INTERNAL MEDICINE

□ PICTURES IN CLINICAL MEDICINE □

Markedly Ring-enhanced Optic Nerves Due to Metastasis of Signet-ring Cell Gastric Carcinoma

Yuichi Hayashi, Takehiro Kato, Yuji Tanaka, Megumi Yamada, Akihiro Koumura, Akio Kimura, Isao Hozumi and Takashi Inuzuka

Key words: MRI, signet-ring cell carcinoma, optic nerve, cerebrospinal fluid, optic neuropathy, leptomeningeal carcinomatosis

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Picture 1. Coronal fat-suppressed MRI with gadolinium showed a marked ring enhancement of the surrounding optic nerves (arrows).

Signet-ring cell carcinoma frequently causes leptomeningeal carcinomatosis, one cause of optic neuropathy in elderly patients. A 77-year-old woman had shown progressive bilateral blindness for one month without any other symptoms. Coronal fat-suppressed MRI with gadolinium showed a marked ring enhancement of the surrounding optic nerves (Picture 1). CSF cytodiagnosis and histopathological examination of the gastric biopsy samples revealed signet-ring cell carcinoma. The patient was diagnosed with leptomeningeal carcinomatosis due to gastric cancer. She died 2 weeks after the diagnosis. Coronal fat-suppressed MRI with gadolinium is useful for the differential diagnosis of optic neuropathy, especially leptomeningeal carcinomatosis (1, 2).

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INTERNAL MEDICINE

□ CASE REPORT □

Serial Monitoring of Basal Metabolic Rate for Therapeutic Evaluation in an Isaacs' Syndrome Patient with Chronic Fluctuating Symptoms

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Abstract

A 52-year-old man presented with hyperhydrosis, painful pseudomyotonia and gait disturbance. The condition was diagnosed as Isaacs' syndrome on the basis of characteristic findings noted on an electromyogram. Carbamazepine treatment was only partially and transiently effective. Intravenous immunoglobulin therapy was effective. The basal metabolic rate (BMR) was serially monitored using an automatic integrated system for breath analysis. Serial monitoring of the BMR facilitates therapeutic evaluation in an Isaacs' syndrome patient with chronic fluctuating symptoms.

Key words: Isaacs' syndrome, intravenous immunoglobulin, basal metabolic rate, automatic integrated system for breath analysis, carbamazepine

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Introduction

Isaacs' syndrome (IS) is characterized by spontaneous and continuous activity of muscle fibers (1). Most cases of IS occur sporadically, and only 38% of all patients with IS test positive for anti-voltage-gated potassium channel (VGKC) antibodies (2).

Isaacs reported that the basal metabolic rate (BMR) is elevated in patients with acute-phase IS, but is normalized with treatment (1). Studies involving the serial monitoring of the BMR of these patients have not been performed because of the complicated procedures involved. We present the case of a patient with sporadic IS without anti-VGKC antibodies over a chronic fluctuating course. We serially monitored the patient's BMR for therapeutic evaluation using an automatic integrated system for breath analysis.

Case Report

A 52-year-old man presented with gait disturbance, painful muscle cramps and hyperhydrosis and was admitted to our hospital in February 2008. He had no specific familial history of disease. He began to suffer from hyperhydrosis when he was in his 30s and from gait disturbance in May 2007. He occasionally experienced dysphagia but recovered from it naturally. His clinical course had fluctuated over several months.

Physical examination revealed that the patient was well nourished. He suffered from mild mental retardation. His blood pressure, heart rate and body temperature were all found to be normal. Neurological examination revealed myokimia, hyperhydrosis, and hypertrophy of the leg muscles. Furthermore, the patient experienced muscle cramps at various times during the day and night. Grip myotonia was not detected; however, the patient's fingers spontaneously flexed after they were extended. His reflexes were slightly exaggerated but the Babinski sign was absent. A photograph of the patient showed flexed upper-limbs, forward-bend posture, standing with legs bending outwards, and hypertrophy of the calf muscles (Fig. 1). He walked with such posture. Painful cramps often occurred during walking. His cranial nerves and sensory perception were normal. He had experienced no epilepsy, hallucination, or insomnia.

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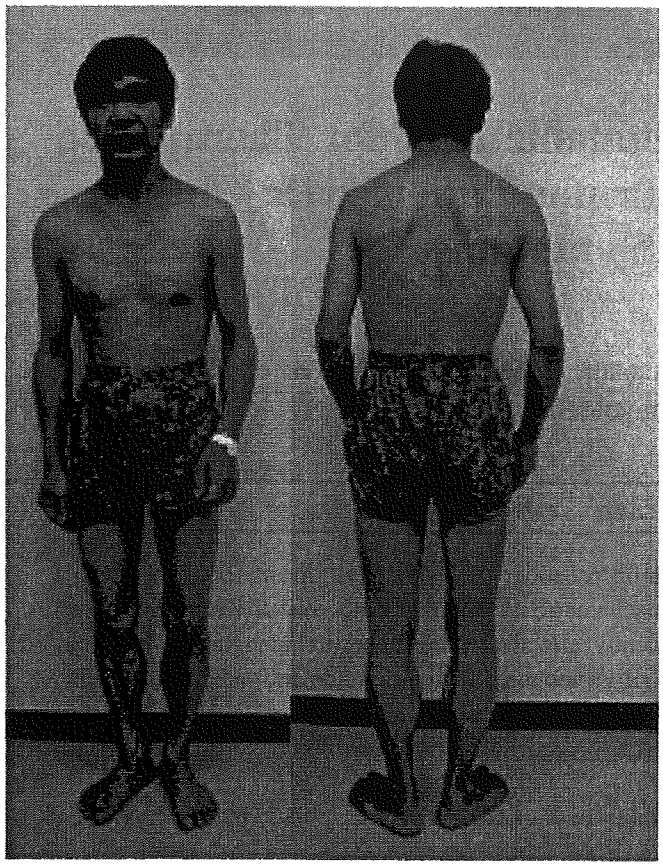

Figure 1. A photograph of the patient.

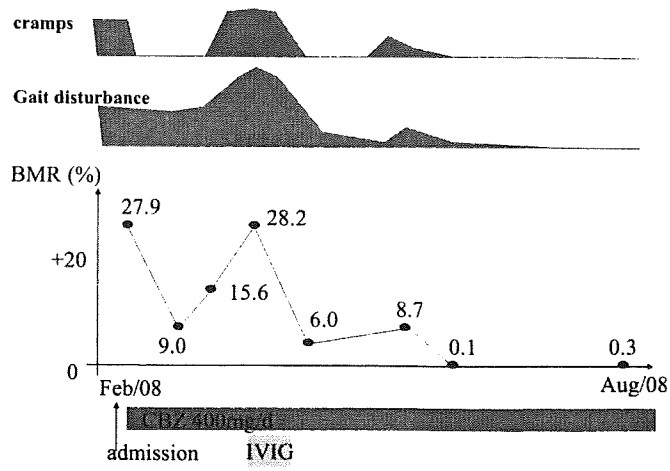

Figure 2. Serial monitoring of basal metabolic rates. We serially monitored the patient's basal metabolic rate (BMR) using an automatic integrated system for breath analysis (FUDAC-77), and found that the clinical symptoms fluctuated in tandem with the BMR. BMR: basal metabolic rate, CBZ: carbamazepine, IVIG: intravenous immunoglobulin (0.4 g/kg/d for 5 d)

Laboratory tests revealed that all the parameters, including the serum creatine kinase (CK) level and the thyroid hormone levels, were within the normal limits. Antibodies against VGKC and glutamic acid decarboxylase antibody were not detected in the serum.

An electromyogram (EMG) of the right biceps brachii, quadriceps and tibialis anterior muscles did not show myotonic discharge. Randomized doublet or triplet muscle waves were observed in the myokimic muscles of the left calf. A surface EMG study showed spontaneous and continuous motor-unit activity in the right biceps and the rectal abdominal muscles and the presence of M-wave afterdischarges in the upper extremities. Nerve conduction study was normal in the upper extremities, but it could not be assessed in the tibial nerves because of muscle cramps. Brain MRI and electroencephalography (EEG) showed no obvious abnormal findings. CT of the muscles showed hypertrophy of the calf muscles.

The BMR was measured at 9 a.m. while the patient was at rest and before breakfast, using an automatic integrated system for breath analysis (FUDAC-77, Fukuda Denshi, Tokyo, Japan). The BMR was 27.9% higher than the upper limit of the normal range for men in their 50s.

IS was diagnosed on the basis of the characteristic findings noted on the EMG, and the patient was administered oral carbamazepine (CBZ: 400 mg/d). The frequency of muscle cramps was reduced with the treatment. Furthermore, the BMR was reduced to 9.0% higher than the upper limit of the normal range. However, the gait disturbance did not improve. Shortly thereafter, the patient's symptoms deteriorated once again, and despite CBZ treatment, he frequently experienced muscle cramps all over his body, both during the day and at night. As was expected with the exacerbation of the symptoms, the BMR was increased to 28.2% higher than the upper limit of the normal range.

We initiated intravenous immunoglobulin (IVIG) therapy (0.4 g/kg/d for 5d). The patient's symptoms improved with IVIG, and the BMR was normalized and maintained for at least 6 months (Fig. 2).

Discussion

We present the case of a patient with chronic fluctuating symptoms of IS, not accompanied by any hormonal disease. The BMR was serially assessed, and it was found that the patient's clinical symptoms fluctuated in tandem with the BMR (Fig. 2).

The BMR is associated with many factors: age, sex, race, and thyroid hormone level (3). The major factors causing an increase in the BMR are hormonal disease and physiological factors; pregnancy, diet, a high environmental temperature, exercise, or a state of excitement (3). When we measure the BMR, the observed value reflects the result of total oxygen consumption of the whole body. The oxygen consumption of the brain and muscles at rest is estimated to be 23 and 20% of that of the whole body, respectively. The oxygen consumption of muscles at exercise reaches maximally 60 times that at rest (4). In general, the BMR test is not used as an indicator of chronic muscle activity; however, secondary chronic muscle activity due to an underlying disease may cause fluctuation in the BMR.

It is reported that IS is sometimes accompanied with dis-

turbance of the central nervous system (CNS), such as Morvan syndrome (5) or limbic encephalitis (6). However, the findings of brain MRI and EEG in the present case suggested no accompaniment of such a CNS disease. The increasing value of BMR in our case mainly reflected the oxygen consumption of muscles, not that of the brain.

In 1961, Isaacs first reported the cases of patients with acute-phase IS, whose BMR was elevated because of continuous or spontaneous muscle fiber activity, but was normalized with treatment (1). However, at that time, serial monitoring of the BMR was not performed because the methods available were complicated.

A closed-circuit respiratory device has been used to calculate the BMR (3). In the method that has traditionally been used to determine the BMR, the patient is required to breathe through the mouth into the analyzer for 6 minutes while at rest and before breakfast, and a skilled medical technologist analyzes the resting end-respiratory volumes for 6 minutes and manually draws a straight line to calculate the BMR.

Recently, an automatic integrated system for breath analysis (FUDAC-77) has been developed; this device automatically calculates the correct BMR by application of the method of least squares (a straight line experiment) (7), and remarkably facilitates its monitoring.

Anti-convulsion drug treatment (1), IVIG therapy (8), and plasma exchange (9) are reported to be effective modalities for IS patients; however, no study thus far has performed an objective therapeutic evaluation of the parameters that reflect the symptoms, such as the BMR. The condition of IS patients is reflected in real time in the BMR.

In the present case, CBZ treatment reduced spontaneous muscle activities, i.e., painful cramps. The BMR was reduced in tandem. However, the gait disturbance and posture did not improve because of completely uncontrolled continuous muscle fiber activities. Spontaneous and continuous muscle fiber activities were finally controlled by IVIG treatment.

Improvement shown by patients treated with immunomodulatory treatments is observed neurophysiologically, but quantitative assessment is also necessary. Serial BMR monitoring is a well-tolerated, quantitative assessment for IS patients with such a fluctuating course.

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Antibodies to the $GABA_B$ receptor in limbic encephalitis with seizures: case series and characterisation of the antigen

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Summary

Background Some encephalitides or seizure disorders once thought idiopathic now seem to be immune mediated. We aimed to describe the clinical features of one such disorder and to identify the autoantigen involved.

Methods 15 patients who were suspected to have paraneoplastic or immune-mediated limbic encephalitis were clinically assessed. Confocal microscopy, immunoprecipitation, and mass spectrometry were used to characterise the autoantigen. An assay of HEK293 cells transfected with rodent $GABA_{B1}$ or $GABA_{B2}$ receptor subunits was used as a serological test. 91 patients with encephalitis suspected to be paraneoplastic or immune mediated and 13 individuals with syndromes associated with antibodies to glutamic acid decarboxylase 65 were used as controls.

Findings All patients presented with early or prominent seizures; other symptoms, MRI, and electroencephalography findings were consistent with predominant limbic dysfunction. All patients had antibodies (mainly IgG1) against a neuronal cell-surface antigen; in three patients antibodies were detected only in CSF. Immunoprecipitation and mass spectrometry showed that the antibodies recognise the B1 subunit of the $GABA_B$ receptor, an inhibitory receptor that has been associated with seizures and memory dysfunction when disrupted. Confocal microscopy showed colocalisation of the antibody with $GABA_B$ receptors. Seven of 15 patients had tumours, five of which were small-cell lung cancer, and seven patients had non-neuronal autoantibodies. Although nine of ten patients who received immunotherapy and cancer treatment (when a tumour was found) showed neurological improvement, none of the four patients who were not similarly treated improved ($p=0\cdot005$). Low levels of $GABA_{B1}$ receptor antibodies were identified in two of 104 controls ($p<0\cdot0001$).

Interpretation $GABA_B$ receptor autoimmune encephalitis is a potentially treatable disorder characterised by seizures and, in some patients, associated with small-cell lung cancer and with other autoantibodies.

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Introduction

Synaptic plasticity is an essential property of neurons that is involved in memory, learning, and cognition. Plasticity depends on the interactions of ion channels and synaptic receptors, including excitatory glutamate NMDA receptors and AMPA receptors, and inhibitory $GABA_B$ receptors.[1,2] In animal models, pharmacological or genetic disruption of these receptors result in seizures and changes in memory, learning, and behaviour.[3–6] Immune responses against these receptors would therefore be expected to result in similar symptoms. Indeed, two disorders, one associated with antibodies to extracellular epitopes of the NR1 subunit of NMDA receptors[7] and the other associated with antibodies to GluR1/2 subunits of AMPA receptors,[8] have recently been identified. These disorders result in encephalitis with prominent psychiatric, behavioural, and memory problems, often accompanied by seizures. The antibodies implicated in these two autoimmune disorders cause a decrease in the amounts of the target receptor in cultured neurons, suggesting the antibodies are pathogenic. Patients with these syndromes often respond to treatment, and in some patients the immune response occurs as a paraneoplastic event. These findings, as well as the prevalence of some of these disorders (eg, anti-NMDA receptor encephalitis[7,9,10]), have raised the possibility that other syndromes in which memory and behaviour are impaired and seizures are common could also be immune mediated. In some of these syndromes an immune-mediated pathogenesis is suggested by the clinical response to immunotherapy, the CSF and MRI findings suggesting limbic encephalitis, and the detection of antibodies to unknown neuronal cell-surface antigens. We aimed to identify the autoantigen involved in a new disorder that has most of these suggestive features.

Methods

Study population

Between January, 2006, and June, 2009, we studied 410 patients with encephalitis suspected to be paraneoplastic or immune mediated. These patients were seen by the authors or by clinicians at other institutions and the patients' sera and CSF were sent for analysis of novel autoantibodies to the Center for Paraneoplastic Disorders at the University of Pennsylvania (PA, USA). We identified autoantibodies in the serum or CSF of 357 patients, including 275 patients with antibodies to NMDA receptors (including 75 patients previously reported[7]), 27 with antibodies to voltage-gated potassium

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channels, 19 with antibodies to glutamic acid decarboxylase 65 (GAD65), 15 with antibodies to AMPA receptors (including ten patients previously reported[8]), 11 with anti-Ma2 antibodies, eight with anti-HuD antibodies, and two with anti-CRMP5 antibodies (patients each had only one of these antibodies). Of the remaining 53 patients, 15 had serum or CSF antibodies with reactivity against neuronal cell-surface antigens predominantly in the neuropil of sectioned rat brain. Because of the serum and CSF findings and the response to immunotherapy and cancer treatment of the first of these patients to be clinically and immunologically studied (the index patient), we focused on these 15 patients. Clinical information about the patients was obtained by the investigators or provided by referring physicians. Patients were said to have neurologically improved if they were able to function independently or with little assistance when they returned home. Control samples were CSF or serum from 104 patients, including 91 randomly selected by use of an online random integer generator from the 410 individuals with encephalitis and 13 who had syndromes associated with GAD65 antibodies and who were not included in the group of 410 patients. These 13 patients were seen either by the study investigators or their serum, CSF, and clinical information were sent from other institutions to the primary investigator (JD) for study of disorders of unknown cause.

Studies were approved by the University of Pennsylvania Institutional Review Board, and written informed consent was obtained from all patients or their representatives.

Procedures

To establish whether serum or CSF contained antibodies to neural tissue, sagittal sections were taken from the brains of adult female Wistar rats; brains had been immersed in 4% paraformaldehyde at 4°C for 2 h, cryoprotected with 40% sucrose for 24 h, and snap frozen in chilled isopentane. Paraffin-embedded tumour tissue from patients was deparaffinised and the antigens retrieved.[11] 7 μm thick frozen (or 4 μm paraffin) tissue sections were incubated with 0·3% hydrogen peroxide for 20 min, with 10% goat serum in PBS for 1 h, and with patients' or control individuals' serum (1:250) or CSF (1:10) or a guineapig polyclonal antibody against an intracellular epitope of the $GABA_{B1}$ receptor (1:200; AB2256, Millipore, Billerica, MA, USA) at 4°C overnight. After using the appropriate secondary antibodies (all 1:2000, diluted in PBS with 5% goat serum), labelling was developed with the avidin–biotin–peroxidase method. Results were photographed under a fluorescence microscope using Zeiss Axiovision software (Zeiss, Thornwood, NY, USA).

Immunohistochemistry with human tissue (small-cell lung cancer) was done by use of IgG purified from patients' or control individuals' sera and labelled with biotin.[12] No secondary antibody was needed, thus avoiding background labelling caused by other human IgG in the tissue.

To identify the antigen and its localisation on cells in vitro, rat hippocampal neuronal cultures were prepared as reported previously.[13] Live neurons grown on coverslips were incubated for 1 h at 37°C with patient or control serum (final dilution 1:200) or CSF (1:10). After removing the media and washing with PBS, neurons were fixed with 4% paraformaldehyde and were made permeable with 0·1% Triton X-100 (Sigma-Aldrich, St Louis, MO, USA). Neurons were single or double immunolabelled with a guineapig polyclonal $GABA_{B1}$ receptor antibody (1:200), followed by the corresponding Alexa Fluor secondary antibodies (1:2000; Molecular Probes, Invitrogen, Eugene, OR, USA). Results were photographed as detailed above.

Rat hippocampal neurons were grown in 100 mm wells (10^6 neurons per well) and incubated at 37°C with filtered serum (1:500) for 1 h. Neurons were then washed with PBS, lysed with buffer (sodium chloride 150 mM, EDTA [edetic acid] 1 mM, tris(hydroxymethyl) aminomethane [Tris]-hydrochloric acid 100 mM, deoxycholate acid 0·5%, 1% Triton X-100, pH 7·5) containing protease inhibitors (P8340; Sigma-Aldrich), and centrifuged at $16·1 \times 10^3$ gravities for 20 min at 4°C. The supernatant was retained and incubated with protein A/G agarose beads (20423; Pierce, Rockford, IL, USA) overnight at 4°C, centrifuged, and the pellet containing the beads with patients' antibodies bound to the target cell-surface antigen was washed with PBS, aliquoted, and kept at –80°C. A 25 μL aliquot of this pellet was resuspended in Laemmli buffer, boiled for 10 min, separated in 4–15% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), and the proteins visualised with EZBlue gel staining (G1041; Sigma-Aldrich). Protein bands from the gels were cut and sent for mass spectrometry to the Proteomics Core Facility of the Genomics Institute at the Abramson Cancer Center (University of Pennsylvania, PA, USA). Protein bands were trypsin digested and analysed with a nanoLC/nanospray/LTQ mass spectrometer (Thermo Electron Corporation, San Jose, CA, USA) as reported previously.[14] Briefly, a 3 μL trypsin-digested sample was injected with autosampler (Eksigent, Dublin, CA, USA). The digested samples were separated on a 10 cm C18 column, using nanoLC (Eksigent) with a 200 μL/min flow rate, and a 45 min gradient. Online nanospray was used to spray the separated peptides into a linear trap quadrupole, and raw data were obtained with Xcalibur software (Thermo Scientific, Waltham, MA, USA). The raw data files were searched against the National Center for Biotechnology Information and Swiss-Prot (Swiss Institute of Bioinformatics, Basel, Switzerland) databases with Mascot (Matrix Science, Boston, MA, USA). The cutoff score for definite protein identification was 70 or more.

After characterisation of the antigen, frozen samples of the pellets were separated in SDS-PAGE, transferred to nitrocellulose (162-0115; Bio-Rad, Hercules, CA, USA),

and blotted with the polyclonal antibodies against $GABA_{B1}$ (1:2000) or $GABA_{B2}$ (1:1000) receptor subunits. The reactivity was developed by use of biotinylated anti-guineapig IgG made in goat (1:2000; Vector Laboratories, Burlingame, CA, USA) and the avidin–biotin–peroxidase diaminobenzidine method.

To determine the sensitivity and specificity of patients' antibodies for the $GABA_B$ receptor, we used a semi-quantitative confocal microscopy analysis similar to that used for other synaptic receptors.[7,8] Live rat hippocampal neurons cultured for 14–21 days in vitro were incubated with patients' CSF (1:30 dilution in Neurobasal B27 medium; GIBCO, Invitrogen, Carlsbad, CA, USA) for 24 h, washed in PBS, fixed in paraformaldehyde (4% paraformaldehyde, 4% sucrose in PBS) for 5 min, made permeable with 0·25% Triton X-100 for 10 min, and blocked with 5% normal goat serum for 1 h. Neurons were incubated with a guineapig polyclonal antibody against an intracellular epitope of the $GABA_B$ receptor (1:1000; Invitrogen) and a mouse monoclonal antibody against the presynaptic marker Bassoon (1:200; Stressgen, Victoria, BC, Canada), washed, and incubated with the appropriate fluorescent-conjugated secondary antibodies (1:1000, Molecular Probes).

A laser-scanning confocal microscope (Leica TCS SP2; Leica, Deerfield, IL, USA) was used to obtain images. For each image, laser light levels and detector gain and offset were adjusted so that no pixel values were saturated. Images were automatically segmented with an iterative thresholding approach that finds maxima of fluorescence intensity,[15] and areas of interest containing dendrites were selected, and the number of individual clusters along dendrites was quantified by use of ImageJ interactive software (Research Services Branch, National Institute of Mental Health, Bethesda, MD, USA) as described previously.[7,8] The colocalisation of clusters

	Sex	Age (years)	Tumour by imaging or pathology	Presenting symptoms	Other clinical and immunological features
Patient					
1	Female	60	SCLC	Subacute onset of complex partial seizures, confusion, memory impairment	SIADH
2	Male	66	SCLC	Subacute onset of seizures, confusion, memory deficit, behavioural problems	N-type VGCC antibodies
3	Female	53	SCLC	Rapidly progressive memory deficits, abnormal sleeping habits, followed by frequent seizures (focal, secondarily generalised), confusion, decline in mental status leading to coma	Pruritic rash with initial weakness
4	Male	75	Mediastinal adenopathy	Subacute onset of seizures, confusion, memory deficit, psychosis, encephalitis; died soon after presentation, before definitive diagnosis or treatment	Poor respiratory status, refused intubation
5	Male	68	Neuroendocrine tumour of the lung	Subacute onset of seizures, status epilepticus, confusion, memory deficit	..
6	Female	43	CT and FDG/PET negative	Subacute onset of secondarily generalised tonic-clonic seizures, confusion, bizarre behaviours, delusions, paranoia, memory impairment	N-type VGCC antibodies
7	Male	69	CT and FDG/PET negative	Subacute onset of seizures, status epilepticus, severe encephalopathy, severe memory deficit, confusion	History of bipolar disorder
8	Female	24	CT and FDG/PET negative	Subacute onset of seizures, status epilepticus, confusion, memory deficit, fever; required intubation and ventilation owing to poor level of consciousness and airway protection	N-type VGCC antibodies
9	Male	63	CT and FDG/PET negative	Subacute onset of seizures, confusion, memory deficit, paranoia, psychosis, gustatory hallucinations	TPO and GAD65 antibodies; hypothyroidism and type 2 diabetes mellitus
10	Female	45	Benign ovarian mass	Subacute onset of complex partial and generalised seizures, confusion, short-term memory deficits	TPO and thyroglobulin antibodies in serum (not in CSF); no endocrinopathy
11	Female	62	CT chest, abdomen, pelvis negative	Subacute onset of generalised seizures, confusion, memory deficit, decreased level of consciousness, fluent aphasia, abnormal orolingual movements	..
12	Male	29	CT and FDG/PET negative	Subacute onset of temporal lobe and generalised tonic-clonic seizures, confusion, memory deficits; no cognitive deterioration	Childhood seizures
13	Female	30	CT and FDG/PET negative	3-month history of severe memory deficit, confusion, followed by seizures (generalised, subclinical)	GAD65 antibodies without endocrinopathy
14	Male	69	SCLC	Subacute onset of generalised tonic-clonic seizures, worsened short-term memory deficit, confusion	Mild short-term memory deficit from past history of subarachnoid haemorrhage
15	Male	70	SCLC	Subacute onset of seizures (partial motor and generalised); severe short-term memory loss, confusion, confabulation, visual hallucinations, disorientation, agitation	GAD65, TPO, and SOX1 antibodies; no endocrinopathy
Control					
1	Female	63	CT and FDG/PET negative	1 year progression of cerebellar ataxia; normal mental status, no seizures, no muscle spasms or stiffness	GAD65 antibodies, adult-onset insulin-dependent diabetes mellitus
2	Female	61	CT and FDG/PET negative	6 week history of gait disturbance, lower extremity myoclonus and stiffness; dysphagia, dysarthria, nystagmus, left gaze palsy. No seizures or cognitive symptoms	GAD65, TPO, and thyroglobulin antibodies (mild thyroid dysfunction)

SCLC=small-cell lung cancer. SIADH=syndrome of inappropriate antidiuretic hormone. VGCC=voltage-gated calcium channel. FDG=fluorodeoxyglucose. TPO=thyroid peroxidase. GAD65=glutamic acid decarboxylase 65. SOX1=sex determining region Y-box 1.

Table 1: **Demographic features and symptoms**

labelled with patients' antibodies, commercial $GABA_B$ antibodies, and Bassoon was quantified using a software macro (written by EH) in ImageJ.

Owing to the reactivity of patients' antibodies with rat tissue and hippocampal neuronal cultures, and the homology between human and rat $GABA_B$ receptor sequences (the B1 receptor subunit has 91·3% cDNA sequence identity and 98·6% amino acid sequence identity in the two species),[16] HEK293 cells were transfected with plasmids containing rodent $GABA_{B1}$ or $GABA_{B2}$ or plasmids without an insert (control), by use of a method previously reported.[7] In other experiments, cells were transfected with $GABA_{B1}$ and $GABA_{B2}$ in equimolar ratios. Cells were then grown for 24 h before assessment. Transfected cells were fixed in 4% paraformaldehyde, made permeable with 0·1% Triton X-100, and then incubated overnight at 4°C with patients' serum (1:200) or CSF (100%) and the guineapig polyclonal $GABA_{B1}$ receptor antibody (1:20000) or a polyclonal $GABA_{B2}$ receptor antibody (1:10000, generated by SJM), washed in PBS, and incubated with the appropriate Alexa Fluor secondary antibodies (1:2000). Results were photographed as before.

Antibody titres were obtained by use of HEK293 cells expressing $GABA_{B1/B2}$ incubated with serial dilutions of serum and CSF, starting at 1:1 dilution. Patients' antibody IgG subtypes in serum or CSF were identified by use of the HEK293 transfected cells and secondary anti-human

	MRI	CSF	Serum antibody titres*	CSF antibody titres*	Chronological list of treatments	Outcome (duration of follow-up)
Patient						
1	FLAIR/T2 increased signal in medial temporal lobes	9 WBC per μL; protein 350 mg/L; no OCBs	640	160	IVIg, corticosteroids, chemotherapy	Substantial improvement; mild residual short-term memory deficit; lives independently; seizure free (12 months)
2	Normal	Normal	1280	..	Corticosteroids, IVIg, chemotherapy	Substantial improvement; died of metastatic disease (15 months)
3	FLAIR/T2 increased signal in medial temporal lobes	..	160	..	Tumour removal (lobectomy), IVIg	Partial improvement after tumour removal and IVIg (4 months); lost to follow-up
4	Normal	..	2560	640	None	Died soon after presentation of rapidly progressive respiratory failure
5	FLAIR/T2 increased signal in medial temporal lobes	..	1280	..	Supportive	Died 6 months after symptom presentation; $GABA_B$ antibodies detected after patient's death in archived serum
6	FLAIR/T2 increased signal in small area of corpus callosum	95 WBC per μL; protein 1040 mg/L; increased IgG index	..	640	Corticosteroids, mycophenylate mofetil	Substantial improvement; lives independently; seizure free (9 months)
7	FLAIR/T2 increased signal in left medial temporal lobe	..	..	640	Corticosteroids, plasma exchange	Initial substantial response to corticosteroids; relapsed 1 month later; died after 5 months in ICU with refractory seizures, status epilepticus, and systemic complications; $GABA_B$ antibodies detected after patient's death in archived serum
8	FLAIR/T2 increased signal in medial temporal lobes	19 WBC per μL; protein 460 mg/L	5120	2560	Corticosteroids, plasma exchange	Substantial improvement; mild residual short-term memory deficit; seizure free (3 months)
9	FLAIR/T2 increased signal in medial temporal lobes	75 WBC per μL; protein 260 mg/L; OCBs present	Negative	4	Corticosteroids	Full recovery (41 months)
10	FLAIR/T2 increased signal in medial temporal lobes	81 WBC per μL; protein 300 mg/L	10240	..	Corticosteroids	Substantial improvement. Residual short-term memory deficit. Lives independently. Seizure free (72 months)
11	Normal	20 WBC per μL; protein 220 mg/L	40	40	Corticosteroids	Full recovery (6 months)
12	FLAIR/T2 increased signal in left medial temporal lobe and insula	950 WBC per μL; OCBs present	Negative	10	Symptomatic	Temporal lobe biopsy 20 months after symptom presentation showing reactive astrocytosis, without inflammation; no follow-up available after biopsy
13	FLAIR/T2 increased signal in medial temporal lobes	4 WBC per μL; protein 1090 mg/L; 6 OCBs	Negative	4	Corticosteroids	Full recovery, except for infrequent brief episodes of visual hallucinations (10 months)
14	FLAIR/T2 increased signal in left medial temporal lobe	Traumatic; negative cytology	..	80	Chemotherapy	Residual short-term memory deficit; seizures controlled; died of sepsis (3 months)
15	Normal	0 WBC per μL; protein 950 mg/L	..	640	IVIg, corticosteroids, chemotherapy	Seizures responded to antiepileptics; memory deficit persisted; died of cancer-related treatment (2 months)
Control						
1	Normal	3 WBC per μL; protein 780 mg/L; 1 OCB	Negative	2	IVIg	No seizures or cognitive deficits; limited response of cerebellar ataxia to IVIg (12 months)
2	Normal	2 WBC per μL; protein 520 mg/L; OCBs present	Negative	2	IVIg, corticosteroids	No seizures or cognitive deficits; full recovery after steroids and IVIg (12 months)

*Titres defined as the reciprocal of the maximal dilution that gave positive immunostaining. FLAIR=fluid-attenuated inversion recovery. WBC=white blood cells (normal <4 per μL). OCB=oligoclonal band. IVIg=intravenous immunoglobulin. ICU=intensive care unit.

Table 2: **Diagnostic tests, treatment, and outcome**

antibodies specific for IgG1, IgG2, IgG3, or IgG4 (all 1:50; Sigma-Aldrich) as reported.[17]

Statistical analysis

The association between $GABA_B$ receptor antibodies and other autoantibodies (GAD65, N-type voltage-gated calcium channel, thyroid peroxidase, thyroglobulin, or SOX1) and that between neurological improvement and cancer treatment or immunotherapy were analysed with Fisher's two-sided exact test. The colocalisation of patients' antibodies with the polyclonal $GABA_B$ receptor antibodies or antibodies to the synaptic marker Bassoon was analysed with the Student's *t* test.

Role of the funding source

The study sponsor had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

In May, 2008, a 60-year-old woman with a long history of smoking was admitted to hospital with confusion, memory problems, and new-onset generalised tonic-clonic and partial complex seizures refractory to treatment (index patient; patient 1). At examination, she was confused about the time and where she was and had poor concentration and short-term memory (table 1). Although she had saccadic pursuits with lateral gaze, no cranial nerve abnormalities were noted. Strength, sensation, reflexes, and coordination were normal. MRI of the brain showed increased fluid-attenuated inversion recovery (FLAIR) signal in the medial temporal lobe of both hemispheres, compatible with limbic encephalitis (table 2, figure 1A). Diffuse slowing and bilateral periodic lateralised epileptiform discharges were seen on encephalography (EEG). In the CSF there were nine white blood cells per μL, total protein concentration was 350 mg/L, and glucose concentration was 3·94 mmol/L; there were no oligoclonal bands and cytological findings were normal. PCR for herpes simplex virus, West Nile virus, and St Louis encephalitis were negative. The patient had hyponatraemia (119 mEq/L) caused by syndrome of inappropriate antidiuretic hormone secretion. Combined CT and fluorodeoxyglucose-PET showed mediastinal lymphadenopathy, which was proven by biopsy to be small-cell lung cancer. The patient was treated with antiepileptic drugs (levetiracetam, valproic acid, and phenytoin) and immunotherapy (intravenous immunoglobulins and corticosteroids), immediately followed by chemotherapy with cisplatin and etoposide. The patient's short-term memory and cognition improved, and seizures resolved. After chemotherapy the patient had standard prophylactic whole-brain radiation therapy. Brain MRI 1 month after symptom presentation showed improvement of the abnormal FLAIR signal (figure 1B); MRI at 3 months and 9 months were unchanged except for progressive general atrophy, probably secondary to radiation (figure 1C, D). 1 year after symptom presentation, the patient had only mild deficits in memory and cognition and lived independently.

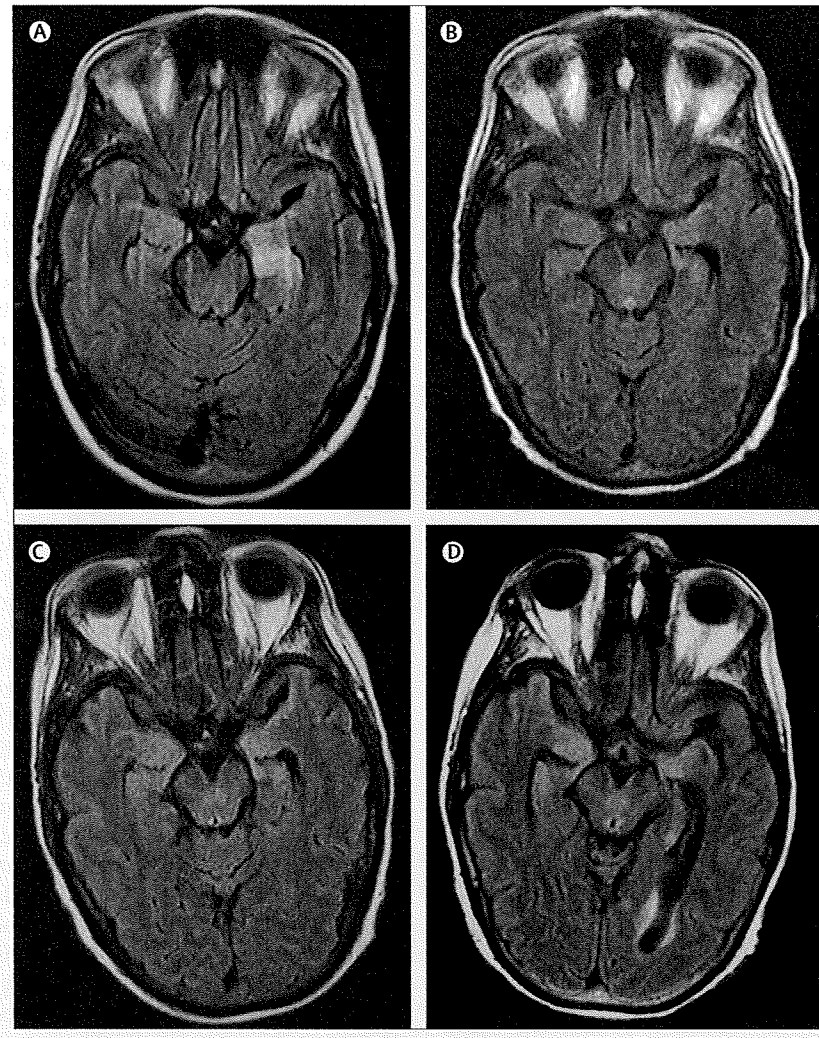

Figure 1: **MRI of a patient with $GABA_B$ receptor antibodies and limbic encephalitis**
Axial fluid-attenuated inversion recovery (FLAIR) MRI from patient 1 at presentation (A) showed increased signal in the medial temporal lobes, which was more pronounced on the left. Repeat study at 1 month (B) showed improvement of the FLAIR signal that remained stable at 3 months and 9 months (C, D), with development of mild generalised atrophy (the patient received standard whole-brain radiation therapy as prophylaxis for small-cell lung cancer metastases).

Sera and CSF from the index patient and the 14 other patients (patients 1–15) showed a pattern of reactivity with the neuropil of rat brain (figure 2) that was different from that reported with antibodies against NR1 subunits of the NMDA receptor, GluR1/2 subunits of the AMPA receptor, or voltage-gated potassium channels.[7,8,18] When non-fixed and non-permeabilised cultures of rat hippocampal neurons were incubated with patients' serum or CSF, intense reactivity with the cell surface was seen (figure 3A). Similar studies with serum or CSF from

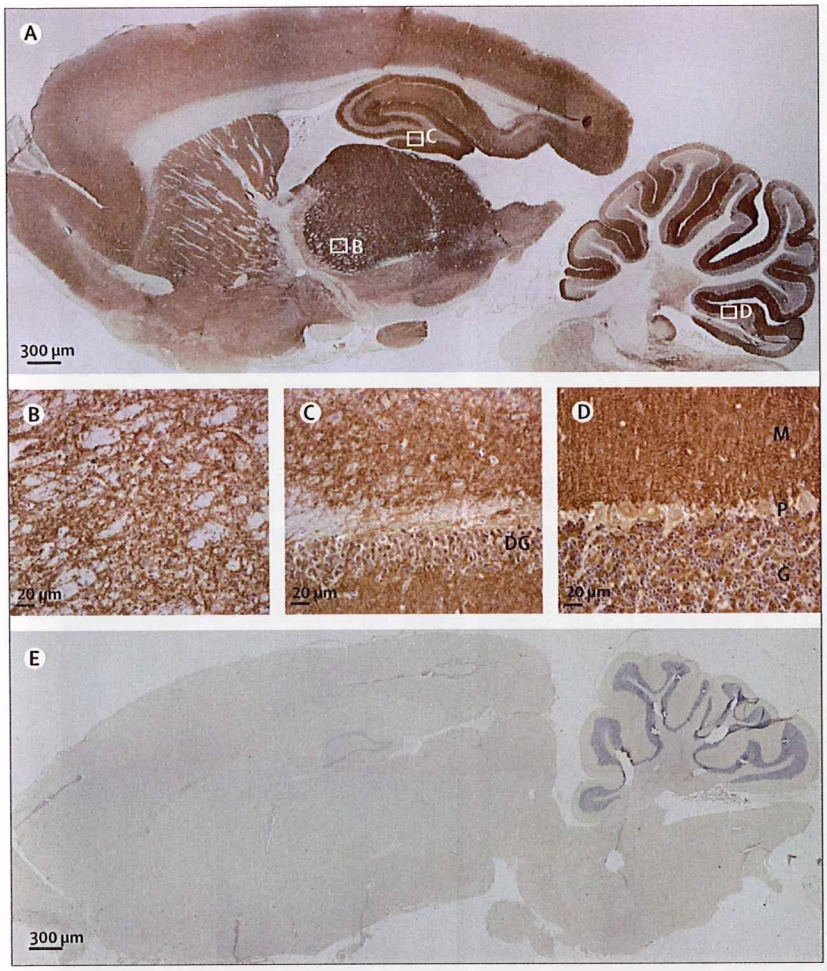

Figure 2: **Immunolabelling of rat brain with patients' antibodies**
Sagittal section of rat brain immunolabelled with CSF of a patient with limbic encephalitis (A) and a control individual (E). Note the extensive staining in A of the neuropil of thalamus (B), hippocampus (C), cerebellum (D), and cerebral cortex. DG=dentate gyrus. M=molecular layer. P=Purkinje cell layer. G=granular cell layer. Avidin–biotin–peroxidase method; sections counterstained with haematoxylin.

control individuals showed no reactivity with rat brain tissue (figure 2E) or cultures of neurons (figure 3B).

The $GABA_B$ receptor was identified as the target antigen by immunoprecipitation of the antigen with patients' serum samples and peptide sequence recognition ($GABA_{B1}$ and $GABA_{B2}$) by mass spectrometry (webappendix). Immunoprecipitates were obtained using the serum from four patients. Electrophoresis of the immunoprecipitates showed similar protein bands at about 90 kDa and 105 kDa (Figure 3C). The indicated protein bands contained sequences derived from $GABA_{B1}$ and $GABA_{B2}$ (protein scores for B1: 160, 225, 342, and 178; protein scores for B2: 1094, 1784, 1946, and 2653).

See Online for webappendix

The results were confirmed by immunoblotting the immunoprecipitates with antibodies specific for $GABA_{B1}$ and $GABA_{B2}$. Immunoblot analysis confirmed that the band at about 105 kDa was recognised by anti-$GABA_{B1}$ and anti-$GABA_{B2}$ antibodies, and the band at about 90 kDa was recognised by anti-$GABA_{B1}$ antibodies (figure 3D).

Colocalisation of patients' antibodies with the $GABA_B$ receptor and the synaptic and extrasynaptic location of the target receptors were noted on confocal microscopy. The colocalisation of patients' antibody clusters with $GABA_B$ receptor clusters (figure 4) was quantified for the dendrites of 23 neurons on four separate coverslips. 103% (SE 0·8%) of the clusters labelled with antibodies from patients colocalised with clusters labelled by the guineapig polyclonal $GABA_B$ receptor antibody, and 107% (SE 0·7%) of guineapig antibody-labelled clusters colocalised with those labelled by patients' antibodies (numbers slightly higher than 100% occur because of overlapping of a few clusters labelled by patient antibodies with two guineapig antibody-labelled clusters and vice versa). These results suggest that all patients' antineuronal cell-surface antibodies target the $GABA_B$ receptors and that almost all neuronal $GABA_B$ receptors are labelled by patients' antibodies. 62% (SE 1·3%) of $GABA_B$ receptor clusters labelled by patients' antibodies were also labelled by Bassoon, significantly fewer than those also labelled by guineapig $GABA_B$ receptor antibodies (Student's t test, $p<0·0001$), suggesting that patient antibodies bind both synaptic and extrasynaptic $GABA_B$ receptors.

The location of the epitope in $GABA_{B1}$ was identified with HEK293 cells transfected with $GABA_{B1}$, $GABA_{B2}$, or both $GABA_B$ receptor subunits. All 15 patients had serum or CSF antibodies that reacted with $GABA_{B1}$ (figure 5), and one had additional reactivity with the $GABA_{B2}$ subunit (data not shown). Similar studies with the 104 control individuals showed that two patients, both with syndromes attributed to GAD65 autoimmunity, had $GABA_{B1}$ receptor antibodies at low titres (CSF 1:2, serum negative), which did not bind at detectable levels to sections of rat brain (Fisher's exact test, $p<0·0001$, data not shown).

Samples from the six patients for whom sufficient serum or CSF was available were analysed for antibody IgG subtypes. All six patients had IgG1 $GABA_{B1}$ antibodies, two had additional IgG3, and one had IgG2 antibodies.

Table 1 shows demographic features and symptoms of the 15 patients and the two control individuals who had antibodies to GAD65. Among the 15 patients, median age was 62 years (range 24–75); eight were men. All patients had seizures, confusion, and memory deficits. In 13 patients the seizures were the presenting symptom; in two (patients 3 and 13) memory deficit and confusion were the presenting symptoms. After further clinical assessment most seizures appeared to have a temporal-lobe onset with secondary generalisation, and three patients had status epilepticus.

Ten patients had unilateral or bilateral increases in medial temporal lobe FLAIR/T2 signal consistent with limbic encephalitis, and one had a small area of increased FLAIR signal in the corpus callosum (table 2). Four patients had normal brain MRI.

CSF was abnormal in nine of ten patients for whom data were available. The most common CSF abnormality was lymphocytic pleocytosis in eight patients. EEG results were available from 12 patients: nine had temporal-lobe seizures, epileptiform discharges, or temporal-lobe slowing; two had generalised slowing; and one had no abnormalities. Several types of seizures were noted on EEG, including complex partial seizures (often of temporal-lobe onset), status epilepticus, and subclinical seizures.

The two control individuals with low titre $GABA_{B1}$ antibodies developed different syndromes in association with high titre GAD65 antibodies in serum and CSF. Neither of these two patients developed seizures or limbic dysfunction (table 1). One had progressive cerebellar ataxia, and the other had gait instability, muscle stiffness, rigidity, myoclonus, and dysarthria, categorised as progressive encephalomyelitis with rigidity and myoclonus.

In addition to $GABA_B$ antibodies, seven of 15 patients had antibodies to one or more of the following: GAD65 (3 patients), thyroid peroxidase (3 patients), N-type voltage-gated calcium channels (3 patients), and SOX1 (1 patient). Only one of the three patients with GAD65 antibodies had endocrinopathy, and one of the three patients with voltage-gated calcium channel antibodies had small-cell lung cancer (table 1). The patient with SOX1 antibodies had small-cell lung cancer.

Seven patients had tumours (table 1), detected at the time of neurological symptom presentation. Of these patients, five had small-cell lung cancer, one had a lung tumour of neuroendocrine origin, and one had mediastinal adenopathy. No other systemic tumours were identified. Because most lung tumours were diagnosed by use of needle biopsy, no tissue was available for analysis of $GABA_B$ receptor expression. However, three of four small-cell lung cancers from control individuals without antibodies or encephalitis (archived tissue from the Division of Anatomic Pathology, University of Pennsylvania) showed reactivity with a guineapig polyclonal antibody to $GABA_{B1}$ receptor and patients' biotinylated IgG, suggesting that these receptors are expressed by small-cell lung cancer (webappendix).

Five of the patients were young (median age 30 years, range 24–45), were non-smokers, and had negative cancer screening including CT/fluorodeoxyglucose-PET, and two of these patients had long-term follow-up (41 and 72 months), making the presence of cancer unlikely.

Nine of 15 patients had a neurological response to immunotherapy (six) or treatment of the tumour as well as immunotherapy (three). The median follow-up of these nine patients was 10 months (range 3–72 months). One patient (patient 2) later died of tumour progression (15 months) and one (patient 3) was lost to follow-up at 4 months. Six patients did not have sustained neurological improvement: three patients (patients 4, 14, and 15) died from tumour or chemotherapy-related complications soon after presentation of the disorder, two were diagnosed with $GABA_B$ receptor antibodies after death (patients 5 and 7), and one was lost to follow-up (patient 12). Of the latter three, only patient 7 was thought to have an autoimmune disorder, and therefore this patient received corticosteroids and plasma exchange; the other two patients did not receive immunotherapy. Overall, after excluding one non-assessable patient (patient 12) nine of the ten patients who received immunotherapy and cancer treatment (when a tumour was found) showed neurological improvement, while none of the four patients (patients 4, 5, 14, and 15) who did not receive immunotherapy or whose tumour treatment was not completed showed improvement (Fisher's exact test p=0·005).

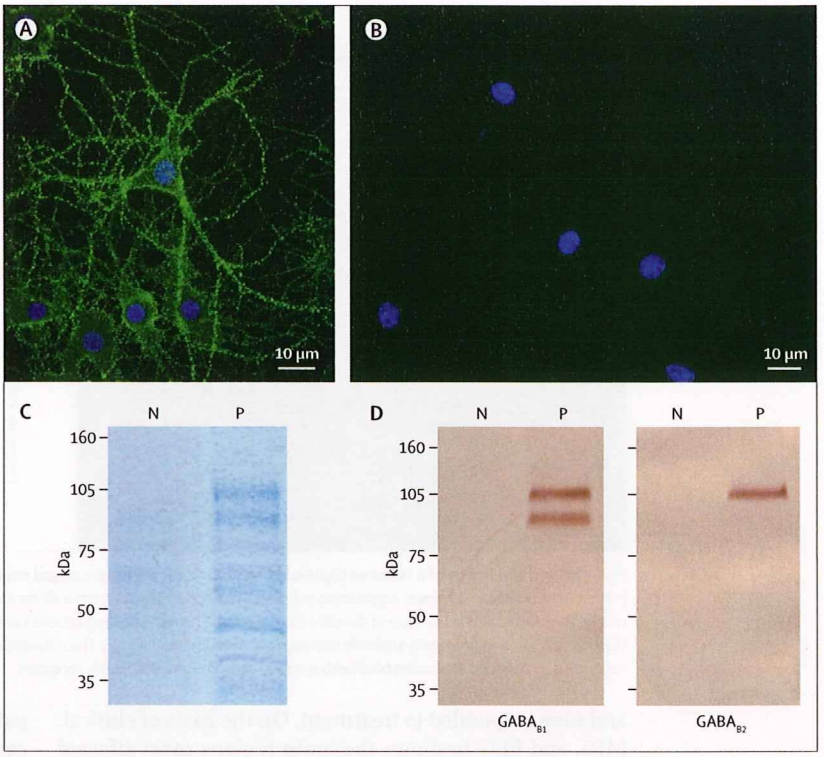

Figure 3: **Culture of rat hippocampal neurons incubated (live, non-permeabilised) with the CSF of a patient with limbic encephalitis and a control individual**
Note the intense punctate reactivity of patient's antibodies with cell surface antigens (A) and the absence of reactivity in the control (B); nuclei of neurons stained with 4',6-diamidino-2-phenylindole (DAPI). The surface antigens were precipitated using the antibodies within the patient's serum, and then electrophoretically separated and visualised with EZBlue (C). Patient's antibodies (P) precipitated two main protein bands at about 105 kDa and 90 kDa; these bands are not seen in the precipitate using serum from a control individual (N). Sequencing of the 105 kDa band by use of mass spectrometry showed it contained the B1 and B2 subunits of the $GABA_B$ receptor (webappendix). The 90 kDa and other smaller bands were proteolytic fragments and patient's IgG products. Subsequent transfer of the gel to nitrocellulose and immunoblotting with antibodies specific for each of the $GABA_B$ (D) subunits confirmed that patient's antibodies precipitated the B1 and B2 subunits (105 kDa) and that the 90 kDa band was a proteolytic fragment of B1.

Discussion

15 patients had autoimmune encephalitis associated with antibodies to extracellular epitopes of the $GABA_B$ receptor

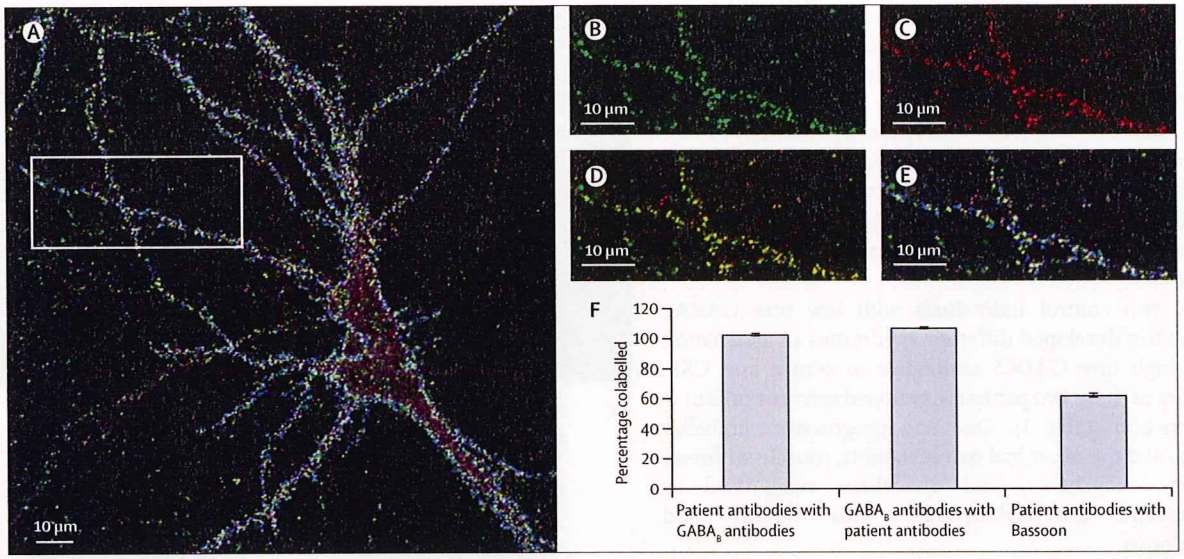

Figure 4: **Confocal image of a cultured triple labelled embryonic rat hippocampal neuron**
Patient's antibodies are in green, a guineapig polyclonal antibody against an intracellular epitope of the GABA$_{B1}$ receptor is in red, and an antibody to the presynaptic marker Bassoon is in blue (A). Area of dendrite from the same neuron showing patient's antibody staining (B), guineapig polyclonal GABA$_{B1}$ receptor antibody staining (C), both patient and guineapig antibody staining (D), and triple staining (E). The colocalisation of labelling of the dendrites of 23 neurons was quantified (F). This suggests that patients' antibodies bind both synaptic and extrasynaptic GABA$_B$ receptors.

and nine responded to treatment. On the basis of clinical, MRI, and EEG findings, the brain regions most affected are the hippocampi and temporal lobes. Thus, it is not surprising that the resulting syndrome is similar to other types of limbic encephalitis (eg, encephalitis associated with antibodies against AMPA receptors or voltage-gated potassium channels), although some clinical and immunological features might suggest GABA$_B$ receptor autoimmunity. We have reported development of seizures in all patients, the association with lung cancer in seven patients (five pathologically confirmed as small-cell lung cancer), and the presence of autoantibodies of unclear relation to this type of limbic encephalitis in seven patients. Disruption of GABA$_B$ receptors by patients' antibodies is a possible explanation for the symptoms because pharmacological[19–21] and genetic[3,4] changes to these receptors in rodents result in phenotypes similar to limbic encephalitis, including prominent seizures, memory deficits, increased anxiety, and mood dysregulation.[22] Moreover, in human beings, some GABA$_B$ receptor polymorphisms are associated with temporal-lobe epilepsy.[23]

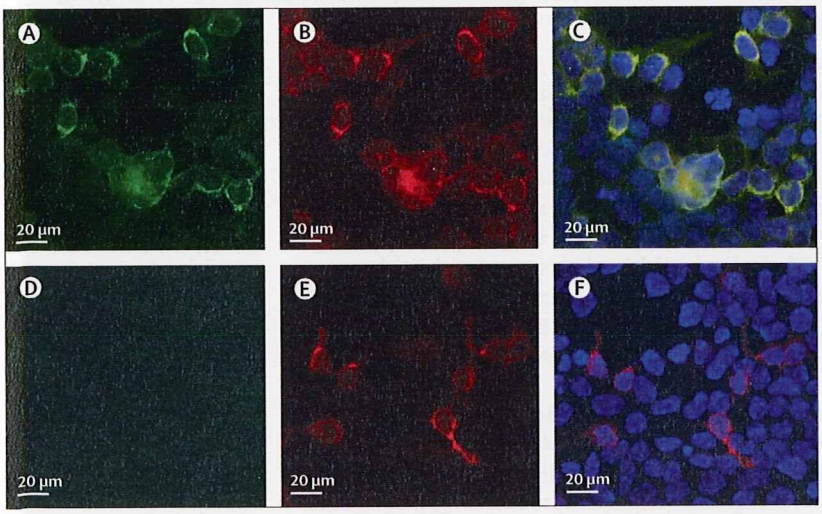

Figure 5: **Detection of antibodies to the GABA$_{B1}$ subunit using a HEK293 cell-based assay**
HEK293 cells transfected with the GABA$_{B1}$ receptor subunit show reactivity with CSF from a patient with limbic encephalitis (A) and a polyclonal antibody against the B1 subunit of the GABA$_B$ receptor (B); both reactivities are merged in C. Similarly transfected cells do not react with CSF from a control individual (D) but do show reactivity with a polyclonal antibody against the B1 subunit of the GABA$_B$ receptor (E); reactivities merged in F. Immunofluorescent method.

GABA$_B$ receptors are G-protein-coupled receptors composed of two subunits, GABA$_{B1}$ and GABA$_{B2}$.[19,24] GABA$_B$ receptors mediate presynaptic inhibition by at least two mechanisms: the activation of G-protein-coupled-inward rectifying potassium channels and the inhibition of calcium channels.[25] These receptors also attenuate presynaptic firing frequencies.[26] Postsynaptic GABA$_B$ receptors mediate inhibition by similar mechanisms[27] and by inducing a slow inhibitory postsynaptic potential.[28] GABA$_B$ receptors limit the duration of network high-activity states, preventing excessive neuronal synchronisation, and allowing new stimuli to break synchronous activity.[29,30] GABA$_B$ receptors are widely distributed in the brain and spinal cord, but the highest levels of GABA$_B$ receptors are found in the hippocampus, thalamus, and cerebellum.[31] In the current study, the corresponding areas of rat brain were more intensely immunolabelled by patients' antibodies. The main antigen recognised by the patients' antibodies, the GABA$_{B1}$ subunit, is necessary for GABA binding and receptor function, whereas the GABA$_{B2}$ subunit is

required for localisation of the receptor to appropriate areas of the cell membrane and G-protein coupling.[32,33]

By use of a HEK293 cell-based assay we showed that the sera or CSF of all 15 patients had antibodies that reacted with $GABA_{B1}$, with additional reactivity to $GABA_{B2}$ in one patient. These findings suggest that HEK293 cells expressing $GABA_{B1/B2}$ or $GABA_{B1}$ could be used as a diagnostic test.

A third of patients with encephalitis and $GABA_B$ receptor antibodies had pathologically confirmed small-cell lung cancer (age range 53–70 years, all smokers). The involvement of this type of tumour in paraneoplastic disorders and its ability to express synaptic proteins, including $GABA_B$ receptors, suggests that it might trigger the immune response against these receptors. In a subgroup of patients with limbic encephalitis and small-cell lung cancer previously thought to be without antibodies or attributed to antibodies against intracellular antigens, $GABA_B$ receptor autoimmunity is probably involved,[34] particularly in patients who improved after treatment of the tumour or immunotherapy.[35,36] Moreover, $GABA_B$ receptor autoimmune encephalitis also seems to develop without cancer association. In this respect, $GABA_B$ receptor autoimmune encephalitis is similar to other synaptic autoimmunities of the CNS (those involving antibodies to NMDA receptors or AMPA receptors)[8–10] or peripheral nervous system (those involving antibodies to acetylcholine receptors or P/Q-type voltage-gated calcium channels) that can develop with or without cancer.[37] As occurs in some of these disorders,[8] almost half of the patients with $GABA_B$ receptor autoimmune encephalitis (including five without tumours) had additional autoantibodies (to TPO, GAD65, SOX1, or N-type voltage-gated calcium channels), suggesting autoimmunity. The overlap with antibodies to GAD65 (an intracellular antigen) suggests that some patients with limbic encephalitis attributed to GAD65 autoimmunity might have $GABA_B$ receptor antibodies as a more likely cause of the symptoms.[38,39] As more relevant cell-surface or synaptic autoantigens are identified, subsets of disorders with unclear definitions, such as steroid-responsive encephalitis or Hashimoto's encephalitis without thyroid peroxidase antibodies in the CSF, will probably be reclassified.

The small number of patients with $GABA_B$ receptor antibodies and the retrospective identification of patients prevented us from assessing the contribution of cancer treatment, immunotherapy, or both, to neurological improvement. Moreover, we were unable to correlate antibody titres with clinical outcome because we did not have serial serum or CSF samples. As this disorder becomes more widely recognised, additional symptoms are likely to be identified. On the basis of the distribution of $GABA_B$ receptors in the brain, one would expect that some patients might develop encephalitis or seizure disorders with less focal limbic dysfunction. This could be tested using HEK293 cells that express $GABA_{B1/B2}$ or $GABA_{B1}$, as described in this paper. By the time antibodies are detected the serum titres can be very low, and we suggest examining both serum and CSF. Identification of these antibodies should prompt the search for a small-cell lung cancer. Recognition of this disorder is important because it is potentially responsive to immunotherapy and treatment of the tumour. The binding of patients' antibodies to the $GABA_B$ receptor in live rat neurons, and the similarity of the syndrome to experimental phenotypes in which this receptor does not function properly, suggest the antibodies are pathogenic. Although $GABA_{B1}$ receptor antibodies are mainly IgG1 and thus able to activate complement, the role of complement-mediated cytotoxicity is questionable in this potentially reversible disorder in which neurons are the main targets. Future studies should focus on the disease mechanism and effects of the antibodies.

Contributors

RC, JR, DF, MBS, WG, AK, KO, TI, MG, FG, and JD designed the study and clinically assessed the patients. EL, ML, XP, EH, and MG did the laboratory studies and prepared the figures. EL, SJM, RB-G, and JD were involved in study design, data analysis, and writing of the report.

Conflicts of interest

RC has received honoraria from Boehringer-Ingelheim and Orion Pharma for projects unrelated to the current study. SJM has received reimbursement for travel and accommodation expenses as well as funding support from Pfizer. JD has received royalties from a patent related to Ma2 autoantibody test and has filed patent applications for NMDA and $GABA_B$ receptor autoantibody tests. JD has received funding from Euroimmun for projects unrelated to the current study. All other authors have no conflicts of interest.

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BRIEF CLINICAL NOTES

胃腸炎後に発症した抗GQ1b抗体陽性の小脳失調を伴わない急性一側性外転神経麻痺の1例*

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Key Words : acute ophthalmoparesis, abducens nerve palsy, anti GQ1b antibody, gastroenteritis

50歳以下の若年成人において外転神経麻痺のみをきたすことは稀であるが，その原因疾患の中には感染後に外転神経麻痺のみを呈する症例が含まれる[1]．われわれは，胃腸炎後に急性に一側性外転神経麻痺をきたし抗GQ1b抗体陽性を示したことから，感染を契機とした免疫学的機序が原因と考えられた症例を経験したので報告する．

症　例

患者：39歳，女性．

主訴：左方注視時の複視，右手足のしびれ．

既往歴：特記すべきことはない．

家族歴：父が高血圧症，糖尿病，母が三叉神経痛，姉が橋本病に罹患．

現病歴：2007年12月下旬に1週間ほど急性胃腸炎に罹患した．12月28日に右手掌のしびれが出現し，30日の昼頃から右足底のしびれが出現した．31日，左方注視時に複視を自覚するようになった．症状が持続するため，2008年2月7日に当科に入院した．

入院時現症：身長151cm，体重37kg．体温36.2℃，血圧95/60mmHg，脈拍78/分・整．リンパ節腫大は認めなかった．心音，呼吸音に異常なく，腹部は平坦かつ軟であった．神経学的所見では意識清明で，脳神経領域では左眼の軽度外転制限を認め，左方視時に複視を自覚した．また，注視方向性のnystagmoid jerkを認めた．運動系では，筋力低下はなく，筋トーヌス，協調運動は正常であった．反射では，両上肢腱反射が亢進しており，病的反射は認めなかった．感覚系では，右手，右足底の異常感覚(ピリピリした感じ)を認めた．他覚的には表在覚，深部感覚に異常は認めなかった．立位・歩行，自律神経系に明らかな異常は認めなかった．

入院時検査所見：検血および一般生化学検査で異常は認めなかった．HbA1cは正常(4.9%，正常4.3～5.8%)で糖尿病は認めなかった．髄液検査では細胞数1/mm³(単核球)，蛋白33mg/dl，糖56mg/dlと正常範囲であり，IgG indexは正常(0.51，正常0.34～0.85)でmyeline basic proteinの上昇はなく，oligoclonal bandは陰性であった．入院時の眼窩MRI画像(造影)では外眼筋や海綿静脈洞，脳幹部に異常信号域，造影効果は認めなかった．Hess chartでは左眼の外転制限を認めた(図1)．末梢神経伝導検査(右正中神経，右尺骨神経，右後脛骨神経，右腓腹神経で施行)，下肢SSPEで異常は認めなかった．

* Acute abducens nerve palsy following gastroenteritis without cerebellar ataxia and with positive anti GQ1b antibody. A case report.(Accepted September 4, 2009).

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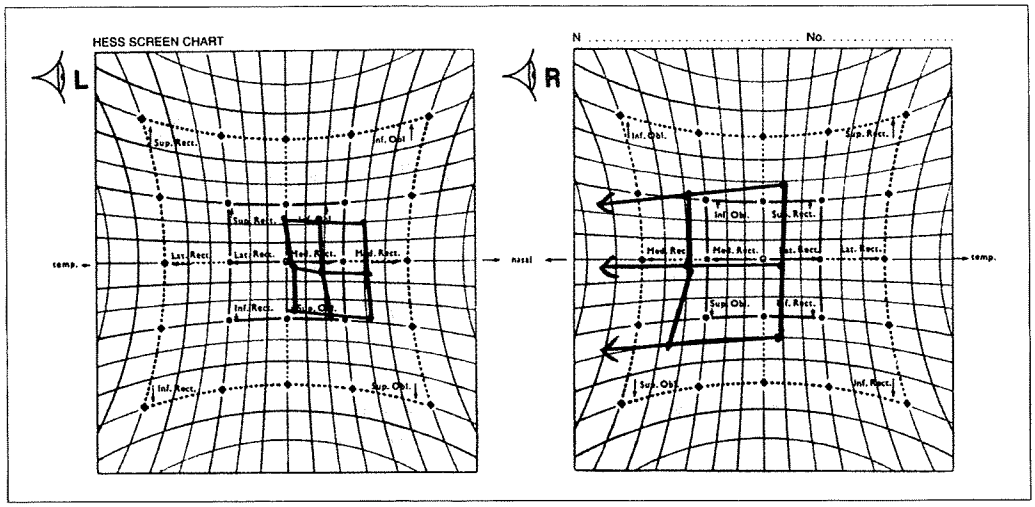

図1 Hess chart
左眼の外転制限を認める．

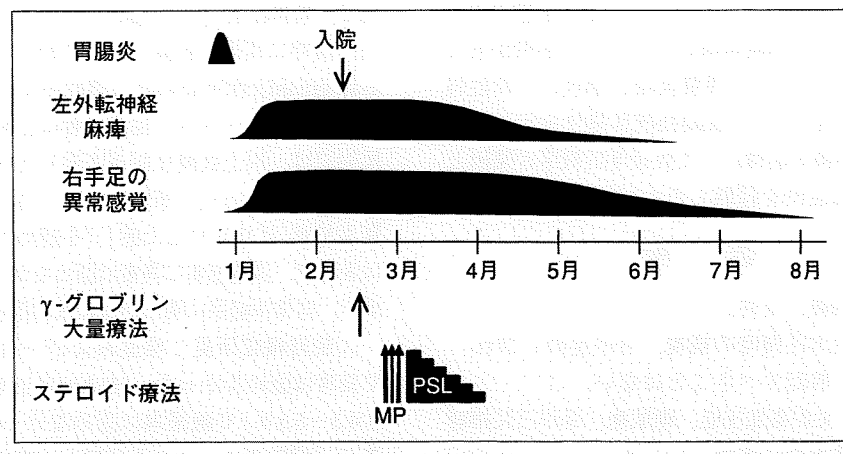

図2 臨床経過
MP：methylprednisolone 1,000mg/day×3days, PSL：prednisolone 60mg/dayから漸減．

入院後経過(図2)：入院後，左外転神経麻痺の原因として感染後の外転神経麻痺を考え，γ-グロブリン大量療法(献血グロベニン-I®)400mg/kg/day×5日間)を施行した．施行直後には明らかな治療効果を認めなかったため，さらにステロイドパルス療法(methylprednisolone 1,000mg/day×3日間)および後療法として経口ステロイド療法(prednisolone 60mg/dayから開始，1カ月で漸減)を行った．その約1週間後から徐々に左眼の外転制限，右手足のしびれは改善した．後に，当科入院時の血清で抗GQ1b-IgG抗体，抗GT1a-IgG抗体が強陽性と判明した．

考　察

本例の特徴は，①胃腸炎後に発症した急性一側性外転神経麻痺で，②小脳失調を伴わず，③後に抗GQ1b抗体陽性が判明した点である．症状，経過などから免疫学的機序の関与が想定される，抗GQ1b抗体陽性の小脳失調を伴わない急性一側性外転神経麻痺と診断した．経過や検査所見などから外転神経麻痺をきたす他疾患は否定した．

外転神経麻痺の原因は，高齢者では微小血管障害が多く，背景に高血圧症，糖尿病，高脂血症を伴うことが多い．しかし，20歳から50歳の若年成

人では外転神経麻痺のみを呈することは稀で，その原因疾患も高齢者とは異なると報告されている．本例でも高血圧症，糖尿病，高脂血症は認められていない．Georgeら[1]は，20歳から50歳の若年成人における外転神経麻痺の原因疾患について検討し，もっとも多い疾患は中枢神経系の腫瘤性病変(33%)で，次いで多発性硬化症(24%)，特発性(13%)，ウイルス感染後(9%)などと報告している．

感染後に外転神経麻痺をきたした100例のまとめ[2]では，平均年齢は42歳，性差はなく，先行感染では上気道炎がもっとも多く(38%)，下痢は14%にみられたとしている．また神経所見では，外転神経麻痺が両側にみられた症例は29%，腱反射低下～消失は27%，遠位部の異常感覚は7%に認め，抗GQ1b-IgG抗体は25%で陽性と報告されている．

感染後の外転神経麻痺の機序に関して，Miller Fisher症候群あるいはGuillain-Barré症候群の不全型として免疫学的な関与が考えられている．Chibaら[3]は，抗GQ1bモノクローナル抗体がヒトの動眼神経，滑車神経，外転神経の，脳幹外の末梢神経の部分に強く反応することを報告している．またShibataら[4]は，抗GQ1b抗体陽性で外転神経麻痺を呈した症例において，造影MRIで外転神経の脳槽内の部分が造影されたことを報告している．これらの報告から抗GQ1b抗体が核下性の外転神経障害に関与することが示唆される．しかし一方でYukiら[5]は，垂直性注視麻痺を伴わず外転障害に内転障害が加わった症例を報告し，その症例における原因病巣として中枢(とくに橋網様体傍正中部など)での障害を推察している．本例では深部腱反射の低下はなく，むしろ両上肢で亢進していたことから中枢性の障害も想定したが，電気生理学的検査，画像所見などにおいて中枢に責任病巣を示唆する変化を指摘できなかった．

本例での異常感覚に関して，電気生理学的検査で異常はなかったものの，本疾患はMiller Fisher症候群あるいはGuillain-Barré症候群の不全型と考えられており，末梢神経障害の可能性が考えられた．

まとめ

胃腸炎後に発症した小脳失調を伴わない急性一側性外転神経麻痺の39歳女性例を経験した．抗GQ1b抗体が陽性であり，免疫学的機序の関与が想定された．抗ガングリオシド抗体の測定がその発症機序の推定や治療に有用と思われた．

抗ガングリオシド抗体を測定していただいた，近畿大学神経内科・楠　進先生に深謝申し上げます．

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<Abstract>

Acute abducens nerve palsy following gastroenteritis without cerebellar ataxia and with postive anti GQ1b antibody. A case report.

by
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A 39-year-old female developed left acute abducens nerve palsy, and dysesthesia in her right hand and sole following gastroenteritis. We diagnosed her as having post-infection abducens nerve palsy and treated her with high-dose intravenous immunoglobulin. Since the treatment did not improve her symptoms, we added steroid therapy. One week later, her symptoms were ameliorated. Laboratory tests showed positive anti-GQ1b-IgG and anti-GT1a-IgG antibodies. The final diagnosis in this case was acute left abducens nerve palsy without cerebellar ataxia induced by anti GQ1b antibody.