Clinical commentary

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Epilepsy with myoclonic atonic seizures and chronic cerebellar symptoms associated with antibodies against glutamate receptors N2B and D2 in serum and cerebrospinal fluid

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ABSTRACT – A 3-year-old boy with normal development presented with acute cerebellitis at one year and 10 months of age. His truncal ataxia resolved without treatment. He experienced a relapse of truncal ataxia and atonic seizures at 2 years and one month of age. Five months later, he experienced myoclonic atonic seizures. By 3 years of age, the truncal ataxia had become severe, and the frequency of myoclonic atonic seizures increased. Compared to controls, we found higher levels of anti-C-terminal GluN2B and anti-N terminal GluD2 antibodies in the serum, and anti-N terminal GluN2B and anti-C terminal GluD2 antibodies in the cerebrospinal fluid (CSF). A cell-based assay revealed the presence of anti-NMDA-type glutamate receptor antibody in the serum, but absence in the CSF. Ictal EEG of myoclonic atonic seizures showed generalized spike and wave complexes. The patient was diagnosed with myoclonic atonic epilepsy. Adrenocorticotrophic hormone therapy resolved the truncal ataxia and myoclonic atonic seizures, along with the decreased serum anti-C-terminal GluN2B and anti-N-terminal GluD2 antibodies, and CSF anti-N-terminal GluN2B and anti-C-terminal anti-GluD2 antibodies. Our results suggest that the anti-GluN2B and anti-GluD2 antibodies may be associated with myoclonic atonic epileptic seizures and chronic cerebellitis.

Key words: epilepsy, myoclonic atonic seizures, cerebellitis, glutamate receptors

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Glutamate is an excitatory neurotransmitter that acts on ionotropic and metabotropic glutamate receptors (GluR). GluR are classified as N-methyl-D-aspartate (NMDA)-type, α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA)-type, and kainite-type. The NMDA-type GluR are composed of two GluN1 and two GluN2 (A-D) or GluN3 (A-B) subunits. The NMDA-type-GluRs play a role in synaptic plasticity and are associated with neural development and learning (Mori *et al*., 1998). The GluN2B subunit has little impact on the kinetics of the receptor, but is essential for the recruitment of signalling molecules required for synaptic plasticity (Delaney *et al*., 2013). GluN2B is prominent in adult hippocampal synapses as an integral part of GluN1, GluN2A, and GluN2B (Rauner and Köhr, 2011). Anti-GluR antibodies are associated with various neurological conditions, such as limbic encephalitis (Mochizuki *et al*., 2006), epilepsy (Yoshino *et al*., 2007), and cerebellitis (Shiihara *et al*., 2007; Shimokaze *et al*., 2007). Antibodies against GluN2B have been found in patients with non-herpetic limbic encephalitis (Mochizuki *et al*., 2006), temporal lobe epilepsy (Yoshino *et al*., 2007), and epilepsia partialis continua (Takahashi *et al*., 2005). GluD2 is predominantly expressed in cerebellar Purkinje cells (Hirai *et al*., 2003). Antibodies against the GluD2 have also been found in patients with chronic cerebellitis (Kubota and Takahashi, 2008). Autoimmune pathophysiology can cause neurological symptoms. Here, we report the case of a 3-year-old boy with myoclonic atonic epilepsy and chronic cerebellar symptoms putatively associated with the presence of anti-GluN2B and anti-GluD2 antibodies in serum and cerebrospinal fluid (CSF).

Case study

A boy with normal development presented with acute cerebellitis at the age of one year and 10 months. Neither he nor his family had a history of epilepsy or autoimmune disease. Truncal ataxia presented at that time had completely resolved without treatment, and the patient appeared normal on brain MRI. However, N-isopropyl (I-123) p-iodoamphetamine (IMP) single-photon emission computed tomography (SPECT) analysis showed hypoperfusion of the cerebellar hemispheres, and EEG showed focal spikes in the left frontal and right central regions of the brain. The patient experienced a relapse of truncal ataxia, dysarthria, and atonic seizures at the age of 2 years and one month. Two months later, he presented with generalized tonic-clonic seizures and was treated with valproate. However, the frequency of seizures increased and at the age of 2 years and 6 months, he was experiencing more than 10 myoclonic atonic seizure episodes per day. He was diagnosed

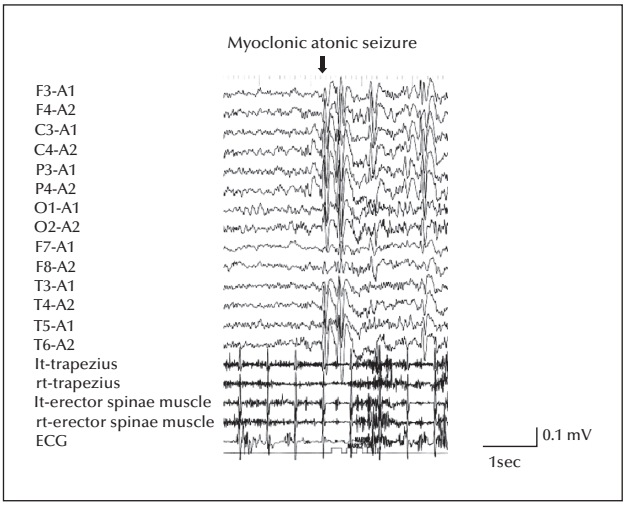

Figure 1. Ictal EEG of myoclonic atonic seizures exhibited generalized spike and wave complexes.

with epilepsy with myoclonic atonic seizures. Daily seizures persisted despite the addition of lamotrigine and clonazepam to his treatment regimen. By the age of three years, he experienced more than 40 myoclonic atonic seizure episodes per day, and the severity of truncal ataxia prevented him from walking without support. His brain MRI findings were normal, but interictal EEG showed high-amplitude (1-2.5 Hz) spike and wave complexes, predominantly in the biparieto-occipital regions of the brain. In addition, ictal EEG of myoclonic atonic seizures showed generalized spike and wave complexes (*figure 1*). Furthermore, 99mTc-ethyl cysteinate dimer (99mTc-ECD)-single-photon emission computed tomography (SPECT) showed hypoperfusion of the cerebellar hemispheres. Laboratory analysis of metabolic parameters yielded normal results. Urine vanilmandelic acid and homovanillic acid levels were within the physiological range and no abnormal masses were detected on abdominal ultrasound. An examination of CSF revealed a monocyte count of 1 cell/μl and a protein concentration of 18 mg/dl. Antibodies against the herpes simplex and measles viruses were not detected. However, the levels of antibodies against the C-terminal of GluN2B (GluN2B-CT) and N-terminal of GluD2 (GluD2-NT) in serum based on an enzyme-linked immunosorbent assay (ELISA) were higher than those of controls (*figure 2*). The levels of antibodies against the N-terminal of GluN2B (GluN2B-NT2) and C-terminal of GluD2 (GluD2-CT) in CSF were also higher than those of controls. An ELISA was performed in accordance with previously reported methods (Fujita *et al*., 2012). The levels of antibodies (mean±standard deviation) against GluN2B-NT2, GluN2B-CT, GluD2-NT, and GluD2-CT in serum were 0.989, 1.288, 1.442, and 1.343, respectively. The control

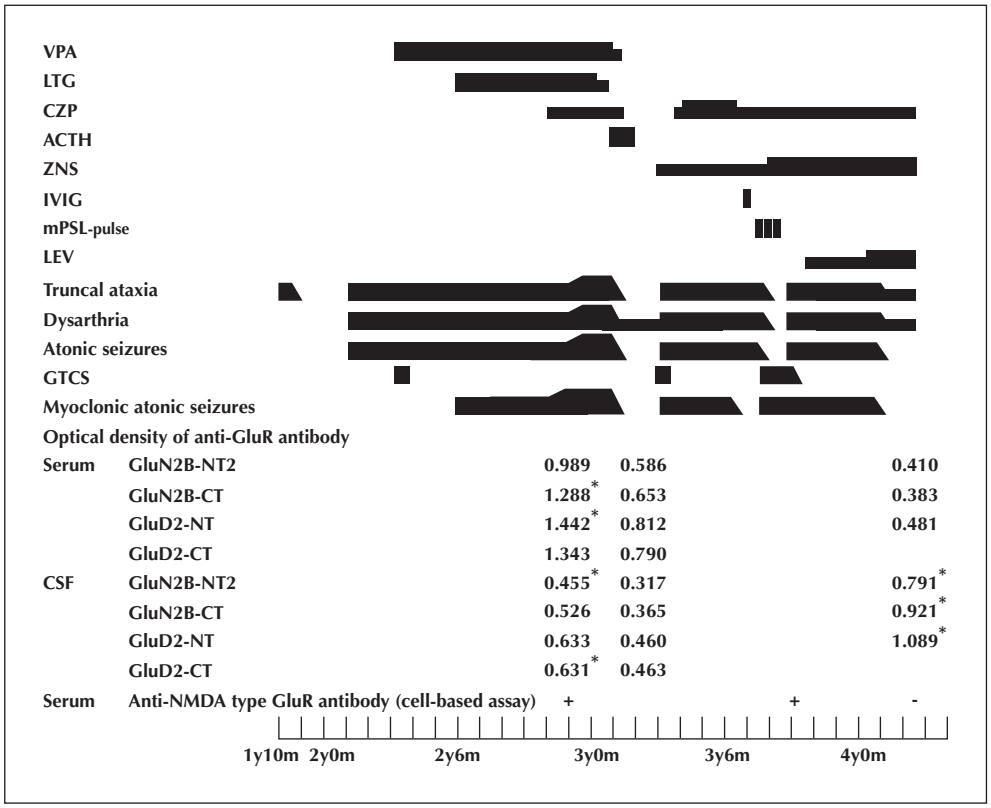

Figure 2. Clinical course of epilepsy with myoclonic atonic seizures and chronic cerebellitis. Asterisks show the titres of antibodies that exceeded +3SD from the mean. Myoclonic atonic seizures resolved concurrently with a decrease in serum anti-GluN2B-CT and anti-GluD2-NT antibodies, and in CSF, anti-GluN2B-NT2 and anti-GluD2-CT, following ACTH therapy.
ACTH: adrenocorticotrophic hormone; CZP: clonazepam; GluR: glutamate receptor; GTCS: generalized tonic-clonic seizures; IVIG: intravenous immunoglobulin; LTG: lamotrigine; LEV: levetiracetam; m: month; mPSL: methylprednisolone; NMDA-type: N-methyl-D-aspartate-type; VPA: valproate; y: year; ZNS: zonisamide.

patients had symptomatic generalized epilepsy, and the antibody levels against GluN2B-NT2, GluN2B-CT, GluD2-NT, and GluD2-CT in the serum of controls were 0.523±0.233, 0.556±0.140, 0.641±0.230, and 0.765±0.429, respectively. In CSF, antibody levels against GluN2B-NT2, GluN2B-CT, GluD2-NT, and GluD2-CT were 0.455, 0.526, 0.633, and 0.631, respectively. In CSF of control patients, antibody levels against GluN2B-NT2, GluN2B-CT, GluD2-NT, and GluD2-CT were 0.202±0.045, 0.252±0.107, 0.260±0.153, and 0.264±0.073, respectively. Based on a cell-based assay, the anti-NMDA-type glutamate receptor antibody was absent in the CSF but present in the serum (according to the method of Takano *et al*., 2011). The patient was finally diagnosed with epilepsy with myoclonic atonic seizures associated with antibodies against glutamate receptors N2B and D2 in serum and CSF. He was treated with adrenocorticotrophic hormone (ACTH) therapy. Synthetic ACTH, as a zinc hydroxide suspension of tetracosactide acetate (Cortrosyn Z [Daiichi-Sankyo Company limited, Tokyo, Japan]), was administered intramuscularly at a dose of 0.010 mg/kg/day for three consecutive days, then at 0.015 mg/kg/day for the next 11 consecutive days, and every other day for one week (three administrations in total) for tapering. Seizures were counted based on the reports from the parents and a nurse at our hospital. Myoclonic atonic seizures disappeared on Day 6 of the ACTH therapy, and valproate and clonazepam administration was terminated. Although dysarthria persisted, the truncal ataxia of the patient resolved and he was able to walk without support. The antibody titres against GluN2B-CT and GluD2-NT in serum decreased. The antibody titres against GluN2B-NT2 and GluD2-CT in CSF also decreased. Interictal EEG testing showed a decrease in spike and wave complex frequency. However, 99mTc-ECD-SPECT continued to show hypoperfusion of the cerebellar hemispheres. The patient was finally diagnosed with epilepsy with myoclonic atonic seizures and chronic cerebellar symptoms, in association with the presence of anti-GluN2B and anti-GluD2 antibodies in serum and CSF. The patient had a relapse of generalized tonic-clonic seizures, myoclonic atonic seizures, truncal ataxia, and dysarthria at the age of

3 years and 3 months. Generalized tonic-clonic seizures were treated with zonisamide. However, clonazepam and high-dose intravenous immunoglobulin therapy were ineffective for myoclonic atonic seizures, truncal ataxia, and dysarthria. The parents rejected further maintenance steroid therapy or the use of an immunosuppression agent due to their possible adverse effects, such as an immunosuppressive state and growth impairment. The patient experienced a cessation of these symptoms for two months after ACTH therapy, thus the parents hoped that the patient would receive high-dose methylprednisolone pulse therapy for a short period. The myoclonic atonic seizures, truncal ataxia, and dysarthria resolved on high-dose methylprednisolone pulse therapy at 30 mg/kg/day for three consecutive days with an interval of one week, repeated three times, at the age of 3 years and 7 months. However, one month later, these symptoms relapsed. Though truncal ataxia and dysarthria persisted with higher antibody titres against GluN2B-NT2, GluN2B-CT, and GluD2-NT in CSF, myoclonic atonic seizures were treated with levetiracetam at the age of 4 years and 2 months.

Antibodies against GluN2B and GluD2 in serum and CSF were measured using an ELISA. Peptides were synthesized from the sequences of GluN2B, amino acids 369 to 382 (KERKWERVGKWKDK) from the extracellular N-terminus, and amino acids 1,153 to 1,166 (DIYKERSDDFKRDS) from the intracellular C-terminus. Maxisorb plates (#468667, Nalge Nunc International, Rochester, New York, USA) were coated overnight with peptide (1 μg/well) in phosphate-buffered saline (PBS) (pH 7.2) and blocked with bovine serum albumin (BSA) (5% w/v) in PBS-Triton X-100 (PBST; 0.05% v/v) for two hours. Serum (100 μl; diluted 1:10 in PBST containing 1% BSA) or CSF (100 μl; undiluted) was then incubated at 37°C for two hours. After washing (in PBST), plates were incubated with a protein A-horseradish peroxidase conjugate (1:10,000) for two hours, and developed using the TMB Microwell Peroxidase Substrate System (#50-76-00, KPL, Gaithersburg, MD, USA). Optical densities (at 450 nm) were measured using a microplate reader.

Discussion

Our report is the first of a case of epilepsy with myoclonic atonic seizures and chronic cerebellitis in association with antibodies against GluN2B and GluD2 in serum and CSF. Due to the cerebellar symptoms, it was initially necessary to detect antibodies associated with cerebellitis, including the GluD2 antibodies. Chronic cerebellitis associated with hypoperfusion of the cerebellar hemispheres may be the result of anti-GluD2 receptor antibodies (Nagamitsu *et al*, 1999, Kubota and Takahashi, 2008). A cell-based assay for antibodies to GluD2 has not been established, therefore we used an ELISA to detect antibodies to GluD2, and similarly measured other antibodies to the NMDA-type GluR. The results of our report refine and support two significant hypotheses. The first hypothesis is that autoimmune pathophysiology can lead to myoclonic atonic seizures. In our case, myoclonic atonic seizures resolved concurrently with a decrease in serum anti-GluN2B-CT and anti-GluD2-NT antibodies, and in CSF, anti-GluN2B-NT2 and anti-GluD2-CT, following ACTH therapy (*figure 2*). We conclude that ACTH therapy may be effective in patients with myoclonic atonic seizures and anti-GluN2B and anti-GluD2 antibodies in serum and CSF (Takahashi *et al*., 2008). Autoimmune epilepsy, in which autoimmune antibodies target the extracellular domain of proteins, responds to immunological therapy with improved clinical symptoms and decreases in autoimmune antibody titre (Irani *et al*., 2011). For example, patients who are suspected of having autoimmune epilepsy often present with anti-neuronal antibodies, and early immunological therapy has led to favourable outcomes associated with decreases in anti-neuronal antibody titres (Quek *et al*., 2012). In our case, anti-GluN2B antibody titres in serum and CSF were correlated with the frequency of myoclonic atonic seizures, which showed high titres during exacerbation and low titres during remission. We hypothesize that the anti-GluN2B antibody impairs the cerebral cortex and limbic system, and contributes to the onset of myoclonic atonic seizures. Recently, we reported that rabbit antibodies to human GluN2B-NT2 peptides induce excitable behaviour and memory dysfunction in mice via passive transfer (Takahashi *et al*., 2015). The levels of antibodies against GluN2B-NT2, GluN2B-CT, and GluN1-NT in CSF of patients with epileptic spasms were higher than those in controls (Mori *et al*., 2016). These data suggest that antibodies to GluN2B-NT2, based on ELISA detection, might contribute to the pathophysiology in our case.

Our data support a second hypothesis that anti-GluD2 in serum and CSF is associated with chronic cerebellitis. In our case, ACTH therapy resolved truncal ataxia and resulted in the disappearance of anti-GluD2-NT antibodies in serum and anti-GluD2-CT antibodies in CSF. When truncal ataxia and dysarthria persisted at the age of 4 years and 3 months, the titre of anti-GluD2-NT antibody in CSF was higher than that of controls. This finding is consistent with reports that have demonstrated the presence of anti-GluD2 antibody in patients with acute cerebellitis and chronic cerebellitis after antecedent infection (Shiihara *et al*., 2007; Shimokaze *et al*., 2007; Kubota and Takahashi, 2008), and p-iodoamphetamine (IMP) SPECT showing decreased regional cerebral blood flow in the cerebellum of patients with post-infectious acute cerebellar

ataxia (Nagamitsu *et al*., 1999). Acute cerebellitis and chronic cerebellitis have been shown to resolve early and without any sequelae following high-dose steroid therapy with a corresponding decrease in anti-GluD2 antibody (Shimokaze *et al*., 2007; Kubota and Takahashi, 2008). Anti-GluD2 antibodies impair the function of cerebellar Purkinje cells, which we presume led to chronic cerebellitis in our patient. Our report therefore adds to evidence suggesting that chronic cerebellitis and hypoperfusion of the cerebellar hemispheres is associated with the presence of anti-GluD2 antibody in CSF.

A limitation of our report is that anti-NMDA-type GluR was not detected in CSF using a cell-based assay. A diagnosis of NMDA encephalitis must demonstrate the presence of anti-NMDA-type GluR in CSF using a cell-based assay. Our case could not be defined as NMDA encephalitis, as further confirmation based on immunochemistry of hippocampal or other brain tissues is required.

In our case, the correlation of clinical symptoms with autoimmune antibodies was important for elucidating autoimmune pathophysiology. These antibodies should be included for differential diagnosis in patients with myoclonic atonic epilepsy when the patient has an intractable tendency toward antiepileptic drugs and cerebellar symptoms, and a correlation between improvement in symptoms and a decrease in anti-GluR antibodies in CSF. These results underscore the necessity of systematic screening for autoimmune antibodies when autoimmune pathophysiology is suspected, as the treatment described here may be used to successfully treat other patients. The role of anti-GluR antibodies is still unknown. Further investigation is required to elucidate their role in the pathophysiology of autoimmune epilepsy and cerebellitis.

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