Antibodies to GABA<sub>A</sub> receptor α1 and γ2 subunits

Clinical and serologic characterization

**ABSTRACT**

**Objective:** To search for antibodies against neuronal cell surface proteins.

**Methods:** Using immunoprecipitation from neuronal cultures and tandem mass spectrometry, we identified antibodies against the α1 subunit of the γ-aminobutyric acid A receptor (GABA<sub>A</sub>R) in a patient whose immunoglobulin G (IgG) antibodies bound to hippocampal neurons. We searched 2,548 sera for antibodies binding to GABA<sub>A</sub>R α, β, and γ subunits on live HEK293 cells and identified the class, subclass, and GABA<sub>A</sub>R subunit specificities of the positive samples.

**Results:** GABA<sub>A</sub>R-Abs were identified in 40 of 2,046 (2%) referred sera previously found negative for neuronal antibodies, in 5/502 (1%) previously positive for other neuronal surface antibodies, but not in 92 healthy individuals. The antibodies in 40% bound to either the α1 (9/45, 20%) or the γ2 subunits (9/45, 20%) and were of IgG1 (94%) or IgG3 (6%) subclass. The remaining 60% had lower antibody titers (p = 0.0005), which were mainly immunoglobulin M (IgM) (p = 0.0025), and showed no defined subunit specificity. Incubation of primary hippocampal neurons with GABA<sub>A</sub>R IgG1 sera reduced surface GABA<sub>A</sub>R membrane expression. The clinical features of 15 patients (GABA<sub>A</sub>R α1 n = 6, γ2 n = 5, undefined n = 4) included seizures (47%), memory impairment (47%), hallucinations (33%), or anxiety (20%). Most patients had not been given immunotherapies, but one with new-onset treatment-resistant catatonia made substantial improvement after plasma exchange.

**Conclusions:** The GABA<sub>A</sub>R α1 and γ2 are new targets for antibodies in autoimmune neurologic disease. The full spectrum of clinical features, treatment responses, correlation with antibody specificity, and in particular the role of the IgM antibodies will need to be assessed in future studies. *Neurology*® 2015;84:1233-1241

**GLOSSARY**

AMPAR = α-amino-3-hydroxy-5-methyl-4-isoxazol-propionic acid receptor; BFCRS = Bush Frances Catatonia Rating Scale; CASPR2 = contactin-associated protein-like 2; CBA = cell-based assay; FAB = Frontal Assessment Battery; GABA<sub>A</sub>R = γ-aminobutyric acid A receptor; HEK293 = human embryonic kidney 293; IgG = immunoglobulin G; IgM = immunoglobulin M; LGI1 = leucine-rich, glioma-inactivated 1; NMDAR = NMDA receptor; PEX = plasma exchange; SDS-PAGE = sodium dodecyl sulfate polyacrylamide gel electrophoresis; VGKC = voltage-gated potassium channel.

Antibodies directed against proteins expressed in the CNS have been identified in a number of neurologic disorders including various encephalopathies<sup>1–3</sup> as well as subgroups of patients with epilepsy<sup>4,5</sup> or psychiatric disease.<sup>6,7</sup> The antibodies, usually immunoglobulin G (IgG), are directed against extracellular epitopes of proteins expressed on the surface of neuronal cells, including the NMDA receptor (NMDAR), leucine-rich, glioma-inactivated 1 (LGI1), and contactin-associated protein-like 2 (CASPR2), and less frequently against γ-aminobutyric acid B receptor (GABA<sub>B</sub>R), α-amino-3-hydroxy-5-methyl-4-isoxazol-propanionic acid receptor (AMPAR), or glycine receptors.<sup>8</sup> The majority of the patients have a favorable response to immunotherapies<sup>3,11</sup> and detection of the antibodies in patient sera and CSF have altered diagnosis and management.

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Antibodies to the α1 and β3 subunits of GABA₉R, 2 subunits of the heteropentameric ligand gated ion channel that mediates the majority of inhibitory neurotransmission in the brain, were recently reported in 18 patients. The 6 patients with high serum and CSF GABA₉R-Abs presented mainly with seizures and refractory status epilepticus, whereas lower serum titers, without CSF antibodies, were observed in 12 patients with broader neurologic diagnoses including stiff-person syndrome and adult-onset opsoclonus myoclonus syndrome.

We independently identified the GABA₉α1 subunit and the novel γ2 subunit as antibody targets and, using a live cell-based assay, detected them in 45 of a total of 2,548 sera referred for other CNS antibody tests. GABA₉R-Abs fell into 2 broad groups defined by their subunit specificity, titer, and immunoglobulin class or subclass.

**METHODS** Standard protocol approvals, registrations, and patient consents. The research use of referred sera is approved by the Oxfordshire Research Ethics Committee A (07/Q160/X/28). When GABA₉R was identified, and a cell-based assay (CBA) established, 502 sera with voltage-gated potassium channel (VGKC) complex, NMDAR, or other antibodies, 92 healthy and 112 disease control sera (table 1), and a further 2,046 referred sera negative for the requested antibodies were tested for the presence of GABA₉R-Abs. Brief clinical data were requested from referring neurologists of positive sera. Specific written consent was obtained from patient 2 for inclusion of his case report and videos.

**Immunoprecipitation from cortical neurons.** To identify new neuronal antibodies, sera were tested for binding to cultured primary rat hippocampal and cortical neurons. A serum with very strong binding was chosen for further study. The patient’s IgG was bound to rat cortical neurons, and the immune complexes solubilized with 2% digitonin and captured using Protein G-Sepharose beads (Sigma, Dorset, UK). The immunoprecipitate was separated by gel electrophoresis and the GABA₉α1 subunit was identified as the target by mass spectroscopy from a sample of digested bands from the patient, but not healthy control, immunoprecipitate.

**Expression of GABA₉R in transfected human embryonic kidney cells.** As for other antibody tests, individual GABA₉R subunits (α1, β2, β3, or γ2) were individually or coexpressed in human embryonic kidney 293 (HEK293) T cells and cell surface expression examined (e-Methods, tables e-1 and e-2 on the Neurology® Web site at Neurology.org). Antibody reactivity was initially assessed using HEK293 cells coexpressing α1β2γ2 Y27 GABA₉R subunits and binding detected with Alexa Fluor 568 goat antihuman IgG (H + L) (1:750, A-21090, Invitrogen, Paisley, UK). All sera were scored (0, negative; 1, low positive; 2–4 positive) and colocalized with a commercial antibody against the α1 subunit of the GABA₉R (1:500, clone N95/35, Antibodies Inc., Davis, CA). Endpoint dilution titers were established by determining the last dilution at which binding was scored as 1. To determine subunit specificities, all positive sera were tested by CBA for binding to different GABA₉R subunit combinations.

GABA₉R IgG subclasses were determined by use of subclass-specific mouse antihuman IgG1, IgG2, IgG3, or IgG4 secondary antibodies (The Binding Site, Birmingham, UK), before washing, fixing, and visualization with Alexa Fluor 568 goat antihuman IgG (H + L) (1:750, A-11001, Invitrogen). Immunoglobulin M (IgM)-specific antibodies were determined using an Alexa Fluor 488 goat antihuman IgM antibody (1:750, A-2125, Invitrogen).

**Effects of patient antibodies on GABA₉R expression in vitro.** Primary P0 rat neuronal cultures (DIV 7) were incubated for 3 days with patient or healthy control serum (1:100; heated at 56°C for 30 minutes to inactivate complement). Subsequently, surface proteins were biotinylated, the cells lysed, and biotinylated surface proteins isolated on a NeutrAvidin agarose column (89881, Pierce Biotechnology, Rockford, IL). Isolated membrane proteins were eluted in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) buffer (Invitrogen) containing 50 mM dithiothreitol; equal amounts of samples were then analyzed by SDS-PAGE and Western blot probing for the α1 and γ2 subunits of GABA₉R. Antibody to the transferrin receptor (1:3-6800, Invitrogen) was used as a cell surface fraction loading control. Quantification of GABA₉R receptor loss was determined by densitometric analysis of the Western blots using ImageJ software, and calculated as the ratio of α1:transferrin receptor and γ2:transferrin receptor.

**RESULTS** Identification of GABA₉R as a target of autoimmunity. Patient 1 had a history of neuropsychological changes including elements of obsessive-compulsive disorder and was febrile on presentation. Patient 2 had a history of neuropsychological changes including elements of obsessive-compulsive disorder and was febrile on presentation. Patient 3 had a history of neuropsychological changes including elements of obsessive-compulsive disorder and was febrile on presentation. Patient 4 had a history of neuropsychological changes including elements of obsessive-compulsive disorder and was febrile on presentation. Patient 5 had a history of neuropsychological changes including elements of obsessive-compulsive disorder and was febrile on presentation. Patient 6 had a history of neuropsychological changes including elements of obsessive-compulsive disorder and was febrile on presentation. Patient 7 had a history of neuropsychological changes including elements of obsessive-compulsive disorder and was febrile on presentation. Patient 8 had a history of neuropsychological changes including elements of obsessive-compulsive disorder and was febrile on presentation. Patient 9 had a history of neuropsychological changes including elements of obsessive-compulsive disorder and was febrile on presentation. Patient 10 had a history of neuropsychological changes including elements of obsessive-compulsive disorder and was febrile on presentation. Patient 11 had a history of neuropsychological changes including elements of obsessive-compulsive disorder and was febrile on presentation. Patient 12 had a history of neuropsychological changes including elements of obsessive-compulsive disorder and was febrile on presentation. Patient 13 had a history of neuropsychological changes including elements of obsessive-compulsive disorder and was febrile on presentation. Patient 14 had a history of neuropsychological changes including elements of obsessive-compulsive disorder and was febrile on presentation. Patient 15 had a history of neuropsychological changes including elements of obsessive-compulsive disorder and was febrile on presentation. Patient 16 had a history of neuropsychological changes including elements of obsessive-compulsive disorder and was febrile on presentation. Patient 17 had a history of neuropsychological changes including elements of obsessive-compulsive disorder and was febrile on presentation. Patient 18 had a history of neuropsychological changes including elements of obsessive-compulsive disorder and was febrile on presentation.
disorder and increased anxiety but without psychosis. She was seen by a neurologist but there was no objective evidence of encephalitis and she returned to her care home. Subsequently, serum VGKC complex antibodies were reported (1938 pM), but all other antibody tests (antibodies to LGI1, CASPR2, NMDAR, AMPAR, GABA\textsubscript{a}R, glycine R) were negative. However, her serum IgG bound intensely to the surface of both hippocampal and cortical neuronal cultures, indicating the presence of a potentially pathogenic antibody against a neuronal surface protein (figure 1A). Using immunoprecipitation and mass spectrometry (see Methods), her serum antibodies were found to bind to the \(\alpha_1\) subunit of GABA\textsubscript{a}R (figure e-1A). GABA\textsubscript{a}R in the immunoprecipitent from the patient, but not from a healthy individual, was confirmed by Western blotting (figure 1B).

Detection of GABA\textsubscript{a}R-Abs in patient sera. In vivo, GABA\textsubscript{a}R is composed of multiple subunits (\(\alpha_1-6, \beta_1-3, \gamma_1-3, \pi, \varepsilon, \theta\)), which combine to form heteropentamers with a central pore; the \(\alpha_1\beta_2\gamma_2\) is the most abundant neuronal GABA\textsubscript{a}R subtype.\textsuperscript{16} Individual homomeric GABA\textsubscript{a}R subunits and heteropentameric GABA\textsubscript{a}Rss (\(\alpha_1\beta_2\gamma_2\) subunits) were expressed in HEK cells and their cell surface expression assessed. Immunostaining of permeabilized fixed cells showed intracellular pools of all of the GABA\textsubscript{a}R subunits (figure e-1B), but surface GABA\textsubscript{a}R expression was only found with cotransfection of all 3 GABA\textsubscript{a}R subunits, and we used \(\alpha_1\beta_2\gamma_2\) to establish the CBA. Patient 1’s antibody bound to the surface of live GABA\textsubscript{a}R-transfected cells, colocalizing with commercial GABA\textsubscript{a}R-\(\alpha_1\) subunit antibody (figure 1C).

GABA\textsubscript{a}R-Abs in patients and controls. Sera from healthy and disease controls (table 1) did not bind to GABA\textsubscript{a}R-transfected cells (healthy control mean \(+3\) SD = 0.28, figure 1D). Only 2 of 108 (1.9%) additional sera positive for VGKC complex antibodies were positive for GABA\textsubscript{a}R-Abs, and adsorption of patient 1’s serum showed that GABA\textsubscript{a}R was not a

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**Figure 1** Identification of the GABA\textsubscript{a} receptor as an antibody target in CNS disease

(A) The index patient sera showed antibody binding (green) to the surface of live hippocampal neurons that were identified postpermeabilization with the neuronal marker MAP-2 (red). (B) After identification of \(\gamma\)-aminobutyric acid A receptor (GABA\textsubscript{a}R) peptides by tandem mass spectroscopy of the immunoprecipitate from cultured neurons, its presence was confirmed by Western blotting using a commercial antibody against the \(\alpha_1\) subunit of GABA\textsubscript{a}R (52 kDa); cortical brain homogenate (Cx) was used as positive control. (C) A cell-based assay was developed using human embryonic kidney 293 cells cotransfected with the \(\alpha_1, \beta_2, \text{and} \, \gamma_2\) subunits of GABA\textsubscript{a}R. Antibody binding to GABA\textsubscript{a}R was demonstrated with serum from patient 1 (red), which colocalized with commercial antibody against the \(\alpha_1\) subunit (green; upper row). Immunoglobulin G (IgG) immunoreactivity to GABA\textsubscript{a}R was not observed with control serum (lower row). (D) GABA\textsubscript{a}R-Abs were identified in 5/502 sera with known antibodies (3 voltage-gated potassium channel complex, 2 NMDAR-Abs), and 40/2,046 sera previously found negative in other routine antibody tests. Samples scoring above 1 (dotted line) are considered positive. GABA\textsubscript{a}R-Abs were not present in healthy (\(n = 92\)) or disease (\(n = 112\)) control sera. Scale bars are 30 \(\mu\)m.
component of the VGKC complex (figure e-2). GABA$_A$R $\alpha_1\beta_2\gamma_2$ antibodies were detected in only 2 of 393 (0.5%) sera positive for other known neuronal surface antibodies but were present in 40 of 2,046 (2%) sera previously found negative for NMDAR, AMPAR, or GABA$_A$R antibodies (table 1). Serum endpoint titers were between 1:80 and 1:4,860, and all 45 GABA$_A$R-Abs-positive sera bound to live hippocampal neurons (as in figure 1A). There were no CSF samples available for testing from these patients.

Subunit specificities of GABA$_A$R-Abs. We tested reactivity to HEK293 cells after substitution of individual subunits ($\alpha_1\beta_2\gamma_2$, $\alpha_2\beta_2\gamma_2$, $\alpha_3\beta_2\gamma_2$, $\alpha_5\beta_2\gamma_2$, $\alpha_1\beta_2\gamma_1$, $\alpha_1\beta_3\gamma_2$, $\alpha_3\beta_3\gamma_2$) of the GABA$_A$R heteropentamer (for examples, see figure 2A). Replacing the $\alpha_1$ subunit with the $\alpha_2$, $\alpha_3$, or $\alpha_5$ subunits abrogated the binding of serum antibody from 9 patients (20%, including patient 1), demonstrating specificity for the $\alpha_1$ subunit. Replacing $\gamma_2$ with $\gamma_1$ abolished binding in a further 9 sera (20%), indicating $\gamma_2$ antibody specificity. However, neither these substitutions nor replacing $\beta_2$ with $\beta_3$ affected binding in the remaining 28 sera. Notably, patients with $\alpha_1$- or $\gamma_2$-specific antibodies had higher antibody titers than patients lacking subunit specificity (Mann-Whitney $p = 0.0005$, figure 2B).

IgG class and subclass of GABA$_A$R-Abs. The antihuman IgG (H + L, A-21090, Invitrogen) is widely used for

![Figure 2](specific-gaba-ar-receptor-subunit-reactivities-and-immunoglobulin-classes)

[A] In the index patient 1, substitution of the $\alpha_1$ subunit with the $\alpha_2$, $\alpha_3$, or $\alpha_5$ subunit ablated binding to the $\gamma$-aminobutyric acid A receptor (GABA$_A$R)-transfected cells, illustrating that the $\alpha_1$ subunit was the antigenic target. $\alpha_1$-Specific antibodies were observed in a further 8 patients (20% of total 45). Case 8 illustrates 1 of 9 sera (20%) that bound only to GABA$_A$Rs containing the $\gamma_2$ subunit, but not the $\gamma_1$ subunit (second row). The third row shows sera from patient 15, which bound to all GABA$_A$Rs without a defined subunit specificity. (B) Sera with subunit-specific GABA$_A$R-Abs ($\alpha_1$ and $\gamma_2$) had significantly higher antibody titers than sera without a distinct subunit reactivity (Mann-Whitney, $p = 0.0005$). (C) Sera with specific $\alpha_1$ (n = 9, red) or $\gamma_2$ subunit (n = 8, blue) antibody reactivities had IgG1 (16) or IgG3 (1) antibodies, compared to only 2/20 sera without a defined GABA$_A$R subunit (green) antibody reactivity (both IgG3), $p < 0.0001$, whose antibodies were predominantly immunoglobulin M (IgM), $p = 0.025$ (18/20). IgG = immunoglobulin G.
Patient antibodies reduce GABA<sub>R</sub> expression on cultured neurons. As patient sera bound to primary cortical neurons, we investigated the effects of 4 sera with high titers (endpoint dilutions 1:540–1:4,860) of IgG1 GABA<sub>R</sub> α1 (n = 2, patients 1 and 2) or γ2 subunits (n = 2, patients 8 and 9) on GABA<sub>R</sub> expression in vitro, as described by others. Neuronal cultures were exposed to the heat-inactivated sera (1:100) for 72 hours, and GABA<sub>R</sub> expression assessed by Western blot. All 4 patient sera caused a reduction in the surface expression of both α1 and γ2 subunits when compared to neurons treated with 2 control sera (p = 0.0023 and p = 0.0067, respectively, figure 3A and B).

Clinical features of patients with GABA<sub>R</sub>-Abs. Overall, there were 22 male participants (age 2–76 years, median 51) and 23 female participants (age 13–80 years, median 54.5). Eight patients (3 M:5 F) were <20 years of age and 9 patients were young adults (21–30 years; 5 M:4 F). GABA<sub>R</sub>-Abs subunit specificity did not correlate with sex (p = 0.7631, Fisher exact test) or age (p = 0.6444, Mann-Whitney test).

We subsequently obtained brief clinical data for 15 representative patients, 6 with α1 and 5 with γ2 GABA<sub>R</sub>-Abs specificity, and 4 with undefined subunit specificity (table 2). The most common presenting features were seizures (n = 7, 47%), memory impairment (n = 7, 47%) with confusion or disorientation (n = 4, 27%), or psychiatric features (n = 5, 33%) with hallucinations (n = 2, 33%) or anxiety (n = 4, 27%). One 13-year-old girl had a dysembryoplastic neuroepithelial tumor resected earlier in life, with established neurodevelopmental problems, but presented with unexplained onset of behavioral disturbance.

CSF had been examined at presentation in only 4/15 patients; 3/4 were normal. MRIs were normal in 4/9 or not performed (6/15). In the few with informative MRIs, patient 3 (α1-specific antibodies) had unilateral hippocampal high signal but this was thought at the time to be due to temporal lobe seizures rather than autoimmune encephalitis. Patient 10 (γ2-specific antibodies), with a small head of caudate matter lesions. Patient 14 (subunit undefined) was in remission from non-Hodgkin lymphoma after treatment when she presented with personality changes, memory loss, and confusion. CSF showed lymphocytic pleocytosis and oligoclonal bands, and there was temporal lobe high signal on MRI. The changes were consistent with a paraneoplastic limbic encephalitis rather than direct infiltration. However, there was mediastinal recurrence of the lymphoma and she failed to respond to intrathecal chemotherapy or immunotherapies, dying soon after. At further follow-up (either verbal or at clinic visit, up to 12 months after reporting the antibodies), 4 patients had done well on symptomatic treatment only, and a further 2 appeared to have improved spontaneously. Of the 3 patients who received some immunotherapy, one (patient 2, α1-specific antibodies) clearly improved (see below) and one showed modest improvement but one was declining after a short course. The others were either lost to follow-up or had died (see table 2).
A 17-year-old boy presented to Neurology 84 March 24, 2015

**Table 2** Summary of clinical data from 15 patients

| No. | Sex/age, y/onset/igG and titer/subunit | History; presenting features; investigations | Neurologist's diagnosis | Treatments given; follow-up |
|-----|--------------------------------------|---------------------------------------------|-------------------------|----------------------------|
| 1   | F/72/insidious/igG1 1:4,860/v1        | Increasing obsessive-compulsive disorder and increased anxiety; facial twitches; MRI, CSF not done, VGKC complex antibodies 1,938 pM | Unclear diagnosis | Patient reluctant to attend hospital |
| 2   | M/17/1 mol/igG1 and igM 1:540/v1     | Behavioral changes, disturbed thought, incontinence, apraxia, orthostatic hypotension, dystonia, tetraparesis, respondents to PEX | Catatonia of unknown etiology | PEX x 2 with good responses; 20 mo |
| 3   | M/56/1 mol/igG1 and igM 1:1,800/v1   | Type 2 DM, frequent visual unformed hallucinations with eye and head deviation, focal partial seizures; temporary MRI FLAIR lesion; CSF normal | Partial status epilepticus | No IT, seizure-free on low-dose Keppra; 2 mo |
| 4   | M/47/12 mol/igG1 1:540/v1           | HIV on cART, splenectomy for ITP, weight loss, impaired memory, verbal fluency, depression; GCs; MRI, CSF, EEG no abnormalities | Neurodegenerative disorder? | Possible slow response to PEX and Aza; 18 mo |
| 5   | F/19/1 wk/igG1 1:6,200/v1            | GTCS following drugs and sleep deprivation; also antibodies to NMDAR, CASPR2, and VGKC complex; did not attend EEG, MRI, or tumor search; CSF nd | Drugs/alcohol-related seizures | No IT, Keppra only required; no FU |
| 6   | M/23/12 y/igG3 1:540/v1             | Weekly loss of consciousness for up to 1 h; MRI normal, CSF, EEG nd | Nonepileptic attacks | No IT; no FU |
| 7   | M/47/2 mol/igG1 and igM 1:540/v2     | Gluten sensitivity, alcohol; severe amnesia, confusion, hallucinations, seizures, ataxia; MRI, cerebellar atrophy, EEG abnormal but no seizures, CSF nd | Celiac/autoimmune encephalitis, alcohol | No IT; improved and did not want FU |
| 8   | F/13/4 mol/igG1 and igM 1:540/v2     | DNET, epilepsy, and learning disability; disorientation, behavioral change, violence, absences, nocturnal GTCS; tumor size unchanged, MRI, CSF nd | Possible psychological disorder | No IT, AED and antipsychotic only; 16 mo |
| 9   | M/62/3 y/igG1 1:6,200/v1             | Amnesia only; MRI few scattered white matter lesions only; EEG, CSF nd | Mild cognitive impairment | No IT, some improvement; 8 mo |
| 10  | F/58/3 mol/igM 1:180/v2              | Anxiety, dizziness, dysphagia, weak legs; appendicular and axial dyskinesias (choreiform) at rest; caudate heads small on brain MRI, CSF normal, EEG nd | Autoimmune or Huntington disease | No IT; Huntington disease confirmed 4 mo |
| 11  | F/47/18 mol/igG1 1:540/v2            | Anterograde amnesia, spatial disorientation; GTCS; MRI and CSF nd, EEG generalized tonic pattern, intermittent L frontaltemporal spike/sharp waves | Focal epilepsy or limbic encephalitis | Pred and IVig but steady decline; 18 mo |
| 12  | M/68/0 mol/1:60/undefined            | Epilepsy, prostatic cancer; amnesia, self-neglect, depression, sleepiness, GTCS with status epilepticus; MRI, R frontal and temporal lobe atrophy; EEG, CSF nd | Delirium on the background of dementia | No IT; died |
| 13  | M/27/9 y/igM 1:180/undefined         | Anxiety, poor concentration, hallucinations; MRI, CSF, EEG nd | Paraneoplastic autoimmune encephalitis | Pred, intrathecal chemotherapy; died |
| 14  | F/65/5 mol/igM 1:180/undefined       | Headache, confusion, memory loss, anxiety; non-Hodgkin lymphoma with limbic changes on MRI, 11 lymphocytes and OCB in CSF | Paraneoplastic autoimmune encephalitis | Pred, intrathecal chemotherapy; died |
| 15  | M/62/15 mol/igM 1:540/undefined      | Hypertension, hyperlipidemia, prostatism; partial complex seizures only; MRI, few white patches only, VGKC complex Abs 550 pM | Focal epilepsy | No IT; some amnesia developing 18 mo |

Abbreviations: Abs = antibodies; AED = antiepileptic drugs; Aza = azathioprine; BFCRS = Bush Frances Catatonia Rating Scale; CART = combination antiretroviral therapy; DM = diabetes mellitus; DNET = dysembryoplastic neuroepithelial tumor; FLAIR = fluid-attenuated inversion recovery; FU = follow-up; GTCS = generalized tonic-clonic seizures; IgG = immunoglobulin G; IgM = immunoglobulin M; IT = immunotherapy; ITP = idiopathic thrombocytopenia; IVIg = IV immunoglobulins; nd = not done; NMDAR = NMDA receptor; OCB = oligoclonal bands; PEX = plasma exchange; Pred = prednisolone; VGKC = voltage-gated potassium channel.

All samples were tested for VGKC complex, NMDAR, and GAD antibodies.

Patient 2 presentation and clinical response to immunotherapy. A 17-year-old boy presented to psychiatrists with a 1-month history of forgetfulness and behavioral changes (disturbed thoughts, including harming others, requesting a sex change, paranoid delusions, and attempts to self-harm). He had occasional tachycardia (up to 120 beats/minute) at rest, but neurologic examination and investigation with PET, CSF analysis, MRI, and 2 EEGs had normal results. He displayed intermittent drooling and long periods of staring and verbiage; at other times he was verbally unresponsive, and sat abnormally still, with grimacing and posturing for more than 1 minute (video 1). He was given a diagnosis of catatonia of unknown etiology and admitted to a psychiatric unit, where he required continuous supervision; His Bush Frances Catatonia Rating Scale (BFCRS) score was 13 (moderate >9, normal 0). He was unresponsive to antidepressant (sertraline, fluoxetine), antipsychotic (olanzapine, haloperidol, quetiapine), and anxiolytic (lorazepam, clonazepam) treatment. He had a Frontal Assessment Battery (FAB) score of 6/18, indicating severe frontal dysfunction. Three months after presentation, GABA\textsubscript{A}R-Abs was detected in his serum (x1-subunit specific; 1:540, IgG1-isotype) and a possible autoimmune etiology was proposed. After consideration of the evidence implicating GABA\textsubscript{A}R in catatonia,\textsuperscript{18,19} the patient received 4 days of plasma exchange (PEX), after which antibodies were no longer detected in his serum and his frontal dysfunction and catatonia resolved within 2 weeks (BFCRS 0, FAB 18/18).
We identified a new antibody target,
both IgM and in another 12 with a variety of
IgA and IgG antibodies in 40% of patients were IgG1 or IgG3, bound
to GABA Rs containing α1 or γ2 subunits, and all 4 sera tested were able to reduce GABA R expression on live cortical neurons. In the remaining 60%, however, the titers were lower, the antibodies were mainly IgM, and they did not show subunit specificity, although the sera also bound to hippocampal neurons in culture. The clinical features of 15 representative patients included seizures, psychiatric and cognitive problems, and only one had a relevant malignancy. GABA R-Abs are relatively common (up to 2% of referrals for CNS autoantibodies) and IgA NMDAR-Abs have previously been reported, and in 2 patients a functional or psychogenic condition was suspected initially. Nevertheless, the large number of referrals for CNS autoantibodies (over 6,000 per year from the United Kingdom) and heterogeneity of the patients described here illustrates the increasing interest in identifying antibodies in patients with subacute onset of unexplained seizures or cognitive or psychiatric features.

GABA R-Abs, binding the α1 or β3 subunits, were identified recently in 6 patients with refractory status epilepticus or epilepsy partialis continua and a change in cognition/behavior with extensive imaging abnormalities and in another 12 with a variety of phenotypes and lower titers. The authors did not report γ2 subunit specificity or examine the immunoglobulin classes and subclasses. IgG GABA R β3 antibodies were also recently reported in 2 patients with thymoma-associated encephalopathies. Both IgM and IgA NMDAR-Abs have previously been reported to be pathogenic in vitro, but their clinical relevance is not clear; however, the serum GABA R-IgM-Abs identified here, although low titers, were not observed in 92 healthy control sera, and they also bound to live hippocampal neurons. This suggests that they could be pathogenic in vivo if they are able to reach the brain parenchyma, or are synthesized intrathecially. However, these possibilities clearly need further study.

DISCUSSION We identified a new antibody target, GABA R, established a CBA using HEK cells expressing heteropentameric GABA Rs, and identified a total of 45 patients with GABA R-Abs. The antibodies in 40% of patients were IgG1 or IgG3, bound to GABA Rs containing α1 or γ2 subunits, and all 4 sera tested were able to reduce GABA R expression on live cortical neurons. In the remaining 60%, however, the titers were lower, the antibodies were mainly IgM, and they did not show subunit specificity, although the sera also bound to hippocampal neurons in culture. The clinical features of 15 representative patients included seizures, psychiatric and cognitive problems, and only one had a relevant malignancy. GABA R-Abs are relatively common (up to 2% of referrals for CNS autoantibodies) and IgA NMDAR-Abs have previously been reported, and in 2 patients a functional or psychogenic condition was suspected initially. Nevertheless, the large number of referrals for CNS autoantibodies (over 6,000 per year from the United Kingdom) and heterogeneity of the patients described here illustrates the increasing interest in identifying antibodies in patients with subacute onset of unexplained seizures or cognitive or psychiatric features.

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As this study was retrospective in design, there are several limitations, in particular the lack of available CSF samples and limited or no immunotherapy intervention in all but 2 of the patients. Nevertheless, this study, in suggesting that a potentially pathogenic antibody can associate with clinical features that are less characteristic of the well-known autoimmune encephalitis syndromes, could have implications for the field. Future prospective studies, detecting GABA<sub>A</sub>R-Abs at onset and testing CSF, with judicious use of immunotherapy, and in vitro and in vivo experiments comparing the effects of IgG and IgM antibodies, will be important in determining their clinical relevance.

**AUTHOR CONTRIBUTIONS**
Philippa Pettingill: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, accepts responsibility for conduct of research and final approval, contribution of vital reagents/tools/patients, acquisition of data, statistical analysis. Holger Kramer: drafting/revising the manuscript, analysis or interpretation of data, accepts responsibility for conduct of research and final approval, contribution of vital reagents/tools/patients, acquisition of data. Jan Adrian Coebergh: drafting/revising the manuscript, analysis or interpretation of data, accepts responsibility for conduct of research and final approval, acquisition of data. Rosemary Pettigill: study concept or design, accepts responsibility for conduct of research and final approval, acquisition of data. Susan Maxwell: analysis or interpretation of data, accepts responsibility for conduct of research and final approval, contribution of vital reagents/tools/patients, acquisition of data. Andrea Malaspina: drafting/revising the manuscript, analysis or interpretation of data, accepts responsibility for conduct of research and final approval. Andrea Malaspina: drafting/revising the manuscript, accepts responsibility for conduct of research and final approval, acquisition of data. Andrea Malaspina: drafting/revising the manuscript, accepts responsibility for conduct of research and final approval, acquisition of data. Andrea Malaspina: drafting/revising the manuscript, accepts responsibility for conduct of research and final approval, acquisition of data.

**REFERENCES**
1. Dalmau J, Tunon E, Wu H, Majtaj J. Paraneoplastic anti-N-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. Ann Neurol 2007;61:25–36.
2. Lai M, Hughes EG, Peng X, et al. AMPA receptor antibodies in limbic encephalitis alter synaptic receptor location. Ann Neurol 2009;65:424–434.
3. Lancaster E, Lai M, Peng X, et al. Antibodies to the GABAB receptor in limbic encephalitis with seizures: case series and characterisation of the antigen. Lancet Neurol 2010;9:67–76.
4. Irani S, Michell A, Lang B, Pettigill P. Faciobrachial dystonic seizures precede Lgi1 antibody limbic encephalitis. Ann Neurol 2011;69:1–9.
5. Brenner T, Sills GJ, Hart Y, et al. Prevalence of neurologic autoantibodies in cohorts of patients with new and established epilepsy. Epilepsia 2013;54:1028–1035.
6. Steiner J, Walter M, Glanz W, et al. Increased prevalence of diverse N-methyl-D-aspartate glutamate receptor antibodies in patients with an initial diagnosis of schizophrenia: specific relevance of IgG NR1a antibodies for distinction from N-methyl-D-aspartate glutamate receptor encephalitis. JAMA Psychiatry 2013;70:271–278.
7. Trottini K, Kanthabay T, Tanaka K, et al. Anti-NMDA receptor antibody detected in encephalitis, schizophrenia, and narcolepsy with psychic features. BMC Psychiatry 2012;12:57.
8. Vincent A, Bien CG, Irani SR, Waters P. Autoantibodies associated with diseases of the CNS: new developments and future challenges. Lancet Neurol 2011;10:759–772.
9. Vincent A, Buckley C, Schott JM, et al. Potassium channel antibody-associated encephalopathy: a potentially immunotherapy-responsive form of limbic encephalitis. Brain 2004;127:701–712.
10. Dalmau J, Gleichman AJ, Hughes EG, et al. Anti-NMDA receptor encephalitis: case series and analysis of the effects of antibodies. Lancet Neurol 2008;7:1091–1098.
11. Tinuera MJ, McCracken L, Gabilondo I, et al. Treatment and prognostic factors for long-term outcome in patients with anti-NMDA receptor encephalitis: an observational cohort study. Lancet Neurol 2013;12:157–165.
12. Petit-Pedrol M, Armande T, Peng X, et al. Encephalitis with refractory seizures, status epilepticus, and antibodies to the GABAA receptor: a case series, characterisation of the antigen, and analysis of the effects of antibodies. Lancet Neurol 2014;13:276–286.

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**DISCLOSURE**
P. Pettigill, H. Kramer, J. Coebergh, R. Pettigill, S. Maxwell, A. Nibber, and A. Malaspina report no disclosures relevant to the manuscript. A. Jacob has received honoraria as a speaker on neuromyelitis optica from Biogen Idec and Chugai and is on clinical trial advisory boards for Chugai and Alexion Pharmaceuticals. S. Irani is a coapplicant and receives royalties on patent application WO/2010/046716 titled “Neurological autoimmune disorders.” The patent has been licensed to Euroimmun AG for the development of assays for LGI1 and other VGKC complex antibodies. C. Buckley and D. Beson report no disclosures relevant to the manuscript. P. Waters has received speaker honoraria from Biogen Idec and Euroimmun AG. A. Vincent and the Nuffield Department of Clinical Neurosciences in Oxford receive royalties and payments for antibody assays and A. Vincent is the named inventor on patent application WO/2010/046716 titled “Neurological autoimmune disorders.” The patent has been licensed to Euroimmun AG for the development of assays for LGI1 and other VGKC complex antibodies. S.R. Irani, P. Waters, and B. Lang are inventors and have received royalties. A patent for the detection of GABA<sub>A</sub>/Y2 receptor antibodies has been filed. Go to Neurology.org for full disclosures.
This Week’s Neurology® Podcast

Antibodies to GABA<sub>A</sub> receptor α1 and γ2 subunits: Clinical and serologic characterization (see p. 1233)

This podcast begins and closes with Dr. Robert Gross, Editor-in-Chief, briefly discussing highlighted articles from the March 24, 2015, issue of Neurology. In the second segment, Dr. Lara Marcuse talks with Dr. Angela Vincent about her paper on the clinical and serologic characterization of the antibodies to GABA<sub>A</sub> receptor α1 and γ2 subunits. Dr. James Addington then reads the e-Pearl of the week about navigating painful neuropathies. In the next part of the podcast, Dr. Michelle Johansen focuses her interview with Dr. Steve Zeiler on the topic of stroke in the setting of CNS vasculitis.

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