Subsequently, LGI1 (leucine-rich glioma inactivated 1) was identified as an additional and major target in limbic encephalitis (LE), in a few cases with MoS,[10,11] and in all acutely tested cases with the recently described syndrome of faciobrachial dystonic seizures (FBDS).[12] LGI1 antibodies were concurrently identified in LE[13] and CASPR2 antibodies in a few cases with MoS or NMT.[14] In addition, a minority of patients have antibodies directed against the third identified antigenic component of the VGKC complex, contactin-2.[11]

To date, there has been no substantive review of patients with MoS or characterization of their antibodies and their reactivity with different brain regions. Here we describe 29 patients with a diagnosis of MoS and present in vitro data to support putative sites of antibody action.

Patients and Methods

Clinical Data

Thirty-two patients were identified from referral correspondence, sent to the Oxford laboratory between 2000 and 2010, indicating a probable diagnosis of MoS and requesting VGKC antibodies. The referring clinicians were subsequently asked to confirm or refute this diagnosis and to complete questionnaires (n = 26, as in Irani et al[11]), or the questionnaires were completed from e-mails/clinic letters or telephone interviews by S.R.I. or A.V. (n = 6). Three patients were given an alternative final diagnosis by their referring clinician. Twenty-seven of 29 sera were available for further assays. Eight of the patients have been reported previously.[4,5,11,15–17]

Cell-Based Techniques, Radioimmunoassays, and Brain Immunohistochemistry

All sera were tested for VGKC-complex antibodies using the radioimmunoprecipitation assay.[7–11,18] Cell-based assays (CBAs) for LGI1, CASPR2, and contactin-2 antibodies were performed using human embryonic kidney (HEK) cells transfected with cDNAs encoding the relevant proteins.[11,12] The sera (1:20–1:100 dilution) were incubated with live transfected cells; these were washed, fixed, and surface-bound immunoglobulin G (IgG) visualized with a fluorophore-conjugated secondary antibody. Endpoint dilutions were determined for available sera. All sera were also tested against untransfected cells and/or cells transfected with an unrelated antigen, aquaporin-4 (AQP4). For adsorption of the specific antibodies, limiting quantities of 6 sera were adsorbed 3× sequentially against 4 × 10^7 CASPR2, LGI1, or AQP4-transfected live HEK cells in solution, and the adsorbed sera were tested as above to confirm complete adsorption. The antibody subclasses and their ability to activate complement were determined.[19,20] Immunostaining of mouse brain sections was performed using available representative sera as previously described in detail elsewhere,[11,21] and summarized in the Supplementary Methods. Details of the commercial antibodies used are given in Supplementary Table 1.

Results

Demographics, Country of Origin, and Preceding Events

The 29 MoS sera were from the United Kingdom (n = 5), Italy (n = 5), Germany (n = 2), Spain (n = 2), Turkey (n = 2), Hungary (n = 1), Cyprus (n = 1), and Norway (n = 1) in Europe; Japan (n = 2), South Korea (n = 1), and India (n = 3) in Asia; and Argentina (n = 3) and New Zealand (n = 1) in the Southern Hemisphere. Twenty-seven of the 29 patients (93.1%) were male, with age at onset from 19 to 80 years (median, 57). In 6 patients, the symptoms were first noted within days to weeks after thymoma chemotherapy (n = 1), thymectomy (n = 1), knee surgery (n = 1), angioedema (n = 1), and drainage of a scrotal hydrocele (n = 2). There were no reports of a preceding infection. Systemic features included weight loss (48.2%), skin lesions/itching (22.2%), and fever (20.1%).

Peripheral Nerve Involvement and Pain

The clinical features are summarized in Table 1. Clinical NMT was the presenting feature in 13 (44.8%), subsequently noted in all cases and confirmed electrophysiologically in 96.6%. Eighteen patients (62.1%) complained of neuropathic pain in the feet and/or legs (n = 15) and back (n = 3). Other peripheral nerve features included areflexia (n = 8) and/or a stocking-type sensory loss (n = 12).

Autonomic System Dysfunction

Autonomic dysfunction was evident in 93.1% of patients; hyperhidrosis (86.2%) and cardiovascular instability (48.3%) were most common. Tachycardia was seen in 11 patients, of whom 6 also had blood pressure abnormalities, and 3 of the 6 cases developed arrhythmias (2 with QT interval prolongation). Eight patients had urinary complaints, and 7 of these also had constipation.

Encephalopathy

Insomnia was the commonest sleep disturbance, seen in 89.7%. Overall, only 2 cases (6.9%) had no sleep disturbance. Neuropsychiatric features were present in 28 patients. Of the 10 cases with generalized tonic–clonic seizures, 2 had complex partial seizures consistent with FBDS,[12] which in 1 patient preceded the onset of MoS.

Tumors

Thymomas were present in 11 patients (37.9%), 9 of whom had a history of acetylcholine receptor antibodies and myasthenia gravis (MG). In 9 cases, the thymoma was recurrent or previously palliatively treated with

TABLE 1: Comparison of MoS with VGKC-Complex Antibody-Positive LE and NMT

Characteristic	MoS, n= 29 (%)	LE, n = 64 (%)	Differences between LE and MoS[a]	NMT, n = 58 (%)	Differences between NMT and MoS[a]
Tumor	12 (41.4)	0 (0.0)	<0.0001	19 (32.8)	NS
Males	27 (93.1)	44 (68.8)	0.0013	37 (63.8)	0.0039
Myasthenia gravis	9 (31.0)	1 (1.6)	<0.0001	11 (19.0)	NS
Peripheral nerve					
Neuromyotonia	29 (100.0)	0 (0.0)	NA	58 (100.0)	NS
EMG-proven neuromyotonic discharges	28 (96.6)	0 (0.0)	NA	55 (94.8)	NS
Pain	18 (62.1)	3 (4.7)	<0.0001	12 (20.7)	0.0002
Peripheral neuropathy features	15 (51.7)	1 (1.6)	<0.0001	5 (8.6)	<0.0001
Autonomic					
Dysautonomia (any)	27 (93.1)	7 (10.9)	<0.0001	32 (55.2)	0.0002
Hyperhidrosis	25 (86.2)	6 (9.4)	<0.0001	29 (50.0)	0.0010
Tachycardia	11 (37.9)	0 (0.0)	<0.0001	1 (1.7)	<0.0001
Blood pressure abnormalities	9 (33.3)	0 (0.0)	<0.0001	1 (1.7)	0.0002
Urinary features	8 (29.6)	0 (0.0)	<0.0001	1 (1.7)	0.0005
Sleep					
Insomnia	26 (89.7)	6 (9.4)	<0.0001	4 (6.9)	<0.0001
Neuropsychiatric					
Any	28 (96.6)	64 (100.0)	NS	12 (20.7)	<0.0001
Disorientation/confusion	19 (65.5)	64 (100.0)	<0.0001	0 (0.0)	<0.0001
Amnesia	15 (55.6)	64 (100.0)	<0.0001	0 (0.0)	<0.0001
Hallucinations	14 (51.9)	11 (17.2)	0.0016	1 (1.7)	<0.0001
Agitation	10 (34.5)	4 (6.3)	0.0051	1 (1.7)	<0.0001
Delusions	7 (25.9)	14 (21.9)	NS	1 (1.7)	0.0016
Seizures					
Generalized tonic–clonic	10 (34.5)	59 (92.2)	<0.0001	0 (0.0)	<0.0001
Systemic features					
Weight loss	13 (48.2)	1 (1.6)	<0.0001	2 (3.4)	<0.0001
Skin lesions or itching	6 (22.2)	0 (0.0)	0.0004	0 (0.0)	0.0009
Investigations					
Normal MRI	23 of 25 (92.0)	24 (37.5)	<0.0001	10 of 10 (100.0)	NS
Normal CSF	11 of 21 (52.3)	43 (67.2)	NS	20 of 31 (64.5)	NS
Serum hyponatremia	7 of 28 (25.0)	38 (59.4)	0.0031	0 (0.0)	0.0002
Death	9 (31.0)	0 (0.0)	<0.0001	4 (6.9)	0.0079

The LE and NMT data are extracted from previous publications.[10,22,23]

Eleven tumors were thymomas, and 1 was a non–small-cell lung carcinoma. Blood pressure abnormalities included hypertension (n = 4), hypotension (n = 1), orthostatic hypotension (n = 3), and blood pressure lability (n = 1). Other features included constipation (n = 7, 25.9%); change in personality (n = 6, 22.2%), change in mood (n = 6 [2 elevated, 4 reduced], 22.2%); hypersalivation, ataxia, fever, and daytime hypersomnolence (n = 5, 17.2%); impotence (n = 4, 14.8%); arrhythmias, anxiety, coma, myoclonus, and startle (n = 3, 10.3%); and hyperlacrimation, hypothermia, small-joint arthralgia, and relapses (n = 2, 6.9%). Three patients developed complex sleep behaviors (sleepwalking/talking); 1 of these also had rapid eye movement sleep behavior disorder, and another patient reported vivid dreams. Two patients with complex partial seizures (both likely faciobrachial dystonic seizures[12]) also had generalized tonic–clonic seizures.

[a]*p* values = Fisher *t* test. Revised *p* value for multiple comparisons is 0.002. CSF = cerebrospinal fluid; EMG = electromyography; LE = limbic encephalitis; MoS = Morvan syndrome; MRI = magnetic resonance imaging; NA = not applicable; NMT = neuromyotonia; NS = not significant; VGKC = voltage-gated potassium channel.

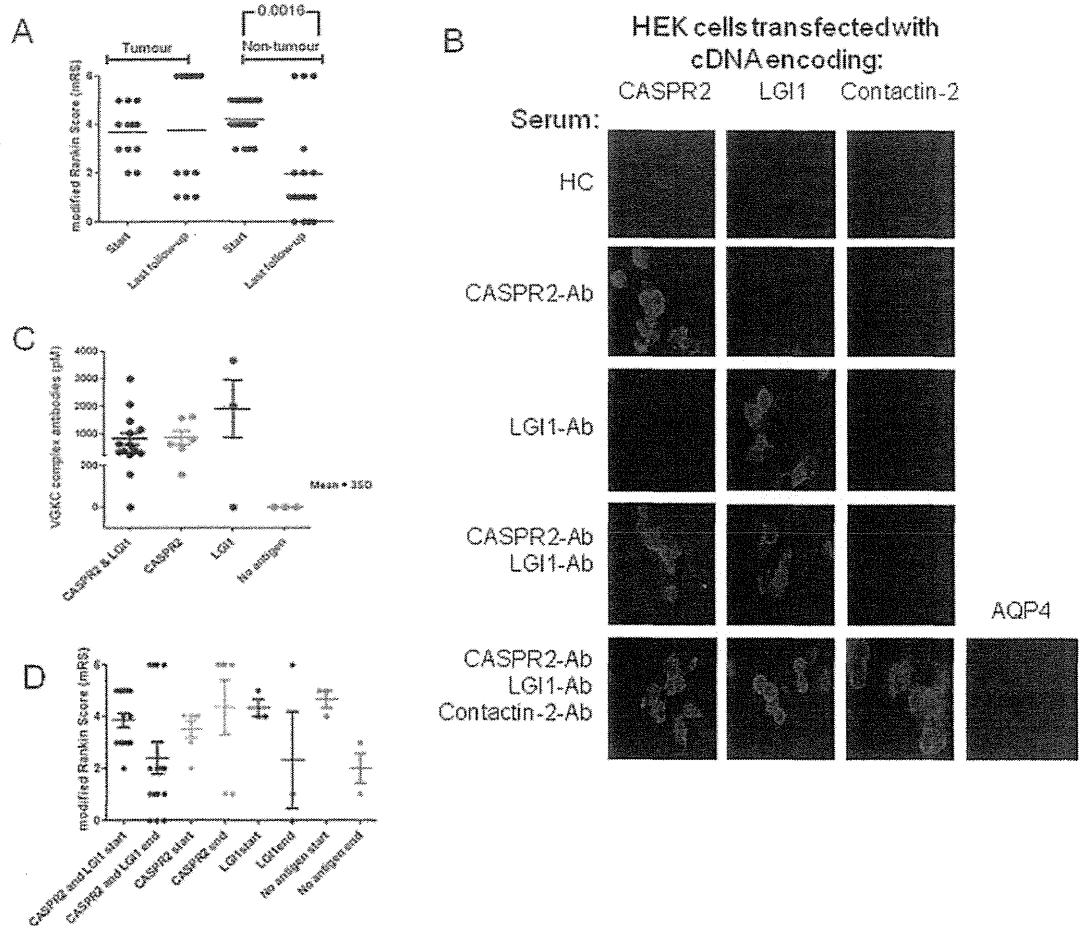

FIGURE 1: Clinical outcomes and relationship between voltage-gated potassium channel (VGKC) complex antibodies and antibody specificity. (A) Clinical outcomes (modified Rankin Score) are shown according to tumor status. Mann–Whitney p = 0.0016 for non-tumor cases. (B) Cell-based assays show results from representative sera with antibodies (Ab) against CASPR2; LGI1; both CASPR2 and LGI1; and CASPR2, LGI1, and contactin-2. The specificity of the antibody binding was demonstrated by lack of reactivity with untransfected (not shown) or aquaporin-4 (AQP4)-transfected cells. (C) VGKC-complex titers (determined using VGKC-complex radioimmunoassay) are grouped by antibody specificities. The cut-off indicated by the dotted line represents the mean plus 3 standard deviations (SD; 100pM) of healthy control values. (D) Modified Rankin Scores at disease onset (start) and at latest follow-up (end) are divided according to antigenic specificities.

chemotherapy, but in 2 patients it was found after the onset of MoS. Two thymomas were not observed on initial chest imaging (computed tomography [CT] and positron emission tomography [PET]) but were noted with subsequent CT.

Comparison of MoS with LE and NMT

Table 1 compares the common clinical features, investigations, and outcomes in the MoS cases to previously reported patients with LE (n = 64)[11] or NMT (n = 58).[22,23] When compared to LE, the neuropsychiatric manifestations in MoS showed significantly less amnesia and confusion/disorientation and fewer seizures, but more hallucinations and agitation. Dysautonomia, peripheral neuropathic features, insomnia, and tumors, although found in a proportion of NMT patients, were significantly more common in MoS and infrequently seen in LE, as were the proportion of males, weight loss, and skin involvement.

Paraclinical Investigations

VGKC-complex antibody serum levels were raised (>100pM) in 23 of 29 (79.3%). Magnetic resonance imaging (MRI) of the brain was normal in 92% of MoS, significantly more frequently than in LE (see Table 1). One patient showed right frontal T2 hyperintensity, and another had bilateral hippocampal T2 high signal that progressed to atrophy. Abnormal cerebral PET was found in the 4 cases examined (focal and generalized hyper- and hypometabolism), all with normal MRI. The cerebrospinal fluid was abnormal in 10 of 21 (47.7%); 4 showed mild to moderate lymphocytosis (range, 6–25/mm^3), 5 had raised protein (0.6–1.6g/l, 3 with lymphocytosis), and 4 had unmatched oligoclonal bands.

TABLE 2: Clinical and Investigation Features Grouped by the Specificity of the Abs Determined by Cell-Based Assays

Feature	CASPR2 Abs only, n = 6	LGI1 and CASPR2 Abs, n = 15	LGI1 Abs only, n = 3
Tumor	3 (50.0%)	8 (53.3%)	0
Myasthenia gravis	3 (50.0%)	6 (40.0%)	0
Weight loss	5 (83.3%)	5 (33.3%)	0
Delusions	0	3 (20.0%)	2 (66.7%)
FBDS/myoclonus[a]	0	2 (13.3%)	1 (33.3%)
Change in mood	1 (16.7%)	1 (6.7%)	2 (66.7%)[b]
Serum hyponatremia	0	5 (33.3%)	2 (66.7%)
Hallucinations	2 (33.3%)	8 (53.3%)	3 (100.0%)
Anxiety	0	3 (20.0%)	0
Agitation	1 (16.7%)	7 (46.7%)	1 (33.3%)
Confusion/disorientation	5 (83.3%)	7 (46.7%)	3 (100.0%)
Amnesia	4 (66.7%)	5 (33.3%)	3 (100.0%)
Seizures	2 (33.3%)	4 (26.7%)	2 (66.7%)
Peripheral neuropathy	3 (50.0%)	7 (46.7%)	3 (100.0%)
Pain	5 (83.3%)	10 (66.7%)	3 (100.0%)

The most common features of Morvan syndrome are not shown, as they were seen in almost all patients. Two cases without serum available are excluded from the analysis. The 3 patients with contactin-2 antibodies (in addition to CASPR2 and LGI1) have not been analyzed as a subgroup, but all 3 had tachycardia and changes in blood pressure (2 high, 1 low).
[a]FBDS may be mistaken for myoclonus.[12]
[b]Elevated mood was noted exclusively in 2 cases with LGI1 Abs. Ab = antibody; FBDS = faciobrachial dystonic seizures.

Electroencephalography was abnormal in 11 of 17 (64.7%) cases; 10 showed diffuse slowing, and 2 showed temporal lobe spikes (both had clinical seizures; 1 also had slowing). Serum sodium was low in 7 cases (range, 125–130; normal range, 135–145mmol/l), and the syndrome of inappropriate antidiuretic hormone (ADH) secretion (SIADH) was confirmed in 5 of 5 for whom osmolarity levels were available.

Treatments and Outcomes

All but 2 patients were treated with immunotherapies that included plasma exchange (n = 16), corticosteroids (n = 14), intravenous immunoglobulins (n = 13), azathioprine (n = 6), cyclosporin (n = 1), and/or cyclophosphamide (n = 1). Two patients without tumors were not administered immunotherapies; 1 died (from respiratory failure), and 1 improved spontaneously (modified Rankin Score [mRS][11] from 3 to 0).

Of the 12 cases with tumors, 6 died from respiratory failure/aspiration pneumonia (n = 2), direct tumor invasion (n = 2), sudden cardiac death (n = 1), and sepsis (n = 1). Six improved by 1 to 4 points, but overall there was no change in mRS within this group (Fig 1A). By contrast, only 3 of the 17 non-tumor cases died (large bowel volvulus, respiratory failure, and left ventricular failure; 17.6%), and 12 made a good (mRS fall >2) recovery ($p = 0.0016$). Two patients suffered relapses. In 1, this occurred after discontinuing prednisolone and was associated with a return of VGKC-complex antibodies.

CASPR2, LGI1, and Contactin-2 (VGKC-Complex Antigens) Antibodies and Associated Clinical Features

Twenty-seven of 29 samples were available to test for the VGKC-complex antigens, LGI1, CASPR2, and contactin-2, using CBAs.[11] Surprisingly, 3 patients had antibodies to all 3 antigens, 12 patients had both CASPR2 and LGI1 antibodies, 6 had only CASPR2 antibodies, and 3 had only LGI1 antibodies. Examples of different specificities are shown in Figure 1B and related to the titers of VGKC-complex antibodies in Figure 1C. Of the 5 samples negative for radioimmunoprecipitation of VGKC complexes, 1 was positive for LGI1 antibodies and 1 for both LGI1 and CASPR2 antibodies, leaving

TABLE 3: Expression of Caspr2 and Lgi1 in Mouse Brain Sections Determined with Use of Commercial Antibodies

Antigen	Hippocampus-CA3	Cerebellum	LC	Raphe	Hypothalamus	Thalamus	Caspr2$^{-/-}$ CNS sections	Conclusion
CASPR2	++ radiatum, − MF	++ mol, (+) PC, + GCL neuropil, ++ WM jxpns	++ neuropil, (+) neurons	++ neuropil, (+) neurons	++ neuropil, − neurons	++ neuropil	Lack of specific staining	Expressed in neuropil and jxpns throughout CNS, mostly not in neurons except weakly in some (PC, raphe, LC)
LGI1[a]	+ MF, + radiatum, + pyr	++ PC, ++ GCL, + mol, + pinceau, (+) WM	++ neurons, (+) neuropil	++ neurons, (+) neuropil	++ neurons including ORX and ADH (but not limited to those)	++ neurons	Same as in Caspr2$^{+/+}$	Expressed in neurons throughout CNS, and some axon terminals (MF, pinceau)

[a]Anti-Lgi1 rabbit antibody shows stronger neuronal staining, whereas the goat antibody shows stronger staining of the pinceau.ADH = antidiuretic hormone; CNS = central nervous system; GCL = granule cell layer; jxpns = juxtaparanodes; LC = locus coeruleus; MF = mossy fiber layer; mol = molecular layer; ORX = orexin; PC = Purkinje cells; pyr = pyramidal cells; WM = white matter. Caspr2$^{-/-}$ is used here and elsewhere for the mouse knock-out tissue.

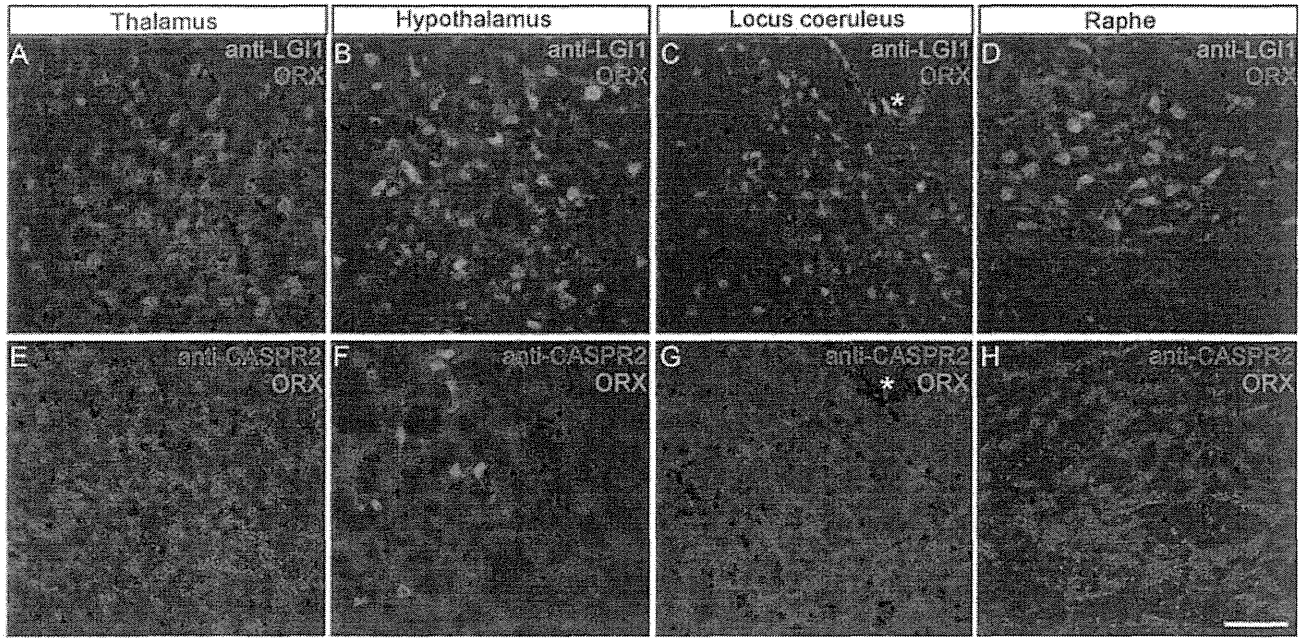

FIGURE 2: Expression of LGI1 and CASPR2 in mouse brain. Immunofluorescence labeling was used with commercial antibodies against LGI1 (A–D; anti-LGI1, green) or CASPR2 (E–H; anti-CASPR2, red) combined with anti-orexin (ORX) commercial antibodies (red [A–D] and green [E–H]). Cell nuclei were stained with DAPI (blue). LGI1 is expressed mainly in neuronal cell bodies throughout the central nervous system (CNS; A–D), including thalamic neurons (A) and the orexin neurons of the hypothalamus (B), as well as neurons in the locus coeruleus (LC; C) and the raphe (D). By contrast, CASPR2 is expressed mainly in the neuropil and juxtaparanodes throughout the CNS (E–H), including the thalamus and hypothalamus, as well as LC and raphe. Mild CASPR2 immunoreactivity is also present in thalamic (E) and raphe (H) neurons, but not in orexin neurons (F). Scale bar = 50μm. *IVth ventricle.

only 3 sera without detectable antibodies. Endpoint titrations of binding to the cells showed that CASPR2 antibodies were higher titer than LGI1 antibodies, except in 1 patient (Supplementary Fig 1A, $p = 0.0067$),[17] as also demonstrated by a fluorescent immunoprecipitation assay using 4 sera with both CASPR2 and LGI1 antibodies that were available in sufficient quantities (see Supplementary Fig 1B). The CASPR2 antibodies were IgG1 more than IgG4, whereas a reverse trend was seen for LGI1 antibodies; both were able to fix complement on the surface of transfected cells (eg, Supplementary Fig 2).

As shown in Table 2, tumors, MG, and weight loss were only found in the presence of CASPR2 antibodies, and the 2 patients with spontaneous resolution of mediastinal lymphadenopathy were CASPR2 antibody positive. Interestingly, the 7 cases with serum hyponatremia all had LGI1 antibodies (5 also with CASPR2 antibodies). Delusions and mood changes were more common with LGI1 antibodies, and myoclonus (probably FBDS[12]) was only seen with LGI1 antibodies. All 3 patients who had CASPR2, LGI1, and contactin-2 antibodies developed tachycardia and alterations in blood pressure. Four of 6 (66.7%) patients with only CASPR2 antibodies died; outcomes in this group were poorest (see Fig 1D).

MoS Sera Contain Distinct Antibody Reactivities and Bind to Brain Regions Relevant to the Localization of the Clinical Features

As there were 15 patients with both CASPR2 and LGI1 antibodies, it was possible that the antibodies might bind a common epitope in the VGKC complex. We first confirmed in monospecific sera that adsorptions against cells transfected only with the target antigen, depleted both VGKC-complex (data not shown) and CBA reactivity (Supplementary Fig 3). We then tested 2 sera that contained both LGI1 and CASPR2 antibodies. After serum adsorption against LGI1, binding to CASPR2 was retained, and conversely, binding to LGI1 was retained after adsorption against CASPR2 (see Supplementary Fig 3).

To determine the distribution of LGI1 and CASPR2 in brain tissue, we examined the reactivity of commercial antibodies throughout the rodent brain with a focus on selected regions, specifically the locus coeruleus (LC), raphe nuclei, thalamus, and lateral hypothalamus as putative generators of insomnia and multisystem dysautonomia. These results are summarized in Table 3 and illustrated in Figure 2. In the hippocampus and cerebellum, LGI1 was detected in some axon terminals such as the mossy fiber layer of the hippocampus and in the cerebellar pinceau, as previously shown.[11,13] In the other

TABLE 4: Examples of MoS Sera Binding to Rodent Brain Tissue

Serum/Sample	Cell-Based Assay Result[a]	LC	Raphe Nucleus	Hypothalamus	Thalamus	Caspr2$^{-/-}$ CNS Sections	Conclusion
MoS1	CASPR2 negative; LGI1, 1:540	++ neurons	+ neurons	++ neurons (including ORX)	++ neurons	ND	LGI1
MoS2	CASPR2 1:14,800; LGI1 negative	++ neuropil, (+) neurons	++ neuropil	++ neuropil, − neurons	++ neuropil, − neurons	Loss of specific neuropil staining	CASPR2
MoS3	CASPR2 1:1,620; LGI1, 1:540	++ neurons, + neuropil	+ neurons, + neuropil	+ neuropil, (+) neurons	+ neurons	ND	LGI1, CASPR2
MoS4	CASPR2 1:1,620; LGI1, 1:180; contactin-2 1:100	++ neurons, + neuropil	++ neurons, + neuropil	++ neurons (including ORX), + neuropil	+ neurons, + neuropil	ND	CASPR2, contactin-2, LGI1
MoS4, adsorbed against LGI1 and CASPR2	CASPR2 negative LGI1 negative	+ neurons	+ neurons	+ neurons	(+) neurons	ND	Contactin-2
MoS5	CASPR2 1:4,860; LGI1 1:180	++ neurons, + neuropil	++ neurons, + neuropil	+ neurons (including ADH and ORX)	++ neurons (surface)	Unchanged neuronal but decreased neuropil staining	CASPR2, LGI1, plus another antigen
MoS5 adsorbed against LGI1 and CASPR2	CASPR2 negative; LGI1 negative	+ neurons	+ neurons	++ neurons (surface)	++ neurons (surface)	Unchanged	Another (non-LGI1/ CASPR2) neuronal surface antigen
MoS6	CASPR2 1:43,740; LGI1, 1:540	++ neuropil, + neurons	++ neuropil, + neurons	+ neuropil, (+) neurons	+ neuropil, (+) neurons	Neuropil binding abolished but neuronal cell body remaining	CASPR2, LGI1
MoS6 adsorbed against LGI1	CASPR2 1:48,740; LGI1, negative	++ neuropil	++ neuropil	+ neuropil	++ neuropil	Neuropil binding abolished	CASPR2

TABLE 4 (Continued)

Serum/Sample	Cell-Based Assay Result[a]	LC	Raphe Nucleus	Hypothalamus	Thalamus	Caspr2$^{-/-}$ CNS Sections	Conclusion
MoS6 adsorbed against CASPR2 and LGI1	CASPR2 negative; LGI1 negative	(+) neurons	(+) neurons	(+) neurons	(+)	ND	Residual weak neuronal pattern suggesting additional antigen

[a]Only MoS4 had contactin-2 antibodies. ADH = antidiuretic hormone (neurons); CNS = central nervous system; LC = locus coeruleus; MoS = Morvan syndrome; ND = not determined; ORX = orexin. Caspr2$^{-/-}$ is used here and elsewhere for the mouse knock-out tissue.

regions examined, commercial antibodies to LGI1, but not to CASPR2, bound to all neuron cell bodies including orexin neurons in the lateral hypothalamus (see Fig 2) as well as to vasopressin/ADH neurons in the medial hypothalamus. By contrast, CASPR2 was expressed mainly in the juxtaparanodes of the white matter[11] and in the neuropil, and in some neurons in the raphe and LC nuclei, but did not colocalize with orexin or vasopressin neurons. This CASPR2 commercial antibody binding was lost when Caspr2$^{-/-}$ tissue was used (see Table 3). We then tested 4 coded MoS sera for reactivity with these regions (summarized in Table 4). MoS1 and MoS2 sera showed reactivities that reflected their known specificities, confirming that LGI1 antibodies bound mainly to the neuron cell bodies, whereas CASPR2 antibodies bound mainly to the neuropil in the regions of interest (Fig 3 and Supplementary Fig 4). MoS3 and MoS4 had both reactivities and showed a mixed pattern of tissue binding, as predicted. MoS4 also had reactivity to contactin-2, consistent with the residual binding to neuronal cell bodies following adsorption against both LGI1 and CASPR2 antigens (see Table 4), and loss of reactivity after adsorption by contactin-2–expressing cells (data not shown).

To investigate further the specificities, we first tested 2 LE sera that were monospecific for either LGI1 or CASPR2 antibodies. LE1 (LGI1 antibody positive) showed binding that corresponded to the observed LGI1 expression pattern (as in Fig 2), including binding to the orexin and ADH neurons in the hypothalamus, and this binding remained unchanged in Caspr2$^{-/-}$ tissue (Supplementary Table 2). LE2 (CASPR2 antibody positive) bound mainly to the neuropil in a similar manner to commercial CASPR2 antibodies, and this binding was abolished in Caspr2$^{-/-}$ tissue (see Supplementary Table 2). Furthermore, after adsorption against the surface of LGI1- and CASPR2-transfected cells, respectively, LE1 and LE2 were negative on all regions, confirming that these sera did not appear to have additional reactivities (Supplementary Fig 5 and Supplementary Table 2). We then tested MoS5 and MoS6 sera, which had both CBA-determined CASPR2 and LGI1 reactivities. They reacted with both neuropil and neuronal cell bodies as expected (eg, Fig 4), and the neuropil (CASPR2) reactivity was reduced or abolished on Caspr2$^{-/-}$ tissue (Fig 5). However, even after adsorption against both antigens, there was still reactivity with neurons, including Purkinje cells, and thalamic and brainstem neurons, with apparent surface binding to neuronal cell bodies (see Figs 4 and 5 and Table 4), suggesting the existence in these sera of additional reactivities. These other reactivities were retained in Caspr2$^{-/-}$ brains as shown for MoS5 and MoS6 in Figure 5 and summarized in Table 4.

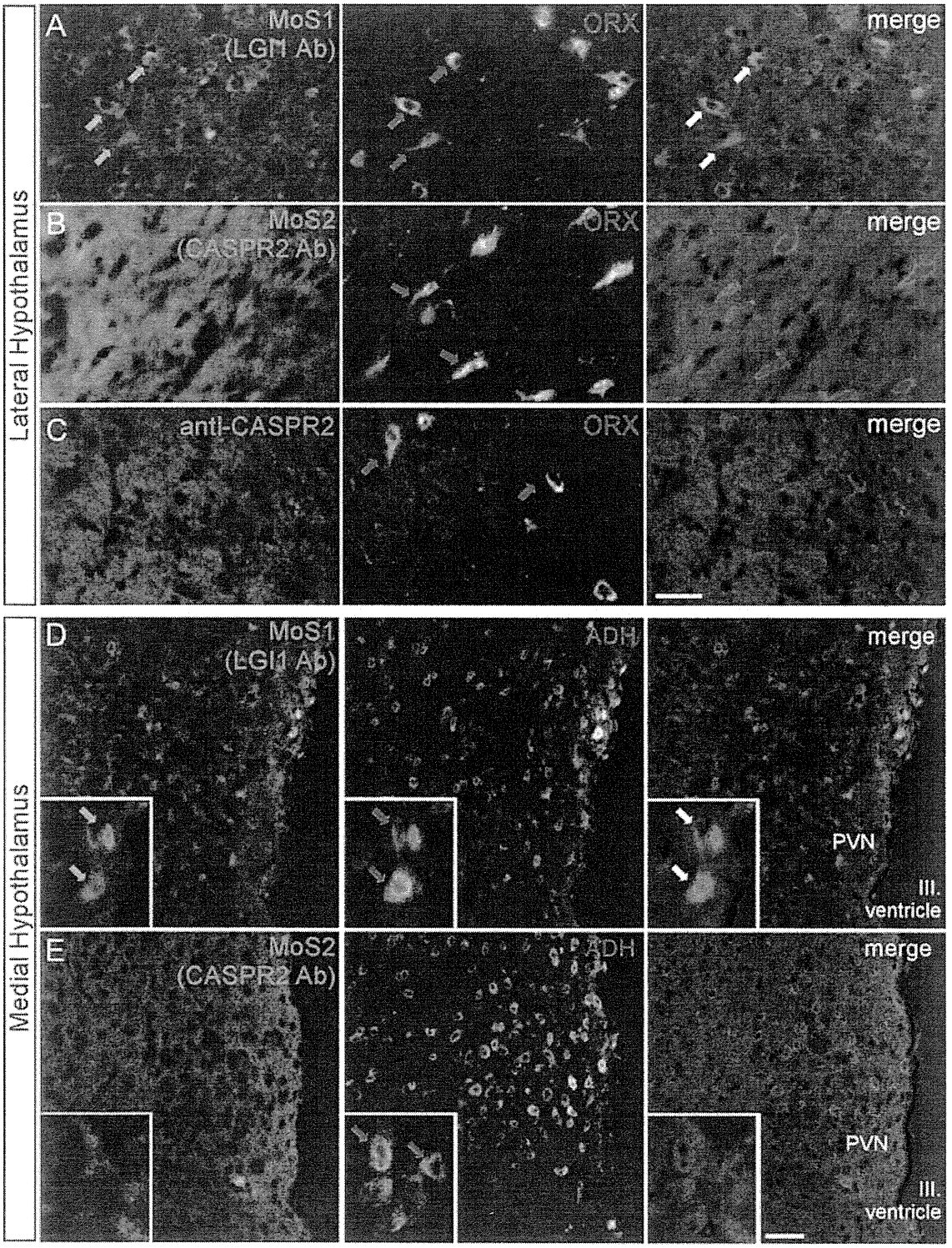

FIGURE 3: Binding of Morvan syndrome (MoS) sera to orexin (ORX) and vasopressin neurons in the hypothalamus. The lateral (A–C) and medial (D–E) hypothalamus were double labeled with MoS1 serum (green) from an LGI1 antibody (Ab)-positive patient (A, D), with MoS2 serum from a CASPR2 Ab-positive patient (B, E), or with anti-CASPR2 commercial antibodies (green in C), combined with commercial antibodies against ORX (A–C) or vasopressin/antidiuretic hormone (ADH; D–E; red). Both separate channels and merged images are shown as indicated. MoS1, similar to commercial LGI1 antibodies (Fig 2B), binds neurons that express ORX in the lateral hypothalamus (*arrows* in A), as well as ORX-negative neurons. By contrast, MoS2 (B) stains the neuropil but not the neurons, similar to commercial antibodies against CASPR2 (Fig 2E–H) (C). In the paraventricular nucleus (PVN) of the medial hypothalamus, MoS1 shows binding to ADH-positive neurons (*arrows* in D *inset*) adjacent to the third (III) ventricle, whereas MoS2 (E) binds the neuropil but not the ADH neurons. Scale bar = 30μm.

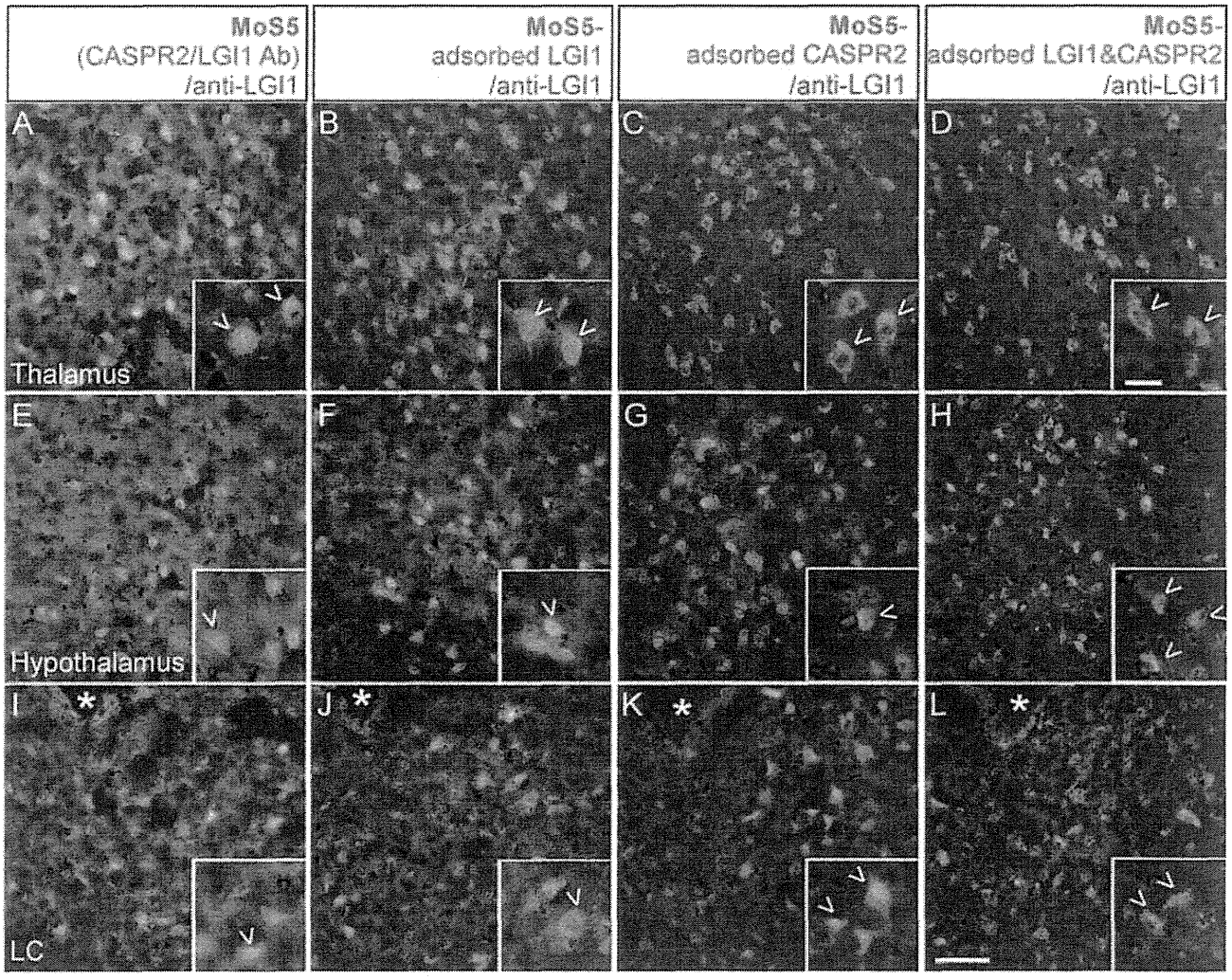

FIGURE 4: Evidence of Morvan syndrome (MoS) serum reactivity against additional antigens. Merged images are shown of thalamus (A-D), hypothalamus (E-H), and locus coeruleus (LC; I-L) double stained with anti-LGI1 commercial antibodies (Abs; red) and from MoS5 serum containing both LGI1 and CASPR2 reactivity (A, E, I) or the adsorbed samples from the same serum, either against LGI1 (B, F, J), against CASPR2 (C, G, K), or against both antigens (D, H, L), as indicated (green). MoS5 binds both neurons (*arrowheads* in *insets*) and neuropil in all areas (A, E, I). After adsorption against LGI1 (B, F, J), there is some reduction of neuronal binding, but residual neuronal, mostly surface, binding in all areas, distinct from that of commercial anti-LGI1 antibody binding (*insets* in B, F, J). Neuropil staining remains unchanged after LGI1 adsorption. When MoS5 is adsorbed against CASPR2 (C, G, K), only the neuropil staining is reduced. Finally, adsorption against both antigens (D, H, L) does not abolish the neuronal surface binding, indicating the presence of at least 1 additional antigen specificity in MoS5. Scale bars: in L = 50μm; in insets = 10μm. *IVth ventricle.

Discussion

MoS is a rare complex disease that combines neuromyotonia with multiorgan autonomic disturbance, insomnia, and encephalopathy. Although first described in 1890, it was the recognition that patients with MoS often have VGKC-complex antibodies and may respond to immunotherapies that has led to greater interest in this condition.[3–7,11,14–17] This study of 29 patients with MoS shows it to be recognized worldwide and almost exclusively seen in males. VGKC-complex antibodies were present in 90% of patients, and although these were directed against LGI1, CASPR2, or commonly both, CASPR2 antibodies predominated and were always found in thymoma cases. Immunostaining of brain tissue showed that these antibodies target subtly different regions of the brain likely to be involved in the localization of the distinctive clinical features seen in MoS, and that additional antibodies and antigenic targets are likely to be involved in some patients.

This study, based on sera referred over many years from different centers, might not be entirely representative of the full spectrum of MoS, but there was excellent agreement between the features reported in these 29 patients and the 25 cases of MoS summarized from the English literature (Supplementary Table 3). Overall, all had clinical neuromyotonia, and the majority of

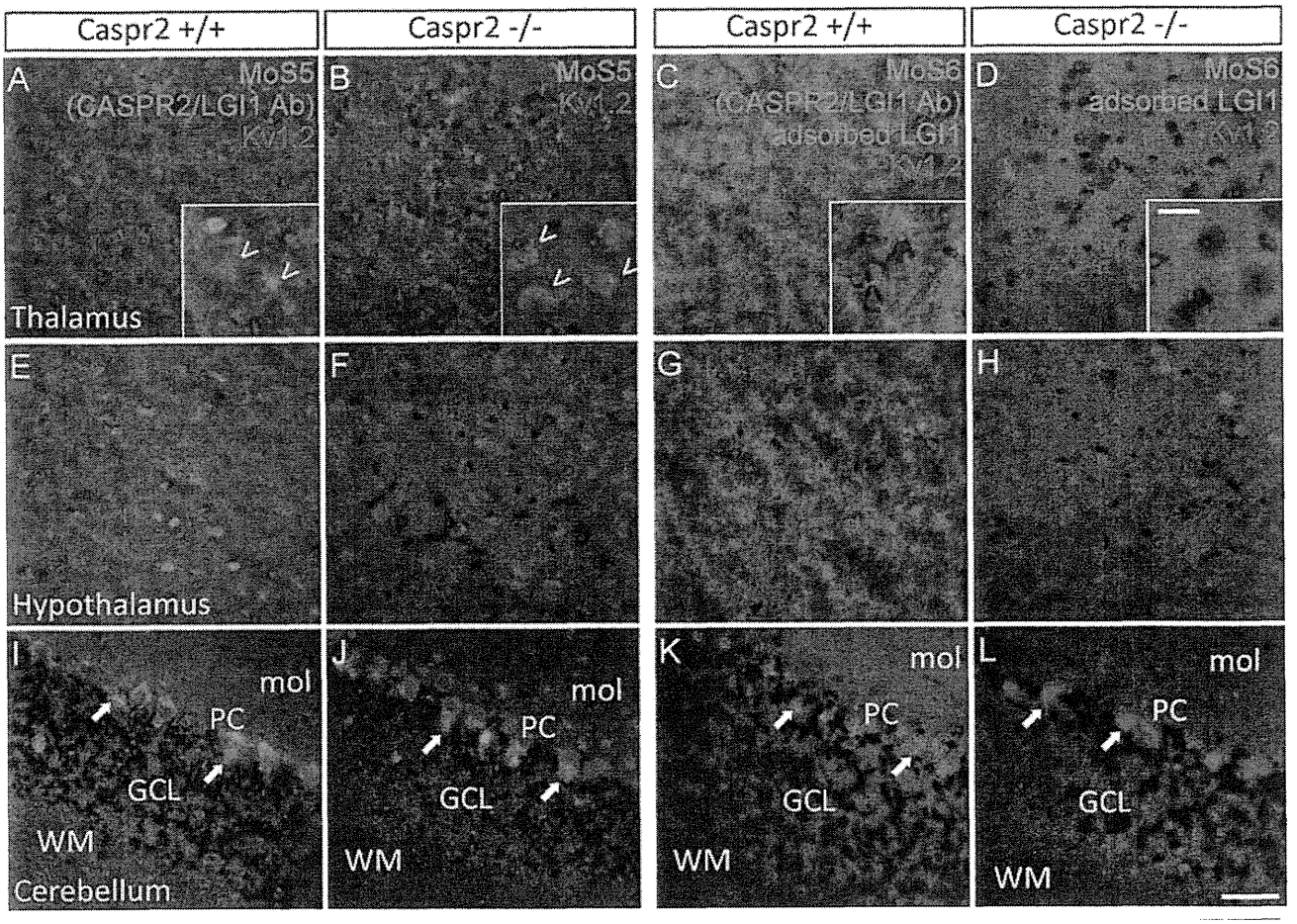

FIGURE 5: Loss of CASPR2-specific sera binding in Caspr2$^{-/-}$ tissue and residual specificities. Morvan syndrome (MoS) 5 (with CASPR2, LGI1, plus another reactivity) and MoS6 from a patient with CASPR2 plus LGI1 reactivity that has been adsorbed against LGI1 (retaining only CASPR2 reactivity; see Table 4; green channel) combined with anti-Kv1.2 commercial antibodies (red channel) were tested on Caspr2$^{+/+}$ and Caspr2$^{-/-}$ tissues including the thalamus (A–D), hypothalamus (E–H), and cerebellum (I–L) to demonstrate the specific loss of CASPR2 reactivity. Kv1.2 shows strong expression mostly in the neuropil in all areas, similar to CASPR2, as well as characteristic strong expression in the cerebellar pinceau (*arrows* in I-L), and this expression is not altered in Caspr2$^{-/-}$ tissue. MoS5 shows binding to both neuronal cell bodies (*arrowheads* in A, B *insets*) and neuropil in the thalamus and hypothalamus in Caspr2$^{+/+}$ (A, E), whereas in Caspr2$^{-/-}$ the neuropil binding is lost but the neuronal binding is unchanged (B, F). In the same areas, the LGI1-adsorbed MoS6 shows mostly neuropil staining in Caspr2$^{+/+}$ (C, G), which is lost in Caspr2$^{-/-}$ (D, H). In the cerebellum, the CASPR2-like binding of both sera to the molecular layer (mol), neuropil of granule cell layer (GCL), and white matter (WM) is abolished in Caspr2$^{-/-}$ tissue, but the binding of MoS5 to Purkinje cells (PC), as well as an additional distinct binding in the GCL, remains unchanged (summarized in Table 4). Scale bar: in L = 30μm; in insets = 10μm. NB. The mouse equivalent of CASPR2 is Caspr2 and this form is used for the comparison between binding to wild type and to knock-out tissues.

patients had a complex dysautonomia and insomnia with an encephalopathy typified by confusion, hallucinations, and agitation with infrequent seizures. Additional features that distinguished these patients from classical LE were the presence of a neuropathic lower limb pain, weight loss, male gender, and thymoma (±MG), although thymomas can also be found in rare cases diagnosed with LE.[24] Six patients who each lacked 1 of the core features of NMT, autonomic disturbance, and insomnia (Supplementary Table 4), and others previously reported,[1,25–27] suggest the existence of conditions with only 2 of these 3 core components.

The striking male preponderance and thymoma association are intriguing. One report has shown CASPR2 mRNA in the prostate,[28] and it may be that the male reproductive system is a rich source of the antigen required to break tolerance, consistent with MoS onset after scrotal drainage in 2 of our cases (and 5 additional cases; S. Sharma, personal communication). In addition, thymectomy and thymoma chemotherapy were likely disease triggers, suggesting that thymic tumors may also harbor the antigenic targets, particularly CASPR2.[11,23,29] Although a few cases with thymomas showed a good outcome, the overall prognosis of MoS-associated thymoma was worse than pure MG-related thymoma or even recurrent thymoma.[30,31] CASPR2 has recently been proposed as a tumor suppressor gene.[32] The data here, the literature review (see Supplementary

Table 3), and a recent study of sleep abnormalities in VGKC-complex antibody-positive patients[33] show around 50% of MoS cases to be associated with tumors.

The frequent combination of LGI1 and CASPR2 antibodies in MoS could contribute to the distinctive multifocal phenotype. Insomnia, dysautonomia, and less frequently hyponatremia are likely due to disturbance of monoaminergic diencephalic and brainstem nuclei involved in arousal and autonomic homeostasis. Neuronal dysfunction anywhere along the arousal system, including the lateral hypothalamic orexin neurons, locus coeruleus, raphe nuclei, and thalamus, could produce insomnia.[4,33–35] The dysautonomia, often with combinations of cardiovascular, cutaneous, and sphincter involvement, is likely to have a central generator, possibly within the hypothalamus and raphe nuclei.[36] We found that LGI1 or CASPR2 antibodies bind all these regions and appear to have differential subcellular specificities that may determine the relative functional significance of each antibody. Interestingly, LGI1 antibodies bound the orexin neurons that are lost in narcolepsy.[37] In addition, hyponatremia secondary to SIADH was found only in those patients with LGI1 antibodies, and LGI1 antibody-positive sera bound to hypothalamic paraventricular nucleus neurons that produce ADH, which mediates water retention. This suggests that LGI1 antibody binding may increase ADH secretion to generate the hyponatremia,[38] although some patients with CASPR2 antibodies do have low plasma sodium.[11] The in vitro binding of patient sera to these relevant central nervous system areas provides a basis for explaining the cardinal manifestations of MoS, but it is clear from this and previous studies[11,14] that the major target antigens are also expressed more widely in the brain, and that sera bind to other areas that are not typically involved in MoS, such as the cerebellum. Thus, besides the target antigen distribution, other factors, including the accessibility to circulating antibodies and physiological properties of neuronal populations, may determine the clinical manifestations.

The coexistence of CASPR2 and LGI1 antibodies in half of the patients contrasts with previous findings in LE.[11,13,14] Moreover, although the combination of CASPR2 and LGI1 antibodies could explain many aspects of the clinical phenotype, they are not necessarily the only targets for antibodies in MoS. Nine MoS patients only had 1 of these antibodies, 3 had none detected, and other patients with CASPR2 antibodies and NMT showed no sleep disturbances.[11,23] It is possible that some sera harbor antibodies directed against other VGKC-complex (or uncomplexed) antigens,[39] which would help to explain the multifocal localization of the phenotype. Indeed, 3 patients also had contactin-2 antibodies, which are only rarely found in LE[11]; contactin-2 is expressed in cardiac conduction tissue,[40] and these 3 patients had cardiovascular instability. Moreover, 2 MoS sera that we examined in detail had reactivities that were not consistent with LGI1, CASPR2, or contactin-2, confirming our suspicion that other antibody reactivities are present in some of these patients. Unfortunately, insufficient volumes of sera were available from other patients for further experiments, which will need to be performed on future samples.

There are similarities between the effects of mutations or drugs targeting Kv1 VGKCs and features of the diseases associated with antibodies to these proteins,[33,41–44] and it is likely that the antibodies reduce VGKC function in vivo. Whether the antibodies directly interfere with a modulatory function, or act via internalization of the target antigens, with or without cointernalization or dispersion of Kv1 potassium channels, is not yet known. Moreover, it seems possible, as in the 3 patients who had CASPR2 or LGI1 antibodies with normal VGKC-complex titers, that these antibodies can bind to their antigens independently of VGKC complexes, raising the possibility of involvement in different clinical syndromes; CASPR2 antibodies have recently been detected in 9 patients with unexplained cerebellar ataxia, only 1 of whom had VGKC-complex antibodies.[45]

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Authorship

S.R.I., P.P., and K.A.K. are joint first authors.

Potential Conflicts of Interest

S.R.I.: grants/grants pending, Fulbright-MS Society. C.M.: travel expenses, CADIMI (Centro de Miastenia). L.Z.: grants/grants pending, European Federation of Neurological Societies Fellowship. A.V.: consultancy, Athena Diagnostics; employment, Oxford University, University College London. A.V. and the Department of Clinical Neurology in Oxford receive royalties and payments for antibody assays, and A.V. is the named inventor on patent application WO/2010/046716 entitled "Neurological Autoimmune Disorders." The patent has been licensed to Euroimmun AG for the development of assays for LGI1, CASPR2 and other VGKC-complex antibodies. S.R.I., P.W., and B.L. are coinventors and may also receive future royalties.

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LETTER TO THE EDITORS

Voltage-gated potassium channel complex antibodies in Creutzfeldt-Jakob disease

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Dear Sirs,

Clinical presentations of Creutzfeldt-Jakob disease (CJD) can be mimicked by those of immune-mediated encephalopathies, including limbic encephalitis with anti-voltage-gated potassium channel (VGKC) complex antibodies [1, 2]. To date, anti-VGKC complex antibodies have been reported to be negative in CJD [1], thereby regarded as important to differentiate non-CJD dementia from CJD [3]. Here we report a patient with definite CJD who had serum anti-VGKC complex antibodies.

A 60-year-old seaman acutely presented with blurred vision, disturbance in depth perception and light discrimination, difficulty recalling Chinese characters, and right-left disorientation. An ophthalmologist found no abnormality in his eyes. Two months later, he underwent a brain magnetic resonance imaging including diffusion-weighted imaging, which showed hyperintensity signals in the bilateral occipital and parietal cortices. Neurologically, there were object agnosia, left hemineglect, dressing apraxia, Gerstmann syndrome, ideomotor apraxia, and instant memory disturbance. He also developed visual hallucinations such as flaming fire, and got agitated. Three months after onset, he got confused after taking one tablet of zolpidem and was admitted to a psychiatric department.

Neurological examination on admission demonstrated the following findings: fluctuating levels of consciousness, difficulty in word recall, extinction; visual disturbance, poor pursuit eye movement, mild dysarthria; increased muscle tonus and myoclonus predominantly in the left; Myerson sign, snout reflex, increased jaw jerk and deep tendon reflexes predominantly in the left, bilateral Babinski and Chaddock signs; and he was bedridden. Serum antinuclear, anti-thyroid peroxidase, anti-thyroglobulin antibodies were negative and sodium levels were normal. No malignant tumors were found. Cell count was 2/mm^3, protein level was 27 mg/dl, 14-3-3 protein was positive, and total tau protein level was 3,420 pg/ml (cut-off value, 1,300 pg/ml) in the cerebrospinal fluid. Initial electroencephalography revealed slow waves, and periodic sharp wave complexes were present 23 weeks after onset. The analysis of *PRNP* revealed a substitution of methionine to arginine at codon 232. He received no immunotherapy. He died 8.5 months after onset and underwent necropsy of the left parietotemporal lobe. Neuropathological examination revealed neuronal loss, spongiform change, gemistocytic astrocytosis (Fig. 1a), and synaptic deposition of PrP (Fig. 1b) in the cerebral cortex, confirming the diagnosis of definite CJD. Western-blot analysis showed type 1 PrP^{Sc}. The titer of anti-VGKC complex antibodies, measured with radioimmunoassay using rabbit brain homogenates and

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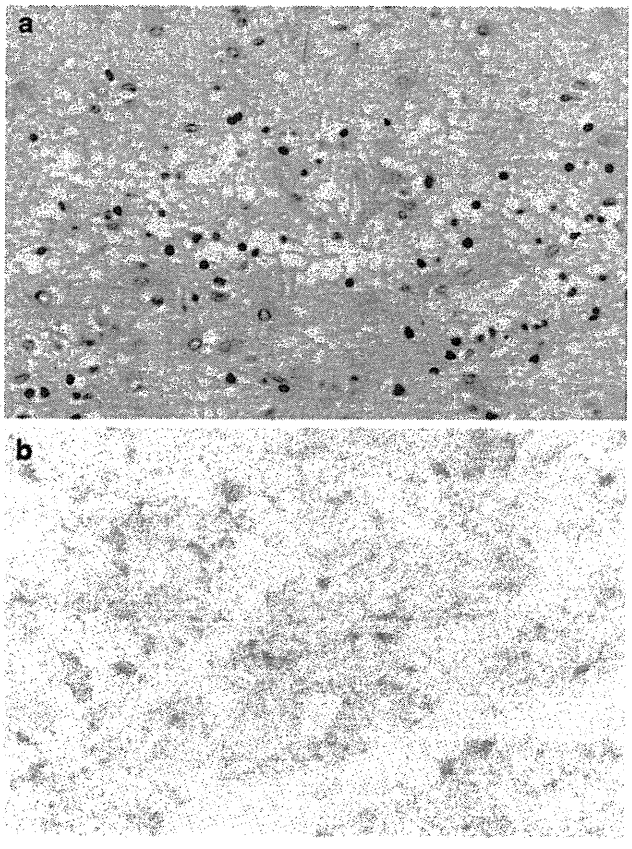

Fig. 1 Neuropathological findings. **a** Hematoxylin and eosin (H&E) staining shows neuronal loss, spongiform change, and gemistocytic astrocytosis in the left parietotemporal cortex. **b** Anti-PrP immunohistochemistry demonstrates synaptic deposition of PrP in the corresponding region

^{125}I-α-dendrotoxin as previously described [4], were 603.5 pM (cut-off values: for limbic encephalitis, 400 pM; for neuromyotonia, 100 pM) in the stored serum obtained 6 months after onset (the earliest time point of sampling). The titer was 0 pM in the serum obtained 8 months after onset. This study was approved by the ethics committee of the Tokushima University Hospital and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

To our knowledge, this is the first report of a CJD patient with anti-VGKC complex antibodies. Rapidly progressive dementia, myoclonus, extrapyramidal dysfunction, visual hallucinations, and psychiatric disturbance can be shared by CJD and anti-VGKC complex limbic encephalitis [1], although we could not specify antibody-related features in the present case. It has been reported that anti-VGKC complex antibodies are true markers of neurologic autoimmunity, because the antibodies were absent in ten patients with histologically confirmed CJD (nine sporadic and one familial) [1]. However, our results showing the antibodies in a pathologically confirmed CJD case indicate that the antibodies could not differentiate autoimmune limbic encephalitis and CJD. The real antigens of anti-VGKC complex antibodies can be leucine-rich, glioma-inactivated 1 (LGI1) or contactin-associated protein-like 2 (caspr2), which form complexes with VGKC [5, 6]. Unfortunately, we have not tested whether the antibodies were directed against LGI1 or caspr2 in the present case. In conclusion, our findings suggest limitation of anti-VGKC complex antibodies test and thus warrant further investigation for the prevalence of the antibodies in CJD patients.

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Conflicts of interest The authors declare that they have no conflicts of interest.

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LETTER TO THE EDITORS

Anti-voltage-gated potassium channel antibody is associated with chronic autonomic and sensory neuropathy

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Dear Sirs,

Anti-voltage-gated potassium channel (VGKC) antibodies have been found in patients with various neurological disorders, including acquired neuromyotonia (Isaac's syndrome), Morvan's syndrome, limbic encephalitis, and dysautonomic phenomena [1]. The spectrum of neurological manifestations involving VGKCs is thus thought to be broad [2]. We report a case of chronic autonomic and sensory neuropathy (ASN) associated with anti-VGKC antibodies presenting as long lesions in the posterior column of the spinal cord.

A healthy 57-year-old man presented with a 2-year history of severe heartburn and vomiting after eating. He subsequently felt numbness with pain in the distal parts of the upper limbs and in the soles of the feet. After the clinical course proved refractory to conventional painkillers and his gait became staggering, he was referred to our hospital. Neurological examination on admission revealed impaired vibratory and joint positional sensation over all extremities, accompanied by pseudoathetosis and sensory ataxia. Superficial sensory disturbance of the glove and stocking type and orthostatic hypotension were also observed. Deep tendon reflexes were totally abolished. Cranial nerve palsy, muscle weakness, and myokymia were not observed. Blood examination including blood sugars, hemoglobin A1c, vitamin B12 (834 pg/ml; normal range 233–914 pg/ml), vitamin E (1.17 mg/dl; normal range 0.75–1.41 mg/dl), *Treponema pallidum*, and angiotensin-converting enzyme and examination of cerebrospinal fluid all yielded normal results. The patient was negative for anti-SS-A (below 5.0; normal range 0–9.9), SS-B (below 5.0; normal range 0–14.9), acetylcholine receptor, glutamic acid decarboxylase, and ganglioside antibodies, but positive results were obtained for serum anti-VGKC antibody (222.8 pM; cut-off values of 400 pM for limbic encephalitis and 100 pM for neuromyotonia), measured by radioimmunoassay using rabbit brain homogenates and ^{125}I-a-dendrotoxin as previously described [3]. Nerve conduction studies were unremarkable except for the disappearance of sensory nerve action potentials in the median, ulnar, and sural nerves. The coefficient of variation of RR intervals was significantly decreased, at 1.35 %. Chest computed tomography showed enlargement of the esophagus in addition to an anterior mediastinal tumor. Spinal magnetic resonance imaging (MRI) showed signal hyperintensities on T2-weighted imaging in the posterior column from the upper cervical cord to the conus medullaris, which were not highlighted by gadolinium-based contrast material (Fig. 1). Two courses of steroid pulse therapy (methylprednisolone 1,000 mg/day, 3 days) showed no efficacy for treating symptoms. We therefore performed plasma exchanges four times over two weeks, followed by oral administration of prednisolone

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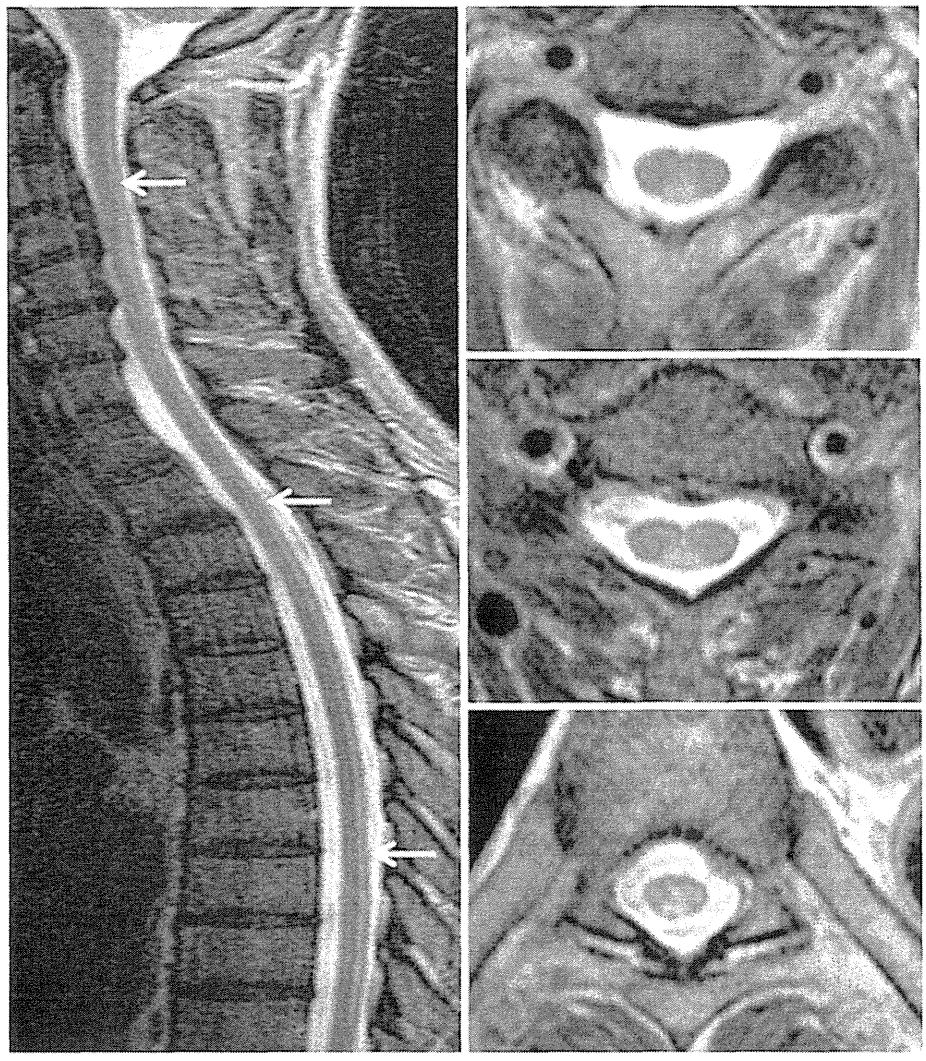

Fig. 1 Spinal MRI. T2-weighted imaging shows signal hyperintensity in the posterior column of the cervicothoracic cord. This lesion was not highlighted by contrast material (data not shown)

(30 mg/day). Following these treatments, severe heartburn and vomiting after eating gradually improved, but other symptoms such as orthostatic hypotension and gait disturbance and abnormal signals in the spinal cord persisted.

We believe that the clinical manifestations in this patient can be attributed to posterior column lesions spreading throughout the spinal cord. Four of 15 acute ASN patients reportedly showed abnormal intensity on T2-weighted imaging in the posterior column [4], suggesting that posterior column involvement is not rare with ASN. However, no previous reports have mentioned the possible roles of anti-VGKC antibody in the pathogenesis.

Anti-VGKC antibodies are known to bind to a limited number of potassium channel subunits (Kv1.1, Kv1.2 and Kv1.6). Sensory nerve cells in the posterior root ganglion express Kv1.1 and Kv1.2 [5], indicating that anti-VGKC antibody-mediated malfunction of Kv1.1 and Kv1.2 in sensory nerve cells could be considered as the underlying mechanism. In fact, cell loss in thoracic posterior root ganglia was observed in an autopsy case with chronic ASN [6]. The MRI abnormalities in the present patient may represent Wallerian degeneration from posterior root ganglia to the posterior column. Although further investigation with a larger numbers of samples is needed, anti-VGKC antibody could serve as a novel diagnostic marker for ASN with posterior column involvement.

Conflicts of interest The authors declare that they have no conflict of interest.

Ethical standard This study has been approved by the appropriate ethics committee and has therefore been performed in accordance with the ethical standards laid down in 1964 declaration of Helsinki.

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症例報告

Faciobrachial dystonic seizuresを呈した抗電位依存性カリウムチャンネル複合体（LGI-1）抗体関連辺縁系脳炎の1例

A Case of Anti-voltage-gated Potassium Channel Complex (LGI-1) Associated Limbic Encephalitis Manifesting Faciobrachial Dystonic Seizures

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高木　俊輔[3)]　中村　康子[4)]　渡辺　裕貴[1)]　渡辺　雅子[1)]

要旨：高齢発症側頭葉てんかんにおいて、健忘症、気分障害、睡眠障害、排尿障害、唾液分泌過多、低ナトリウム血症を認め、抗電位依存性カリウムチャンネル複合体（voltage-gated potassium channel：VGKC-complex（leucine-rich glioma inactivated 1 protein：LGI-1））抗体陽性から、抗VGKC複合体抗体関連辺縁系脳炎（VGKC-LE）と診断した。本例は数秒間こみあげ息がつまる発作が1日100回と頻発し左上肢を強直させることがあった。Iraniらは、VGKC-LEの中で抗LGI-1抗体を有するものは、3～5秒間顔面をしかめ上腕を強直させるfaciobrachial dystonic seizures（FBDS）を報告しており、本発作は診断の一助となると考えられた。本例は、本邦において抗LGI-1抗体とFBDSの関連を指摘した最初の報告である。

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Key Words：faciobrachial dystonic seizures, limbic encephalitis, voltage-gated potassium channel complex antibody, LGI-1

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序　論

抗電位依存性カリウムチャンネル（voltage-gated potassium channel：VGKC）抗体関連辺縁系脳炎は、亜急性から慢性に経過する、てんかん、記憶障害を中核症状とする非腫瘍性自己免疫性脳炎である[1,2)]。壮年期から高齢の男性に多く、高率に低Na血症を伴う。ステロイド、血漿交換、および

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