Epilepsy Center between 1991 and 2010 (Table 1). In 4 patients (Patients 1, 5, 12 and 14), histological findings of samples collected at surgical intervention confirmed the diagnosis. Of the 18 patients, 14 attended the National Epilepsy Center and RS was diagnosed by the characteristics of EEG, clinical evolution, seizure semiology and neuroimaging. The remaining four patients visited other hospitals and were suspected of having RS, and their clinical data were sent to the National Epilepsy Center for measurement of serum and CSF antibodies to GluRs. Their characteristics were compatible with RS. Patients who fulfilled the diagnostic criteria and gave written informed consent according to procedures approved by the institutional ethical committee were included in this study.

The average onset age (mean ± SD) of RS patients was 7.7 ± 9.5 years (0.2–42 years). Samples were obtained between 5 and 180 months after onset. Patients who had partial epilepsy without infectious etiology or progressive clinical course served as disease controls ($n = 23$). In disease controls, the average age at examination (mean ± SD) was 11.1 ± 11.7 years (0.7–48 years) and the average duration of epilepsy at examination (mean ± SD) was 39 ± 35 months (0–144 months), which corresponded to those of RS patients. These control patients had seizures at different frequencies ranging from daily to yearly.

Antigen peptides for ELISA

Peptides encoding the extracellular and intracellular domains of the human GluN2B (GenBank accession number U88963) and GluN1 (GenBank accession number Q05586) were synthesized. The sequences were KERKWERVGKWK DK (AA369-382) for N-terminal of GluN2B (GluN2B-NT2), DIYKERSD DFKRDS (AA 11153-1166) for C-terminal of GluN2B (GluN2B-CT), QKRLETLLEERESK (AA177-190) for N-terminal of GluN1 (GluN1-NT), SS FKRRRSSKDTST (AA889-902) for C-terminal of GluN1 (GluN1-CT) (Greiner Bio-One, GmbH, Frickenhausen, Germany).

Methods for detection of antibodies by ELISA

CSF samples collected from patients and controls were centrifuged and the supernatants were frozen immediately and stored at −80 °C. ELISA was performed using previously reported methods (Fujita et al., 2012). Ninety-six-well ELISA plates (Nalge Nunc International, Rochester, NY, USA) were coated overnight at 4 °C with 100 μl per well of 10 μg/ml of each peptide in phosphate buffered saline (PBS). After blocking unreacted sited of the plates with PBS containing 5% bovine serum albumin (BSA), CSF was added (100 μl/well) and incubated for 2 h at 37 °C. After washing vigorously, horseradish peroxidase (HRP)-conjugated protein A (1:10,000) (Funakoshi, Tokyo, Japan) was added and detection was performed with 100 μl of HRP substrate (TMB Microwell Peroxidase Substrate, Funakoshi). All tests were done in duplicate. All samples were assayed simultaneously for antibodies to the four peptides under identical conditions using plates from a single lot. Preliminary experiment suggested that assays using undiluted CSF were the most sensitive and specific. Optical densities (OD) at 450 nm were measured using a microplate reader (Bio-Rad, Hercules, CA, USA). The well containing PBS with 1% BSA (no CSF; reagent blank) was measured for nonspecific binding, and this OD was subtracted from the OD value of each sample. Antibody level is expressed in OD unit.

MR images

A total of 34 MRI scans from 17 patients were evaluated by three medical doctors independently. Patient 5 was excluded from analysis of the relationships between MRI findings and antibodies to GluN1 and GluN2B, because the CSF sample was collected after surgery. MRI were evaluated according to the MRI staging proposed by Bien et al. (2002b). MRI stage 0 is characterized by normal cortical volume and normal T2/FLAIR signal; stage 1 by cortical swelling with hyperintense T2/FLAIR signal; stage 2 by normal cortical volume and hyper intense T2/FLAIR signal; stage 3 by cortical atrophy and hyperintense T2/FLAIR signal, and stage 4 by progressive cortical atrophy and normal T2/FLAIR signal.

Clinical stages

The clinical course was dividing into four stages: first stage; within 11months, second stage; 12 to 23 months, third stage; 24 to 47 months, and fourth stage; over 48 months from epilepsy onset.

Statistical analysis

Non-parametric Mann–Whitney *U*-test was used to compare the semi-quantitative variables between two groups. A p value less than 0.05 was considered to indicate a statistically significant difference.

Results

All data of antibodies to GluN2B & GluN1 in CSF from RS patients

Forty samples from 18 RS patients (mean number of samples per patient; 2.2 ± 1.2, range 1–5) and 23 samples from 23 disease controls were assayed for autoantibodies against N-terminal and C-terminal of GluN2B (GluN2B-NT2 and GluN2B-CT, respectively) and GluN1 (GluN1-NT and GluN1-CT) (Table 1, Fig. 1). In 12 patients, samples collected at different stages of the clinical course were evaluated (2 to 5 serial samples).

Anti-GluN2B-NT2 and anti-GluN2B-CT antibody levels [mean ± SD (minimum–maximum)] in the CSF of RS patients were 0.542 ± 0.468 (0.081–2.216) and 0.648 ± 0.484 (0.076–2.440), respectively. These levels were significantly higher than the corresponding CSF levels in disease controls [0.252 ± 0.103 (0.102–0.585) and 0.273 ± 0.103 (0.13–0.544); $p = 0.002$ and <0.001, respectively]. Anti-GluN1-NT and GluN1-CT antibody levels in the CSF of RS patients were 0.710 ± 0.494 (0.103–2.347) and 0.709 ± 0.466 (0.104–2.383), respectively, and were also significantly higher than those in the CSF of disease controls [0.404 ± 0.219 (0.103–0.947) and 0.415 ± 0.223 (0.092–0.917); $p = 0.004$ and 0.004, respectively].

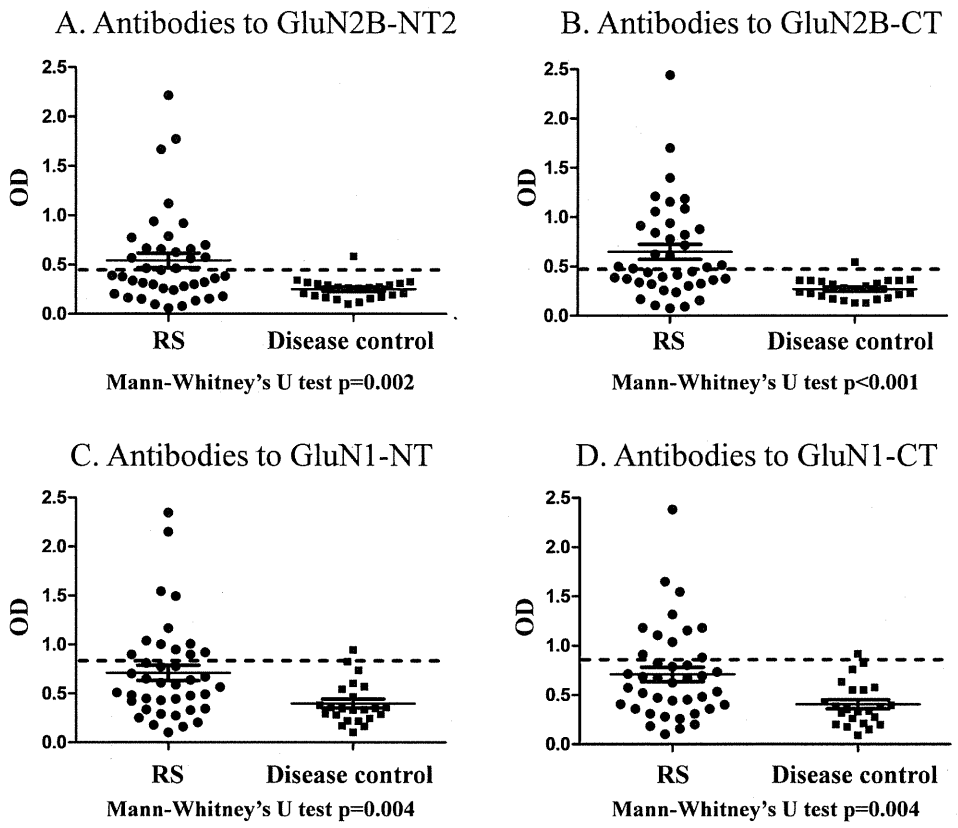

Fig. 1 *Cerebrospinal fluid levels of antibodies to N-methyl-D-aspartate (NMDA) type glutamate receptors (GluR) in patients with Rasmussen syndrome (RS) and disease controls (non-inflammatory partial epilepsy).* Levels of antibodies to N-terminal (NT) and C-terminal (CT) of two GluR subunits; GluN2B and GluN1, in cerebrospinal fluid were measured by ELISA (A) GluN2B-NT2, (B) GluN2B-CT, (C) GluN1-NT, (D) GluN1-CT. Data are expressed as optical density (OD). Solid horizontal lines denote mean and standard error of the mean. Dotted line denotes mean + 2 standard deviations of disease controls.

Relationship between antibodies to NMDA-type GluR and clinical features

The relations between CSF levels of the antibodies and clinical factors at the time of CSF sampling were assessed as follows.

Changes over time after epilepsy onset

We evaluated the changes in antibody levels over the clinical course of 40 CSF samples collected in four clinical stages (Table 1, Fig. 2). First, we determined the cut-off levels for positivity of these antibodies as mean + 2 standard deviations (SD) of the levels in disease controls. The proportions of samples with levels above cut-off in the first stage (≦11 months) were 20% for both anti-GluN2B-NT2 and anti-GluN2B-CT, and 0% for both anti-GluN1-NT and anti-GluN1-CT antibodies. The proportions in the second stage (12–23 months) were 70% for anti-GluN2B-NT2 and anti-GluN2B-CT antibodies, and 50% for anti-GluN1-NT and anti-GluN1-CT antibodies. In patient 1, CSF was negative for anti-GluN1-NT and anti-GluN1-CT antibodies in the first stage, but became positive in the second stage. In patient 4, CSF was negative for all four antibodies in the first stage, but became positive in the second stage. In the third stage (24–47 months), the proportions of samples with positive antibodies were 45% for both anti-GluN2B-NT2 and anti-GluN2B-CT, 27% for both anti-GluN1-NT and anti-GluN1-CT antibodies. In the fourth stage (48< months), the proportions were 36% for anti-GluN2B-NT2, 57% for anti-GluN2B-CT, 29% for anti-GluN1-NT, and 21% for anti-GluN1-CT antibodies. In patient 1, CSF was positive for anti-GluN1-NT and anti-GluN1-CT antibodies in the second stage, but became negative in the third stage. In patient 10, CSF was positive for anti-GluN2B-NT2 antibodies in the second stage, but became negative in the fourth stage. In patient 12, CSF was positive for anti-GluN2B-NT2 and anti-GluN2B-CT antibodies in the second stage, but became negative in the third stage.

Seizure frequency during one month before CSF sampling

Of 40 CSF samples examined for anti-GluN2B and anti-GluN1 antibodies, 31 were collected when seizure frequency was recorded as daily, 2 as weekly, and 6 as monthly, and 1 as seizure-free. The relations between seizure frequency and CSF antibody levels are shown in Table 1 and Fig. 3.

Anti-GluN2B-NT2 and anti-GluN2B-CT antibody levels in CSF sampled when seizures occurred daily were significantly higher than those when seizures occurred less frequently (weekly, monthly and seizure free) ($p = 0.004$ and 0.008, respectively). Likewise, anti-GluN1-NT and anti-GluN1-CT

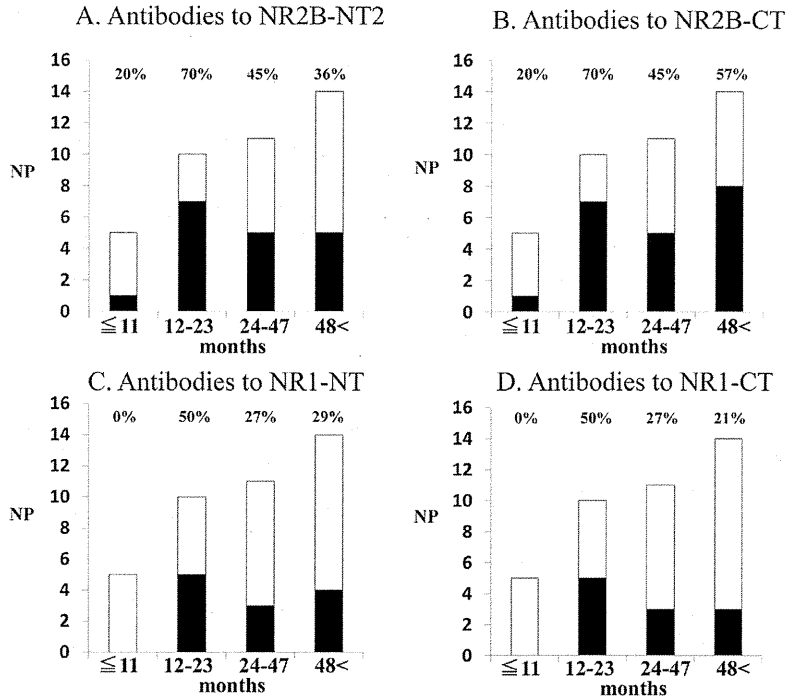

Fig. 2 *Antibodies to N-methyl-D-aspartate (NMDA) type glutamate receptors (GluR) in cerebrospinal fluid during the clinical course of Rasmussen syndrome (RS).* Antibodies against N-terminal (NT) and C-terminal (CT) of two GluR subunits; GluN2B and GluN1, are shown (A) GluN2B-NT2, (B) GluN2B-CT, (C) GluN1-NT, (D) GluN1-CT. The clinical course is divided into four clinical stages (within 11 months, 12 to 23 months, 24 to 47 months, and over 48 months from epilepsy onset). The cut-off levels of optimal density (OD) for antibody positivity was defined as mean + 2 standard deviations of the disease controls. Bar graphs show number of antibody-positive (black columns) and -negative samples (white columns) in each stage (*Y* axis on the left). The proportions of antibody-positive samples (%) in each stage was shown above bar graphs.

antibody levels in CSF sampled when seizure occurred daily were significantly higher than when seizures occurred less frequently ($p = 0.005$ and 0.009, respectively).

MRI stage

Of 40 CSF samples, 33 were sampled at known MRI stage. Eight samples were collected at MRI stage 0, 3 at stage 2, 16 at stage 3, and 6 at stage 4. The relations of MRI stage with CSF antibody levels are shown in Table 1 and Fig. 4.

Anti-GluN2B-NT2 and anti-GluN2B-CT antibody levels in CSF samples at MRI stage 3 were significantly higher than those at MRI stage 0 ($p = 0.001$ and 0.001, respectively), those at MRI stage 2 ($p = 0.029$ and 0.039), and those at MRI stage 4 ($p = 0.014$ and 0.014). The CSF anti-GluN1-NT and anti-GluN1-CT antibody levels at MRI stage 3 were significantly higher than those at MRI stage 0 ($p < 0.001$ and 0.001, respectively), and those at MRI stage 4 ($p = 0.009$ and 0.002, respectively). While CSF anti-GluN1-NT antibody level at MRI stage 3 was significantly higher than that at MRI stage 2 ($p = 0.012$), anti-GluN1-CT antibody level only tended to be higher with marginal significance ($p = 0.050$).

Discussion

We examined antibodies to GluN2B and GluN1 by ELISA in 40 CSF samples collected from 18 RS patients. RS was diagnosed based mainly on clinical criteria (Bien et al., 2005) because consent for brain biopsy cannot be obtained in Japan due to cultural reasons. Our data revealed that CSF of RS patients had autoantibodies not only to GluN2B, but also to GluN1, and that the autoantibodies recognized broad domains from N-terminal to C-terminal of GluN2B and GluN1. These findings suggest that antibodies to NMDA-type GluR in the CSF of RS patients are not cross-reacting antibodies against microbes, but autoantibodies against NMDA-type GluR complexes. Heterogeneous polyclonal antibodies against peptide-specific antibodies to GluR3 have been detected by ELISA in sera from RS patients (Wiendl et al., 2001). Autoantibodies against neuronal acetylcholine receptor α7 subunit (Watson et al., 2005) and Munc-18 (Yang et al., 2000) have also been reported in RS. These data suggest that autoantibodies in RS have broad epitopes for various neuronal molecules. Gahring et al. (2001) provided a potential link between GluR3 autoantibody and the contribution of CTL to RS. They found that the immunogenic domains of the GluR3 protein are exposed to the immune system only after cleavage of GluR3 by granzyme B, a protease produced by activated T cells. Therefore, CTL may be involved in the production of antibodies to broad epitopes of various neuronal molecules, by causing neuronal death in CNS releasing of various neuronal antigens. Although the cytotoxic T lymphocytes appear to play a major role in the pathophysiology of RS, the role of autoantibodies in the primary disease mechanism of RS is still unclear.

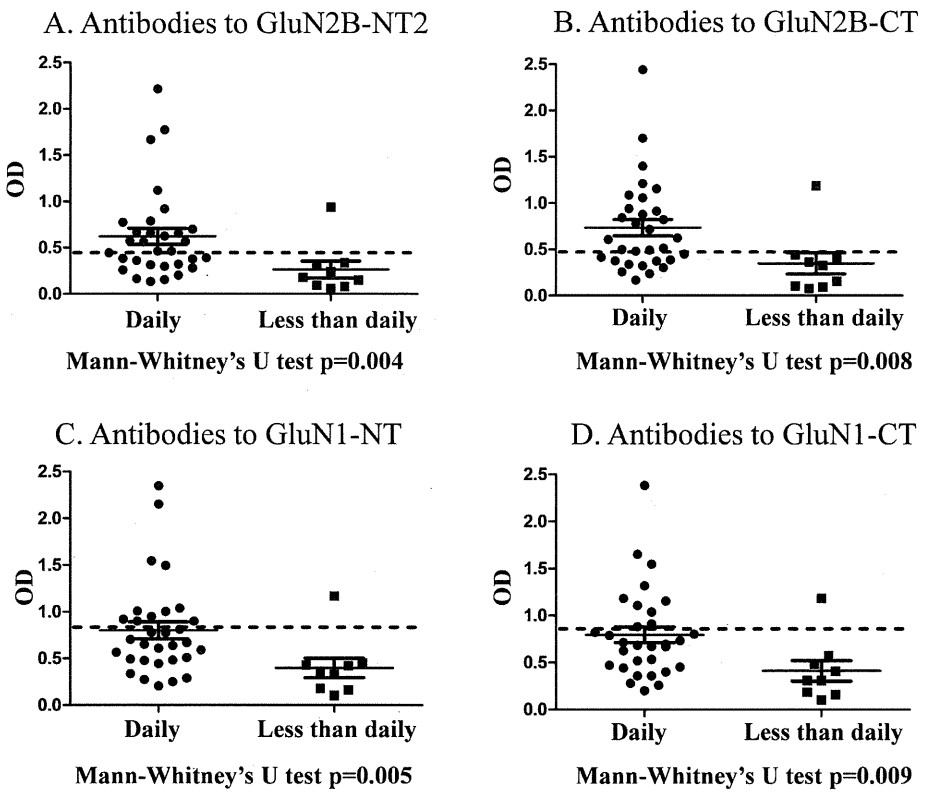

Fig. 3 *Relationship between antibodies to N-methyl-D-aspartate (NMDA) type glutamate receptors (GluR) in cerebrospinal fluid and seizure frequencies in Rasmussen syndrome (RS).* Relation between seizure frequency during one month prior to cerebrospinal fluid sampling. The levels of antibodies against N-terminal (NT) and C-terminal (CT) of two GluR subunits; GluN2B and GluN1, are shown: (A) GluN2B-NT2, (B) GluN2B-CT, (C) GluN1-NT, (D) GluN1-CT. Data are expressed as optical density obtained from ELISA. Seizure frequency was recorded during one month prior to cerebrospinal fluid sampling. Solid horizontal lines denote mean and standard error of the mean. Dotted line denotes mean + 2 standard deviations of disease controls.

The proportions of CSF samples positive for anti-GluN2B and anti-GluN1 antibodies were highest in the clinical stage from 12 to 23 months after epilepsy onset, and declined after 24 months. In addition, CSF levels of antibodies to NMDA-type GluR were high when seizures occurred daily and when MRI FLAIR showed cortical atrophy and hyperintense lesion. These data suggest that antibodies to NMDA-type GluR may be produced, during active progression of RS, and not before onset. Bien et al. (2002b) reported that the median densities of CD3+ T lymphocytes, CD68+ microglial nodules, and GFAP+ astrocytes in the cortical parenchyma were higher within 24 months from onset than later stages, and T cells and reactive astrocytes were more abundant in the MRI stage before the late stage (atrophy only). These findings also support that antibodies against NMDA-type GluR subunits are produced in the CNS after cytotoxic T cell-mediated neuronal damages.

In our previous immunoblot study, we observed that antibodies against GluN2B (GluRε2) in the CSF of RS patients were predominantly against C-terminal and rarely against N-terminal of GluN2B (Takahashi et al., 2003). In the present study using ELISA, the mean anti-GluN2B-NT2 antibody level (OD; 0.542) was lower than the mean anti-GluN2B-CT antibody level (OD; 0.648), while the mean anti-GluN1-NT antibody level was almost the same as the mean anti-GluN1-CT antibody level. The GluRs are transmembrane proteins with the N-terminal in the extracellular domain and the C-terminal in the intracellular domain. Therefore, in the CSF, antibodies to N-terminal of NMDA-type GluR subunits have access to their epitopes, but antibodies to C-terminal do not. The difference in quantity between antibodies to N-terminal and those to C-terminal of NMDA-type GluR subunits in CSF may reflect the amount of antibodies bound to extracellular N-terminal domains of NMDA-type GluRs. These data may suggest that anti-GluN2B-NT2 antibodies are more likely to bind to the extracellular domains of NMDA-type GluR complexes, compared with anti-GluN1-NT antibodies to.

In this study, we detected not only anti-GluN2B antibodies but also anti-GluN1 antibodies in the CSF of RS patients by ELISA. Patients with anti-NMDA receptor (NMDAR) encephalitis, which is causally related to anti-NMDAR antibodies that recognize NMDAR complex (mainly NR1 subunits), present with psychiatric symptoms, seizures, memory deficits, and impaired consciousness (Dalmau et al., 2007, 2008, 2011; Florance et al., 2009; Irani et al., 2010). Anti-NMDAR antibodies cause internalization of surface NMDA-type GluR complex, resulting in abrogation of NMDA-type GluR-mediated synaptic function (Dalmau et al., 2011; Hughes

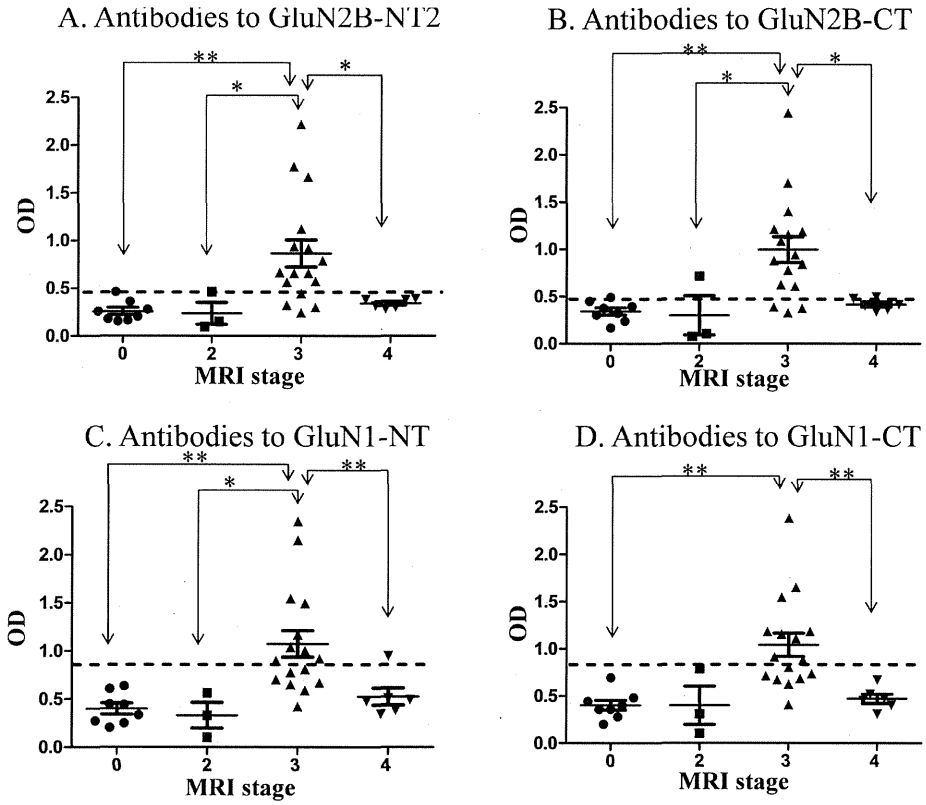

Fig. 4 *Relationship between antibodies to N-methyl-D-aspartate (NMDA) type glutamate receptors (GluR) in cerebrospinal fluid and MRI stages in Rasmussen syndrome (RS).* The levels of antibodies against N-terminal (NT) and C-terminal (CT) of two GluR subunits; GluN2B and GluN1, are shown: (A) GluN2B-NT2, (B) GluN2B-CT, (C) GluN1-NT, (D) GluN1-CT. Data are expressed as optical density obtained from ELISA. Solid horizontal lines denote mean and standard error of the mean. Dotted line denotes mean + 2 standard deviations of disease controls. * $p < 0.05$, ** $p < 0.01$ by Mann–Whitney's *U* test

et al., 2010; Takano et al., 2011). While RS patients have unfavorable outcome if not treated appropriately, the majority of patients with anti-NMDAR encephalitis recover from severe CNS dysfunction in the acute stage. Autopsies of these patients revealed IgG deposits in the hippocampus, extensive microgliosis, and rare T cell infiltrates (Dalmau et al., 2007). Our study on RS, suggests that N-terminal antigen is bound to a greater extent by anti-GluN2B antibodies than by anti-GluN1 antibodies. If we hypothesize that antibodies to GluN1 is mainly involved in internalization of NMDA-type GluR, then little binding by anti-GluN1 antibodies in CSF of RS patients would lead to little internalization of NMDA-type GluR complex, resulting in greater excitotoxic effect by NMDA-type GluR complexes.

In disease controls of this study, we detected relatively few amounts of antibodies to GluRs. These control patients had seizures at different frequencies ranging from daily to yearly. They may include autoimmune epilepsy patients causally related with smoldering chronic encephalitis (Niehusmann et al., 2009) or pediatric epilepsy patients with "unknown cause", (Suleiman et al., 2013).

The following limitation of our studies must be considered, ELISA for antibodies to NMDA-type GluRs was semi-quantitative with optical density, and samplings of CSF were not controlled systematically, and treatment strategies varied among patients. Especially among the clinical stage from 12 to 23 months after epilepsy onset with clustering seizures, various treatment including immunomodulatory treatment, anesthesia, and AEDs, may affect the levels of the antibodies and seizure frequencies. We have not done studies including passive transfer of the antibodies to mice that may reveal the pivotal roles of the antibodies in RS.

The present study confirms the presence of antibodies to GluN2B and GluN1 in the CSF of RS patients, and suggests that these antibodies may affect the pathologic mechanisms of RS in the most active stage (from early stage up to several years after onset of epilepsy) and that these antibodies could be a marker for active inflammation in RS. Further studies are required to examine the pathogenic effects of those antibodies in RS in order to improve the outcome of RS.

Conflict of interest statement

None of the authors has any conflict of interest to disclose.

Ethical approval

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Original article

Three patients manifesting early infantile epileptic spasms associated with 2q24.3 microduplications

Shinsaku Yoshitomi [a,*], Yukitoshi Takahashi [a], Mamiko Ishizuka [a], Tokito Yamaguchi [a], Akito Watanabe [a], Hirosato Nasu [a], Yuki Ueda [a], Hideyuki Ohtani [a], Hiroko Ikeda [a], Katsumi Imai [a], Hideo Shigematsu [a], Yushi Inoue [a], Yoshihiro Tanahashi [b], Kaori Aiba [c], Hodaka Ohta [d], Shino Shimada [e,f], Toshiyuki Yamamoto [e,f]

[a] *National Epilepsy Center, Shizuoka Institute of Epilepsy and Neurological Disorders, Shizuoka, Japan*
[b] *Ogaki Municipal Hospital, Ogaki, Japan*
[c] *Toyohashi Municipal Hospital, Toyohashi, Japan*
[d] *Mie Prefectural General Medical Center, Yokkaichi, Japan*
[e] *Institute of Medical Genetics, Tokyo Women's Medical University, Tokyo, Japan*
[f] *Tokyo Women's Medical University Institute for Integrated Medical Sciences, Tokyo, Japan*

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Abstract

Background: Recent development of genetic analyses enabled us to reveal underlying genetic causes of the patients with epileptic encephalopathy in infancy. Mutations of voltage-gated sodium channel type I alpha subunit gene (*SCN1A*) are to be causally related with several phenotypes of epilepsy, generalized epilepsy with febrile seizure plus (GEFS+), Dravet syndrome, and other infantile epileptic encephalopathies. In addition to *SCN1A*, contiguous genes such as *SCN2A* and *SCN3A* in 2q24.3 are also reported to have contribution to epileptic seizures. Therefore, gene abnormality involving this region is reasonable to contribute to epilepsy manifestation.

Results: We encountered three patients with 2q24.3 microduplication diagnosed by Array comparative genomic hybridization array (aCGH). They developed partial seizures and epileptic spasms in their early infantile periods and showed remarkable developmental delay, although their seizures disappeared from 11 to 14 months of age. One of three patients had 2q24.3 microduplication which excludes *SCN1A*. Therefore, characteristics of epilepsy with 2q24.3 microduplication do not necessarily need duplication of *SCN1A*. This study suggested that 2q24.3 microduplication is one of the causes for early infantile epileptic spasms. Epileptic spasms associated with 2q24.3 microduplications may have better seizure outcome comparing with other etiologies.

Keywords: 2q24.3 microduplication; Epileptic spasms; Array comparative genomic hybridization array (aCGH); *SCN1A*; *SCN2A*; *SCN3A*

1. Introduction

Recent development of genetic analyses enabled us to reveal underlying genetic causes of the patients with epileptic encephalopathy in infancy effectively. Several genes including the syntaxin binding protein 1 gene (*STXBP1*) [1,2], the cyclin-dependent kinase-like 5 gene (*CDKL5*) [3], the potassium channel subfamily T member 1 gene (*KCNT1*) [4], the protocadherin 19 gene (*PCDH19*) [5], the aristaless related homeobox gene

* Corresponding author.

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(*ARX*) [6], the potassium voltage-gated channel, KQT-like subfamily, member 2 gene (*KCNQ2*) [7], the Cdc42 guanine nucleotide exchange factor 9 gene (*ARHGEF9*) [8], the gamma-aminobutyric acid A receptor, alpha 1 gene (*GABRA1*) [9] and the spectrin, alpha, non-erythrocytic 1 gene (*SPTAN1*) [10] have been identified as the major genes responsible for epileptic encephalopathy in infancy. Although the voltage-gated sodium channel type I alpha subunit gene (*SCN1A*) is not only related to epileptic encephalopathy in infancy, i.e., Dravet syndrome, but to other epileptic syndromes, i.e., generalized epilepsy with febrile seizure plus (GEFS+), etc., *SCN1A* is located on chromosomal region of 2q24.3, which includes clustering of other sodium channel genes, i.e., *SCN2A* and *SCN3A*. Latter two genes are also reported to have contribution to epileptic seizures. Although missense mutations in *SCN2A* have been reported to be associated with benign familial neonatal–infantile seizures (BFNIS) [11–13], recent reports also revealed *SCN2A* is one of causal genes for early-onset epileptic encephalopathies such as Ohtahara syndrome and West syndrome [14]. Furthermore, *SCN3A* is confirmed to be correlated to focal epilepsy in children [15,16]. Therefore, contiguous gene abnormality involving the region of 2q24.3 is reasonable to contribute to epilepsy manifestation. Here, we report three patients with 2q24.3 duplications associated with epileptic encephalopathy in infancy.

2. Patient reports

2.1. Patient 1

A male infant was born at 38 weeks of gestation by caesarean section with no distress, because of repeated caesarean section. At the birth his weight was 2790 g, and neurologic examination was normal, and no dysmorphic feature was noticed. He had no particular family history except for his elder brother's simple febrile seizure. He developed partial seizures consisting of tonic posture and deviation of the eyes to the left or right at 7 days after birth. He developed combined epileptic spasms occurring in cluster at 30 days of age, with the interictal EEG showing the bursts of high voltage slow and spikes, and following diffuse background attenuation (suppression-burst pattern) (Fig. 1A). His seizures did not respond to valproate (VPA), pyridoxal phosphate, zonisamide, levetiracetam (LEV), or ACTH. Carbamazepine (CBZ) was added to LEV at 8 months of age, because of asymmetric spasms and combined partial seizures. Thereafter both types of seizures disappeared from 11 months of age. His EEG at 12 months of age showed remarkable improvement without epileptic discharge (Fig. 1A). His development was remarkably delayed, and head control was incomplete at even 12 months. Brain magnetic resonance imaging (MRI) at 12 months showed nonspecific mild brain atrophy, large cavum septi pellucidi and cavum vergae (Fig. 2A).

2.2. Patient 2

A female infant was born at 38 weeks of gestation by caesarean section with no distress. Her birth weight was 2728 g, head circumference was 34.5 cm. Neurologic examination was normal, and no dysmorphic feature was noticed. She had no family history of neurological disease. She developed partial seizures with deviation of the eyes to the right at 3 days after birth, and combined epileptic spasms occurring in cluster joined at the age of 30 days. Her EEG at 2 months of age showed multifocal spikes, sharp waves, and high voltage slow background (Fig. 1B). Treatment with ZNS and VPA resulted in gradual control of seizures by 11 months of age. Her EEG at 12 months of age showed improvement without epileptic discharge (Fig. 1B). Her development was considerably delayed, with no head control at the age of 18 months. Brain MRI at the age of 18 months showed moderate atrophy of bilateral frontal lobes (Fig. 2B).

2.3. Patient 3

A female infant was born at 37 weeks of gestation without distress and dysmorphic features. Her birth weight was 2934 g, and head circumference was 31.5 cm. She had no family history of neurological disease. She developed clustering epileptic spasms and partial seizures with upward deviation of the eyes from several hours after birth. Her EEG at 5 months of age showed spikes at right parietal and left occipital regions (Fig. 1C). At 5 months of age, new partial seizure developed, consisting of eyes opening and slow deviation of eyes to the left during sleep, and then disappeared by 7 months. After the addition of lamotrigine (LTG) to VPA from 8 months, epileptic spasms disappeared gradually, and she became seizure-free at 14 months of age. Her brain MRI at 12 months showed hypoplastic corpus callosum, bilateral diffuse atrophy and possible delay of myelination (Fig. 2C). Her development was extremely delayed, with no complete head control at the age of 14 months.

2.4. Molecular cytogenetic analysis

Chromosomal microarray testing was performed according to the method described previously [18]. The present three patients showed genomic copy number gains in 2q24.3 region (Fig. 3). Fluorescence in situ hybridization (FISH) analyses confirmed the signal duplications of this region on the same chromosome, indicating no translocation (data not shown). All of

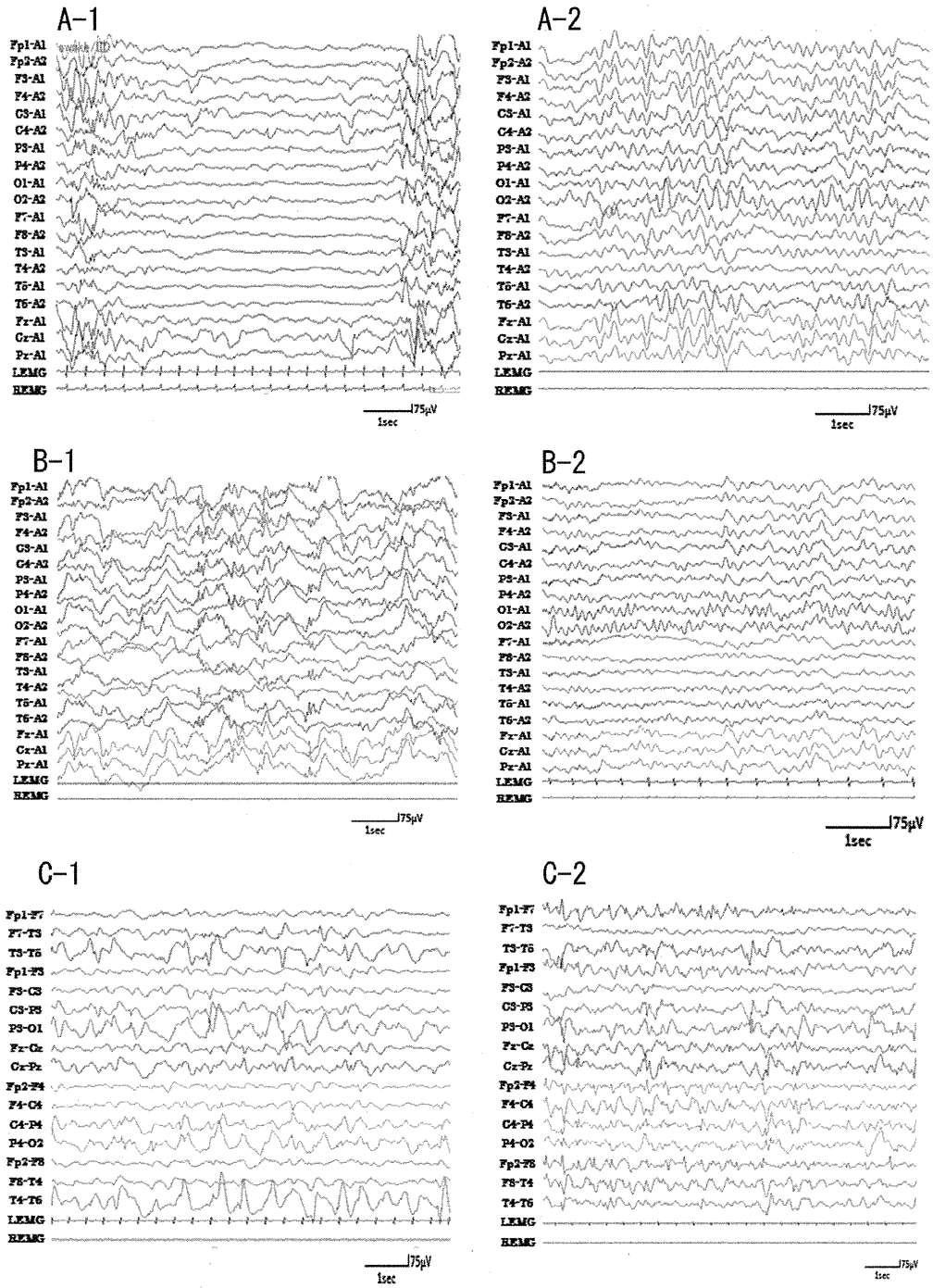

Fig. 1. Interictal EEG of the three patients. (A): EEG of Patient 1 showing suppression-burst pattern, containing the bursts of high voltage slow and spikes, and alternate diffuse background attenuation (A-1). At 12 months of age, EEG findings have improved to the level of few spikes in O2 (A-2). (B): EEG of Patient 2 showing multifocal spikes, sharp waves, and high voltage slow background at 2 months of age (B-1). At 12 months of age, epileptic discharges seen before have greatly decreased (B-2). (C): EEG of Patient 3 showing spikes in bilateral parietal, anterotemporal, and occipital regions at 5 months of age. In this figure, spikes in P3, P4, and T6 are seen (C-1). At 12 months of age, the findings of multifocal spikes continued (C-2).

the parents of three patients were negative for 2q24.3 duplications by either of chromosomal microarray testing or FISH. This indicates de novo occurrence of 2q24.3 duplications in all patients.

3. Discussion

We identified 2q24.3 microduplications in three patients with early infantile epileptic spasms and partial

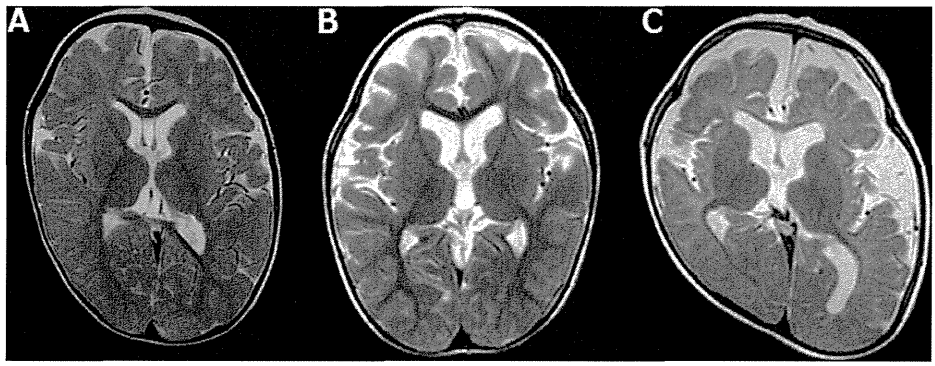

Fig. 2. MRI findings of the three patients. (A): T2WI MRI in Patient 1 (12 months of age) showing mild brain atrophy, large cavum septi pellucidi and cavum vergae. (B): T2WI MRI in Patient 2 (18 months of age) with remarkable atrophy of bilateral frontal lobes. (C): T2WI MRI in Patient 3 (12 months of age) showing hypoplastic corpus callosum, severe bilateral diffuse atrophy and possible delay of myelination.

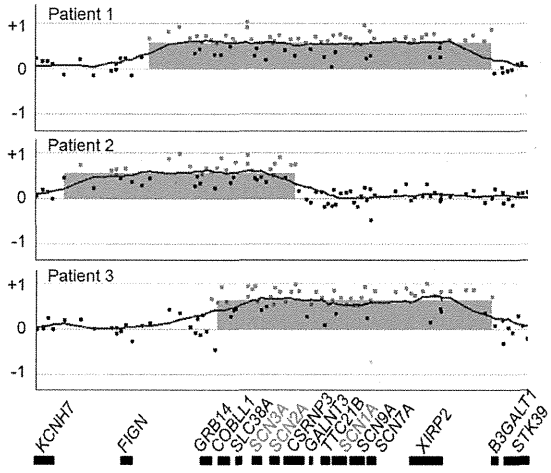

Fig. 3. Chromosomal microarray testing identified 2q24.3 duplications in three patients in this study. The identified duplicated regions are expanded by Gene Views of Agilent. Patient 1 has duplication from 164,842,075 to 168,690,006 (size, 3,847,931), and Patient 2 has duplication from 163,875,903 to 166,478,766 (size, 2,602,863), and, Patient 3 has duplication from 165,600,128 to 168,690,006 (size, 3,089,878). Genomic locations indicate the February 2009 human reference sequence (GRCh37). Genes included in the duplicated regions of each patients except for *SCN1A*, *SCN2A*, *SCN3A* are as follows. *FIGN* (fidgetin, a member of the AAA (ATPases associated with diverse cellular activities) family of ATPases, *GRB14* (Growth Factor Receptor-Bound Protein 14 gene)), *SLC38A* (Solute Carrier Family 38, Member), *CSRNP3* (Cysteine–Serine-Rich Nuclear Protein 3 gene), *GALNT3* (UDP-N-Acetyl-Alpha-D-Galactosamine:Polypeptide N-Acetylgalactosaminyltransferase 3), *TTC21B* (Tetratricopeptide Repeat Domain 21B), *SCN9A* (Sodium Channel, Voltage-Gated, Type IX, Alpha Subunit), *SCN7A* (Sodium Channel, Voltage-Gated, Type VII, Alpha Subunit), *XIRP2* (Xin Actin-Binding Repeat Containing 2).

seizures. This indicates that 24.3 microduplication is one of the causes of this phenotype. Such genomic copy number aberrations especially copy number gains are hard to be detected by means of the targeted resequencing method using next generation sequencer. Therefore, it would be important to screen whole genome copy number aberrations by use of microarray system.

Three patients had common characteristics of epileptic spasms and partial seizures with early infantile onset, relatively better seizure outcome, and severe developmental outcome. These characteristics were quite different from Dravet syndrome with mutation of *SCN1A* in 2q24.3.

As far as we know, the microduplications of this region have been reported in at least 10 patients [17–22]. Because there is a variation of genomic duplicated regions in these patients, we studied genotype–phenotype relationship in these patients. Chromosomal region of 2q24.3 includes the clustering of sodium channel genes, including *SCN1A*. These patients with 2q24.3 duplications rarely show epileptic spasms, and *SCN1A* is not necessarily included in the duplicated region [17–22]. There are two reports of 2q24.3 microduplications which do not include *SCN1A* [20,22]. In our study, Patient 2 had a 2q24.3 microduplication which excludes *SCN1A* as the patient reported by Vecchi et al. [22]. Among these patients with 2q24.3 microduplications sparing *SCN1A*, the familial patients reported by Heron et al. showed seizure onset at 2–18 days [20] and Patient 2 in our study had seizure onset at 3 days after birth, as similar as the other patients with 2q24.3 duplications including *SCN1A*. The patient reported by Vecchi et al. showed seizure onset at 3 months of age [22], which is relatively later than other patients having 2q24.3 duplications. From these findings, we suspect that 2q24.3 microduplications associated with epileptic spasms does not necessarily include *SCN1A*, and whether *SCN1A* is included in the duplication or not does not affect the timing of seizure onset.

The duplicated region in the three patients included some genes other than *SCN1A, SCN2A, SCN3A*. The duplication of *SCN7A* and *SCN9A*, which have been suspected to be causal genes for epilepsy, were detected in Patients 1 and 3. Some authors have suggested the association between *SCN7A* [23] and temporal lobe epilepsy with hippocampal sclerosis, and others have

reported mutation of *SCN9A* may attribute to the Dravet syndrome without *SCN1A* mutation [24]. Because genotype and phenotype of our patients were quite different from those of these reports, the relation between the duplication of *SCN7A* or *SCN9A* and epilepsy are remained to be solved.

Epileptic spasms associated with 2q24.3 microduplications may have better seizure outcome comparing with other etiologies. Although carbamazepine (CBZ) and lamotrigine (LTG) are known to exacerbate seizures in Dravet syndrome, CBZ has been reported to achieve good seizure outcome in cases of 2q24.3 duplication [19,22]. We prescribed CBZ and LTG as the additional treatment in Patients 1 and 3, respectively, and the seizures decreased gradually, resulting in disappearance in a few months after starting CBZ or LTG. However, the effectiveness of CBZ and LTG cannot be concluded at this moment, because of the possibility of natural remission. Three patients in this study had no fever-induced epileptic seizures, consistent with the other reported patients. This is one of the definitive differences between 2q24.3 microduplication and Dravet syndrome or GEFS+. Furthermore, patients with 2q24.3 microduplication syndrome has relatively better seizure outcome than Dravet syndrome.

From the review of three patients of our study and previous reports [17–22], manifestations of partial seizures were various and interictal EEG findings were also nonspecific. We reported three patients with partial seizures and spasms, and previous papers reported many kinds of seizures, for example, GTCS, absence, myoclonic seizures, clonic seizures, focal tonic seizures, autonomic seizures, secondary GTCS, etc. A variety of EEG features have also been reported on 2q24.3 duplications. In interictal phase, some patients showed diffuse spike and slow waves, others showed focal spike, or severe abnormal findings containing frequent independent spike, spike and waves, resembling hypsarrhythmia. In our patients, EEG findings of Patient 1 showed suppression-burst pattern, which suggested the diagnosis of early infantile epileptic encephalopathy (EIEE). Lim et al. also reported the case diagnosed as EIEE with 2q24.3 duplication [18].

The response to antiepileptic drugs also seems to be heterogeneous. Some authors reported that phenobarbital, VPA, CBZ, oxcarbazepine, vigabatrin were effective. Seizures of three patients in our study showed improvement after taking LEV and VPA, ZNS and VPA, or LTG and VPA, as previously mentioned.

No structural abnormality of brain has been reported except for one case of hypoplastic corpus callosum by MRI [20]. In our reports, Patient 1 had large cavum septi pellucidi and Patient 2 had remarkable atrophy of bilateral frontal lobes at the age of 18 months, and Patient 3 had hypoplastic corpus callosum. These data may suggest that some patient with 2q24.3 duplication have abnormality of corpus callosum. There have been no imaging reports of 2q24.3 duplication cases after 2 years of age when myelination could have finished. In three patients, the last MRI was taken before 2 years of age, so we are going to follow up their MRI.

As with three patients in our study showed severe development delay, almost all patients with 2q24.3 duplication showed poor developmental outcome [17–22].

The patients with neonatal or early infantile seizures associated with developmental delay should be examined by aCGH. More cases have to be studied to unveil the phenotype–genotype correlation, clinical course, and optimal treatment.

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Case Report

Usefulness of ketogenic diet in a girl with migrating partial seizures in infancy

Tatsuo Mori [a,*], Katsumi Imai [a], Taikan Oboshi [a], Yuh Fujiwara [b], Saoko Takeshita [b], Hirotomo Saitsu [c], Naomichi Matsumoto [c], Yukitoshi Takahashi [a], Yushi Inoue [a]

[a] *National Epilepsy Center, NHO, Shizuoka Institute of Epilepsy and Neurological Disorders, Japan*
[b] *Department of Pediatrics, Yokohama City University Medical Center, Japan*
[c] *Department of Human Genetics, Yokohama City University Graduate School of Medicine, Japan*

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Abstract

Migrating partial seizures in infancy (MPSI) are an age-specific epilepsy syndrome characterized by migrating focal seizures, which are intractable to various antiepileptic drugs and cause severe developmental delay. We report a case of MPSI with heterozygous missense mutation in *KCNT1*, which was successfully managed by ketogenic diet. At age 2 months, the patient developed epilepsy initially manifesting focal seizures with eye deviation and apnea, then evolving to secondarily generalized clonic convulsion. Various antiepileptic drugs including phenytoin, valproic acid, zonisamide, clobazam, levetiracetam, vitamin B6, and carbamazepine were not effective, but high-dose phenobarbital allowed discontinuation of midazolam infusion. Ictal scalp electroencephalogram showed migrating focal seizures. MPSI was suspected and she was transferred to our hospital for further treatment. Potassium bromide (KBr) was partially effective, but the effect was transient. High-dose KBr caused severe adverse effects such as over-sedation and hypercapnia, with no further effects on the seizures. At age 9 months, we started a ketogenic diet, which improved seizure frequency and severity without obvious adverse effects, allowing her to be discharged from hospital. Ketogenic diet should be tried in patients with MPSI unresponsive to antiepileptic drugs. In MPSI, the difference in treatment response in patients with and those without *KCNT1* mutation remains unknown. Accumulation of case reports would contribute to establish effective treatment options for MPSI.

Keywords: Migrating partial seizures in infancy (MPSI); Ketogenic diet; *Potassium channel subfamily T member 1 (KCNT1)*; Bromide

1. Introduction

Migrating partial seizures in infancy (MPSI) were originally reported by Coppola et al. [1]. Important characteristics of MPSI include onset of focal seizures within the first 6 months of life associated with autonomic features and developmental arrest. Ictal EEG reveals migrating ictal discharges. Most of the MPSI were intractable to various therapies [2].

In this report, we describe a case of MPSI with heterozygous missense mutation in *Potassium channel subfamily T member(KCNT)1*, which did not respond to most antiepileptic drugs but improved by ketogenic diet.

* Corresponding author at: Department of Pediatrics, University of Tokushima Graduate School, 3-18-15 Kuramoto-cho, Tokushima City, Tokushima 770-8503, Japan. Tel.: +81 88 633 7135; fax: +81 88 631 8697.
E-mail address: mori.tatsuo@tokushima-u.ac.jp (T. Mori).

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2. Case report

The girl was born at 39 weeks of gestation with birth weight of 3006 g and head circumference of 32 cm. She showed normal development until the onset of epilepsy.

At age 2 months, she developed focal seizures with eye deviation, later associated with apnea and secondarily generalized clonic convulsions. She was admitted to a local hospital. Various antiepileptic drugs including phenytoin (PHT), valproic acid (VPA), zonisamide (ZNS), clobazam (CLB), levetiracetam (LEV), vitamin B6, and carbamazepine (CBZ) were not effective. High-dose phenobarbital (PB) (serum concentration 50 μg/ml) allowed discontinuation of midazolam infusion.

At age 6 months, MPSI was suspected by ictal scalp EEG and she was transferred to our hospital for further investigations and treatment. At admission, her mental development had regressed. She could not drink and lost the ability of eye tracking and smile while being dandled. Seizure frequency while on PB, ZNS and CLB was 40 to 80 times per day. Epileptic seizures consisted of autonomic seizures such as apnea, and inconspicuous focal motor seizures such as head and eye deviations. Apnea during seizures occurred approximately 20 times per day, and oxygen saturation decreased to around 60% during apnea. Interictal scalp EEG showed multifocal spikes with background slowing. Ictal scalp EEG showed rhythmic fast discharges arising from the migrating foci, which continued for approximately 5–10 min (Fig. 1). Brain MRI showed diffuse atrophy with hypomyelination in the bilateral occipital lobes (Fig. 2A). Genetic screening conducted by the referring doctor revealed heterozygous missense mutation in *KCNT1* [NM_020822.2:c2800G>A(p.Ala934Thr)] in the patient but no abnormality in her parents. Finally, MPSI was diagnosed.

Three weeks after the initiation of potassium bromide (KBr, 40 mg/kg/day), epileptic seizures decreased to around 30 times a day. However, seizure frequency

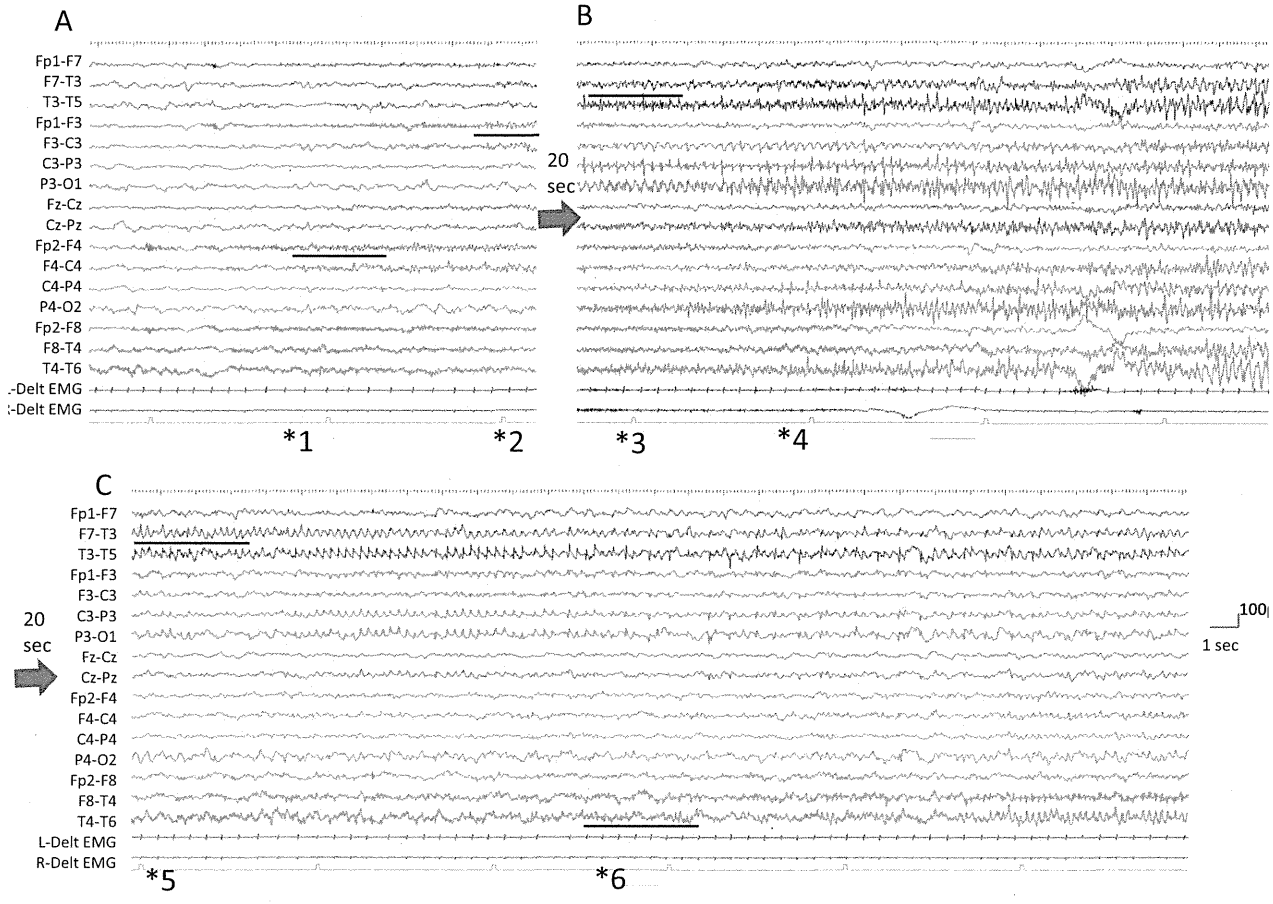

Fig. 1. A series of ictal scalp EEG findings showing migrating ictal discharges (A → B → C). (A) Ictal epileptic discharges started at F4 (*1), becoming dominant at F3 (*2). In this early period, epileptic symptoms consisted of inconspicuous focal motor seizures such as head and eye deviations. (B) Twenty seconds later, ictal discharges migrated to P3 (*3) and then to O2 (*4), accompanied by apnea. (C) Twenty seconds later, ictal discharges migrated to T3 (*5) and evolved to P3-O1. Before the disappearance of P3-O1 spikes, T4 spikes started (*6). Such migrating discharges continued for approximately 5–10 min, associated with apnea and inconspicuous focal motor seizures.

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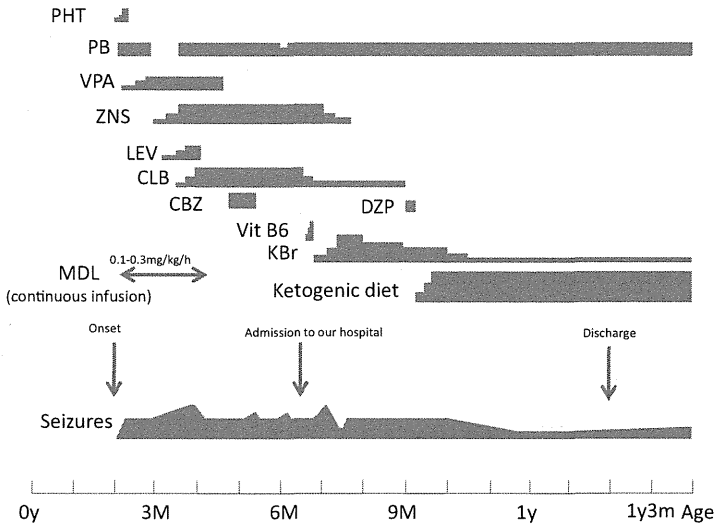

Fig. 2. Clinical course of the present case. PHT, phenytoin; PB, phenobarbital; VPA, valproic acid; ZNS, zonisamide; LEV, levetiracetam; CLB, clobazam; CBZ, carbamazepine; DZP, diazepam; VitB6, vitamin B6; MDL, midazolam; KBr, potassium bromide.

increased again after two weeks of improvement. The KBr dose was increased to 60 mg/kg/day, which caused severe adverse effects such as over-sedation and hypercapnia ($pCO_2 > 60$ mmHg) with no further effects on the seizures.

At age 9 months, we started ketogenic diet using a ketogenic formula (Ketone Formula ⅰ, Meiji Co., Ltd., Tokyo) fed via a nasogastric tube. The diet was started with a ketogenic ratio of 1:1 mixed with regular milk and gradually increased at weekly intervals to 2:1 and 3:1. Total calorie was 440 kcal, which was almost the same as that before the start of ketogenic diet. Serum beta-hydroxybutyrate was monitored at one- or two-week intervals, and serum level exceeded 4 mmol/L after 2 weeks of 3:1 ketogenic diet. After 1 week on 3:1 ketogenic diet, both seizure frequency and severity were dramatically improved without obvious adverse effect. The ketogenic diet allowed reduction of the KBr dose to 11 mg/kg/day, resulting in disappearance of KBr-related severe adverse effects. Eventually, discharge from hospital was possible while on the ketogenic diet.

During the last follow-up at age 15 months, she regained the ability of eye tracking. Seizure frequency remained reduced at 20–40 times per day. Apnea with reduced oxygen saturation was also reduced to 0–3 times per day, and she did not need oxygenation during seizures. The duration of seizures was also shortened to within 30 s, with no migrating ictal discharges on EEG. Brain MRI performed at age 14 months showed no aggravation of brain atrophy compared with that of age 6 months.

Clinical course of the present case is illustrated in Fig. 2.

3. Discussion

Potassium bromide is one of the most commonly used antiepileptic drugs for MPSI, with some reports of complete seizure remission [3–5]. However, similar to our case, some patients suffer from severe adverse effects such as bromoderma and over-sedation [2,6]. Favorable responses to stiripentol [1,7], acetazolamide [8], and rufinamide [9] have also been reported. In the present case, however, we selected a ketogenic diet preferentially, because the patient already suffered from severe adverse effects of KBr, and we were concerned about further adverse effects of antiepileptic drugs.

There are a few reports of ketogenic diet for MPSI in the literature. McTague et al. [2] treated 9 of 14 patients with MPSI by ketogenic diet, and reported partial response in two patients when used in combination with topiramate or prednisolone. Caraballo et al. [10] treated three MPSI patients with ketogenic diet, and reported good response in two of the three children, with one of them becoming seizure-free. Sugai [11] reported three patients treated by ketogenic diet. The diet was transiently effective in only one of three patients, but the patient could not continue ketogenic diet because of ileus. To the best of our knowledge, the present patient is the first detailed case report in Japan on the effectiveness of ketogenic diet for MPSI. Although the antiepileptic mechanisms of ketogenic diet are still poorly delineated, experiment using a mouse model suggested that an inhibitory amino acid GABA in cerebrospinal fluid, which is regulated by ketogenic body, plays a role in the anticonvulsant mechanism [12]. In our case, seizures responded slightly to midazolam,

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KBr and PB. The common mechanism of action for these drugs is enhancement of the inhibitory neural pathway by promoting Cl^- inflow via the gamma-aminobutyric acid (GABA) receptor. This observation may suggest that one antiepileptic mechanism of ketogenic diet for MPSI may be enhancement of the inhibitory neural pathway.

McTague et al. [2] found an abnormality in *KCNT1* in 2 of 14 patients with MPSI. *KCNT1* encodes the pore-forming alpha subunit of a sodium activated potassium channel that is highly expressed in both neurons and cardiomyocytes, and all reported *KCNT1* mutations in MPSI were gain-of-function mutations. In a recent report of a case of MPSI, treatment with quinidine resulted in marked reduction of epileptic seizures [13]. However, quinidine is not yet approved for the treatment of epilepsy. In MPSI, the difference in response to treatment between patients with and those without *KCNT1* mutation remains unknown, and the efficacy of therapy may differ in individual cases of MPSI.

In conclusion, ketogenic diet should be tried in patients with MPSI intractable to various antiepileptic drugs. Accumulation of case reports would contribute to establish effective treatment options for MPSI.

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Original article with video sequences

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Characteristic phasic evolution of convulsive seizure in *PCDH19*-related epilepsy*

Hiroko Ikeda[1], Katsumi Imai[1], Hitoshi Ikeda[1], Hideo Shigematsu[1], Yukitoshi Takahashi[1], Yushi Inoue[1], Norimichi Higurashi[2,3], Shinichi Hirose[3,4]

[1] National Epilepsy Center, Shizuoka Institute of Epilepsy and Neurological Disorders, NHO
[2] Department of Pediatrics, Jikei University School of Medicine
[3] The Central Research Institute for the Molecular Pathomechanisms of Epilepsy of Fukuoka University
[4] Department of Pediatrics, Fukuoka University, School of Medicine, Japan

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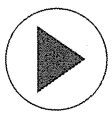
ABSTRACT – *PCDH19*-related epilepsy is a genetic disorder that was first described in 1971, then referred to as "epilepsy and mental retardation limited to females". *PCDH19* has recently been identified as the responsible gene, but a detailed characterization of the seizure manifestation based on video-EEG recording is still limited. The purpose of this study was to elucidate features of the seizure semiology in children with *PCDH19*-related epilepsy. To do this, ictal video-EEG recordings of 26 convulsive seizures in three girls with *PCDH19*-related epilepsy were analysed. All seizures occurred in clusters, mainly during sleep accompanied by fever. The motor manifestations consisted of six sequential phases: "jerk", "reactive", "mild tonic", "fluttering", "mild clonic", and "postictal". Some phases were brief or lacking in some seizures, whereas others were long or pronounced. In the reactive phase, the patients looked fearful or startled with sudden jerks and turned over reactively. The tonic and clonic components were less intense compared with those of typical tonic-clonic seizures in other types of epilepsy. The fluttering phase was characterised initially by asymmetric, less rhythmic, and less synchronous tremulous movement and was then followed by the subtle clonic phase. Subtle oral automatism was observed in the postictal phase. The reactive, mild tonic, fluttering and mild clonic phases were most characteristic of seizures of *PCDH19*-related epilepsy. Ictal EEG started bilaterally and was symmetric in some patients but asymmetric in others. It showed asymmetric rhythmic discharges in some seizures at later phases. The electroclinical pattern of the phasic evolution of convulsive seizure suggests a focal onset seizure with secondary generalisation. Based on our findings, we propose that the six unique sequential phases in convulsive seizures suggest the diagnosis of *PCDH19*-related epilepsy when occurring in clusters with or without high fever in girls. [*Published with video sequences online*]

Key words: *PCDH19*-related epilepsy, *PCDH19*, focal-onset convulsive seizures, EEG



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Correspondence:
Hiroko Ikeda
National Epilepsy Center,
Shizuoka Institute of Epilepsy and Neurological Disorders,
886 Urushiyama,
Shizuoka 420-8688, Japan
<ikedahiroko7@gmail.com>

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The disorder "epilepsy and mental retardation limited to females" (EFMR) presents seizures in infancy, often in combination with intellectual disability or autism (Juberg and Hellman, 1971; Ryan *et al.*, 1997; Scheffer *et al.*, 2008). Recently, it has been reported that mutations in the X chromosome-encoded *PCDH19* gene cause EFMR (Dibbens *et al.*, 2008). As such, the term "EFMR" has been replaced by *PCDH19*-related epilepsy (PCDH19RE) in recent reports. Subsequent studies revealed sporadic cases with *de novo* mutations (Jamal *et al.*, 2010; Higurashi *et al.*, 2011; Hynes *et al.*, 2010; Specchio *et al.*, 2011), but the genetic mechanism underlying the phenotypic expression of EFMR remains elusive. The onset of seizure is early and seizures tend to occur in clusters, which are often induced by fever (Higurashi *et al.*, 2011; Specchio *et al.*, 2011; Marini *et al.*, 2012; Higurashi *et al.*, 2013). Here, we report the video-EEG recordings of convulsive seizures in three patients with PCDH19RE, aiming to elucidate the characteristic features of convulsive seizures associated with PCDH19RE.

Methods

Analysis of convulsive seizure semiology was conducted by recording and reviewing ictal video-EEG. Family history, precipitation by fever, frequency and duration of seizures, interictal EEG, brain imaging, treatments, and cognitive and behavioural assessments were reviewed from the medical records. Epileptic seizures were classified according to the International League Against Epilepsy criteria. Genetic analyses of *PCDH19* and *SCN1A* were performed at Fukuoka University, as described previously (Higurashi *et al.*, 2011). The GenBank accession numbers used as reference sequences are the complete human *PCDH19* mRNA and protein EF676096.1, and the complete human *SCN1A* coding sequence and protein AB093548.

The patients and their parents were informed and they consented to participate in this study. The *PCDH19* and *SCN1A* genetic tests were approved by the institutional ethical committee.

Results

Five patients with *PCDH19* mutations were identified in our hospital. Among these five patients, we detected 47 seizures by EEG, of which 43 occurred during sleep. Twenty-six convulsive seizures from three patients were recorded by video-EEG and analysed in detail.

Patient 1

Patient 1 was a 14-year-old girl whose mother had severe toxaemia during pregnancy. The patient had no medical history of neurological diseases. The psychomotor development was normal before seizure onset. Her initial seizure occurred at 10 months of age, induced by high fever. Since then, intellectual disability has been observed. Her IQ was 62 at 11 years of age. MRI and computed tomography (CT) revealed slightly diffuse brain atrophy, however, not pathological for her age.

The patient's mother also had epilepsy and intellectual disability. She used to have clusters of seizures similar to her daughter until the age of 31 years when valproate (VPA) was introduced, which she took throughout her pregnancy. VPA was successfully discontinued at the age of 45 years. The patient's father committed suicide due to depression. A *PCDH19* missense mutation (c.416C>T/p.Ser139Leu) was detected in this patient and also in her mother. No *SCN1A* mutation was detected.

The convulsive seizures occurred in clusters with fever. Each seizure lasted for one minute. The seizures recurred every one or two hours, five to 10 times a day. She never had seizures without fever. The patient was treated initially with phenobarbital (PB) at 10 months of age and then carbamazepine (CBZ), but the seizures could not be controlled. When she first visited our hospital at the age of 10 years, we discontinued CBZ and introduced VPA (with concomitant PB). The patient has been seizure-free for two years since then, even when she had a high fever. Interictal EEG showed normal alpha rhythm without paroxysmal epileptic discharges, but with some frontal dominant slow waves. She showed a photoparoxysmal response without clinical correlates by intermittent photic stimulation.

Six convulsive seizures were recorded by video-EEG recording, all of which occurred during sleep. They began with several jerks of the body, resulting in cough-like movement, after which her body and limbs became mildly tonic. The patient's right lower extremity became tonic earlier than her left extremities. She then turned over and both lower extremities showed tonic extension. The right hand and fingers were widely extended, whereas the left hand was flexed. The fingers on both hands and the lower extremities repeatedly moved in a less synchronous, less rhythmic, and less intense manner. The mild trembling/fluttering feature of the seizures was more marked in the distal part of the extremities. Thereafter, this movement gradually became more synchronized, nearly clonic, but less intense, synchronous, and asymmetric. In the postictal state, the patient gasped with oral automatism. Seizures were 1.5 to 3 minutes in duration and

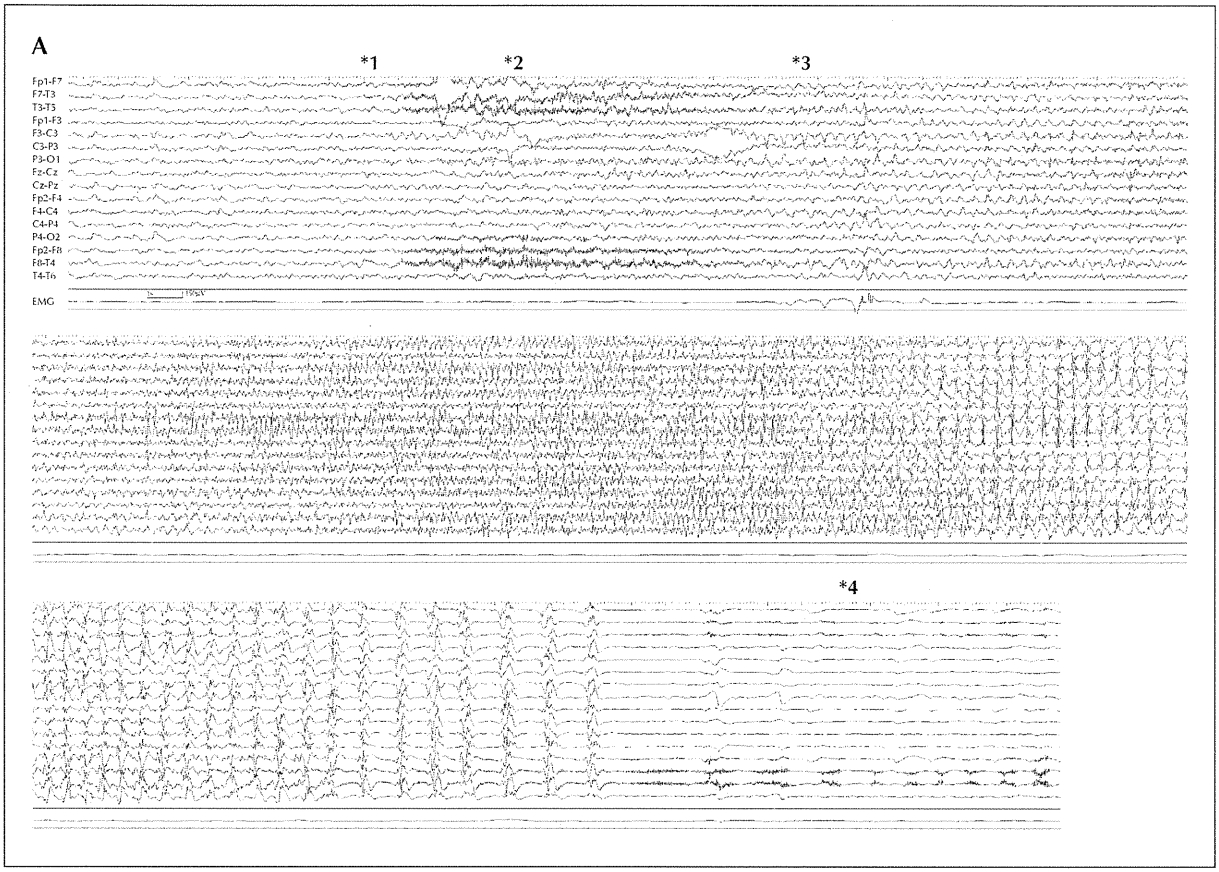

Figure 1. Ictal EEG of Patient 1. (A) Initial 8-Hz rhythmic waves with Cz and C3 predominance (*1) were followed by 13-Hz rhythmic waves predominantly at Cz (*2), which then gradually changed to diffuse slow waves (*3). These rhythmic waves terminated as high-amplitude, diffuse, slow wave bursts with spikes over the bilateral frontal area. Motion artefacts due to postictal oral automatism were present on bilateral temporal traces (*4).

tended to repeat every seven to 10 minutes for one to two hours. On ictal EEG (*figure 1A*), alpha-band waves with a slight predominance of the Cz and C3 regions were followed by rhythmic waves appearing from the Cz, which soon generalised. Another ictal EEG (*figure 1B*) during the same cluster showed different sequential rhythmic waves predominantly on the left side. Though ictal EEG mimicked typical secondary generalised tonic-clonic seizures (sGTC), surface electromyograms (EMGs) of both deltoid muscles showed only subtle discharges, suggesting that proximal muscles were less involved than peripheral ones (*video sequence 1*).

Patient 2

Patient 2 was a six-year-old girl who was born after an uneventful pregnancy and had no specific medical history. Her development was normal at 6 months of age, but became retarded after the onset of epilepsy. Her full-scale IQ score was 49 at 6 years of age, and autistic symptoms had also been noted. MRI/CT showed slightly diffuse brain atrophy. This proband's brother, brother-in-law on the mother's side, and cousin on the father's side each had a history of a single febrile seizure. The patient and her father had a *PCDH19* missense mutation (c.1787A>T/p.Asp596Val), but this was asymptomatic in the father. Since 6 months of age, the patient had experienced clusters of seizures. The seizures occurred under afebrile conditions initially, but later also under febrile conditions, in a cluster consisting of more than 10 seizures. The seizure clusters occurred a few times a day, and recurred at intervals of three weeks to four months. Despite initial treatment with CBZ and clonazepam (CZP), the seizure cluster repeated. Treatment with zonisamide, clobazam (CLB), and VPA aggravated seizures. Midazolam was given intravenously and frequently until CBZ and CLB were given in combination. The interictal EEG showed slightly irregular theta rhythms over both occipital-parietal central regions