Clinical commentary

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A *de novo GABRB2* variant associated with myoclonic status epilepticus and rhythmic high-amplitude delta with superimposed (poly) spikes (RHADS)

Aiko Nishikawa [1], Yui Otani [1], Susumu Ito [1], Satoru Nagata [1], Mutsuki Shiota [2], Jun-ichi Takanashi [3], Mitsuko Nakashima [4], Hirotomo Saitsu [4], Naomichi Matsumoto [5], Hirokazu Oguni [1]

[1] Department of Pediatrics, Tokyo Women's Medical University, Tokyo, Japan
[2] Department of Pediatric Neurology, Fuji-Toranomon seikeigeka hospital, Shizuoka, Japan
[3] Department of Pediatrics, Tokyo Women's Medical University, Yachiyo Medical Center, Chiba, Japan
[4] Biochemistry Department, Hamamatsu University School of Medicine, Hamamatsu, Japan
[5] Department of Human Genetics, Yokohama City University Graduate School of Medicine, Yokohama, Japan

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ABSTRACT – We report a child who developed myoclonic status epilepticus (MSE) at four months of age, associated with rhythmic high-amplitude delta and superimposed (poly) spikes (RHADS), harbouring a *GABRB2* (β2 subunit of the *GABA A* receptor) variant. The patient was treated under a presumptive diagnosis of neonatal-onset Alpers syndrome (AS) and underwent targeted sequence analysis for *POLG1* (polymerase gamma 1) and subsequent whole-exome sequence analysis (WES). The patient is currently a 10-year, eight-month-old boy, suffering from daily MSE associated with RHADS and severe global developmental delay from early infancy. Although *POLG1* mutation was negative, WES revealed a *de novo* missense variant of *GABRB2* (NM_021911.2: c.784G>T, p.[Val262Phe]). Based on a review of case series with *GABRB2* variants, we found that five of the 18 cases shared the clinical and EEG characteristics associated with our patient. In summary, this *de novo GABRB2* variant was associated with an AS phenotype, characterized by treatment-resistant MSE and RHADS, and may represent an alternative aetiology for neonatal-onset AS without *POLG1* mutation [*Published with video sequence*].

Key words: Alpers syndrome, *GABRB2*, *POLG1*, myoclonic status epilepticus, RHADS

VIDEO ONLINE

Correspondence:
Hirokazu Oguni
Department of Pediatrics,
Tokyo Women's Medical University,
8-1 Kawada-Cho,
Shinjuku-ku,
Tokyo 162-8666, Japan
<oguni.hirokazu@twmu.ac.jp>

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Alpers syndrome (AS) is an early-onset neurodegenerative disorder with a poor prognosis, characterized by the triad of developmental regression, intractable epilepsy, and hepatic dysfunction (Sofou *et al*., 2012; Rose and Al Khalili, 2019). The neuropathological examination demonstrates neuronal loss, status spongiosus, and astrocytosis, affecting the cerebral cortex (Harding *et al*., 1986). The most common age at onset is between one month and six years of age, although a few prenatal- and adult-onset cases have been reported. As an early symptom, epileptic seizures resistant to antiepileptic drug (AED) treatment develop in the form of focal, multifocal or myoclonic seizures evolving into epilepsia partialis continua (EPC) or myoclonic status epilepticus (MSE), which markedly increase in frequency over months or years (Wolf *et al*., 2009; Rose and Al Khalili, 2019). Among the laboratory findings, the emergence of rhythmic high-amplitude delta with superimposed (poly) spikes (RHADS) was demonstrated to be specific for this syndrome (Boyd *et al*., 1986; Wolf *et al*., 2009; van Westrhenen *et al*., 2018). In order to make an early diagnosis and avoid the use of sodium valproate (VPA), which may accelerate fatal hepatic failure, the *POLG1* (polymerase gamma 1) mutation test is recommended (Saneto *et al*., 2010). We report a child presenting with daily MSE and RHADS, associated with a *de novo* missense variant of the β2 subunit of GABA A receptor (*GABRB2)*.

Case study

The patient is currently a 10-year, eight-month-old boy. His family history was unremarkable. He was born by Caesarean section uneventfully due to pelvic position at 38 weeks of gestation. His birth weight, length, and head circumference were 2,880 g (-0.18 SD), 47.6 cm (-0.45 SD), and 33.0 cm (-0.30 SD), respectively, which were appropriate for his gestational age. After birth, he was crying a little and feeding poorly. At the one-month medical check-up, hypertonia, poor feeding, poor weight gain, and ptosis were noted. He maintained a rigid posture with flexion of both arms at the elbow and extension of both legs, and became opisthotonic during the traction manoeuvre. At four months of age, rhythmic myoclonus affecting his face and extremities developed which gradually increased in frequency (*see video sequence*). At 10 months of age, he was transferred to another hospital for further examination and microcephaly (-3.6 SD), hypotonia and no neck fixation, mild rigidity, and frequent rhythmic myoclonus of the extremities were observed. At the age of one year and seven months, he underwent gastrostomy and Nissen's operation because of disabling gastroesophageal regurgitation.

At three years and two months of age, he was referred to our hospital. He was 95 cm (+ 0.28 SD) tall, weighed 13.9 kg (+ 0 SD) and had a head circumference of 43.5 cm (-4.0 SD). He was unable to hold his head or sit and had marked generalized hypotonia with mild spasticity of the extremities. He had ptosis, no visual fixation or following, and no language ability. Sensory stimulation, such as sudden touch or sudden noise, easily provoked the rhythmic myoclonus of the face and all four extremities synchronously lasting for several minutes to a few hours. Polygraphic analysis demonstrated that the rhythmic myoclonus corresponding to RHADS localized to both midfrontal-central-parietal regions (*figure 1A*). In addition, the jerked-locked back-averaging revealed that the RHADS-polyspike component was time-locked to myoclonic EMG potentials and preceded their onset by 8 msec. (*figure 1B*). He had no respiratory compromise nor autonomic symptoms during the attack, and no postictal EEG slowing after the seizures. Thus, the rhythmic myoclonus was considered to be either bilateral EPC or MSE. He also had daily autonomic seizures originating from the left or right temporal region.

The interictal EEG exhibited persistent diffuse slow waves with multifocal spikes, frequently interrupted by RHADS during wakefulness, and an intermittent suppression-burst (SB) pattern during sleep (*figure 2*). On brain MRI, moderate brain atrophy with diffuse T2 high intensity in the white matter was observed (*figure 3*).

The protein, lactic acid and pyruvate levels on CSF examination were normal. No abnormalities were found for blood amino acids, urine organic acids, or tandem mass screening tests. Array CGH results were normal. Visual and sensory evoked potential studies showed a normal response and poor separation of N20 component, respectively. Only a mild increase in liver enzymes was found by liver function tests. Based on the clinical and EEG findings, as well as the progressive nature of the disorder, AS was strongly suspected. No pathological or biochemical abnormalities were found on muscle biopsy. At present, he receives artificial respiratory assistance and is cared for at home. MSE has continued, and the suppression-burst EEG pattern has gradually become dominant on EEG during sleep and wakefulness.

Gene analysis

The targeted sequence analysis for *POLG1* was performed at four years of age, but no pathogenic *POLG1* variants were found. At six years and five months of age, whole-exome sequencing was carried out, as previously described (Nakashima *et al*., 2019), for the patient and his parents, which revealed a *de novo GABRB2* variant (NM_021911.2: c.784G>T, p.[Val262Phe]) in the patient. The variant was not observed in exome data of

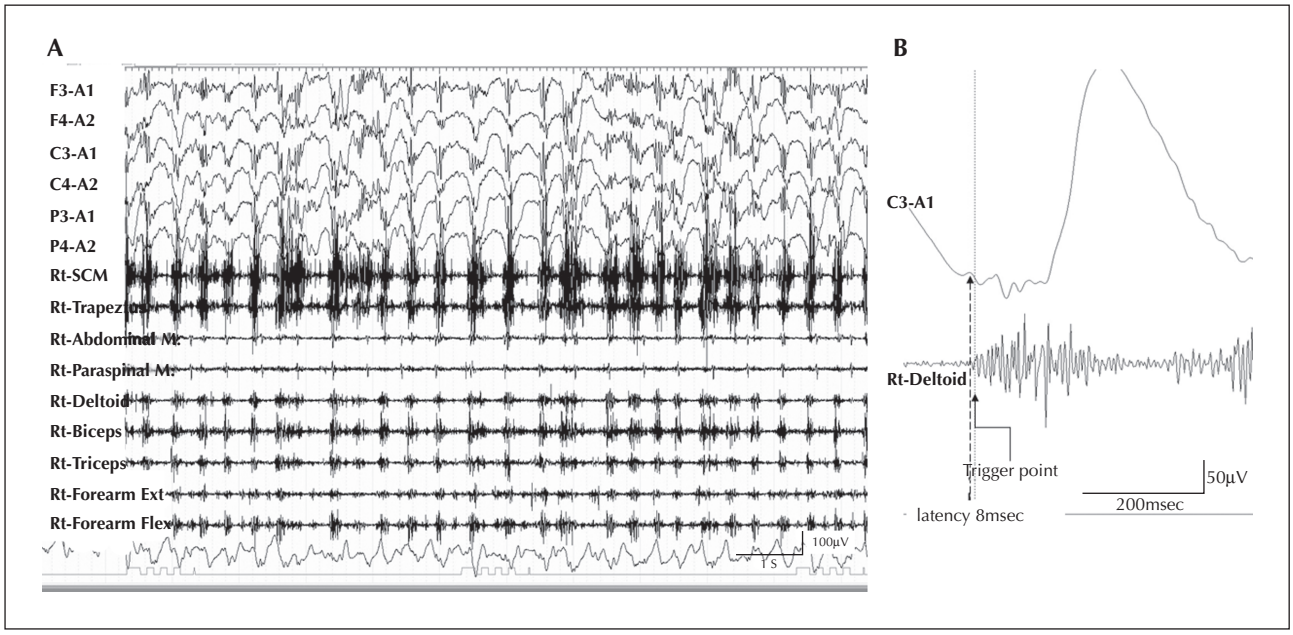

Figure 1. Polygraph of myoclonic status epilepticus (at three years and two months of age). The recording was performed at a sampling frequency of 512 Hz using a digital electroencephalograph manufactured by Nippon Denshi Kogyo and analysed by the FOCUS program developed by Nippon Denshi Kogyo. (A) Rhythmic EMG discharges correspond to rhythmic high-amplitude delta with superimposed (poly) spikes (RHADS) localized to both midfrontal-central-parietal regions with right-sided dominance. (B) The polygraph was averaged (*n*=11) at the trigger point, placing the onset of myoclonic EMG potentials at the right deltoid muscle. As a result, the first spike component of RHADS preceded the onset of myoclonic EMG potentials by 8 mseconds, suggesting that the myoclonic status epilepticus was derived from the contralateral primary motor cortex.

575 in-house Japanese controls or gnomAD, another database comprising 125,748 exome sequencing and 15,708 genome sequencing samples (Karczewski *et al*., 2019). This variant was judged to be likely pathogenic (PS2+PM2+PP3) according to the American College of Medical Genetics and Genomics (ACMG) guidelines (Richards *et al*., 2015).

Informed consent was received from all participants in accordance with the Japanese regulations. The study was approved by the institutional review boards of the Yokohama City University School of Medicine.

Discussion

In our case, neurological abnormalities were already observed in the neonatal period, and MSE appeared from four months after birth, suggesting neonatal-onset AS. Sofou *et al*. (2012) examined the phenotype-genotype association in 19 patients meeting the clinical definition of AS. When the phenotypes were compared between the *POLG1*-positive mutation group (*n*=6) and *POLG1*-negative mutation group (*n*=13), the former group was characterized by a median age at neurological onset of 6.5 months, pharmacoresistant epileptic seizures as the first or second main clinical symptom, and accompanying ataxia and stroke-like episodes. On the other hand, in the *POLG*-negative mutation group, symptom onset often occurred at the perinatal stage, and microcephaly and spasticity were common. EPC, status epilepticus, and myoclonus were more often observed in the former group, whereas neonatal seizures and infantile spasms were more common in the latter group. Furthermore, liver dysfunction was more severe in the former group. In our patient, the clinical findings were similar to those of the *POLG1*-negative mutation group, such as neonatal onset and absence of *POLG1* mutation, but the seizure characteristics were similar to those of the *POLG1*-positive mutation group. A *de novo GABRB2* missense variant was identified as an underlying cause.

To date, 18 patients with developmental disorders or developmental and epileptic encephalopathies associated with *GABRB2* missense variants have been reported, all exhibiting global developmental delay, intellectual disability and epilepsy (Hamdan *et al*., 2017). As there have been no case reports of *GABRB2* truncating variants or copy number variations, and a loss of receptor function has also been confirmed by functional studies in three patients with different *GABRB2* missense variants, it is possible that dominant-negative effects of p.(Val262Phe) caused the developmental and epileptic encephalopathy in this patient (Ishii *et al*., 2017; May *et al*., 2018). Detailed clinical information was described for 13

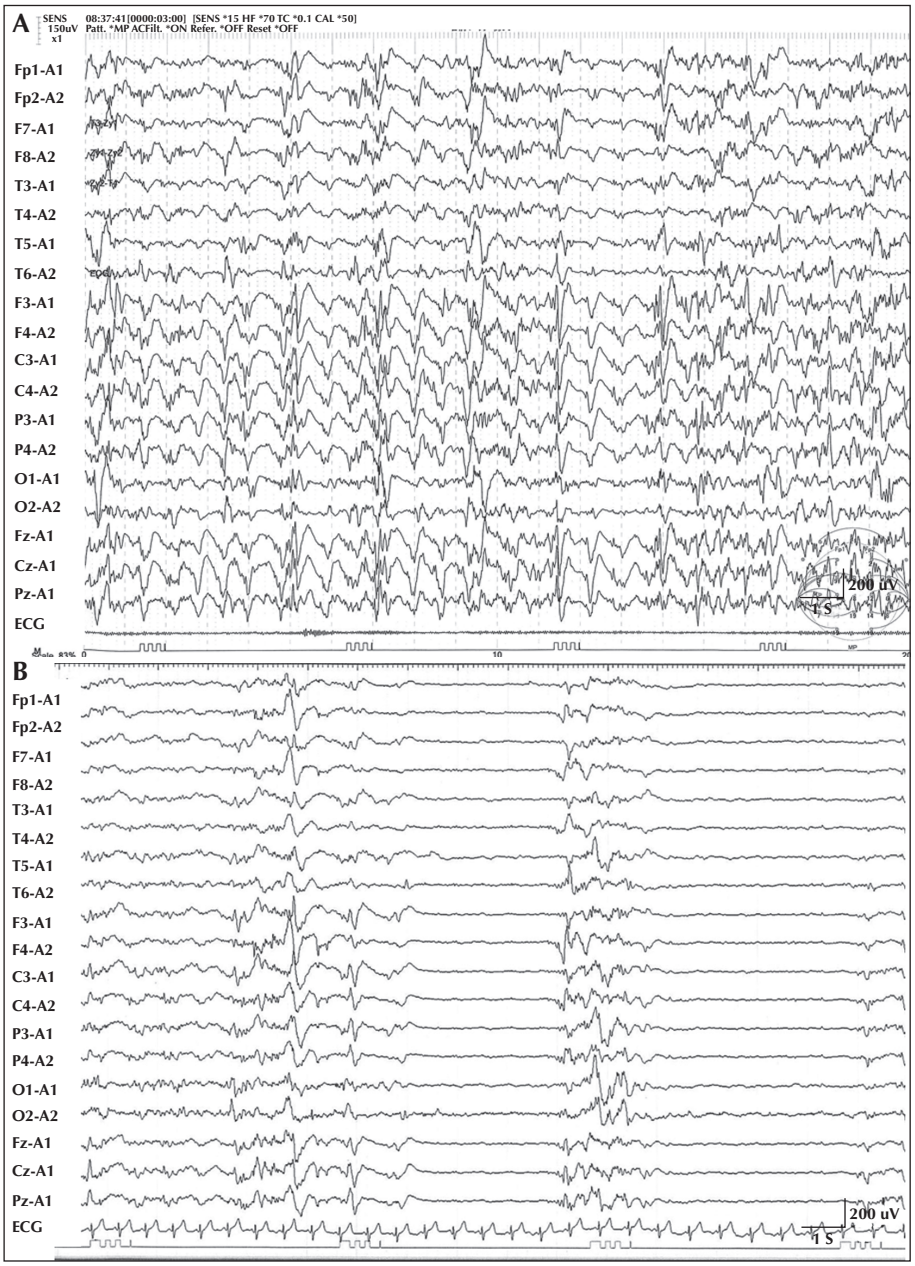

Figure 2. Interictal awake and sleep EEG at three years and two months of age. During wakefulness, the EEG frequently showed RHADS, localized predominantly to the bilateral central-parietal regions (A), whereas no physiological sleep discharges were observed on sleep EEG (B), however, the suppression-burst pattern was noted as sleep progressed.

patients; two developed early myoclonic encephalopathy (EME) and three others had myoclonic epileptic encephalopathies (Hamdan *et al*., 2017; Ishii *et al*., 2017) presenting with MSE. In addition, the descriptions of EEG findings for two patients were similar to that of RHADS.

EPC is a variant of simple focal motor status epilepticus in which frequent repetitive muscle jerks, usually arrhythmic, continue over a prolonged period of time. The jerks are often stereotyped, affecting single muscles, muscle groups, entire limbs or large parts of a hemibody. Previous clinical and electrophysiological studies suggested that focal cortical myoclonus, EPC and focal motor seizures, bilateral EPC, and MSE comprise a spectrum from a single jerk to repetitive jerks and clonic motor seizures, and from focal jerks to generalized jerks through the intrahemispheric and transcallosal spreading of abnormal sensorimotor

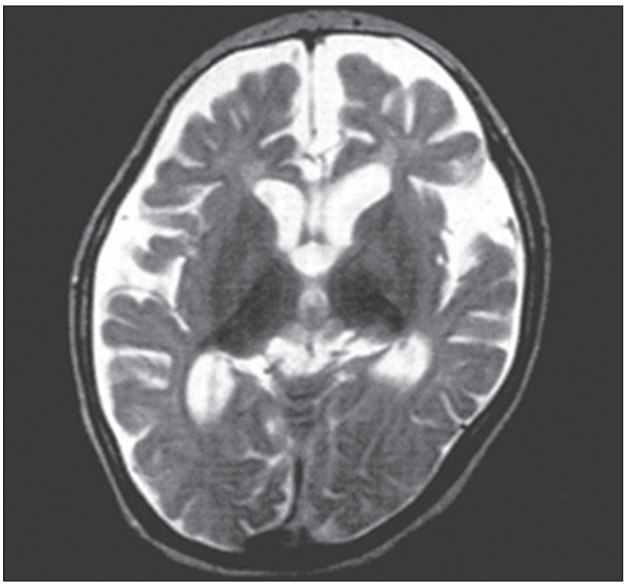

Figure 3. Brain MRI (T2 weighted) at three years and two months of age, revealing moderate brain atrophy with diffuse T2 high intensity in the white matter.

cortical discharges (Donaldson *et al*., 2012). MSE in our patient was easily provoked by sensory stimulation, indicating a neurophysiological characteristic of cortical-reflex myoclonus (Hallett *et al*., 1979).

According to the polygraphic study, the MSE corresponded to RHADS recorded from both centro-parietal regions. RHADS was first reported be a specific EEG finding for AS in 1986 and has been confirmed by many case series (Boyd *et al*., 1986; Wolf *et al*., 2009; van Westrhenen *et al*., 2018). RHADS was characterized by asymmetry between both hemispheres, intermittent appearance, a large slow wave with an amplitude ranging from 200 to 1,000 μV and a frequency at 1 to 3 Hz preceded by small polyspike discharges. Some mitochondrial diseases, such as myoclonic epilepsy with ragged-red fibres (MERRF), mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) and AS, are widely known to often associate with EPC, presumably affecting primary sensorimotor neurons (Lamperti and Zeviani, 2016). The *GABRB2* variants are also suspected to excite primary sensorimotor neurons through reduced GABA inhibitory function (Ishii *et al*., 2017). In conclusion, the *GABRB2* variants are associated with an AS phenotype characterized by treatment-resistant MSE and RHADS. In addition, they may represent an alternative aetiology of neonatal-onset AS without *POLG1* mutation (Sofou *et al*., 2012). □

Legend for video sequence

Video-polygraph of myoclonic status epilepticus (at three years and two months of age), showing rhythmic myoclonus of the face and all four extremities synchronously (myoclonic status epilepticus), lasting for several minutes to a few hours. Forcibly raising both legs at times stopped or lessened the myoclonic status epilepticus.

Key words for video research on www.epilepticdisorders.com

Phenomenology: myoclonic status epilepticus
Localisation: rhythmic high-amplitude delta with superimposed (poly) spikes (RHADS) localized to both midfrontal-central-parietal regions with right-sided dominance
Syndrome: epileptic encephalopathy not otherwise classified
Aetiology: genetic disorder

Supplementary data.
Summary didactic slides are available on the www.epilepticdisorders.com website.

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We certify that we have read the journal's position regarding issues pertaining to ethical publications and confirm that this report is consistent with those guidelines.
None of the authors have any conflict of interest to declare.

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TEST YOURSELF

(1) What seizure types and EEG findings are specific to Alpers syndrome?

(2) What is the role of GABA-A receptors in the brain and what effect do *GABA-A* receptor mutations have regarding the aetiology of epileptic encephalopathies?

Note: Reading the manuscript provides an answer to all questions. Correct answers may be accessed on the website, www.epilepticdisorders.com, under the section "The EpiCentre".