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

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/

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.A301fsX380)	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

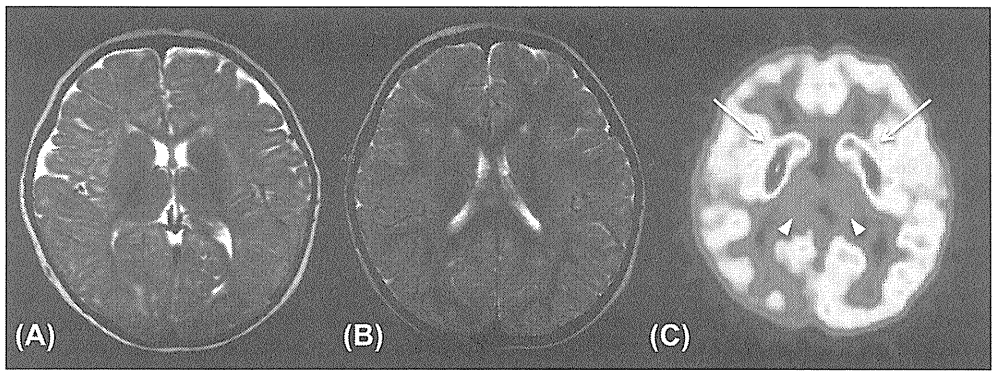

Fig. 1. MRI and FDG-PET (fludeoxyglucose-positron emission tomography) findings of a patient with GLUT-1 DS. (A) T2-weighted image at 12 months of age shows high intense signal in the widespread subcortical and deep white matters, suggesting delayed myelination. (B) T2-weighted image at 2 years and 9 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

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

[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,

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.

Acknowledgments

We are grateful to Naoko Ishihara MD, Yoshiteru Azuma MD, and Ikuko Kato MD and all of the doctors who collaborated and sent valuable information on their patients for this study. This study was funded in part by Research Grants (059) for the Specified Disease Treatment Research Program from the Ministry of Health, Labor, and Welfare. We certify that we have read the Journal's position regarding issues pertaining to ethical publications, and affirm that this report is consistent with those guidelines.

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Brain & Development 37 (2015) 698–703

BRAIN & DEVELOPMENT
Official Journal of the Japanese Society of Child Neurology

www.elsevier.com/locate/braindev

Original article

Carnitine–acylcarnitine translocase deficiency: Two neonatal cases with common splicing mutation and *in vitro* bezafibrate response

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Received 28 July 2014; received in revised form 7 October 2014; accepted 8 October 2014

Abstract

Background: Mitochondrial fatty acid oxidation (FAO) disorders are among the causes of acute encephalopathy- or myopathy-like illness. Carnitine–acylcarnitine translocase (CACT) deficiency is a rare FAO disorder, which represent an energy production insufficiency during prolonged fasting, febrile illness, or increased muscular activity. CACT deficiency is caused by mutations of the *SLC25A20* gene. Most patients developed severe metabolic decompensation in the neonatal period and died in infancy despite aggressive treatment.

Patients and methods: We herein report the clinical findings of two unrelated cases of CACT deficiency with mutation confirmation, and *in vitro* bezafibrate responses using *in vitro* probe acylcarnitine (IVP) assay. Patients 1 and 2 are products of nonconsanguineous parents. Both patients developed cardiac arrest at day 3 of life but survived the initial events. Their blood chemistry revealed hypoglycemia and metabolic acidosis. The acylcarnitine profiles in both patients demonstrated increased long-chain acylcarnitines, suggesting CACT or carnitine palmitoyltransferase-2 (CPT2) deficiency.

Results: The mutation analysis identified homozygous IVS2-10T>G in the *SLC25A20* gene in both patients, confirming the diagnosis of CACT deficiency. The IVP assay revealed increased C16, C16:1, but decreased C2 with improvement by bezafibrate in the cultured fibroblasts. The short-term clinical trial of bezafibrate in Patient 1 did not show clinical improvement, and died after starting the trial for 6 months.

Conclusion: This splicing mutation has been identified in other Asian populations indicating a possible founder effect. IVP assay of cultured fibroblasts could determine a response to bezafibrate treatment. A long-term clinical trial of more enrolled patients is required for evaluation of this therapy.

Keywords: CACT deficiency; *SLC25A20* mutation; IVP assay; Bezafibrate

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http://dx.doi.org/10.1016/j.braindev.2014.10.005
0387-7604/

1. Introduction

Mitochondrial fatty acid oxidation (FAO) disorders are among the causes of neuromuscular symptoms as well as acute encephalopathy or even sudden death. In particular, the carnitine cycle is important in energy-producing pathway for cardiac and skeletal muscle and for preventing from hypoglycemia especially during prolonged fasting or increased muscular exercise. Carnitine–acylcarnitine translocase (CACT, EC 2.3.1.21) is one of the enzymes in the carnitine cycle, which catalyzes the transfer of the long-chain fatty acylcarnitines across the inner mitochondrial membrane in exchange of free carnitine. CACT deficiency (OMIM 212138) was first described in 1992 [1]. It is an autosomal-recessive disease caused by mutations of the *SLC25A20* gene located in chromosome 3p21.31 [2]. The gene consists of 9 exons and encodes protein comprising 301 amino acids [3]. CACT deficiency is a very rare disorder with so far as approximately 30 patients have been described, and accounted for 10% of patients with FAO disorders in French population [4]. However, it might be a common FAO disorder in some East Asian countries such as Hong Kong with the estimated incidence of 1 in 60,000 live births, and accounted for 33% of patients with FAO disorders [5]. Most patients develop neonatal-onset encephalopathy with nonketotic hypoglycemia, hyperammonemia, and hypothermia, or sudden death from cardiac arrhythmias. Cardiomyopathy and hepatic dysfunction may be the associated complications. CACT deficiency could be detected by elevations of C16 and C18 acylcarnitines, and low free carnitine in acylcarnitine profiles. However, the same profile could be found in neonatal carnitine palmitoyltransferase-2 (CPT2) deficiency. Therefore, confirmation of diagnosis requires CACT enzyme assay or molecular analysis of the *SLC25A20* gene [6]. Treatment includes intravenous glucose for acute decompensation, and avoidance of long fasting with frequent meals. Long-chain fatty acids may be restricted in diet, but medium-chain triglyceride (MCT) oil is supplemented instead. Carnitine therapy is still controversial. Despite aggressive treatment, most patients still died in infancy [7]. However, there have been some patients who received early treatment with good outcomes [8,9]. Novel therapy for FAOD using bezafibrate, which is a hypolipedimic drug acting as a peroxisome proliferator-activated receptor (PPAR) agonist has been reported. The clinical trials of bezafibrate showed clinical improvement in adult patients with CPT2 deficiency [10], and a child with glutaric acidemia type 2 (GA2) [11]. *In vitro* probe acylcarnitine (IVP) assay can be used to evaluate FAO disorders [12], and determine the effect of bezafibrate [13]. We herein report the clinical findings of two unrelated cases with neonatal-onset CACT deficiency, and *in vitro* bezafibrate response using the IVP assay.

2. Patients and methods

2.1. Patients

2.1.1. Case 1

This patient was the first child of possibly consanguineous parents from the southern province of Thailand. He was born at 37 weeks of gestation with birth weight of 2460 g (25th percentile), length 48 cm (3rd percentile), and head circumference 30 cm (<3rd percentile). He developed hypothermia at 10 h of age. Sepsis was suspected, but the patient rapidly responded to rewarming treatment. However, after rooming-in with the mother, he developed hypothermia again. At 60 h after birth, he had cardiac arrest. On physical examination, no abnormalities were found. Serum glucose was 1.2 mmol/L and acetoacetate was 0 mmol/L. Venous blood pH was 7.24 and serum bicarbonate was 13 mmol/L with an anion gap of 20. Plasma ammonia was 471 μmol/L (normal, <110 μmol/L). There were mildly elevated liver enzymes aspartate aminotransferase (AST) (97 U/L; normal, 0–32) and alanine aminotransferase (ALT) (78 U/L; normal, 0–33). Serum creatine kinase was 4439 U/L (normal, <190). He had a good response to treatment with intravenous glucose administration. Urine organic acids were unremarkable. A dried blood spot acylcarnitine profile by tandem mass spectrometry (MS/MS) showed free carnitine (C0), 5.26 μM (10–60); C16-acylcarnitine, 14.14 μM (0.6–7); C18-acylcarnitine, 2.71 μM (0.15–2.1); C18:1-acylcarnitine, 4.3 μM (0.3–3.2); and a (C16 + C18)/C0 ratio, 3.21 (0.007–0.5). The profile was consistent with CPT2 or CACT deficiency. The patient has been treated with a modular medical formula, which has been composed of modified fats (long-chain fatty acid restriction along with supplementation of 83% of fat as medium-chain triglyceride oil), protein, maltodextrins, minerals, and fat-, and water-soluble vitamins. L-Carnitine at a daily dosage of 100–150 mg/kg has been supplemented. Thereafter, he has had several episodes of hypoglycemia, hyperammonemia, and metabolic acidosis following infections. At 8 months of age, he developed cholestasis and hepatomegaly. At 9 months of age, an echocardiogram revealed hypertrophic cardiomyopathy. At the age of 15 months, he had mild developmental delay and generalized hypotonia. He could stand with support, put block in cup, and say one word. Then he had a metabolic crisis, and developed generalized weakness. After he recovered from encephalopathy, neurologic examination revealed normal cranial nerves, muscle weakness (grade 3/5), and decreased muscle tone and deep tendon reflexes (1+) in all extremities. A brain computed tomography scan was normal. Serum creatine kinase was elevated (1419 U/L). A nerve conduction study showed no evidence of demyelination. He had been ventilator-dependent since then. At 2½ years of

age, he had several complications including chronic liver disease, upper gastrointestinal bleeding, and osteoporosis. He died at the age of 2 years and 8 months from upper gastrointestinal bleeding and metabolic decompensation.

2.1.2. Case 2

The patient was the first child of nonconsanguineous parents. She was born at 35 weeks of gestation with a birth weight of 2.3 kg (50th percentile), length 44 cm (25th percentile), and head circumference 30 cm (10th percentile). At 2 days after birth, she developed lethargy, poor feeding, and cardiac arrest. Blood glucose was 0.56 mmol/L. She responded to cardiac resuscitation and intravenous glucose infusion. Serum acetoacetate was 0 mmol/L. Venous blood pH was 7.39 and serum bicarbonate was 13 mmol/L with an anion gap of 20. Plasma ammonia was 157 μmol/L (normal, <110 μmol/L). There were elevated liver enzymes AST (638 U/L; normal, 0–32) and ALT (83 U/L; normal, 0–33). Plasma lactate dehydrogenase (LDH) was 522 U/L (normal, 240–480). An echocardiogram revealed no cardiomyopathy. A dried blood spot acylcarnitine profile by MS/MS analysis showed C0, 13.8 μM (10–60); C16-acylcanitine, 15 μM (0.6–7); C18-acylcarnitine, 4.3 μM (0.15–2.1); C18:1-acylcarnitine, 5.9 μM (0.3–3.2); and a (C16 + C18)/C0 ratio, 1.4 (0.007–0.5). The profile was consistent with either CPT2 or CACT deficiency. The patient had been treated with a high-MCT formula (Portagen®, Mead Johnson Nutritionals), and 100 mg/kg/day of L-carnitine. At 1 month of age, she developed anemia from Hb AE Bart's disease – a thalassemia intermedia resulting from the interaction between α-thalassemia and heterozygous Hb E, which required monthly blood transfusion. At the age of 4 months, she had poor feeding and cardiac arrest. Blood glucose was 0.5 mmol/L. The patient died without any response to resuscitation. An autopsy revealed left ventricular hypertrophy, micro/macrovesicular steatosis of the liver with focal areas of bridging fibrosis, and abnormal lipid accumulation in skeletal muscles and the proximal renal tubules.

2.2. Materials and methods

This study was approved by the Siriraj Institutional Review Board. The written informed consents for the mutation analysis, IVP assay, and bezafibrate trial were obtained from the parents. Genomic DNA was extracted from leukocytes. Mutation analyses of the *CPT2* and *SLC25A20* genes were performed in case 1, and only *SLC25A20* gene in case 2. All coding exons and their flanking intron sequences (up to 20 bases for both sides) of the *CPT2* and *SLC25A20* genes were PCR-amplified and directly sequenced according to the previously described method [14]. The IVP assay was performed using the skin fibroblasts in the absence and presence of bezafibrate according to the previously described method [11].

3. Results

3.1. Mutation analysis and IVP assay

Mutation analysis of the *SLC25A20* gene identified homozygous c.199-10T>G (IVS2-10T>G) mutation in both patients, and heterozygous mutation in their parents (Fig. 1). Mutation analysis of the *CPT2* gene revealed no pathogenic mutation in Case 1. The IVP assay profiles revealed increased C16, C16:1 acylcarnitines, and decreased C2 (acetylcarnitine) indicating a typical pattern of CPT2 or CACT deficiency, with substantial reduction of long-chain acylcarnitines by the presence of bezafibrate in the cultured fibroblasts from both patients (Fig. 2). However, C2 acylcarnitine did not increase as expected.

3.2. Clinical trial of bezafibrate

We started a clinical trial of bezafibrate in case 1 at age of 2 years and 2 months, after the IVP assay which

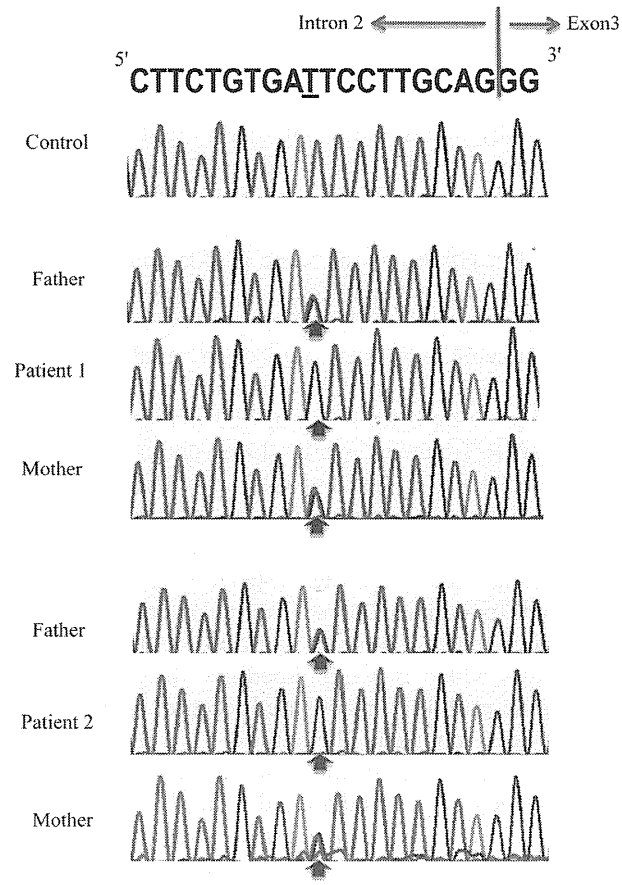

Fig. 1. The reference DNA sequence of an intron 2/exon 3 boundary of the *SLC25A20* gene, and the IVS2-10T>G mutation identified in both patients and their parents denoted by black arrows and the underlined letter.

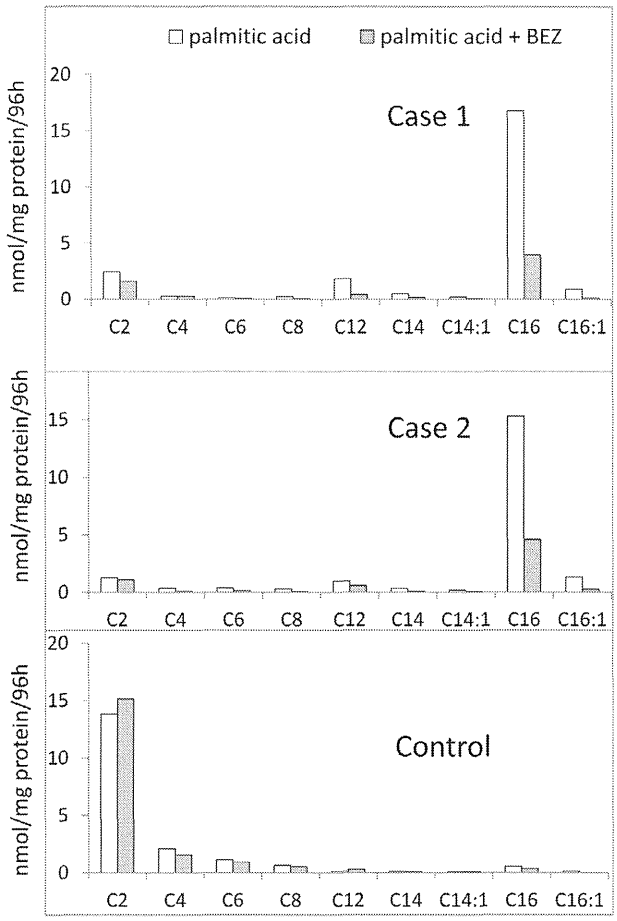

Fig. 2. Acylcarnitine profiles of IVP assay in the presence and absence of bezafibrate (BEZ) of cases 1, 2, and normal control respectively. Unit of vertical lines, nmol/mg protein of acylcarnitines (ACs); the horizontal lines represent acylcarnitines from C2, C4, C6, C8, C12, C14, C14:1, C16, and C16:1. The experiments for each were performed in triplicate, and the mean values of ACs are illustrated with bars.

showed some improvement in acylcarnitine profiles with bezafibrate. We used a dosage of 17–25 mg/kg/day as previously described [11]. Monitoring of liver functions, lactate dehydrogenase (LDH), creatine kinase (CK), and lipid profiles showed no adverse effects of bezafibrate. A short-term evaluation, after 6 months of the trial, did not show clinical improvement except for slightly increased back muscle strength noted by the mother. An echocardiography showed stable but no improvement in a left ventricular mass index. Acylcarnitine profiles in dried blood spots and other biochemical parameters did not show improvement (data not shown). Case 2 died before a clinical trial was considered.

4. Discussion

We report 2 unrelated cases of CACT deficiency with molecular confirmation first identified in Thailand. The c.199-10T>G (IVS2-10T>G) nucleotide change was the most prevalent mutation and identified in 14 out of 76 mutant alleles [15]. This mutation was homozygously identified in three Vietnamese and three Chinese patients. In the present study, in spite that two families had no consanguineous history, both patients were also a homozygotes of the c.199-10T>G mutation. In Japan, three CACT deficient patients have been described. Among them the same mutation was identified heterozygously in only one patient [14]. We propose that this mutation is a founder mutation in Asian populations. Clinical history of the three Chinese patients with homozygous c.199-10T>G mutation were reported [16]. All of them developed cardiac arrest within two days of age, as well as our two patients. Hence the phenotype of homozygotes of c.199-10T>G mutation is severe. This mutation was suggested to reside at a consensus lariat branch point sequence resulting in skipping of exons 3 and 4 or exon 3 alone, which leads to truncation of the protein [17].

Although our cases 1 and 2 were homozygotes of the same mutation, Case 1 survived until 2 years and 8 months and Case 2 died at 4 months of age. Several factors might attribute to their different clinical outcomes: (1) Thalassemia disease in case 2 which required repeated blood transfusions might affect cardiac functions by chronic hypoxia, iron overload, or decreased carnitine [18]; (2) differences in possible modifier genes such as *SLC25A29* gene (CACT-like, CACL) which has palmitoyl-carnitine transporting activity [19]; and (3) different formulas using in our cases, one is a synthetic modular formula and the other is a commercial formula. However, the rationale of both special formulas for diet therapy is a reduction in long-chain fatty acids together with supplementation of medium-chain triglyceride oil to be a caloric source shunting an obstruction of long-chain fatty acid β-oxidation.

Although increased FAO flux induced by bezafibrate was clearly shown in fibroblasts only from patients with mild phenotypes of FAO disorders, increased mRNA expression after bezafibrate exposure also occurred in cell lines from patients with severe phenotypes [20]. This could explain *in vitro* response to bezafibrate observed in fibroblasts of patient 1 and 2. Despite the severe genotype leading to barely detectable enzyme activity [21], we believe that there should be some FAO flux which could be enhanced by bezafibrate in these patients. Our hypothesis is if there is entirely absent FAO flux in these patients, they should have anomalies like those found in a lethal neonatal form of CPT2 deficiency or GA2 [22], even though there has been no report of such findings in CACT deficiency. To our knowledge, patient 1 is the first case of neonatal-onset CACT deficiency who underwent a clinical trial of bezafibrate after showing an *in vitro* response by IVP assay. However, no beneficial short-term effect was shown. This might indicate the irreversible damage of the affected organs esp. the cardiac and skeletal muscles, and liver. Moreover, the difference between the *in vitro* and *in vivo* responses is

probably due to the difference of bezafibrate concentration used in the IVP assay (400 μmol/L) and typical concentrations obtained in patients on bezafibrate therapy (50–200 μmol/L) [23]. Another possible reason is inadequate acetyl-CoA production despite bezafibrate treatment. This hypothesis is supported by persistently low C2 acylcarnitines in IVP assays of our cases and a previous case with CACT deficiency [11]. Moreover, C16 acylcarnitine did not decrease to the control level after bezafibrate treatment. Overall, although some improvement of acylcarnitine profile was shown in the patient 1 and 2's fibroblasts in IVP assay with bezafibrate, the effect of bezafibrate was less than those in fibroblasts from patients with mild forms of FAO disorders [11,24]. Hence clinical improvement in this patient was thought to be limited. Since CACT-deficient patients who developed metabolic decompensation in early neonatal period had poor prognosis with routine management [7], we decided to use bezafibrate treatment in patient 1. He survived until two years of age with bezafibrate treatment. However, it is uncertain whether this longer survival owed to the effect of bezafibrate treatment or not, since no apparent improvement of clinical laboratory data was obtained.

In conclusion, CACT deficiency may be a common FAO disorder in East Asian populations probably from a founder effect. IVP assay of fibroblasts could determine a response to bezafibrate treatment. A long-term clinical trial and more enrolled patients are required for evaluation of this therapy.

Acknowledgements

We would like to thank the patients and their families for their participation in this study. N.V. is a recipient of the Siriraj Chalermprakiat Fund.

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Brain & Development xxx (2015) xxx–xxx

BRAIN & DEVELOPMENT
Official Journal of the Japanese Society of Child Neurology

www.elsevier.com/locate/braindev

Original article

Surgical versus medical treatment for children with epileptic encephalopathy in infancy and early childhood: Results of an international multicenter cohort study in Far-East Asia (the FACE study)

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Received 25 August 2015; received in revised form 11 November 2015; accepted 13 November 2015

Summary

Objective: To compare the seizure and developmental outcomes in infants and young children with epileptic encephalopathy who have undergone surgical and medical treatments.

Methods: An international, multicenter, observational cohort study was undertaken. A total of 317 children aged <6 years, who had frequent disabling seizures despite intensive medical treatments, were registered. Among the enrolled children, 250 were treated medically (medical group), 31 underwent resective surgery (resective group), and 36 underwent palliative surgery [callosotomy ($n = 30$) or vagal nerve stimulation ($n = 6$); palliative group] on admission. Seizure and developmental outcomes were obtained for 230 children during the 3-year follow-up period. Cox proportional hazard model was used to adjust for clinical backgrounds among treatment groups when comparing the seizure-free survival rates.

Results: At the 3-year follow-up, seizure-free survival was 15.7%, 32.1%, and 52.4% in the medical, palliative, and resective groups, respectively. The adjusted hazard ratios for seizure recurrence in the resective and palliative groups versus the medical group

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http://dx.doi.org/10.1016/j.braindev.2015.11.004

Please cite this article in press as: Otsuki T et al. Surgical versus medical treatment for children with epileptic encephalopathy in infancy and early childhood: Results of an international multicenter cohort study in Far-East Asia (the FACE study). Brain Dev (2015), http://dx.doi.org/10.1016/j.braindev.2015.11.004

were 0.43 (95% CI, 0.21–0.87, $P = 0.019$) and 0.82 (95% CI, 0.46–1.46, $P = 0.50$), respectively; the former was statistically significant. Regarding the developmental outcome, the mean DQs in the resective group increased significantly compared to those in the medical group during the follow-up ($P < 0.01$). As for subgroup analysis, better seizure and development outcomes were demonstrated in the resective group compared to the medical group in children with nonsyndromic epilepsies (those to which no known epilepsy syndromes were applicable).

Significance: These results suggest that surgical treatments, particularly resective surgeries, are associated with better seizure and developmental outcomes compared with successive medical treatment. The present observations may facilitate the identification of infants and young children with epileptic encephalopathy who could benefit from surgery.

Keywords: Epileptic encephalopathy; Epilepsy surgery; Prospective cohort study; Seizure outcome; Developmental quotient

1. Introduction

Intractable epilepsy during infancy and early childhood is characterized by frequent seizures and epileptic encephalopathy, and often results in a progressive and severe developmental delay [1–3]. The etiology varies and includes cortical dysplasia, perinatal insults, dysplastic tumors, genetic abnormalities, or unknown causes [4]. Although it was reported that surgical intervention in early life could resolve epileptic encephalopathy and improve the developmental prognosis in some cases [5–9], no systematic cohort study has been undertaken to date.

The pathophysiology of epileptic encephalopathy in infancy and early childhood may be multifactorial. Because the resection of focal cortical epileptogenic pathology in children often ameliorates epileptic encephalopathy, the presence of abnormal underlying cortical–subcortical–cortical circuits has been proposed [10,11]; i.e., cortical epileptic activities alter subcortical function, which in turn results in a widespread, generalized functional disturbance in otherwise normal cortices. In fact, bilateral generalized neocortical hyperexcitability was demonstrated by producing a unilateral epileptogenic lesion in an animal model of infantile spasms [12]. Alternatively, epileptic encephalopathy may arise from the cortical interneuron dysfunction that is caused by genetic abnormalities, including mutations in the sodium channel, voltage-gated type I alpha subunit (SCN1A), and aristaless-related homeobox X-linked gene (ARX) [13,14].

Epileptic encephalopathy could itself cause the progressive deterioration of cerebral function. Spontaneous seizures in early life may result in maladaptive synaptic plasticity, which in turn could produce imbalances in excitation and inhibition that contribute to learning and behavioral difficulties [15]. Prolonged epileptic activity in electrical status epilepticus during sleep has been reported to interfere with normal physiological sleep processes, and may disrupt normal cortical processing and alter synaptic connections [16].

To select the most appropriate surgical candidates among children with epileptic encephalopathy, it is necessary to compare the outcomes between surgical and medical treatment in a controlled cohort study [17]. However, such children are generally unsuitable for controlled studies because they require urgent multidisciplinary treatment; furthermore, the condition is rare, which results in treatment being concentrated to specialized pediatric epilepsy centers [18]. Therefore, we performed an observational cohort study of infants and young children with epileptic encephalopathy who were treated at major pediatric epilepsy centers in East Asia. Because patient demography has already been reported [19], this study mainly focused on the seizure and developmental prognoses of these children.

2. Methods

2.1. Study design

An international and multicenter observational cohort study, namely, the Far-East Asia Catastrophic Epilepsy (FACE) study was undertaken in children with medically intractable epilepsy. These children were admitted to 12 collaborating pediatric epilepsy centers in three East Asian countries between April 1, 2009 and March 31, 2010. Children aged <6 years on admission with a history of frequent disabling epileptic seizures (at least 10/month), which were not suppressed (or that were recurrent) despite intensive medical treatment with more than two major antiepileptic drugs (AEDs), adrenocorticotropic hormone (ACTH), or a ketogenic diet before admission, were enrolled. Diagnostic examinations, including electroencephalography (EEG), magnetic resonance imaging (MRI), and developmental assessments were performed routinely in all patients. Patients with nonepileptic conditions, atypical forms of benign epilepsy, severe physical comorbidities, and those with a history of surgery for epilepsy were excluded.

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Following approval of the institutional review boards of the participating institutions, patients were registered and written informed consent was obtained between July 1, 2009 and March 31, 2010. Follow-up surveys were completed 1- and 3-years after discharge between April 1, 2010 and September 30, 2013.

2.2. Investigation format

All data were evaluated in each hospital according to the study protocol. Anonymous data were collected digitally in an Excel™ data form developed for this study. The collected data included epilepsy syndrome, seizure type and frequency, EEG findings, etiology, MRI findings, psychomotor development, medical and surgical treatment undertaken, and complications. Details of the format were reported previously [19].

2.3. Epilepsy syndrome

The epilepsy syndrome was classified based on the International League Against Epilepsy classification [20]. The term "nonsyndromic epilepsies" was used to define epilepsies for patients in whom no corresponding established epilepsy syndrome was identified (http://www.ilae.org/Comission/Class). This term included both neocortical and unclassified epilepsies in this study.

2.4. Seizure assessment

For each patient, seizure type and seizure frequency of the most disabling seizure was assessed. Seizure frequency was categorized into the following 6 classes: 1. >10/day, 2. daily (>1/day), 3. weekly (1–6 days/week), 4. monthly (1–4 days/month), 5. yearly (>1–11 days/year) and 6. (<1 day/year). At follow-up, seizure outcome was evaluated based on ILAE classification. Seizure frequency of the sixth category (<1 day/year) corresponded to ILAE class 1.

2.5. Developmental assessment

The developmental status of all patients was evaluated using the Vineland Adaptive Behavior Scale, Kinder Infant Development Scale, Enjoji Scale of Infant Analytical Development, and Tanaka–Binet test. To address the difference in the availability of assessment scales among countries, we developed a specific assessment scale (the FACE developmental scale) for this study by integrating the established development assessments. This developmental scale contained an assessment of communication, daily living, socialization, and motor skills (Supplementary Table 1A). The correlation of this scale with other established developmental scales, including the Vineland Adaptive Behavior Scale, was validated using data obtained at the time of registration (Supplementary Table 1B).

The FACE developmental scale was used to calculate the developmental quotient (DQ). The DQ was defined as the ratio of developmental age (DA) in months divided by the calendar age (CA) in months multiplied by 100 ($DQ = [DA/CA] \times 100$). Because the developmental scale measured DA from 0 to 67 months, children who were expected to reach a DA of 67 months during the study (i.e., children with a DA > 31 months on admission) were excluded from the analysis of sequential DQ changes over 3 years.

The gross developmental status of patients before onset was determined from assessments made by individual physicians in clinical records, and categorized as borderline to normal (corresponding to a DQ of $\geqslant 70$), mild to moderate delay (DQ of 35–69), and severe delay (DQ of <35).

2.6. Comparison among treatments

Patients were categorized into three groups based on the treatment they received at initial admission: a medical group (medical treatment only), a resective group (resective epilepsy surgery), and a palliative group [palliative surgery, i.e., callosotomy or vagal nerve stimulation (VNS)]. The seizure and developmental outcomes were compared among the three groups.

2.7. Statistical analysis

Kaplan–Meier analysis was undertaken to assess the seizure-free survival time during the follow-up period using the seizure outcome data and the interval from discharge to the follow-up date. Cox proportional hazard models were used to adjust for the different clinical backgrounds of the treatment groups when comparing seizure-free survival rates. Changes in the DQ during the follow-up period were compared using analysis of covariance (ANCOVA) adjusted for the baseline DQ. Statistical analyses were performed using SAS V9.2 (SAS Institute Inc., Cary, NC, USA) and IBM SPSS 22.0 (IBM Corp, Armonk, NY, USA).

3. Results

A total of 317 children (184 boys and 133 girls) were registered into the study. Among these, 250 were treated medically (medical group), 36 underwent palliative surgery (palliative group), and 31 underwent resective surgery (resective group) at initial admission. The seizure and developmental outcomes were obtained for 281 children (88.6%) at 1-year follow-up, and 230 children (72.6%) at 3-year follow-up (179 in the medical group, 29 in the palliative group, and 22 in the resective group).

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3.1. Pre-admission medical treatment

Before initial admission patients were administered a mean of 3.6 AEDs [standard deviation (SD), 2.4]. ACTH treatment was used in 54 patients (17.0%), and 24 patients (7.6%) received a ketogenic diet. Other medical agents, including oral steroids, liposteroids, thyrotropin-releasing hormone (TRH), and gammaglobulin, were administered to 24 patients (7.6%).

3.2. Diagnostic evaluations on admission

Interictal EEGs were obtained for all children, and ictal video-EEGs were recorded in 243 children (76.7%). MRI was conducted in 310 children (98.1%), of whom 80 (25.7%) underwent high magnetic field ($\geqslant$3 Tesla) MRI, 229 (73.6%) underwent intermediate magnetic field (1.0–1.5 Tesla) MRI, and two (0.6%) underwent low magnetic field ($\leqslant$0.5 Tesla) MRI. Interictal and ictal single photon emission computed tomography was performed in 124 children (39.1%) and 50 children (15.8%), respectively. Fluorodeoxyglucose positron emission tomography and magnetoencephalography were conducted in 34 (10.7%) and 33 children (10.4%), respectively, in specific institutions. Genetic analysis for the SCN1A gene was undertaken in 13 children (4.1%).

3.3. Patient demographics

3.3.1. Age

The mean age at seizure onset was 10.2 months (SD, 12.8) in the medical group, 12.4 (SD, 17.9) in the resective group, and 8.2 (SD, 6.9) in the palliative group (Table 1). There were no statistically significant differences in the age at seizure onset among treatment groups.

3.3.2. Epilepsy syndrome

In the medical group, regarding epilepsy syndromes, West syndrome was most common (accounting for 37.2%) followed by Lennox–Gastaut syndrome (8.8%), Dravet syndrome (5.6%) and other syndromic epilepsies (6.8%). On the other hand, non-syndromic epilepsies such as neocortical and unclassified epilepsies accounted for 41.6%. In the palliative group, West syndrome was most common (accounting for 50.0%), followed by Lennox–Gastaut syndrome (27.8%) and non-syndromic epilepsies (19.5%). In the resective group, non-syndromic epilepsies were the most common (51.7%), followed by Lennox–Gastaut syndrome (16.1%) and West syndrome (9.7%).

3.3.3. Seizure types, EEG findings, etiology, and MRI findings

Regarding seizure types, epileptic spasms were the most common type of seizure in the medical group (accounting for 39.2%), followed by generalized tonic seizures (18.4%). In the palliative group, the percentage of both seizure types was comparable (36.1%), whereas generalized tonic seizures were the most common in the resective group (25.8%), followed by epileptic spasms (19.4%).

Regarding the major EEG findings on admission, hypsarrhythmia was the most common in the medical group (28.8%), followed by bilateral multifocal epileptic activities (23.6%). In the palliative group, generalized slow spike-wave burst was the most common EEG finding (58.3%), followed by bilateral multifocal epileptic activities (16.7%) and bilateral generalized synchronous epileptic activities (16.7%). In the resective group, generalized slow spike-wave burst (25.8%) and bilateral multifocal epileptic activities (25.8%) were the most common.

Although the etiology was unknown in most cases (44.4%), genetic or chromosomal abnormalities were predominant in the medical group (13.6%). In contrast, the most common etiology in the palliative and resective groups was cortical dysplasia (22.2% and 45.2%, respectively).

Bilateral abnormalities were the most common MRI findings in the medical and palliative groups (46.4% and 41.7%, respectively). In contrast, focal or unilateral abnormalities were the most common findings in the resective group (80.6%).

3.4. Treatment

3.4.1. Medical group

Among the 250 patients in the medical group, a mean of 2.9 AEDs (SD, 1.5) was administered during admission. In addition, ACTH (mean total dose, 0.4 mg/kg) was used in 11.9% of cases, and a ketogenic diet was administered to 10.3% of cases. Other medical agents, including oral steroids, liposteroids, TRH, and gammaglobulin, were administered to 11.1% of all patients.

3.4.2. Resective group

Among the 31 patients in the resective group, 10 hemispherectomies, five multilobar resections, 10 lobectomies, and eight focal resections were undertaken.

3.4.3. Palliative group

Among the 36 patients in the palliative group, 30 callosotomies (28 total and two anterior two-thirds), and six VNSs were performed.

3.5. Additional epilepsy surgery

During the follow-up period after discharge, additional epilepsy surgery was undertaken in 24 patients, including 16 patients in the medical group, five patients in the resective group, and three patients in the palliative group. In the medical group, 10 patients required

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Table 1
Patient demographics in each treatment group.

Treatment group	Medical group	Resective group	Palliative group
Total	250	31	36
Sex			
Male	138	19	27
Female	112	12	9
Age of seizure onset (month)			
n	250	31	36
Mean (SD)	10.2 (12.8)	12.4 (17.9)	8.2 (6.9)
Median	5	2	6
Min, max	0, 68	0, 63	0, 32
Age of initial AED treatment (month)			
n	250	31	36
Mean (SD)	12.2 (14.4)	14.4 (18.0)	11.0 (8.7)
Median	7	4	8
Min, max	0, 75	0, 63	2, 39
Age of admission (month)			
n	250	31	36
Mean (SD)	28.3 (21.1)	36.6 (24.5)	39.2 (18.2)
Median	23	38	37
Min, max	0, 83	2, 71	7, 69
Epilepsy syndrome on admission			
West syndrome	93	3	18
Lennox–Gastaut syndrome	22	5	10
Ohtahara syndrome	3		
Dravet syndrome	14		
Doose syndrome	4		
Rasmussen syndrome	3	3	
Sturge–Weber syndrome	1	1	
Other epilepsy syndromes	6	3	1
Nonsyndromic epilepsies	104	16	7
Major etiology			
Hemimegalencephaly	1	1	
Cortical dysplasias	29	14	8
Genetic/chromosomal abnormalities	34	5	3
Tumors		2	1
Vascular lesions	12	1	
Infection	8	4	2
Head trauma	3		
Hypoxic encephalopathy	33	1	2
Others	19		3
Unknown	111	3	17
Seizure type on admission (overlapped)			
Epileptic spasms	108	6	15
Head nodding	27	3	12
Generalized tonic	67	10	18
Generalized clonic	10	2	
Generalized tonic clonic	108	10	13
Generalized absence (atypical/typical)	25	1	7
Generalized myoclonic (positive/negative)	34	1	3
Generalized atonic	6	1	2
Partial simple motor	20	4	1
Partial complex motor	73	6	
Hypomotor seizure	7	5	4
Gelastic seizures	2	3	
EEG findings on admission (overlapped)			
Suppression-burst (EIEE)	7		
Hypsarrhythmia	74	3	3
Slow spike-wave burst	39	8	21

(*continued on next page*)

Please cite this article in press as: Otsuki T et al. Surgical versus medical treatment for children with epileptic encephalopathy in infancy and early childhood: Results of an international multicenter cohort study in Far-East Asia (the FACE study). Brain Dev (2015), http://dx.doi.org/10.1016/j.braindev.2015.11.004