

Antibodies to GABA_A receptor α 1 and γ 2 subunits

Clinical and serologic characterization

OPEN 

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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 α 1 subunit of the γ -aminobutyric acid A receptor (GABA_AR) in a patient whose immunoglobulin G (IgG) antibodies bound to hippocampal neurons. We searched 2,548 sera for antibodies binding to GABA_AR α , β , and γ subunits on live HEK293 cells and identified the class, subclass, and GABA_AR subunit specificities of the positive samples.

Results: GABA_AR-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 α 1 (9/45, 20%) or the γ 2 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_AR IgG1 sera reduced surface GABA_AR membrane expression. The clinical features of 15 patients (GABA_AR α 1 $n = 6$, γ 2 $n = 5$, 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_AR α 1 and γ 2 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

AMPA = α -amino-3-hydroxy-5-methyl-4-isoxazol-propionic acid receptor; **BFCS** = Bush Frances Catatonia Rating Scale; **CASPR2** = contactin-associated protein-like 2; **CBA** = cell-based assay; **FAB** = Frontal Assessment Battery; **GABA_AR** = 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 encephalopathies¹⁻³ as well as subgroups of patients with epilepsy^{4,5} or psychiatric disease.^{6,7} 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_BR), α -amino-3-hydroxy-5-methyl-4-isoxazol-propionic acid receptor (AMPA), or glycine receptors.⁸ The majority of the patients have a favorable response to immunotherapies⁹⁻¹¹ and detection of the antibodies in patient sera and CSF have altered diagnosis and management.

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Supplemental data
at Neurology.org

From the Nuffield Department of Clinical Neurosciences (P.P., R.P., S.M., A.N., S.R.I., C.B., D.B., B.L., P.W., A.V.) and the Department of Physiology, Anatomy and Genetics (H.B.K.), University of Oxford; St George's Healthcare NHS Trust (J.A.C.), Tooting, London; the Centre for Neuroscience & Trauma, Blizard Institute (A.M.), Barts and The London School of Medicine and Dentistry, Queen Mary University of London; and The Walton Centre Foundation Trust (A.J.), Liverpool, UK.

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Table 1 Summary of serum samples screened for GABA_AR antibodies

Serum samples tested for GABA _A R-Abs	No. (%) positive for GABA _A R-Abs
Healthy controls (n = 92)	0/92 (0)
Disease controls (n = 112) ^a	0/112 (0)
Sera positive for other antibodies	
VGKC complex (LG11/CASPR2/contactin-2 negative) (n = 109)	3 ^b (2.8)
NMDA receptor (n = 290)	2 ^c (0.7)
LG11/CASPR2 (n = 47)	1 ^c (2.1)
Glycine receptor (n = 39)	0 (0)
AMPA (n = 11)	0 (0)
GABA _B receptor (n = 6)	0 (0)
Total of samples positive for other antibodies (n = 502)	5 (1)
Samples negative for other antibodies (n = 2,046)	40 (2)

Abbreviations: AMPAR = α -amino-3-hydroxy-5-methyl-4-isoxazol-propionic acid receptor; CASPR2 = contactin-associated protein-like 2; GABA_AR = γ -aminobutyric acid A receptor; LG11 = leucine-rich, glioma-inactivated 1; VGKC = voltage-gated potassium channel.

^aRasmussen syndrome (suspected and biopsy-proven) n = 51, epilepsy n = 17, opsoclonus myoclonus n = 44.

^bPatient 1 serum and 2 other VGKC complex antibody-positive sera (152 pM and 550 pM).

^cOne patient had NMDA receptor (NMDAR)-Abs (1:540) and another had both NMDAR-Abs (1:1,000) and CASPR2-Abs (1:180).

Antibodies to the α 1 and β 3 subunits of GABA_AR, 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.¹² The 6 patients with high serum and CSF GABA_AR-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 GABA_A α 1 subunit and the novel γ 2 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. GABA_AR-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

Q160X/28). When GABA_AR 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 GABA_AR-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 GABA_AR α 1 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 GABA_AR in transfected human embryonic kidney cells. As for other antibody tests,¹³⁻¹⁵ individual GABA_AR subunits (α 1, β 2, β 3, or γ 2) 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 α 1 β 2 γ 2 GABA_AR 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 α 1 subunit of the GABA_AR (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 CBAs for binding to different GABA_AR subunit combinations.

GABA_AR 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 antimouse 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 GABA_AR 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 α 1 and γ 2 subunits of GABA_AR. Antibody to the transferrin receptor (13-6800, Invitrogen) was used as a cell surface fraction loading control. Quantification of GABA_AR receptor loss was determined by densitometric analysis of the Western blots using ImageJ software, and calculated as the ratio of α 1:transferrin receptor and γ 2:transferrin receptor.

RESULTS Identification of GABA_AR as a target of autoimmunity. Patient 1 had a history of neuropsychological changes including elements of obsessive-compulsive

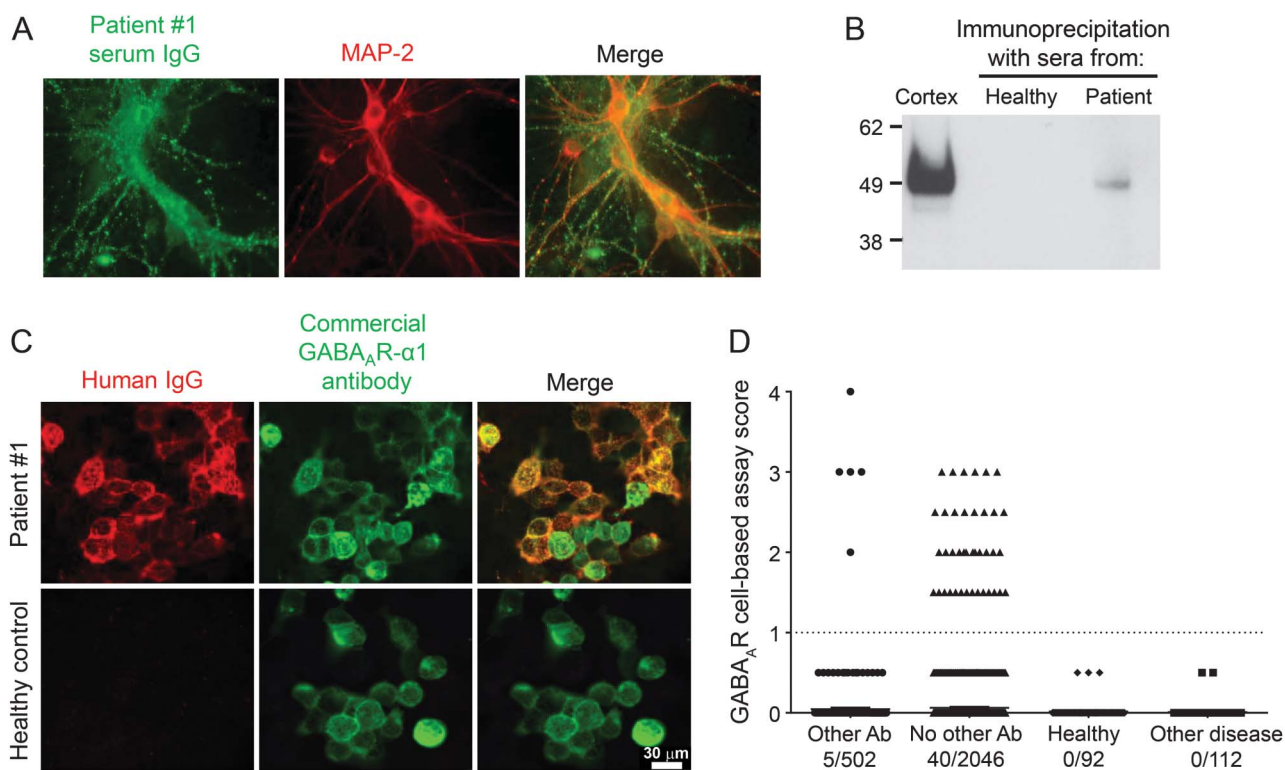
disorder and increased anxiety but without psychosis. She was seen by a neurologist but there was no objective evidence of encephalitis and she returned to her care home. Subsequently, serum VGKC complex antibodies were reported (1938 pM), but all other antibody tests (antibodies to LGI1, CASPR2, NMDAR, AMPAR, GABA_BR, 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_AR (figure e-1A). GABA_AR in the immunoprecipitate from the patient, but not from a healthy individual, was confirmed by Western blotting (figure 1B).

Detection of GABA_AR-Abs in patient sera. In vivo, GABA_AR is composed of multiple subunits ($\alpha 1-6$, $\beta 1-3$, $\gamma 1-3$, π , ϵ , θ), which combine to form

heteropentamers with a central pore; the $\alpha 1\beta 2\gamma 2$ is the most abundant neuronal GABA_AR subtype.¹⁶ Individual homomeric GABA_AR subunits and heteropentameric GABA_AR ($\alpha 1\beta 2\gamma 2$ 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_AR subunits (figure e-1B), but surface GABA_AR expression was only found with cotransfection of all 3 GABA_AR 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_AR-transfected cells, colocalizing with commercial GABA_AR $\alpha 1$ subunit antibody (figure 1C).

GABA_AR-Abs in patients and controls. Sera from healthy and disease controls (table 1) did not bind to GABA_AR-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_AR-Abs, and adsorption of patient 1's serum showed that GABA_AR was not a

Figure 1 Identification of the GABA_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 γ -aminobutyric acid A receptor (GABA_AR) 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_AR (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 the $\alpha 1$, $\beta 2$, and $\gamma 2$ subunits of GABA_AR. Antibody binding to GABA_AR 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_AR was not observed with control serum (lower row). (D) GABA_AR-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_AR-Abs were not present in healthy (n = 92) or disease (n = 112) control sera. Scale bars are 30 μ m.

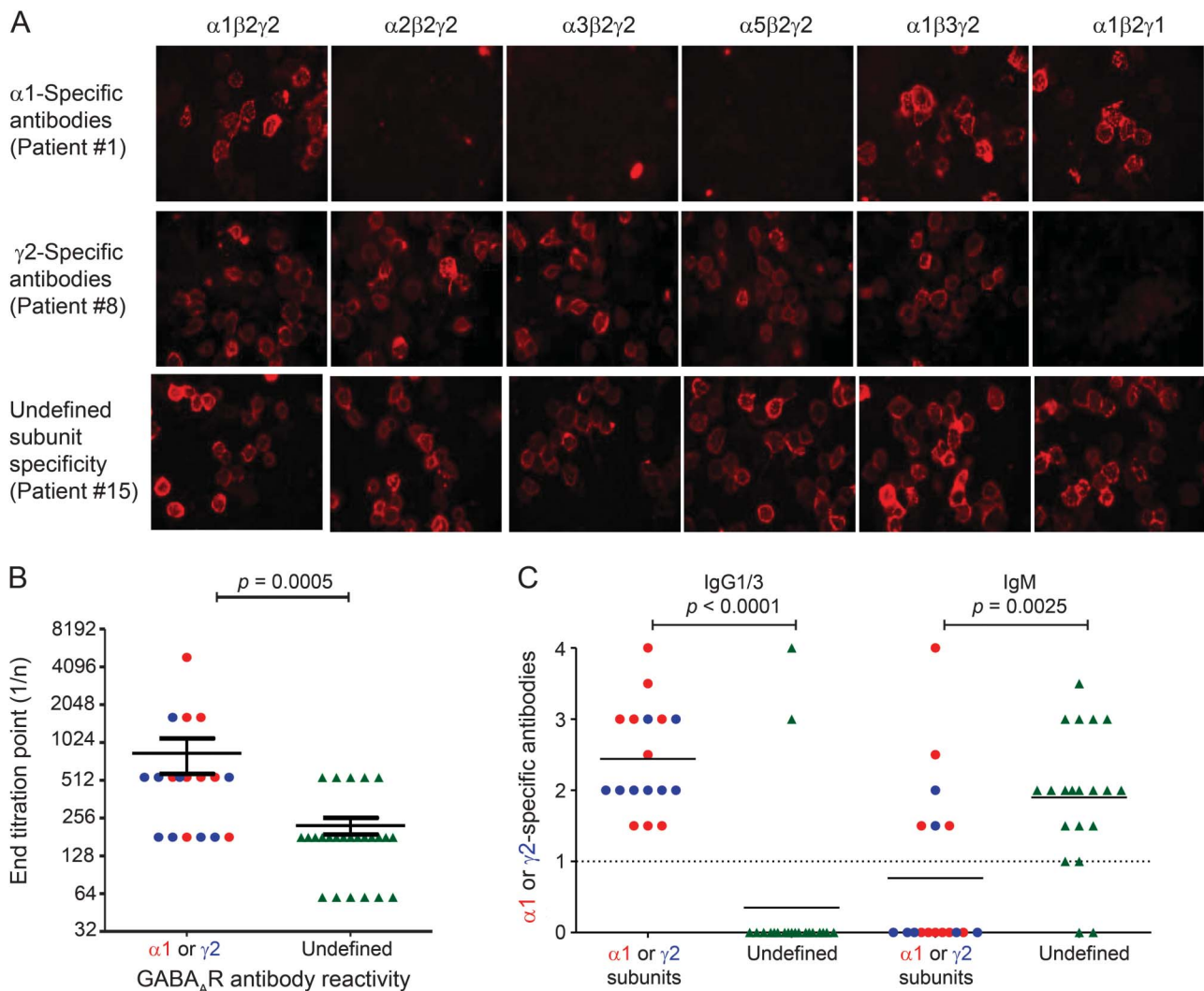
component of the VGKC complex (figure e-2). GABA_AR α1β2γ2 antibodies were detected in only 2 of 393 (0.5%) sera positive for other known neuronal surface antibodies but were present in 40 of 2,046 (2%) sera previously found negative for NMDAR, AMPAR, or GABA_BR antibodies (table 1). Serum endpoint titers were between 1:80 and 1:4,860, and all 45 GABA_AR-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_AR-Abs. We tested reactivity to HEK293 cells after substitution of individual subunits (α1β2γ2, α2β2γ2, α3β2γ2, α5β2γ2, α1β2γ1, α1β3γ2, α3β3γ2) of the GABA_AR

heteropentamer (for examples, see figure 2A). Replacing the α1 subunit with the α2, α3, or α5 subunits abrogated the binding of serum antibody from 9 patients (20%, including patient 1), demonstrating specificity for the α1 subunit. Replacing γ2 with γ1 abolished binding in a further 9 sera (20%), indicating γ2 antibody specificity. However, neither these substitutions nor replacing β2 with β3 affected binding in the remaining 28 sera. Notably, patients with α1- or γ2-specific antibodies had higher antibody titers than patients lacking subunit specificity (Mann-Whitney $p = 0.0005$, figure 2B).

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

Figure 2 Specific GABA_A receptor subunit reactivities and immunoglobulin classes



(A) In the index patient 1, substitution of the α1 subunit with the α2, α3, or α5 subunit ablated binding to the γ-aminobutyric acid A receptor (GABA_AR)-transfected cells, illustrating that the α1 subunit was the antigenic target. α1-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_AR containing the γ2 subunit, but not the γ1 subunit (second row). The third row shows sera from patient 15, which bound to all GABA_AR without a defined subunit specificity. (B) Sera with subunit-specific GABA_AR-Abs (α1 and γ2) had significantly higher antibody titers than sera without a distinct subunit reactivity (Mann-Whitney, $p = 0.0005$). (C) Sera with specific α1 ($n = 9$, red) or γ2 subunit ($n = 8$, blue) antibody reactivities had IgG1 (16) or IgG3 (1) antibodies, compared to only 2/20 sera without a defined GABA_AR subunit (green) antibody reactivity (both IgG1), $p < 0.0001$, whose antibodies were predominantly immunoglobulin M (IgM), $p = 0.025$ (18/20). IgG = immunoglobulin G.

detection of human IgG binding but as it is directed against total IgG, including both heavy and light chains, it can crossreact with the light chains shared with other classes of antibody. Using anti-IgG or anti-IgM subclass-specific secondary antibodies only 19/37 (51.4%) available sera contained IgG1 (n = 18) or IgG3 (n = 1) GABA_AR-Abs. Seventeen of the IgG antibodies were specific for GABA_AR α1 or γ2 subunits; 6 of these also had IgM antibodies. By contrast, 18 of the 20 remaining sera, without a defined subunit specificity, were exclusively IgM-GABA_AR-Abs ($p = 0.0025$, Mann-Whitney; figure 2C).

GABA_AR-Abs reduce surface GABA_AR 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_AR α1 (n = 2, patients 1 and 2) or γ2 subunits (n = 2, patients 8 and 9) on GABA_AR expression in vitro, as described by others.¹⁷ Neuronal cultures were exposed to the heat-inactivated sera (1:100) for 72 hours, and GABA_AR 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

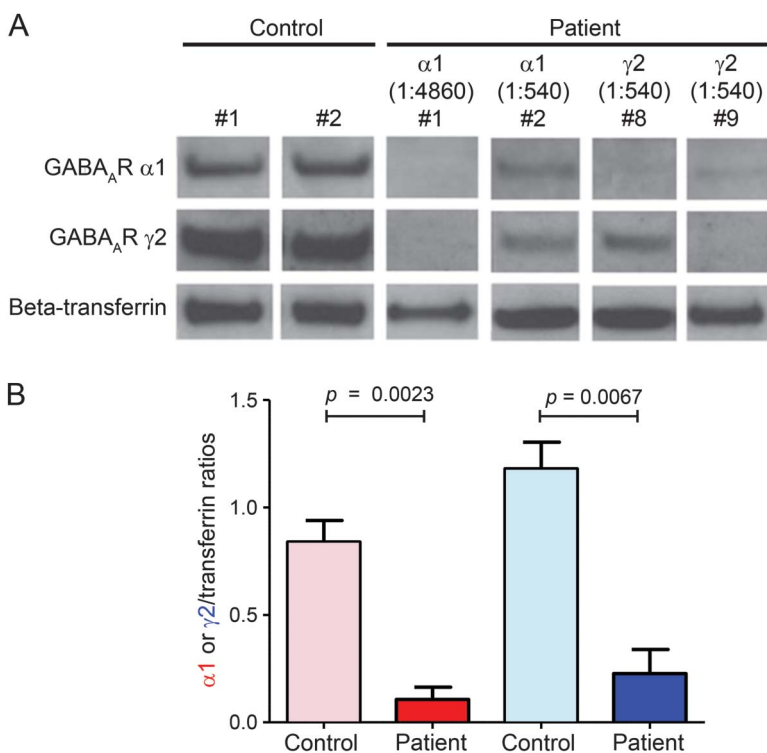
2 control sera ($p = 0.0023$ and $p = 0.0067$, respectively, figure 3, A and B).

Clinical features of patients with GABA_AR-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_AR-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_AR-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 neurodevelopment 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 on MRI, subsequently was diagnosed with Huntington disease. In 2 other patients there were nonspecific white 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).

Figure 3 Patient antibodies reduce GABA_A receptor surface expression in neuronal cultures



Primary cortical neuronal cultures (day 7 in vitro) were treated with serum (1:100) from γ-aminobutyric acid A receptor patients (n = 4) or healthy controls (n = 2) for 72 hours. (A) Western blot analysis showed that patient serum but not control decreased GABA_AR levels (α1 [red] and γ2 [blue]) on the neuronal surface. β-Transferrin was used as a loading control. (B) Densitometry analysis showed that this loss was significant for both α1 and γ2 subunits treated with patient sera relative to healthy control ($p = 0.0023$ and $p = 0.0067$, respectively).

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/ α 1	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 mo/IgG1 and IgM 1:540/ α 1	Behavioral changes, disturbed thought, incontinence, grimacing, posturing; BFCRS moderately severe catatonia; MRI, 2 EEGs normal, CSF nd	Catatonia of unknown etiology	PEX \times 2 with good responses; 20 mo
3	M/56/1 mo/IgG1 and IgM 1:180/ α 1	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 mo/IgG1 1:540/ α 1	HIV on cART, splenectomy for ITP, weight loss, impaired memory, verbal fluency, depression; GTCS; MRI, CSF, EEG no abnormalities	Neurodegenerative disorder?	Possible slow response to PEX and Aza; 18 mo
5	F/19/1 wk/IgG1 1:1,620/ α 1	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/ α 1	Weekly loss of consciousness for up to 1 h; MRI normal, CSF, EEG nd	Nonepileptic attacks	No IT; no FU
7	M/47/2 mo/IgG1 and IgM 1:540/ γ 2	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 mo/IgG1 and IgM 1:540/ γ 2	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 1:1,620/ γ 2	Amnesia only; MRI few scattered white matter lesions only; EEG, CSF nd	Mild cognitive impairment	No IT, some improvement; 8 mo
10	F/56/3 mo/IgM 1:180/ γ 2	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	No IT; Huntington disease confirmed 4 mo
11	F/47/18 mo/IgG1 1:540/ γ 2	Anterograde amnesia, spatial disorientation; GTCS; MRI and CSF nd, EEG generalized tonic pattern, intermittent L frontotemporal spike/sharp waves	Focal epilepsy or limbic encephalitis	Pred and IVIg but steady decline; 18 mo
12	M/68/2 mo/1:60/undefined	Epilepsy, prostatic cancer; amnesia, self-neglect, depression, sleepiness, GTCS with status epilepticus; MRI, R frontal and temporal lobe atrophy; CSF, EEG 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	Paranoid schizophrenia	No IT, clozapine only; improved at 18 mo
14	F/65/1 mo/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 mo/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 verbigeration; 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_AR-Abs was detected in his serum (α 1-subunit specific; 1:540, IgG1-isotype) and a possible autoimmune etiology was proposed. After consideration of the evidence implicating GABA_AR in catatonia,^{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).

Six months after PEX, the patient relapsed with bizarre and unpredictable behavior (e.g., sudden onset intense handwashing for a few days followed by new-onset praying) and GABA_AR-Abs were once again detected in his serum (1:180), but were undetectable in his CSF. There were subtle motor symptoms of catatonia and subtle frontal symptoms (BFCRS 5/FAB 11), and he received PEX again; symptoms of catatonia resolved within 2 weeks. He continued to have reduced verbal fluency, severe apathy, emotional and social withdrawal, blunted affect, and difficulty in abstract thinking (FAB 9/18). He received 5 days of methylprednisolone 1,000 mg IV and 5 days of immunoglobulins in January 2014 and was started on 1 mg/kg/OD prednisolone, which was weaned down to 10 mg/day in August 2014. His FAB returned to 17/18 in April 2014 (video 2). These symptoms improved slowly in the following months but did not disappear, perhaps due to the continued use of olanzapine and fluoxetine and an underlying diagnosis of mild Asperger syndrome made at age 11. His last GABA_AR-Abs levels in April 2014 were undetectable. At this time his FAB was 17/18. His symptoms of catatonia and frontal dysfunction twice improved, strongly linked to disappearance of GABA_AR-Abs with immunotherapy.

DISCUSSION We identified a new antibody target, GABA_AR, established a CBA using HEK cells expressing heteropentameric GABA_ARs, and identified a total of 45 patients with GABA_AR-Abs. The antibodies in 40% of patients were IgG1 or IgG3, bound to GABA_ARs containing α 1 or γ 2 subunits, and all 4 sera tested were able to reduce GABA_AR 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_AR-Abs are relatively common (up to 2% of referred sera compared with around 4% identified with NMDAR-Abs over the same time period), are potentially pathogenic, and associate with seizure and behavioral phenotypes. However, although the clinical features were variable and the paraclinical findings often normal, one boy with severe catatonia twice improved substantially following immunotherapy in parallel with normalization of his GABA_AR-Abs.

GABA_ARs are ionotropic cell surface receptors that predominantly mediate the fast-inhibitory neurotransmission in the brain, and are usually assembled as heteropentamers. On activation, influx of chloride ions results in hyperpolarization and stabilization of the neuronal membrane potential. The GABA_ARs are

the therapeutic target of many clinically important drugs, such as barbiturates, benzodiazepines, and topiramate, with anticonvulsant, anxiolytic, sedative, cognitive, and mood-altering properties (reviewed in reference 20). In 18 patients, we identified α 1 or γ 2 subunits as the main targets and showed that 4 of these were able to reduce GABA_AR complexes from the neuronal surface in vitro, most likely through antibody cross-linking and internalization, as described for NMDAR and AMPAR antibodies,^{2,16} supporting the idea that these antibodies are pathogenic.

This study was initially designed to identify the target for antibodies in a patient with VGKC complex antibody of 1,938 pM. Despite preadsorption of GABA_AR-Abs, the patient sera still bound to cultured neurons, indicating the presence of a second cell surface neuronal antibody. Thus VGKC complex antibodies that are negative for binding LGI1/CASPR2/Contactin-2 but bind cultured neurons require further study to identify their specific targets and to explore their pathogenicity.

The sera positive for GABA_AR-Abs had all been sent for other CNS antibody tests. Although many of the patients had seizures, or cognitive or neuropsychiatric problems, they were given a range of tentative diagnoses (table 2). In most there was little to suggest a classical immune-mediated disease such as limbic encephalitis or NMDAR-Abs encephalitis, 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_AR-Abs, binding the α 1 or β 3 subunits, were identified recently in 6 patients with refractory status epilepticus or epilepsy partialis continua and a change in cognition/behavior with extensive imaging abnormalities¹² and in another 12 with a variety of phenotypes and lower titers. The authors did not report γ 2 subunit specificity or examine the immunoglobulin classes and subclasses. IgG GABA_A β 3 antibodies were also recently reported in 2 patients with thymoma-associated encephalopathies.²¹ Both IgM and IgA NMDAR-Abs have previously been reported to be pathogenic in vitro, but their clinical relevance is not clear^{22,23}; however, the serum GABA_AR-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 intrathecally. However, these possibilities clearly need further study.

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_AR-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 Adriaan Coebergh: drafting/revising the manuscript, analysis or interpretation of data, accepts responsibility for conduct of research and final approval, acquisition of data. Rosemary Pettingill: 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. Anjan Nibber: analysis or interpretation of data, accepts responsibility for conduct of research and final approval, acquisition of data. Andrea Malaspina: drafting/revising the manuscript, analysis or interpretation of data, accepts responsibility for conduct of research and final approval. Anu Jacob: drafting/revising the manuscript, accepts responsibility for conduct of research and final approval, acquisition of data. Sarosh R. Irani: drafting/revising the manuscript, study concept or design, accepts responsibility for conduct of research and final approval, contribution of vital reagents/tools/patients, study supervision, obtaining funding. David Beeson: study concept or design, accepts responsibility for conduct of research and final approval, contribution of vital reagents/tools/patients, study supervision, obtaining funding. Bethan Lang: 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. Patrick Waters: 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. Angela Vincent: 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, study supervision, obtaining funding.

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DISCLOSURE

P. Pettingill, H. Kramer, J. Coebergh, R. Pettingill, 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. Beeson 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 coinventors and have received royalties. A patent for the detection of GABA_Aγ2 receptor antibodies has been filed. Go to Neurology.org for full disclosures.

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This Week’s *Neurology*[®] Podcast



Antibodies to GABA_A receptor α 1 and γ 2 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_A receptor α 1 and γ 2 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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