mirtazapine: Enantioselective effects of the CYP2D6 ultra rapid metabolizer genotype and correlation with adverse effects. *Clin Pharmacol Therap*, **2007**, *81*:699–707.

Cannon TD, Keller MC. Endophenotypes in the genetic analyses of mental disorders. *Annu Rev Clin Psychol*, **2006**, *2*:267–290.

Chen YW, Dilsaver SC. Lifetime rates of suicide attempts among subjects with bipolar and unipolar disorders relative to subjects with other Axis I disorders. *Biol Psychiatry,* **1996**, *39*:896–899.

Cryan JF, Holmes A. The ascent of mouse: Advances in modelling human depression and anxiety. *Nature Rev,* **2005**, *4*:775–790.

Danish University Antidepressant Group (DUAG). Clomipramine dose-effect study in patients with depression: clinical end points and pharmacokinetics. *Clin Pharmacol Ther*, **1999**, *66*:152–165.

Delgado PL, Price LH, Miller HL, *et al*. Rapid serotonin depletion as a provocative challenge test for patients with major depression: Relevance to antidepressant action and the neurobiology of depression. *Psychopharmacol Bull,* **1991**, *27*: 321–330.

DeRubeis RJ, Siegle GJ, Hollon SD. Cognitive therapy versus medication for depression: Treatment outcomes and neural mechanisms. *Nat Rev Neurosci,* **2008**, *9*:788–796.

Entsuah AR, Huang H, Thase ME. Response and remission rates in different subpopulations with major depressive disorder administered venlafaxine, selective serotonin reuptake inhibitors, or placebo. *J Clin Psychiatry,* **2001**, *62*:869–877.

Foley KF, DeSanty KP, Kast RE. Bupropion: Pharmacology and therapeutic applications. *Expert Rev Neurother,* **2006**, *6*: 1249–1265.

Frazer A. Pharmacology of antidepressants. *J Clin Psychopharmacol,* **1997**, *17(Suppl 1)*:2S–18S.

Gibbons RD, Brown CH, Hur K, *et al*. Early evidence on the effects of regulators' suicidality warnings on SSRI prescriptions and suicide in children and adolescents. *Am J Psychiatry,* **2007**, *164*:1356–1363.

Goldberg JF, Truman CJ. Antidepressant-induced mania: An overview of current controversies. *Bipolar Disord,* **2003**, *5*:407–420.

Goodman WK. Selecting pharmacotherapy for generalized anxiety disorder. *J Clin Psychiatry,* **2004**, *65(Suppl 13)*:8–13.

Hiemke C, Hartter S. Pharmacokinetics of selective serotonin reuptake inhibitors. *Pharmacol Ther,* **2000**, *85*:11–28.

Hollister LE. Current antidepressant drugs: Their clinical use. *Drugs,* **1981**, *22*:129–152.

Jarema M. Atypical antipsychotics in the treatment of mood disorders. *Curr Opin Psychiatry,* **2007**, *20*:23–29.

Katz MM, Tekell JL, Bowden CL, *et al*. Onset and early behavioral effects of pharmacologically different antidepressants and placebo in depression. *Neuropsychopharmacology,* **2004**, *29*:566–579.

Kessler RC, McGonagle KA, Zhao S, *et al*. Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States. Results from the National Comorbidity Survey. *Arch Gen Psychiatry*, **1994**, *51*:8–19.

Leonard BE, Richelson E. Synaptic effects of anitdepressants. In, *Schizophrenia and Mood Disorders: The New Drug Therapies in Clinical Practice.* (Buckley PF, Waddington JL, eds.) Butterworth-Heinemann, Boston, **2000**, pp. 67–84.

Livingston MG, Livingston HM. Monoamine oxidase inhibitors. An update on drug interactions. Drug Saf, **1996**, *14*:219–227.

Manganas LN, Zhang X, Li Y, *et al*. Magnetic resonance spectroscopy identifies neural progenitor cells in the live human brain. *Science*, **2007**, *318*:980–985.

Mann JJ, Emslie G, Baldessarini RJ, *et al*. ACNP Task Force report on SSRIs and suicidal behavior in youth. *Neuropsychopharmacology,* **2006**, *31*:473–492.

Millan MJ. The neurobiology and control of anxious states. *Prog Neurobiol,* **2003**, *70*:83–244.

Millan MJ. Multi-target strategies for the improved treatment of depressive states: Conceptual foundations and neuronal substrates, drug discovery and therapeutic application. *Pharmacol Ther,* **2006**, *110*:135–370.

Miller HL, Delgado PL, Salomon RM, *et al*. Effects of alpha-methyl-para-tyrosine (AMPT) in drug-free depressed patients. *Neuropsychopharmacology,* **1996**, *14*:151–157.

Miller EJ, Saint Marie LR, Breier MR, Swerdlow NR. Pathways from the ventral hippocampus and caudal amygdala to forebrain regions that regulate sensorimotor gating in the rat. *Neuroscience*, **2010**, *165*:601–611.

O'Donnell JM, Zhang HT. Antidepressant effects of inhibitors of cAMP phosphodiesterase (PDE4). *Trends Pharmacol Sci*, **2004**, *25*:158–163.

Owens MJ, Morgan WN, Plott SJ, Nemeroff CB. Neurotransmitter receptor and transporter binding profile of antidepressants and their metabolites. *J Pharmacol Exp Ther,* **1997**, *283*:1305–1322.

Rakofsky JJ, Holtzheimer PE, Nemeroff CB. Emerging targets for antidepressant therapies. *Curr Opin Chem Biol,* **2009**, *13*:291–302.

Rudorfer MV, Potter WZ. Metabolism of tricyclic antidepressants. *Cell Mol Neurobiol,* **1999**, *19*:373–409.

Rush AJ, Trivedi MH, Wisniewski SR, *et al*. Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: A STAR*D report. *Am J Psychiatry,* **2006**, *163*:1905–1917.

Santarelli L, Saxe M, Gross C, *et al*. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science*, **2003**, *301*:805–809.

Schmidt HD, Duman RS. The role of neurotrophic factors in adult hippocampal neurogenesis, antidepressant treatments and animal models of depressive-like behavior. *Behav Pharmacol,* **2007**, *18*:391–418.

Sen S, Duman R, Sanacora G. Serum brain-derived neurotrophic factor, depression, and antidepressant medications: Meta-analyses and implications. *Biol Psychiatry,* **2008**, *64*: 527–532.

Shelton RC. Cellular mechanisms in the vulnerability to depression and response to antidepressants. *Psychiatr Clin North Am,* **2000**, *23*:713–729.

Shelton RC. Augmentation strategies to increase antidepressant efficacy. *J Clin Psychiatry,* **2007**, *68(Suppl 10)*:18–22.

Shelton RC, Lester N. SSRIs and newer antidepressants, In, *APA Textbook of Mood Disorders*. APA Press, Washington, D.C., **2006**.

Simon GE, Savarino J, Operskalski B, Wang PS. Suicide risk during antidepressant treatment. *Am J Psychiatry,* **2006**, *163*:41–47.

Suominen KH, Isometsa ET, Henriksson MM, *et al*. Inadequate treatment for major depression both before and after attempted suicide. *Am J Psychiatry,* **1998**, *155*:1778–1780.

Vaswani M, Linda FK, Ramesh S. Role of selective serotonin reuptake inhibitors in psychiatric disorders: A comprehensive review. *Prog Neuropsychopharmacol Biol Psychiatry*, **2003**, *27*:85–102.

Witkin JM, Marek GJ, Johnson BG, Schoepp DD. Metabotropic glutamate receptors in the control of mood disorders. *CNS Neurol Disord Drug Targets,* **2007**, *6*:87–100.

Zarate CA Jr., Singh JB, Carlson PJ, *et al*. A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. *Arch Gen Psychiatry,* **2006**, *63*:856–864.

Zhao Z, Baros AM, Zhang HT, *et al*. Norepinephrine transporter regulation mediates the long-term behavioral effects of the antidepressant desipramine. *Neuropsychopharmacology,* **2008**, *33*:3190–3200.

Zhao Z, Zhang HT, Bootzin E, *et al*. Association of changes in norepinephrine and serotonin transporter expression with the long-term behavioral effects of antidepressant drugs. *Neuropsychopharmacology*, **2009**, *34*:1467–1481.

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Case Report

Cerebellar symptoms in a case of acute limbic encephalitis associated with autoantibodies to glutamate receptors δ2 and ε2

Ryuta Kinno[a,*], Takahiro Yamazaki[a], Masahiro Yamamoto[a], Yukitoshi Takahashi[b], Toshiya Fukui[a], Eriko Kinugasa[a]

[a] Department of Internal Medicine, Showa University Northern Yokohama Hospital, Kanagawa, Japan
[b] National Epilepsy Center, Shizuoka Institute of Epilepsy and Neurological Disorders, Shizuoka, Japan

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1. Introduction

Glutamate, the major excitatory neurotransmitter in the mammalian central nervous system, activates both ion-channel-forming (ionotropic) and G-protein-coupled (metabotropic) glutamate receptors (GluRs). Pharmacological and molecular techniques have been used to identify a large variety of ionotropic and metabotropic GluRs. Ionotropic GluRs can be grouped into the following categories according to their agonist selectivity and sequence homology: α-amino-3-hydroxy-5-methyl-4-isoxazole propionate-type receptors (GluRα), N-methyl-D-aspartate (NMDA)-type receptors (GluRζ, GluRχ, and GluRε), kainate receptors (GluRβ and GluRγ), and GluRδ2. Within these GluRs, autoantibodies to NMDA-type GluR have recently been recognized as biomarkers for non-herpetic acute limbic encephalitis (NHALE) [1]. Indeed, the autoantibody to GluRε2 has been detected in patients with NHALE, who typically show psychiatric symptoms. However, the cerebellar symptoms associated with autoantibodies to GluR have not been emphasized in the literature. We herein report a rare case of acute limbic encephalitis with cerebellar symptoms associated with autoantibodies to GluRε2 and δ2.

2. Case report

A 36-year-old woman had lower back pain with fever, and 2 days later presented with a flat mood. She consulted our department 4 days after the onset of emotional disturbances. On admission, her temperature was 38.7 °C; other vital signs and general examination were normal. Cranial nerve examination revealed no abnormalities. Seizure-like movements of the extremities were observed. No cerebellar sign was detected. Her muscle power was full, with normal tendon reflexes. No sensory deficits or evidence of meningeal signs were observed. Her blood examination was notable for a mildly elevated in white blood cell count (12,750/μL with 83% neutrophils). A cerebrospinal fluid (CSF) examination showed a mild elevation in cell count of 21/μL (95% lymphocytes) with normal glucose level (75 mg/dL) and protein level (26 mg/dL). Both the oligoclonal immunoglobulin G (IgG) band and myelin basic protein were negative. Brain magnetic resonance imaging (MRI) showed no abnormalities. After admission, she progressively lost consciousness and a generalized seizure followed, which was resolved by propofol. An electroencephalogram showed slow-wave abnormalities (2–6 Hz polymorphic delta and theta activity) without epileptic activity. Mechanical ventilation was required because she developed hypoventilation. On day 7, brain MRI showed a hyperintense area in the right insula on a fluid attenuated inversion recovery (FLAIR) image (Fig. 1A) and in the cerebellum on a diffusion weighted image (DWI). Abdominal MRI showed no abnormalities, including ovarian teratoma. Aciclovir 10 mg/kg body weight every 8 h was started for possible herpes simplex virus (HSV) encephalitis. IgG and IgM

* Corresponding author at: Department of Internal Medicine, Showa University Northern Yokohama Hospital, 35-1 Chigasaki-chuo Tsuzuki, Yokohama, Kanagawa 224-8503, Japan. Tel.: +81 45 949 7845; fax: +81 45 949 7927.
E-mail address: kinno@med.showa-u.ac.jp (R. Kinno).

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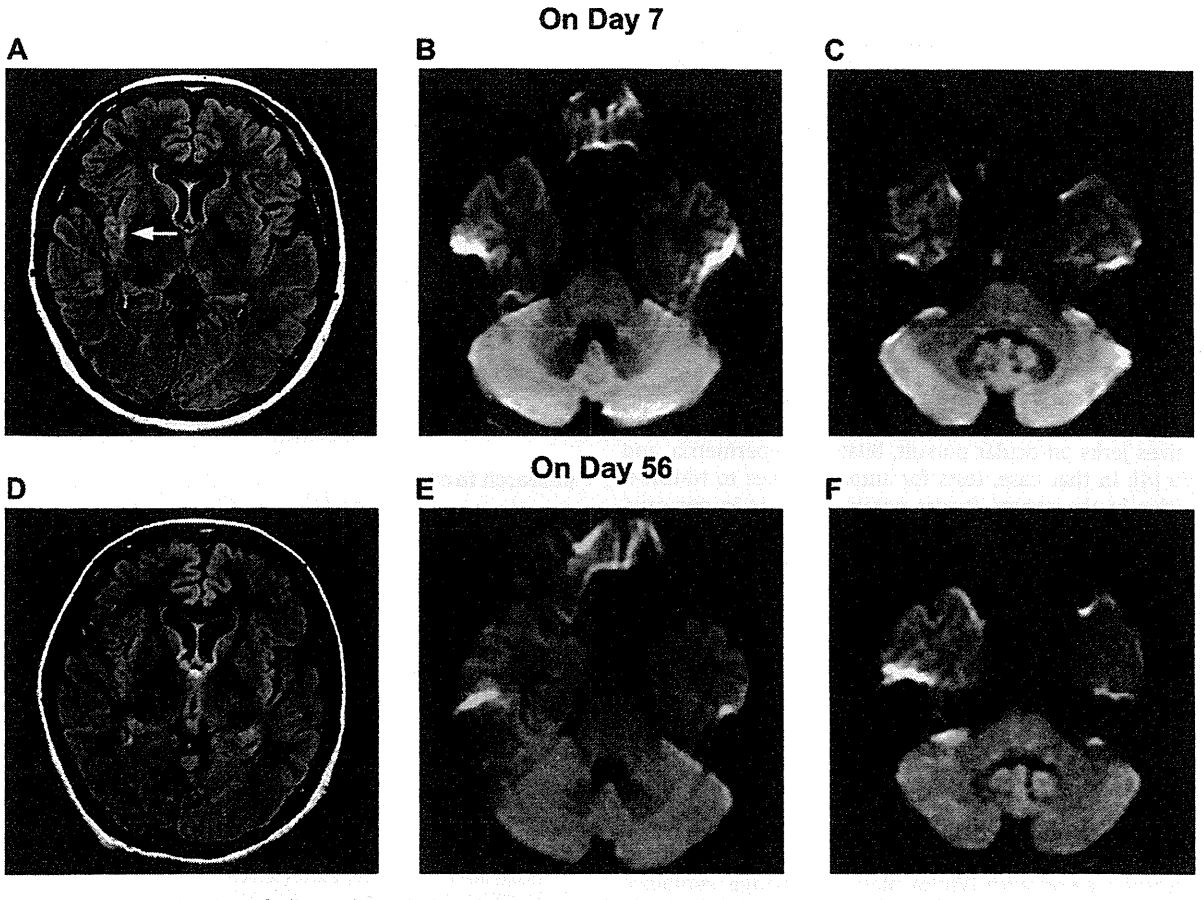

Fig. 1. Serial MRI of the patient. (A–C) MRI finding on day 7. The hyperintense area in the right insula (white arrow) was observed on a FLAIR image (A), and the hyperintense area in the cerebellum was observed on a DWI (B, C). (D–F) MRI findings on day 56. There were no abnormalities in MRI findings.

antibodies to HSV were negative in both the CSF and serum, and aciclovir was discontinued. Tests for autoantibodies to GluR δ2 and ε2 were positive in the CSF (sampled on day 3) (Table 1).

We subsequently performed plasma exchange for possible autoimmune limbic encephalitis. After the fifth plasma exchange (on day 30), the patient gradually regained consciousness and the generalized seizures subsided, allowing the discontinuation of propofol. However, she subsequently presented with cerebellar signs. She exhibited ocular overshoot, gaze-evoked nystagmus, and dysarthria with scanning speech. There was severe limb and truncal ataxia with intention tremor. Finger-to-nose and heel-to-knee test showed mild dysmetria and decomposition of limbs. Muscle tone was hypotonic; and deep tendon reflexes were normal. She could not sit upright or maintain an erect posture without support due to severe cerebellar symptoms. After the seventh plasma exchange, her neurological complications were resolved, and she became able to sit upright or maintain an erect posture without support. On day 56, a brain MRI showed no apparent abnormities in either the insula (Fig. 1D) or the cerebellum (Fig. 1E and F). We made a diagnosis of acute limbic encephalitis with cerebellar symptoms associated with autoantibodies to GluRδ2 and ε2. She was discharged on day 73.

3. Discussion

To our knowledge, this is the first case of acute limbic encephalitis with cerebellar symptoms associated with autoantibodies to GluRδ2 and GluRε2. Our case started with prodromal symptoms, followed by early symptoms like emotional disturbances, fever and movement disorders. Later on, she developed loss of consciousness, seizures and hypoventilation. These clinical features resemble those reported in association with anti-NMDA receptor (NMDA-R) encephalitis. Previous studies suggested that NMDA-R encephalitis is associated with the autoantibody to the NR1–NR2 heteromers (GluRζ1/GluRε2), and the main epitope targeted by this autoantibody is in the extracellular N-terminal (NT) domain of the NR1 or NR2 subunits [1]. The autoantibody to GluRε2-NT2 was positive in our case (Table 1) while the autoantibody to the NR1–NR2 heteromers was not detected. The cerebellar symptoms became clinically evident after this classical pattern of NMDA-R encephalitis disappeared. The CFS and MRI findings in our case suggest that cerebellar symptoms become visible after other symptoms resolve. Taken these findings together, it is supposed that acute limbic encephalitis with cerebellar symptoms is an atypical form of anti-NMDA-R encephalitis.

Table 1
Optical density of antibody to GluR.

	GluRε2-NT2	GluRε2-CT1	GluRδ2-NT	GluRδ2-CT
CFS	0.823 (0.162 ± 0.055)	0.909 (0.189 ± 0.061)	0.961 (0.172 ± 0.086)	0.847 (0.261 ± 0.100)
Serum	0.346 (0.432 ± 0.133)	0.517 (0.556 ± 0.140)	0.502 (0.583 ± 0.148)	0.817 (0.638 ± 0.202)

Optical densities assessed by ELISA are shown (mean ± standard deviation for control data).

Regarding the relationship between the cerebellum and GluR, it is known that GluRδ2 is expressed predominantly in cerebellar Purkinje cells and plays a crucial role in cerebellar functions [2]. In addition, a previous study suggested that 6% of NMDA-R encephalitis had MRI-abnormalities of the cerebellum, although cerebellar symptoms have not been noted in the literature [1]. In contrast to GluRδ2, the expression of GluRε2 mRNA is restricted to the forebrain including the cerebral cortex and limbic system after birth, and GluRε2 is associated with memory and learning [3]. We thus inferred that the cerebellar symptoms were associated with the autoantibody to GluRδ2. Our case provides clinical evidence for the anatomical and functional significance of GluRδ2 for the cerebellum. Further studies should establish the relation between deficits in cerebellar functions and the autoantibody to GluRδ2.

Recent literature has reported a case of anti-NMDA-R encephalitis presenting with cerebellar symptoms such as scanning speech, square-waves jerks on ocular pursuit, bilateral hypermetria, and gait ataxia [4]. In that case, tests for autoantibodies to NMDA-R were positive in the serum. It was notable that, as in our case, the cerebellar symptoms became clinically evident only after the patient regained consciousness. The authors concluded that the cerebellar symptoms observed in that case could be explained by a disabling action on glutamate NMDA-R by the antibody to NMDA-R. However, the significance of the autoantibody to GluRδ2 in NHALE presenting with cerebellar symptoms was not emphasized in this report. Therefore, our case is a novel one that may have important implications: namely, that the autoantibody to GluRδ2 is critical for cerebellar symptoms in NHALE including anti-NMDA-R encephalitis.

It has been supposed that immunotherapy such as plasma exchange, corticosteroids, and intravenous immunoglobulin (IVIg) is an effective therapy for NMDA-R encephalitis [1]. A recent study suggests starting with IVIg or plasma exchange, combined with steroids. If no proper reaction is seen in 10–14 days, they suggest continuing with secondary therapy like rituximab or cyclophosphamide [5]. Indeed, our case showed relatively rapid recovery from her neurological complications after the seventh plasma exchange, although there was no apparent malignancy on clinical examination. These observations suggest the effectiveness of plasma exchange for resolving cerebellar as well as psychiatric symptoms, although spontaneous improvement cannot be completely ruled out.

4. Conclusion

We report a case of acute limbic encephalitis having cerebellar symptoms associated with autoantibodies to GluRδ2 and GluRε2. Acute limbic encephalitis with cerebellar symptoms is presumably an atypical form of anti-NMDA-R encephalitis. Extensive assessments of cerebellar symptoms would be valuable for planning effective rehabilitation programs for these patients.

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References

[1] Dalmau J, Gleichman AJ, Hughes EG, Rossi JE, Peng X, Lai M, et al. Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. Lancet Neurology 2008;7:1091–8.
[2] Yuzaki M. The delta2 glutamate receptor: a key molecule controlling synaptic plasticity and structure in Purkinje cells. Cerebellum 2004;3:89–93.
[3] Tang YP, Shimizu E, Dube GR, Rampon C, Kerchner GA, Zhuo M, et al. Genetic enhancement of learning and memory in mice. Nature 1999;401:63–9.
[4] Naeije G, de Hemptinne Q, Depondt C, Pandolfo M, Legros B. Acute behavioural change in a young woman evolving towards cerebellar syndrome. Clinical Neurology and Neurosurgery 2010;112:509–11.
[5] Rosenfeld MR, Dalmau J. Anti-NMDA-receptor encephalitis and other synaptic autoimmune disorders. Current Treatment Options in Neurology 2011;13:324–32.

CLINICAL REPORT

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Interstitial Duplication of 2q32.1–q33.3 in a Patient With Epilepsy, Developmental Delay, and Autistic Behavior

Daisuke Usui,[1] Shino Shimada,[2] Keiko Shimojima,[2] Midori Sugawara,[2] Hajime Kawasaki,[3] Hideo Shigematu,[1] Yukitoshi Takahashi,[1] Yushi Inoue,[1] Katsumi Imai,[1] and Toshiyuki Yamamoto[2]*

[1]National Epilepsy Center, Shizuoka Institute of Epilepsy and Neurological Disorders, Shizuoka, Japan

[2]Tokyo Women's Medical University Institute for Integrated Medical Sciences, Tokyo, Japan

[3]Kuwana East Medical Center, Kuwana, Japan

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Duplications of the 2q33 region are rare; to date, only 13 patients have been reported to have this chromosomal abnormality. The reported duplications are of varying size, and the patients shared developmental delay and minor dysmorphic findings. In this study, we identified a duplication of 2q32.1–q33.3 in a patient with psychomotor developmental delay, epilepsy, and autistic behavior. The duplicated region of this patient was reciprocal to the 2q32–q33 deletion syndrome. Chromosomal microarray testing confirmed the 19.5 Mb of duplication that includes over 100 genes, some of which could have functional relevance to the neurological features of this patient. The SATB homeobox 2 gene (*SATB2*)—the primary gene responsible for the 2q32–q33 deletion syndrome—may be one of them, because of its expression in the cortical projection neurons of the developing brain. The duplication of the potassium channel tetramerisation domain-containing 18 gene (*KCTD18*) and the ADAM metallopeptidase domain 23 gene (*ADAM23*) may also contribute to the phenotype. FISH analysis confirmed a tandem configuration of the duplicated segments. This result is in agreement with our previous study, in which we observed that duplicated segments as interstitial duplications are generally inserted in the tandem configuration.

Key words: 2q32–q33 duplication; psychomotor developmental delay; epilepsy; autistic behavior; *SATB2*

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INTRODUCTION

Genomic copy number aberrations often cause neurological impairments from the early infantile period, thereby resulting in developmental delay, intellectual disability, epilepsy, and other impairments [Lee and Lupski, 2006]. Chromosomal analysis is therefore the routine diagnostic examination for patients with neurological disorders. Chromosomal duplications of the 2q33 region are rare; only 13 cases of patients having duplications of the 2q33 region have been reported previously (Table I, Fig. 3) [Couturier et al., 1977; Dennis et al., 1978; Schumacher et al., 1983; Ramer et al., 1990; Romain et al., 1994; Barnicoat et al., 1997; Matos et al., 1997; Lukusa et al., 1999; Seidahmed et al., 1999; Bird and Mascarello, 2001; Slavotinek et al., 2003; Sebold et al., 2005; Elbracht et al., 2009]. The phenotypic features commonly observed in patients with 2q33 duplications are developmental delay and constellations of minor anomalies.

We recently investigated a patient with intellectual disability and epilepsy associated with a chromosomal duplication of 2q32.1–q33.3. The genes that may be related to the phenotypic features of this patient, and the mechanism underlying this patient's interstitial chromosomal duplication are discussed in this report.

MATERIALS AND METHODS

Patient Report

A 5-year- and 9-month-old boy (DECIPHER #TWM264219; https://decipher.sanger.ac.uk/) is the second child of healthy and genetically unrelated father and mother, age 40 and 28 years,

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*Correspondence to:
Toshiyuki Yamamoto, M.D., Ph.D., Tokyo Women's Medical University Institute for Integrated Medical Sciences, 8-1 Kawada-cho, Shinjuku-ward, Tokyo 162-8666, Japan. E-mail: yamamoto.toshiyuki@twmu.ac.jp

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TABLE I. Clinical Features of Patients With a Partial Duplication of Chromosome 2q Including/Overlapping 2q32.1–q33.32

	1	2	3		4	5	6	Present case	7		8	9	10	11	12	13
	Schumacher et al. [1983]	Matos et al. [1997]	Barnicoat et al. [1997]		Couturier et al. [1977]	Ramer et al. [1990]	Romain et al. [1994]		Lukusa et al. [1999]		Seidahmed et al. [1999]	Dennis et al. [1978]	Elbracht et al. [2009]	Sebold et al. [2005]	Bird and Mascarello [2001]	Slavotinek et al. [2003]
Mechanism	dup	dup	ins		ins	ins	dup	dup	ins		inv/dup	ins	trans	dup	inv/dup	inv/dup
Duplicated segment																
Proximal breakpoint	q21	q21	q24.4		q24	q31	q31.1	q32.1	q32.1		q32	q33	q33	q33.1	q33.3	q33
Terminal breakpoint	q33	q33	q32.1		q34	q33	q35	q33.3	q35		q37	q37	qter	q35	q37.1	q37.3
Age at present (at death)	20 d	5 min			8 y	6 y	22 y	5 y	3 y	18 mo	4 mo	18 mo	2 y	16 mo	6 mo	8 w
Gender	F	M	M	F	M	F	F	M	M	M	F	M	F	F	M	F
Gestation age at birth (wks)	36	33	Term	Term	35	Term	Term	39w	Term	Term	Term	42	NA	40	40.8	39
Physical findings																
Birth weight (g)	2,370	1,650	4,250	4,000	3,000	2,630	2,600	2,988	3,100	2,840	2,400	3,030	3,030	2,693	2,920	2,700
Birth length (cm)						47	47	50.7	49.5	49.5	54	48	51		49.7	48
Birth OFC (cm)	36						31	32		35	32.5		33		31.8	32
Pre-natal growth retardation			−	−			+							−		+
Post-natal growth retardation					+	+	+				+				+	+
Microcephaly					−	+	−	−			−				+	
Head																
Brachycephaly/flat occiput	+		+	+		+		−	+	+		+		+		
Frontal bossing	+	+	+	+	+			−	+	+	+	+	+	−		+
Trigonocephaly									+	+		+				
Anterior frontanelle size	Large							−					+		Small	
Face																
Hairline low					Ant	Ant/Post		−			+					
Midface retrusion			+	+	+	+		−							+	
Hypertelorism	−	+	+	+	+		+	+				−		−	+	+
Palpebral fissure slant	Up		Up	Up	Up			Up				Up		−	−	Down
Epicanthal folds			+	+	+	+	+	−		+	+				+	+
Iris coloboma				+	+			−							+	
Esotropia								−						Left		
Proptosis								−			+				+	
Ears malformed	+					−		−	Tag/Pits		+			−	+	+
Ears low-set	+	+				−		+	+		+	+		+	+	
Ears postrotated		+				−		−	+	+			+		+	
Ear lobes creased			+	+				−	+	+	+					
Nasal bridge low	+		+	+	+	+	+	+	+	+	+	+		+	+	+
Nasal tip narrow			+	+				−								
Nose broad	+	+	+	+	+			+		+				+		+
Nose short					+			−	+	+		+		Small	+	+
Nose beaked								−					+			
Nares anteverted	+		+	+	+			−			+	+			+	+
Philtrum long	+		+	+		−	+	−	+	+	+	+	+	+	+	+
Philtrum smooth			+	+		−	−	−	+	+						+
Upper lip thin			+	+	+	−	+	−	+	+	+				+	
Cupid's bow					+		+	+						+		
Abnormal mouth size					Large			Normal								Small
Mouth corners turn down								−				+				+
Micrognathia						−		+			+	−		+	+	+
Alveolar ridge broad/ palate narrow and high arched	−					+		+			+				+	−/+
Dental caries			+	+				−								
Neck/Chest																
Neck short		+					+	+				−				+
Nuchal skin excessive								−							+	
Nipple spacing	Wide							−						Wide		

downwardly	Wide																
Nipple inverted								−		+							
Breast tisue hypoplastic															+		
Petus excavatum			Mild	Mild	+			−							−		
11 pairs ribs								−			+						
Extremeties																	
Legs thin					+	−		−									
Fingers short								−								+	
Palmar flexion creases abnormal	+		+			−		−			+	−			+		
5th finger clinodactyly			+	+		−	+	+						+		+	
Finger camptodactyly			+	+		−		−				4th			3rd to 5th		
Thumbs proximal					+	−		−					+				
Preaxial polydactyly		+				−		−									
Fingers tapered								−	+	+	+						
Fetal finger pad								−	+	+							
Hallux, lateral deviation								−							+		
Feet equinovarus		+			+			−									
Pes cavus								−							+		
Nail hypoplasia	Toe				+			−			−	Toes					
Nails narrow								−							+		
Overlap toes	+					+		−									
Extra genitalia																	
Labial hypoplasia	+																
Inguinal hernia								−				Right					
Shawl scrotum								−				Left					
Cryptorchidsm					+			−							+		
Small testis								−				−					
Hypospadias		+						−				+			−		
Penis small								−				−			+		
Sacro-coccygeal dimple	+	Fistula		+	Fistula			−	+			Linear					
Skin																	
Dry skin/Scaring rash								−							+		
Loose skin at birth								−							+		
Hypertricosis								−			+						
Neurological																	
Muscular hypotonia					+			Mild	+	+	+			+	+		
Mental reterdation			+	+	IQ < 25	+	IQ < 30	+	+	+		D057		+	+		
Feeding problems								−			+				+		
Brain anomaly	+	+						−			+		+				
Autistic behavior								+	+								
Epilepsy	+							+									
Crardiac defect	+	+						−				−		+	+	−	
Urinary tract anomaly	+	+						−				?			+	+	
Kyphosis/scoliosiE					+		+	−								−	
Other features	Incomplete lung lobation, small lung, accessory spleen					Hirshspung disease, freaquent otitis media, prognathism					Blue sclerae, long fingers, advanced bone age, adducted thumbs, fisted hand	Aplasia cutis scalp, alae nasi small	Skeltal anomaly		Large halluces, freaquent otitis media		

dup, de novo interstitial duplication; ins, duplication derived from parental balanced insertion; inv/dup, inverted-duplication-deletion; trans, partial trisomy derived from parental balanced translocation; d, days; min, minutes; y, years; mo, months; w, weeks; M, male; F, female; Term, birth at term; NA, not available; ant, anterior; post, posterior; IQ, intelligence quotient; DQ, development quotient.

respectively. He has a healthy sister who is 2 years older to him. His family history and the findings during the pregnancy period were unremarkable. He was born by normal delivery at 39 weeks of gestation. His birth weight was 2,988 g (−0.5 SD), length was 50.7 cm (+0.6 SD), and occipito-frontal circumference (OFC) was 32.0 cm (+1.1 SD). His development was delayed, with head control attained at 9 months, sitting posture at 11 months, and his first steps were taken at 52 months. Conventional karyotyping was performed at the age of 1 year because of his developmental delay. The results indicated 46,XY,ins(2:?)(q33;?). Because his parents showed normal karyotypes, the patient's chromosomal rearrangement was a de novo occurrence.

At the age of 25 months, the patient had a complex partial seizure. After this episode, similar seizures were observed multiple times, and they became more frequent after the age of 38 months. His seizures were easily triggered by high fever. Initial treatment with carbamazepine (CBZ) did not cause complete sedation, and similar seizures recurred on a near-weekly basis after 57 months of age.

At the age of 5 years and 9 months, the patient was admitted to our facility for better seizure control. At that time, his weight was 18.7 kg (+0.2 SD), height was 108.3 cm (+0.1 SD), and OFC was 50.0 cm (−0.9 SD). He exhibited severely delayed psychomotor development, spoke no meaningful words, and had a staggering gait. His activities of daily living were at the level of a 1-year-old child: using diapers and requiring full assistance for eating, clothing, and bathing. Autistic behavior was noted with respect to poor eye contact and stereotyped behavior. His facial features included hypertelorism, a depressed nasal bridge, broad nose, high palate, low-set ears, short neck, micrognathia, and bilateral clinodactyly of the fifth digit (Fig. 1A,B). Ophthalmological and otolaryngological examinations revealed no abnormalities. Brain magnetic resonance imaging (MRI) revealed a mildly hypoplastic cerebrum (Fig. 1C,D). Single-photon emission computed tomography with *N*-isopropyl-[^{123}I]-*p*-iodoamphetamine (IMP-SPECT) showed hypoperfusion of the left frontal lobe (See Supplemental eFig. S1 in Supporting Information online). There were no abnormal findings in chest and abdominal ultrasonography. The results of the routine laboratory examinations were unremarkable. An electroencephalogram (EEG) taken during sleep showed left side-predominant spike and wave complexes (Supplemental eFig. S2 in Supporting Information online). After initiation of valproic acid (VPA), the clinical seizures disappeared.

Chromosomal Microarray Testing and Fluorescence In-Situ Hybridization (FISH)

Although his initial conventional karyotyping showed chromosomal abnormalities, the origin of the duplicated segment

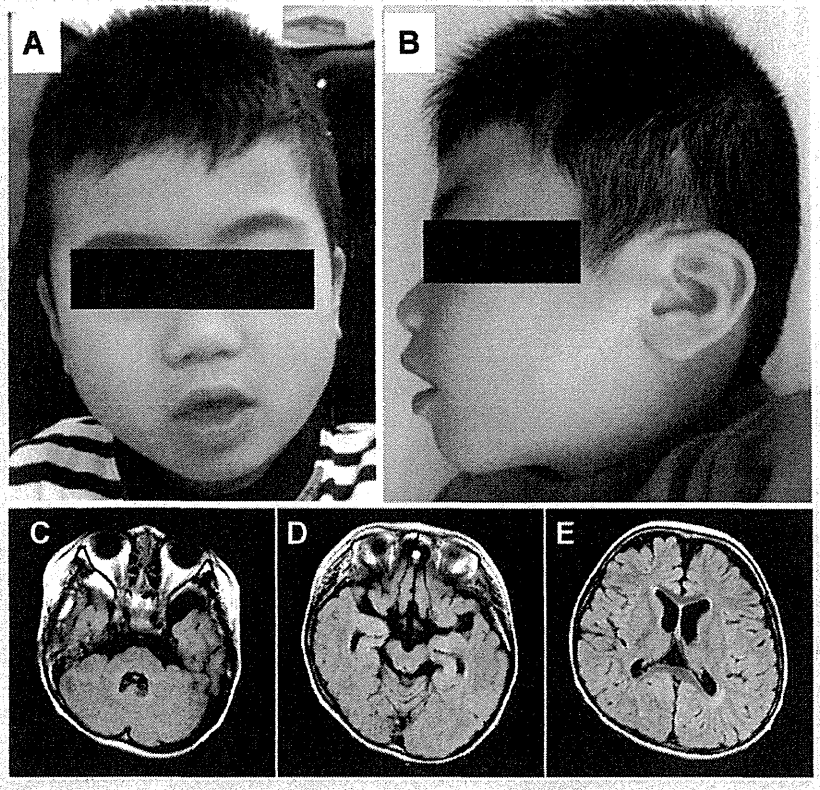

FIG. 1. Clinical data of the present patient. Frontal view (A) and lateral view (B) of the present patient indicating hypertelorism, depressed nasal bridge, broad nose, low-set ears, short neck, and micrognathia. C–E: Axial fluid attenuation inversion recovery (FLAIR) image of the brain MRI demonstrates mildly reduced volume of the frontal region. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmga]

was undetermined. We therefore re-examined his karyotype by chromosomal microarray testing using the Agilent SurePrint G3 Hmn CGH 60K Oligo Microarray Kit (Agilent Technologies, Santa Clara, CA), according to the method described in previous studies[Shimojima et al., 2010, 2011, 2009]. FISH analysis using BAC clones selected from the UCSC Genome Browser (http://genome.ucsc.edu/) was performed as previously described [Shimojima et al., 2010, 2011, 2009].

RESULTS

The gain of the genomic copy numbers was confirmed by chromosomal microarray testing indicated by arr 2q32.1q33.3-(189,162,857–208,631,364) × 3 dn (Fig. 2A,B) with a size of 19.5 Mb. FISH analyses using two BAC clones—RP11-655A7 at 2q32.2 as the target and RP11-664N22 at 2p25.3 as a marker for chromosome 2—confirmed duplicated signals in the same chromosome 2 (Fig. 2C). Then, to confirm the direction of the duplicated segment, further FISH analysis using 2 BAC clones in the duplicated region—RP11-655A7 at 2q32.2 and RP11-17I6 at 2q33.3—was performed, and a tandem configuration of the duplicated segment was detected (Fig. 2D).

DISCUSSION

In this study, we identified a rare chromosomal duplication of 2q32.1–q33.3 in a patient with psychomotor developmental delay and epilepsy. Previously, only 13 cases of patients have been

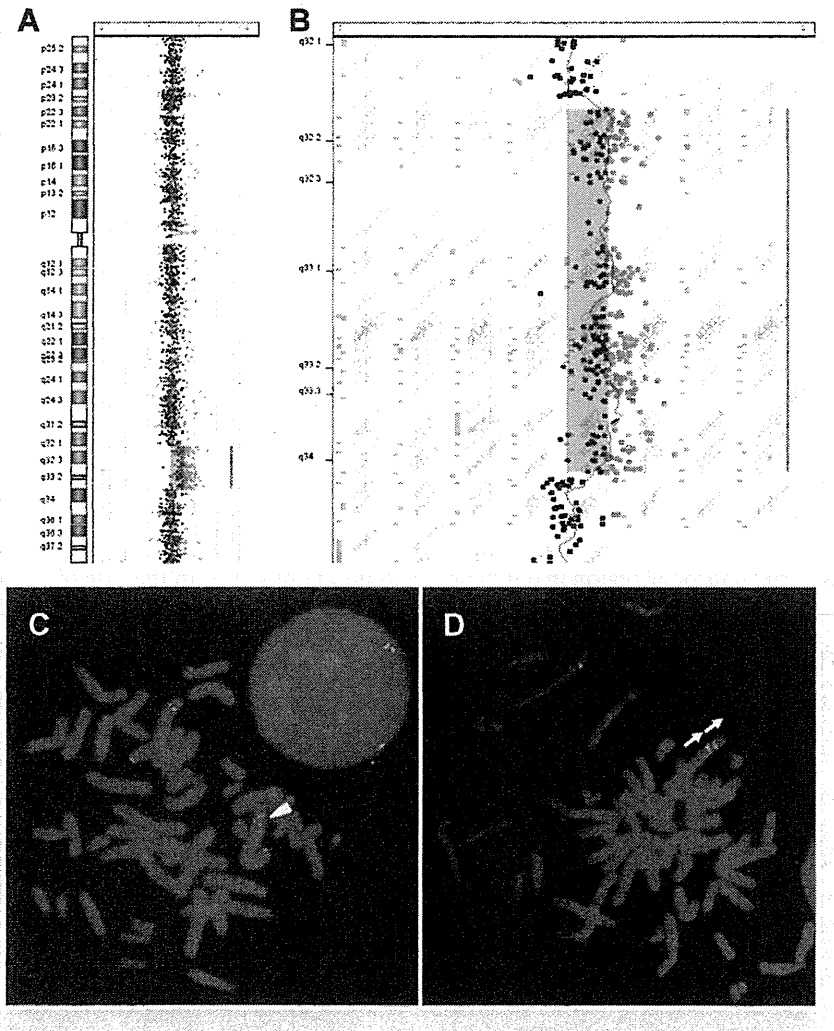

FIG. 2. Molecular and cytogenetic karyotyping. Duplication of 2q32.1—q33.3 is shown by in Chromosome view (A) and Gene view (B) in Agilent Genomic Workbench ver 6.5 (Agilent Techonologies). FISH analysis using 2 BAC clones—RP11-655A7 at 2q32.2 labeled with Spectrum Green as the target and RP11-644N22 at 2p25.3 labeled with Spectrum Orange—confirm duplicated signals on the same chromosome 2 (arrow head; C). Further FISH analysis using 2 BAC clones—RP11-655A7 at 2q32.2 labeled with spectrum orange and RP11-17I6 at 2q33.3 labeled with spectrum green—confirms a tandem configuration of the duplicated segments (arrows; D). [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmga]

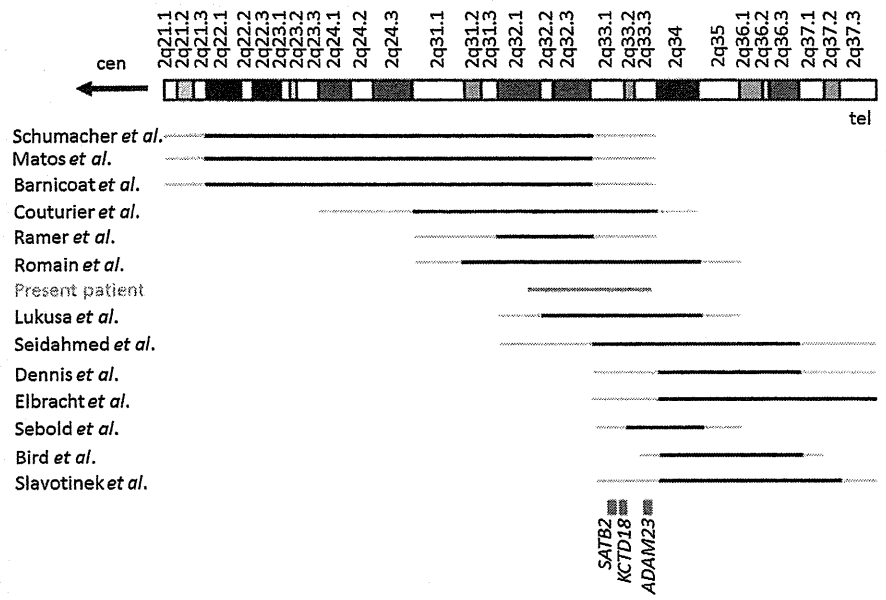

FIG. 3. Schematic representation of the previously reported duplications of 2q. The black bar and gray bar indicate certain and uncertain regions of the duplications, respectively. The red bar indicates the duplicated region, as confirmed by chromosomal microarray testing. The blue rectangles indicate the locations of the indicated genes. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmga]

reported with the duplications of this region (Table I, Fig. 3) [Couturier et al., 1977; Dennis et al., 1978; Schumacher et al., 1983; Ramer et al., 1990; Romain et al., 1994; Barnicoat et al., 1997; Matos et al., 1997; Lukusa et al., 1999; Seidahmed et al., 1999; Bird and Mascarello, 2001; Slavotinek et al., 2003; Sebold et al., 2005; Elbracht et al., 2009]. The phenotypic features commonly observed in these patients are developmental delay and constellations of minor anomalies.

Intriguingly, the duplicated chromosomal region in our patient was reciprocal to the 2q32–q33 deletion syndrome reported by Van Buggenhout et al. [2005]. There are some reports of the chromosomal deletion of 2q31–2q33 [Mencarelli et al., 2007; Prontera et al., 2009]. Among them, the most frequently overlapped region was the 2q33.1 region, in which the SATB homeobox 2 gene (*SATB2*) was located. SATB2 is a DNA-binding protein that regulates chromatin organization, and it is expressed in the cortical projection neurons in the developing brain [Alcamo et al., 2008]. A previous study showed that SATB2 has expressed predominantly in the upper layer of the cortex [Britanova et al., 2008]. *SATB2* is now known to be the gene responsible for the phenotype of 2q32-q33 deletion syndrome, manifesting in intellectual disability, neurological features including epilepsy, and cleft lips [Britanova et al., 2006; Rosenfeld et al., 2009].

Many of neurodevelopmental disorders can be caused by genomic rearrangements resulting in altered gene dosage through either deletion or duplication of dosage-sensitive gene(s) contained within the rearranged genomic interval [Lee and Lupski, 2006; Gu and Lupski, 2008]. The majority of such neurodevelopmental features stem from genomic copy number changes of only a single gene included in the region. The patients with genomic copy number loss and gain of 17p11.2 are recognized as Smith–Magenis syndrome (SMS) and Potocki–Lupski syndrome (PLS) patients, respectively, and the gene primarily responsible for both syndromes is known as the retinoic acid induced 1 gene (*RAI1*), indicating that *RAI1* is a dosage-sensitive gene. Similarly, many other microdeletions and their reciprocal duplications are known to be the cause of neurodevelopmental disorders [Lee and Lupski, 2006; Gu and Lupski, 2008]. Thus, genes may be dosage-sensitive if clinical manifestations are observed in the case of their haploinsufficiency. Because the loss of function of *SATB2* is responsible for neurological manifestations, we suspected that a gain of *SATB2* copy number may contribute to the neurological manifestations in this patient.

The duplicated region of this patient includes more than 100 genes, including *SATB2*. The other potential candidate genes that may contribute to the phenotype of this patient are the potassium channel tetramerisation domain-containing 18 gene (*KCTD18*) and the ADAM metallopeptidase domain 23 gene (*ADAM23*). Because *ADAM23* is highly expressed in the brain, it may have some functional relevance to neurological features [Sagane et al., 1998]. We should accumulate more information for a better understanding of the genotype–phenotype correlation of 2q33 duplication.

Regarding the mechanisms of 2q duplications, previously reported patients showed the following four types of chromosomal rearrangements (Table I): (1) four patients, including the present patient, showed de novo occurrences of intra-chromosomal interstitial duplication of 2q and (2) five patients showed segmental 2q duplications derived from parental balanced insertions into other chromosomes. Unlike these two mechanisms, the other two mechanisms were characteristic of the subtelomere region: (3) three

patients showed inverted-duplication-deletion of 2q as a consequence of U-fiber exchanges [Rowe et al., 2009; Zuffardi et al., 2009] and (4) the remaining patients exhibited a partial trisomy that included the 2q33 region as a consequence of parental balanced translocation. Although the previously reported duplications that occurred as de novo intra-chromosomal duplications were not analyzed for the relative directions of the duplicated segments, the FISH analyses in this study revealed that the duplicated segments were aligned in the tandem configuration. This result is in agreement with that of our previous study in which we observed that duplicated segments as the interstitial duplications are generally inserted in the tandem configurations [Shimojima et al., 2009, 2010].

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REFERENCES

Alcamo EA, Chirivella L, Dautzenberg M, Dobreva G, Farinas I, Grosschedl R, McConnell SK. 2008. Satb2 regulates callosal projection neuron identity in the developing cerebral cortex. Neuron 57:364–377.

Barnicoat AJ, Abusaad I, Mackie CM, Robards MF. 1997. Two sibs with partial trisomy 2q. Am J Med Genet 70:166–170.

Bird LM, Mascarello JT. 2001. Chromosome 2q duplications: Case report of a de novo interstitial duplication and review of the literature. Am J Med Genet 100:13–24.

Britanova O, Depew MJ, Schwark M, Thomas BL, Miletich I, Sharpe P, Tarabykin V. 2006. Satb2 haploinsufficiency phenocopies 2q32-q33 deletions, whereas loss suggests a fundamental role in the coordination of jaw development. Am J Hum Genet 79:668–678.

Britanova O, de Juan Romero C, Cheung A, Kwan KY, Schwark M, Gyorgy A, Vogel T, Akopov S, Mitkovski M, Agoston D, Sestan N, Molnar Z, Tarabykin V. 2008. Satb2 is a postmitotic determinant for upper-layer neuron specification in the neocortex. Neuron 57:378–392.

Couturier J, Aurias A, Prieur M, Barois A. 1977. Partial trisomy for the long arm of chromosome 2 due to malsegregation of a maternal insertion: ins(6;2)(p22;q24q34). Ann Genet 20:52–55.

Dennis NR, Neu RL, Bannerman RM. 1978. Duplication 2q33 leads to 2q37 due to paternal ins (12;2) translocation. Am J Med Genet 1:271–277.

Elbracht M, Roos A, Schonherr N, Busse S, Damen R, Zerres K, Rudnik-Schoneborn S, Schuler HM. 2009. Pure distal trisomy 2q: A rare chromosomal abnormality with recognizable phenotype. Am J Med Genet Part A 149A:2547–2550.

Gu W, Lupski JR. 2008. CNV and nervous system diseases–What's new? Cytogenet Genome Res 123:54–64.

Lee JA, Lupski JR. 2006. Genomic rearrangements and gene copy-number alterations as a cause of nervous system disorders. Neuron 52:103–121.

Lukusa T, Devriendt K, Jaeken J, Fryns JP. 1999. Mild dysmorphic signs in two male sibs with partial trisomy 2q32.1–>q35 due to maternal ins(14;2) translocation. Clin Dysmorphol 8:47–51.

Matos A, Nogueira A, Criado B, Pereira S, Castedo S, Montenegro N. 1997. Prenatal diagnosis of partial trisomy 2q. Case report. Prenat Diagn 17:874–876.

Mencarelli MA, Caselli R, Pescucci C, Hayek G, Zappella M, Renieri A, Mari F. 2007. Clinical and molecular characterization of a patient with a 2q31.2–32.3 deletion identified by array-CGH. Am J Med Genet Part A 143A:858–865.

Prontera P, Bernardini L, Stangoni G, Capalbo A, Rogaia D, Ardisia C, Novelli A, Dallapiccola B, Donti E. 2009. 2q31.2q32.3 deletion syndrome: Report of an adult patient. Am J Med Genet Part A 149A:706–712.

Ramer JC, Mowrey PN, Robins DB, Ligato S, Towfighi J, Ladda RL. 1990. Five children with del (2)(q31q33) and one individual with dup (2)-(q31q33) from a single family: Review of brain, cardiac, and limb malformations. Am J Med Genet 37:392–400.

Romain DR, Mackenzie NG, Moss D, Columbano-Green LM, Smythe RH, Parfitt RG, Dixon JW. 1994. Partial trisomy for 2q in a patient with dir dup (2) (q33.1q35). J Med Genet 31:652–653.

Rosenfeld JA, Ballif BC, Lucas A, Spence EJ, Powell C, Aylsworth AS, Torchia BA, Shaffer LG. 2009. Small deletions of SATB2 cause some of the clinical features of the 2q33.1 microdeletion syndrome. PLoS ONE 4:e6568.

Rowe LR, Lee JY, Rector L, Kaminsky EB, Brothman AR, Martin CL, South ST. 2009. U-type exchange is the most frequent mechanism for inverted duplication with terminal deletion rearrangements. J Med Genet 46:694–702.

Sagane K, Ohya Y, Hasegawa Y, Tanaka I. 1998. Metalloproteinase-like, disintegrin-like, cysteine-rich proteins MDC2 and MDC3: Novel human cellular disintegrins highly expressed in the brain. Biochem J 334:93–98.

Schumacher RE, Rocchini AP, Wilson GN. 1983. Partial trisomy 2q. Clin Genet 23:191–194.

Sebold CD, Romie S, Szymanska J, Torres-Martinez W, Thurston V, Muesing C, Vance GH. 2005. Partial trisomy 2q: Report of a patient with dup (2)(q33.1q35). Am J Med Genet Part A 134A:80–83.

Seidahmed MZ, Rooney DE, Salih MA, Basit OB, Shaheed MM, Abdullah MA, Abomelha A. 1999. Case of partial trisomy 2q3 with clinical manifestations of Marshall–Smith syndrome. Am J Med Genet 85: 185–188.

Shimojima K, Tanaka K, Yamamoto T. 2009. A de novo intra-chromosomal tandem duplication at 22q13.1q13.31 including the Rubinstein–Taybi region but with no bipolar disorder. Am J Med Genet Part A 149A:1359–1363.

Shimojima K, Imai K, Yamamoto T. 2010. A de novo 22q11.22q11.23 interchromosomal tandem duplication in a boy with developmental delay, hyperactivity, and epilepsy. Am J Med Genet Part A 152A: 2820–2826.

Shimojima K, Okamoto N, Inazu T, Yamamoto T. 2011. Tandem configurations of variably duplicated segments of 22q11.2 confirmed by fiber-FISH analysis. J Hum Genet 56:810–812.

Slavotinek AM, Boles D, Lacbawan F. 2003. A female infant with duplication of chromosome 2q33 to 2q37.3. Clin Dysmorphol 12: 251–256.

Van Buggenhout G, Van Ravenswaaij-Arts C, Mc Maas N, Thoelen R, Vogels A, Smeets D, Salden I, Matthijs G, Fryns JP, Vermeesch JR. 2005. The del(2)(q32.2q33) deletion syndrome defined by clinical and molecular characterization of four patients. Eur J Med Genet 48:276–289.

Zuffardi O, Bonaglia M, Ciccone R, Giorda R. 2009. Inverted duplications deletions: Underdiagnosed rearrangements? Clin Genet 75:505–513.

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FULL-LENGTH ORIGINAL RESEARCH

Risk factors for hyperammonemia in pediatric patients with epilepsy

*†Yoshiaki Yamamoto, *Yukitoshi Takahashi, *Katsumi Imai, *Nobuyuki Mishima, ‡Rei Yazawa, ‡Kazuyuki Inoue, ‡Kunihiko Itoh, †Yoshiyuki Kagawa, and *Yushi Inoue

*Department of Clinical Research, National Epilepsy Center, Shizuoka Institute of Epilepsy and Neurological Disorders, Shizuoka, Japan; †Department of Clinical Pharmaceutics, Graduate School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan; and ‡Department of Clinical Pharmacology and Genetics, Graduate School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan

Summary

Purpose: To identify risk factors for hyperammonemia in pediatric patients with epilepsy.

Methods: A total of 2,944 pediatric patients (ages 0–15 years) were classified into the following three groups: a group without drug treatment (n = 445, group I), a group receiving antiepileptic drugs other than valproic acid (VPA) (n = 673, group II), and a VPA-treated group (n = 1,826, group III). Hyperammonemia was defined as a plasma ammonia level exceeding 100 μg/dl with reference to the standard range and previous reports.

Key Findings: The mean ammonia level of groups I, II, and III was 36.0, 56.0, and 86.8 μg/dl, respectively, and the incidence of hyperammonemia was 1.6%, 7.7%, and 31.7%, respectively. In each group, the mean ammonia level of patients aged 3 years or younger was significantly higher than that of patients aged 4–15 years. In group II, concomitant use of topiramate and zonisamide were risk factors for hyperammonemia (adjusted odds ratio [OR] 3.9, 95% confidence interval [CI] 1.7–9.2, and OR 3.5, 95% CI 1.9–6.5, respectively). In group III, the ammonia level increased in a VPA dose–dependent manner. At a VPA dose of 30 mg/kg, there was 4.3-fold increase in the incidence of hyperammonemia. The other significant risk factors identified were female gender (OR 1.3, 95% CI 1.0–1.6), symptomatic generalized epilepsy (OR 1.4, 95% CI 1.1–1.8), and the concomitant use of phenytoin (OR 4.7, 95% CI 3.3–6.9), phenobarbital (OR 2.2. 95% CI 1.6–3.2), acetazolamide (OR 6.6, 95% CI 2.5–17.2), topiramate, or zonisamide.

Significance: A young age and concomitant use of carbonic anhydrase inhibitors are associated with an increased risk of hyperammonemia regardless of whether the patient is taking VPA. In patients receiving VPA, concomitant use of phenytoin and/or phenobarbital enhances the risk of hyperammonemia. An increase in ammonia can be caused by multiple factors. Our results may help clinicians to avoid problems of hyperammonemia.

KEY WORDS: Hyperammonemia, Epilepsy, Children, Risk factor, Valproic acid, Phenytoin.

Hyperammonemia is a frequent problem associated with antiepileptic drugs (AEDs) that can lead to vomiting, aggression, ataxia, and exacerbation of seizures. Acute hyperammonemia can also cause cerebral edema and severe brain damage (encephalopathy), whereas chronic hyperammonemia due to metabolic disorders is associated with developmental delay and intellectual disability (Cagnon & Braissant, 2007; Lichter-Konecki, 2008). Among the AEDs, valproic acid (VPA) is recommended as a first-line treatment for generalized epilepsy. Although VPA can cause an increase of the blood ammonia level, VPA therapy is rarely associated with hyperammonemic encephalopathy.

The exact relationship between symptoms and the ammonia level remains unclear. Murphy and Marquardt (1982) reported that patients with hyperammonemia not exceeding 240 μg/dl were asymptomatic. In contrast, Coulter and Allen (1981) recommended reducing the VPA dose when the ammonia level exceeded 100 μg/dl. Our data (see later) indicate that hyperammonemia is common, but clinicians may be unaware of it and may attribute the clinical consequences to other causes.

Our previous study established that high-dose VPA therapy, concomitant use of hepatic enzyme inducers, and concomitant use of topiramate (TPM) were risk factors for an increase of the blood ammonia level in adult patients with epilepsy who were treated with VPA (Yamamoto et al.,

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Address correspondence to Yoshiaki Yamamoto, Department of Clinical Research, National Epilepsy Center, Shizuoka Institute of Epilepsy and Neurological Disorders, 886, Urushiyama, Aoi-ku, Shizuoka-shi, Shizuoka 420-8688, Japan. E-mail: yamamoty@szec.hosp.go.jp

2012). There have also been some reports regarding the risk of hyperammonemia in pediatric patients with epilepsy (Coulter & Allen, 1981; Batshaw & Brusilow, 1982; Murphy & Marquardt, 1982; Ohtani et al., 1982; Haidukewych et al., 1985; Laub, 1986; Iinuma et al., 1988; Thom et al., 1991; Kondo et al., 1992; Altunbaşak et al., 1997; Sharma et al., 2011). Verrotti et al. (1999) found that the VPA dose, the duration of treatment, and polytherapy were associated with hyperammonemia. In addition, Haidukewych et al. (1985) and Sharma et al. (2011) reported that the blood concentration of VPA was correlated positively with the ammonia level, but other studies have not detected a significant association. The number of subjects in most of these previous studies was fewer than 100, which may have contributed to the differing results. In addition, most of the previous studies were conducted before 2000, so it remains unknown whether new AEDs, such as zonisamide (ZNS), TPM, gabapentin, lamotrigine, and levetiracetam, can increase the ammonia level.

VPA is metabolized mainly by uridine diphosphate glucuronosyltransferases (UGTs) and partially via β-oxidation and various cytochrome P450 (CYP) enzymes. Metabolites of VPA, such as valpronyl–coenzyme A (CoA), propionate, and 2-n-propyl-4-pentenoic acid (4-en-VPA), reduce the activity of enzymes involved in the urea cycle, resulting in the accumulation of ammonia (Kondo et al., 1992; Verbiest et al., 1992; Aires et al., 2011). Growth and development of pediatric patients may influence the metabolism of VPA, leading to age-related differences in the risk of hyperammonemia. Altunbaşak et al. (1997) reported that children younger than 2 years old had a higher risk of hyperammonemia related to VPA therapy, but other authors have not found a relationship between age and the blood level of ammonia. This may be because patient background factors varied widely among the studies. To identify risk factors for hyperammonemia, a large-scale study of pediatric patients covering a wide age range is needed.

Although hyperammonemia is generally associated with VPA, monotherapy with phenytoin (PHT), carbamazepine (CBZ), primidone, or acetazolamide can occasionally cause hyperammonemia (Ambrosetto et al., 1984; Katano et al., 2002; Kim et al., 2007; Adams et al., 2009; Labib et al., 2011). In addition, ZNS, TPM, acetazolamide, and sulthiame inhibit carbonic anhydrase and block bicarbonate re-uptake, so drug-induced metabolic acidosis could lead to an increase of the blood ammonia level in patients taking these AEDs. However, there is limited information regarding the risk of elevation of the ammonia level due to AED therapy other than VPA.

In recent years, several reports have indicated that generalized tonic–clonic (GTC) seizures can cause hyperammonemia (Liu & Su, 2008; Yanagawa et al., 2008; Hung et al., 2011; Liu et al., 2011). Therefore, it seems that an increase of ammonia could be caused by multiple factors, including those related to the patient's profile, seizure control, AED regimen, and drug doses. Accordingly, the aim of the present study was to evaluate the risk factors and prevalence of hyperammonemia among pediatric patients in relation to their AED therapy.

Methods

Subjects

This was a retrospective study of 2,944 pediatric patients aged 0–15 years who consulted the National Epilepsy Center (Shizuoka, Japan) from January 2006 to December 2011. These patients were classified into three groups. Group I was 445 patients with newly diagnosed or suspected epilepsy or intellectual disability who had not received any prior AED therapy. The other 2,499 patients were taking AEDs including 673 patients treated with AEDs other than VPA (group II) and 1,826 patients treated with VPA (group III). Patients with serious hepatic dysfunction (aspartate aminotransferase or alanine aminotransferase >200 U/L), metabolic disorders, or severe infections were excluded. The study protocol was approved by the ethics committee of our hospital (protocol No. 2012–11).

Venous blood samples were obtained from the patients at 2–6 h after a meal. Measurement of the plasma ammonia level was done by the method described previously (Yamamoto et al., 2012). If multiple measurements were performed during the study period, the highest blood ammonia level was used. If VPA was added or discontinued during the study, we obtained two blood samples from the relevant patient. At our hospital, the normal range of plasma ammonia is 16–76 μg/dl. In this study, hyperammonemia was defined as a plasma ammonia level exceeding 100 μg/dl with reference to the above standard range and previous reports.

PHT, phenobarbital (PB), and CBZ are enzyme-inducing drugs; in this study, they will be referred to as inducers. Because primidone is converted to PB, we considered it to be equivalent to PB. ZNS, TPM, acetazolamide, and sulthiame were classified as carbonic anhydrase inhibitors (CAIs).

Statistical analysis

Quantitative variables were analyzed by the unpaired *t*-test or analysis of variance (ANOVA) with a post hoc Scheffe's multiple comparison test. Nominal variables were analyzed by the chi-square test.

To extract the factors influencing the plasma ammonia level, stepwise multiple regression analysis was performed using the age, gender, type of epilepsy (classified as focal epilepsy, idiopathic generalized epilepsy, symptomatic generalized epilepsy, or Dravet syndrome), and 14 AEDs (PHT, CBZ, PB, TPM, ZNS, clobazam, clonazepam, nitrazepam, acetazolamide, sulthiame, gabapentin, lamotrigine, levetiracetam, and ethosuximide) as the factors. In addition, the dose of VPA was added to the multiple regression model in group III. Finally, multiple logistic regression analysis was performed to calculate adjusted odds ratios for hyperammonemia,

which was defined as a maximum ammonia level exceeding 100 or 150 μg/dl.

Results are expressed as the mean ± standard error. Statistical analyses were conducted with SPSS software Ver 19.0 (IBM Japan, Tokyo, Japan).

Results

Patient characteristics

Table 1 shows the 2,944 pediatric patients classified into three groups based on their treatment. There was no difference in age among the three groups, but group I had the highest percentage of males (60.4%). The mean ammonia level of group I was 36.0 μg/dl, which was significantly lower than that of the patients treated with AEDs (groups II and III). Among the 1,826 patients treated with VPA, 578 patients (31.7%) had ammonia levels >100 μg/dl and 110 of these 578 patients (19.0%) required L-carnitine therapy. In this study, symptoms of hyperammonemia were not investigated in all of the patients, but 37 of 166 patients with ammonia levels exceeding 150 μg/dl had symptoms such as somnolence, lethargy, nausea, vomiting, and anorexia.

Epilepsy was classified as focal epilepsy in 1,492 (50.7%) patients, idiopathic generalized epilepsy in 168 (5.7%) patients, symptomatic generalized epilepsy in 523 (17.8%) patients, and Dravet syndrome in 108 (3.7%) patients. Other categories were unclassified epilepsy, situation-related syndrome, and undiagnosed epilepsy type/syndrome.

Influence of age

Figure 1 displays the plasma ammonia levels of the patients classified into four age groups. The mean ammonia level varied across the age groups (ANOVA: $p < 0.001$). In all three treatment groups, patients aged from 0 to 3 years had the highest ammonia levels of all age groups (Scheffe's test; $p < 0.001$). There was also a statistical difference between patients aged 4–7 and 12–15 years in groups I and III ($p < 0.005$).

Influence of AED monotherapy or combination therapy

Table 2 shows the effect of AED monotherapy on the blood ammonia level. The mean ammonia levels in patients receiving any type of monotherapy were significantly higher than those of the untreated group. Among the inducers, PHT monotherapy was associated with higher blood ammonia levels despite the patients having the greatest mean age, but there were no significant differences. The mean ammonia level of patients taking CAIs was significantly higher than that of patients taking CBZ ($p < 0.005$), and hyperammonemia was significantly more frequent (chi-square test: $p < 0.05$).

Table 3 shows the blood ammonia levels of patients receiving VPA monotherapy or VPA plus other AEDs. The mean ammonia level of the patients on VPA plus AEDs was significantly higher than that of those on VPA monotherapy, but the combined effects of VPA and each AED were different. Concomitant use of PHT, PB, or CAIs was associated with a significantly higher blood ammonia level compared with concomitant CBZ. The combination of VPA plus PHT, VPA plus PB, or VPA plus CAIs was associated with a higher incidence of hyperammonemia than the other AED regimens ($p < 0.001$). In particular, concomitant use of PHT was most likely to cause hyperammonemia exceeding 150 μg/dl.

Relation between the ammonia level and the VPA dose

There was a significant positive correlation between the plasma ammonia level and the dose of VPA in the patients receiving VPA monotherapy (Pearson's

Table 1. Characteristics of the subjects

	Group I (non-AED group)	Group II (non-VPA group)	Group III (VPA group)	p-Value
No. of patients	445	673	1,826	
Gender (female/male)	176/269	310/363	848/978	<0.05
Age (years)	7.2 ± 0.20	6.9 ± 0.17	7.1 ± 0.11	NS
Ammonia level (μg/dl)	36.0 ± 1.0*†	56.0 ± 1.1†	85.8 ± 1.0	<0.001
>100 μg/dl	7	52	578	<0.001
>150 μg/dl	1	5	160	<0.001
>200 μg/dl	0	0	42	<0.001
AED therapy				
VPA dose (mg/kg)	–	–	21.4 ± 0.23	
Concomitant AEDs				
Hepatic enzyme inducers	–	519	566	<0.005
Carbonic anhydrase inhibitors	–	256	534	NS
Benzodiazepines	–	186	483	NS
New AEDs	–	106	209	NS
Other AEDs	–	46	156	NS

Carbonic anhydrase inhibitors were zonisamide, topiramate, acetazolamide, and sulthiame. New AEDs were gabapentin, lamotrigine, and levetiracetam. Benzodiazepines were clobazam, clonazepam, nitrazepam, and diazepam. Other AEDs were ethosuximide, bromide, stiripentol, and ethotoin.
Significance was determined by ANOVA, the unpaired *t*-test or the chi-square test, Scheffe's post hoc test; *$p < 0.001$ versus group II, †$p < 0.001$ versus group III.

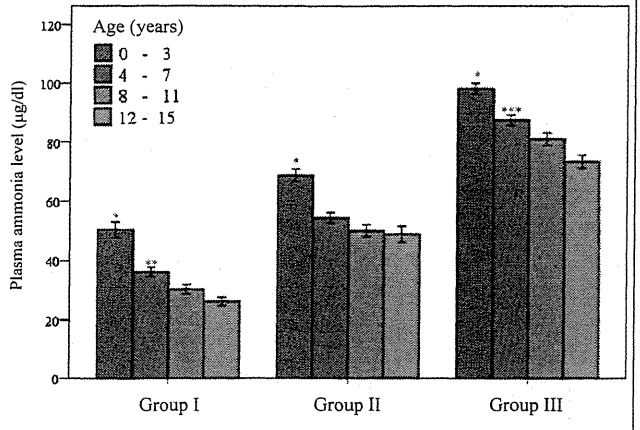

Figure 1.
Plasma ammonia level stratified by age. Significance was determined by ANOVA ($p < 0.001$) among groups I, II, and III. Scheffe's post hoc test; *$p < 0.001$ versus ages 4–7, 8–11, and 12–15, **$p < 0.005$ versus age 12–15, ***$p < 0.001$ versus age 12–15.
Epilepsia © ILAE

correlation coefficient analysis, $r = 0.44$, $p < 0.001$). When patients were classified into four VPA dose groups: <10 mg/kg, ≥10 and <20 mg/kg, ≥20 and <30 mg/kg, and ≥30 mg/kg, the mean plasma ammonia levels increased in a VPA dose–dependent manner (46.8, 60.4, 85.7, and 92.6 μg/dl, respectively, ANOVA: $p < 0.001$). In particular, there was significant difference between the dose ranges of <10 mg/kg and ≥20 mg/kg ($p < 0.001$, See Fig. S1.).

Risk factors for hyperammonemia in patients with or without VPA therapy

By stepwise multiple regression analysis, the factors influencing the ammonia level were extracted (see Table S1). These factors were then used as independent variables for multiple logistic regression analysis.

In group II, age and the concomitant use of PHT, PB, ZNS, TPM, or levetiracetam had a significant influence on the ammonia level, and these factors were entered as independent variables in multiple logistic regression analysis. Age and concomitant use of ZNS and TPM were found to be significant risk factors for hyperammonemia (Table 4). PHT and PB were also risk factors, but were not statistically significant.

In group III, the age, symptomatic generalized epilepsy, VPA dose, and concomitant use of PHT, PB, TPM, ZNS, or acetazolamide were all found to be significant risk factors (Table 5). In contrast, the VPA dose and concomitant use of PHT, PB, or TPM were significantly associated with an increased risk of an ammonia level exceeding 150 μg/dl), with high-dose VPA and concomitant PHT being the most important factors.

Discussion

The present cross-sectional study of 2,944 pediatric patients demonstrated that the mean ammonia level was 2.4-fold higher in patients treated with VPA than in patients without AED therapy (Table 1). A literature review performed by Chicharro and Kanner (2007) identi-

Table 2. Effect of AED monotherapy on the plasma ammonia level

Regimen	No AEDs (reference)	PHT	PB	CBZ	CAIs	BZs	p-Value[a]
No. patients	445	17	29	187	58	15	
Age (years)	7.2 ± 0.2	10.8 ± 0.6	4.2 ± 0.8[c]	7.7 ± 0.3[e]	6.2 ± 0.6[d]	7.0 ± 1.2	<0.001
Ammonia level (μg/dl)[b]	36.0 ± 1.0	59.9 ± 4.9	52.5 ± 5.1	44.9 ± 1.6	60.0 ± 3.3[f]	48.5 ± 8.5	<0.001
>100, n (%)	7 (1.6)	0 (0)	1 (3.4)	3 (1.6)	6 (10.3)	1 (6.7)	<0.05

CAIs; carbonic anhydrase inhibitors, BZs; benzodiazepines.
[a]Significance was determined by ANOVA or the chi-square test.
[b]Dunnett's test; non AED versus PHT, PB, CBZ, CAIs; $p < 0.001$.
Post hoc Scheffe's multiple comparison test; [c]$p < 0.001$ versus PHT, [d]$p < 0.005$ versus PHT, [e]$p < 0.05$ versus PB, [f]$p < 0.005$ versus CBZ.

Table 3. Effect of VPA plus other AEDs on the plasma ammonia level

Regimen	VPA mono (reference)	VPA + PHT	VPA + PB	VPA + CBZ	VPA + CAIs	VPA + BZs	p- Value[a]
No. patients	560	53	39	156	201	126	
Age (years)	7.8 ± 0.2	8.0 ± 0.6	5.2 ± 0.7	7.9 ± 0.4[e]	6.1 ± 0.3[h]	6.8 ± 0.4	<0.001
Ammonia level (μg/dl)	67.0 ± 1.5	115.0 ± 8.0	107.1 ± 7.9	76.6 ± 3.0[b,d]	93.7 ± 2.5[c,g]	74.2 ± 3.1[b,d,i]	<0.001
>100, n (%)	87 (15.5)	29 (54.7)	21 (53.8)	38 (24.4)	84 (41.8)	21 (16.7)	<0.001
>150, n (%)	18 (3.2)	15 (28.3)	6 (15.4)	6 (3.8)	15 (7.5)	4 (3.2)	<0.001
VPA dose (mg/kg)	17.5 ± 0.4	24.2 ± 1.7	26.5 ± 2.2	19.6 ± 0.8[f]	23.2 ± 0.7[h]	21.5 ± 0.7	<0.001

[a]Significance was determined by ANOVA or the chi-square test.
Post hoc Scheffe's multiple comparison test; [b]$p < 0.001$ versus VPA + PHT, [c]$p < 0.05$ versus VPA + PHT, [d]$p < 0.005$ versus VPA + PB, [e]$p < 0.05$ versus VPA + PB, [f]$p < 0.01$ versus VPA + PB, [g]$p < 0.005$ versus VPA + CBZ, [h]$p < 0.05$ versus VPA + CBZ, [i]$p < 0.001$ versus VPA + CAIs.

Table 4. Multiple logistic regression analysis of risk factors for hyperammonemia in group II (non-VPA group)

	Plasma ammonia level (>100)	
Risk factor	OR (95% CI)	p value
Age (per year)	0.89 (0.82–0.96)	<0.005
Concomitant AEDs		
Phenytoin	2.00 (0.97–4.13)	0.060
Phenobarbital	1.67 (0.85–3.27)	0.13
Zonisamide	3.48 (1.88–6.53)	<0.001
Topiramate	3.93 (1.68–9.20)	<0.005
Levetiracetam	0.96 (0.21–4.37)	0.96

OR, adjusted odds ratio; CI, confidence interval.

fied a significant difference between the VPA group and the control group in 11 of 17 cross-sectional studies, whereas the other six studies showed no difference. The large sample size of the present study allowed us to detect significant differences of the ammonia level among groups I, II, and III.

Our data also suggested that inducers and CAIs had the potential to increase the ammonia level in patients who were not taking VPA (Tables 2 and 4). Several previous studies have found a decrease of carnitine in patients treated with PHT, PB, or CBZ rather than VPA. Hug et al. (1991) reported that the mean total carnitine level was 57.8 nmol/ml in healthy children, whereas the carnitine level of pediatric patients with epilepsy who were receiving VPA, PB, PHT, or CBZ was 35.6, 32.7, 39.7, and 41.5 nmol/ml, respectively. Reduction of the blood carnitine level by these AEDs may cause mitochondrial dysfunction, resulting in an increase of ammonia.

Tables 2 and 3 show that VPA monotherapy was more closely associated with hyperammonemia in comparison with monotherapy taking PHT, PB, or CBZ (15.5% vs. 0%, 3.4%, and 1.6%). Figure S1 shows that the increase of ammonia in our pediatric patients with epilepsy was dependent on the daily dose of VPA. Several authors have reported that the blood carnitine level decreases with an increase in the VPA dose or concentration (Hamed & Abdella, 2009; Nakajima et al., 2011). Another possible mechanism of hyperammonemia related to VPA therapy is inhibition of carbamoyl phosphate synthase 1 and *N*-acetylglutamate synthase by metabolites of VPA such as valpronyl-CoA, propionate, and 4-en-VPA. That is, high-dose VPA monotherapy could increase harmful metabolites and reduce the carnitine level, resulting in an increased risk of hyperammonemia.

In patients receiving VPA with PHT or PB, a high incidence of hyperammonemia was observed (Table 3). According to our multivariate model, combined therapy with VPA and PHT was the strongest risk factor for hyperammonemia in the pediatric patients with epilepsy (Table 5). These findings agree with the results of a previous study in adult patients with epilepsy (Yamamoto et al., 2012). Although hyperammonemia associated with VPA + PHT and/or PB therapy has long been reported, the mechanism remains obscure. PHT and PB increase the activity of CYP enzymes and UGTs, which would facilitate metabolism of VPA. Among the CYP enzymes, CYP2C9, CYP2A6, and CYP2B6 are involved in the synthesis of 4-en-VPA and propionate, which inhibits urea cycle enzymes. In contrast, a number of studies have shown that the blood carnitine concentration is lower in patients receiving VPA combined with inducers than in patients receiving VPA monotherapy (Ohtani et al., 1982; Coulter 1991). Hyperammonemia in patients receiving VPA + PHT and/or PB therapy could be caused by a combination of these mechanisms. Among the inducers, CBZ had the smallest impact on the plasma ammonia level. Measurement of VPA metabolites and the carnitine concentration could be valuable for clarifying the mechanisms of hyperammonemia associated with concomitant use of VPA and inducers.

The influence of new AEDs such as ZNS, TPM, lamotrigine, and levetiracetam on the blood ammonia level

Table 5. Multiple logistic regression analysis of risk factors for hyperammonemia in group III (VPA group)

	Plasma ammonia level			
	>100		>150	
Risk factor	OR (95% CI)	p-Value	OR (95% CI)	p-Value
Age (per year)	0.97 (0.94–0.99)	<0.05	1.00 (0.96–1.04)	0.91
Gender (female = 1)	1.30 (1.04–1.61)	<0.05	1.55 (1.10–2.20)	<0.05
Symptomatic generalized epilepsy	1.40 (1.09–1.80)	<0.01	1.17 (0.79–1.73)	0.44
VPA dose (per mg/kg)	1.05 (1.03–1.06)	<0.001	1.05 (1.03–1.07)	<0.001
Concomitant AEDs				
Phenytoin	4.73 (3.25–6.88)	<0.001	5.45 (3.54–8.40)	<0.001
Phenobarbital	2.22 (1.59–3.12)	<0.001	2.44 (1.57–3.79)	<0.001
Zonisamide	1.72 (1.33–2.23)	<0.001	1.42 (0.95–2.11)	0.089
Topiramate	2.69 (1.78–4.05)	<0.001	2.13 (1.20–3.76)	<0.05
Acetazolamide	6.55 (2.50–17.2)	<0.001	2.51 (0.91–6.95)	0.076

OR, odds ratio; CI, confidence interval.

remains poorly understood. Several case reports have been published about hyperammonemia in patients on TPM therapy (Hamer et al., 2000; Longin et al., 2002; Knudsen et al., 2008; Deutsch et al., 2009), but there have been none about the other new AEDs. The present study demonstrated that concomitant use of ZNS and TPM was an important risk factor for an increase of the ammonia level, irrespective of whether the patient was being treated with VPA. Coppola et al. (2006) reported that lamotrigine and TPM did not have a significant effect on the serum carnitine level. In contrast, ZNS and TPM inhibit carbonic anhydrase and block bicarbonate reuptake, resulting in metabolic acidosis that may lead to an increase of the plasma ammonia level regardless of concomitant VPA therapy. We also found that treatment with acetazolamide had the potential to increase the ammonia level, presumably via the same mechanism as that for TPM and ZNS. However, there were only five patients receiving acetazolamide in group II, contributing to the lack of a significant result. In this study, bicarbonate was not measured, so further studies will be needed to determine the association between bicarbonate and ammonia levels.

Our study demonstrated that pediatric patients with epilepsy aged 3 years or younger who were treated with VPA had an increased risk of hyperammonemia, confirming a previous report by Altunbaşak et al. (1997). The same trend was also seen in the untreated and non-VPA groups, suggesting that ammonia metabolism differs among infants, children, and adolescents. Galal et al. (2010) reported that there was no relationship between age and the ammonia level in patients attending a pediatric emergency department. In contrast, Colombo et al. (1984) reported that the plasma ammonia level was higher in neonates than in children aged 2–6 years (range 30–144 vs. 24–48 μmol/l). Because of the large number of patients in our study it was possible to establish a more reliable indication of the impact of age on the plasma ammonia level.

Among patients receiving VPA, female gender was associated with a risk of hyperammonemia. In group III, female patients had a significantly lower mean body weight than males (24.5 vs. 26.7 kg, $p < 0.001$), suggesting that female patients would have greater hepatic blood flow and VPA clearance. Furthermore, a review by Pleym et al. (2003) suggested that UGT activity is higher in male patients than female, indicating that CYP-derived metabolites may be more important in female patients. Therefore, gender-based differences of body weight and UGT activity presumably resulted in differences of VPA metabolism and the ammonia level.

Continuous muscle contraction due to GTC seizures can elevate the blood ammonia level by deamination of adenosine monophosphate in the purine nucleotide cycle (Mutch & Banister, 1983; Wilkinson et al., 2010). Several reports have indicated that the risk of hyperammonemia in adult patients with epilepsy is significantly related to GTC seizures (Liu & Su, 2008; Yanagawa et al., 2008; Hung et al., 2011; Liu et al., 2011). These studies included patients without AEDs and excluded patients taking VPA. In contrast, our study showed that symptomatic generalized epilepsy was one of the risk factors for hyperammonemia in group III (with VPA), but not in group II (without VPA). Because VPA is a first-line treatment for GTC seizures, the prevalence of generalized epilepsy was significantly higher in group III than in group II (23.2% vs. 11.9%), so this lower prevalence may have contributed to the lack of any association with GTC seizures in group II.

Our previous study showed that 40.7% of adult patients on VPA therapy had symptomatic hyperammonemia (Yamamoto et al., 2012). In the present study, 37 (22.3%) of 166 patients with an ammonia level exceeding 150 μg/dl had symptoms of hyperammonemia. Therefore, careful attention should be paid to whether symptoms are related to hyperammonemia in patients with an ammonia level >100 μg/dl.

There were several limitations of the present study. Although the dose of VPA showed a high partial regression coefficient, blood samples were not collected at a specific time and VPA trough concentrations were not obtained. In addition, if multiple measurements were obtained from same patients, the highest ammonia level was used, which may have contributed to selection bias. Long-term VPA therapy reduces the blood carnitine level, but we did not investigate the duration of AED therapy. Furthermore, the retrospective design of this study meant that we could not confirm seizure frequency, nitrogen intake, and symptoms at the point of examination. We were also unable to investigate the symptoms of hyperammonemia in all 2,944 patients and could not set clinical criteria for symptomatic hyperammonemia. In particular, the relationship between symptoms and the ammonia level remains unclear, although it is known that chronic hyperammonemia can have harmful effects on brain development and seizure control. Therefore, further studies will be required to evaluate the ammonia level below which there will be no influence on seizure control and brain development.

In conclusion, our study established a number of risk factors for hyperammonemia in pediatric patients with epilepsy who are on AED therapy. We emphasize the need for measurement of ammonia when patients have multiple risk factors, especially those receiving high-dose VPA and regimens such as VPA plus PHT and/or PB. In addition, use of CAIs and a younger age can be associated with an increase of ammonia regardless of whether a patient is receiving VPA. These results have several implications for minimizing the risk of hyperammonemia in pediatric patients with epilepsy treated with VPA-based AED therapy.

Disclosure

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

References

Adams EN, Marks A, Lizer MH. (2009) Carbamazepine-induced hyperammonemia. *Am J Health Syst Pharm* 15:1468–1470.

Aires CC, van Cruchten A, Ijlst L, de Almeida IT, Duran M, Wanders RJ, Silva MF. (2011) New insights on the mechanisms of valproate-induced hyperammonemia: inhibition of hepatic *N*-acetylglutamate synthase activity by valproyl-Coa. *J Hepatol* 55:426–434.

Altunbaşak S, Baytok V, Tasouji M, Hergüner O, Burgut R, Kayrin L. (1997) Asymptomatic hyperammonemia in children treated with valproic acid. *J Child Neurol* 12:461–463.

Ambrosetto G, Riva R, Baruzzi A. (1984) Hyperammonemia in asterixis induced by carbamazepine: two case reports. *Acta Neurol Scand* 69: 186–189.

Batshaw ML, Brusilow SW. (1982) Valproate-induced hyperammonemia. *Ann Neurol* 11:319–321.

Cagnon L, Braissant O. (2007) Hyperammonemia-induced toxicity for the developing central nervous system. *Brain Res Rev* 56:183–197.

Chicharro AV, Kanner AM. (2007) The measurement of ammonia blood levels in patients taking valproic acid: looking for problems where they do not exist? *Epilepsy Behav* 11:361–366.

Colombo JP, Peheim E, Kretschmer R, Dauwalder H, Sidiropoulos D. (1984) Plasma ammonia concentrations in newborns and children. *Clin Chim Acta* 138:283–291.

Coppola G, Epifanio G, Auricchio G, Federico RR, Resicato G, Pascotto A. (2006) Plasma free carnitine in epilepsy children, adolescents and young adults treated with old and new antiepileptic drugs with or without ketogenic diet. *Brain Dev* 28:358–365.

Coulter DL. (1991) Carnitine, valproate, and toxicity. *J Child Neurol* 6: 7–14.

Coulter DL, Allen RJ. (1981) Hyperammonemia with valproic acid therapy. *J Pediatr* 99:317–319.

Deutsch SI, Burket JA, Rosse RB. (2009) Valproate-induced hyperammonemic encephalopathy and normal liver functions: possible synergism with topiramate. *Clin Neuropharmacol* 32:350–352.

Galal NM, Fouad HM, Saied A, Dabnon M. (2010) Hyperammonemia in the pediatric emergency care setting. *Pediatr Emerg Care* 26:888–891.

Haidukewych D, John G, Zielinski JJ, Rodin EA. (1985) Chronic valproic acid therapy and incidence of increases in venous plasma ammonia. *Ther Drug Monit* 7:290–294.

Hamed SA, Abdella MM. (2009) The risk of asymptomatic hyperammonemia in children with idiopathic epilepsy treated with valproate: relationship to blood carnitine status. *Epilepsy Res* 86:32–41.

Hamer HM, Knake S, Schomburg U, Rosenow F. (2000) Valproate-induced hyperammonemic encephalopathy in the presence of topiramate. *Neurology* 54:230–232.

Hug G, McGraw CA, Bates SR, Landrigan EA. (1991) Reduction of serum carnitine concentrations during anticonvulsant therapy with phenobarbital, valproic acid, phenytoin, and carbamazepine in children. *J Pediatr* 119:799–802.

Hung TY, Chen CC, Wang TL, Su CF, Wang RF. (2011) Transient hyperammonemia in seizures: a prospective study. *Epilepsia* 52: 2043–2049.

Iinuma K, Hayasaka K, Narisawa K, Tada K, Hori K. (1988) Hyperamino-acidaemia and hyperammonaemia in epileptic children treated with valproic acid. *Eur J Pediatr* 148:267–269.

Katano H, Fukushima T, Karasawa K, Sugiyama N, Ohkura A, Kamiya K. (2002) Primidone-induced hyperammonemic encephalopathy in a patient with cerebral astrocytoma. *J Clin Neurosci* 9:79–81.

Kim JM, Ryu WS, Hwang YH, Kim JS. (2007) Aggravation of ataxia due to acetazolamide induced hyperammonaemia in episodic ataxia. *J Neurol Neurosurg Psychiatry* 78:771–772.

Knudsen JF, Sokol GH, Flowers CM. (2008) Adjunctive topiramate enhances the risk of hypothermia associated with valproic acid therapy. *J Clin Pharm Ther* 33:513–519.

Kondo T, Ishida M, Kaneko S, Hirano T, Otani K, Fukushima Y, Muranaka H, Koide N, Yokoyama M, Nakata S. (1992) Is 2-propyl-4-pentenoic acid, a hepatotoxic metabolite of valproate, responsible for valproate-induced hyperammonemia? *Epilepsia* 33:550–554.

Labib PL, Wing S, Bhowmik A. (2011) Transient hyperammonaemia in a patient with confusion: challenges with the differential diagnosis. *BMJ Case Rep* Sep 4 [Epub ahead of print].

Laub MC. (1986) Nutritional influence on serum ammonia in young patients receiving sodium valproate. *Epilepsia* 27:55–59.

Lichter-Konecki U. (2008) Profiling of astrocyte properties in the hyperammonaemic brain: shedding new light on the pathophysiology of the brain damage in hyperammonaemia. *J Inherit Metab Dis* 31:492–502.

Liu KT, Su CS. (2008) Postictal transient hyperammonemia. *Am J Emerg Med* 26(388):e1–e2.

Liu KT, Yang SC, Yeh IJ, Lin TJ, Lee CW. (2011) Transient hyperammonemia associated with postictal state in generalized convulsion. *Kaohsiung J Med Sci* 27:453–456.

Longin E, Teich M, Koelfen W, König S. (2002) Topiramate enhances the risk of valproate-associated side effects in three children. *Epilepsia* 43:451–454.

Murphy JV, Marquardt K. (1982) Asymptomatic hyperammonemia in patients receiving valproic acid. *Arch Neurol* 39:591–592.

Mutch BJ, Banister EW. (1983) Ammonia metabolism in exercise and fatigue: a review. *Med Sci Sports Exerc* 15:41–50.

Nakajima Y, Ito T, Maeda Y, Ichiki S, Kobayashi S, Ando N, Hussein MH, Kurono Y, Sugiyama N, Togari H. (2011) Evaluation of valproate effects on acylcarnitine in epileptic children by LC-MS/MS. *Brain Dev* 33:816–823.

Ohtani Y, Endo F, Matsuda I. (1982) Carnitine deficiency and hyperammonemia associated with valproic acid therapy. *J Pediatr* 101:782–785.

Pleym H, Spigset O, Kharasch ED, Dale O. (2003) Gender differences in drug effects: implications for anesthesiologists. *Acta Anaesthesiol Scand* 47:241–259.

Sharma S, Gulati S, Kabra M, Kalra V, Vasisht S, Gupta YK. (2011) Blood ammonia levels in epileptic children on 2 dose ranges of valproic acid monotherapy: a cross-sectional study. *J Child Neurol* 26:109–112.

Thom H, Carter PE, Cole GF, Stevenson KL. (1991) Ammonia and carnitine concentrations in children treated with sodium valproate compared with other anticonvulsant drugs. *Dev Med Child Neurol* 33:795–802.

Verbiest HB, Straver JS, Colombo JP, van der Vijver JC, van Woerkom TC. (1992) Carbamyl phosphate synthetase-1 deficiency discovered after valproic acid-induced coma. *Acta Neurol Scand* 86:275–279.

Verrotti A, Greco R, Morgese G, Chiarelli F. (1999) Carnitine deficiency and hyperammonemia in children receiving valproic acid with and without other anticonvulsant drugs. *Int J Clin Lab Res* 29:36–40.

Wilkinson DJ, Smeeton NJ, Watt PW. (2010) Ammonia metabolism, the brain and fatigue; revisiting the link. *Prog Neurobiol* 91:200–219.

Yamamoto Y, Takahashi Y, Suzuki E, Mishima N, Inoue K, Itoh K, Kagawa Y, Inoue Y. (2012) Risk factors for hyperammonemia associated with valproic acid therapy in adult epilepsy patients. *Epilepsy Res* 101:202–209.

Yanagawa Y, Nishi K, Sakamoto T. (2008) Hyperammonemia is associated with generalized convulsion. *Intern Med* 47:21–23.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Factors influencing the ammonia level in Groups II and III on multiple regression analysis.

Figure S1. Relation between VPA dose and plasma ammonia level in patients on VPA monotherapy.

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Original article

Immunomodulatory therapy versus surgery for Rasmussen syndrome in early childhood ☆

Yukitoshi Takahashi [a,b,*], Etsuko Yamazaki [a], Jun Mine [a], Yuko Kubota [a], Katsumi Imai [a], Yuki Mogami [a], Koichi Baba [a], Kazumi Matsuda [a], Hirokazu Oguni [c], Kenji Sugai [d], Yoko Ohtsuka [e], Tateki Fujiwara [a], Yushi Inoue [a]

[a] *National Epilepsy Center, Shizuoka Institute of Epilepsy and Neurological Disorder, Shizuoka, Japan*
[b] *Department of Pediatrics, Gifu University School of Medicine, Japan*
[c] *Department of Pediatrics, Tokyo Women's Medical University, Japan*
[d] *Department of Child Neurology, National Center of Neurology and Psychiatry, Japan*
[e] *Department of Pediatric Neurology, Okayama University School of Medicine, Japan*

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Abstract

We examined seizure, cognitive, and motor outcomes in patients with Rasmussen syndrome or Rasmussen encephalitis (RS), after recent initiation of immunomodulatory therapies. Among 53 patients with a diagnosis of RS referred from all over Japan, 49 patients (male 22, female 27) with symptoms and findings characteristic of RS were evaluated. Regular intravenous immunoglobulin (IVIg) therapy was administered at a dose of 100 mg/kg/day, etc. Regular steroid pulse therapy was conducted with methylprednisolone at a dose of 30 mg/kg/day (children) or 1000 mg/day (adults) for 3 days. Tacrolimus was given at an initial dose of 0.1 mg/kg/day (children). Mean onset age was 8.7 ± 10.5 years. Seizure-free rate was 71% after treatment by functional hemispherectomy (FH), and response rate for seizures was 81% by regular steroid pulse therapy, 42% by tacrolimus therapy, and 23% by regular IVIg therapy. Rate of patients with IQ higher than 80 (R80) was 50% by regular steroid pulse therapy, 43% by regular IVIg therapy, 29% by tacrolimus therapy, and 0% by FH. R80 after regular steroid pulse therapy was 100% in patients without MRI lesions, and 37% in those with advanced MRI lesions. Improvement of motor function (paresis) was observed only by immunomodulatory therapy. Motor function was aggravated in 100% of patients treated by FH, 62% by regular IVIg, and 10% by regular steroid pulse therapy. We suggest a new treatment strategy for RS using early immunomodulatory therapy: initiation of regular steroid pulse therapy after early diagnosis indicated by biomarkers, then switching to tacrolimus therapy after several months.

Keywords: Rasmussen syndrome; Steroid-pulse therapy; IVIG therapy; Tacrolimus; Functional hemispherectomy; Seizure outcome; Cognitive outcome; Motor outcome

1. Introduction

Rasmussen syndrome or Rasmussen encephalitis (RS) is a slowly progressive, autoimmune chronic inflammatory disease of the central nervous systems [1–3]. Preceding infection occurring around two weeks before onset is observed in 38% of patients [3]. Histological examination usually shows inflammatory lesions with T cell infiltration. Cytotoxic T cells (CTLs) contrib-

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* Corresponding author. Address: National Epilepsy Center, Shizuoka Institute of Epilepsy and Neurological Disorders, 886 Urushiyama, Aoi-ku, Shizuoka 420-8688, Japan. Tel.: +81 54 245 5446; fax: +81 54 247 9781.
E-mail address: takahashi-ped@umin.ac.jp (Y. Takahashi).

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ute to the immunopathology of RS [4]. The IFNγ, IL-12, and granzyme B levels in CSF are elevated suggesting immunological involvement, especially in the early stage of the disease [5].

In RS, the initial symptom is usually intractable partial seizures, and epilepsia partialis continua (EPC) develops in 58.8% of the patients. Soon after the onset of RS, treatment with antiepileptic drugs (AEDs) is usually initiated because partial seizures are predominant symptoms. In a few years after onset, unihemispheric cortical dysfunctions (such as hemiplegia and cognitive deficit) become apparent [6,7]. RS is suspected when unilateral cortical deficit, unihemispheric EEG slowing, and unihemispheric cortical atrophy on MRI appear evolutionally. Before the availability of immunopathology, functional hemispherectomy (hemispherotomy) (FH) was the only treatment to achieve complete control of epileptic seizures. Therefore, in patients with involvement of the non-dominant hemisphere, FH is considered after the appearance of motor deficits. On the other hand, in those with disease involving the dominant hemisphere, immunomodulatory therapies using corticosteroids, intravenous immunoglobulin (IVIg), plasma pheresis (PEX) or immunoabsorption, and tacrolimus have been tried [2].

In considering treatment strategies for RS, comprehensive consideration of seizure outcome, neurological outcome, cognitive outcome, and motor outcome is necessary. In making a decision to undergo FH, the parents of patients with RS desire to achieve complete control of seizures and normal cognitive development at the sacrifice of hemiplegia.

With recent developments of many kinds of immunomodulatory therapies, we compared the treatment results of Japanese RS patients treated by surgery and/or immunomodulatory therapies, by evaluating their seizure, cognitive, and motor outcomes.

2. Methods

2.1. Patients

We identified 53 patients with a diagnosis of RS referred to the National Epilepsy Center from all over Japan between 1991 and 2010, and reviewed them basically according to the European diagnostic criteria for RS (Fig. 1) [2]. Of 53 patients, three patients who had no frequent partial seizures, and eight patients who had no unihemispheric cortical dysfunction were initially excluded from a diagnosis of RS. From the eight patients without unihemispheric cortical dysfunction, seven patients were subsequently diagnosed as having RS based on characteristic histology, elevated granzyme B in CSF, or high intensity lesion on MRI characteristic of RS [2,8]. RS was staged into three MRI categories: no lesion, high intensity lesion, and advanced MRI lesion.

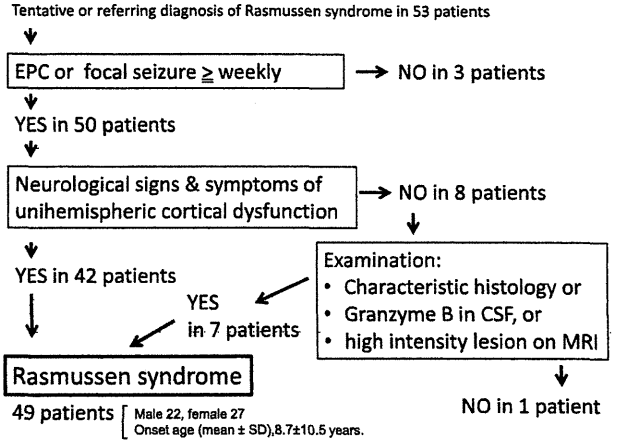

Fig. 1. Patient disposition. EPC, epilepsia partialis continua.

2.2. Evaluation

Seizure outcome was classified according to the change in seizure frequency before and after treatments into seizure-free (free), >50% seizure reduction (responder) (decreased), between <50% reduction and <50% increase (stable), >50% seizure increase (aggravated). In patients with EPC and solitary partial seizures, change in frequency of solitary partial seizures was evaluated. Cognitive outcome was measured by intelligence quotient (IQ) or developmental quotient (DQ). IQ was measured by Tanaka–Binet, WISCIII, and WAISIII, dependent on the age at examination. DQ was measured by MCC-baby test, KIDS-test, and other scales. We used full scale IQ (FSIQ) measured by WISC or WAIS for evaluation. Cognitive outcome was classified into FSIQ/DQ increase >10 (improved), between <10 increase and <10 decrease (stable), FSIQ/DQ decrease >10 (aggravated), and uncertain (uncertain). Rate of FSIQ/DQ preservation was calculated as number of patients (improved + stable)/number of patients (improved + stable + decreased). Motor outcome was classified into improved, stable and aggravated.

2.3. IVIg therapy

The protocol for regular IVIg therapy was a dose of either 100 mg/kg/day for several days, 400 mg/kg/day for several days, or 1 g/kg for one day, at an interval of once a month for several months to several years depending on response.

2.4. Steroid pulse therapy

The protocol for regular steroid pulse therapy with methylprednisolone was doses ranging from 30 mg/kg/day (for children) to 1000 mg/day (for adults) for 3 days, at an interval of once in a month for several months to several years depending on response. Only patients who had received more than 3 cycles were evaluated.