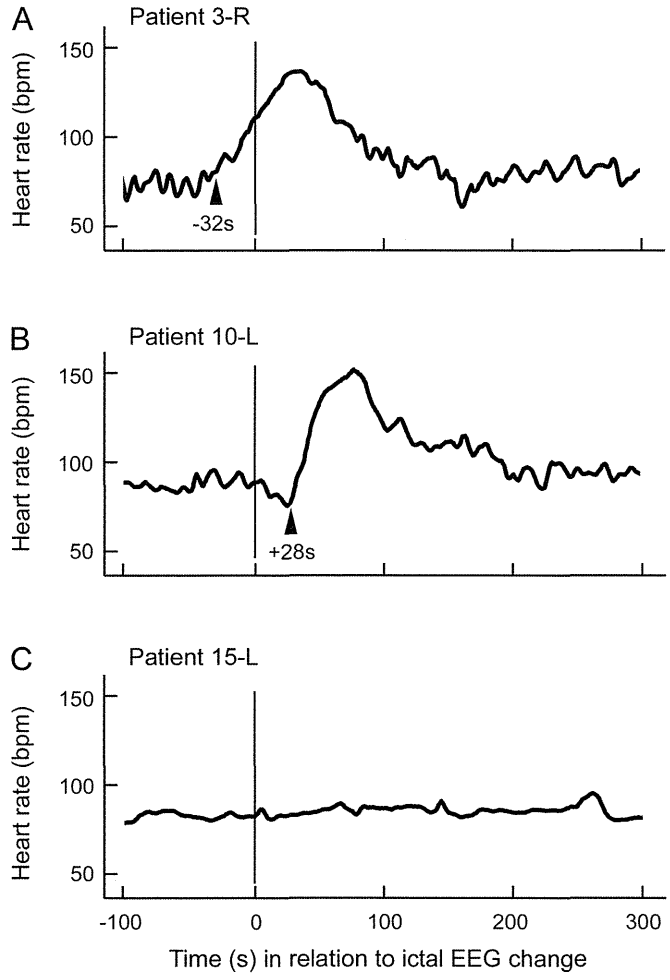
Figure 1 Typical examples of ictal HR change in relation to ictal EEG onset



(A) Right temporal seizure in patient 3-R. Heart rate (HR) started to increase 32 seconds before ictal EEG onset. (B) Left temporal seizure in patient 10-L. HR started to increase 28 seconds after ictal EEG onset. (C) Left temporal seizure in patient 15-L. No obvious HR changes were seen.

Maximum HR changes from baseline showed no significant difference between right and left seizures (47.5 ± 19.1 vs 40.8 ± 20.0/min; Student t test, $p = 0.8387$).

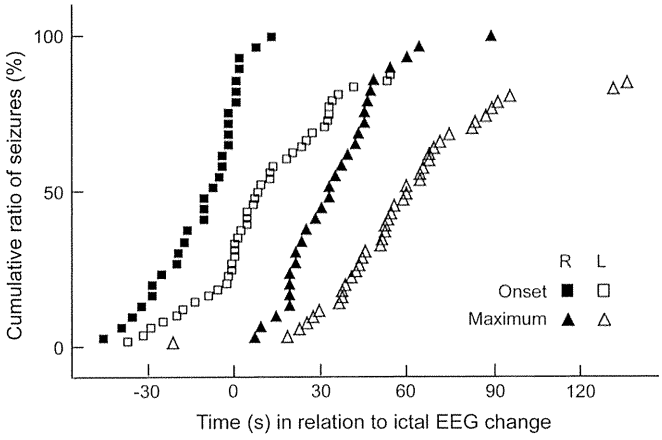
Table 2 shows the numbers of seizures with HR increase starting before and after ictal EEG onset in right and left mTLEs. HR increased before ictal EEG onset in 22 right mTLE seizures and increased after ictal EEG onset in 7 seizures. HR increased before ictal EEG onset in 11 left mTLE seizures and after in 31 seizures. Sensitivity and specificity of "HR increase before ictal EEG" were 75.9% and 73.8%, respectively, calculated as a lateralization sign of the right mTLE seizures. Positive and negative predictive values were 66.7% and 81.6%, respectively. Odds ratio was 8.857 (95% confidence interval [CI], 3.101–28.136; $p < 0.0001$).

DISCUSSION In the present study, our results revealed that HR increase was inevitable in right mTLE whereas not in left mTLE, and that onset time of HR increase was significantly earlier in right than left mTLE seizures. Laterality findings of the ictal tachycardia were more evident in the present study than any previous reports, probably because we strictly selected patients with mTLE who had an MRI lesion in the mesial temporal structures ipsilateral to ictal EEG onset. Another possibility is that our methodology to determine tachycardia and to define the onset time of HR increase may explain this disparity in the results.

Early tachycardia in right temporal seizures can be explained by sympathetic cardiovascular regulation activated by seizures. Hemispheric lateralization of autonomic cardiovascular control has been demonstrated by animal studies with electrical stimulation, extracellular recording, and lesion placement in the insular cortex,[13–15] as well as by human studies with electrical stimulation of the insular cortex, intracarotid amobarbital procedure, and clinical evaluation of patients with stroke.[16–19] These studies indicated that the right posterior insular cortex was critical to HR increase. The sympathetic network from the brain to the spine is mainly ipsilateral, as indicated by an anatomical study using retrograde tracing with viral strains,[20] and a physiologic study with microinjection of drugs to the dorsomedial hypothalamic nucleus that is important in coordinating the autonomic system.[21] Laterality of autonomic innervation to the heart is also well known,[22,23] which results from looping and rotation of the heart tube during cardiac development. The sympathetic fibers from the right thoracic cord innervate the anterior base of the heart, whereas those from the left thoracic cord innervate the apical and posterior portions of the heart. Previous studies showed that injection of β-adrenergic blocker abolished the tachycardia induced by stimulation of the insular cortex or dorsomedial hypothalamic nucleus,[15,24] implying the dominant effect of sympathetic activity. Ictal elevation of serum catecholamines may be one of the augmenting factors for ictal tachycardia. However, brainstem stimulation in cats caused no prominent elevation of serum catecholamine, at least within a few minutes.[25] For all the reasons above, we consider that a right temporal seizure results in early tachycardia through sympathetic activation transmitted from the right hemisphere to the heart base.

As discussed above, the left hemisphere has a lesser effect on cardiac sympathetic control than the right hemisphere. We consider that the delayed HR increase in the left temporal seizures observed in this study resulted from seizure propagation to the right temporal lobe. Several human studies demonstrated that interhemispheric propagation took approximately 20 seconds in temporal lobe epilepsy,[26,27]

Figure 2 Cumulative distribution of "onset time of HR increase" and "time of maximum HR"



Time axis is related to time of ictal EEG onset. Closed and open squares represent onset of ictal heart rate (HR) increase in right and left temporal seizures, respectively (mean ± SD, −11.5 ± 14.8 vs 9.2 ± 21.7 seconds; mean difference, 20.7 seconds; 95% confidence interval, 12.1–29.4 seconds; $p < 0.0001$). Closed and open triangles represent time of maximum HR in right and left temporal seizures, respectively (36.0 ± 18.1 vs 58.0 ± 28.7 seconds; mean difference, 22.1 seconds; 95% confidence interval, 10.9–33.3 seconds; $p < 0.0001$). The distribution of left temporal seizures (open markers) did not reach 100% because 6 left temporal seizures were accompanied by no obvious HR increase.

corresponding to our finding that the mean onset time of HR increase was 20.7 seconds later in left compared with right temporal seizures. The absence of a clear HR increase in 6 left seizures can be explained by either no seizure propagation to the right hemisphere, or inhibition of HR increase by parasympathetic activation in the left mTLE seizures. The present study observed no seizures with ictal bradycardia, which are known to be rare events.[1]

Timing of HR increase may be a valuable lateralizing sign of mTLE. The present study found high odds ratio and negative predictive value. This new lateralizing sign may be termed "preceding tachycardia sign" to ictal onset in scalp EEG. Further studies with bilateral intracranial electrodes are required to prove our hypothesis that abrupt tachycardia is a direct phenomenon of right temporal seizures, and delayed HR increase is a secondary phenomenon after left-to-right seizure propagation.

Table 2 Onset time of HR increase before and after onset time of EEG seizures

Onset of HR increase	R mTLE	L mTLE	Total
Before ictal EEG onset	22	11	33
After ictal EEG onset	7	31	38
No HR change	0	6	6
Total	29	48	77

Abbreviations: HR = heart rate; mTLE = mesial temporal lobe epilepsy.

AUTHOR CONTRIBUTIONS

K. Kato: undertook analysis, conceived and designed the study, and wrote the manuscript. K. Jin: conceived and designed the study and wrote the manuscript. H. Itabashi: contributed to writing of the manuscript. M. Iwasaki: contributed to the study design and writing of the manuscript. Y. Kakisaka: contributed to writing of the manuscript. M. Aoki: contributed to writing of the manuscript. N. Nakasato: conceived and designed the study and wrote the manuscript.

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REFERENCES

1. Sevcencu C, Struijk JJ. Autonomic alterations and cardiac changes in epilepsy. Epilepsia 2010;51:725–737.
2. Garcia M, D'Giano C, Estelles S. Ictal tachycardia: its discriminating potential between temporal and extratemporal seizure foci. Seizure 2001;10:415–419.
3. Leutmezer F, Schernthaner C, Lurger S, Pötzelberger K, Baumgartner C. Electrocardiographic changes at the onset of epileptic seizures. Epilepsia 2003;44:348–354.
4. Frysinger RC, Harper RM. Cardiac and respiratory correlations with unit discharge in human amygdala and hippocampus. Electroencephalogr Clin Neurophysiol 1989; 72:463–470.
5. Kirchner A, Pauli E, Hilz M, Neundorfer B, Stefan H. Sex differences and lateral asymmetry in heart rate modulation in patients with temporal lobe epilepsy. J Neurol Neurosurg Psychiatry 2002;73:73–75.
6. Mayer H, Benninger F, Urak L, Plattner B, Geldner J, Feucht M. EKG abnormalities in children and adolescents with symptomatic temporal lobe epilepsy. Neurology 2004;63:324–328.
7. Tinuper P, Bisulli F, Cerullo A, et al. Ictal bradycardia in partial epileptic seizures: autonomic investigation in three cases and literature review. Brain 2001;124: 2361–2371.
8. Di Gennaro G, Quarato PP, Sebastiano F, et al. Ictal heart rate increase precedes EEG discharge in drug-resistant mesial temporal lobe seizures. Clin Neurophysiol 2004; 115:1169–1177.
9. Britton JW, Ghearing GR, Benarroch EE, Cascino GD. The ictal bradycardia syndrome: localization and lateralization. Epilepsia 2006;47:737–744.
10. Nei M, Ho RT, Abou-Khalil BW, et al. EEG and ECG in sudden unexplained death in epilepsy. Epilepsia 2004;45: 338–345.
11. Rugg-Gunn FJ, Simister RJ, Squirrell M, Holdright DR, Duncan JS. Cardiac arrhythmias in focal epilepsy: a prospective long-term study. Lancet 2004;364:2212–2219.
12. van Elmpt WJ, Nijsen TM, Griep PA, Arends JB. A model of heart rate changes to detect seizures in severe epilepsy. Seizure 2006;15:366–375.

13. Zhang Z, Oppenheimer SM. Characterization, distribution and lateralization of baroreceptor-related neurons in the rat insular cortex. Brain Res 1997;760:243–250.
14. Zhang ZH, Rashba S, Oppenheimer SM. Insular cortex lesions alter baroreceptor sensitivity in the urethane-anesthetized rat. Brain Res 1998;813:73–81.
15. Oppenheimer SM, Cechetto DF. Cardiac chronotropic organization of the rat insular cortex. Brain Res 1990; 533:66–72.
16. Colivicchi F, Bassi A, Santini M, Caltagirone C. Cardiac autonomic derangement and arrhythmias in right-sided stroke with insular involvement. Stroke 2004;35:2094–2098.
17. Oppenheimer S, Gelb A, Girvin J, Hachinski V. Cardiovascular effects of human insular cortex stimulation. Neurology 1992;42:1727–1732.
18. Zamrini EY, Meador KJ, Loring DW, et al. Unilateral cerebral inactivation produces differential left/right heart rate responses. Neurology 1990;40:1408–1411.
19. Hilz MJ, Dütsch M, Perrine K, Nelson PK, Rauhut U, Devinsky O. Hemispheric influence on autonomic modulation and baroreflex sensitivity. Ann Neurol 2001;49: 575–584.
20. Farkas E, Jansen AS, Loewy AD. Periaqueductal gray matter input to cardiac-related sympathetic premotor neurons. Brain Res 1998;792:179–192.
21. Xavier CH, Nalivaiko E, Beig MI, et al. Functional asymmetry in the descending cardiovascular pathways from dorsomedial hypothalamic nucleus. Neuroscience 2009; 164:1360–1368.
22. Fujiki A, Masuda A, Inoue H. Effects of unilateral stellate ganglion block on the spectral characteristics of heart rate variability. Jpn Circ J 1999;63:854–858.
23. Yanowitz F, Preston JB, Abildskov JA. Functional distribution of right and left stellate innervation to the ventricles: production of neurogenic electrocardiographic changes by unilateral alteration of sympathetic tone. Circ Res 1966;18:416–428.
24. Fontes MA, Tagawa T, Polson JW, Cavanagh SJ, Dampney RA. Descending pathways mediating cardiovascular response from dorsomedial hypothalamic nucleus. Am J Physiol Heart Circ Physiol 2001;280:H2891–H2901.
25. Bereiter DA, Engeland WC, Gann DS. Peripheral venous catecholamines versus adrenal secretory rates after brain stem stimulation in cats. Am J Physiol 1986;251: E14–E20.
26. Gloor P, Salanova V, Olivier A, Quesney LF. The human dorsal hippocampal commissure: an anatomically identifiable and functional pathway. Brain 1993;116:1249–1273.
27. Weinand M, Serxner B, Labiner D, Ahern G. Interhemispheric propagation time and temporal lobe epileptogenicity. Pathophysiology 2009;16:39–42.

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Long-term follow-up of cortical hyperexcitability in Japanese Unverricht–Lundborg disease

Katsuya Kobayashi [a], Takefumi Hitomi [a,b,1], Riki Matsumoto [a,c], Takayuki Kondo [a], Jun Kawamata [a,2], Masao Matsuhashi [d], Shuji Hashimoto [e], Hitoshi Ikeda [f], Yasumichi Koide [f], Yushi Inoue [f], Ryosuke Takahashi [a], Akio Ikeda [a,c,*]

[a] Department of Neurology, Kyoto University Graduate School of Medicine, Japan
[b] Department of Respiratory Care and Sleep Control Medicine, Kyoto University Graduate School of Medicine, Japan
[c] Department of Epilepsy, Movement Disorders and Physiology, Kyoto University Graduate School of Medicine, Japan
[d] Human Brain Research Center, Kyoto University Graduate School of Medicine, Japan
[e] Department of Neurology, Tenri Hospital, Japan
[f] National Epilepsy Center, Shizuoka Institute of Epilepsy and Neurological Disorders, Japan

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ABSTRACT

Purpose: To delineate chronological changes of cortical hyperexcitability by long-term follow-up of the amplitudes of somatosensory evoked potentials (SEPs) in patients with Japanese Unverricht–Lundborg disease (ULD).
Method: SEPs to median nerve stimulation were repeatedly examined in 7 genetically diagnosed ULD patients with the mean interval of 11.9 years. The degree of temporal changes in the amplitude of 3 early cortical components, N20, P25 and N35, to the age was analyzed and compared with that of healthy subjects.
Results: Their clinical course was almost stable during the follow-up period, namely cessation of generalized tonic-clonic seizures and little or no progression of myoclonus. SEP amplitudes of P25 and N35 were enlarged in all patients and were gradually decreased with aging in ULD on average. The degree of temporal changes of P25 and N35 in ULD was similar or even lower than that of healthy subjects.
Conclusion: Enlarged but relatively stable SEP amplitudes had a consistency with so-called self-limited clinical course in Japanese ULD. SEP amplitude could be one of the surrogate markers of the degree of cortical hyperexcitability in ULD during the long-term follow-up period.

1. Introduction

Unverricht–Lundborg disease (ULD) is one of the progressive myoclonus epilepsies that is characterized by myoclonus, epileptic seizures and cerebellar ataxia, and nowadays is called progressive myoclonus epilepsy type 1 (EPM1).[1] In ULD patients, the somatosensory evoked potentials (SEPs) usually show enhanced cortical components after N20 (i.e., giants SEPs) as a marker of cortical hyperexcitability to the external stimuli.[2,3] Although the term "progressive" was assigned, it was reported that the European ULD patients showed self-limited progression in the later life.[4] By investigating neurophysiological correlates of this clinical course, we just recently reported 2 Japanese ULD patients who showed clinically no or little progressive course. Their bimodal waveforms of N35 in the previous SEPs became unimodal over the course of 16 years, and it suggests decreased cortico-cortical connectivity and/or cortical excitability.[5] We also reported the amplitudes of the early SEP components (N20, P25 and N35) were almost stable, although these components were already enlarged in the previous SEP. However, it is not certain whether waveform change of N35 is common or not because we studied only 2 patients. We could not evaluate the chronological changes in amplitudes of each cortical SEP component, either. Herewith we extensively evaluated 5 more genetically proven ULD patients.

Abbreviations: ULD, Unverricht–Lundborg disease; EPM1, progressive myoclonus epilepsy type 1; SEP, somatosensory evoked potential; GTCS, generalized tonic clonic seizure.

* Corresponding author at: Corresponding author. Department of Epilepsy, Movement Disorders and Physiology, Kyoto University Graduate School of Medicine, 54 Shogoin-Kawaharacho, Sakyo-ku, Kyoto 606-8507, Japan.
Tel.: +81 75 751 3772; fax: +81 75 751 9416.
E-mail address: akio@kuhp.kyoto-u.ac.jp (A. Ikeda).
[1] Present address: Department of Clinical Laboratory Medicine, Kyoto University Graduate School of Medicine, Japan.
[2] Present address: Department of Neurology, Sapporo Medical University School of Medicine, Japan.

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In this report, we further described the temporal changes in SEP amplitudes of a total of 7 Japanese ULD patients to delineate the chronological changes of responsive, cortical hyperexcitability. Only abstract is available for this study.[6]

2. Patients and methods

We recruited 7 Japanese patients with genetically diagnosed ULD (4 men and 3 women; mean age at the first SEP examination of 27.6 years, ranged from 17 to 42 years). During the follow-up period, we collected the changes in the frequency of generalized tonic clonic seizures (GTCSs), and the degree of myoclonus for each patient by using the simplified myoclonus rating scale as follows,[4] 0 = no myoclonus; 1 = minor myoclonus, no interference with daily living; 2 = mild myoclonus, interference with fine movements and/or speech, no interference with walking; 3 = moderate myoclonus, patient still able to walk without support; 4 = moderate to severe myoclonus, patient able to stand, unable to walk without support; 5 = severe myoclonus, patient wheelchair-bound or bedridden.

The SEPs were not recorded with completely the same equipment and condition, since the patients belonged to several institutes and the repeated examination were done with the mean interval of 11.9 years (ranged from 4 to 16 years). Recording conditions were quite consistent at least within each patient. Furthermore, as compared with previous study,[5] the overall recording condition was essentially consistent except for stimulus frequency in several patients as follows. The median nerve was stimulated at the wrist at a fixed rate of 1.1–3.0 Hz (1.1 Hz in 3 patients and 2.0 Hz in 4 for the previous recording; 1.1 Hz in 5 and 3.0 in 2 for the present recording) and the stimulus intensity was adjusted to produce a clear twitch of the thenar muscle. Short latency SEPs were recorded from C3/C4 according to the International 10–20 System or CP3/CP4 by 10-10 System of the American EEG Society. The electrode impedance was kept below 5 kΩ. The ipsilateral earlobe to the stimulated hand (A1 or A2) or the linked earlobes (A1 + A2) were used as the reference. The bandpass filter was within the range of 0.5–3000 Hz. At least 200 responses were averaged.

We adopted 3 components of N20, P25 and N35. Amplitudes of P25 and N35 were measured from the preceding opposite peak and those of N20 were measured from the baseline. An SEP was judged as "giant" when P25 was more than 6.3 μV or N35 was more than 9.8 μV.[7] We also compared the amplitudes of the 3 components of ULD patients in the previous and present states with those of healthy subjects ($N = 19$; 11 men and 8 women; mean age at the SEP examination of 49 years, ranged from 22 to 74 years) by Mann–Whitney U test. A tangential component of N30–P30 was not adopted in this study, since we could not detect the reliable N30–P30 from shortage of the number of the recorded electrodes.

In order to evaluate the chronological changes in SEP amplitude in ULD, we compared the early cortical components of SEPs between previous and present states by Wilcoxon signed-rank test. In addition, we analyzed the relationship between the age at SEP recording and its amplitudes. Namely we calculated the averaged value of the ages and amplitudes of 7 patients both at previous and present SEP recording, and then extrapolated the chronological changes. Since SEP amplitude increased with aging even in healthy subjects,[8,9] we compared the gradient of ULD patients with those of 19 healthy subjects as reported previously.[9]

3. Results

As for the clinical symptoms, all the patients showed a cessation of GTCSs and a stable or little worsened myoclonus. The medications at the previous and present SEP recording are shown in Table 1. During the follow-up period, the dosage of the medications did not show clear tendency. Namely, medication was increased in 4 patients (Patient 3, 4, 5, and 6) whereas it was decreased in 3 patients (Patient 1, 2, and 7). The averaged simplified myoclonus rating scale of 7 patients indicated almost stable but moderately severe, 3.4 and 3.9 at the previous and present recording, respectively.

In the previous recording, N20, P25 and N35 amplitudes (mean ± S.D.) in 7 ULD patients were 0.9 ± 0.9 μV, 10.5 ± 6.4 μV and 12.9 ± 7.0 μV, and those in the present evaluation were 2.3 ± 1.6 μV, 9.9 ± 6.1 μV and 10.8 ± 4.0 μV, respectively (Table 2). Both in the previous and present recording, the amplitudes of N20 in ULD were

Table 1
Medications at the previous and present SEP recording in 7 patients (mg/day).

	Previous		Present	
	Age at SEP		Age at SEP	
Patient 1	42	VPA 1,400, CZP 4.5, PB 120, Piracetam 15,000	58	VPA 700, CZP 6, PB 60, Piracetam 6,000
Patient 2	32	VPA 3,600, CZP 12, PHT 300, CBZ 400	48	VPA 2,000, CZP 8, ZNS 300, DZP 5
Patient 3	24	VPA 1,100, CZP 6, ZNS 200	34	VPA 1,800, CZP 6, ZNS 200, Piracetam 33,000
Patient 4	31	VPA 1,200, PB 90, ZNS 200	42	VPA 1,200, CZP 6, PB 60, ZNS 200, Piracetam 27,000
Patient 5	17	VPA 1,200, CZP 1.3, PB 40, PRM 400	34	VPA 1,800, CZP 4, ZNS 600, PRM 300, AZM 250
Patient 6	24	VPA 800, CBZ 200	33	VPA 1,400, PB 50, ZNS 200
Patient 7	23	VPA 800, CZP 2, Piracetam 12,000	27	VPA 600, CZP 2, Piracetam 12,000

VPA: valproic acid, CZP: clonazepam, PB: phenobarbital, PHT: phenytoin, CBZ: carbamazepine, ZNS: zonisamide, DZP: diazepam, PRM: primidone, AZM: acetazolamide.

Table 2
Latency and amplitude of cortical components of SEPs in 7 patients, latency (ms)/amplitude (μV).

	Previous				Present			
	Age at SEP	N20	P25	N35	Age at SEP	N20	P25	N35
Patient 1	42	20.6/1.3	26.6/6.6*	30.9/10.3*	58	20.1/2.5	25.4/9.5*	30.8/10.9*
Patient 2	32	19.2/0.2	24.8/5.5	31.5/11.8*	48	20.7/0.8	24.9/4.7	32.3/13.0*
Patient 3	24	23.7/0.0	29.1/5.2	37.9/8.3	34	20.6/2.0	25.3/6.0	31.4/10.0*
Patient 4	31	23.8/0.4	32.0/17.4*	38.7/19.7*	42	20.9/5.1	26.6/22.4*	33.1/18.2*
Patient 5	17	20.0/0.2	26.0/21.0*	32.6/24.8*	34	20.3/1.1	25.8/6.6*	31.0/5.6
Patient 6	24	20.4/2.3	26.6/5.8	34.9/4.7	33	20.5/1.3	27.4/7.8*	35.3/7.6
Patient 7	23	14.2/1.8	20.2/11.9*	31.8/10.4*	27	18.5/3.6	24.0/12.3*	32.7/10.3*
Average ± S.D.	27.6	20.3 ± 3.2/0.9 ± 0.9	26.5 ± 3.6/10.5 ± 6.4	34.0 ± 3.2/12.9 ± 7.0	39.4	20.2 ± 0.8/2.3 ± 1.6	25.6 ± 1.1/9.9 ± 6.1	32.4 ± 1.5/10.8 ± 4.0

* Giant SEP: P25 > 6.3 or N35 > 9.8 μV [7]

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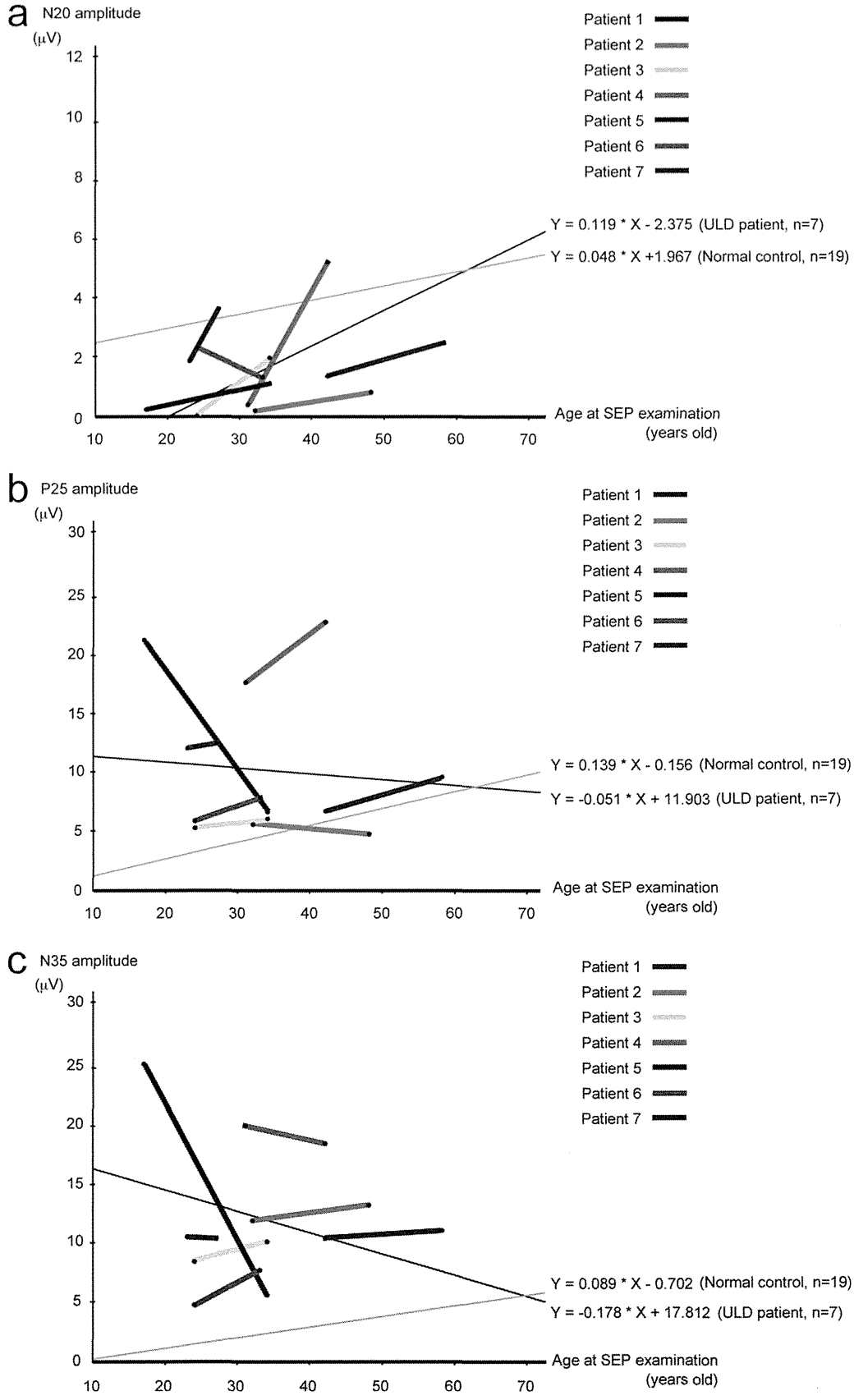

Fig. 1. Correlation between the age at SEP recording and its amplitudes in 7 ULD patients. Values of N20 (A), P25 (B) and N35 (C) (different colored lines) and the gradients of their temporal changes drawn by calculating the averaged value of the ages and amplitudes between previous and present SEP recordings (black lines) in 7 patients are compared with linear regression curves of 19 healthy subjects (gray lines). N20 amplitudes were much smaller and P25 and N35 amplitudes larger in ULD patients. As for the components associated with giant SEP (i.e., P25 and N35), 7 ULD patients showed a tendency of slight decrease in amplitude during the follow-up period, and the degree of its tendency was even lower than that of healthy subjects. (For interpretation of reference to color in this figure legend, the reader is referred to the web version of this article.)

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lower than those of healthy subjects ($p < 0.05$). On the other hand, the amplitudes of P25 and N35 in ULD were higher than those of healthy subjects and only N35 amplitudes reached statistical significance ($p < 0.05$). All 7 patients fulfilled the criteria of the giant SEPs at least once during their clinical course.

The amplitudes of P25 and N35 in some of the ULD patients tended to increase during the follow-up period, but no significant difference in amplitude was found between previous and present data by Wilcoxon signed-rank test. Whereas, only 1 patient (Patient 5) showed relatively drastic decrease in SEP amplitude for P25 and N35 (Fig. 1(b) and (c)). In total, the gradients of temporal changes of N20, P25 and N35 amplitudes for age in the 7 ULD patients were 0.119, −0.051, and −0.178, respectively. As compared with healthy subjects (N20: 0.048, P25: 0.139, N35: 0.089), that of P25 and N35, were even lower than those of healthy subjects (Fig. 1). Namely, enhanced amplitude of P25 and N35 reflects the responsive, cortical hyperexcitability across all the 7 patients, but the degree of its gradient in temporal change for aging is not increased at all. When 1 patient (Patient 5) was excluded because she showed dramatically decrease in amplitudes, the gradients of temporal changes of N20, P25 and N35 amplitudes for age in the 6 ULD patients were quite similar to those of aging in healthy subjects, i.e., 0.141, 0.156, and 0.073, respectively (not shown in the figure).

The waveform change of N35 from bimodal to unimodal over the course was observed only in 2 out of 7 patients, both of whom we reported in the previous report.[5]

4. Discussion

We demonstrated that SEP amplitudes were enlarged or giant in genetically diagnosed ULD patients throughout the follow-up period, that was quite consistent with previous reports.

We also showed that once enlarged SEP amplitude of P25 and N35 gradually decreased on average with aging in ULD. Indeed, 5 patients for P25 and 4 patients for N35 demonstrated that enlarged SEP amplitude gradually increased with aging, but the degree of increment was quite comparable to that of aging in healthy subjects (Fig. 1). Namely, most likely we were able to demonstrate that responsive cortical hyperexcitability was not progressive for aging but relatively stable in the primary sensori-motor cortices in Japanese ULD. These electrophysiological findings in quite parallel with clinical course in our patients were quite consistent with self-limited clinical course as reported previously.[4] Therefore, SEP amplitude could be one of the surrogate markers of the degree of cortical excitability and at least a part of pathophysiology in ULD during the long-term follow-up period. However, it is unclear that self-limited progression was observed in all Japanese ULD patients, since one of the patients (Patient 5), who showed a little worsened myoclonus, showed relatively drastic decrease in SEP amplitude. It might be explained by the paradoxical association between giant SEP and the degree of myoclonus, which was clearly reported in patients with cortical myoclonus previously.[10]

In addition, some ULD patients in other reports with genetically diagnosed show continuous progression in terms of clinical symptoms as well as electrophysiological results.[11] In our previous report we mentioned the waveform change of N35 from bimodal to unimodal could reflect a lesser degree of cortico-cortical connectivity and/or decreased cortical hyperexcitability in ULD patients. The additionally recruited 5 patients did not show similar waveform change of N35 component, which suggests not only this waveform change but also chronological changes in SEP amplitudes contribute to self-limited progression.

Further case accumulation showing variable clinical course is indispensable to further clarify the clinico-electorophysiological correlates and to solve these clinical questions in patients with ULD. The current long-term observation in this study started mostly after age 20 years in 6 out of 7 patients, but it was done until before 50 years in 6 out of 7 patients. Therefore, it may not be completely excluded that ULD patients in much older age more than 50 years are actually so stable. Thus, it is important to continue the evaluation of our patients to see if changes in SEP can manifest in older patients.

There are several limitations in this study. Firstly, the change of SEP amplitude over a decade might be due to chronic exposure to antiepileptic or antimyoclonic drugs. In general, most antiepileptic drugs seem to decrease the SEP amplitude.[12] The dosages of these drugs were not completely the same during the follow-up period as well. Secondly, as mentioned in Section 2, the recording conditions were not completely the same among studied patients and within each patient. However, except stimulus rate, we consider that the influence of the different recording conditions on the SEP amplitude was quite small. With regard to the stimulus rate, in the previous reports, the amplitude of P25 decreased and N35 was not significantly altered once the stimulus rate gradually increased from 1.0 to 5.0 Hz.[13,14] In our study, the stimulus rate increased from 2.0 Hz to 3.0 Hz in 2 patients (Patients 5 and 6), and that decreased from 2.0 Hz to 1.1 Hz in the other 2 patients (Patient 3 and 4) between previous and present recordings. The change of amplitude, such as decreased amplitude of P25 only in Patient 5, might be partly influenced by the change of stimulus rate. Despite these limitations, our study has some value in evaluating long-term clinico-electorophysiological changes in Japanese ULD.

5. Conclusions

We demonstrated that SEP amplitudes were enlarged or giant in genetically diagnosed ULD patients throughout the follow-up period. We also showed that once enlarged SEP amplitude of P25 and N35 gradually decreased with aging in ULD. Enlarged but relatively stable or gradually decreased SEP amplitudes had a consistency with so-called self-limited clinical course in ULD. SEP amplitude could be one of the surrogate markers of the degree of cortical hyperexcitability in ULD during long-term follow-up period.

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References

1. Kälviäinen R, Khyuppenen J, Koskenkorva P, Eriksson K, Vanninen R, Marvaala E. Clinical picture of EPM1 – Unverricht–Lundborg disease. *Epilepsia* 2008;**49**: 549–56.
2. Shibasaki H, Yamashita Y, Neshige R, Tobimatsu S, Fukui R. Pathogenesis of giant somatosensory evoked potentials in progressive myoclonic epilepsy. *Brain* 1985;**108**:225–40.
3. Canafoglia L, Ciano C, Panzica F, Scaioli V, Zucca C, Agazzi P, et al. Sensorimotor cortex excitability in Unverricht–Lundborg disease and Lafora body disease. *Neurology* 2004;**63**:2309–15.
4. Magaudda A, Ferlazzo E, Nguyen VH, Genton P. Unverricht–Lundborg disease, a condition with self-limited progression: long-term follow-up of 20 patients. *Epilepsia* 2006;**47**:860–6.

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5. Kobayashi K, Matsumoto R, Kondo T, Kawamata J, Hitomi T, Inouchi M, et al. Decreased cortical excitability in Unverricht–Lundborg disease in the long-term follow-up: a consecutive SEP study. *Clin Neurophysiol* 2011;**122**: 1617–21.
6. Kobayashi K, Hitomi T, Matsumoto R, Kondo T, Kawamata J, Takahashi R, et al. Giant somatosensory evoked potentials in patients with Unverricht–Lundborg disease. *J Jpn Epilep Soc* 2010;**28**(304 (abstract in Japanese).
7. Ikeda A, Shibasaki H, Nagamine T, Xu X, Terada K, Mima T, et al. Peri-rolandic and fronto-parietal components of scalp-recorded giant SEPs in cortical myoclonus. *Electroencephalogr Clin Neurophysiol* 1995;**96**:300–9.
8. Lüders H. The effects of aging on the wave form of the somatosensory cortical evoked potential. *Electroencephalogr Clin Neurophysiol* 1970;**29**:450–60.
9. Hitomi T, Ikeda A, Kondo T, Imamura H, Inouchi M, Matsumoto R, et al. Increased cortical hyperexcitability and exaggerated myoclonus with aging in benign adult familial myoclonus epilepsy. *Mov Disord* 2011;**26**:1509–14.
10. Rothwell JC, Obeso JA, Marsden CD. On the significance of giant somatosensory evoked potentials in cortical myoclonus. *J Neurol Neurosurg Psychiatry* 1984;**47**:33–42.
11. Chew NK, Mir P, Edwards MJ, Cordivari C, Martino D, Schneider SA, et al. The natural history of Unverricht–Lundborg disease: a report of eight genetically proven cases. *Mov Disord* 2008;**23**:107–13.
12. Erdem H, Yigit A, Culcuoglu A, Mutluer N. Effect of sodium valproate on somatosensory evoked potentials in juvenile myoclonic epilepsy. *Ups J Med Sci* 2001;**106**:197–203.
13. Huttunen J, Homberg V. Influence of stimulus repetition rate on cortical somatosensory potentials evoked by median nerve stimulation: implications for generation mechanisms. *J Neurol Sci* 1991;**105**:37–43.
14. Garcia Larrea L, Bastuji H, Mauguiere F. Unmasking of cortical SEP components by changes in stimulus rate: a topographic study. *Electroencephalogr Clin Neurophysiol* 1992;**84**:71–83.

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Original article

Nationwide survey of glucose transporter-1 deficiency syndrome (GLUT-1DS) in Japan

Yasushi Ito [a], Satoru Takahashi [b], Kuriko Kagitani-Shimono [c], Jun Natsume [d], Keiko Yanagihara [e], Tatsuya Fujii [f], Hirokazu Oguni [a,*]

[a] *Department of Pediatrics, Tokyo Women's Medical University, Japan*
[b] *Department of Pediatrics, Asahikawa Medical University, Japan*
[c] *United Graduate School of Child Development, Osaka University Graduate School of Medicine, Japan*
[d] *Department of Pediatrics, Nagoya University, Japan*
[e] *Section of Pediatric Neurology, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan*
[f] *Shiga Medical Center for Children, Shiga, Japan*

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Abstract

Objectives: We conducted a nationwide survey of glucose transporter type-1 deficiency syndrome (GLUT-1DS) in Japan in order to clarify its incidence as well as clinical and laboratory information.

Subjects and methods: A questionnaire to survey the number of genetically and clinically confirmed cases of GLUT-1DS was sent to 1018 board-certified pediatric neurologists, which resulted in 57 patients being reported. We obtained the clinical and laboratory data of 33 patients through a secondary questionnaire.

Results: The age of the 33 patients (male: 15, female: 18) at the time of the study ranged between 3 and 35 years (mean: 13.5 years). The age of these patients at the onset of initial neurological symptoms ranged between the neonatal period and 48 months (mean: 9.4 months). GLUT-1DS was diagnosed at a mean age of 8.4 years (range: 1 year to 33 years). The initial symptom was convulsive seizures, which occurred in 15 cases, and was followed by abnormal eye movements in 7 cases and apneic or cyanotic attacks in 4 cases. The latter two symptoms most frequently occurred early in infancy. Thirty-two patients (97%) exhibited some type of epileptic seizure. Neurological findings revealed that most patients had muscle hypotonia, cerebellar ataxia, dystonia, and spastic paralysis. Mild to severe mental retardation was detected in all 33 cases. Furthermore, paroxysmal episodes of ataxia, dystonia/dyskinesia, and motor paralysis were described in approximately 1/3 of all patients. The factors that frequently aggravated these events were hunger, exercise, fever, and fatigue, in that order. The mean CSF/blood glucose ratio was 0.36 (0.28–0.48). Pathological mutations in the *SLC2A1* gene were identified in 28 out of 32 cases (87.5%).

Conclusion: The results described herein provided an insight into the early diagnosis of GLUT1-DS, including unexplained paroxysmal abnormal eye movements, apneic/cyanotic attacks, and convulsive seizures in infancy, as well as uncommon paroxysmal events (ataxia, atonia, and motor paralysis) in childhood.

Keywords: Glucose transporter type-1; Hypoglycorrhachia; Epilepsy; Movement disorders; Ketogenic diet

* Corresponding author at: Department of Pediatrics, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 162-8666, Japan. Tel.: +81 3 3353 8111; fax: +81 3 5269 7338.
E-mail address: hoguni@ped.twmu.ac.jp (H. Oguni).

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1. Introduction

Glucose transporter-1 deficiency syndrome (GLUT-1DS, OMIM 606777) is a metabolic disorder in the brain that results from the central nervous system being unable to effectively utilize glucose, which is the main substrate providing energy to the brain under physiological conditions [1–3]. In 1991, De Vivo et al. first reported two patients with the disorder who presented with infantile seizures, developmental delays, acquired microcephaly, and unexplained hypoglycorrhachia [4]. Based on the findings of studies published to date, typical cases exhibit the onset of paroxysmal eye movements and epileptic seizures in early infancy and later present with symptoms such as developmental delays, hypotonia, spastic paralysis, cerebellar ataxia, and dystonia [1–5]. Most patients with GLUT-1DS have been sporadic in occurrence; however, some familial cases of GLUT-1DS with autosomal-dominant inheritance and less often with autosomal-recessive inheritance have also been identified [6,7]. Heterozygous *de novo* mutations in the *SLC2A1* gene (gene locus 1p35-31.3) were previously identified to be a causative gene and have been reported in a large number of cases [8,9]. Although patients with missense mutations often exhibit mild symptoms (especially mental retardation), a clear genotype-phenotype relationship has not yet been established. *SLC2A1* gene mutations have also recently been identified in some patients with paroxysmal exercise-induced dyskinesia, early-onset absence epilepsy, and myoclonic-astatic epilepsy, and GLUT-1DS is now considered to have a wide spectrum of phenotypes [3,10–16]. Approximately 200 cases of GLUT-1DS have been reported to date, mainly in the US and Europe [17]. There have also been sporadic reports in Japan since 1991 [18–21]. Although epileptic seizures in GLUT-1DS patients are often refractory to antiepileptic drug treatments, ketogenic diet therapy (KD) is an effective and causal therapeutic method that can supply ketone bodies instead of glucose as a source of brain energy [22–24]. If KD can be started early, a chronic state of glucose deficiency in the brain causing impairment of CNS function can be prevented. Brain development is especially pronounced during infancy and early childhood, and the treatable nature of this disease should not be overlooked. Therefore, guidelines need to be established for the early diagnosis and treatment of this disorder. In the present study, we conducted a nationwide survey of this disease in order to clarify the number of GLUT-1DS patients in Japan and their clinical and laboratory information.

2. Subjects and methods

Genetically confirmed as well as clinically diagnosed patients with GLUT-1DS who were treated at medical facilities throughout Japan participated in this study. Clinically diagnosed GLUT-1DS patients were defined as those in whom hypoglycorrhachia was present in association with typical neurological symptoms, but a genetic diagnosis or erythrocyte glucose uptake assay either had not been performed or was negative. In this study, hypoglycorrhachia was defined as the ratio of CSF glucose vs. blood glucose concentration sampling being simultaneously lower than 0.45 in spite of the absence of meningitis [17]. Typical neurological symptoms were described elsewhere [2,5,9,17]. A primary questionnaire to survey the actual number of genetically and clinically confirmed cases of GLUT-1DS was initially sent to 1018 board-certified pediatric neurologists of the Japanese Society of Child Neurology who were working at university hospitals, children's hospitals, national sanitariums, and other relevant institutes. After the primary questionnaire was returned, a secondary questionnaire was sent to the physicians who had patients and who agreed to participate in the secondary questionnaire. The clinical data collected from the secondary questionnaire included perinatal history, age at the onset of seizures, types of seizures, neurological complications, electroencephalogram (EEG) findings, relationship between diet and seizures, eating habits, biochemical findings, genetic diagnosis, brain imaging diagnosis, developmental assessment, and treatment. The following statistical analysis was also performed; mental outcomes at the last follow-up in relation to the onset age of the first symptoms as well as CSF/blood glucose ratio, and types of *SLC2A* mutations in relation to the onset age of the first symptoms as well as CSF/blood glucose ratio and mental outcomes. Mental outcomes were subclassified into borderline to mild ($80 > IQ \geqslant 55$), moderate ($55 > IQ \geqslant 35$), and severe ($IQ < 35$) retardation. When IQ was difficult to estimate, developmental quotient (DQ) was alternatively used to assess the mental outcome.

Mutation screenings were performed by the direct sequencing of PCR fragments spanning the entire coding region and exon–intron boundaries of *SCL2A1*. If direct sequencing yielded normal results, large rearrangements of *SCL2A1* were examined using the Multiple Ligation Probe Amplification (MLPA) method. The details of these methods were described elsewhere [20,21].

This study was conducted with the approval of the Ethics Committee of Tokyo Women's Medical University with which the principle investigators were affiliated and the Ethics Committees of the facilities with which the subinvestigators were affiliated.

2.1. Statistical analyses

The chi-squared test, *t*-test, and one-way ANOVA were employed to compare results between two or three

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variables. A P value of <0.05 was regarded as significant.

3. Results

3.1. Patient demographic data

Responses to the primary questionnaire were received from 499 pediatric neurologists. Of these, 28 pediatric neurologists had a total of 57 patients with GLUT-1DS. Conversely, 471 physicians responded that they had no experience with GLUT-1DS. All 28 physicians agreed to participate in the study and were sent the secondary questionnaire. Detailed responses to the questionnaire survey were received for 33 of the 57 patients and subsequently submitted for analysis. Two families comprising 4 patients were included.

The age of the 33 patients at the time of the study ranged between 3 and 35 years with a mean age of 13.5 years (Table 1). Four patients were 20 years or older. The age at the onset of the initial neurological symptoms ranged between the neonatal period and 48 months after birth, with a mean age of 9.4 months. The age at which GLUT-1DS was diagnosed was a mean age of 8.4 years, ranging between 1 year 0 months and 33 years. Fifteen subjects were male (45%), and 18 were female (55%). Their mean ages of the onset of neurological symptoms were 9.1 months and 9.6 months, respectively ($P < 0.05$). The gestational age during pregnancy was a mean of 38.5 weeks, ranging between 33 and 40 weeks. The average weight at birth was 2940 g. The mean head circumference at birth, which was recorded in 18 cases, was 32.8 cm.

3.2. Clinical analysis of first symptoms and epileptic seizures

The most frequently reported initial symptom was a convulsive seizure, which occurred in 15 cases, and was followed by abnormal eye movements in 7 cases, apneic/cyanotic attacks in 4 cases, developmental delays in 3 cases, and atonic episodes with poor response/eye-rolling-up in 3 cases. Abnormal eye movements were observed in a total of 12 cases, including 5 cases who manifested these movements following other neurological symptoms, but were all observed in infancy. Abnormal eye movements were described as opsoclonus in 6 cases and (rotatory) nystagmus in the remaining 6 cases, with the mean age of onset being 6.1 months. Apneic/cyanotic attacks were reported in 5 cases during the infant period, including one case in which an apneic attack appeared following other symptoms. Since it could not be determined whether apneic/cyanotic attacks were epileptic or nonepileptic in origin, they were distinguished from epileptic seizures in this study.

Thirty-two patients (97%) exhibited some type of epileptic seizure. The seizure type could be classified in 27 out of 32 cases. The epileptic seizure types observed in infancy aged between 0 and 2 years were generalized tonic–clonic seizures (GTCS) in 10 cases, partial seizures in 6 cases, astatic seizures in 3 cases, myoclonic seizures in 2 cases, and febrile seizures in 1 case (Table 1). The epileptic seizure types noted in early childhood aged between 2 years and 6 years ($n = 26$) were GTCS in 9 cases, partial seizures in 7 cases, absence seizures in 6 cases, astatic seizures in 5 cases, and myoclonic, febrile, and autonomic seizures (ictal vomiting) in 2 cases each. In later childhood aged older than 7 years ($n = 15$), absence seizures were reported in 7 cases, astatic seizures, partial seizures, and GTCS in 4 cases each, and febrile and autonomic seizures in 1 case each. The most frequent epileptic seizure type observed in adolescence and thereafter was absence seizures, which were noted in 4 patients. Of these, the epileptic seizure types were confirmed by video-EEG studies only in 6 patients, 5 of whom had absence seizures and 1 of whom had atonic seizures associated with a diffuse spike-and-wave complex.

3.3. Neurological abnormalities other than epileptic seizures

Neurological findings revealed muscle hypotonia and cerebellar ataxia in 59.4% and 90.9% of patients, respectively, at the final examination. Spastic paralysis of various degrees was also reported in 63.6% of all cases, with the frequency of diplegia, paraplegia, and quadriplegia increasing in that order. Otherwise, dystonia was observed in 45.5% of patients, with other involuntary movements being noted in 6.1%. Dysarthria was present in 63.6% of cases, and sensory disturbance was seen in 18.2%. However, no dysphagia or brainstem disorder was identified. Acquired microcephaly and a short stature were observed in 30.3% and 39.4% of cases, respectively.

Mild to severe mental retardation was recognized in all 33 cases at the last follow-up period (borderline to mild:12, moderate:9, severe:12). No significant differences were observed in the age at the onset of neurological symptoms or CSF/blood glucose ratio ($P > 0.05$) among those with mild, moderate and severe retardation, whereas the age at the onset of neurological symptoms was youngest in those with severe mental retardation (Table 2). Learning disabilities and attention deficit hyperactive disorders were each observed in 24.2% of patients at some time during the clinical course. However, no autistic disorders were detected. Patients had relatively social and friendly personalities.

Regarding paroxysmal symptoms other than epileptic seizures, paroxysmal ataxia, paroxysmal dyskinesia/dystonia, and paroxysmal motor paralysis (hemiplegia/

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Table 1
Clinical, genetic, and laboratory data of the 33 patients.

Pt No	Gender	Age at the time of the study (years/months)	Mutation Nucleotide change (amino acid change)	Type of mutation	Age at onset (months)	Initial symptoms	Seizure type (age at the initial seizure, years/months)	Age at diagnosis (years/months)	Neurological sign						Mental retardation	Interictal EEG findings	Cyclic vomiting	Microcephalus	Short stature
									CSF/blood glucose ratio	Ataxia	Dystonia	Hypotonia	Pyramidal sign	Paroxysmal episodes (complex movement disorders)					
1*	M	9/8	c.971C > T (p.Ser324Leu)	Missense	12	Developmental delay	PS (2/10)	7/5	0.37	Y	N	N	Mild paraplegia	Exercise-induced dyskinesia	Mild, TKBIQ = 65	F spikes from infancy to school age	N	N	N
2	M	12/0	c.988C > T (p.Arg330X)	Nonsense	4	Rotatory nystagmus	AS (0/7) ~ infancy, GCS/late infancy	7/0	0.36	Y	Y	Y	Diplegia	N	Severe	F spikes during preschool age	Y	N	Y
3	M	16/1	c.745_746insC (p.Arg249 fs)	Frameshift	4	Myoclonic seizures	AS/infancy ~ present	6/9	NE (29 mg/dl)	Y	Y	Y	Diplegia	Rt hemiparesis	Severe (TKBinet = 30)	GSW from infancy	N	N	Y
4	M	16/3	c.1199G > A (p.Arg400His)	Missense	8	Horizontal & rotatory nystagmus	MS (2/0) ~ Ab/school age	12/2	0.45	Y	Y	Y	Paraparesis	N	Moderate. (FSIQ = 48)	GSW during school age	Y	N	N
5	M	18/8	c.579delC (p.Ile193IlefsX36)	Frameshift	6	Opsoclonus	PS (1/9), Ab/adolescence	14/7	0.29	Y	Y	Y	Diplegia	Hemiplegia/dyskinesia	Severe	GSW during school age	Y	N	Y
6	M	20/5	c.84C > G (p.Tyr28X)	Nonsense	2	Myoclonic seizures	MS/infancy, CPS/late infancy~	11/7	0.3	Y	Y	Y	Tetraplegia	N	Severe	No epileptic abnormality	Y	N	Y
7*	F	35/2	c.971C > T (p.Ser324Leu)	Missense	15	Febrile seizure	GTCS/early childhood	33/3	NE	N	N	N	N	Dystonia	Mild	GSW during school age	N	N	N
8	F	10/7	c.997C > T (p.Arg333Trp)	Missense	15	Myoclonic seizures	MS + GTS/early childhood	4/7	0.34	Y	Y	Y	Diplegia	N	Moderate DQ = 46	F spikes from infancy to school age	Y	Y	Y
9	M	12/7	c.902_903insC (p.A301IfsX380)	Frameshift	3	Absence attack	AS + GTCS/infancy, AA + GTCS/early childhood, AA,PS/school age	6/6	0.31	Y	Y	Y	Tetraplegia	N	Severe DQ = 13	F spikes from infancy to school age	Y	N	N
10	F	12/4	Not identified		1	Eye-rolling up with no response	CPS, Ab/infancy, AS/early childhood, AA, AS/school age, AS/adolescent	5/4	NE (30 mg/dl)	Y	Y	Y	Paraplegia	N	Severe	GSW during preschool age	Y	Y	Y
11	F	12/10	c.679 + 1G > A	Splice site mutation	9	Focal twitching of the face with cyanosis for 2–3 min	PS (monthly)/infancy	5/3	0.36	Y	Y	Y	Paraplegia	N	Severe	GSW + C spikes from infancy	N	Y	Y
12	M	14/0	Not identified		2	Opsoclonus	PS/infancy, GTSA + AS (daily)/early childhood, GTCS (yearly)/adolescent	7/0	0.39	Y	Y	Y	Paraplegia	N	Moderate	ND	Y	Y	Y
13	F	14/0	Not identified		5	Opsoclonus	GTS/infancy, AA/early childhood, AS/adolescent	3/0	0.39	Y	Y	Y	N	N	Mild	2–3GSW from infancy	Y	Y	N
14	F	7/0	Not identified		3	Opsoclonus	Ab/infancy, AS/early childhood, AS/adolescent	5/6	0.38	Y	N	Y	N	N	Mild	No epileptic abnormality	Y	N	Y
15	F	4/9	c.227G > C (p.Gly76Ala)	Missense	36	Eye rolling-up with motion arrest and LOC	AA/early childhood	4/7	NE (38 mg/dl)	N	N	N	N	Ataxia	Borderline	GSW during preschool age	Y	N.D.	N
16	M	9/6	c.997C > T p.Arg333Trp	Missense	3	GTS	PS + GTS/infancy, GTCS + vomiting/early childhood	1/9	0.34	Y	N	N	N	Ataxia	Mild	No epileptic abnormality	Y	N	N
17	M	3/1	c.1279_1280ins26 (p.Gln427 fs)	Frameshift	Neonatal period	Paroxysmal nystagmus	Nystagmus with LOC/infancy	2/7	0.32	Y	N	N.D.	N	Ataxia	Moderate	F spikes during preschool age	N	N	N
18	F	16/0	c.884C > T (p.Thr295Met)	Missense	5	GTCS	GTCS/infancy, AA + vomiting/early childhood, GTCS, CPS/adolescent	8/4	0.4	Y	N	N	Diplegia	Ataxia/hemiplegia/tetraplegia/balisms, chorea	Mild	C spikes during infancy	Y	Y	N
19	F	7/6	c.376C > T (p.Arg126Cys)	Missense	17	GTCS	2GTCS/early childhood, main features were paroxysmal hypotonia with LOC	4/3	0.29	Y	N	N	N	Atonia with LOC	Mild, KABC = 60	46 months/GSW, CT spikes from school age	N	N	Y
20	F	6/2	p.Asp326Glyfs	Frameshift	6	GTCS	GTCS, PS/infancy early childhood	3/11	0.32	Y	Y	N	N	N	Mild TKBinetIQ = 74	No epileptic abnormality	N	N	N
21	F	5/2	c.431_432delTG (p.Val144GlyfsX2)	Frameshift	9	Tonic stiffening during crying	GTS/infancy, AA/early childhood	2/11	0.3	Y	N	N	N	N	Moderate DQ = 49	GSW, CT spikes	N	N	N
22	M	29/10	Not done		13	GTCS	GTCS/infancy, AA/school age	22/10	0.4	Y	Y	Y	Diplegia	Ataxia, myoclonus	Moderate WISCIII FSIQ < 50	Epileptic abnormality during adolescent age	Y	Y	N
23	F	8/10	c.997C > T (p.Arg333Trp)	Missense	17	PS	PS/early childhood, Ab/school age	4/3	0.35	Y	N	N	N	Ataxia (4/1)/atonia	Mild	Epileptic abnormality during preschool age	N	N	N
24	F	27/5	c.1198_1199insTCCAC (p.Arg400Leufs)	Frameshift	5	Developmental delay	CPS + CSGTCS/early childhood	21/4	0.43	Y	N	N	Diplegia	Atonia	Mild	Epileptic abnormality during school age	N	N.D.	N.D.
25	F	16/0	c.988C > T (p.Arg330X)	Nonsense	8	Prolonged atonia with LOC	CPS/infancy & early childhood only	9/7	0.39	Y	Y	N	Diplegia	Atonia with dystonic posture	Severe	Epileptic abnormality during school age	N	N	N
26	M	6/10	c.1279–1G > A	Splice site mutation	4	Cyanotic attack	CPS & FS/early childhood	4/11	0.36	Y	N	Y	Paraplegia	N	Severe	Focal spikes during preschool age	N	Y	N
27	F	15/8	c.835C > T (p.Gln279X)	Nonsense	2	GTCS	Prolonged atonia with LOC/school age	14/11	0.336	Y	N	Y	N	N	Severe	ND	N	N.D.	N
28	F	18/4	c.988C > T (p.Arg330X)	Nonsense	2	Apneic attack	GTS/once in infancy, PS/adolescent	16/7	0.34	Y	N	N	Diplegia	Right hemiplegia/atonia	Mild	Epileptic abnormality from adolescent age	N	N	N
29	F	7/9	c.113delA (p.Lys38ArgfsX2)	Frameshift	1	Apneic attack	CPS (monthly)/infancy to school age	3/4	0.38	Y	N	Y	Y	Atonia	Severe	F spikes from adolescent age	N	N	N
30	M	14/8	c1272T > A (p.Tyr424X)	Nonsense	3	Apneic attack	CPS (yearly)/9 m. Ab (daily)/early childhood, GTCS (yearly)/adolescent	7/11	0.35	Y	Y	Y	Paraplegia	N	Moderate	GSW from school age	Y	Y	Y
31**	M	14/1	c.1031T > C (p.Met344Thr)	Missense	3	GTS frequent & resistant	GTS/infancy, GTS + AS/school age, GTS/adolescent	10/9	0.48	Y	N	Y	Paraplegia	N	Severe	GSW from school age	N	Y	Y
32**	M	11/4	c.1031T > C (p.Met344Thr)	Missense	48	GTCS	GTCS (monthly)/early childhood ~ school age	7/11	0.47	N	N	N	N	N	Moderate	No epileptic abnormality	N	N	N
33	F	14/11	Ex1_8delEx9_10dup	Deletion/duplication	6	Ataxia & hypotonia	Lt hemiconvulsion & GTCS/infancy, CPS/early childhood, AS, AA/school age	8/5	0.36	Y	N	Y	Y	N	Moderate, TKBinetIQ = 39	GSW from school age	N	N	N

Abbreviations: F: female, M: male, GTCS: generalized tonic clonic seizures, GTS: generalized tonic seizures, LOC: loss of consciousness, AS: atonic seizures, AA: atypical absence seizures, CPS: complex partial seizures, MS: myoclonic seizures, PS: partial seizures, NE: not examined, CSF/BG: CSF glucose value/blood glucose value, F: frontal, GSW: generalized spike-and-wave complex, ND: not detected, CT: centrotemporal.

* Mother–child case.

** Proband–sibling case.

Table 2
Relationship between mental outcomes at the last follow-up and the onset age of the first symptoms as well as CSF/blood glucose ratio.

	Borderline to Mild (80 > IQ≧55)	Moderate (55 > IQ≧35)	Severe (IQ < 35)	*P* values
N	12	9	12	
Onset age of the first neurological symptoms (months)	10.50 ± 9.78	11.61 ± 14.49	3.92 ± 2.58	0.1439
CSF/blood glucose ratio	0.361 ± 0.041 ($N = 10$)*	0.376 ± 0.056	0.357 ± 0.055 ($N = 10$)*	0.7092

* CSF glucose levels were only examined in 2 cases for each.

tetraplegia) were reported in 30.3%, 39.4%, and 33.3% of patients, respectively. One patient (case 7) was diagnosed with paroxysmal exercised-induced dyskinesia because the attacks were brief and mostly occurred during physical exercise. Otherwise, cyclic vomiting (45.5%) and paroxysmal headaches (6.1%) were also reported.

The factors that most frequently aggravated these paroxysmal and static neurological symptoms were hunger, exercise, fever, and fatigue, in that order, as well as temperature changes, bathing, and drug-associated factors (2 patients each by phenobarbital and triclofos sodium, one each by clonazepam and theophylline). Contrary to our expectations, no aggravation by specific foods or beverages was reported. Furthermore, the factors that most frequently improved abnormal neurological symptoms were eating, sleeping, and resting, in that order. Several patients were previously shown to have recovered immediately from neurological abnormalities following intravenous glucose drip infusion therapy at a hospital [25]. It was also reported that patients were more likely to exhibit gradual neurological improvements over the long term as they got older.

3.4. Neuroimaging findings

Of the 33 patients analyzed, 14 underwent computed tomography (CT) of the head. One case exhibited mild atrophy of the cerebrum and cerebellum. Thirty patients underwent magnetic resonance imaging (MRI) of the head, with 12 exhibiting abnormalities. Various degrees of cerebral atrophy and ventriculomegaly were detected in 6 cases. Furthermore, diffuse delays in myelination and high-signal foci at the subcortical white matter were identified in 7 cases by T2-weighted or fluid-attenuated inversion recovery imaging. In these 7 patients, abnormal findings were all detected at 8 years old or younger. Abnormalities were observed in 8 out of the 16 patients who underwent cerebral blood flow single-photon emission computed tomography (SPECT), demonstrating nonspecific localized reduced blood flow in various cerebral regions. Abnormal findings were identified in 15 out of the 16 patients who underwent fludeoxyglucose-positron emission tomography (FDG-PET). FDG-PET revealed that glucose uptake by the cerebral cortex was reduced at various locations. Additionally, the relatively elevated uptake of glucose by the basal ganglia was detected in 9 cases, whereas that by the thalamus was reduced in 4 cases. Taken together, these results indicated that the myelination delay and high-signal foci in the subcortical white matter observed on T2-weighted magnetic resonance images as well as the relatively enhanced accumulation of glucose by the basal ganglia and reduced uptake of glucose by the thalamus observed in FDG-PET study were characteristic of this disorder (Fig. 1).

3.5. EEG findings

In the interictal EEG examination, the slowing of background activity was detected in 12 out of the 33 cases (64%). These background activity abnormalities were improved by eating (15 cases) and glucose injections (2 cases). In infancy, an epileptiform EEG abnormality was reported in 5 out of 17 cases (29.4%) who underwent the EEG examination. In early childhood, childhood, and adolescent and beyond, an epileptiform EEG abnormality were identified in 15 cases (62.5%), 20 cases (80%), and 7 cases (63.6%), respectively (Table 3). Regarding the types of epileptiform EEG abnormalities, focal epileptiform discharges were frequently observed in the infancy and early childhood periods, while generalized spike–wave discharges at 2.5–4 Hz were more frequently seen in the childhood and adolescent periods. The focal epileptiform EEG abnormality in early and later childhood was often localized in the frontal region. In conclusion, the epileptiform abnormality was not frequently detected during infancy, but was increasingly identified from the early childhood to adolescent periods in the form of generalized spike–wave discharges. Other neurophysiological tests were performed in 15 cases, and abnormal auditory brainstem responses (ABR) were noted in 3 cases, abnormal visual-evoked potentials (VEP) in 2 cases, and abnormal somatosensory-evoked potentials (SEP) in 3 cases. A peripheral nerve conduction test was performed in 9 cases with no abnormal findings.

3.6. Biochemical findings

No abnormal findings that were specific to this disease were observed in general blood and urine

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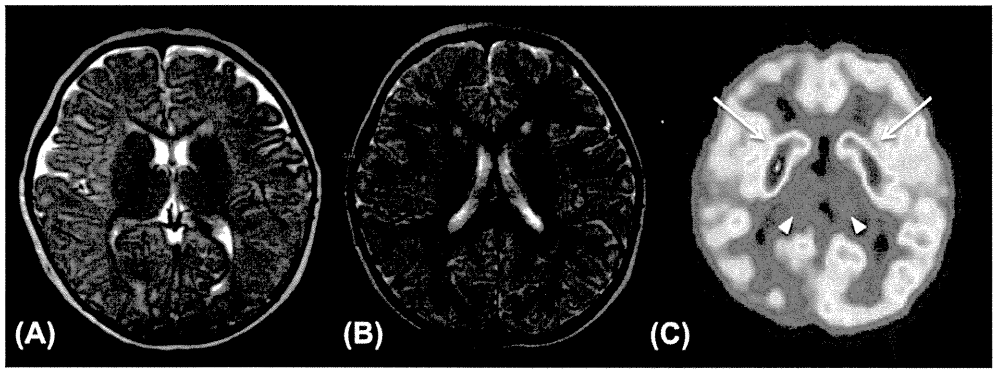

Fig. 1. MRI and FDG-PET (fludeoxyglucose-positron emission tomography) findings of a patient with GLUT-1 DS. (A) T2-weighted image at 12 months of age shows high intense signal in the widespread subcortical and deep white matters, suggesting delayed myelination. (B) T2-weighted image at 2 years and 9 months of age shows multiple subcortical high intense areas. (C) FDG-PET at 3 years and 4 months of age revealed relatively enhanced accumulation of glucose in the bilateral basal ganglia (white arrows) and hypometabolism in the bilateral thalami (white arrow heads). The right occipital lobe is also hypometabolic.

Table 3
Routine EEG findings according to the age period.

Age period	N	Epileptiform EEG discharges (%)	Focal epileptiform discharges (%)	Generalized spike–wave discharges (%)
Infancy (<12 months)	17	5 (29.4)	3 (60)	3 (60)
Early childhood (1–6 years)	24	15 (62.5)	11 (73)	8 (53)
Childhood (6–12 years)	25	20 (80)	9 (45)	10 (50)
Adolescence and beyond (12 years <)	11	7 (63.6)	3 (28)	9 (82)

The total number of patient showing focal and generalized epileptiform discharges exceeded those having epileptiform EEG discharges because some patients had both focal and generalized EEG discharges.

laboratory tests. Bone age was assessed in 9 cases, and 4 of these exhibited some delay. In a growth hormone-loading test, 4 cases tested positive for the insufficient secretion of growth hormones.

3.6.1. Cerebrospinal fluid (CSF) examination

CSF was analyzed in 32 cases, excluding one case of an adult patient (mother of an affected boy, case 7). In this case, the proband (case 1) was unexpectedly found to have GLUT-1DS by the CSF analysis, which was performed to identify the cause of paroxysmal dyskinesia. The mean CSF glucose level was 32.4 mg/dL (26–42 mg/dL), and mean CSF (C)/blood (B) glucose ratio (C/B ratio) was 0.36 (0.28–0.48). When the cut-off values for diagnosis (CSF glucose level less than 40 mg/dL and C/B ratio less than 0.45) were applied, CSF glucose levels below 40 mg/dL were observed in 91% of all 45 CSF tests, and C/B ratios were below 0.45 in 89% of all 44 CSF tests. Mean lactate and pyruvate levels were 9.6 mg/dL (6.0–13.1 mg/dL) and 0.68 mg/dL (0.39–1.5 mg/dL), respectively. The mean lactate/pyruvate ratio was 15.4 (7.1–23.0).

3.7. Genetic diagnosis

Pathological mutations in the *SLC2A1* gene were identified in 28 out of the 32 cases (87.5%) who underwent genetic testing. The remaining 4 patients (12.5%) who were given a clinical diagnosis of GLUT-1DS based on a low CSF C/B ratio were negative for the *SLC2A1* gene mutation. Genetic testing has not yet been conducted in the one patient. The genetic mutations identified were missense (11 cases), frame shift (8 cases), nonsense (6 cases), and splice site mutations (2 cases), as well as a large scale deletion (1 case) (Table 1). Arg333 and Arg330 mutations, which were detected in multiple nonconsanguineous family lines, were thought to be prevalent mutation sites. Familial cases were identified in two family lines (cases 1 and 7, cases 31 and 32), with the details of one being described elsewhere [20]. Three cases of GLUT-1DS with *SLC2A1* mutations (case 18, 31, 32) did not exhibit the low uptake of 3-*O*-methyl-D-glucose by erythrocytes.

Regarding the types of mutation (missense vs. truncating mutation) in relation to clinical symptoms, the significantly later onset of the initial symptoms, higher C/B ratios, and better mental outcomes were observed in patients with missense mutations ($P < 0.05$)(Table 4).

4. Discussion

Since we reported the first case of GLUT-1DS in Japan in 2004, the number of cases has gradually accumulated [18,20,24]. This study is a first nation-wide

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Table 4
Relationship between types of SLC2A mutation and the onset age of the first neurological symptoms as well as CSF/blood glucose ratio and mental outcomes.

	Missense mutation	Truncating mutation**	*P* values
N	11	17	
Onset age of the first neurological symptoms (months)	16.27 ± 14.02	4.38 ± 2.64	0.001
CSF/blood glucose ratio	0.388 ± 0.066	0.344 ± 0.037	0.0225
Mental outcomes			
Borderline to Mild	7	3	
Moderate	3	4	0.0166
Severe	1	10	
Epilepsy	11	16	0.6071
Paroxysmal episodes (Complex movement disorders)	7	7	0.2200
Pyramidal sign	5	13	0.1027
Postnatal microcephaly	3/10*	3/15*	0.4553
Short statue	3	6/16*	0.4488
Cyclic vomoting	5	5	0.3205

* A number of cases excluded those without the information.
** It included nonsense mutation, frame shift mutation, splice site mutation and deletion/duplication.

survey of GLUT-1DS in Japan to elucidate the prevalence, clinical characteristics and prognosis of the Japanese patients. This survey found a total of 57 genetically confirmed or clinically diagnosed cases of GLUT-1DS and the clinical data of 33 of these were submitted for analyses.

As GLUT-1DS is an epileptic encephalopathy that can be treated with ketogenic diet therapy (KD), an early diagnosis as well as early introduction of the KD to prevent a chronic glucose deficiency in the brain is expected [5,17]. A special form of ketone milk (Ketone Formula, Meiji Co., Ltd., Tokyo, Japan) can be started to treat patients from the neonatal period in Japan. Therefore, establishing guidelines for the early diagnosis of GLUT-1DS is important for the early introduction of KD. In the present study, we investigated the clinical and neurological symptoms of patients with GLUT-1DS in detail in order to identify key symptoms specific to this syndrome.

In this study, most patients with GLUT-1DS developed the initial clinical symptoms from early infancy, which is consistent with previous findings [2,4,5]. Convulsive seizures, paroxysmal attacks of abnormal eye movements, and apnea/cyanotic attacks were most frequent manifestations in that order up to 12 months of age. Furthermore, patients had diverse epileptic seizure types, including GTCS, myoclonic, absence, atonic, and partial seizures. As previously reported, GTCS and absence seizures were identified as the two most frequent seizure types observed in all ages [26]. Myoclonic and astatic seizures were also reported. As absence, myoclonic, and astatic seizures are generally produced by generalized spike and wave complexes, the frequent occurrence of interictal generalized 2.5–4 Hz spike–wave EEG discharges in our patients was consistent with these seizure phenotypes [27].

Complex movement disorders (paroxysmal episodes of ataxia, dystonia/dyskinesia, and motor paralysis) including one case of paroxysmal exercised-induced dyskinesia were noted in 45% of cases and mostly after infancy. Not only paroxysmal symptoms important, but also abnormal neurological findings were considered important, including hypotonia, cerebellar ataxia, dystonia, and dysarthria of different degrees. Another important result was that neurological abnormalities were aggravated by hunger (especially morning fasting), exercise, body temperature elevations (fever, hot weather, and bathing), and fatigue, and were improved by eating, sleeping, and resting. However, the fluctuations in neurological abnormalities observed in association with hunger or eating may not necessarily be clear in the early clinical course of the disease.

In the present study, CSF tests were the most effective diagnostic method and should be conducted whenever the aforementioned paroxysmal and nonparoxysmal neurological abnormalities are noted [28]. It was important for CSF glucose levels to be 40 mg/dL (2.2 mMol/L) or less and the C/B ratio to be 0.45 or less (mean, 0.35) despite normal blood glucose levels. Furthermore, lactate levels should be normal to low. The results of this study indicated that the recommended CSF glucose levels of less than 40 mg/dL and CB ratios of less than 0.45 were appropriate [2,17]. However, the time between the collection of cerebrospinal fluid and blood must be strictly defined, and should more closely follow the recommendation of Klepper et al., who stated that blood sampling should be performed 4–6 h after breakfast when blood glucose levels have stabilized and also that blood glucose should be measured first in order to avoid high blood glucose levels due to the stress of a lumbar puncture [17].

A number of atypical groups, which lack neither frequent epileptic seizures nor severe neurological symptoms despite the typical laboratory and genetic abnormalities, have recently been described

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[3,10,12,29–31]. Leen et al. [9] classified GLUT-1DS into three clinical phenotypes based on an analysis of genetically-confirmed 57 cases: (1) classic clinical phenotype: accounting for 85% of all cases, this group was classified into an early-onset type (onset at 2 years of age or younger) and delayed type (older than 2 years of age); (2) atypical phenotype: accounting for 15%, this group was characterized by mental retardation and paroxysmal kinesigenic choreoathetosis (paroxysmal exertion-induced dyskinesia) and the absence of epilepsy; and (3) adult phenotype with only subtle symptoms. Moreover, *SLC2A1* gene mutations were also found in various cases with established epileptic as well as neurological syndromes, such as early-onset absence epilepsy, myoclonic-astatic epilepsy, and alternating hemiplegia syndrome, underscoring the diversity of the clinical manifestations of GLUT-1DS [10–12,30]. In our study, 31 cases or 94% of all cases were subclassified into classical phenotype, while the remaining two cases, one was a child and another was his mother, were subclassified into atypical phenotype and adult phenotype, respectively. It was difficult to refer to the details of epileptic phenotypes in order to determine whether established epileptic syndromes were included in our study because of the limitation of the questionnaire study. However, as far as the GLUT-1DS diagnosis is concerned, the presence of the characteristic neurological and neurocognitive abnormalities, in addition to the epileptic seizures regardless of seizure types, can help to distinguish GLUT-1DS from specific epileptic syndromes.

Our study included two families comprising 4 cases. Familial cases with autosomal dominant and recessive transmission have recently been described and included a parent or siblings having the same *SLC2A1* mutation to a proband who exhibited the typical clinical characteristics, but almost no clinical symptoms or mild symptoms [9,20,30,32]. Hashimoto et al. already reported the details of the two families included in the present study, in which both families included an affected parent and sibling and mild clinical symptoms in the mother were undiagnosed until the proband was diagnosed [20].

EEG findings have frequently been examined in children with GLUT-1DS because epileptic seizures have been identified as the primary symptoms in most patients [18]. In this study, the slowing of background activity, epileptic focal spike discharge, especially from the frontal region, and generalized 2.5–4 Hz spike–wave discharges were detected from early childhood and most frequently during the adolescent and later period. The epileptic EEG abnormality was generally infrequent during infancy and increased in frequency after early childhood. However, the most important EEG finding was the improvement observed in both epileptic abnormalities and background activities by the consumption of food or administration of a glucose injection [18,25]. The EEG examination can be performed on an outpatient basis such that the first EEG can be recorded in the morning after a whole night of fasting and the second EEG can be performed 30 min after breakfast, thereby showing marked EEG improvements and providing important information for an accurate diagnosis of GLUT-1DS.

Neuroimaging abnormalities including cerebral atrophy, myelination delay, and high-signal foci in the subcortical white matter (T2-weighted fluid-attenuated inversion recovery imaging) were reported; however, none of these were specific to this syndrome by themselves. In contrast, the relatively enhanced accumulation of glucose by the basal ganglia and reduced uptake of glucose by the thalamus on FDG-PET are considered to be very specific to GLUT-1DS [33]. However, FDG-PET scanning is not highly recommended when attempting to diagnose GLUT-1DS because it is expensive and its usefulness in early diagnosis has not been proven.

Heterozygous *de novo* mutations in the *SLC2A1* gene (1p35-31:3, OMIM 138140) have been detected in approximately 70%–80% of patients with GLUT-1DS, and causes this syndrome due to haploinsufficiency [8,9,34]. Genetic mutations were not detected in 10%–20% of cases, which is consistent with our results. Five patients in the present study including 4 without identifiable *SLC2A1* gene mutations were clinically diagnosed with GLUT-1DS based on their clinical symptoms and low CSF glucose levels as well as good responses to KD. A previous study speculated that potential disease mechanisms in patients without mutations in the coding regions of the *SLC2A1* gene could be posttranscriptional modifications such as alternative splicing, defects in N-glycosylation, GLUT-1 trafficking and GLUT-1 assembly, which affect the GLUT-1 function[35]. Patients with a missense mutation frequently exhibit milder clinical symptoms; however, no clear genotype-phenotype relationship has yet been established [9]. The results of this study also showed that patients with a missense mutation had better mental outcomes, later onset age of the first symptoms, and milder C/B ratio. This study included 3 cases of GLUT-1DS with *SLC2A1* missense mutations who did not exhibit the low uptake of 3-*O*-methyl-D-glucose by erythrocytes. Recent study demonstrated that the specific type of mutation alter GLUT confirmation and asymmetrically affects glucose flux across the cell by perturbing efflux (glucose release in CSF) than influx (uptake of glucose) [36]. A diversity of phenotypes was observed among patients with the same mutation and even within autosomal dominant family members, suggesting a complex onset pathomechanism. The results of our study were also consistent with findings reported in the US and Europe.

In conclusion, this study demonstrated that unexplained paroxysmal abnormal eye movements, apneic/cyanotic attacks, and convulsive seizures in infancy in combination with complex movement disorders (ataxia,

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dystonia/dyskinesia, and motor paralysis) in early childhood and thereafter were important clinical manifestations for suspected GLUT-1DS. An early CSF study is recommended for these patients and the early introduction of KD or ketone milk is important to prevent irreversible brain dysfunction due to the chronic depletion of glucose in the brain. Further studies are warranted to identify key clinical and laboratory information that will lead to a CSF study and accurate diagnosis of GLUT-1DS at as early an age as possible. We need a more accumulation of GLUT-1DS cases whose early clinical and EEG information can be obtained in details to make an accurate diagnostic and treatment guideline. We also need to confirm whether the early introduction of KD can improve brain function and prevent secondary brain disorders.

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References

[1] Wang D, Pascual JM, De Vivo D. Glucose Transporter Type 1 Deficiency Syndrome. In: Pagon RA, Adam MP, Ardinger HH, Bird TD, Dolan CR, Fong CT, et al., editors. GeneReviews® Seattle (WA): University of Washington; 1993.

[2] Klepper J, Voit T. Facilitated glucose transporter protein type 1 (GLUT1) deficiency syndrome: impaired glucose transport into brain–a review. Eur J Pediatr 2002;161:295–304.

[3] Brockmann K. The expanding phenotype of GLUT1-deficiency syndrome. Brain Dev 2009;31:545–52.

[4] De Vivo DC, Trifiletti RR, Jacobson RI, Ronen GM, Behmand RA, Harik SI. Defective glucose transport across the blood-brain barrier as a cause of persistent hypoglycorrhachia, seizures, and developmental delay. N Engl J Med 1991;325:703–9.

[5] Verrotti A, D'Egidio C, Agostinelli S, Gobbi G. Glut1 deficiency: when to suspect and how to diagnose? Eur J Paediatr Neurol 2012;16:3–9.

[6] Rotstein M, Engelstad K, Yang H, Wang D, Levy B, Chung WK, et al. Glut1 deficiency: inheritance pattern determined by haploinsufficiency. Ann Neurol 2010;68:955–8.

[7] Klepper J, Scheffer H, Elsaid MF, Kamsteeg EJ, Leferink M, Ben-Omran T. Autosomal recessive inheritance of GLUT1 deficiency syndrome. Neuropediatrics 2009;40:207–10.

[8] Seidner G, Alvarez MG, Yeh JI, O'Driscoll KR, Klepper J, Stump TS, et al. GLUT-1 deficiency syndrome caused by haploinsufficiency of the blood-brain barrier hexose carrier. Nat Genet 1998;18:188–91.

[9] Leen WG, Klepper J, Verbeek MM, Leferink M, Hofste T, van Engelen BG, et al. Glucose transporter-1 deficiency syndrome: the expanding clinical and genetic spectrum of a treatable disorder. Brain 2010;133:655–70.

[10] Arsov T, Mullen SA, Damiano JA, Lawrence KM, Huh LL, Nolan M, et al. Early onset absence epilepsy: 1 in 10 cases is caused by GLUT1 deficiency. Epilepsia 2012;53:e204–7.

[11] Gokben S, Yilmaz S, Klepper J, Serdaroglu G, Tekgul H. Video/EEG recording of myoclonic absences in GLUT1 deficiency syndrome with a hot-spot R126C mutation in the SLC2A1 gene. Epilepsy Behav 2011;21:200–2.

[12] Mullen SA, Marini C, Suls A, Mei D, Della Giustina E, Buti D, et al. Glucose transporter 1 deficiency as a treatable cause of myoclonic astatic epilepsy. Arch Neurol 2011;68:1152–5.

[13] Perez-Duenas B, Prior C, Ma Q, Fernandez-Alvarez E, Setoain X, Artuch R, et al. Childhood chorea with cerebral hypotrophy: a treatable GLUT1 energy failure syndrome. Arch Neurol 2009;66:1410–4.

[14] Roubergue A, Apartis E, Mesnage V, Doummar D, Trocello JM, Roze E, et al. Dystonic tremor caused by mutation of the glucose transporter gene GLUT1. J Inherit Metab Dis 2011;34:483–8.

[15] Schneider SA, Paisan-Ruiz C, Garcia-Gorostiaga I, Quinn NP, Weber YG, Lerche H, et al. GLUT1 gene mutations cause sporadic paroxysmal exercise-induced dyskinesias. Mov Disord 2009;24:1684–8.

[16] Suls A, Dedeken P, Goffin K, Van Esch H, Dupont P, Cassiman D, et al. Paroxysmal exercise-induced dyskinesia and epilepsy is due to mutations in *SLC2A1*, encoding the glucose transporter GLUT1. Brain 2008;131:1831–44.

[17] Klepper J. GLUT1 deficiency syndrome in clinical practice. Epilepsy Res 2012;100:272–7.

[18] Ito Y, Gertsen E, Oguni H, Nakayama T, Matsuo M, Funatsuka M, et al. Clinical presentation, EEG studies, and novel mutations in two cases of GLUT1 deficiency syndrome in Japan. Brain Dev 2005;27:311–7.

[19] Fujii T, Morimoto M, Yoshioka H, Ho YY, Law PP, Wang D, et al. T295M-associated Glut1 deficiency syndrome with normal erythrocyte 3-OMG uptake. Brain Dev 2011;33:316–20.

[20] Hashimoto N, Kagitani-Shimono K, Sakai N, Otomo T, Tominaga K, Nabatame S, et al. *SLC2A1* gene analysis of Japanese patients with glucose transporter 1 deficiency syndrome. J Hum Genet 2011;56:846–51.

[21] Takahashi S, Ohinata J, Suzuki N, Amamiya S, Kajihama A, Sugai R, et al. Molecular analysis and anticonvulsant therapy in two patients with glucose transporter 1 deficiency syndrome: a successful use of zonisamide for controlling the seizures. Epilepsy Res 2008;80:18–22.

[22] Klepper J, Engelbrecht V, Scheffer H, van der Knaap MS, Fiedler A. GLUT1 deficiency with delayed myelination responding to ketogenic diet. Pediatr Neurol 2007;37:130–3.

[23] Klepper J. Glucose transporter deficiency syndrome (GLUT1DS) and the ketogenic diet. Epilepsia 2008;49(Suppl 8):46–9.

[24] Ito Y, Oguni H, Ito S, Oguni M, Osawa M. A modified Atkins diet is promising as a treatment for glucose transporter type 1 deficiency syndrome. Dev Med Child Neurol 2011;53:658–63.

[25] Akman CI, Engelstad K, Hinton VJ, Ullner P, Koenigsberger D, Leary L, et al. Acute hyperglycemia produces transient improvement in glucose transporter type 1 deficiency. Ann Neurol 2010;67:31–40.

[26] Pong AW, Geary BR, Engelstad KM, Natarajan A, Yang H, De Vivo DC. Glucose transporter type 1 deficiency syndrome: epilepsy phenotypes and outcomes. Epilepsia 2012;53:1503–10.

[27] Hirano Y, Oguni H, Funatsuka M, Imai K, Osawa M. Differentiation of myoclonic seizures in epileptic syndromes: a video-polygraphic study of 26 patients. Epilepsia 2009;50:1525–35 (in eng).

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[28] Leen WG, Wevers RA, Kamsteeg EJ, Scheffer H, Verbeek MM, Willemsen MA. Cerebrospinal fluid analysis in the workup of GLUT1 deficiency syndrome: a systematic review. JAMA Neurol 2013;70:1440–4.
[29] Anand G, Padeniya A, Hanrahan D, Scheffer H, Zaiwalla Z, Cox D, et al. Milder phenotypes of glucose transporter type 1 deficiency syndrome. Dev Med Child Neurol 2011;53:664–8.
[30] Arsov T, Mullen SA, Rogers S, Phillips AM, Lawrence KM, Damiano JA, et al. Glucose transporter 1 deficiency in the idiopathic generalized epilepsies. Ann Neurol 2012;72:807–15.
[31] Koy A, Assmann B, Klepper J, Mayatepek E. Glucose transporter type 1 deficiency syndrome with carbohydrate-responsive symptoms but without epilepsy. Dev Med Child Neurol 2011;53:1154–6.
[32] Striano P, Weber YG, Toliat MR, Schubert J, Leu C, Chaimana R, et al. GLUT1 mutations are a rare cause of familial idiopathic generalized epilepsy. Neurology 2012;78:557–62.
[33] Pascual JM, Van Heertum RL, Wang D, Engelstad K, De Vivo DC. Imaging the metabolic footprint of Glut1 deficiency on the brain. Ann Neurol 2002;52:458–64.
[34] Klepper J. Absence of *SLC2A1* mutations does not exclude Glut1 deficiency syndrome. Neuropediatrics 2013;44:235–6.
[35] Klepper J, Leiendecker B. GLUT1 deficiency syndrome–2007 update. Dev Med Child Neurol 2007;49:707–16.
[36] Wang D, Yang H, Shi L, Ma L, Fujii T, Engelstad K, et al. Functional studies of the T295M mutation causing Glut1 deficiency: glucose efflux preferentially affected by T295M. Pediatr Res 2008;64:538–43.

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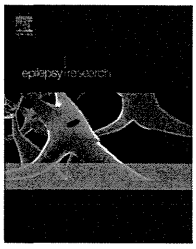

Association of nonsense mutation in *GABRG2* with abnormal trafficking of $GABA_A$ receptors in severe epilepsy

Atsushi Ishii[a,d], Takeshi Kanaumi[a,d], Miwa Sohda[e], Yoshio Misumi[b], Bo Zhang[c], Naoto Kakinuma[f], Yoshiko Haga[g], Kazuyoshi Watanabe[h], Sen Takeda[f], Motohiro Okada[i], Shinya Ueno[j], Sunao Kaneko[k,m], Sachio Takashima[l], Shinichi Hirose[a,d,*]

[a] *Department of Pediatrics, Fukuoka University, Fukuoka, Japan*
[b] *Department of Cell Biology, Fukuoka University, Fukuoka, Japan*
[c] *Department of Biochemistry, Fukuoka University, Fukuoka, Japan*
[d] *Central Research Institute for the Molecular Pathomechanisms of Epilepsy, Fukuoka University, Fukuoka, Japan*
[e] *Division of Oral Biochemistry, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan*
[f] *Department of Anatomy and Cell Biology, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Chuo, Japan*
[g] *Department of Pediatrics, Nagoya University Graduate School of Medicine, Nagoya, Japan*
[h] *Faculty of Health and Medical Sciences, Aichi Shukutoku University, Nagakute, Japan*
[i] *Division of Neuroscience, Graduate School of Medicine, Mie University, Tsu, Japan*
[j] *Rehabilitation Medicine, Institute of Brain Science, Japan*
[k] *Department of Neuropsychiatry, Hirosaki University, Hirosaki, Japan*
[l] *Yanagawa Institute for Developmental Disabilities, Child Neurology, International University of Health and Welfare, Yanagawa, Japan*
[m] *North Tohoku Epilepsy Center, Minato Hospital, Hachinohe, Japan*

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Summary Mutations in *GABRG2*, which encodes the γ2 subunit of $GABA_A$ receptors, can cause both genetic epilepsy with febrile seizures plus (GEFS+) and Dravet syndrome. Most *GABRG2* truncating mutations associated with Dravet syndrome result in premature termination codons

Abbreviations: GEFS+, genetic epilepsy with febrile seizures plus; PTCs, premature termination codons; NMD, nonsense-mediated decay.

* Corresponding author at: Department of Pediatrics, School of Medicine, Fukuoka University, 45-1, 7-chome Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan. Tel.: +81 92 801 1011; fax: +81 92 862 6955.
E-mail address: hirose@fukuoka-u.ac.jp (S. Hirose).

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Trafficking;
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(PTCs) and are stably translated into mutant proteins with potential dominant-negative effects. This study involved search for mutations in candidate genes for Dravet syndrome, namely *SCN1A*, *2A*, *1B*, *2B*, *GABRA1*, *B2*, and *G2*. A heterozygous nonsense mutation (c.118C>T, p.Q40X) in *GABRG2* was identified in dizygotic twin girls with Dravet syndrome and their apparently healthy father. Electrophysiological studies with the reconstituted $GABA_A$ receptors in HEK cells showed reduced GABA-induced currents when mutated γ2 DNA was cotransfected with wild-type α1 and β2 subunits. In this case, immunohistochemistry using antibodies to the α1 and γ2 subunits of $GABA_A$ receptor showed granular staining in the soma. In addition, microinjection of mutated γ2 subunit cDNA into HEK cells severely inhibited intracellular trafficking of $GABA_A$ receptor subunits α1 and β2, and retention of these proteins in the endoplasmic reticulum. The mutated γ2 subunit-expressing neurons also showed impaired axonal transport of the α1 and β2 subunits. Our findings suggested that different phenotypes of epilepsy, e.g., GEFS+ and Dravet syndrome (which share similar abnormalities in causative genes) are likely due to impaired axonal transport associated with the dominant-negative effects of *GABRG2*.

Introduction

Epilepsy is associated with various gene mutations. However, the exact molecular mechanisms underlying the pleomorphic phenotypes of this disease remain unclear. There are two major epilepsy phenotypes associated with mutations in the $GABA_A$ receptor. The first is genetic epilepsy with febrile seizures plus (GEFS+). Individuals with GEFS+ have a missense mutation in the gene encoding the γ2 subunit of the $GABA_A$ receptor, *GABRG2* (Baulac et al., 2001). The second phenotype is Dravet syndrome, which is a malignant epilepsy condition characterized by refractory seizures and psychomotor developmental arrest.

Compared with Dravet syndrome, GEFS+ is relatively benign. However, these seemingly different syndromes are considered part of a single disease spectrum as mutations detected in both syndromes (Mulley et al., 2005; Singh et al., 2001) lie on the same genes, namely *GABRG2* (Baulac et al., 2001; Harkin et al., 2002), the gene encoding the α1 subunit of the neuronal voltage-gated sodium channel, *SCN1A* (Abou-Khalil et al., 2001; Claes et al., 2001, 2003; Escayg et al., 2000, 2001; Fujiwara et al., 2003; Fukuma et al., 2004; Gennaro et al., 2003; Kimura et al., 2005; Nabbout et al., 2003; Ohmori et al., 2002; Sugawara et al., 2001, 2002; Wallace et al., 2003), and the gene encoding the α2 subunit of the neuronal voltage-gated sodium channel, *SCN2A* (Shi et al., 2009; Kamiya et al., 2004; Sugawara et al., 2001). Most of the mutations initially identified in Dravet syndrome were truncation mutations (Claes et al., 2001; Sugawara et al., 2002). On the other hand, mutations identified in GEFS+ were exclusively missense mutations (Abou-Khalil et al., 2001; Escayg et al., 2000; Sugawara et al., 2001). Based on these studies and the more malignant nature of Dravet syndrome, it has been since postulated that mutations found in Dravet syndrome are associated with the more severe phenotypes due to a more significant genetic loss compared to the usually milder effects of missense mutations. Intriguingly, subsequent analysis revealed that missense mutations are also associated with Dravet syndrome (Fujiwara et al., 2003; Fukuma et al., 2004; Gennaro et al., 2003; Ohmori et al., 2002).

Since most of the nonsense mutations are located in the 5′ end of *SCN1A*, the mutant transcripts go through a nonsense-mediated decay (NMD) pathway (Holbrook et al., 2004). Thus, NMD processing underlies the distinct phenotypes resulting from truncation mutations and, further, may explain the variety of phenotypes associated with different mutations in the same gene. However, the evidence that GEFS+ and Dravet syndrome occupy the same spectrum of disorders or are allelic variants is not compelling.

In this study, we identified a nonsense mutation (c.118C>T, p.Q40X) in the *GABRG2* gene in individuals with Dravet syndrome. To specify the molecular mechanisms underlying the phenotypes of GEFS+ and Dravet syndrome resulting from allelic *GABRG2* truncation mutations, we focused on intracellular trafficking since several mutants are retained in the endoplasmic reticulum (ER) (Hirose, 2006; Gallagher et al., 2005; Hales et al., 2005; Harkin et al., 2002; Kang and Macdonald, 2004; Macdonald et al., 2004).

Materials and methods

Ethics standards

The study methodologies conformed to the standards set by the Declaration of Helsinki; and the study methodologies were approved by the Ethics Review Committees of Fukuoka University. The parents of patient and her sibling provided signed informed consent with the understanding before the study.

Patients

We studied a Japanese family, including dizygotic twin girls with the epilepsy phenotype of Dravet syndrome. The twins had experienced seizures from 2 months of age, and one of the twins died in bed at 3 years and 5 months of age. The twins had a novel nonsense mutation of the gene encoding the $GABA_A$ receptor γ2 subunit. We found the same mutation in their father, who did not have any seizure episodes. In contrast, their mother did not have the mutation, but had several seizure episodes in early childhood.