kinase concentration was extremely high (45149 IU/L) on the day of birth without any anomaly. Serum lactate level, plasma amino acid profiles, and carnitine profiles were normal. Urinary organic acid profiles showed no specific abnormalities. The patient suddenly suffered from severe lactic acidosis, hyperglycemia, and acute heart failure at day 17. Levels of lactate and pyruvate in the CSF were 4.9 mM and 0.21 mM. A mitochondrial disorder was suspected and treatment was started with carnitine, ubiquinone, and other vitamins in addition to cardiotonics and insulin. The infant's condition improved, but he subsequently presented with poor feeding, muscle weakness, and hypotonia at 1 month. Hypertrophic cardiomyopathy occurred at 3 months and cardiopulmonary function worsened after repeated lactic acidosis, and he required mechanical ventilation from the age of 6 months. He presented with an enlarged head circumference and a tense anterior fontanelle at 12 months, and died of pneumonia at 17 months.

Magnetic resonance imaging (MRI)at 2 months revealed cerebellar cysts, pachygyria, and T2-hyperintense lesions in white matter and the brainstem, but basal ganglia were normal (Fig. 1A). A follow-up investigation at 4 months indicated extended T2-hyperintense lesions (Fig. 1B). A brain computed tomography (CT) scan at 14 months showed severe hydrocephalus and extensive cerebral atrophy (Fig. 1C).

Cerebellar cysts and pachygyria are characteristic of FCMD, genetic testing for *FCMD* was performed. We examined retrotransposal insertion into the 3′-untranslated region (UTR) of the *FCMD* gene using a polymerase chain reaction (PCR)-based diagnostic method involving peripheral blood leukocytes of this case and his parents [1]. A homozygous mutation of this case and heterozygous mutation of his parents were detected. Repeated lactic acidosis and brain stem lesions led us to suspect LS. A skin biopsy was performed for mitochondrial analysis at 1 month. Activities of mitochondrial respiratory chain complex (Co) I, II, III, and IV were assayed from skin fibroblasts, as described previously [3]. The activities were also calculated as the percent relative to citrate synthetase (CS), a mitochondrial enzyme marker and to Co II activity, and evaluated according to the diagnostic criteria [4]. Respiratory chain complex I and II activities were very low, but CS, Co III, and Co IV activities were normal (Table 1). Expression of the mitochondrial respiratory chain CoI, II, III, and IV proteins was concurrently examined by Western blotting

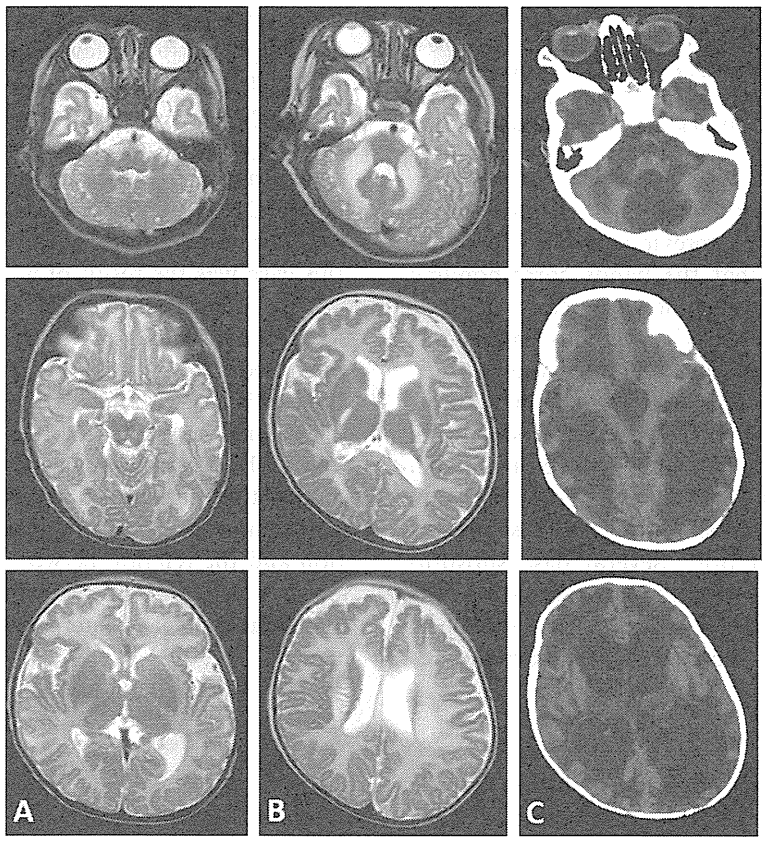

Fig. 1. Magnetic resonance image (MRI) at the age of 2 months (A) shows cerebellar cysts (A, top), bilateral symmetrical lesions in the brainstem (A, middle), pachygyria, and T2-hyperintensity in white matter, predominantly in the frontal lobes (A, bottom). An MRI at 4 months of age indicated T2-hyperintensity extending into the middle cerebellar peduncles, posterior limb of the internal capsule, and the corona radiata (B). A brain computed tomography scan at 14 months of age showed severe hydrocephalus, widespread hypodensity of white matter, and extensive cerebral atrophy (C).

Table 1
Activities of mitochondrial respiratory chain complex (Co) I, II, III, and IV; citrate synthase (CS) from skin fibroblasts. Enzyme activities are expressed as a percentage of mean relative activity of 35 normal controls and relative to CS and Co II.

	Co I	Co II	Co II + III	Co III	Co IV	CS
Crude activity (%)	32	18	21	56	45	80
CS ratio (%)	38	21	24	65	55	
Co II ratio (%)	177		112	312	259	

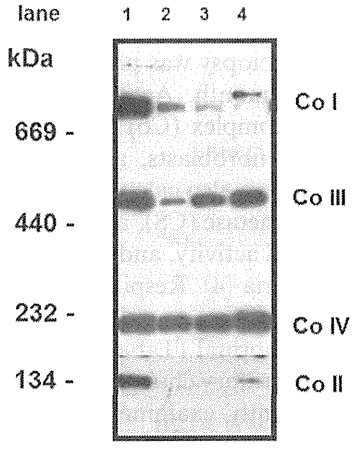

Fig. 2. Blue native polyacrylamide gel electrophoresis (BN-PAGE) analysis of skin fibroblasts 1: control; 2: this case; 3: a double of lane 2; 4: a triple of lane 2. The bands corresponding to Co II were almost invisible and those corresponding to Co I were markedly weak, whereas the intensities of the Co III and IV bands gradually became strong.

using blue native polyacrylamide gel electrophoresis (BN-PAGE), as described previously [5]. The BN-PAGE analysis showed that the bands corresponding to Co II were almost invisible and those corresponding to Co I were markedly weak (Fig. 2). This finding was in agreement with the enzyme activity assay results. The mitoSEQr™ system (Applied Biosystems, Foster City, CA, USA) was used for the entire mitochondrial DNA analysis. Genomic DNA was extracted from skin fibroblasts. Data were analyzed with SeqScape Software v2.5 and compared with mitochondrial DNA sequences (Mitomap: www.mitomap.org). Several base substitutions were detected, but no pathogenic mutation was detected in the entire mitochondrial DNA sequence. High resolution chromosome analysis was normal.

2.2. Family history

The index case's older brother had repeated afebrile convulsions since the age of 2 months, but brain MRI findings were normal. Laboratory tests showed elevated level of lactate (2.92 mM) in CSF and a normal level of serum creatine kinase (75 IU/L). He died of sudden cardiac dysfunction at 4 months. The second child was a healthy girl with normal development.

3. Discussion

A case of FCMD and mitochondrial respiratory chain disorder (MRCD) has never been reported. The pathophysiology of FCMD and MRCD is quite different, therefore, low activities of the respiratory chain complexes in this case were probably not due to FCMD. LS is clinically characterized by a wide variety of manifestations involving multiple organs in infancy or early childhood. Thus, the early onset of his symptoms suggested that LS was the main cause.

White matter abnormalities in patients with FCMD are often detected by MRI as transient T2-hyperintensity. Kato et al. reported that the pathological origin of white matter lesions is dysmyelination and that the lesions are masked by brain development [6]. In this case, the extended signal abnormalities had different features compared with those of FCMD. Some cases of complex II deficiency with extensive T2-hyperintensities in white matter have been reported [7,8]. The white matter abnormalities in our case may have been associated with the complex II deficiency. The patient presented with progressive hydrocephalus, but he had no prior clinical signs of intraventricular hemorrhage or infection in CSF. Patients with FCMD, who are homozygotes for the insertion mutation with hydrocephalus have never been reported [9]. A few patients with LS develop cerebellar atrophy or ventricular enlargement [10].

Many cases of combined complex deficiencies have been reported, but a case with a complex I + II deficiency has rarely been reported. The entire mitochondrial DNA sequencing in this case showed no pathogenic mutation. These findings suggest that LS in this case was the result of a nuclear gene mutation.

The genes responsible for mitochondrial disease located contiguous to the FCMD gene have not been identified. The infant's older brother was suspected to have MRCD without obvious clinical signs of FCMD. Therefore, we speculated that the present case was unlikely to be a contiguous gene syndrome. We are investigating this patient's fibroblasts using next-generation sequencing to identify the causative nuclear gene mutation and the relation between the two diseases.

Potential conflict of interest report

The authors indicated no potential conflict of interest.

Acknowledgments

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Original article

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

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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.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

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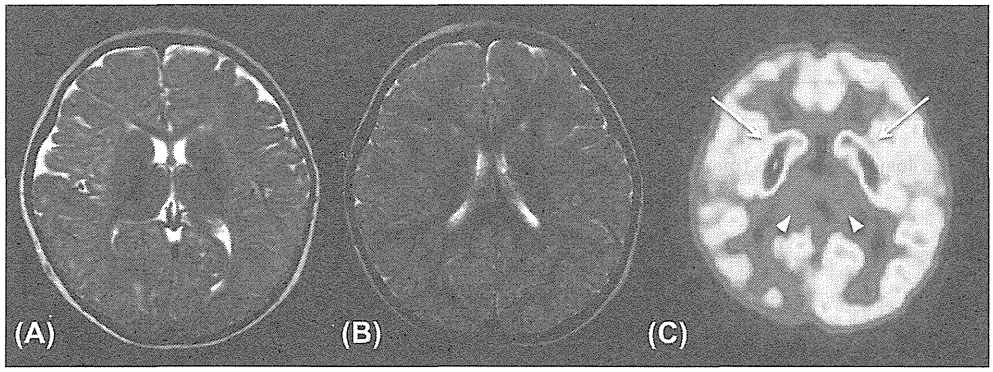

Fig. 1. MRI and FDG-PET (fludeoxyglucose-positron emission tomography) findings of a patient with GLUT-1 DS. (A) T2-weighted image at 12 months of age shows high intense signal in the widespread subcortical and deep white matters, suggesting delayed myelination. (B) T2-weighted image at 2 years and 9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