

# Investigations of Caspr2, an Autoantigen of Encephalitis and Neuromyotonia

Eric Lancaster, MD, PhD,<sup>1</sup> Maartje G. M. Huijbers, BS,<sup>1</sup> Vered Bar, PhD,<sup>2</sup> Anna Boronat, BS,<sup>1</sup> Andrew Wong, BS,<sup>1</sup> Eugenia Martinez-Hernandez, MD,<sup>1</sup> Christina Wilson, MD, PhD,<sup>1</sup> Dina Jacobs, MD,<sup>1</sup> Meizan Lai, MD,<sup>1</sup> Russell W. Walker, MD,<sup>3</sup> Francesc Graus, MD,<sup>4</sup> Luis Bataller, MD,<sup>5</sup> Isabel Illa, MD,<sup>6</sup> Sander Markx, MD,<sup>7</sup> Kevin A. Strauss, MD,<sup>8,9,10</sup> Elicor Peles, PhD,<sup>2</sup> Steven S. Scherer, MD, PhD,<sup>1</sup> and Josep Dalmau, MD, PhD<sup>1</sup>

**Objective:** To report clinical and immunological investigations of contactin-associated protein-like 2 (Caspr2), an autoantigen of encephalitis and peripheral nerve hyperexcitability (PNH) previously attributed to voltage-gated potassium channels (VGKC).

**Methods:** Clinical analysis was performed on patients with encephalitis, PNH, or both. Immunoprecipitation and mass spectrometry were used to identify the antigen and to develop an assay with Caspr2-expressing cells. Immunoabsorption with Caspr2 and comparative immunostaining of brain and peripheral nerve of wild-type and Caspr2-null mice were used to assess antibody specificity.

**Results:** Using Caspr2-expressing cells, antibodies were identified in 8 patients but not in 140 patients with several types of autoimmune or viral encephalitis, PNH, or mutations of the Caspr2-encoding gene. Patients' antibodies reacted with brain and peripheral nerve in a pattern that colocalized with Caspr2. This reactivity was abrogated after immunoabsorption with Caspr2 and was absent in tissues from Caspr2-null mice. Of the 8 patients with Caspr2 antibodies, 7 had encephalopathy or seizures, 5 neuropathy or PNH, and 1 isolated PNH. Three patients also had myasthenia gravis, bulbar weakness, or symptoms that initially suggested motor neuron disease. None of the patients had active cancer; 7 responded to immunotherapy and were healthy or only mildly disabled at last follow-up (median, 8 months; range, 6–84 months).

**Interpretation:** Caspr2 is an autoantigen of encephalitis and PNH previously attributed to VGKC antibodies. The occurrence of other autoantibodies may result in a complex syndrome that at presentation could be mistaken for a motor neuron disorder. Recognition of this disorder is important, because it responds to immunotherapy.

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We recently reported that leucine-rich glioma inactivated 1 (Lgi1) is the main autoantigen of limbic encephalitis previously attributed to voltage-gated potassium channels (VGKC).<sup>1</sup> In that study, we also demonstrated that neither Lgi1 nor VGKC are the target antigens in patients with disorders other than limbic encephalitis who also have antibodies previously attrib-

uted to VGKC. A direct immunoprecipitation technique showed that 1 of these antigens is contactin-associated protein-like 2 (Caspr2), but the clinical and immunological associations of these antibodies were not further examined. Here we describe the index patient whose serum was used to isolate Caspr2, and examine the clinical and immunological associations in 7 additional patients

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Address correspondence to Dr Dalmau, Department of Neurology, 3 W. Gates (Division of Neuro-oncology), University of Pennsylvania, 3400 Spruce Street, Philadelphia, PA 19104. E-mail: [josep.dalmau@uphs.upenn.edu](mailto:josep.dalmau@uphs.upenn.edu)

From the <sup>1</sup>Department of Neurology, University of Pennsylvania School of Medicine, Philadelphia, PA; <sup>2</sup>Department of Molecular Cellular Biology, Weizmann Institute of Science, Rehovot, Israel; <sup>3</sup>Barrow Neurological Institute, St. Joseph's Hospital and Medical Center, Phoenix, AZ; <sup>4</sup>Service of Neurology, Hospital Clinic, University of Barcelona and Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelonà, Spain; <sup>5</sup>Department of Neurology, University Hospital La Fe, Valencia, Spain; <sup>6</sup>Department of Neurology, Hospital de Sant Pau, Autònoma University of Barcelona, Barcelona, Spain; <sup>7</sup>Department of Psychiatry, Columbia University, College of Physicians and Surgeons, New York, NY; <sup>8</sup>Clinic for Special Children, Strasburg, PA; <sup>9</sup>Department of Biology, Franklin and Marshall College, Lancaster, PA; and <sup>10</sup>Lancaster General Hospital, Lancaster, PA.

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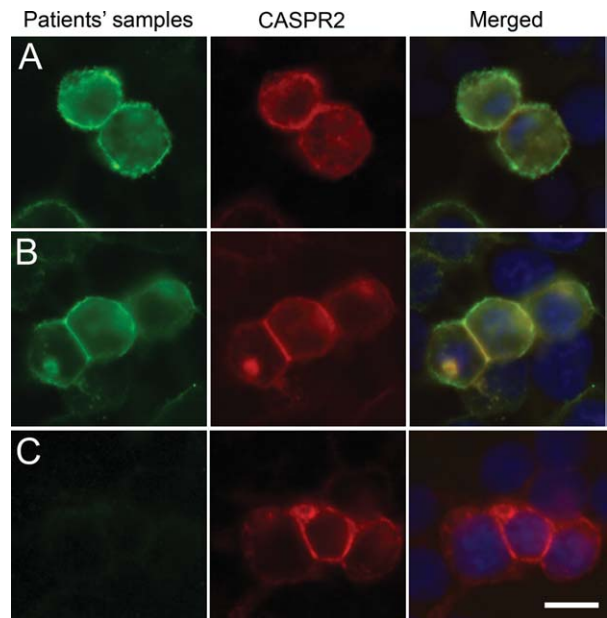
with this autoimmunity. We also demonstrate that antibodies to Caspr2 specifically account for the reactivity of these patients' cerebrospinal fluid (CSF) or sera against brain and peripheral nerve.

## Patients and Methods

The term *antibodies attributed to VGKC* is used in this study to define antibodies identified by a radioimmunoassay based on the immunoprecipitation of brain protein complexes containing VGKC labeled with  $^{125}\text{I}$ - $\alpha$ -dendrotoxin ( $^{125}\text{I}$ - $\alpha$ -dendrotoxin RIA) or by immunohistochemical methods.<sup>2-4</sup> Because specific assays show that in patients with limbic encephalitis and antibodies attributed to VGKC the main target antigen is Lgi1, and because Caspr2 was precipitated with serum of a patient with encephalitis and peripheral neuropathy,<sup>1</sup> a goal of the current study was to determine whether patients with encephalitis or peripheral nerve dysfunction suspected to be related to VGKC antibodies in fact had Caspr2 antibodies. The term *peripheral nerve hyperexcitability* (PNH) was used to include acquired neuromyotonia (Isaacs syndrome) or partial manifestations of this disorder such as cramps and fasciculations without clear evidence of neuromyotonia. The groups of patients examined to identify the 7 additional cases included 49 patients with limbic encephalitis previously attributed to VGKC antibodies, 18 with acquired PNH, 15 with encephalitis and atypical antibodies against the neuropil of hippocampus, and 6 with Morvan syndrome. As control samples, we used serum or CSF from 19 patients with other autoimmune encephalitis associated with N-methyl-d-aspartate (n = 7),  $\alpha$ -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (n = 6), or gamma-aminobutyric acid B (n = 6) receptor antibodies, 13 patients with Rasmussen encephalitis, and 6 patients with viral encephalitis. Additionally, because some disorders with genetic mutations (eg, hereditary ataxias) are associated with a tendency to autoimmunity,<sup>5</sup> and patients with mutations of *CNTNAP2* (the gene that codes for Caspr2) develop symptoms resembling those of patients with Caspr2 antibodies, including seizures, hyperactivity, encephalopathy,<sup>6</sup> and sometimes neuromyotonia (discussed later), we examined 21 patients with polymorphisms or mutations of *CNTNAP2* for antibodies to Caspr2. Studies were approved by the University of Pennsylvania Institutional Review Board.

### Immunocytochemistry on Caspr2-Expressing Cells

To generate a diagnostic cell-based assay, human embryonic kidney 293 cells (HEK293) were transiently transfected using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) and a plasmid containing human Caspr2.<sup>1</sup> Patients' CSF (diluted 1:5) or sera (1:200) and a commercial rabbit antibody to Caspr2 (1:10,000; Abcam, Cambridge, MA; ab93228) were applied to cells, followed by fluorescein isothiocyanate (FITC)-conjugated antihuman immunoglobulin (Ig)G (1:1,000; Molecular Probes, Eugene, OR) and tetramethylrhodamine isothiocyanate (TRITC)-conjugated antirabbit secondary antibodies (1:1,000; Jackson Laboratories, Bar Harbor, ME). Images of transfected cells were captured with an epifluorescence microscope using Zeiss Axiovision software (Zeiss, Thornwood, NY).



**FIGURE 1:** Patients' antibodies react with cells expressing Caspr2. HEK cells were transiently transfected to express human Caspr2, and labeled with the index patient's cerebrospinal fluid (CSF) (A; green) or serum from a different patient (B; green) and a rabbit antibody to Caspr2 (A, B; red), and counterstained with DAPI. Merged images (A, B; yellow) demonstrate the overlap of patients' antibody staining with Caspr2 expression. (C) CSF from a control patient did not react with cells expressing Caspr2. Scale bar = 20  $\mu\text{m}$ .

### Immunohistochemistry on Brain and Peripheral Nerve

Adult rat brains were prepared for immunohistochemical screening.<sup>4</sup> Female Wistar rats were sacrificed without perfusion, the brains removed and immersed in 4% paraformaldehyde at 4°C for 1 hour, cryoprotected in 40% sucrose for 24 hours, snap frozen in chilled isopentane, and sectioned. *Caspr2*-null mice and their wild-type littermates were generated and genotyped as reported,<sup>7</sup> and the brains were prepared and sectioned as above. For studies labeling peripheral nerve with patients' antibodies, unfixed teased rat or mouse sciatic nerve fibers were prepared as previously described for paraformaldehyde fixed nerves.<sup>8</sup>

Immunohistochemistry was done using a standard avidin-biotin peroxidase method, using serum (1:200) or CSF (1:5) on 7  $\mu\text{m}$  sagittal brain sections followed by the appropriate secondary antibodies.<sup>4</sup>

Teased nerve fibers were permeabilized with acetone (−20°C for 10 minutes), washed with phosphate buffered saline (5 minutes  $\times$  3), blocked with 5% goat serum (1 hour, room temperature), then incubated with serum (1:200 to 1:4,000) or CSF (1:5) and a rabbit antibody to Caspr2 (Abcam; ab93228; diluted 1:300) overnight at 4°C, followed by FITC-conjugated antihuman IgG (1:200; Molecular Probes) and TRITC-conjugated antirabbit secondary antibody (1:300; Jackson Laboratories) for 1 hour at room temperature. An antihuman IgM antibody (1:200; Molecular Probes) was used for some experiments to confirm that patients' antibodies were of the IgG type. In some

**TABLE 1: Demographic and Clinical Features**

Patient	Age, yr	Sex	Symptoms/Signs	Other Clinical and Immunological Features
1	68	M	3 years of thermal allodynia, 2 years of intermittent complex partial seizures, and 1 year of progressive memory difficulties.	VGKC antibodies. <sup>a</sup> CSF: 2 WBC/ $\mu$ l, protein 44mg/dl, glucose 65mg/dl. Oligoclonal bands negative.
2	46	F	2 years of progressive fatigue, personality changes, increased sweating, dysphagia with substantial weight loss, dysarthria, and intermittent ptosis and diplopia. Progressed to prolonged respiratory failure, visual hallucinations, and delusions of persecution.	Diagnosed with myasthenia gravis. MuSK antibodies 89nmol/l (normal <0.5). AChR antibodies 247nmol/l (normal <0.5). VGKC antibodies. <sup>a</sup>
3	66	M	1 year of progressive lower extremity cramps, worse with exercise, and sensory-predominant polyneuropathy.	VGKC antibodies not detected. <sup>a</sup>
4	52	M	5 years of progressive encephalopathy, myokymia, and weakness. Progressed to critical illness with seizures, severe bulbar weakness, and encephalitis.	VGKC antibodies. <sup>a</sup> GAD65 antibodies, AChR antibodies. SIADH, peripheral neuropathy (type 1 diabetes).
5	77	M	1 year of 70 pounds weight loss, diffuse sweating, twitching of hands and feet. Progressed to severe jerking at night, even during sleep, difficulty walking, falls, difficulty concentrating, confusion, and psychomotor slowing.	VGKC antibodies. <sup>a</sup> Serum sodium 129 mmol. CSF: no cells, protein 34mg/dl, glucose 58mg/dl.
6	60	M	Agitation, amnesic periods, seizures. No peripheral nervous system symptoms.	VGKC antibodies not tested. CSF: 4 WBC/ $\mu$ l, protein 35mg/dl, glucose 75mg/dl. Elevated PSA-negative prostate biopsy, negative PET scan and CT of abdomen and pelvis.
7	61	M	Myokymia, fasciculations, cramps, involuntary lingual movements. Developed seizures and encephalitis after 1 year. Progressive dysphagia, dysarthria, myoclonus, and ataxia after 6 years.	VGKC antibodies. <sup>a</sup> CSF: 15 WBC/ $\mu$ l, normal protein and glucose.
8	59	M	Generalized seizures and severe amnesic syndrome.	VGKC antibodies. <sup>a</sup>

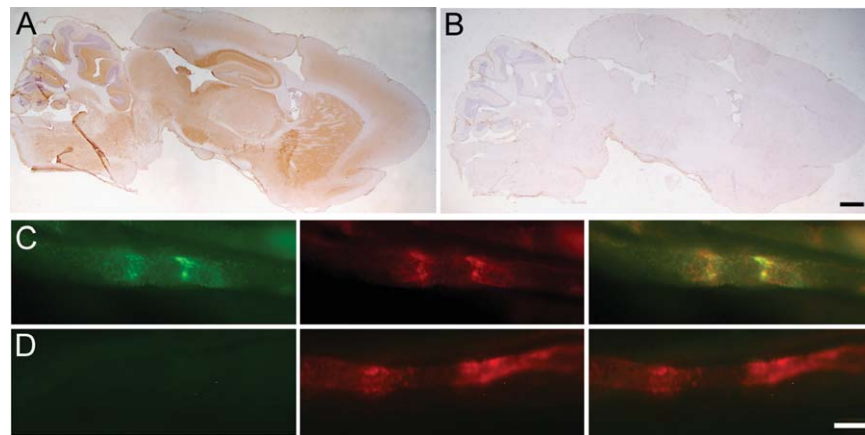
<sup>a</sup>VGKC antibodies detected by the <sup>125</sup>I- $\alpha$ -dendrotoxin radioimmunoassay; normal values differed in different laboratories. VGKC = voltage-gated potassium channels; CSF = cerebrospinal fluid; WBC = white blood cells; MuSK = muscle-specific kinase; AChR = acetylcholine receptor; SIADH = syndrome of inappropriate antidiuretic hormone secretion; PSA = prostate-specific antigen; PET = positron emission tomography; CT = computed tomography.

experiments, paranodes were labeled with a monoclonal antibody to Caspr (1:50) described previously,<sup>9</sup> followed by a Cy5-conjugated goat antimouse secondary antibody (Jackson Laboratories; 1:200). Images of peripheral nerve were captured with an epifluorescence microscope using Openlab 3.1.7 software (Perkin Elmer, Waltham, MA). Immunoabsorption was carried out with serial incubations of patient's CSF in 6 wells containing fixed, permeabilized HEK293 cells expressing Caspr2 or a control plasmid without an insert. After sequential passes of 1 hour each, the CSF was applied to brain or peripheral nerve as described above.

## Results

### Index Patient (Case #1)

A 68-year-old man was evaluated for seizures and memory difficulties. Over the previous 2 years he had had 3 seizures, consisting of staring and unresponsiveness followed by generalized tonic-clonic convulsions. Approximately 1 year before evaluation, he developed progressive memory difficulties. He often repeated himself, and had difficulty learning new information and recalling names of close acquaintances. He occasionally became lost while



**FIGURE 2:** Immunoabsorption with Caspr2 abrogates the reactivity of the index patient's antibodies with rodent brain and peripheral nerve. Top row: Rat brain sections were immunolabeled with the cerebrospinal fluid (CSF) of the index patient after immunoabsorption with (A) sham transfected HEK cells or (B) HEK cells transfected to express Caspr2. Scale bar = 300 $\mu$ m. Bottom rows: Teased mouse sciatic nerve fibers were immunolabeled with the CSF (green) of the index patient after immunoabsorption with (C) sham transfected HEK cells or (D) HEK cells transfected to express Caspr2; a rabbit antibody against Caspr2 (red) labels the endogenous Caspr2. Note that the reactivity of the patient's CSF for both brain and teased fibers is abrogated when the CSF is preabsorbed with Caspr2. Scale bar = 10 $\mu$ m.

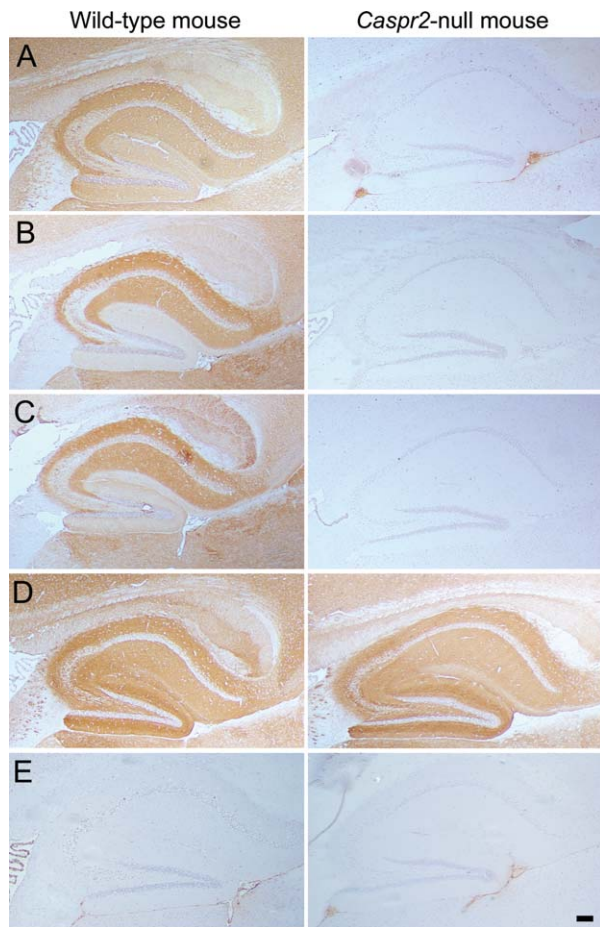
driving in familiar surroundings. Starting 3 years earlier, he had painful hypersensitivity to warm temperatures affecting his hands and feet that persisted for 2 years and spontaneously resolved. Twelve months after the onset of seizures, he had low-grade, noninvasive bladder cancer that was treated with local scraping and cauterization; this has not recurred. He smoked (40–50 packs/year) and drank approximately 8 alcoholic drinks per week.

His Mini Mental State Examination was 29/30 (missed recall of 1 item at 5 minutes). He was able to name 12 of 15 objects on the Boston Naming test. Neuropsychological testing revealed a mild memory deficit, with difficulties in visuospatial and nonverbal memory. Cranial nerve function, strength, and muscle tone were normal. Sensation of vibration was decreased on the toes, but sensation of cold, proprioception, and pinprick was intact. Reflexes were 2/5 and symmetrical. His gait was slightly wide-based, and he had difficulty performing tandem gait.

Brain magnetic resonance imaging (MRI) demonstrated bilateral mesial temporal lobe abnormalities, particularly in the amygdala, with extension posteriorly through the hippocampus, as well as mild cortical atrophy. An electroencephalogram (EEG) demonstrated left frontal polar and bitemporal epileptiform discharges but no electrographic seizures. A lumbar puncture revealed an opening pressure of 156mm H<sub>2</sub>O, 2 white blood cells/ $\mu$ l, 0 red blood cells/ $\mu$ l, protein 44mg/dl, and glucose 65mg/dl. CSF cytology and flow cytometry were negative, and there were no oligoclonal bands. <sup>125</sup>I- $\alpha$ -dendrotoxin RIA for VGKC antibodies in serum was positive (0.19nmol/l, upper limit of normal, 0.02nmol/l). CSF polymerase chain reaction was negative for West Nile vi-

rus, *Tropheryma whipplei*, and arboviruses. Serum vitamin B12 level was normal, and the following antibodies were negative: ANA, SSB, ds-DNA, RNP, Scl 70, and Jo-1. Elevated SSA/Ro antibodies (3U; upper limit of normal, 0.9U) were noted in serum; complement C3/C4 was within normal limits. Tumor markers CEA, CA19-9, CA125,  $\alpha$ -fetoprotein,  $\beta$ -hCG, and paraneoplastic antibodies (Hu, Ma2, CRMP5, amphiphysin, Yo, Ri) were all negative. Computed tomography and fluorodeoxyglucose positron emission tomography did not reveal a neoplasm. Nerve conduction velocities showed no evidence of polyneuropathy, and needle electromyogram (EMG) showed no evidence of PNH.

One month following this evaluation, he presented with a cluster of 3 generalized tonic-clonic seizures, including 1 lasting 20 minutes, followed by non-convulsive status epilepticus. His seizures were controlled with lorazepam, levetiracetam, valproic acid, and phenytoin. Repeat CSF and brain MRI studies were unchanged. A 5-day course of methylprednisolone (1g daily) was initiated, with marked improvement in his cognitive function. He was slowly tapered from prednisone 60mg to 40mg, which led to increased confusion. Prednisone 60mg was reinitiated, with some improvement in mental function. In an effort to decrease his steroid dependence, he was initiated on weekly rituximab for a 4-week cycle. At the last visit (3 months after rituximab), he was seizure free for >6 months and was able to fluently converse, balance a check book, and independently manage his daily affairs. His chief concern was continuing difficulty recalling events from several years ago.



**FIGURE 3:** Patients' antibodies react with wild-type but not *Caspr2*-null mouse brains. The (A) cerebrospinal fluid (CSF) and (B) serum from 2 different patients with *Caspr2* antibodies immunostain the hippocampus of a wild-type mouse in a pattern identical to that obtained with (C) a rabbit polyclonal antibody against *Caspr2*. This staining is not seen in sections from a *Caspr2*-null mouse (A–C, columns on the right). (D) The pattern of staining with a CSF from a patient with limbic encephalitis associated with *Lgi1* antibodies is subtly different from *Caspr2*, and it is not affected using wild-type or *Caspr2*-null mice. (E) The CSF of a control individual without *Caspr2* or *Lgi1* antibodies does not label either sample. Scale bar = 100 $\mu$ m.

### Identification of *Caspr2* as a Target Autoantigen

The serum and the CSF from the index case reacted with a neuronal surface antigen expressed on live, nonpermeabilized rat hippocampal neurons (data not shown) that was precipitated and characterized by mass spectrometry as *Caspr2*.<sup>1</sup> Subsequently, HEK293 cells transfected to express human *Caspr2* were used as a diagnostic assay that confirmed the presence of *Caspr2* antibodies in the index patient (Fig 1), and served to identify 7 additional cases among the indicated cohort of patients (Table 1). These 7 patients' sera did not react with nontransfected cells, cells transfected with the volt-

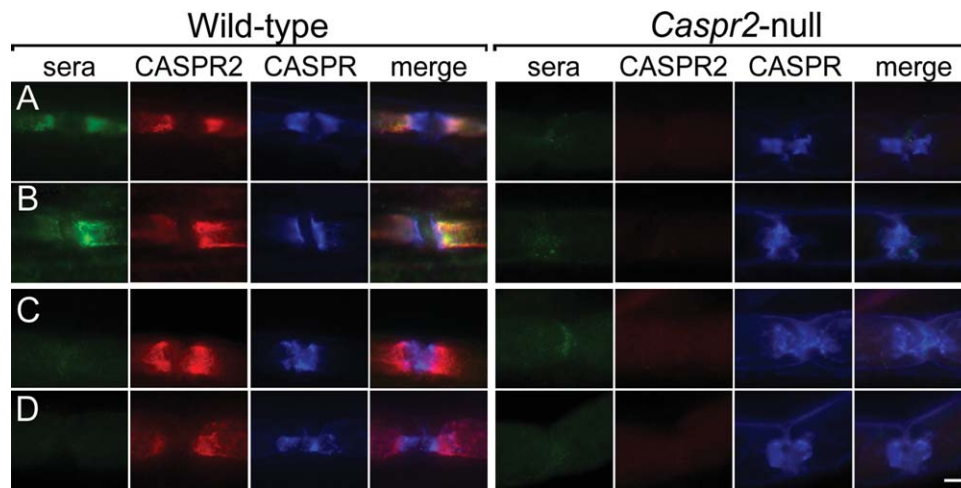
age-gated potassium channel subunits Kv1.1 and Kv1.4, or cells transfected with *Lgi1* (data not shown).

### Patients' Antibodies React with Peripheral Nerve and Colocalize with the Juxtaparanodal Expression of *Caspr2*

Because *Caspr2* is concentrated at the juxtaparanodal region of myelinated axons<sup>7</sup> and the sera of some patients with antibodies previously attributed to VGKC labeled myelinated axons,<sup>10</sup> we examined whether patients' antibodies colocalized with *Caspr2* at the juxtaparanodal region of mouse sciatic nerve fibers. The patients' serum and CSF, but not those from controls, robustly stained the juxtaparanodal regions of myelinated fibers in a pattern that overlapped with that of *Caspr2* (Supplementary Fig). Patients' antibodies bound to *Caspr2* at the juxtaparanodal region were labeled by a secondary antihuman IgG antibody but not a secondary antihuman IgM antibody, indicating that they are of the IgG type (data not shown).

### *Caspr2* Is the Target Autoantigen in Brain and Nerve

We next used 2 different approaches to demonstrate that the reactivity of patients' antibodies with brain and nerve is directed against *Caspr2*. First, we immunoabsorbed a patient's CSF with *Caspr2*, and found that this specifically abrogated the reactivity of patient's antibodies with brain and peripheral nerve (Fig 2). As a further test, we immunostained brains and teased nerve fibers from *Caspr2*-null mice and their wild-type littermates. Patients with coexisting antibodies to other antigens (acetylcholine receptor [AChR], muscle-specific kinase [MuSK], glutamic acid decarboxylase) were excluded from this analysis (#2 and #4). These studies showed that sera from the remaining 5 patients immunostained the neuropil of brain in a pattern expected for *Caspr2* (Fig 3). Similar experiments with peripheral nerve showed that patients' sera also labeled the juxtaparanodes of myelinated peripheral nerve fibers of wild-type mice in a pattern expected for *Caspr2* reactivity (Fig 4). In contrast, these patterns of reactivity were not seen in *Caspr2*-null mice, indicating that the immunostaining was due to specific *Caspr2* antibodies (see Figs 3 and 4). Peripheral nerve fibers of wild-type and *Caspr2*-null mice were costained with a monoclonal antibody to the paranodal protein *Caspr*, which is related to *Caspr2*.<sup>9</sup> *Caspr* staining did not colocalize with patients' antibody staining and was unchanged in *Caspr2*-null mice, further confirming the specificity of patients' antibodies for *Caspr2*.



**FIGURE 4:** Patients' antibodies label teased fibers from wild-type but not *Caspr2*-null nerves. (A, B) The sera (green) of 2 patients with *Caspr2* antibodies immunolabel the juxtaparanodes of teased sciatic nerve fibers from wild-type mice in a pattern similar to that of a rabbit antibody against *Caspr2* (red), but different from the paranodal staining seen with a mouse monoclonal against *Caspr* (blue). In contrast, neither these patients' sera nor the rabbit antibody against *Caspr2* label teased sciatic nerve fibers from *Caspr2*-null mice (columns on the right). (C, D) Sera from 2 controls do not label teased nerve fibers. Scale bar = 5  $\mu$ m.

### Characteristics of Patients with *Caspr2* Antibodies

The clinical characteristics of the patients with *Caspr2* antibodies are described in Tables 1 and 2. Five patients had symptoms involving both the central nervous system (CNS) (encephalitis, seizures) and peripheral nervous system (PNS) (hyperexcitability, neuropathy, thermal allodynia). Two additional patients had a purely CNS syndrome. Only 1 patient (#3) had acquired neuromyotonia without CNS symptoms. Two patients (#2 and #4) had bulbar weakness consistent with myasthenia gravis, and other autoantibodies against the skeletal muscle AchR and/or MuSK. One of these patients (#2) was described in a case report prior to the identification of *Caspr2* antibodies.<sup>11</sup> Case #7 had severe progressive bulbar weakness but was not evaluated for myasthenia. A diagnosis of motor neuron disease was initially considered in these 3 patients (#2, #4, and #7).

Of the 5 patients with CNS symptoms who had brain MRI, 2 had T2 hyperintensities affecting the medial temporal lobes, 1 developed generalized atrophy, and 2 were normal. EEG studies of these 5 patients showed focal epileptiform activity in 3, diffuse slowing in 1, and no abnormality in 1. Of the 5 patients with PNS symptoms who had EMG/nerve conduction studies, 4 had electrophysiological evidence of PNH, and 1 (who only had transient thermal allodynia) had a normal study.

Six of 7 patients tested for VGKC antibodies using the <sup>125</sup>I- $\alpha$ -dendrotoxin RIA had positive results, although none of them recognized HEK293 cells transfected with VGKC subunits (data not shown). None of the patients had a tumor except for past history of low-grade bladder

cancer in the index case. Seven patients improved with immunotherapy and were either healthy or only mildly disabled at the last follow-up (median, 8 months; range, 6–84 months). The patient with poor outcome (#7) had progressive bulbar weakness, never received immunotherapy, and was lost to follow-up after 7 years.

### Other Groups of Patients

Among the groups of patients studied, *Caspr2* antibodies were not identified in 2 of 6 patients with Morvan syndrome, 17 of 18 patients with acquired PNH, and 13 of 15 patients with encephalitis associated with atypical antibodies against the neuropil of hippocampus. None of the 49 patients with limbic encephalitis and antibodies attributed to VGKC had *Caspr2* antibodies; however, they all had *Lgi1* antibodies. Two patients with PNH and 1 patient with Morvan syndrome without *Caspr2* or *Lgi1* antibodies had a positive <sup>125</sup>I- $\alpha$ -dendrotoxin RIA, suggesting they had antibodies against other components of the VGKC-protein complex.

### Discussion

This study demonstrates that *Caspr2* is a brain and peripheral nerve autoantigen in a subgroup of disorders previously attributed to VGKC antibodies, including encephalitis, peripheral nerve dysfunction, or a combination of both (Morvan syndrome). Symptoms may include cognitive impairment, memory loss, hallucinations, delusions, seizures, PNH, and axonal sensorimotor neuropathy. The presence of other autoantibodies may result in a complex syndrome with clinical and electrophysiological

**TABLE 2: Imaging, Clinical Electrophysiology, Treatment, and Outcome**

Patient	Brain MRI	EEG	EMG/NCS	Treatments	Outcome (duration of follow-up)
1	Bilateral increased T2 signal involving mesial temporal lobes, and mild cortical atrophy.	Left frontal polar and bitemporal epileptiform discharges but no electrographic seizures.	Normal motor and sensory responses (aside from median neuropathy at the wrist). No evidence of peripheral nerve hyperexcitability.	Corticosteroids, rituximab.	Improving cognition; mild residual memory deficits; seizures controlled with antiepileptics (6 months).
2	Normal.	—	Normal motor and sensory nerve conduction studies. Needle EMG showed spontaneous motor unit discharges and fibrillation potentials, but no neuromyotonia. Repetitive stimulation was normal.	Prednisone, cyclosporine, thymectomy, rituximab.	All symptoms resolved. Mental status normal (5 years).
3	—	—	Decreased sensory and motor amplitudes, normal conduction velocities, fasciculations, 5–150Hz spontaneous motor unit discharges, increased motor unit duration, amplitude, and polyphasic motor units.	Carbamazepine, amitriptyline, pregabalin (all for peripheral nerve hyperexcitability).	Mild disability from continuing sensory-motor polyneuropathy and cramps (4 years).
4	Normal.	Mild diffuse background slowing.		Methylprednisolone, plasma exchange, IVIG, low-dose cyclophosphamide, rituximab.	Markedly improved cognition, seizures controlled, bulbar weakness improved (6 months).
5	Age-appropriate atrophy.	Normal.	At presentation: myokymia, fasciculations, chronic and acute denervation. After 1 month of treatment: marked improvement of myokymia, no signs of denervation.	Corticosteroids, plasma exchange.	Cognitive symptoms persist (not oriented to time), improving muscle movements (12 months).
6	T2 increased intensities affecting left medial temporal lobe.	Focal slowing and sharp waves over left temporal region.		Methylprednisolone.	Recovered from seizures and memory deficits; mild mood instability (6 months).
7	At presentation: normal. At 2 years: diffuse cortical and subcortical atrophy.	Right temporal sharp waves.	At presentation: normal. At 5 years: normal NCS, diffuse fasciculations, normal motor unit morphology. At 7 years: normal sensory responses, decreased motor amplitudes, normal conduction velocities, widespread fasciculations, fibrillation potentials, and 5–150Hz motor unit discharges.	None.	Lost to follow-up after 7 years of worsening symptoms.
8	Normal.	—	—	IVIg (6-month cycles).	Normal (6 months).

MRI = magnetic resonance imaging; EEG = electroencephalography; EMG = electromyography; NCS = nerve conduction studies; IVIG = intravenous immunoglobulin.

features suggesting a motor neuron disorder. Three different sets of experiments establish Caspr2 as an autoantigen of these disorders, (1) specific immunostaining of HEK293 cells expressing Caspr2 with serum or CSF of patients; (2) specific abrogation of patients' serum or CSF antibody reactivity after immunoabsorption with Caspr2-expressing cells; and (3) comparative brain and nerve immunostaining of wild-type and *Caspr2*-null mice, demonstrating lack of reactivity of patients' serum and CSF with *Caspr2*-null mice.

Caspr2 has a critical role in concentrating VGKC and other proteins in the juxtaparanodal region of myelinated axons in both the PNS and the CNS.<sup>9,12</sup> One patient with homozygous deletion of *CNTNAP2* (OMIM 604569), the human gene that encodes Caspr2, had a history of seizures and developed over 6 months progressive painful peripheral neuropathy and neuromyotonia to the point that it interfered with the patient's gait (K. Strauss, unpublished data). However, knockout mice with disruption of the murine homolog encoding Caspr2<sup>7</sup> had normal peripheral nerve conduction, suggesting that in patients with genetic or autoimmune disruption of Caspr2 the occurrence of peripheral neuropathy may be due to the involvement of other proteins that interact with Caspr2. Caspr2 is also expressed in the hippocampus,<sup>13</sup> and mutations and polymorphisms of *CNTNAP2* have been linked to schizophrenia, psychosis, intractable focal seizures, autism, mental retardation, and cortical dysplasia.<sup>6,14–16</sup> In this respect, the CNS symptoms of patients with antibodies to Caspr2 recapitulate some of the clinical features of genetic disruption of the gene. In 1 patient with mutated *CNTNAP2*, the resemblance of symptoms with "VGKC-antibody associated encephalitis" led his neurologist to suspect this disorder (S. Markx, unpublished observation). Subsequent testing for VGKC antibodies using <sup>125</sup>I- $\alpha$ -dendrotoxin RIA as well as for Caspr2 antibodies produced negative results. We examined 20 additional patients with *CNTNAP2* mutations or polymorphisms, and all were negative for Caspr2 antibodies.

Juxtaparanodal immunostaining of peripheral nerve by sera of patients with PNH has been previously attributed to antibodies against VGKC.<sup>10</sup> Our data show that an actual antigenic target is Caspr2. Other investigators recently confirmed that Lgi1 is a major CNS target of VGKC complex-binding autoantibodies, and independently reported the paranodal protein Caspr2 as a second antigenic target.<sup>17</sup> In clinical practice, the diagnosis of patients with Caspr2 antibodies can be more complicated than that of patients with classical limbic encephalitis and Lgi1 antibodies. This is due to the frequent occurrence of Caspr2 with other autoantibodies, resulting in a

complex disorder that can manifest with motor weakness, atrophy, fasciculations, and bulbar symptoms, leading one to suspect an irreversible motor neuron disorder (see pictures and detailed information of case #2 in Diaz-Manera et al<sup>11</sup>). However, this patient and most patients of the current study had dramatic responses to immunotherapy. It has been suggested that Caspr2 antibodies often occur in association with tumors (mostly thymoma) but our study shows otherwise.<sup>17</sup> Extensive tumor screening, sometimes with a long follow-up, did not reveal a tumor in most patients, an observation that is emphasized by our referral pattern that likely favors patients with paraneoplastic disorders.

Our findings help to clarify the perplexing diversity of symptoms in patients previously diagnosed with VGKC antibodies. We suspect that other components of the Lgi1 or Caspr2 protein complex may be target antigens in other subgroups of patients pending characterization. For example, Lgi1 is a neuronally secreted protein that interacts with pre- and postsynaptic proteins, organizing a trans-synaptic protein complex with multiple components.<sup>18</sup> There are cases of epilepsy or rapidly progressive dementia, different from limbic encephalitis, reported in association with antibodies to VGKC.<sup>19</sup> It is unclear whether in these cases the target antigen is Lgi1, Caspr2, or another cell surface protein. Because only 1 of 18 patients with acquired PNH had Caspr2 antibodies and another 2 had positive <sup>125</sup>I- $\alpha$ -dendrotoxin RIA, other autoantigens may account for the majority of these cases.

Additional work is required to clarify the pathogenic mechanisms of autoantibodies to Caspr2, and determine how they may cause central and peripheral nerve dysfunction. Based upon the response of most patients with Caspr2 antibodies to immunotherapy and what is known about the direct effects of other cell surface antibodies on the target antigens,<sup>20–22</sup> functional disruption of Caspr2, as opposed to neuronal destruction, is a likely mechanism.

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## Authorship

E.L. and M.G.M.H. contributed equally.



## Potential Conflicts of Interest

E.L. has received grant support from Talecris, a company that sells human immunoglobulin. A patent application for the use of Lgi1 antibody detection in patients' CSF and sera has been filed by J.D., whose laboratory has received grant support from Euroimmun. No other authors have conflicts of interest.

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