Antibodies to metabotropic glutamate receptor 5 in the Ophelia syndrome

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ABSTRACT

Objective: To report the metabotropic glutamate receptor 5 (mGluR5) as the autoantigen of antibodies from 2 patients with Hodgkin lymphoma (HL) and limbic encephalopathy (Ophelia syndrome).

Methods: Immunohistochemistry with brain tissue and cultures of rat hippocampal neurons were used to demonstrate antibodies. Immunoprecipitation, mass spectrometry, and *mGluR5*-null mice served to identify the antigen. HEK293 cells transfected with mGluR5 or mGluR1 were used to determine immunologic crossreactivity.

Results: Both patients developed symptoms consistent with limbic encephalopathy; one had MRI findings typical of this disorder and the other had more extensive radiologic involvement, including parietal and occipital cortex. Patients' sera had antibodies that predominantly reacted with the neuropil of hippocampus and cell surface of live hippocampal neurons. Immunoprecipitation from cultured neurons and mass spectrometry demonstrated that the antigen was mGluR5, a receptor involved in processes of learning and memory. The reactivity of patients' sera was abrogated in brain of *mGluR5*-null mice, further confirming the antibody specificity. Studies with a large number of controls including 2 patients with cerebellar ataxia and mGluR1 antibodies showed that mGluR5 was only identified by sera of the 2 patients with the Ophelia syndrome, and that despite the homology of this receptor with mGluR1 each autoantigen was specific for a distinct syndrome.

Conclusions: Antibodies to mGluR5 should be considered in patients with symptoms of limbic encephalitis and HL (Ophelia syndrome). Recognition of this disorder is important because it can affect young individuals and is reversible. **Neurology® 2011;77:1698-1701**

GLOSSARY

AVBD = doxorubicin, vinblastine, bleomycin, and dacarbazine; **FDG** = fluorodeoxyglucose; **GAD** = glutamic acid decarboxylase; **HL** = Hodgkin lymphoma; **LTD** = long-term depression; **mGluR5** = metabotropic glutamate receptor 5.

In 1982, Ian Carr¹ described an unusual neuropsychiatric disorder in his daughter, who was then 15.5 years old. He noted that one day “a little of the sparkling precision of her conversation had gone.” This was followed by progressive loss of memory, depression, hallucinations, and bizarre behavior. A Hodgkin lymphoma (HL) was detected and successfully treated resulting in neurologic recovery except for “a neatly excised piece of memory for about eighteen months.” Dr. Carr postulated that a humorally mediated mechanism, “perhaps a circulating neurotransmitter-like molecule secreted by the tumor,” caused the disorder, which he named “Ophelia syndrome.” We report 2 patients with a similar syndrome and the characterization of the metabotropic glutamate receptor 5 (mGluR5) as the target antigen of patients' antibodies.

PATIENTS Patient 1. In April 2006, a 46-year-old woman was evaluated for depression and personality change. Examination showed enlarged cervical lymph nodes, and a CT scan revealed a right lung mass and

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cervical lymphadenopathy, which biopsy and tumor staging demonstrated HL (IIIA). In May 2006, she had a seizure, and brain MRI showed subtle increase in T2 signal in the right mesial temporal area. CSF analysis had normal results. She was treated with doxorubicin, vinblastine, bleomycin, and dacarbazine (AVBD) with improvement of the cervical lymphadenopathy. In June 2006, she developed more seizures and her mental status worsened. On examination she was alert but fearful and tremulous; she had short-term memory deficit and delusions, and was emotionally labile. Frequent myoclonic jerks were noted. Cranial nerves, strength, sensation, and coordination were normal. Repeated brain MRI showed increased T2 signal in the mesial temporal lobes, cingulate gyrus, insular regions, and right thalamus. Repeat CSF analysis showed 23 white blood cells/ μ L (90% lymphocytes), total protein 55 mg/dL, glucose 57 mg/dL, and negative cytology. Antibodies to onconeural antigens and glutamic acid decarboxylase (GAD) were negative. The patient continued with AVBD chemotherapy and she also received a course of IV methylprednisolone followed by a slow steroid taper. Her seizures remitted and her mental status improved to normal over several months. A follow-up 4 years after presentation showed that her mental status was normal. Analysis of archived serum (CSF not available) demonstrated mGluR5 antibodies.

Patient 2. In 2009, a 15-year-old boy developed headache and nausea followed 5 days later by confusion, anxiety, extreme agitation, and auditory and visual hallucinations. He frequently expressed the idea that death will soon arrive. On hospital day 1, CSF revealed 114 leukocytes/ μ L (90% lymphocytes) and protein concentration of 0.40 g/L. Because of generalized seizures, he required nasotracheal intubation and received IV midazolam, fentanyl, and acyclovir. On day 4, CSF showed 64 leukocytes/ μ L and protein concentration of 0.40 g/L. Studies for varicella zoster, herpes and parvovirus B19, onconeural, and NMDA receptor antibodies were negative. On day 9, MRI diffusion sequences showed bilateral hyperintensities in the posterior parietal-occipital cortex. On day 12, sedation was discontinued and although his neurologic status was improving he required diazepam and risperidone. On day 25, the CSF showed 2 leukocytes/ μ L and protein concentration of 0.39 g/L along with intrathecal synthesis of immunoglobulin G. On day 32, he was transferred to a rehabilitation center, where he adapted well with other children and performed school tasks normally. He appeared distant and unconcerned by the hospital admission. Memory tests had normal results, but attention and verbal fluency were decreased. On day

35, a fluorodeoxyglucose (FDG) PET scan showed mediastinal lymph node uptake; biopsy and staging revealed HL (IIA). Mediastinal radiotherapy resulted in complete oncologic and neurologic recovery. Two years later, the patient remains neurologically normal. Analysis of archived serum (CSF not available) demonstrated mGluR5 antibodies.

Antibody and antigen characterization. The characterization of patients' antibodies and the identification of mGluR5 as the target antigen were performed combining immunohistochemistry of rat brain, cultured rat hippocampal neurons, immunoprecipitation, and mass spectrometry, as reported (appendix e-1 and figure e-1 on the *Neurology*[®] Web site at www.neurology.org).² HEK293 cells transfected with a plasmid coding for rat mGluR5 were specifically labeled by the 2 patients' sera (figure 1) but not by sera of 104 controls that included 2 patients with mGluR1 antibodies (one of these patients has not been previously reported and is described in appendix e-1). Despite the high homology between mGluR5 and mGluR1, the sera of the 2 patients with the Ophelia syndrome did not react with mGluR1 (figure e-2B), and vice versa, the sera of 2 patients with mGluR1 antibodies (both with cerebellar ataxia) did not react with mGluR5 (figure 1E). Further demonstration that mGluR5 was the only antigen related to the Ophelia syndrome was provided by abrogation of patients' sera reactivity with brain of *mGluR5*-null mice.³

Standard protocol approvals, registrations, and patient consents. Studies were approved by the Institutional Review Board of the University of Pennsylvania. Written consent for studies was provided by guardians of patients.

DISCUSSION This study identifies mGluR5 as a target antigen of antibodies from 2 patients with the Ophelia syndrome. Three features characterize this disorder: 1) the epitopes are in the extracellular domain of the receptor, as shown by patients' antibody binding to the surface of live neurons; 2) the antibodies specifically react with mGluR5, as demonstrated by the abrogation of reactivity with brain of *mGluR5*-null mice and lack of cross-reactivity with mGluR1; and 3) the neurologic disorder is reversible, as occurred in our 2 patients.

There are 8 mammalian mGluR receptors classified in 3 groups: group 1 (mGluR1 and mGluR5), group 2 (mGluR2 and mGluR3), and group 3 (mGluR4, mGluR6, mGluR7, and mGluR8). All these receptors modulate neuronal activity by the activation of intracellular signaling pathways. The mGluR5 shares 85% amino acid sequence homology

Figure 1 Patients' antibodies recognize metabotropic glutamate receptor 5 (mGluR5) in transfected cells and rodent brain

Left panels show HEK cells transfected to express mGluR5, reacted with a commercial antibody to mGluR5 (A, D, G), serum from a patient with the Ophelia syndrome (B), serum from a patient with cerebellar ataxia and mGluR1 antibodies (E), and serum from a normal individual (H). Merged reactivities are shown in the column of the right where the nuclei of the cells are labeled blue with DAPI (C, F, I). Note that mGluR5 is only recognized by serum of the patient with Ophelia syndrome. Scale is 10 μ m. Right panels show that the serum of a patient with Ophelia syndrome and mGluR5 antibodies intensively reacts with wild-type mouse brain, especially the hippocampus (J), but the reactivity is abrogated in *mGluR5*-null mouse brain (L). In contrast, the serum of a patient with ataxia and mGluR1 antibodies intensively reacts with *mGluR5*-null mouse brain, mainly the cerebellum (N; an identical reactivity was noted with the wild-type brain, not shown). A predominant reactivity with the hippocampus of wild-type brain (K) is also demonstrated with a polyclonal rabbit antibody to mGluR5 (06-541, Milipore, Billerica, MA; 1:500). The reactivity of this antibody is abrogated in the brain of *mGluR5*-null mouse (M). Scale is 1 mm.

with mGluR1 and both act via calcium/IP3 signaling to modulate synaptic functions including long-term depression (LTD).⁴ While mGluR5 is more important for LTD in the hippocampus,⁵ mGluR1 is necessary for rapid dendritic signaling in Purkinje cells.⁶ Genetic disruption of mGluR5 impairs certain types of behavioral learning, particularly those involving extinction of nonreinforced behaviors.⁷ In contrast, disruption of mGluR1 alters cerebellar development, synaptic plasticity, and motor coordination.⁸

Despite the homology between mGluR1 and mGluR5, each receptor has a different brain distribution and associates with a clinically and immunologically distinct disorder. For example, in this study we identified a new patient with cerebellar ataxia and mGluR1 antibodies, representing the fourth case reported with this disorder, and the second without HL.⁹ These antibodies are important because they alter LTD in cerebellar synapses, and cause ataxia when intrathecally injected to animals.¹⁰ While sera of patients with mGluR1 antibodies shows robust staining of the cerebellum, and this autoimmunity associates with cerebellar ataxia, the sera of patients with mGluR5 antibodies shows intense immunolabeling of the hippocampus and other brain regions (with the lowest reactivity in brainstem and cerebellum), consistent with the predominant limbic dys-

function that characterizes the Ophelia syndrome. Therefore, these 2 immune-mediated phenotypes are consistent with the distribution, function, and models of genetic disruption of the corresponding receptor.

Given that HL has a bimodal distribution of age incidence (peaking at 15–34 years, and >55 years),^{e1} and the Ophelia syndrome usually precedes the identification of HL, mGluR5 autoimmunity should be considered in patients of all ages, including children and teenagers with symptoms of limbic encephalopathy of unclear etiology. In contrast with classic paraneoplastic syndromes, but in line with other autoimmune synaptic encephalitis,^{e2} patients with mGluR5 antibody-associated encephalitis may start to improve before the tumor is treated. Although one of our patients did not receive immunotherapy and the other only corticosteroids, both patients had prompt and successful tumor treatment, suggesting that the main concern of the physician should be to recognize this disorder and search for HL. Future studies should clarify the role of immunotherapy.

AUTHOR CONTRIBUTIONS

Dr. Lancaster: drafted manuscript, performed experiments, analyzed data. Dr. Martinez-Hernandez: performed experiments, analyzed data. Dr. Titulaer: performed experiments, analyzed data. Dr. Boulos: provided information, serum samples, and analyzed data. Dr. Weaver: provided information, serum samples, and analyzed data. Dr. Antoine: provided

information, serum samples, and analyzed data. Dr. Leibers: provided patient information, analyzed data. Dr. Kornblum: provided information, serum samples, and analyzed data. Dr. Bein: provided information, serum samples, and analyzed data. Dr. Honnorat: provided patients' samples, analyzed data. Dr. Wong: performed experiments, analyzed data. Dr. Contractor: provided *mGluR*-null mouse, analyzed data. Dr. Xu: provided *mGluR*-null mouse, analyzed data. Dr. Balice-Gordon: interpreted data, revised manuscript. Dr. Dalmau: designed study, analyzed data, drafted and revised manuscript.

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DISCLOSURE

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