DPPX antibody–associated encephalitis

Main syndrome and antibody effects

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ABSTRACT

Objective: To report the main syndrome of dipeptidyl-peptidase–like protein 6 (DPPX) antibody–associated encephalitis, immunoglobulin G (IgG) subclass, and the antibody effects on DPPX/Kv4.2 potassium channels.

Methods: A retrospective analysis of new patients and cases reported since 2013 was performed. IgG subclass and effects of antibodies on cultured neurons were determined with described techniques.

Results: Nine new patients were identified (median age 57 years, range 36–69 years). All developed severe prodromal weight loss or diarrhea followed by cognitive dysfunction (9), memory deficits (5), CNS hyperexcitability (8; hyperekplexia, myoclonus, tremor, or seizures), or brainstem or cerebellar dysfunction (7). The peak of the disease was reached 8 months (range 1–54 months) after onset. All patients had both IgG4 and IgG1 DPPX antibodies. In cultured neurons, the antibodies caused a decrease of DPPX clusters and Kv4.2 protein that was reversible on removal of the antibodies. Considering the current series and previously reported cases (total 39), 67% developed the triad: weight loss (median 20 kg; range 8–53 kg)/gastrointestinal symptoms, cognitive-mental dysfunction, and CNS hyperexcitability. Outcome was available from 35 patients (8 not treated with immunotherapy): 60% had substantial or moderate improvement, 23% had no improvement (most of them not treated), and 17% died. Relapses occurred in 8 of 35 patients (23%) and were responsive to immunotherapy.

Conclusions: DPPX antibodies are predominantly IgG1 and IgG4 and associate with cognitive-mental deficits and symptoms of CNS hyperexcitability that are usually preceded by diarrhea, other gastrointestinal symptoms, and weight loss. The disorder is responsive to immunotherapy, and this is supported by the reversibility of the antibody effects in cultured neurons. ***Neurology*®** **2017;88:1340–1348**

GLOSSARY

AMPAR = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic receptor; **DPPX** = dipeptidyl-peptidase–like protein 6; **IgG** = immunoglobulin G; **mRS** = modified Rankin Scale; **NMDAR** = NMDA receptor; **PERM** = progressive encephalomyelitis with rigidity and myoclonus.

In 2013, we described 4 patients with a disorder that occurs with antibodies against dipeptidyl-peptidase–like protein 6 (DPPX), a regulatory protein of the Kv4.2 potassium channels that are involved in somatodendritic signal integration and attenuation of back-propagation of action potentials.[1] The clinical picture was consistent with a syndrome of CNS hyperexcitability including hyperekplexia, myoclonus, tremor, or seizures that in 3 patients were preceded by unexplained weight loss and diarrhea. Subsequent studies confirmed and expanded these findings, suggesting that the course of the disease can be protracted and that in some patients the syndrome may occur in association with systemic lymphoma.[2] Less frequently, the presence of myoclonus and hyperekplexia was found to be associated with a syndrome resembling progressive encephalomyelitis with rigidity and myoclonus (PERM),[3] but the frequency of this

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presentation and potential symptom similarity between the main syndrome related to DPPX antibodies and PERM could not be investigated because of the small number of cases. Additional studies showed that patients' antibodies increased neuronal excitability in preparations of myenteric neurons and reduced cell membrane protein levels of DPPX/Kv4.2 in cultured neurons[4]; the potential reversibility of these effects was not investigated.

Here, we report 9 additional patients and review all previously reported cases to determine whether the anti-DPPX syndrome can be recognized clinically and discerned from PERM. In addition, we have determined the main immunoglobulin G (IgG) subclass, the antibody effects on neuronal cell-surface clusters of DPPX and protein levels of Kv4.2 channels, and whether the antibody effects are reversible.

METHODS

Standard protocol approvals, registrations, and patient consents. The study was approved by the institutional review board of the Hospital Clinic (Barcelona, Spain). All patients gave written informed consent for use of serum, CSF, and clinical information for research purposes.

Patients and serologic testing. Patients investigated in the laboratory of Clinical and Experimental Neuroimmunology (Hospital Clinic, University of Barcelona) and University of Pennsylvania (Philadelphia) whose serum and CSF were found positive for DPPX antibodies were included in the study. The study period includes all new patients identified after the initial report of 2013 until May 30, 2016. During this time 9,798 patients were studied for encephalitis and a variety of disorders of the CNS suspected to be autoimmune, including among others 121 patients with stiff-person syndrome spectrum disorders.[5] Criteria for the presence of DPPX antibodies included brain tissue immunostaining similar to that reported for human DPPX antibodies[1] and cell-based assay with human embryonic kidney 293 cells transfected with DPPX, as reported.[1] The presence of other antibodies was determined with in house cell-based assay specific for NMDA receptor (NMDAR),[6] α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic receptor (AMPAR),[7] $GABA_A$ receptor,[8] $GABA_B$ receptor,[9] LGI1,[10] CASPR2,[10] glycine receptor,[5] mGluR1,[11] mGluR5,[11] IgLON5,[12] and neurexin-3α.[13]

Clinical information was obtained from the authors or referring physicians through a structured written questionnaire. Neurologic disability was measured with the modified Rankin Scale (mRS), and treatment effect was assessed with the mRS score.[14]

Cultures of neurons, antibody effects on DPPX and Kv4.2, and confocal microscopy. Details of the methods used to determine a mode of action of the antibodies on cultured neurons are provided in appendix e-1 at Neurology.org. Briefly, patients' IgG (including IgG1 and IgG4) antibodies were purified from serum with protein A/G Sepharose columns. Hippocampal neurons were prepared from isolated rat hippocampi of E18 embryos, and cultured neurons were treated with purified patient or control IgG (final concentration 50 μg/mL media) for 3 days to assess the antibody effects on cell-surface DPPX clusters and concentration of Kv4.2 channels. In parallel experiments, neurons similarly treated were washed and allowed to recover using media without DPPX antibodies for 4 or 7 days. The changes in surface DPPX and Kv4.2 were quantitatively analyzed with confocal microscopy and immunoblot of biotinylated surface proteins, respectively.

Review of previously reported patients with DPPX antibodies. To assess the spectrum of symptoms, response to treatment, presence of associated tumors, and outcome of patients with DPPX antibodies, we reviewed the current data along with all previously reported cases with these antibodies.[1–4,15,16]

Statistical analysis. Confocal DPPX cluster density and quantitative immunoblot analysis among IgG-treated groups were given as the median with interquartile range and the mean with SEM, respectively. Statistical significance was analyzed with the Kruskal-Wallis test followed by the Dunn post hoc test for nonnormally distributed data. A value of $p < 0.05$ was considered significant in post hoc testing after correction for multiple comparisons (Dunn test). All tests were done with GraphPad Prism version 7 (GraphPad Software Inc, La Jolla, CA).

RESULTS

Symptoms associated with DPPX antibodies. A summary of the clinical information of the 9 patients identified since 2013 is shown in the table. Eight were male, and the median age at onset was 57 years (range 36–69 years). All 9 patients had prodromal weight loss (median 20 kg, range 8–53 kg), and 7 had severe diarrhea that preceded the development of neurologic symptoms in a median of 4 months (range 2–60 months). Four of these patients also had mood changes or depression preceding other neurologic symptoms (median 5 months, range 3–60 months).

The neurologic disorder progressed for a median of 8 months (range 1–54 months) before reaching the peak of the disease, and in one patient (case 4), the symptom progression was subacute, reaching the maximum disability in 1 month. The progression of neurologic symptoms included cognitive dysfunction or memory loss (all patients) accompanied by mood or personality disorders in 4 and psychosis in 3. Symptoms of CNS hyperexcitability occurred in 8 patients, including hyperekplexia (6), myoclonus (7), tremor (5), muscle rigidity or stiffness (4), or seizures (2). Additionally, 7 patients developed brainstem or cerebellar dysfunction, 4 had sleep disorders, and 3 developed sensory symptoms (2 dysesthesia and 1 pruritus). During the course of the disease, 2 patients developed orthostatic hypotension or urinary incontinence; none of the 9 patients had cardiac dysrhythmia.

CSF, brain MRI, and EEG findings are shown in the table. Pleocytosis (median 12 white blood cells/mm^3, range, 6–130 white blood cells/mm^3) occurred in 5, and intrathecal IgG synthesis was confirmed in 2 of 5 patients. Brain MRI showed nonspecific T2/fluid-attenuated inversion recovery white matter

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Table Demographic, clinical, and immunologic data in 9 new patients with anti-DPPX antibodies

Patient sex/age, y	Prodromal symptoms; months to neurologic onset/DPPX antibody test	Main neurologic symptoms	Months to maximal deficit[a]; maximum mRS	Ancillary tests (IgG1/IgG4 titers)	Immunotherapy (effect)	Months of FU,[a] last mRS, recovery
1. F/36	Weight loss (8 kg), mood disorder[b]; 60/84	Memory loss, tremor, hyperekplexia, myoclonus, hyperreflexia, ataxic gait, dysarthria, nystagmus, truncal dystonia, RBD	25, mRS 4	CSF[c] and MRI: normal; EEG: diffuse slowing, focal paroxysmal discharge; serum (1/1,280; 1/2,560); CSF (1/80; 1/80)	IVMP, IVIg (no effect); RTX (marked improvement)	36, mRS 2, memory and mood recovered
2. M/52	Diarrhea, weight loss (53 kg), mood disorder; 6/72[d]	Memory loss, executive dysfunction, visual hallucination, delusion, hyperekplexia, myoclonus, seizures, muscle cramps	8, mRS 5	CSF: 22 WBCs, OCB+; EEG: diffuse slowing; serum (1/320; 1/10,240); CSF (1/10; 1/160)	IVIg (transient), IVMP, oral steroids (marked improvement); relapsed 1 y after steroid withdrawal	24, mRS 6, died of pneumonia during the relapse
3. M/68	Diarrhea, weight loss (20 kg); 4/11	Memory loss, topographic disorientation, tremor, cerebellar ataxia, rigidity of the arms, sleep disorder (hypersomnia)	11, mRS 3–4	CSF: normal; MRI: nonspecific microangiopathic PVH on T2WI; EEG: focal slowing on left frontal area; serum (1/640; 1/20,480); CSF (NA)	IVMP, oral steroids, IVIg (marked improvement)	22, mRS 1, almost; premorbid status
4. M/67	Diarrhea, weight loss (10 kg), mood disorder; 3/3	Memory loss, hyperekplexia, tremulous jaw movement, myoclonus, tremor, generalized seizures, unilateral rigidity, sleep disorder (wake-sleep cycle disruption, jerky spasms), hyperesthesia	1, mRS 5	CSF: 6 WBCs; MRI: nonspecific white matter lesions; EEG: bilateral temporal slowing; serum (1/5,120; 1/10,240); CSF (1/320; 1/40)	IVIg, PE (partial, transient); CPA, RTX (marked improvement)	6, mRS 0–1, considering going back to work
5. M/49	Diarrhea, weight loss; 3/12	Memory loss, personality change (disinhibition), agitation, confabulation, myoclonus, tremor	11, mRS 2	CSF, MRI, and EEG: normal; serum (1/1,280; 1/5,120); CSF (1/40; 1/40)	IVMP, oral steroids (marked improvement)	12, mRS 0, fully recovered
6.[e] M/57	Weight loss (20 kg), diarrhea, headache; 5/13	Mild mood and cognitive deficits progressing in 1 mo to severe cognitive deficit, hyperekplexia, myoclonus, progressive cerebellar ataxia	8, mRS 5	CSF: 8 WBCs; MRI: nonspecific white matter lesions; serum (1/10,240; 1/163,840); CSF (1/320; 1/2,560)	IVMP, IVIg (partial); RTX, CPA as a part of R-CHOP (marked improvement with remission of neoplasm)	19, mRS 2, mild cognitive difficulties
7. F/45	Weight loss (40 kg), mood disorder; 4/10	Confusion, unspecified personality change, hallucination, tremor, hyperekplexia, myoclonus, hyperreflexia, nystagmus, axial rigidity, urinary incontinence	5, mRS 5	CSF and MRI normal; EEG: diffuse slowing; other: ANA and anti-Smith+.; serum (1/5,120; 1/2,560); CSF (1/160; 1/40)	IVMP, PE (partial: only improvement of hyperekplexia); RTX, CPA (partial response)	15, mRS 5, bedridden with rigidity, tube feeding
8. M/57	Diarrhea, weight loss; NA/54	2-y Progression of executive dysfunction, mood disorder, hyperekplexia, myoclonic jerks interfering with sleep, cerebellar ataxia, axial rigidity, allodynia, pruritus, orthostatic hypotension (long admissions to neuropsychiatric wards)	54, mRS 4	CSF: 12 WBCs, OCB+; MRI: bilateral temporal atrophy; EEG: diffuse slowing; serum (1/2,560; 1/5,120); CSF (1/40; 1/40)	IVMP, oral steroids, IVIg, PE (partial improvement); AZP, RTX (marked improvement); CPA (maintenance of remission)	72, mRS 2, persisting allodynia and pruritus
9. M/69	Diarrhea, weight loss (8 kg); 2/5	Loss of concentration, visual and auditory hallucinations	3, mRS 4	CSF: 130 WBCs; MRI: normal. serum (1/2,560; 1/1,280); CSF (1/160; 1/160)	Steroids, IVIg (marked improvement)	14, mRS 1, mild cognitive symptoms

Abbreviations: ANA = anti-nuclear antibody; AZP = azathioprine; CPA = cyclophosphamide; FU = follow-up; IgG = immunoglobulin G; IVIg = intravenous immunoglobulins; IVMP = intravenous methylprednisolone; mRS = modified Rankin Scale; NA = not available; OCB = oligoclonal IgG band; PE = plasma exchange; PVH = periventricular hyperintensity; RBD = REM sleep behavior disorder; R-CHOP = rituximab, cyclophosphamide, hydroxydaunorubicin, oncovin, prednisolone; RTX = rituximab; T2WI = T2-weighted image; WBC = white blood cell.

[a] From neurologic symptom onset until maximal disability (excluding mood disorders or weight loss).

[b] Apathy, depression, or appetite loss.

[c] No signs of pleocytosis or OCB.

[d] Samples were retrospectively examined after the patient died.

[e] Mantle cell lymphoma was found during the workup of the neurologic disorder.

abnormalities in 3 patients, indicated atrophy of the temporal lobes in 1 patient, and was normal in the other 5 patients. EEG was available from 7 patients, showing slow background activity in 5 patients, epileptiform discharges in 1 patient, and normal findings in 1 patient. Tumor screening revealed a Mantle cell lymphoma in 1 patient (case 6).

Treatment and outcomes. All patients were treated with immunotherapy. After a median follow-up of 19 months (range 6–72 months), 4 had substantial recovery (mRS score 0–1), 3 had mild disability (mRS score 2), 1 patient did not improve (remained bedbound; mRS score 5), and 1 patient died (table, see below for treatment outcome for the whole series of patients).

Anti-DPPX syndrome spectrum and response to treatment. The number of patients reported with anti-DPPX encephalitis since the discovery of this disorder is 30.[1–4,15,16] Combining these patients with the current series (total 39 patients), the median age was 52 years (range 13–76 years), and 27 (69%) were male. Only 2 patients were <18 years old (13 and 15 years). Prodromal weight loss, diarrhea, or other gastrointestinal symptoms occurred in 30 patients (77%). Cognitive, memory, or mental alterations were identified in 36 patients (92%) and signs or symptoms of hyperexcitability in 30 (77%). Only 3 patients (8%) had a clinical picture compatible with PERM (muscle rigidity, brainstem symptoms, and myoclonus with absent or mild and late cognitive alteration). Overall the triad of prodromal weight loss or gastrointestinal symptoms, altered cognitive or mental functions, and CNS hyperexcitability (myoclonus, hyperekplexia, tremor, or seizures) was identified in 26 patients (67%). The presence of an underlying tumor was identified in 3 patients (all B-cell neoplasms: 1 Mantle-cell lymphoma, 1 B-cell lymphoma, and 1 B-cell chronic lymphocytic leukemia). Immunotherapy was used in 29 patients (74%). The outcome was available for 35 patients, including 8 who did not receive immunotherapy: 16 (46%) had substantial improvement, 5 (14%) had moderate improvement, 8 (23%, 6 of them not treated with immunotherapy) had no improvement or disease progression, and 6 (17%, 2 of them not treated with immunotherapy) died.

Eight of the 35 patients (23%) had clinical relapses, in 7 of them during immunotherapy withdrawal. Three of these 8 patients did not receive additional immunotherapy and had unfavorable outcome (2 died, 1 clinical progression). Among the other 5 patients, outcome was assessable for 4 (1 treated with rituximab; 1 plasma exchange and rituximab; 1 plasma exchange, rituximab, and cyclophosphamide; and 1 steroids and plasma exchange), and all showed clinical improvement (3 substantial and 1 moderate).

Anti-DPPX IgG antibodies are predominantly IgG1 and IgG4. Serum was available from all 9 newly studied patients and CSF from 7, including paired serum/CSF samples in 7 cases. All samples had IgG1 and IgG4 DPPX antibodies (figure 1). Additionally, IgG2 antibodies were identified in 7 of 9 sera and 6 of 6 CSF samples; none of the patients had IgG3 antibodies. The IgG antibody subclass was similar in serum and CSF of all cases examined (5 of 5, not enough CSF sample was available from the other 2 patients).

Patients' antibodies decrease the density of surface DPPX and concentration of cell-surface Kv4.2 protein level. Cultured hippocampal neurons treated for 3 days with purified patients' IgG containing DPPX antibodies showed a significant decrease of the density of surface DPPX clusters compared with neurons treated with control IgG (figure e-1, A–D). Because 2 of 3 commercial antibodies against Kv4.2 are raised against intracellular epitopes and the other commercial antibody produced suboptimal live neuronal cell-surface immunostaining (data not shown), we used quantitative immunoblot analysis of cell-surface biotinylated proteins to determine the effects of patients antibodies on Kv4.2. These studies showed a significant reduction of surface protein levels of Kv4.2 and DPPX in neurons treated with patients IgG compared with control IgG (figure e-1, E and F). In parallel experiments, neurons were similarly treated with patients' or control IgG for 3 days and subsequently washed and allowed to recover for 4 or 7 days in media free of antibodies (figure 2). This experiment showed that the levels of cell-surface DPPX clusters were progressively restored, with normal baseline levels reached 7 days after removal of patients' antibodies (figure 2, A–E). Similarly, quantitative immunoblot studies showed that the cell-surface concentrations of Kv4.2 and DPPX were also restored to normal baseline concentrations (figure 2, F and G). Given that patients' antibodies did not react with Kv4.2 (figure e-2), the effects on this receptor are postulated to be caused by the antibody-mediated reduction of DPPX.

DISCUSSION We report our experience with 9 new patients with anti-DPPX encephalitis and review all previously reported cases, providing the main clinical features that should raise suspicion for this disorder and the profile of symptoms that differentiate it from PERM. We also confirm that symptom progression can be chronic and that a few patients may have an underlying tumor, usually a B-cell neoplasm. In this study we show that the antibodies are predominantly IgG1 and IgG4 and that their pathogenic effects on DPPX and Kv4.2 in cultured neurons are reversible on removal of the antibodies from the media.

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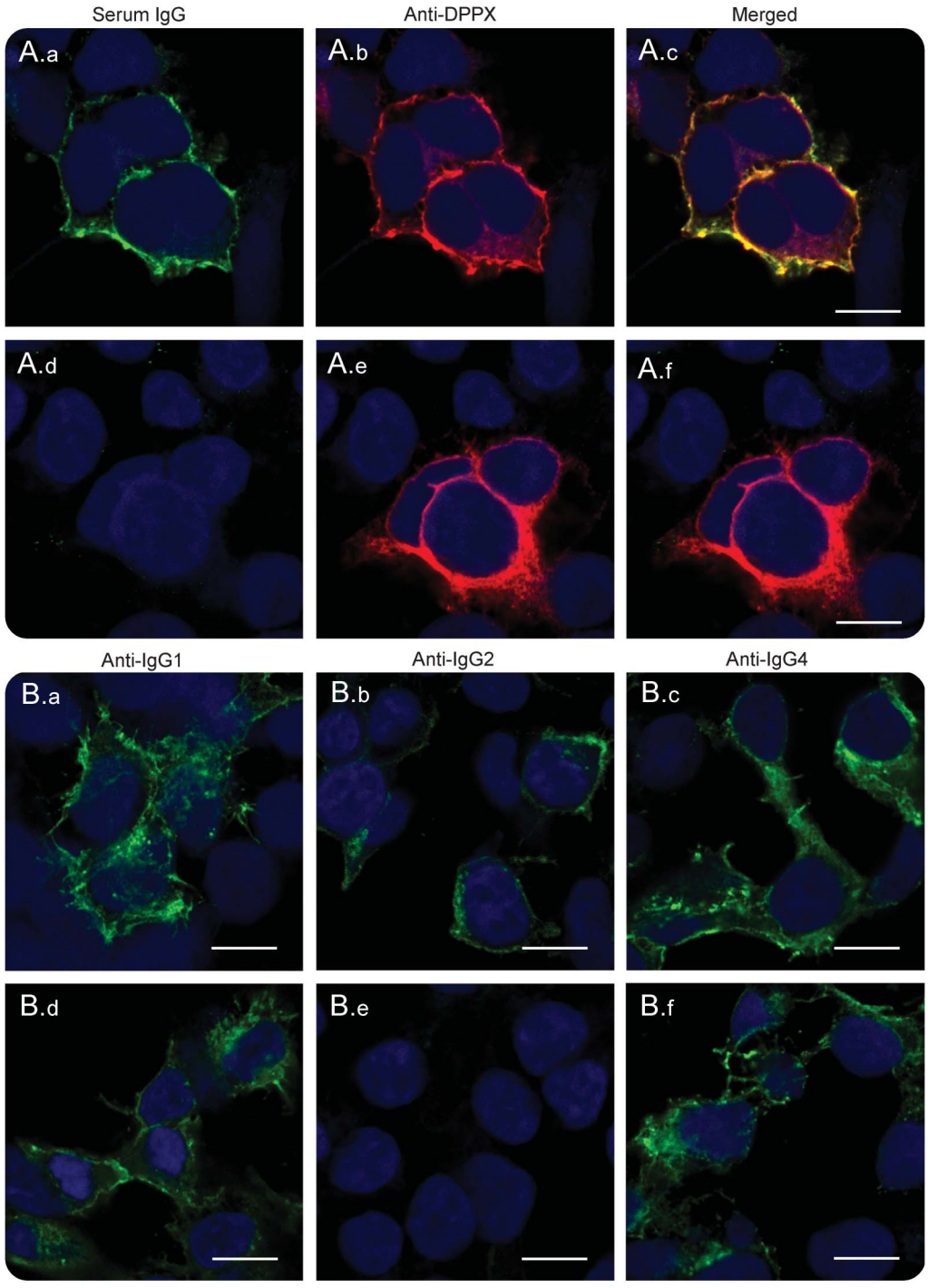
Figure 1 Demonstration of DPPX antibodies and IgG subclasses in a cell-based assay



(A) Serum of a representative case (patient 4) showing reactivity (green, A.a) with human embryonic kidney (HEK) cells expressing dipeptidyl-peptidase–like protein 6 (DPPX). The reactivity of a commercial antibody against DPPX (red, A.b) colocalizes with that of the patient's serum (yellow, A.c). Note that a control serum is negative (A.d–A.f). (B) Determination of immunoglobulin G (IgG) antibody subclasses in 2 cases (patients 4 and 1). Patients' antibodies bound to HEK cells expressing DPPX are demonstrated with secondary anti-human antibodies specific for the indicated subclasses. Patient 4 has DPXX antibodies of the IgG1, IgG2, and IgG4 subclasses (B.a–B.c), whereas patient 1 has IgG1 and IgG4 subclass antibodies (B.d–B.f). Nuclei counterstained with 4′, 6-diamino-2-phenylindole (A and B). Scale bars = 10 μm.

The enriched expression of DPPX in myenteric plexus may explain the frequent gastrointestinal problems, usually accompanied by weight loss.[1] In our experience, this weight loss is not subtle (median 20 kg in the current patients), and it is one of the first symptoms of the disease. Considering the current series and all previously reported cases in which clinical information was assessable (total 39 patients),[1–4,15,16] 67%

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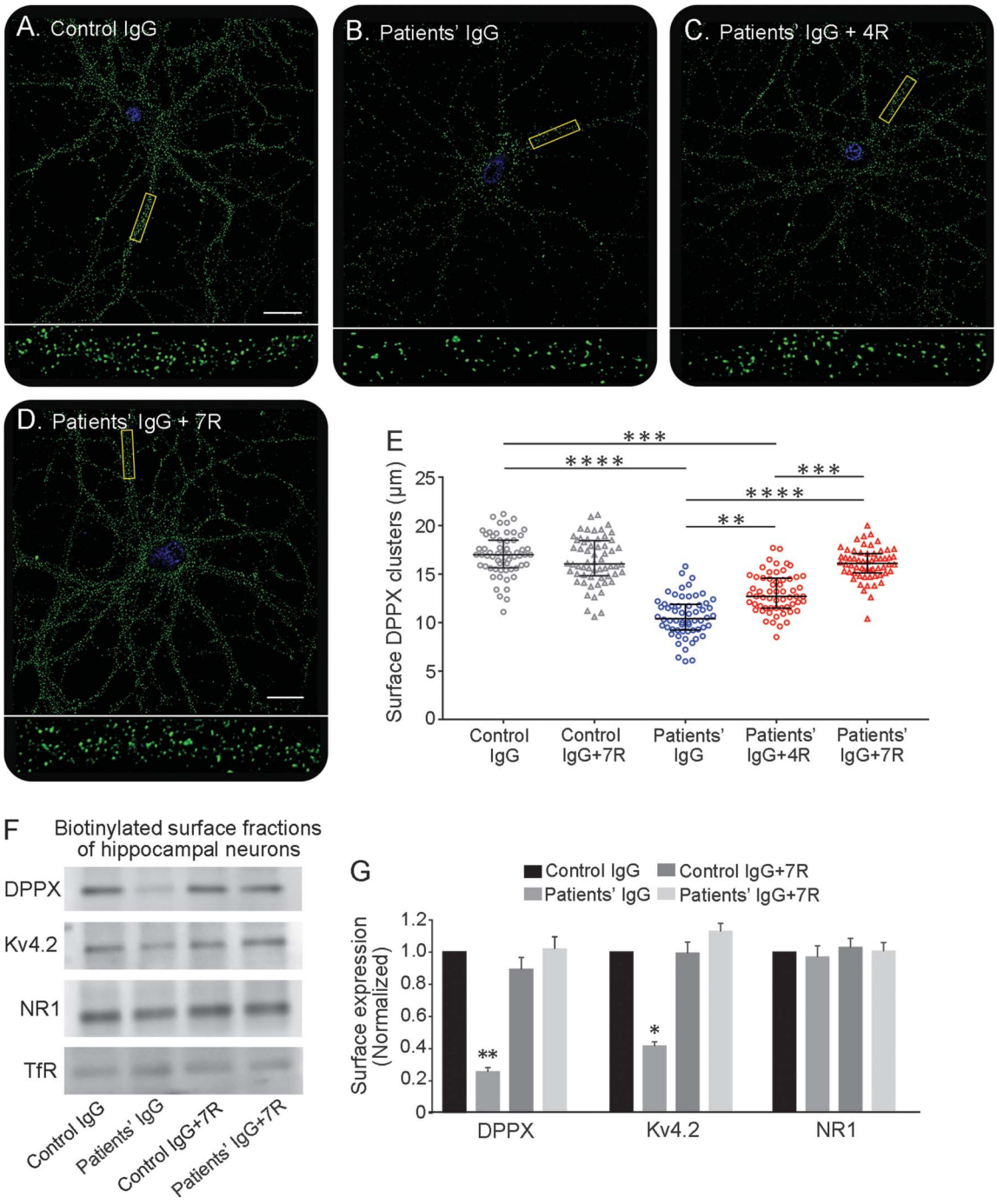
Figure 2 **Effects of patients' antibodies on DPPX and Kv4.2 are reversible**



(A–D) Clusters of dipeptidyl-peptidase–like protein 6 (DPPX) in cultured neurons incubated for 3 days with pooled control immunoglobulin G (IgG), pooled patients' (cases 4 and 7) IgG without recovery, and pooled patients' (cases 4 and 7) IgG for 3 days followed by 4 or 7 days of recovery (fresh media without antibodies). Scale bars = 10 μm. Boxed dendrites are shown at higher magnification below (×100/1.3 numerical aperture oil objective). Note the reduction of clusters of DPPX caused by 3 days' exposure to patients' antibodies and the progressive restoration of the density of DPPX clusters after neurons are allowed to recover for 4 to 7 days. (E) Graphic representation of the density of DPPX clusters (median with interquartile range) after treatment for 3 days with control or patients' IgG and after 4 to 7 days of recovery (4 independent experiments, 15 neurons per experiment in each condition). (F) An immunoblot of biotinylated surface proteins of hippocampal neurons treated with pooled control IgG and pooled patients' IgG for 3 days without or with recovery for 4 to 7 days. The protein bands are demonstrated with commercial antibodies specific for DPPX and Kv4.2; NR1 and transferrin receptor (TfR) are used as loading controls. Note that patients' antibodies cause a substantial decrease of levels of DPPX and Kv4.2 after a 3-day incubation and that the levels of cell-surface proteins are progressively restored after 4 to 7 days. (G) Quantitative densitometry analysis of panel F; the data were normalized to the values of control IgG and are represented as median with SEM of 3 independent experiments (for each experiment, immunoblots were repeated, n = 6). All statistical analyses: Kruskal-Wallis test followed by Dunn test, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

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of all patients had weight loss/gastrointestinal symptoms, cognitive or mental dysfunction, and symptoms of CNS hyperexcitability. This triad of symptoms is unusual in other forms of paraneoplastic or antibody-associated encephalitis (except for anti-Hu associated gastroparesis and encephalitis)[17,18]; therefore, in the context of encephalitis of unclear etiology, it should raise concern for DPPX antibody–associated encephalitis. None of our patients developed cardiac dysrhythmias, but a study in which 3 of 20 patients had ventricular tachycardia (1 case associated with cardiac arrest) suggested that these symptoms could be related to the important role of DPPX in the normal generation of Kv4.3-dependent cardiac rhythms.[2]

The neurologic syndrome associated with DPPX antibodies may resemble that of PERM, a syndrome that was described in the 1970s as a polioencephalomyelitis predominantly involving lower brainstem and spinal cord.[19–22] Recent studies support the concept that PERM is mediated by immune responses against proteins of the GABAergic synapses such as the glycine receptor and, less frequently, amphiphysin or glutamic acid decarboxylase 65.[5,23] The core symptoms of PERM (regardless of antibody association) include prominent muscle stiffness/rigidity in limbs and trunk accompanied by abnormal postures, generalized myoclonus, brainstem dysfunction, and dysautonomia. The clinical presentation is usually slowly progressive, over months, and unlike most patients with DPPX antibody–associated symptoms, patients with PERM do not present with prodromal gastrointestinal symptoms, severe loss of weight, or early and prominent cognitive or mental alterations.[19–22] A previous report described 3 patients with DPPX antibodies who developed a clinical syndrome of PERM,[3] but a subsequent study of 20 patients with DPPX antibodies showed that none developed PERM although 6 had stiffness and rigidity.[2] We did not find this syndrome in any of the patients of the present series or our previous study,[1] which combined include 13 patients. Twelve of these patients had early cognitive or mental changes, and although hyperekplexia was found to be a prominent symptom in 7, only 4 of them had muscle stiffness/rigidity. Nevertheless, case 8 of the current series shows points of convergence between both disorders and the possibility of misdiagnosis. This patient was initially described as having PERM in a cohort of 121 patients with stiff-person syndrome spectrum disorders[5]; however, he had prodromal diarrhea, weight loss, and early executive dysfunction, which are unusual for PERM.[19–22]

In pathologic studies of PERM,[19,24,25] the inflammatory infiltrates predominated in the brainstem and spinal cord; in rare instances in which supratentorial inflammatory infiltrates were identified, they were mild and none of the patients developed cognitive or mental alterations. In contrast, the autopsy (limited to brain) of one of our patients with DPPX antibodies who died of pneumonia during a relapse showed prominent inflammatory infiltrates in the hippocampus, amygdala, cingulum, and temporo-occipital cortex and milder involvement of the pons, cerebellum, and medulla.[16]

Most patients of our series (7 of 9) and 67% of patients from previous studies[2–4,15,16] responded to immunotherapy regardless of the duration of symptoms (range 0–177 months),[2] suggesting that early diagnosis and treatment may further improve outcome. The observation that 9 (4 current and 5 previous[1–3]) of 12 patients who failed first-line immunotherapies responded to rituximab alone or combined with other therapies (5 cyclophosphamide, 1 azathioprine) emphasizes the importance of second-line immunotherapies. Moreover, 7 of 8 patients (1 current and 7 previous[1–3,16]) who developed clinical relapses had not been previously treated with rituximab, and the only case who had received this treatment developed the relapse while treatment was being discontinued. Future studies will clarify the relative contribution of each individual drug, but rituximab was the most frequently used second-line immunotherapy during the initial episode or at relapses, resulting in clinical improvement in 10 of 13 patients (77%). Rituximab has been reported to be highly effective in a number of autoimmune disorders associated with IgG4 antibodies,[26–28] as occurred in our patients.

In addition to IgG4 DPPX antibodies, all our patients had IgG1 antibodies. The distribution of antibody subclass did not change in serum and CSF. Serum IgG (containing both IgG1 and IgG4) from representative patients showed a significant decrease of both cell-surface DPPX and Kv4.2 potassium channels, confirming the findings of a previous study.[4] In addition, our study shows that these effects are reversible on removal of the antibodies from the media, resembling the mode of action of IgG1 antibodies against ion channels (NMDAR,[29] AMPAR[7]). For DPPX/Kv4.2, the process of recovery of cell-surface levels of proteins took longer (7 days) than that observed for antibody-internalized NMDAR (<4 days),[29] suggesting that the underlying mechanisms leading to cell-surface reinsertion of target proteins may be different (e.g., transcriptional for DPPX vs recycling for NMDAR[30]). The limited amount of samples did not allow further studies examining whether isolated IgG4 DPPX had the same effects on decreasing the levels of DPPX/Kv4.2. Given that IgG4 has the unique feature of being hetero-bispecific (continuously undergoing half-antibody exchange) and less effective than IgG1 in crosslinking and internalizing the target antigen,[31,32] we postulate that the

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observed effects were mediated predominantly by IgG1, causing internalization and reduction of levels of DPPX that in turn regulate the cell-surface levels of Kv4.2.[33,34] The internalization of a protein not directly targeted by the antibodies (e.g., Kv4.2) but that interacts with the antigen (DPPX) resembles the effects of LGI1 antibody–associated encephalitis in which the antibodies are specific for LGI1 but cause a decrease of density of the interacting AMPARs.[35]

A limitation of the study is the retrospective assessment of symptoms, but all information (current patients and the 9,798 cases studied since 2013) is similarly collected with a structured questionnaire. Future studies should include EMG evaluation to determine whether patients with DPPX develop continuous motor unit dischargers (such as in PERM), to determine the frequency of other autonomic symptoms, mainly cardiac dysrhythmias, and to confirm the beneficial effect of rituximab. The exact mechanism causing the decrease of DPPX/Kv4.2 and restoration of the levels of these proteins needs to be determined, as well as the individual contributions of IgG1 and IgG4 to the pathogenic effects.

AUTHOR CONTRIBUTIONS

Design/conceptualization of the study, analysis and interpretation of the data: M.H., M.R.R., F.G., J.D.; data collection: M.H., H.A., M.P.-P., L.S., M.J.T., E.M.-H.; statistical analysis: M.H.; figure development: M.H., J.D.; drafting of the manuscript M.H., M.R.R., F.G., J.D.

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DISCLOSURE

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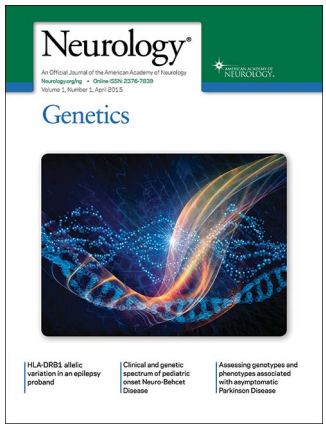

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