ABSTRACT

Background: γ-Aminobutyric acid-B receptor antibodies (GABA\(_B\)-R-ab) were recently described in 15 patients with limbic encephalitis (LE), associated with small-cell lung cancer (SCLC) or with concurrent glutamic acid decarboxylase (GAD) antibodies. We analyzed the frequency of GABA\(_B\)-R-ab in 147 patients with LE or neurologic syndromes associated with GAD-ab.

Methods: We examined the presence of GABA\(_B\)-R-ab in 70 patients with LE (33 paraneoplastic with onconeural antibodies, 18 paraneoplastic without onconeural antibodies [5 with Gad-ab], and 19 idiopathic with either GAD-ab [5 patients] or seronegative) and 77 patients with GAD-ab-associated neurologic syndromes other than LE (29 stiff-person syndrome, 28 cerebellar ataxia, 14 epilepsy, and 6 with diverse paraneoplastic neurologic syndromes). GABA\(_B\)-R-ab were analyzed in serum or CSF by indirect immunofluorescence on HEK293 cells transfected with GABA\(_{B_1}\) and GABA\(_{B_2}\) receptor subunits.

Results: GABA\(_B\)-R-ab were detected in 10 of the 70 patients with LE (14%). Eight had SCLC and 2 were idiopathic. One of the 8 patients with LE with SCLC had an additional onconeural antibody (Hu) and 2 GAD-ab. GABA\(_B\)-R-ab were identified in 7 (70%) of the 10 patients with LE and SCLC without onconeural antibodies. GABA\(_B\)-R-ab antibodies were not found in patients with GAD-ab and stiff-person syndrome, idiopathic cerebellar ataxia, or epilepsy. However, one patient with GAD-ab, paraneoplastic cerebellar ataxia, and anaplastic carcinoid of the thymus also presented GABA\(_B\)-R-ab.

Conclusions: GABA\(_B\)-R-ab are the most common antibodies found in LE associated with SCLC previously considered “seronegative.” In patients with GAD-ab, the frequency of GABA\(_B\)-R-ab is low and only observed in the context of cancer. Neurology® 2011;76:795–800
antibodies are probably pathogenic, and, unlike onconeural antibodies, the presence of these antibodies does not necessarily indicate that the patient has an underlying tumor.1

Antibodies to GABA\textsubscript{B} receptor (GABA\textsubscript{B}R-ab) were recently identified in 15 patients with idiopathic or paraneoplastic LE.4 Seizures were the presenting clinical symptom in 13 patients. Seven of the 15 patients had lung tumors and 5 of these were small-cell lung cancer (SCLC). Three patients also had glutamic acid decarboxylase antibodies (GAD-ab). Ten patients received treatment for the LE and 9 showed neurologic improvement.4

We previously described that the concurrent detection of onconeural antibodies, particularly against amphiphysin, and antibodies against neuronal surface proteins was not unusual in patients with paraneoplastic LE and lung cancer.6 The aim of the present study was to analyze the presence of GABA\textsubscript{B}R-ab in patients with paraneoplastic (with and without onconeural antibodies) and idiopathic LE, and neurologic syndromes associated with GAD-ab.

**METHODS**

**Patients.** We reviewed 147 patients with final diagnosis of LE, or with other neurologic syndromes associated with GAD-ab whose serum or CSF was sent to our laboratory (Barcelona, Spain) for analysis of antineuronal antibodies. LE was defined by the subacute onset of short-term memory loss, behavior change, seizures, and involvement of the temporal lobes by EEG, imaging studies, or postmortem examination. LE was considered definite paraneoplastic if a tumor was diagnosed or the serum presented well-characterized onconeural antibodies (Hu, Yo, Ri, CV2, Ma2, amphiphysin).7 The diagnosis of definite idiopathic LE required the absence of cancer and well-characterized onconeural antibodies, and a follow-up of at least 3 years. Patients with LE with a shorter follow-up were classified as possible idiopathic LE. Patients with GAD-ab were classified, as previously reported, in one of the following groups: stiff-person syndrome (SPS), cerebellar ataxia, isolated epilepsy, and paraneoplastic neurologic syndromes.8 The information was obtained from forms filled out by the referring neurologists, telephone interviews, and review of the clinical records.

**Immunologic studies.** Onconeural antibodies, SOX1-ab, and GAD-ab were screened by immunohistochemistry performed on frozen sections of paraformaldehyde-perfused rat cerebellum using an avidin-biotin immunoperoxidase technique and confirmed by immunoblot when indicated.1 GAD-ab were confirmed by radioimmunoassay.8 Neuropil antibodies were screened by immunohistochemistry on frozen sections of rat brain postfixed with 4% paraformaldehyde.2 The presence of AMPA glutamate receptor antibodies was confirmed by immunofluorescence on HEK-293 cells transfected with plasmids containing the appropriate antigens,3 and voltage-gated potassium channel (VGKC) antibodies were confirmed by radioimmunoassay.

GABA\textsubscript{B}R-ab were screened on HEK293 cells transfected with plasmids containing rodent GABA\textsubscript{B}R\textsubscript{1} and GABA\textsubscript{B}R\textsubscript{2} in equimolar ratios.4 Positive samples were also analyzed by immunocytochemistry of rat hippocampal neuronal cultures. Both techniques have previously been described.4 Briefly, HEK293 transfected cells were incubated with the patients’ serum (dilution 1:20) or CSF (1:2) for 1 hour at 37°C, washed, fixed with 4% paraformaldehyde, permeabilized with triton X, incubated with a rabbit polyclonal GABA\textsubscript{B}R\textsubscript{1} antibody (1:1,000) (Santa Cruz Biotechnology, sc-14006; Santa Cruz, CA) followed by the appropriate Alexa Fluor secondary antibodies (Molecular Probes, Eugene, OR). For immunocytochemistry of rat hippocampal neuronal cultures, live neurons grown on coverslips were incubated with the patients’ serum (1:100) or CSF (1:2) for 1 hour at 37°C, washed, fixed with 4% paraformaldehyde, and immuno-reacted with antihuman immunoglobulin G Alexa Fluor second-

![Figure 1](https://example.com/figure1.png)

**Figure 1** Detection of γ-aminobutyric acid-B receptor antibodies (GABA\textsubscript{B}R-ab) using a HEK293 cell-based assay

HEK293 cells were transfected to express GABA\textsubscript{B}R\textsubscript{1,2} receptor and incubated live, not permeabilized, with a patient’s CSF. Afterwards, cells were fixed, permeabilized, and incubated with a polyclonal antibody against an intracellular epitope of the B1 subunit of the GABA\textsubscript{B} receptor. Note that patient’s CSF stains the cell surface of cells that specifically express GABA\textsubscript{B} receptors (A), as demonstrated by the intracellular reporter antibody (B). Both reactivities are shown merged in C. Nuclei counterstained with DAPI. Scale bar = 20 μm.
ary antibody. Results were photographed under a fluorescence microscope using Zeiss Axiovision software (Zeiss, Thornwood, NY). To confirm the specificity of the neuronal reactivity, all positive samples were preabsorbed with the non-neuronal cell line HEK293 to remove antibodies that could react with non neuronal specific surface antigens.

RESULTS
Eleven of the 147 patients tested positive for GABABR-ab on the screening of HEK293 cells transfected with the B1 and B2 subunits of the GABA_B receptor (figure 1). All positive samples immunoreacted in vivo with primary cultures of hippocampal neurons (figure 2). GABABR-ab were positive in both serum and CSF in the 5 patients from whom paired samples were available. Median titer of GABABR-ab was 1/120 (range 40–2,000) in serum and 1/60 (range 20–640) in the CSF.

We found GABABR-ab in 10 patients with LE. Positive GABABR-ab were identified more frequently in the group of paraneoplastic LE without onconeural antibodies (previously considered “sero-negative”) (table 1). Seven (39%) of the 18 patients were GABABR-ab-positive and all had SCLC. In total, positive GABABR-ab were identified in 7 (70%) of the 10 patients with LE and SCLC without onconeural antibodies. The other 3 patients were positive for AMPAR-ab. We previously demonstrated that 9 (39%) of 23 patients with paraneoplastic LE had concomitant onconeural or other antibodies against intraneuronal antigens, and antibodies against unidentified neuronal surface antigens.6 In this study, we analyzed 33 patients with LE and onconeural antibodies, and only one, with Hu-ab and SCLC, tested positive for GABABR-ab. However, 4 of the 7 patients with LE with GABABR-ab without onconeural antibodies presented antibodies against intracellular antigens (table 2). Two patients had GAD-ab (one also SOX1-ab), one Hu-ab, and, in 2 cases previously reported, one had brain serine/threonine kinase (BRSK)2-ab and the other SOX1 and VGKC-ab.9,10

No tumor was identified in the remaining 2 GABABR-ab-positive patients but the follow-up is too short to classify them as definite idiopathic LE. In the group of 10 patients with LE and GAD-ab, the coincidence of GABABR-ab and GAD-ab occurred in 2 patients with SCLC whereas the other 3 patients with paraneoplastic LE associated with other tumors (thymoma 2, lymphoma) were GABABR-ab-negative. None of the 5 patients with idiopathic LE and GAD-ab were positive for GABABR-ab (table 1).

A summary of the clinical features of the GABABR-ab-positive patients is presented in table 2. Nine of the 10 patients with GABABR-ab and LE were men. Median age was 60 years (range 47–70 years). Seizures were the predominant and presenting symptom in 8 patients and 2 required admission to the intensive care unit for control of the seizures. All patients also presented confusion, disorientation, memory loss, or behavior changes consistent with encephalitis predominantly involving the limbic system. The CSF disclosed mild lymphocyte pleocytosis in 4 patients. Brain MRI showed increased fluid-attenuated inversion recovery signal in one or both hippocampus and amygdala in 7 patients. In 4 of them the initial brain MRI was reported normal.

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>No. of patients</th>
<th>GABABR-ab positive (%)</th>
<th>Comments on positive cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paraneoplastic LE</td>
<td>51</td>
<td>8 (16)</td>
<td></td>
</tr>
<tr>
<td>With onconeural ab</td>
<td>33</td>
<td>1 (3)</td>
<td>Hu-ab with SCLC</td>
</tr>
<tr>
<td>Without onconeural ab</td>
<td>18</td>
<td>7 (39)</td>
<td>All SCLC, GAD-ab: 2</td>
</tr>
<tr>
<td>Idiopathic LE</td>
<td>19</td>
<td>2 (14)</td>
<td>Short follow-up</td>
</tr>
<tr>
<td>GAD-ab-positive, non-LE</td>
<td>77</td>
<td>1 (1)</td>
<td></td>
</tr>
<tr>
<td>Stiff-person syndrome</td>
<td>29</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Cerebellar ataxia</td>
<td>28</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Epilepsy</td>
<td>14</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Paraneoplastic</td>
<td>6</td>
<td>1 (17)</td>
<td>Cerebellar ataxia</td>
</tr>
</tbody>
</table>

Abbreviations: GABABR-ab = γ-aminobutyric acid-B receptor antibodies; LE = limbic encephalitis; SCLC = small-cell lung carcinoma.

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Only one patient had hyponatremia. Seven patients were treated with steroids, IV immunoglobulins, or combination of both drugs. Three of the 8 patients with SCLC were also treated with chemotherapy. Only 2 patients made a complete recovery (one without cancer) and none of them had concurrent antineuronal antibodies. Partial responses to the indicated treatments were achieved in 4 with a relapse in one of them.

GABA<sub>AB</sub>-ab were not detected in 71 patients with GAD-ab and nonparaneoplastic SPS, cerebellar ataxia, or epilepsy. In contrast, one of the 6 patients with paraneoplastic neurologic syndromes and GAD-ab was GABA<sub>AB</sub>-ab positive (table 1). She was a 57-year-old woman with a known anaplastic carcinoid of the thymus and bone metastases. She developed nausea, vomiting, gait instability, and diplopia. Neurologic examination disclosed a normal mental status, bilateral horizontal nystagmus, and cerebellar gait ataxia. The patient was treated with oral steroids and the symptoms slowly resolved over the ensuing 3 months.

The other 5 GAD-ab-positive patients without GABA<sub>AB</sub>-ab presented with paraneoplastic encephalomyelitis associated with pancreatic cancer<sup>11</sup> and cerebellar ataxia with breast cancer (2 patients), non-SCLC, and neuroendocrine thymic carcinoma.<sup>12</sup> To see if GABA<sub>AB</sub>-ab associate with other cases of paraneoplastic cerebellar degeneration (PCD), we analyzed the serum or CSF of a series of 45 patients with PCD and lung cancer (35 with SCLC). The majority (73%) were included in a previous study.<sup>13</sup> These patients had Hu-ab (15%) or voltage-gated calcium channel antibodies (50%). However, all were negative for GABA<sub>AB</sub>-ab.

**DISCUSSION** The current study expands our knowledge of the profile of symptoms and immunologic associations of GABA<sub>AB</sub>-ab. We found that GABA<sub>AB</sub>-ab are the most common antibodies identified in patients with SCLC and LE previously considered “seronegative.” Although the occurrence of GABA<sub>AB</sub>-ab and GAD-ab was observed in an initial series of 15 patients,<sup>4</sup> when we tested a larger series of patients with several...
types of neurologic syndromes associated with GAD-ab, GABA<sub>B</sub>-R-ab were only identified in those who had a paraneoplastic syndrome. At the time of the initial description of Hu-ab as markers of neurologic syndromes associated with SCLC, we observed that up to 50% of patients with LE were “seronegative.” The syndrome of these patients was highly restricted to the limbic system and seemed to improve more often after treatment of the cancer than that of patients with Hu-ab. In the current study, 7 of 10 (70%) patients with LE, SCLC without onconeural antibodies had GABA<sub>B</sub>-R-ab. The other 3 patients were positive for AMPAR-ab. Taken together, all patients with LE and GABA<sub>B</sub>-R-ab is not substantially different from that seen in other autoimmune LE. The high frequency of seizures was noted in the previous study and could be explained by the role of GABA<sub>B</sub> receptors in the development of seizures. Since almost 50% of patients with all types of LE and GABA<sub>B</sub>-R-ab except in the 2 patients with SCLC. Our 5 patients with idiopathic LE and GABA<sub>B</sub>-R-ab were women with a median age of 29 years and 4 presented with seizures. This profile is similar to that recently reported in a series of 9 patients. One of the 2 patients with concurrent GABA<sub>B</sub>-R-ab and GAD-ab had a paraneoplastic neurologic disorder. In the initial series, 2 of the 8 patients with idiopathic LE encephalitis had additional GAD-ab. We did not find GABA<sub>B</sub>-R-ab in patients with LE or isolated epilepsy with GAD-ab except in the 2 patients with SCLC. Our 5 patients with idiopathic LE and GABA<sub>B</sub>-R-ab were women with a median age of 29 years and 4 presented with seizures. This profile is similar to that recently reported in a series of 9 patients. One of the 2 patients with concurrent GABA<sub>B</sub>-R-ab and GAD-ab and idiopathic LE previously reported also had this phenotype. To determine how often both antibodies coincide in patients with idiopathic LE, we suggest routinely looking for GABA<sub>B</sub>-R-ab in all patients with LE suspected to be related to GAD-ab.

The only patient with GAD-ab and GABA<sub>B</sub>-R-ab without LE had a reversible cerebellar ataxia associated with carcinoid of the thymus. GABA<sub>B</sub>-R-ab titers were similar to those of patients with LE. GABA<sub>B</sub> receptors are widely expressed in the brain with the highest levels in the hippocampus, thalamus, and cerebellum. Therefore it is plausible that some patients with GABA<sub>B</sub>-R-ab may develop cerebellar rather than hippocampal dysfunction. Low titers of GABA<sub>B</sub>-R-ab have previously been reported in a patient with nonparaneoplastic cerebellar ataxia and GAD-ab. However, we did not find any case in our series of patients with nonparaneoplastic cerebellar ataxia with GAD-ab or with paraneoplastic cerebellar degeneration and lung cancer, suggesting that GABA<sub>B</sub>-R autoimmunity rarely causes cerebellar dysfunction.

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DISCLOSURE

Dr. Boronat, Dr. Sahater, and Dr. Saiz report no disclosures. Dr. Dalmau has received royalties from a patent re: Ma2 autoantibody test and has patents pending re: NMDA and GABA<sub>B</sub> receptor autoantibody tests (license fee payments received from EUROIMMUN AG); and receives research support from the European Union’s Horizon 2020 research and innovation program under grant agreement No 776371.”
REFERENCES