAbs from CSF clonally expanded B cells of patients with MS were shown to bind on a specific conformational CBA, which combines PLP1 expression with increased coexpression of cholesterol and glycolipids.[15] Using this assay, and differently from our study, PLP1-IgG were found in the CSF of 58.7% of 63 patients with MS tested and in none of 81 controls.[15] The discrepancy between these findings and our results might be attributed to the limited number of patients with non-MS ADDs included in their study or by different performance of the assays used. Indeed, the 2 CBAs could have different analytical sensitivity (which could be important to detect PLP1-IgG in patients with MS) or might detect PLP1-IgG with affinities for different conformational epitopes of the protein, which, in turn, might be differently represented in MS and non-MS phenotypes. Cross-validation of PLP1-IgG–positive samples between laboratories could help to clarify the discrepancies.

PLP1 genetic disorders mainly include CNS demyelinating syndromes, such as Pelizaeus-Merzbacher disease and spastic paraplegia type 2,[29] but additional PNS involvement is observed in the variant with complete PLP1 loss (PLP null syndrome).[30] In our study, 24.1% of patients with CNS + PNS ADD tested (15% in the exploratory cohort and 32% in the validation cohort) were positive for PLP1-IgG. This condition, often referred to as combined central and peripheral demyelination (CCPD), includes a heterogeneous group of disorders characterized by an autoimmune attack against shared CNS and PNS epitopes.[19] Autoantibodies against several targets including NF155, CNTN1, and CASPR1 have been associated with CCPD, but the results of different studies are conflicting.[27-32] It is important to note that none of the PLP1-IgG–positive patients in our study had antibodies against nodal/paranodal proteins.

The PLP gene encodes for 2 transmembrane proteins, differing by 35 amino acids, namely PLP1, mainly expressed by oligodendrocytes as part of the compact myelin sheet, with a probable role in its preservation,[33] and DM20, produced by alternative splicing and mostly represented in Schwann cells, with a not yet fully characterized function.[34] In our series, all patients with a CCPD phenotype had IgG that recognized both PLP1 and DM20, supporting their role as shared targets and suggesting that this subgroup of patients could harbor autoantibodies with slightly different epitope specificities/affinities.

PLP1-IgG were also found in subgroups of ADD patients with MOGAD and with MS. Of interest, these patients seem to present peculiar clinical features that recall those observed in PLP1-IgG–positive other ADDs, such as the PNS involvement found more frequently in PLP1-IgG–positive patients with MOGAD vs PLP1-IgG–negative patients with MOGAD. This feature, rather unexpected because of the exclusive expression of MOG on oligodendrocytes, has been described in less than 10% of patients with MOGAD,[35,36] possibly due to the central myelin representation in proximal spinal roots[36] or to other coexisting autoantibodies against PNS antigens,[35] as suggested by our findings.

Similarly, PLP1-IgG positive MS patients frequently showed additional features considered atypical for MS, but commonly

found in MOGAD and in PLP1-IgG positive other-ADD, namely bilateral ON, optic chiasm lesions, LETM, and TDL.[17] Indeed, PLP1-IgG were consistently found more frequently in both the exploratory and validation cohorts in atypical MS (21.2%) compared with typical MS (1.4%). Therefore, our findings suggest that the presence of PLP1-IgG in MOGAD and MS, although rare, might contribute to their presenting clinical phenotype.

It is important to note that a small subgroup of PLP1-IgG–positive patients with ADD fulfilled the criteria for SN-NMOSD,[2] accounting for 12% of patients with SN-NMOSD tested. In the past, the discovery of MOG-IgG made it possible to move around 30% of patients from the category of AQP4-IgG–negative NMOSD to that of MOGAD.[37] Notwithstanding the small number of patients with SN-NMOSD in our ADD cohort, our data suggest that PLP1-IgG might account for a further proportion of them, more frequently those with coexisting PNS involvement (3 of 4 PLP1-IgG–positive patients with SN-NMOSD in our cohort).

A relevant common feature found across all the PLP1-IgG–positive ADD subgroups was disease severity. All patients with other ADDs accumulated disability after the first attack, with one-third unable to walk at follow-up and one-fourth relapsing. Similarly, patients with MOGAD and PLP-IgG–positive patients with MS had a more severe disease course compared with those PLP1-IgG–negative.

This could be due to a direct pathogenic effect of PLP1-IgG, supported by their prevalent IgG1/IgG3 subclasses and their ability to induce CDC in vitro. In addition, the presence, at least in some patients, of PLP1-IgG intrathecal production suggests a relevant role in the disease pathophysiology. This is in line with previous studies that showed that PLP1-mAbs can induce complement-mediated demyelination and neuronal damage in both in vitro and in vivo models,[15,21] that PLP1 antibodies can worsen demyelinating lesions in EAE, and that they can be produced intrathecally.[10,15,38,39] Alternatively, we cannot exclude that PLP1-IgG might only be epiphenomenal and produced because of myelin damage. However, their absence in AQP4 NMOSD, a highly destructive CNS disorder, makes this hypothesis less convincing.

This study has limitations. First, the validation cohort included heterogeneous patients for whom well-detailed clinical information, especially regarding the follow-up, was not always available. Second, the definition of atypical MS is arbitrary, although supported by literature data.[17] Third, PLP1-IgG conformational binding might depend on complex PLP1 epitopes that need the coexpression of cholesterol and glycolipids on CBA,[15] but we did not directly address this issue nor compared our assays with the many others reported in the literature. Fourth, a proper calculation of intrathecal PLP1-IgG synthesis in our cohort could not be performed because the albumin quotient was not available for all patients. However, the very low serum:CSF PLP1-IgG titer ratio found in many patients and the fact that 3 of them had CSF-only antibodies support the presence of intrathecal PLP1-IgG synthesis, at least in a subgroup of patients. Finally, our data on pathogenicity are very preliminary because CDC was measured only in a small subset of samples, namely those whose volumes were sufficient to cover all the in vitro experiments. Indeed, most of these samples were sent to us in limited amounts primarily for diagnostic purposes. Future studies should focus on validating our results in independent cohorts of patients with MS, MOGAD, and other ADDs and confirming the pathogenicity of patient-derived PLP1-IgG in larger experiments and possibly in animal models.

In conclusion, the data presented here show that conformational PLP1-IgG predominantly identify patients with non-MS ADDs. In clinical practice, they should be tested mainly in those with CNS + PNS ADD, coherently with DM20-IgG coreactivity. In the future, PLP1-IgG could also be investigated as disease modifiers and prognostic markers in MS and MOGAD. Preliminary evidence supports their pathogenic potential through complement activation, suggesting potential therapeutic targets.

Acknowledgment

The authors thank Prof. E.A. Greenfield and Prof. V.-J. Kuchroo for providing the PLP1 monoclonal antibodies, Prof. Markus Reindl for providing the MOG plasmid, and Prof. Stefano Pluchino and Prof. Angela Vincent for critical reading of the manuscript.

Author Contributions

S. Masciocchi: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data. P. Businaro: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data. G. Greco: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data. S. Scaranzin: major role in the acquisition of data. A. Malvaso: major role in the acquisition of data. C. Morandi: major role in the acquisition of data. E. Zardini: major role in the acquisition of data. M. Risi: major role in the acquisition of data. E. Vegezzi: major role in the acquisition of data. L. Diamanti: major role in the acquisition of data. P. Bini: major role in the acquisition of data. S. Siquilini: major role in the acquisition of data. M.P. Giannoccaro: major role in the acquisition of data. L. Morelli: major role in the acquisition of data. Rocco Liguori: major role in the acquisition of data. F. Patti: major role in the acquisition of data. V. De Giuli: major role in the acquisition of data. E. Portaccio: major role in the acquisition of data. C. Zanetta: major role in the acquisition of data. S. Bergamoni: major role in the acquisition of data. A.M. Simone: major role in the acquisition of data. R. Lanzillo: major role in the acquisition of data. G. Bruno: major role in the acquisition of data. A. Gallo: major role in the acquisition of data. A. Bisecco: major role in the acquisition of data. M. Di

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Study Funding

The pilot grant 2019 to the IRCCS Mondino Foundation supported the experiments leading to the antigen discovery. The "ricerca corrente 2022-2024" grant from the Italian Ministry of Health supported the experiments on PLP1-IgG validation and characterization.

Disclosure

M. Gastaldi received compensation for speaking activities and/or consulting services, from Alexion, UCB, and Roche; he is the MG is the co-beneficiary of a patent on the PLP1 CBA currently under evaluation from the UIBIM (IT n. 102023000005046). Prof. M. Filippi is Editor-in-Chief of the Journal of Neurology; Associate Editor of Human Brain Mapping, Neurological Sciences, and Radiology; he received compensation for consulting services from Alexion, Almirall, Biogen, Horizon, Merck, Novartis, Roche, Sanofi; speaking activities from Bayer, Biogen, Celgene, Chiesi Italia SpA, Eli Lilly, Genzyme, Horizon, Janssen, Merck-Serono, Neopharmed Gentili, Novartis, Novo Nordisk, Roche, Sanofi, Takeda, and TEVA; participation in Advisory Boards for Alexion, Biogen, Bristol-Myers Squibb, Horizon, Merck, Novartis, Roche, Sanofi, Sanofi-Aventis, Sanofi-Genzyme, Takeda; scientific direction of educational events for Biogen, Merck, Roche, Celgene, Bristol-Myers Squibb, Lilly, Novartis, Sanofi-Genzyme; he receives research support from Biogen Idec, Merck-Serono, Novartis, Roche, Italian Ministry of Health, Fondazione Italiana Sclerosi Multipla, and ARiSLA (Fondazione Italiana di Ricerca per la SLA). C. Zanetta received compensation for speaking activities, and/or consulting services, from Alexion, Astrazeneca, Biogen, Bristol Myers Squibb, Janssen, Merck, Novartis, Roche, Sandoz, and Sanofi. R. Lanzillo received personal compensations for speaking or consultancy from Biogen, Alexion, Sanofi, Merck, Bristol Myer Squibb, Jansenn, Novartis, Amgen and Roche. The remaining authors report no disclosures. Go to Neurology.org/NN for full disclosures.

Publication History

Received by *Neurology: Neuroimmunology & Neuroinflammation* July 24, 2024. Accepted in final form November 25, 2024. Submitted and externally peer reviewed. The handling editor was Editor Josep O. Dalmau, MD, PhD, FAAN.

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Continued

Appendix *(continued)*

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