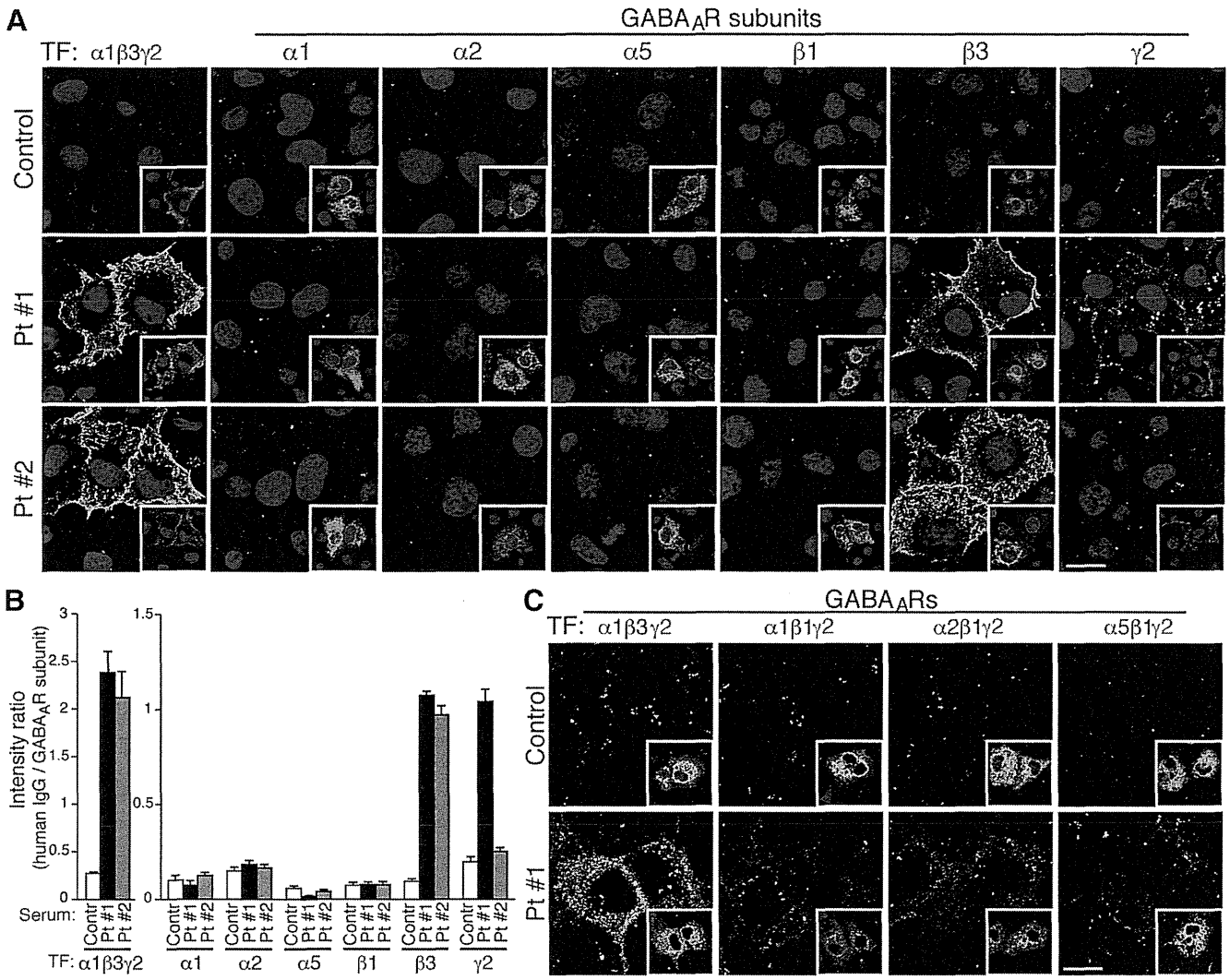


**Figure 2.** Patients' GABA$_A$ receptor antibodies are directed to extracellular epitope of β3 subunit. ***A***, ***B***, COS7 cells were transfected (TF) to surface express the indicated GABA$_A$ receptor subunits. Transfected cells were fixed and doubly stained with the patient sera (Patient 1 or Patient 2; red, human IgG) together with the antibodies specific to the individual expressed subunits (green, insets). Nuclear DNA was stained by Hoechst 33342 (blue) to distinguish untransfected cells. To clearly show the weak binding of the serum from Patient 1 to the γ2 subunit, the detector gain of the red channel is enhanced upon image acquisition (right, middle). The ratio of the human IgG intensity to the GABA$_A$ receptor subunit intensity was graphed (***B***). Error bars indicate SEM; $n = 10$ transfected cells. ***C***, COS7 cells were transfected to surface express the indicated heteromeric GABA$_A$ receptors and tested for binding of serum antibodies (red). Transfected cells were detected by staining with the individual α subunit (green) and γ2 subunit (blue) antibodies. Merged images are shown in insets. Scale bars, 20 μm.

ceptor (α1/β3/γ2; a representative is shown in Fig. 2*A*), although this screening might have missed some serum samples that contained antibodies to other GABA$_A$ receptor subunits than α1/β3/γ2. Because neither serum antibody from the two patients bound to COS7 cells expressing the α subunit alone, one may wonder whether the α subunit might not have been efficiently expressed at the cell surface without other subunits. To further examine the possible involvement of α subunit antibodies in the patient serum, COS7 cells were transfected with various combinations of three subunit genes of the GABA$_A$ receptor, α1/β3/γ2, α1/β1/γ2, α2/β1/γ2, or α5/β1/γ2 (Fig. 2*C*). There were no apparent differences in the weak binding of serum antibodies from Patient 1 to three different GABA$_A$ receptors, α1/β1/γ2, α2/β1/γ2, and α5/β1/γ2, indicating that the binding of serum antibodies was attributed to the γ2 subunit, but not to the α1, α2, or α5 subunits. Serum from Patient 2 did not show any apparent binding to α1/β1/γ2, α2/β1/γ2, or α5/β1/γ2 (data not shown). Together, these results indicate that the two patients with immune-mediated encephalitis had autoantibodies directed against the GABA$_A$ receptor and that the extracellular part of the β3 subunit was the antigenic epitope recognized by the patients' GABA$_A$ receptor antibodies. One of the two patients also had a low level of γ2 autoantibodies (Patient 1), but neither patient had any autoantibodies to the α1, α2, α5, or β1 subunit.

### GABA$_A$ receptor containing β3 subunit is the main target of the patient serum antibodies

We next investigated whether the GABA$_A$ receptor is the main target of the patient serum antibodies in neurons. We took advantage of knock-down approach in cultured rat hippocampal neurons. MicroRNAs (miRNA-β3-211 and miRNA-β3-347) for the GABA$_A$ receptor β3 subunit were first validated by the reduced expression of exogenously expressed rat GABA$_A$ receptor β3 in HEK293T cells (Fig. 3*A*). Then, by cell surface staining with anti-β2/β3 antibody, we quantified the knock-down effect on the β3 subunit expression in neurons. When miRNA-β3-211 for the GABA$_A$ receptor β3 subunit was expressed in neurons, the number of β3 subunit clusters in soma and dendrites that were stained

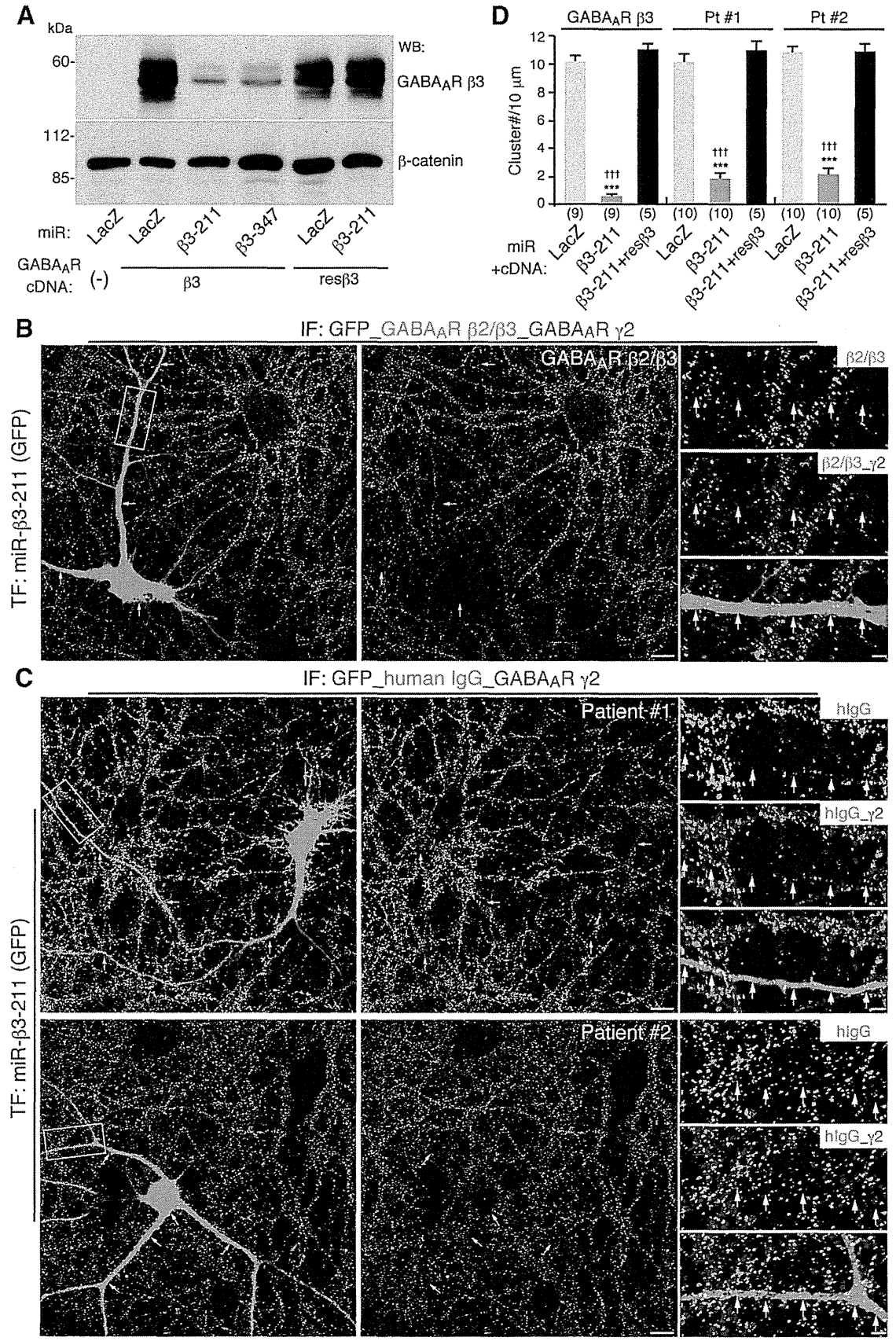


**Figure 3.** GABA$_A$-receptor-containing β3 subunit is a major target of the patients' antibodies in hippocampal neurons. ***A***, Validation of miRNA constructs for the GABA$_A$ receptor β3 subunit. HEK293T cells were cotransfected with the indicated knock-down (miR) and β3 expression vectors. Three days after the transfection, the cell lysates were analyzed by Western blotting with GABA$_A$ receptor β3 and β-catenin antibodies. miR-LacZ, Control miRNA targeting to LacZ; resβ3, miR-211-resistant β3. ***B***, Effective knock down of the endogenous β3 subunit. Cultured rat hippocampal neurons were transfected with the miR-β3 expression vector at 10 DIV. Cell surface GABA$_A$ receptor β2/β3 (red) and γ2 (blue) subunits were stained at 15 DIV. (*Figure legend continues.*)

by anti-β2/β3 antibody was robustly reduced, showing that β3 subunit expression was decreased to 5.47 ± 3.60% (Fig. 3*B,D*). This is consistent with the previous report showing that β2 expression is very low in hippocampal neurons (Pirker et al., 2000). This reduction was not due to off-target effects of miRNA expression because it was completely rescued by coexpression of the knock-down-resistant β3 construct (resβ3) with miRNA-β3. We noted that γ2 subunit clusters were also decreased in neurons in which the β3 subunit was knocked down, confirming an essential role of the β3 subunit in the $GABA_A$ receptor function in hippocampal neurons (DeLorey et al., 1998). Under these conditions, the overall immunoreactivity of the sera from Patient 1 and Patient 2 to the neurons was greatly reduced by the expression of miRNA-β3-211 and rescued by coexpression of the knock-down-resistant β3 construct. Importantly, the residual immunoreactivity upon β3 knock down was 18.2 ± 10.8% for Patient 1 and 19.8 ± 11.7% for Patient 2 (Fig. 3*C,D*). These results indicate that the binding of the patients' antibodies to the neuronal surface was mostly (~80%) attributed to the $GABA_A$ receptor containing the β3 subunit and that the patients had other autoantibodies in addition to $GABA_A$ receptor antibodies.

### Coexisting antibodies with $GABA_A$ receptor antibodies in the patient serum

We therefore performed the cell-based binding assay (Fig. 4*A*) and the cell-based ELISA test, which quantifies the frequent serum antibodies against LGI1, CASPR2, DCC (Ohkawa et al., 2013), and $GABA_A$ receptor β3 (Fig. 4*B*). We found that the serum samples of Patient 1 and Patient 2, but no other tested serum samples, bound to the $GABA_A$ receptor β3 (Fig. 4*A*) and showed similar positive values for $GABA_A$ receptor antibodies (ELISA absorbance = 0.57 for Patient 1; 0.52 for Patient 2; Fig. 4*B*). We also found that Patient 1 had low levels of LGI1 antibodies (absorbance = 0.37) and DCC antibodies (absorbance = 0.26) in addition to $GABA_A$ receptor antibodies, but not CASPR2 antibodies. In contrast, serum from Patient 2 contained CASPR2 antibodies (absorbance = 0.51) and a low level of DCC antibodies (absorbance = 0.21) in addition to $GABA_A$ receptor antibodies, but not LGI1 antibodies. However, the low level of LGI1 antibodies of Patient 1 is unlikely to cause the patient's CNS symptoms, because the value for LGI1 antibodies of Patient 1 was much lower than the cutoff value (absorbance = 0.8) determined for diagnosis of limbic encephalitis (Ohkawa et al., 2013; see Materials and Methods). In fact, in the present study population, patients with limbic encephalitis and monospecific LGI1 antibodies had much higher levels of LGI1 antibodies (average of ELISA absorbance = 1.41 ± 0.36, $n$ = 34 patients; Patient A as a representative) than patients with neuromyotonia (no CNS symptoms) and LGI1 autoantibodies (0.65 ± 0.16, $n$ = 10 patients; Patient C as a representative) (Fig. 4). CASPR2 and DCC antibodies are also unlikely to be causes of the patient's CNS symptoms because CASPR2 and DCC autoantibodies are specifically associated with PNS symptoms of neuromyotonia, but are not associated with CNS symptoms observed in encephalitis (Ohkawa et al., 2013). Together, these quantitative analyses (Figs. 3, 4) strongly suggest that the $GABA_A$ receptor containing the β3 subunit is a primary target of the patients' serum antibodies and is the main contributor to the patients' symptoms.

### Patients' $GABA_A$ receptor antibodies reduce the number of both synaptic and surface $GABA_A$ receptor clusters

Next, we explored a mode of action of patients' $GABA_A$ receptor antibodies. Previous studies showed that autoantibodies against NMDA and AMPA receptors induce the internalization of the corresponding receptors and reduce the number of synaptic receptors (Lai et al., 2009; Hughes et al., 2010). These previous findings inspired us to investigate whether patients' $GABA_A$ receptor antibodies reduce the number of synaptic $GABA_A$ receptors. When hippocampal neurons were treated with the serum from Patient 1 and Patient 2 for 2 d, the number of synaptic $GABA_A$ receptor clusters, represented by γ2 or β3 subunit clusters adjacent to both gephyrin and vGAT, was significantly reduced (Fig. 5*A*). The effect was specifically attributed to the patients' $GABA_A$ receptor antibodies because treatment of neurons with a control serum without detectable autoantibodies or with the serum from the patient (Patient C) with invasive thymoma and neuromyotonia, who had LGI1 and CASPR2 antibodies but not $GABA_A$ receptor antibodies (Fig. 4), did not affect the synaptic $GABA_A$ receptor clusters. The number of surface γ2 subunit clusters, including both synaptic and extrasynaptic $GABA_A$ receptors, was also heavily reduced by treatment with the serum from Patient 1 and Patient 2. The effect of the patients' serum on $GABA_A$ receptor clusters was not complement mediated because the heat-inactivated patient serum reduced the number of both synaptic and surface $GABA_A$ receptor to a similar extent to the non-heat-inactivated patient serum; therefore, we pooled these data. Under these conditions, the number of gephyrin clusters apposed to vGAT was not altered (Fig. 5*A*). The effect of the patients' sera on $GABA_A$ receptor clusters was selective because the same treatment did not affect synaptic or surface AMPA receptor subunit GluA1.

This cell biological results were confirmed by the biochemical experiment: hippocampal neurons were treated with the patient or control serum for 3 d and then the surface-expressed proteins were labeled with biotin and purified by the avidin-conjugated beads (Fig. 5*B*). In the patient serum-treated neurons, the amount of cell surface $GABA_A$ receptor β3 subunits was significantly reduced and the total amount of the β3 subunit tended to be reduced (but not significantly). This effect was specific to the $GABA_A$ receptor because the amount of the surface GluA1 and N-cadherin was not affected. Together, these results indicate that $GABA_A$ receptor autoantibodies cause a selective decrease in $GABA_A$ receptor surface density and synaptic localization, probably by enhancing the receptor internalization.

To determine the relationship between $GABA_A$ receptor antibodies and patient' symptoms, we compared serum samples of Patient 1 at two different time points, from the episode of invasive thymoma and myasthenia gravis (without encephalitis) and from the episode of encephalitis. The sample of Patient 1 before the episode of encephalitis had acetylcholine receptor (AchR) antibodies, but no detectable $GABA_A$ receptor antibodies (Fig. 5*C*, Table 1) and showed no effects on synaptic $GABA_A$ receptor density (Fig. 5*D*). In contrast, the sample of the same patient at the time of symptom presentation of encephalitis had elevated $GABA_A$ receptor antibodies instead of AchR antibodies and decreased synaptic $GABA_A$ receptor density (Fig. 5*C,D*). Therefore, the clinical course of Patient 1

←

(*Figure legend continued.*) MicroRNA-transfected neurons were reported by the GFP expression (green). ***C***, Binding of serum antibodies (Patient 1 and Patient 2; red) was examined in neurons in which the β3 subunit was knocked down (green). Magnified view of the region indicated by a white square (***B***, ***C***). Arrows indicate the soma and dendrites of the neuron in which β3 was knocked down (***B***, ***C***). Scale bars, 10 μm (2 μm, magnified). ***D***, Neurons were cotransfected with the indicated miR and the knock-down-resistant construct (resβ3) or GST (for mock). The number of clusters labeled by β3 antibody or human IgG of patients' serum (Patient 1 and Patient 2) was counted and graphed. One-way ANOVA with Scheffe's *post hoc* analysis, $^{***}p < 0.001$ compared with miR-LacZ; $^{\dagger\dagger\dagger}p < 0.001$ compared with miR-β3–211 + resβ3. Error bars indicate SEM. The number of neurons examined is indicated in parentheses.

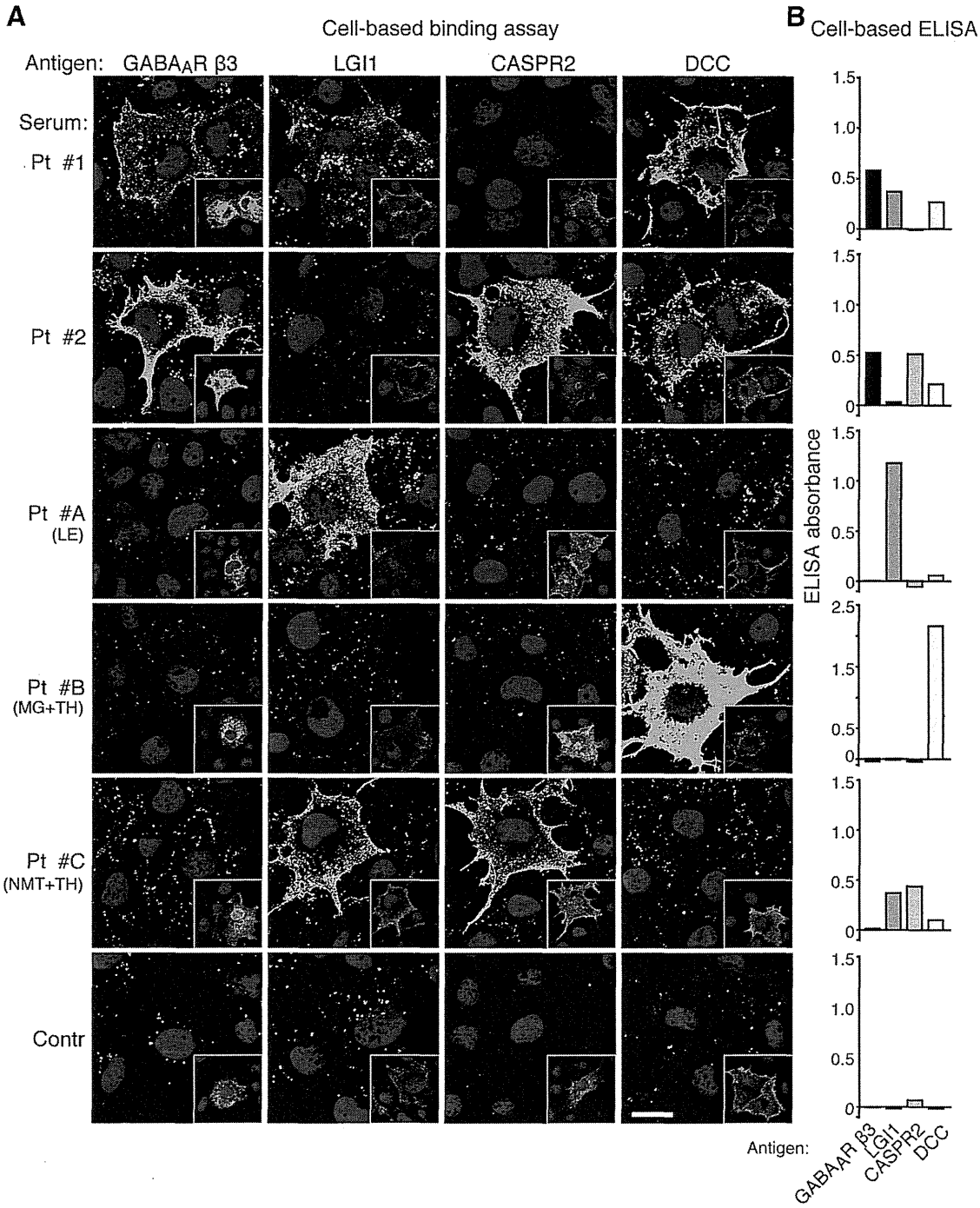


**Figure 4.** Identification of coexisting antibodies with GABA$_A$ receptor antibodies in the patient serum. ***A***, ***B***, Patient 1 and Patient 2 sera from their initial episodes of encephalitis were tested by cell-based binding assay (***A***) and cell-based ELISA tests (***B***) against the GABA$_A$ receptor β3 subunit, LGI1, CASPR2, and DCC. Additional sera were tested: from Patient A with limbic encephalitis (LE), Patient B with myasthenia gravis (MG) and thymoma (TH), and Patient C with neuromyotonia (NMT) and thymoma. Contr, Serum sample from a control patient with a neurodegenerative disease. Scale bar, 20 μm in ***A***. Average values from triplicate measurements of the individual serum are shown in ***B***.

correlates with the levels and effects of the patient's GABA$_A$ receptor antibodies. Although LGI1 antibodies were also detected only at the time of symptom presentation of encephalitis, the low level of LGI1 antibodies is unlikely to cause the patient's CNS symptoms, as described for Figure 4 (also see Discussion).

### Patients' GABA$_A$ receptor antibodies selectively reduce mIPSC amplitude and frequency

Finally, we assessed the effects of two patient sera (Patient 1 and Patient 2) on inhibitory synaptic transmission by whole-cell patch-clamp recording of mIPSCs in rat hippocampal neurons. We found a significant decrease in the mean amplitude of mIPSCs in patients' serum-treated neurons compared with that of control serum-treated neurons (Fig. 6*A*,*B*). This result is consistent with our immunocytochemical data (Fig. 5*A*) showing that the treatment of neurons with patients' serum reduced the number of synaptic clusters of GABA$_A$ receptors. The frequency of mIPSCs was also decreased in patients' serum-treated neurons (Fig. 6*A*,*B*), probably due to the increase in small-amplitude mIPSCs that fell below the threshold of de-

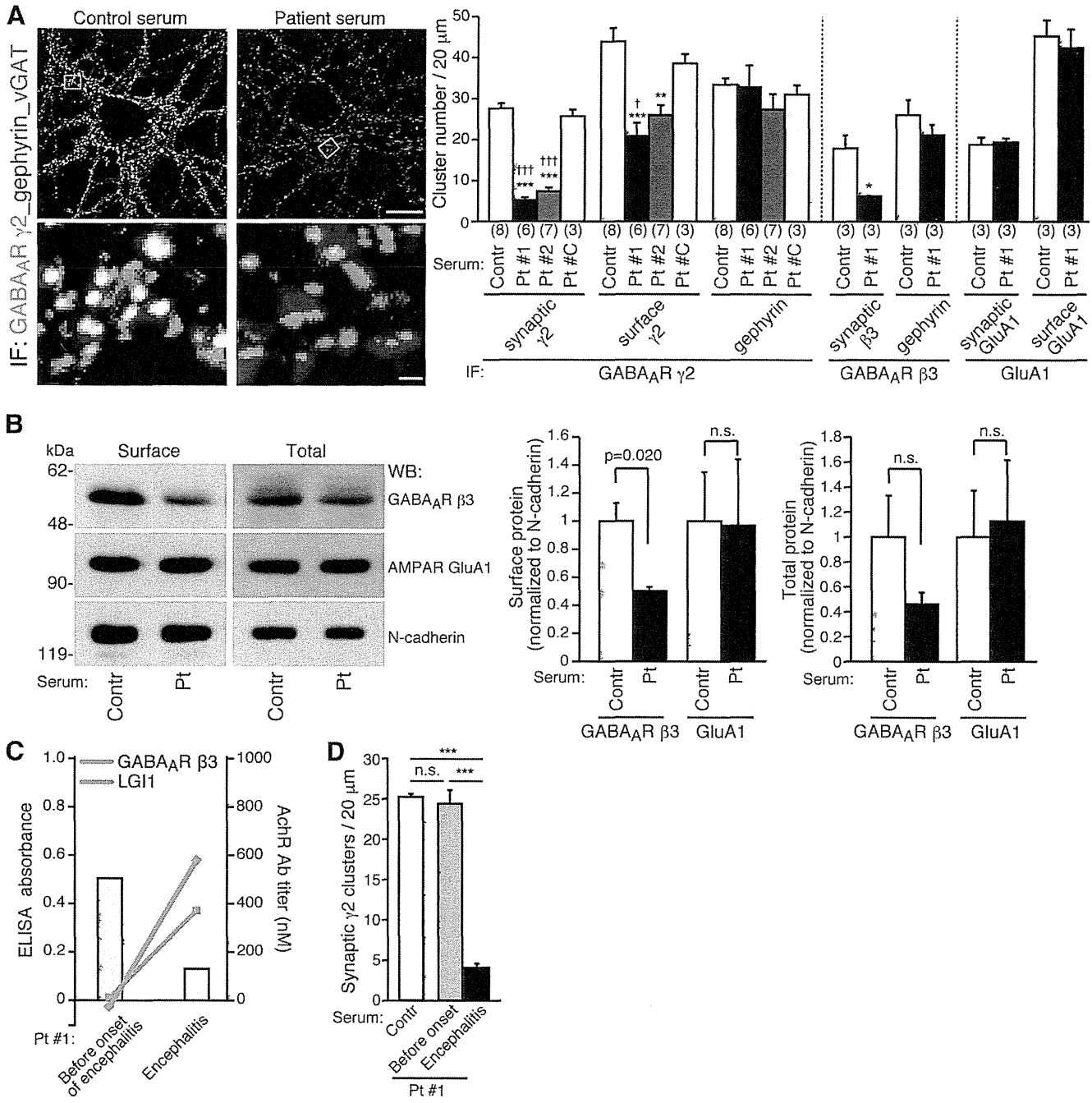


**Figure 5.** Patients' GABA$_A$ receptor antibodies specifically reduce synaptic and cell surface GABA$_A$ receptor density. ***A***, Cultured rat hippocampal neurons were incubated with serum from Patient 1 and Patient 2, serum from Patient C with thymoma that contained both LGI1 and CASPR2 antibodies (Fig. 4), or a control serum for 2 d. Representative images of surface GABA$_A$ receptor clusters in neurons treated with the control or the serum from Patient 1 are shown (left). Bottom, Magnified view of the region indicated by a white square. Synaptic GABA$_A$ receptors, which were γ2 (red) or β3 (data not shown) subunit-positive clusters adjacent to both gephyrin (green) and vGAT (blue), were counted. Surface-expressed GABA$_A$ receptor clusters labeled by the γ2 subunit antibody and gephyrin clusters were also independently counted. In addition, synaptic GluA1 clusters adjacent to both PSD-95 and vGluT1 and surface-expressed GluA1 clusters were counted. Scale bars, 20 μm (1 μm, magnified). Statistical analyses were performed by one-way ANOVA with Scheffe's *post hoc* analysis (γ2 clusters); or by Student's *t* test (β3 and GluA1 clusters). $*p < 0.05$; $**p < 0.01$; $***p < 0.001$ compared with control; $\dagger p < 0.05$, $\dagger\dagger\dagger p < 0.001$ compared with Patient C. Error bars indicate SEM. The number of separate cultures is indicated in parentheses. ***B***, Surface biotinylated and total proteins of the serum from Patient 1 treated hippocampal neurons were analyzed by Western blotting with the indicated antibodies. Student's *t* test; n.s., not significant. Error bars indicate SEM; $n = 3$ separate cultures. ***C***, ***D***, Serum samples of Patient 1 at two different time points, from the episode of thymoma and MG (before onset of encephalitis) and from the episode of encephalitis, were analyzed by cell-based ELISA (***C***), and their effects on synaptic GABA$_A$ receptors were investigated (***D***). Average values for GABA$_A$ receptor β3 and LGI1 ELISA from triplicate measurements of the individual serum samples are shown (***C***). Titer of serum antibodies against AchR is shown (bar graph in ***C***; Miyazaki et al., 2012). Synaptic GABA$_A$ receptors were counted as in ***A***. One-way ANOVA with Tukey's *post hoc* analysis; $***p < 0.001$; n.s., not significant. Error bars indicate SEM; $n = 3$ separate cultures.

tection. In support of this, cumulative distribution of the mIPSC amplitude of the patients' serum-treated neurons showed the significant leftward shift (Fig. 6*C*). In contrast, patients' antibodies did not affect AMPA-receptor-mediated mEPSCs (Fig. 6*A*,*D*), which is also consistent with no effects of the patients' antibodies on synaptic AMPA receptor clusters (Fig. 5*A*). Therefore, patients' antibodies specifically reduce the synaptic GABA$_A$ receptor function.

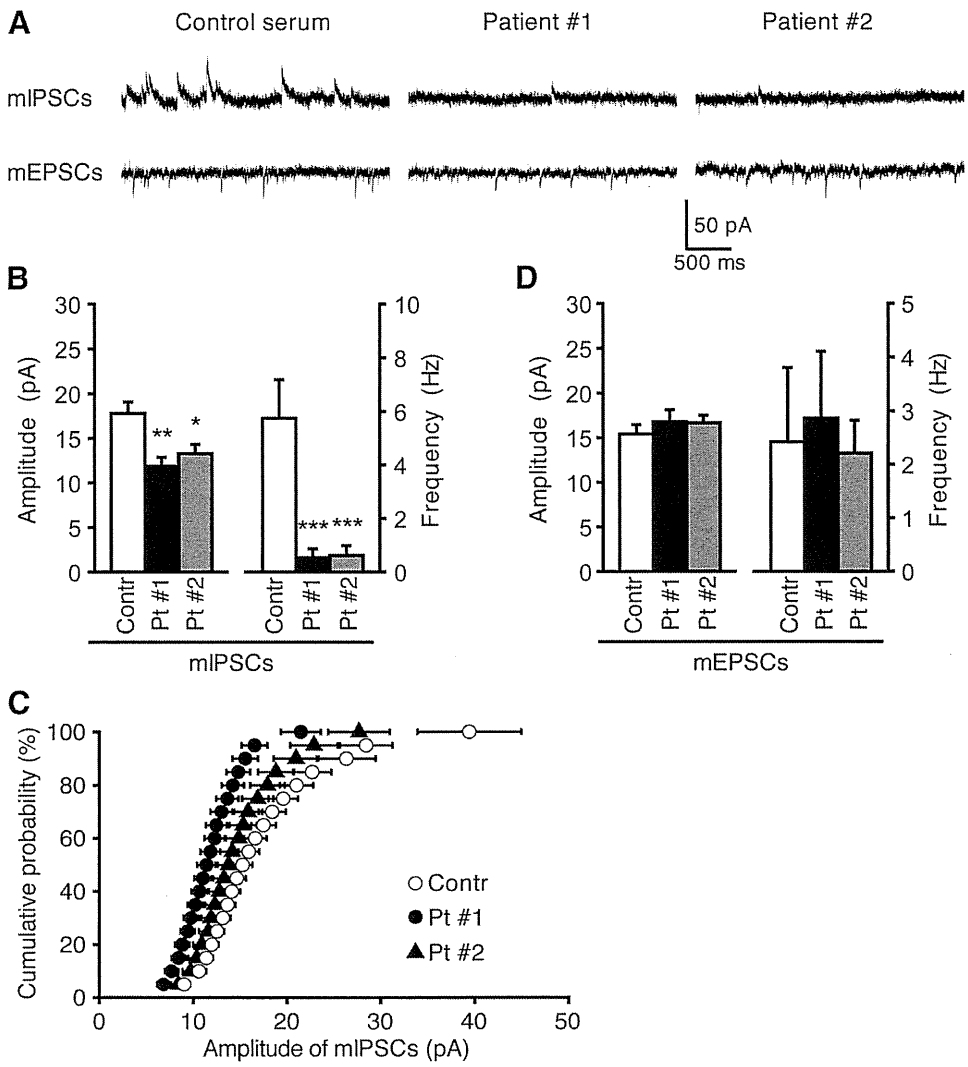


**Figure 6.** Patients' GABA$_A$ receptor antibodies selectively decrease mIPSCs. ***A***, Representative traces of mIPSCs ($V_H = -20$ mV, top) and mEPSCs ($V_H = -80$ mV, bottom) recorded from cultured rat hippocampal neurons, which were incubated with the serum from Patient 1 and Patient 2 or a control individual for 1 d. ***B***, ***D***, Treatment of neurons with the patient serum significantly decreased the amplitude and frequency in mIPSCs (***B***), but did not affect those in mEPSCs (***D***). Statistical analyses were performed by one-way ANOVA with Scheffe's *post hoc* analysis. $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ compared with control. ***C***, Cumulative distribution of the mIPSC amplitude. Note the significant leftward shift in the amplitude distribution in the patients' serum-treated neurons ($F_{(2, 540)} = 62.9$, $p < 0.001$; two-way ANOVA). Error bars indicate SEM; Control, $n = 9$; Patient 1, $n = 10$; Patient 2, $n = 11$ (***B***, ***C***); Control, $n = 9$; Patient 1, $n = 9$; Patient 2, $n = 12$ (***D***). $n$ values indicate the number of neurons examined from two separate cultures.

## Discussion

### Identification of GABA$_A$ receptor autoantibodies in autoimmune encephalitis

GABA is a major inhibitory neurotransmitter and exerts its functions through ionotropic GABA$_A$ receptors and metabotropic GABA$_B$ receptors. GABA$_B$ receptor was recently identified as an autoantigen associated with limbic encephalitis (Lancaster et al., 2010). However, antibodies to the ionotropic GABA$_A$ receptor have not been yet reported in any neuroimmunological disorders. Here, we found the autoantibodies to GABA$_A$ receptors in patients with autoimmune encephalitis and revealed a mode of action of the antibodies. One may wonder why GABA$_A$ receptor autoantibodies have not been found for a long time. One possibility is that the immunoprecipitated band of human IgG (heavy chains) almost completely overlaps with bands of co-isolated GABA$_A$ receptor subunits (all are ~50 kDa) in the SDS-PAGE gel, thereby hindering the detection of GABA$_A$ receptor subunits. Very recently, while this manuscript was under review, a related paper was published reporting the identification of GABA$_A$ receptor autoantibodies in patients with encephalitis showing refractory seizures and/or status epilepticus (Petit-Pedrol et al., 2014). Their history of autoimmunity or cancer seems different from that of our cases. In cases they reported, autoantibodies to GABA$_A$ receptors were sometimes concurrently detected with autoantibodies to GAD65 or GABA$_B$ receptor and were not frequently associated with underlying tumors. In contrast, our cases represent a paraneoplastic subtype of encephalitis with invasive thymoma (Table 1) in which GABA$_A$ receptor autoantibodies coexist with LGI1, CASPR2, or DCC antibodies. It will be worthwhile to test patients presenting with thymoma and encephalitis for GABA$_A$ receptor autoantibodies.

### Link between GABA$_A$ receptor autoantibodies and patient symptoms

We found that the GABA$_A$ receptor antibodies of both Patient 1 and Patient 2 targeted the $\beta$3 subunit of the GABA$_A$ receptor directly. Based on the previous genetic studies showing that mutations in the human GABA$_A$ receptor $\beta$3 subunit cause genetic

epilepsy syndromes (Macdonald et al., 2010) and that the genetic loss of the β3 subunit causes seizures and learning and memory deficits in mice (DeLorey et al., 1998), it is strongly suggested that the patients' $GABA_A$ receptor antibodies are the direct cause of some CNS disorders such as cognitive impairment and/or seizures often observed in encephalitis. Consistently, both the patients' antibodies similarly showed a selective effect on inhibitory synapses (Figs. 5, 6). In addition, only the serum sample from the episode of encephalitis (Patient 1) had decreased synaptic $GABA_A$ receptor density, whereas the sample of the same patient before the onset of encephalitis had no detectable $GABA_A$ receptor antibodies and no effects on synaptic $GABA_A$ receptor density (Fig. 5*C*,*D*). To further strengthen the link between $GABA_A$ receptor antibodies and patients' symptoms, we considered two additional factors of two patients, "thymoma" and "VGKC-complex antibodies," as follows.

The Patient 1 and Patient 2 both had invasive thymoma (Table 1) (Ohshita et al., 2006; Miyazaki et al., 2012). Because patients with (invasive) thymoma often develop multiple autoantibodies due to disturbed self-tolerance, we included patients with thymoma as controls. Our subjects for the screening contained 19 patients with thymoma, but only two patients had $GABA_A$ receptor antibodies, indicating that not all patients with thymoma develop $GABA_A$ receptor antibodies. For example, the Patient B, with invasive thymoma and myasthenia gravis, had monospecifically DCC antibodies without $GABA_A$ receptor antibodies, whereas Patient C, with invasive thymoma and neuromyotonia, had LGI1 and CASPR2 antibodies without $GABA_A$ receptor antibodies (Fig. 4). Treatment of hippocampal neurons with these patients' serum did not affect synaptic $GABA_A$ receptor clusters (Fig. 5*A* for Patient C; cluster number for Patient B serum, 25.0 ± 4.5/20 μm dendrite and control serum, 25.4 ± 4.2, $p = 0.85$, Student's $t$ test; $n = 11$ neurons).

In addition, we included patients with VGKC-complex antibodies as controls because Patient 1 and Patient 2 both had VGKC-complex antibodies (649 pM for Patient 1; 403 pM for Patient 2; Table 1), which are now attributed to LGI1 and/or CASPR2 antibodies, and previous case reports for these patients showed a correlation between patients' symptoms and the follow-up of VGKC-complex antibodies (Ohshita et al., 2006; Miyazaki et al., 2012). Here, we investigated the serum from Patient C as a control because the patient had VGKC-complex antibodies (809 pM; now revealed as LGI1 and CASPR2 antibodies; Fig. 4) but no $GABA_A$ receptor antibodies. Treatment with this patient serum did not affect the synaptic $GABA_A$ receptor clusters (Fig. 5*A*). In addition, we tested another patient serum with VGKC-complex antibodies (2121 pM) and limbic encephalitis. The patient had high level of monospecific LGI1 antibodies (absorbance = 1.86) without $GABA_A$ receptor antibodies. This serum treatment did not affect the synaptic $GABA_A$ receptor clusters (cluster number for control serum, 27.6 ± 1.2; for the patient serum, 24.6 ± 2.3, $p = 0.39$, Student's $t$ test; $n = 3$ separate cultures). These overall results exclude the possibility that coexisting antibodies other than $GABA_A$ receptor antibodies mediate the effects and support the specific role of $GABA_A$ receptor antibodies in the patients' symptoms.

Two patients with $GABA_A$ receptor antibodies shared some clinical features: cognitive impairment, multifocal abnormal brain MRI signals, and invasive thymoma (Table 1). Importantly, Patient 1 had seizures/status epilepticus, but Patient 2 had no seizure episodes. Given that loss of the β3 subunit in mice causes seizures and learning and memory deficits (DeLorey et al., 1998), it is reasonable to expect that loss of function of the $GABA_A$ receptor mediated by $GABA_A$ receptor β3 antibodies may cause seizures in human patients. However, at present, it seems too early to conclude that $GABA_A$ receptor antibodies should always cause seizures in human patients. It is conceivable that the brain regions where the antibodies act and the amount of the antibodies at different regions can be highly variable between patients. In addition, other factors such as medication and coexisting antibodies may modify the clinical features. In fact, Patient 2 had suffered from postherpetic neuralgia and had been under treatment with carbamazepine, an antiepileptic and anti-nerve-pain drug, for 5 years, including the periods of the initial episode and the relapse of encephalitis (Ohshita et al., 2006) (Table 1). This medication might have prevented the patient's seizure onset. The exact relationship between $GABA_A$ receptor antibodies and specific CNS symptoms will be clarified in the future as the number of cases increases.

### Anti-$GABA_A$ receptor encephalitis as a new class of autoimmune encephalitis

The present study indicates that encephalitis associated with $GABA_A$ receptor antibodies shows different clinical features and mechanisms, at least from limbic encephalitis associated with monospecific LGI1 antibodies. Both cases with the $GABA_A$ receptor antibodies showed the similar brain MRI finding, extensive multifocal lesions involving bilateral temporal lobes (Ohshita et al., 2006; Miyazaki et al., 2012). In contrast, limbic encephalitis with LGI1 autoantibodies is featured by the typical MRI finding with the focal lesion of medial temporal lobes (Cash et al., 2011; Lancaster et al., 2011). We previously found that the monospecific serum against LGI1 (ELISA absorbance = 1.86) from a patient with limbic encephalitis significantly reduce synaptic AMPA receptor density of hippocampal neurons (Ohkawa et al., 2013), but the serum did not affect synaptic $GABA_A$ receptor density. Conversely, serum from Patient 1 showed a selective effect on $GABA_A$ receptor function, but did not affect synaptic AMPA receptor density nor mEPSCs regardless of coexisting LGI1 antibodies (absorbance = 0.37; Figs. 5*A*, 6). Unlike NMDA, AMPA, and $GABA_A$ receptor antibodies directly targeting ionotropic receptors to induce the receptor internalization (Lai et al., 2009; Hughes et al., 2010; Fig. 5), LGI1 antibodies need to titrate out endogenous LGI1 to prevent LGI1 from binding to its receptor ADAM22 and then to reduce synaptic AMPA receptors. This indirect mode of action of LGI1 antibodies should require a higher concentration of LGI1 antibodies to be effective. Therefore, the loss of effect of serum from Patient 1 on synaptic AMPA receptors is probably due to the low LGI1 antibody level (Fig. 4*B*) and in turn highlights a predominant role of the $GABA_A$ receptor antibodies in the symptoms experienced by Patient 1. Therefore, it is conceivable that encephalitis with $GABA_A$ receptor antibodies might be distinguished as a new class of autoimmune encephalitis. In addition, we propose that clinical phenotypes of autoimmune anti-$GABA_A$ receptor encephalitis may be further modified by the combination of coexisting autoantibodies such as LGI1, CASPR2, or DCC antibodies, especially if the patient has thymoma. The multiplex ELISA testing to determine the involved autoantibodies will be essential for the accurate diagnosis of a spectrum of autoimmune encephalitis.

In conclusion, we discovered $GABA_A$ receptor autoantibodies associated with autoimmune encephalitis and revealed their pathogenic role, downregulation of the $GABA_A$ receptor function. Given that many agonistic and antagonistic ligands bind to specific sites on the $GABA_A$ receptor, the fine epitope mapping of autoantibodies on the $GABA_A$ receptor β3 subunit may contribute to further understanding the pathogenic mechanism causing

abnormal neuronal excitation in the brain and developing therapeutic interventions.

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Original article

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# Persistent frequent subclinical seizures and memory impairment after clinical remission in smoldering limbic encephalitis

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**ABSTRACT** – *Aim.* To delineate a possible correlation between clinical course and EEG abnormalities in non-infectious "smoldering" limbic encephalitis. *Methods.* Long-term clinical data, including video-EEG monitoring records, were analysed in two patients. *Results.* The two patients were positive for anti-voltage-gated potassium channel complex antibody and unspecified antineuronal antibody, respectively. The latter patient had small cell lung carcinoma. Both patients had memory impairment and clinical seizures. EEG showed frequent subclinical seizure patterns in the bilateral temporal regions. Subclinical seizure patterns and memory impairment persisted over one to two years after clinical seizure remission. Therapy (prednisolone and chemoradiation in the two patients, respectively) resulted in decreased occurrence of subclinical seizure patterns and memory improvement. *Conclusions.* EEG seizure patterns may persist years after clinical seizure remission in "smoldering" limbic encephalitis and lead to memory impairment.

**Key words:** EEG seizure pattern, memory impairment, smoldering limbic encephalitis, voltage-gated potassium-channel complex antibody, antineuronal antibody, epilepsy

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Recent studies of non-infectious limbic encephalitis have reported a number of syndromes associated with various autoantibodies, such as anti-voltage-gated potassium channel (VGKC) complex antibodies (Vincent *et al.*, 2004). Although autoantibodies should be sought

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in non-infectious limbic encephalitis (Pruss *et al.*, 2010), the list of relevant biomarkers or antibodies is so far incomplete. Negative results do not exclude paraneoplastic or autoimmune disorders, and antineuronal antibodies without specific antigen characterisation are sometimes positive based on immunohistochemical analysis. This result would justify immunotherapy in individual patients, and further research on these cases may reveal new antigens in the future (Zuliani *et al.*, 2012).

To our knowledge, despite many reports on immune-mediated limbic encephalitis, chronological change of EEG abnormalities, along with anatomo-functional imaging findings over a period of years, related to a "smoldering" clinical course, even after clinical seizure remission, has not been described in detail in the literature. In the present study, we aimed to delineate a possible correlation in two well-documented patients with non-infectious smoldering limbic encephalitis. We previously reported a case of limbic encephalitis associated with anti-VGKC complex antibodies, to which we referred to as "smoldering limbic encephalitis" (Nakaoku *et al.*, 2013). In the present study, we describe two patients with "smoldering limbic encephalitis" due to the presence of limbic encephalitis with persisting chronic symptoms, such as memory impairment and personality change, as well as other abnormalities such as EEG seizure patterns and/or residual increased glucose metabolism, persisting for more than a year, even after clinical seizure remission. We specifically chose the word "smoldering" due to the suggestion of residual active chronic inflammation based on increased glucose metabolism on 18F-fluorodeoxyglucose-positron emission tomography (FDG-PET) in at least one of the two patients, whereas epilepsy patients, in general, demonstrate glucose hypometabolism.

## Materials and methods

We retrospectively analysed clinical data of two patients with immune-mediated limbic encephalitis, for whom long-term follow-up data with video-EEG records were available.

*Patient 1* was a 62-year-old man with non-infectious smoldering limbic encephalitis without autoimmune comorbidities, such as systemic lupus erythematoses.

*Patient 2* was a 60-year-old man with paraneoplastic limbic encephalitis. Revised Hasegawa's Dementia Scale (Imai and Hasegawa, 2005) was performed for Patient 1, and Mini-Mental State Examination (MMSE) for Patient 2. The Wechsler Adult Intelligence Scale-Revised (WAIS-R) and Wechsler Memory Scale-Revised (WMS-R) were also performed on three and two occasions for Patients 1 and 2, respectively. Laboratory examinations, including autoantibody tests, cerebrospinal fluid (CSF) analysis, magnetic resonance imaging (MRI), and FDG-PET, were performed. Video- and routine EEGs were recorded on multiple occasions over the clinical course of 32 months for Patient 1, and 58 months for Patient 2. Patient 1 was treated with prednisolone and Patient 2 with chemoradiation.

## Results

*Patient 1* was positive for anti-VGKC complex antibodies (in July 2009), but negative for anti-Hu, Yo and Ri antibodies. He had a generalised tonic-clonic seizure, followed by gradual memory impairment in November 2008. He was initially given carbamazepine (CBZ), at 200 mg/day, which was later substituted for valproate (VPA), at 1,200 mg/day, due to skin rash caused by CBZ. Although no seizure recurred, memory impairment worsened (VPA blood concentration: 59.0 μg/mL). He also had a change in personality and became very irritable.

In July 2009, although the WAIS-R showed preserved intelligence, the WMS-R showed moderate memory impairment (*see figure 1 and table 1*). The CSF analysis was normal: 0/mm$^3$ cells; 27.3 mg/dL protein; 57 mg/dL glucose; and an IgG index of 0.45 (reference value: 0.34-0.85). The bilateral mesial temporal lobes showed increased volume and high intensity signal abnormality on MRI fluid-attenuated inversion recovery (FLAIR) images, and increased glucose metabolism on FDG-PET. Video-EEG showed 52 subclinical EEG seizure patterns, independently in the left and right anterior temporal regions over two days, and interictal transient sharp waves in the right temporal region. After methyl-prednisolone pulse therapy (1 g/day for three days), memory and irritability gradually improved.

Follow-up WAIS-R and WMS-R, 10 months later in May 2010, showed improved intelligence and memory. The hippocampi were atrophic on the right, and still hypertrophic on the left, on MRI. The bilateral mesial temporal lobes showed residual, though less prominent, high glucose metabolism on FDG-PET. Video-EEG showed bilateral independent subclinical EEG seizure patterns 10 times over two days, and interictal sharp waves in the left and right temporal regions, independently. After a second methyl-prednisolone pulse therapy, memory impairment remained stable and irritability improved.

His irritability, as well as follow-up WAIS-R and WMS-R scores, remained stable in July 2011. The bilateral hippocampi were atrophic on MRI. The bilateral mesial temporal lobes showed residual high glucose metabolism on FDG-PET, although this had further improved. A third methyl-prednisolone pulse therapy

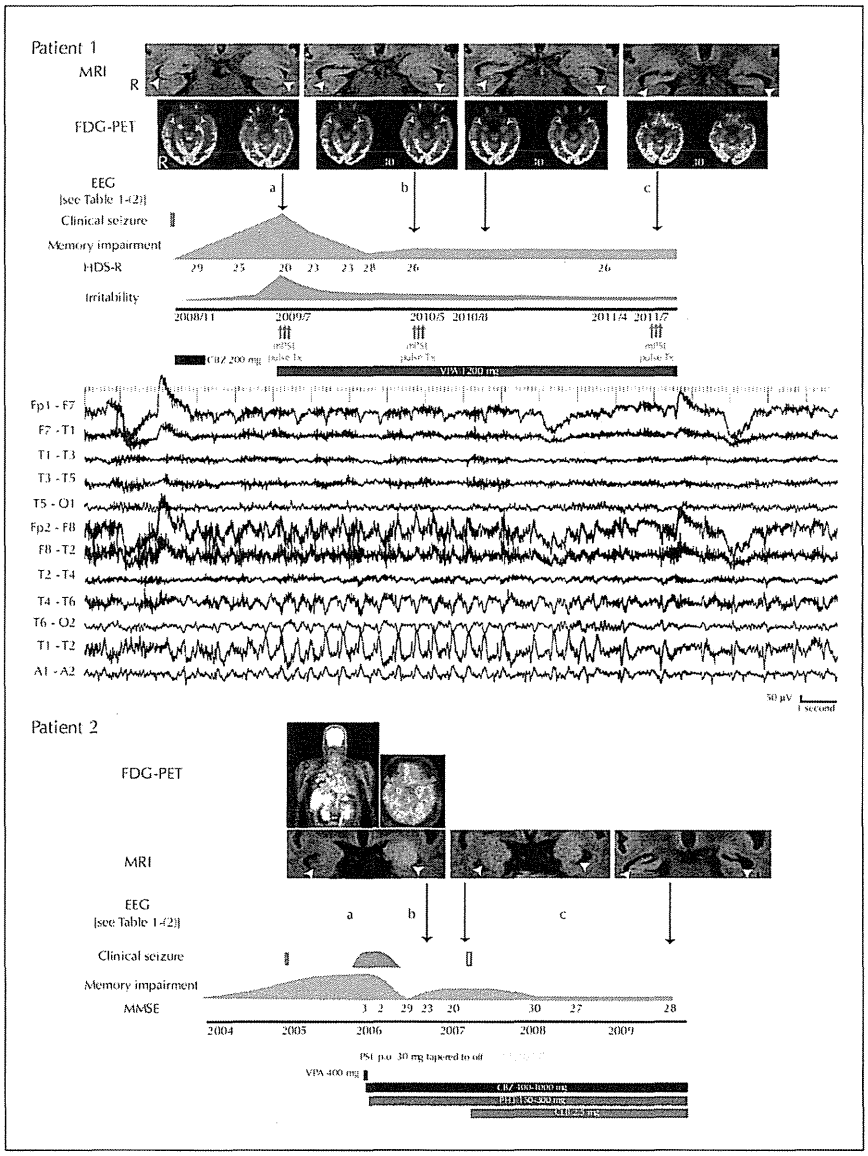


**Figure 1.** Clinical course, imaging findings, and ictal EEG pattern.
The small filled purple rectangles for Patients 1 and 2 indicate isolated generalised seizures, and the small open rectangle in Patient 2 indicates an isolated complex partial seizure. The shaded purple area for Patient 2 indicates frequency of simple partial and secondary generalised tonic-clonic seizures. White arrowheads in the MR image (FLAIR) in Patients 1 and 2 indicate abnormal hippocampi. Red arrowheads in the FDG-PET image in Patients 1 and 2 indicate increased uptake in the mesial temporal lobes. Orange arrows in the FDG-PET image in Patient 2 indicate increased uptake in the mediastinum and subcarinal lymph nodes due to small cell lung carcinoma. Ictal EEG pattern as a subclinical seizure in the right temporal region is shown in Patient 1. Patient 1 had no clinical seizures except one generalised seizure at the beginning of his clinical course. EEG: bipolar montage; sampling rate: 200 Hz; high frequency filter: 60 Hz; TC: 0.3 sec.
CBZ: carbamazepine; CLB: clobazam; FDG-PET: $^{18}$F-fluorodeoxyglucose-positron emission tomography; FLAIR: fluid-attenuated inversion recovery; HDS-R: Revised Hasegawa's Dementia Scale (Imai and Hasegawa, 2005) (consisting of nine simple questions with a maximum score of 30; a widely accepted scale not only for clinical use but also for epidemiological surveys of cognition in Japan [≤21 regarded as cognitive decline]); MMSE: Mini-Mental State Examination; mPSL: methylprednisolone; PHT: phenytoin; p.o.: per oral; PSL: prednisolone; R: right; Tx: therapy; VPA: valproate.

**Table 1.** Chronological change of psychometric examination.

| | | Neuropsychological assessment | | |
|---|---|---|---|---|
| | | **July 2009** | **May 2010** | **July 2011** |
| | WAIS-R: | | | |
| | VIQ | 105 | 113 | 111 |
| | PIQ | 107 | 113 | 113 |
| | FIQ | 106 | 114 | 113 |
| ***Patient 1*** | WMS-R: | | | |
| | Verbal memory | 63 | 88 | 79 |
| | Visual memory | 68 | 97 | 110 |
| | General memory | 59 | 90 | 88 |
| | Attention | 112 | 125 | 71 |
| | Delayed memory | <50 | 65 | 68 |
| | | **October 2006** | **April 2007** | |
| | WAIS-R: | | | |
| | VIQ | 86 | 83 | |
| | PIQ | 101 | 96 | |
| | FIQ | 93 | 89 | |
| ***Patient 2*** | WMS-R: | | | |
| | Verbal memory | 60 | 74 | |
| | Visual memory | 81 | 50 | |
| | General memory | 61 | 61 | |
| | Attention | 101 | 92 | |
| | Delayed memory | 50 | <50 | |

FIQ: full intelligence quotient; PIQ: performance intelligence quotient; VIQ: verbal intelligence quotient; WAIS-R: Wechsler Adult Intelligence Scale-Revised; WMS-R: Wechsler Memory Scale-Revised.

was given and his memory impairment and irritability have remained stable to date.

*Patient 2* was negative for anti-VGKC complex, Hu, Yo, Ri, CV2, Tr, Ma2, and amphiphysin antibodies, but positive for an unspecified antineuronal antibody (immunohistochemical staining using the patient's serum with biotin-labelled antihuman IgG and avidin-biotin complex [Tanaka *et al.*, 1994] gave rise to a diffuse staining pattern associated with the neuropil, rather suggestive of a cell surface antibody). His memory gradually declined from 2004, followed by the development of complex partial and generalised tonic-clonic seizures in 2005. During the initial work-up in January 2006, he stared with a blank look and hardly spoke. MMSE score was 3/30. He continued to have impaired orientation and abnormal behaviour even after he was able to have a conversation. MRI was normal. EEG showed background slowing (6-7 Hz) without paroxysmal discharges (see *figure 1 and table 2*). It was therefore assumed that he most likely had diffuse encephalopathy at that time. He continued to have seizures, simple partial and secondary generalised tonic-clonic, with VPA treatment at 400 mg/day. The CSF analysis was normal: 3/mm$^3$ cells; 37 mg/dL protein; 59 mg/dL glucose; and an IgG index of 0.58. Seizure control improved with phenytoin (PHT) at 150 mg/day and CBZ at 900 mg/day, and his MMSE score improved to 29/30.

His memory impairment recurred in October 2006. The left hippocampus showed increased volume and high intensity signal abnormality on MRI (FLAIR). The bilateral mesial temporal lobes showed increased glucose metabolism on FDG-PET, as well as in the mediastinum and subcarinal lymph nodes. Video- and routine EEGs showed three to four subclinical seizure patterns per hour in the left and right temporal regions, independently, and interictal intermittent rhythmic delta activities in the left temporal region.

Following a diagnosis of small cell lung carcinoma (SCLC), he underwent chemoradiation therapy (cisplatin+etoposide/60 Gy, followed by carboplatin+irinotecan, and then amrubicin). Seizures remitted after the addition of clobazam (CLB) at 2.5 mg/day (blood concentration: 14.9 μg/mL PHT; 6.1 μg/mL CBZ; 13 ng/mL CLB; 345 ng/mL desmethyl-CLB), and his memory improved and stabilised. Follow-up MRI showed increased volume and high intensity signal abnormality also in the right hippocampus (February 2007), followed by bilateral hippocampal atrophy (June 2009). EEG seizure patterns decreased to 1/30 minutes.

**Table 2.** Chronological change of EEG.

| | Record date (refer to *figure 1*) | Recording time | Interictal paroxysmal discharge | Seizure pattern |
|---|---|---|---|---|
| Patient 1 | a | 2 days | transient sharp waves<br>R FT | L = twice<br>R = 50 times |
| | b | 2 days | sharp waves<br>R or L FT | L = once<br>R = 9 times |
| | c | 2 days | transient sharp waves<br>R Fp | L = 6 times<br>R = 4 times |
| Patient 2 | a | 30 minutes | none | none |
| | b | 7.5 hours | TIRDA<br>L mT | L = 29 times<br>R = none |
| | | 30 minutes | none | L = none<br>R = twice |
| | c | 30 minutes | sharp wave<br>L FT | L = once<br>R = none |

FT: fronto-temporal; Fp: fronto-polar; L: left; mT: mid-temporal; R: right; TIRDA: temporal intermittent rhythmic delta activity.

His clinical course was complicated with radiation pneumonitis due to radiotherapy for SCLC. Prednisolone at 30 mg/day was started in July 2007, which was subsequently tapered off. Cancer recurred with hepatic metastasis in April 2008, without further memory deterioration. He died in August 2009.

## Discussion

Autoimmune pathophysiology such as limbic encephalitis has been recently described in relation to both acute (Buckley *et al.*, 2001; Vincent *et al.*, 2004; Dalmau *et al.*, 2007; Quek *et al.*, 2012) and chronic epileptogenicity (Brenner *et al.*, 2013). Autoantibodies such as anti-VGKC complex antibodies and anti-N-methyl D-aspartate (NMDA) receptor antibodies have been reported to be related to seizures mostly with an acute clinical course. The two patients manifested a clinically "smoldering" course with EEG seizure patterns persisting for one to two years even after clinical seizure remission. This phenomenon has not been well documented in the literature, at least for immune-mediated limbic encephalitis.

In the present study, Patients 1 and 2 were positive for anti-VGKC complex antibody and for an unspecified antineuronal antibody, respectively. Their clinical courses correlated with imaging abnormality in the bilateral mesial temporal lobes. Memory impairment was predominant, while clinical seizures were infrequent, despite very frequent EEG seizure patterns. Even after clinical seizure remission, EEG seizure patterns persisted for one to two years, and thus might have contributed to memory impairment, at least partly, in addition to limbic encephalitis itself.

Video-EEG has been reported to be useful in the early diagnosis of acute anti-NMDA receptor encephalitis, as confirmation of NMDA receptor antibodies takes several weeks (Dericioglu *et al.*, 2013; Di Capua *et al.*, 2013; Tan *et al.*, 2013). The present study suggests that video-EEG, even over only one to two days, could also be a clinically useful means to evaluate and follow up a "smoldering" clinical state, and possibly limbic encephalitis itself. Video-EEG could therefore be considered when chronic symptoms, such as memory impairment and personality change, persist even after clinical seizure remission, and the results of laboratory examinations other than video-EEG do not provide a clear explanation for the persistent symptoms.

With regards to immunotherapy for the treatment of epilepsy, in general, plasmapheresis and steroid therapy for Rasmussen encephalitis have been well established, although the immunological basis of the treatment remains to be determined. With regards to immunotherapy for the treatment of epilepsy patients with positive autoantibodies, the response rate to immunotherapy in patients with exclusive or predominant seizure presentation, in whom an autoimmune aetiology was suspected (with a median history of seizure activity of five months [range: 3 weeks to 12 years]), was reported to be 81%, and 67%

patients were seizure-free (Quek *et al.*, 2012). In NMDA receptor encephalitis, second-line immunotherapy (rituximab, cyclophosphamide) has been reported to be effective when first-line therapies (steroids, intravenous immunoglobulin, plasmapheresis) fail (with a time between symptom onset and initiation of immunotherapy of 18 days according to Ikeguchi *et al.* [2012], and 21 days in children and 28 days in adults according to Titulaer *et al.*, [2013]). In the present study, prednisolone in Patient 1 and chemoradiation in Patient 2 resulted in decreased frequency of EEG seizure patterns and memory improvement. Improvement in Patient 2 may have also reflected a favourable effect of oral prednisolone therapy given for radiation pneumonitis.

As a limitation of the present study, the immunological testing was incomplete; although different kinds of autoantibodies were tested, it remains possible that our patients were positive for other untested autoantibodies.

In conclusion, for a chronic course persisting to a milder degree, *i.e.* "smoldering", non-infectious limbic encephalitis should be considered as a possible diagnosis when bilateral independent interictal epileptiform discharges and EEG seizure patterns are observed in elderly patients. These patients need appropriate diagnosis and treatment in terms of autoimmunity. □

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Case Report

# A case of autoimmune epilepsy associated with anti-leucine-rich glioma inactivated subunit 1 antibodies manifesting electrical shock-like sensations and transparent sadness

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ABSTRACT

Autoimmune epilepsy is an isolated phenotype of autoimmune encephalitis, which may be suspected in patients with unexplained adult-onset seizure disorders or resistance to antiepileptic drugs (AEDs). Antibodies against leucine-rich glioma inactivated subunit 1 of the voltage-gated potassium channel (VGKC) complex, recently termed anti-LGI-1 antibodies, are one of the causes of autoimmune epilepsies. Bizarre symptoms with extremely short duration and high frequency are clues to the possible presence of autoimmune epilepsy with anti-LGI-1 antibodies. Precise diagnosis is important because autoimmune epilepsy is treatable and the prognosis can be predicted.



## 1. Introduction

Autoimmune encephalitis is a group of syndromes with the subacute onset of amnesia, confusion, and often prominent seizures [1]. The spectrum of autoimmune encephalitis is widening. Recent retrospective studies have shown an association between antivoltage-gated potassium channel (VGKC) antibodies and the development of a new onset, unexplained seizure disorder in patients with autoimmune limbic encephalitis symptoms [2]. Some patients with isolated seizure syndromes, including drug-resistant epilepsy and temporal lobe epilepsy (TLE), have low titers of anti-VGKC-complex antibodies in their sera [2,3]. Identification of an immune basis is important because adjunctive immunotherapy may improve the clinical conditions in these patients [4].

We report a case of autoimmune epilepsy with a high titer of anti-VGKC-complex antibodies (>400 pM). Bizarre symptoms of brief and frequent limbic seizures were the clues suggesting autoimmune epilepsy.

## 2. Case report

A 53-year-old university professor was referred to our department for investigation of seizures. He had nephrotic syndrome in childhood. There was no history of trauma, inflammation, infection, psychiatric disease, or medication use. He had no relevant past medical history including risk factors for malignancy. His family history was unremarkable. Stereotyped paroxysmal episodes started approximately two years before presentation. In November 2009, he repeatedly experienced an extremely short (<0.5 s) and unexplained sensation like an electric shock in the head. Thereafter, the same sensation recurred several times a day and was triggered by startle stimulus. In December 2009, the electric shock-like sensation increased to 20 to 30 times a day. He experienced unexplained sadness, which he described as "transparent sadness", for a few minutes while watching a play. Thereafter, unexplained sadness occurred when he listened to a certain piece of music. Listening to the same phrase of the music induced the same unexplained sadness. He avoided listening to music. In January 2010, he had a feeling of falling and sensed the odor of burned rubber at the same time. Although the feeling of falling and the sensing of the odor of burned rubber disappeared after only one day, the electric shock-like sensation continued.

He experienced a hot sensation in his lower left arm and lower leg momentarily, followed by pressure behind his left ear and sharp ringing and fullness in the left ear 10 to 20 times a day. He visited several medical and psychiatric departments, but the cause could not be identified. He consulted an epilepsy specialist in a hospital. Epilepsy was suspected, although an electroencephalogram (EEG) showed no epileptic discharge. Treatment with 300 mg of carbamazepine (CBZ) was

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started, and the electric shock-like sensation and hot sensation in his lower left arm and lower leg disappeared. Because of adverse reactions, CBZ was discontinued, and treatment was switched to zonisamide (ZNS). The electric shock-like sensation recurred 20 to 30 times a day. MRI showed no abnormality. In June, water dribbled from the right corner of his mouth while drinking. On the next day (June 24 X—, he had twitching in the right corner of his mouth for a few seconds, which was triggered by eating. Zonisamide was discontinued, but twitching continued. In July, he bit the right edge of his tongue during sleeping. After starting lamotrigine (LTG) and clonazepam (CZP), the electric shock-like sensation and twitching in the right mouth corner disappeared. In September, while on business travel, he was short of medication, and the electric shock-like sensations recurred. In January 2011, LTG was discontinued because of drowsiness, and treatment was switched to levetiracetam (LEV). The electric shock-like sensations recurred and increased to 10 times a day. Adding phenytoin (PHT) was not effective. He had a tactile sensation starting from the fingers on his right hand and spreading to the shoulder, which lasted a few seconds. He saw a bolt of lightning with jagged edges several times. In April, while he was traveling on a short-distance train, a generalized convulsion occurred when he was awakened by the alarm clock that he set on his cellular phone. He sustained a compression fracture of the lumbar spine as a result of the seizure. The tactile sensation disappeared after the generalized convulsion.

In June 2011, he was referred to our hospital. General and physical neurological examinations were unremarkable. He was alert, oriented, and highly intelligent. His Mini-Mental State Examination score was 30 out of 30. Although he had several bizarre symptoms that lasted very short durations, all the symptoms were repetitive and stereotyped, and epileptic seizures were suspected. Intraindividual seizure variability and unusually high seizure frequency observed in this patient suggested autoimmune epilepsy.

Full blood workup included routine tests to assess electrolytes, liver and renal function, vitamins $B_1$ and $B_{12}$, and autoimmune-related antibodies. All the test results were within normal ranges. Cerebrospinal fluid (CSF) examination showed no abnormality. The EEG showed no epileptiform discharges or slow waves (Fig. 1). A magnetic resonance imaging (MRI) (3T) scan was normal. 2-Deoxy-2-[$^{18}$F] fluoro-d-glucose ($^{18}$F-FDG) positron emission tomography/computed tomography (FDG-PET/CT) of the brain and body showed no hyper- or hypometabolism (Fig. 2). Although there were no other features to support an autoimmune etiology, we examined autoantibodies including anti-VGKC complex antibodies and intracellular autoantibodies (anti-Hu, Yo, Ri, Ma2/Ta, and amphiphysin). Serum anti-VGKC complex antibodies were elevated to 2493 pM (normal: <100 pM). The target antigen of VGKC complex antibodies was LGI-1.

Since the patient had no other subjective symptoms including memory impairment, he was observed without initiating immunotherapy. Occult cancer was excluded with whole body FDG-PET/CT.

In November 2011, he became seizure-free. During follow-up over one year, serum concentrations of anti-VGKC complex antibodies declined (768 pM), and the patient remained seizure-free with no memory impairment. In August 2014, anti-VGKC complex antibodies became negative. He became seizure free without antiepileptic drugs.

## 3. Discussion

Autoimmune encephalitis is characterized by seizures, memory disturbance, and/or psychiatric symptoms with or without inflammation revealed by CSF examination and/or MRI. In contrast to the encephalitic-type phenotype of autoimmune encephalitis, the new concept of autoimmune epilepsy is an isolated phenotype of autoimmune encephalitis that lacks a typical "limbic encephalitis" phenotype and resistance to antiepileptic drugs [5]. Recent case series have reported neuronal autoantibodies in approximately 10% of patients who have epilepsy without cognitive or psychiatric features [6]. Normal laboratory and imaging findings (particularly normal CSF analysis and MRI) do not exclude autoimmune encephalitis. Prompt diagnosis and treatment with immunosuppressive therapies improve or even reverse symptoms. If left untreated, retrospective case series show that these conditions can lead to irreversible cognitive deficits, ongoing seizures, and death [7–9]. Bien and Elger suggested that limbic encephalitis should be considered a differential diagnosis in any adult patient with newly developed temporal lobe epilepsy and/or a rather quickly progressing amnestic disturbance [10]. Toledano et al. proposed valuable clinical clues to diagnose autoimmune epilepsy: intraindividual seizure variability or multifocality, an unusually high seizure frequency, antiepileptic drug (AED) resistance, subacute onset, personal or family history of autoimmunity, and recent or past history of neoplasia [11]. Our case manifested multifocal seizure types. We suspect that the electrical shock-like sensation in the head was a cephalic aura originated from the mesial temporal lobe, although the semiology is atypical for temporal lobe epilepsy. Sadness triggered by specific music probably originated from the amygdala and the odor of burned rubber from the uncus. In addition to the temporal lobe seizures, he had other types of seizures including mouth twitching, hot sensation, tactile sensation,

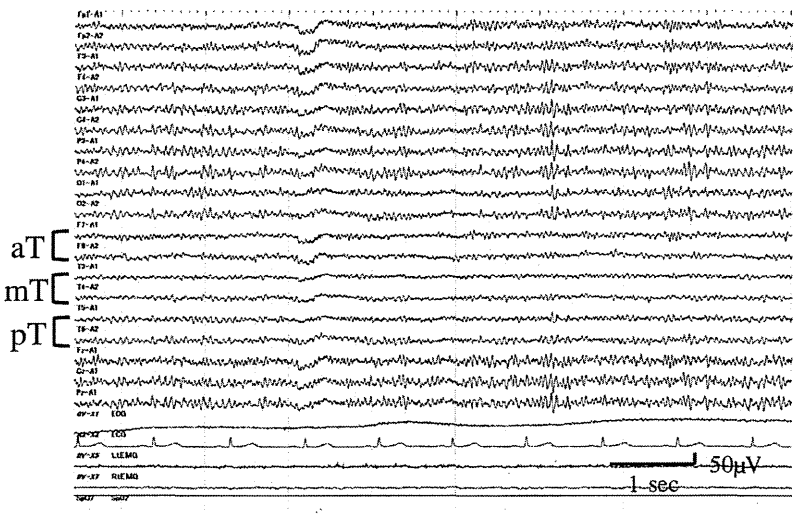


**Fig. 1.** Interictal EEG.The EEG shows epileptiform discharges or slow waves.

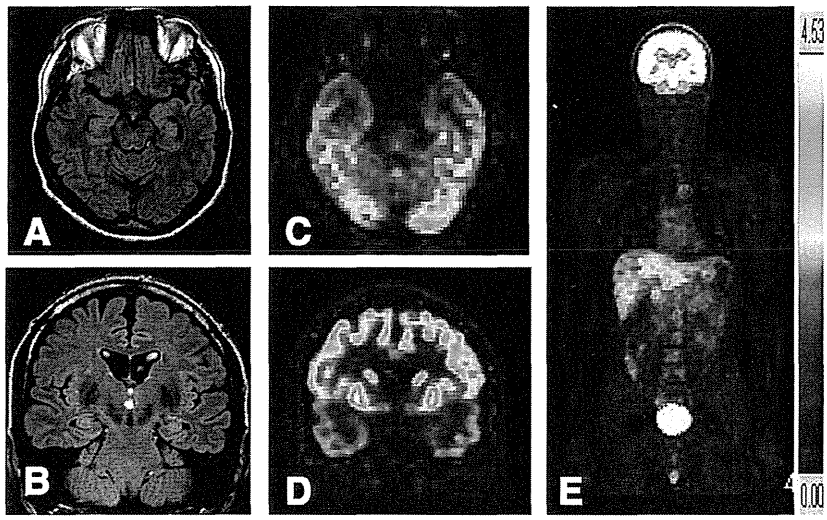


**Fig. 2.** Imaging studies. A: Brain MRI with fluid-attenuated inversion-recovery (FLAIR) sequence, transaxial view. B: Brain MRI with FLAIR sequence, transcoronal view. C: Brain $^{18}$F-FDG PET/CT, transaxial view. D: Brain $^{18}$F-FDG PET/CT, transcoronal view. Brain images (A, B, C, and D) show no abnormalities. E: Whole body $^{18}$F-FDG PET/CT shows no abnormal uptake and excluded underlying paraneoplastic syndrome.

and lightning bolt with jagged edges. We assumed that these seizures originated from the extratemporal lobes. The seizures were frequent and resistant to AEDs. Extremely short duration of seizures was characteristic for this patient, although it was not reported in previous papers [7–9,11]. The frequent and short seizures (FSS) of our case resembles faciobrachial dystonic seizures (FBDS). Faciobrachial dystonic seizures are multiple, brief (<3 s) episodes of simultaneous facial grimacing and arm dystonia with a high frequency of attacks. Faciobrachial dystonic seizures are highly specific for anti-LGI-1 limbic encephalitis and are important for its recognition and diagnosis [12]. Our case report suggests that frequent and short seizures might be an important feature of autoimmune epilepsy associated with anti-LGI-1 antibodies.

In our patient, symptoms disappeared completely without immunotherapy and underwent spontaneous remission. Irani et al. reported that FBDS often preceded the cognitive impairment observed in anti-VGKC-complex antibody-associated limbic encephalitis [13]. Immunotherapy shortened the time to recovery and might prevent subsequent development of cognitive impairment [13]. A few cases of anti-VGKC antibody-associated limbic encephalitis that improved without immunosuppressive therapy have been reported [14–16]. Butler et al. reported that patients with VGKC-associated limbic encephalitis often recovered substantially with immunotherapy despite broad cognitive dysfunction in the acute phase but might have residual permanent anterograde amnesia [17]. Our case showed no cognitive dysfunction and became seizure-free with LTG, although his seizures might have improved earlier with immunotherapy than without immunotherapy. This case demonstrates that high titers of anti-VGKC complex antibodies are not always related to a severe, progressive clinical course.

Autoimmune epilepsy is one of the epilepsies that improves with immunotherapy. Physicians should pay attention to clinical clues such as subacute onset, intraindividual seizure variability or multifocality, AED resistance, personal or family history of autoimmunity, and recent or past history of neoplasia. In addition, FSS might be additional features in autoimmune epilepsy associated with anti-LGI-1 antibodies.

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## Conflict of interest

None of the authors have any conflicts of interest to report.

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# Safety and efficacy of thalidomide in patients with POEMS syndrome: a multicentre, randomised, double-blind, placebo-controlled trial

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## Summary

**Background** Polyneuropathy, organomegaly, endocrinopathy, M-protein, and skin changes (POEMS) syndrome is a rare cause of demyelinating neuropathy, with multi-organ involvement characterised by plasma cell dyscrasia and VEGF overproduction. No treatments have been established for patients with POEMS syndrome who are not eligible for stem-cell transplantation. Thalidomide suppresses VEGF and plasma cell proliferation. We aimed to assess the safety and efficacy of thalidomide for the treatment of POEMS syndrome.

**Methods** We did a randomised, double-blind, placebo-controlled, phase 2/3 trial at 12 hospitals in Japan. Adults (age ≥20 years) with POEMS syndrome who were ineligible for autotransplantation were randomly assigned (1:1) by a minimisation method to treatment with oral dexamethasone (12 mg/m² per day on the first 4 days of every 28-day cycle) plus either oral thalidomide (200 mg daily) or placebo for six cycles. All study personnel and patients were masked to treatment allocation. The primary endpoint was the reduction rate of serum VEGF concentrations at 24 weeks. Analysis was by intention to treat. This study is registered with the UMIN Clinical Trials Registry, UMIN000004179.

**Findings** Between Nov 11, 2010, and July 3, 2014, we randomly assigned 25 patients to receive either thalidomide (n=13) or placebo (n=12); one patient in the placebo group was excluded from analyses because of a protocol violation. The adjusted mean VEGF concentration reduction rate at 24 weeks was 0·39 (SD 0·34) in the thalidomide group compared with −0·02 (0·54) in the placebo group (adjusted mean difference 0·41, 95% CI 0·02–0·80; p=0·04). Mild sinus bradycardia was more frequent in the thalidomide group than in the placebo group (seven [54%] *vs* zero; p=0·006). Five patients had serious adverse events: three in the thalidomide group (transient cardiac arrest, heart failure, and dehydration) and two in the placebo group (ileus and fever). No deaths occurred during the randomised study. In the 48-week open-label study period (n=22), newly developed adverse events were sinus bradycardia (n=4), constipation (n=5), and mild sensory neuropathy (n=5). Two patients died in the open-label study; both patients were initially in the placebo group, and the cause of death was progression of the disease.

**Interpretation** Thalidomide reduces serum VEGF concentrations and represents a new treatment for patients with POEMS syndrome who are not eligible for stem-cell transplantation. Thalidomide treatment poses a risk of bradycardia; however, the benefits are likely to exceed the risk.

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## Introduction

Polyneuropathy, organomegaly, endocrinopathy, M-protein, and skin changes (POEMS) syndrome, also known as Crow-Fukase syndrome, is a rare cause of demyelinating polyneuropathy associated with multi-organ involvement, plasma cell dyscrasia, and VEGF overproduction.[1,2] The pathogenesis of POEMS syndrome is not fully understood; however, upregulated VEGF is assumed to play a major part by its strong action on vascular permeability and neovascularisation. VEGF overproduction is likely to cause many of the symptoms, including capillary leak syndrome (pleural effusion, ascites, and oedema), skin angioma, and presumably peripheral neuropathy. The prevalence of POEMS syndrome in Japan is estimated to be 0·3 per 100 000 people.[3] The prevalence of POEMS syndrome was originally thought to be higher in Japan than in other countries.[4,5] However, findings from recent case series from the USA (n=99),[6] France (n=25),[7] China (n=99),[8] and India (n=29)[9] suggest that POEMS syndrome is widely distributed.

Patients with POEMS syndrome develop disabling polyneuropathy and massive pleural effusion or ascites resulting in multiple organ failure.[2,10] In the 1980s, the mean survival time was 33 months in 34 patients who were mainly treated with corticosteroids.[4] Since the

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## Research in context

### Evidence before the study

We searched PubMed up to March 31, 2016, with the following terms without language restrictions: "POEMS syndrome", "Crow-Fukase syndrome", "Takatsuki disease", "clinical trial", and "treatment". We identified no randomised clinical trials for polyneuropathy, organomegaly, endocrinopathy, M-protein, and skin changes (POEMS) syndrome, only observational or open studies with alkylating drugs, thalidomide, lenalidomide, bortezomib, or autologous peripheral blood stem-cell transplantation.

### Added value of this study

This is, to our knowledge, the first randomised, placebo-controlled clinical trial of the safety and efficacy of any kind of intervention for POEMS syndrome. In our study, we found a significant improvement in serum concentrations of VEGF, motor function, and some measures of quality of life in patients with POEMS syndrome treated with thalidomide compared with those who received placebo. Thalidomide should be considered as an effective treatment for patients with this disorder.

### Implications of all the available evidence

There is an urgent need for treatments for patients with POEMS syndrome. Our findings show that thalidomide is a new treatment option for patients with POEMS syndrome who are not eligible for autologous transplantation.

1990s, the treatment strategy for POEMS syndrome has shifted to suppression of monoclonal plasma cell proliferation; therapeutic interventions used for multiple myeloma have also been used for POEMS syndrome.[2,10,11] Autologous peripheral blood stem-cell transplantation with high-dose chemotherapy can result in substantial clinical improvement and has been suggested as the first-line treatment for appropriate candidates.[11–13] However, transplantation is not indicated in about half of patients because they are older than 65 years or have organ failure. Such patients have been treated with immunomodulatory drugs such as thalidomide,[14] lenalidomide,[15] or low-dose alkylators.[16,17] No randomised controlled trials have been done, mainly because of the rarity and severity of the disorder.[2,10]

Thalidomide was initially developed as a sedative, but was withdrawn from the market because of its teratogenic effects. The drug has since been recognised as having antiangiogenic and anti-inflammatory cytokine properties, and has been used to treat erythema nodosum leprosum and multiple myeloma.[18] Findings from observational studies have suggested that thalidomide use in patients with POEMS syndrome decreases serum VEGF concentrations, improves or stabilises symptoms, and is safe for use in patients who are not eligible for autotransplantation.[14] No standard treatment guidelines exist for POEMS syndrome because of the scarcity of evidence-based clinical trial data. We therefore investigated the safety and efficacy of thalidomide in patients with POEMS syndrome who were not eligible for autotransplantation.

## Methods

### Study design and patients

For the trial protocol see http://opac.ll.chiba-u.jp/da/curator/100005/?lang=1

The POEMS Syndrome Thalidomide (J-POST) Trial was an investigator-led, phase 2/3, randomised, double-blind, placebo-controlled trial at 12 hospitals in Japan. The trial consisted of two periods: a 24-week, double-blind, randomised, comparative study followed by a 48-week, open-label safety study.

The trial protocol has been published elsewhere.[19] Briefly, adults (age ≥20 years) with probable or definite POEMS syndrome according to published diagnostic criteria[11] who were ineligible for autotransplantation during the study period and did not have substantial electrocardiographic abnormalities were eligible for inclusion. Ineligibility for transplantation was defined as age older than 65 years, organ failure (renal, respiratory, or cardiac), or patient refusal. Patients who had unstable disease or received oral or intravenous corticosteroids within 4 weeks of providing informed consent were excluded.

The study protocol was approved by the institutional review board of each hospital. Patients gave written informed consent before enrolment.

### Randomisation and masking

Patients were randomly assigned (1:1) by a minimisation method to receive thalidomide plus dexamethasone or matching placebo plus dexamethasone. The allocation was generated by a computer program located at the registration centre.[20] The allocation coordinators at the registration centre enrolled patients and assigned them to the trial groups, but they had no involvement in the rest of the trial. Assignment was stratified by serum VEGF concentration (≤3000 pg/mL or >3000 pg/mL) and the presence of pleural effusion (yes or no). The placebo capsules were indistinguishable in appearance and taste from the thalidomide capsules. The trial drugs were supplied in numbered containers and were distributed to each hospital at the start of the trial under the responsibility of SaK. All study personnel and patients were masked to treatment group allocation.

### Procedures

This 24-week, double-blind, randomised comparative study consisted of six 28-day cycles. Oral thalidomide or placebo were continuously given after a titration period, and oral dexamethasone was administered at a dose of 12 mg/m² per day on the first 4 days of every cycle. The

starting dose of the trial drug was one capsule containing 100 mg of thalidomide or placebo every 2 days. The titration period was 14 days. The dose increased to one capsule daily on day 8 and two capsules daily on day 15. The dose remained at two capsules daily except in cases of haematological or skin toxicity exceeding grade 3 according to the Common Terminology Criteria for Adverse Events (CTCAE, version 4.0).

Patients were moved from the randomised comparative study to the open-label safety study if they had subacute worsening of POEMS syndrome, defined as subacute capillary-leak-like symptoms (5 kg/month of weight gain due to generalised oedema or pleural effusion increase by predefined criteria) or evident deterioration of neuropathy (increase in overall neuropathy limitation scale score ≥2). Patients received antiplatelet drugs or anticoagulants according to baseline thrombotic risk factors, such as a history of deep vein thrombosis and obesity.

Patients were admitted and assessed at hospital during the titration period and thereafter were assessed at each clinic on day 1 of each cycle and on the last day of the randomised comparative study period. Clinical assessments, VEGF measurements, blood and urine tests, chest radiographs, and electrocardiograms were done at study entry and each visit. CT of the chest and abdomen and nerve conduction studies were done on day 1 of cycles 1 (at entry) and 4, and on the last day of the study. Respiratory function tests and the 36-Item Short-Form Survey (SF-36) were assessed at entry and at the end of the study. Adverse events were assessed during patients' hospital stay and at each visit, and pregnancy tests for all women of reproductive age were done every 28 days.

The randomised comparative trial was followed by a 48-week, open-label safety study. Patients in both the thalidomide and placebo groups in the randomised period were treated with thalidomide (200 mg daily) for 48 weeks in the open-label safety study. Dexamethasone was not given in the open-label study.

### Outcomes

In the randomised study, the primary outcome was the reduction rate in serum VEGF concentration between baseline and the end of 24 weeks of treatment.[19] VEGF concentration reduction was defined as (VEGF concentration at baseline−VEGF concentration at 24 weeks)/VEGF concentration at baseline. Serum VEGF concentrations were measured using ELISA at the central laboratory (SRL Medisearch, Tokyo, Japan), and results were masked to participants and study personnel from the baseline measurement until after the last patient completed the randomised controlled study period. Secondary endpoints were changes in serum VEGF concentrations at 24 weeks, the achievement of a normal range of serum VEGF (<1000 pg/mL; 52·1 pmol/L), motor function (sum of the scores from manual muscle testing, grip strength, and overall neuropathy limitation scale[21]), parameters of nerve conduction studies (motor conduction velocity, compound muscle action potential amplitude, and F-wave latency), M-protein concentrations (serum and urine), pleural effusion, vital capacity, body weight, quality of life (QoL, SF-36), and adverse events. Summary scales of the SF-36 were each scored using weights derived from a national probability sample from Japan.[22] Adverse events were graded for severity according to the CTCAE and relation to the study drug. Secondary endpoints for efficacy were assessed at the end of 24 weeks of treatment and those for safety (adverse events) were assessed during patients' hospital stay and at each visit.

The primary outcome of the open-label study was safety, and the secondary outcomes were the same parameters as in the randomised study. Secondary endpoints for efficacy were assessed at the end of 48 weeks of treatment and those for safety (adverse events) were assessed during patients' hospital stay and at each visit.

### Statistical analysis

The study was powered to detect a mean reduction rate of 0·35 (SD 0·25) between the two treatments at 24 weeks for the primary outcome, with 80% power, a two-sided 5% α level, and allowing for 20% loss to follow-up, producing a target cohort of 24 patients (12 per group).

All analyses were prespecified in a detailed statistical analysis plan. Analyses of the primary and secondary outcomes were done in the intention-to-treat population. For the baseline variables, summary statistics were constructed using frequencies and proportions for categorical data and means and SDs for continuous variables. Patient characteristics were compared using Fisher's exact test for categorical outcomes and Student's *t* test or Wilcoxon rank sum test for continuous variables, as appropriate.

For the **statistical analysis plan** see http://opac.ll.chiba-u.jp/da/curator/100006/?lang=1

For the primary analysis, we compared the thalidomide and placebo groups with an ANCOVA model, taking into account the variation caused by treatment effects and using the baseline serum VEGF concentrations (≤3000 pg/mL or >3000 pg/mL) and evidence of pleural effusion as covariates. The primary analyses were done in the intention-to-treat population with last-on-treatment-observation-carried-forward (LOCF) imputation. For sensitivity analyses, we used the mixed-effect model for repeated measures (MMRM) and worst-observation-carried-forward methods to examine the effect of missing data. The secondary analysis was done in the same manner as for the primary endpoint. All comparisons were planned, and all p values were two sided. A p value of less than 0·05 was deemed significant. All statistical analyses were done using SAS (version 9.4).

The trial has been reported to the Japanese Pharmaceuticals and Medical Devices Agency (number 22-1716) and is registered with the UMIN Clinical Trials Registry, number UMIN000004179.