A modified Atkins diet is promising as a treatment for glucose transporter type 1 deficiency syndrome

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ABBREVIATIONS

GLUT1-DS	Glucose transporter type 1 deficiency syndrome
MAD	Modified Atkins diet

AIM Glucose transporter type 1 deficiency syndrome (GLUT1-DS) is a metabolic encephalopathy that can be effectively treated with a ketogenic diet. The aim of this study was to consolidate the effectiveness of the modified Atkins diet (MAD) as an alternative treatment for GLUT1-DS.
METHOD Six Japanese males with GLUT1-DS were selected for treatment with the MAD. Their age at the time the MAD was instituted ranged from 7 to 16 years and the duration of treatment ranged from 1 to 42 months. All participants had early-onset epilepsy. Each participant's neuropsychological activity, seizure frequency, neurological status, and electroencephalographic (EEG) findings were compared before and after the introduction of the MAD.
RESULTS After initiation of the treatment, all individuals showed +2 to +3 urinary ketosis on a ketostick test check. Epileptic seizures and other paroxysmal events decreased markedly in all individuals. Interictal EEG showed improvement in the background activity and disappearance of epileptic discharges. Along with an increased vigilance level, improvement in motivation and cognitive function was also achieved. Non-paroxysmal permanent ataxia, spasticity, dysarthria, and dystonia were moderately improved in four individuals and slightly improved in the remaining two. Preprandial transient aggravation of neurological symptoms completely disappeared in all participants. There were no significant side effects.
INTERPRETATION For the treatment of GLUT1-DS, the MAD is less restrictive, more palatable, and easier to maintain than the conventional ketogenic diet, but its effectiveness was similar. Thus, MAD treatment is promising for individuals with GLUT1-DS and their families.

Glucose transporter type 1 deficiency syndrome (GLUT1-DS, OMIM 606777) is a treatable metabolic encephalopathy caused by impaired glucose transport into the brain.[1–4] GLUT1-DS was first reported in 1991 by De Vivo et al.,[5] and is characterized by early-onset epilepsy, developmental delay, spasticity, ataxia, dystonia, and other paroxysmal phenomena such as abnormal eye movements.[1–4] The diagnosis of this disorder is suggested by an aggravation of these neurological symptoms due to fasting and/or fever, the presence of hypoglycorrhachia in the presence of a normal blood glucose level, and postprandial improvement in electroencephalographic (EEG) background activity. Molecular analysis of *SLC2A1* (GLUT1) or a 3-*O*-D-methylglucose uptake test is used to make a definite diagnosis of GLUT1-DS.[1–6] This syndrome can be treated effectively with a ketogenic diet, which provides ketones as an alternative fuel for the brain.[1–8]

The Atkins diet is a well-known diet for reducing body weight.[9] Through severe restriction of carbohydrate intake, body fat is believed to be consumed as a fuel source as a substitute for glucose. Since the first publication by Kossoff et al. in 2003, the modified Atkins diet (MAD) has been used in individuals with intractable epilepsy as an alternative to a conventional ketogenic diet.[10,11] The MAD is modified from the typical Atkins diet and consists of approximately 10% carbohydrates, 30% protein, and 60% fat without any restriction of calories or fluids.[10] We previously described the effectiveness of the MAD in a 7-year-old male with GLUT1-DS.[12] The present study included a greater number of individuals with GLUT1-DS and attempted to consolidate the effectiveness of the MAD.

METHOD

Participants

Six Japanese males with GLUT1-DS were included in the study (Table I). Their ages ranged from 8 years 7 months to 19 years 3 months (mean 13y 6mo). The age at onset of disease ranged from 2 months to 1 year (mean 6mo) and their age at diagnosis ranged from 6 years 9 months to 14 years 7 months (mean 9y 11mo).

The initial symptom of GLUT1-DS in these individuals was either abnormal episodic eye movements or infantile seizures. All individuals had early-onset epilepsy as well as some other paroxysmal events and a late-onset complex movement disorder with individual elements of ataxia, spasticity, and

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dystonia. Their cognitive function ranged from mild to profound intellectual disability. The ratio of cerebrospinal fluid to blood glucose ranged from 0.29 to 0.45. GLUT1-DS diagnosis was confirmed in participants 1, 2, 3, 5, and 6 by mutational analyses and in participants 3, 4, and 6 by 3-*O*-D-methylglucose uptake study.

Procedure

The age of participants at starting the diet ranged from 7 years 4 months to 16 years 9 months (Table II) and the duration of treatment ranged from 1 to 42 months (mean 19.6mo). In two individuals (participants 4 and 6) a medium-chain triglycerides–ketogenic diet (2:1) was changed to the MAD because the latter was less restrictive in terms of total proteins and calories consumed, and appeared to be more palatable.

All six individuals were introduced to the MAD following the protocol recommended by Kossoff and Dorward,[11] i.e. starting without a fasting period with no restriction of calories, fluids, or proteins, and with an initial limitation of 10g of carbohydrates per day, and encouraging fat intake. The ketogenic ratio of the MAD in our study stood at nearly 2.5 to 2.1:1. The study was approved by the ethics committee of Tokyo Women's Medical University and written informed consent was obtained from all parents/caregivers.

Analysis

Neuropsychological activity, seizure frequency, neurological status, IQ, and EEG findings were compared before, 1 month after, and 6 months after introduction of the MAD in five individuals, and before and 1 month after in the remaining individual (participant 5) because of a short follow-up period.

To determine neuropsychological activity, we asked parents or caregivers to assess vigilance level (i.e. level of general awareness, alertness or responsiveness), comprehension, concentration, and motivation in daily life as worse, no change, improved, or markedly improved. Neurological status (including ataxia, spasticity, gait disturbance, kinetic dystonia, and slurred speech) was also rated by neurological examination as worse, no change, improved, or markedly improved.

IQ was evaluated by the Wechsler Intelligence Scale for Children – Third Edition in children aged 5 years and over, by the Tanaka–Binet Intelligence Scale (the Japanese version of the Stanford–Binet test) in children aged 2 years and over, and by the Owaki Intelligence Test and the Tsumori-Inage Developmental Questionnaire, which is a scale used in Japan, in very young children. IQ values were compared before and after the MAD in four individuals.

What this paper adds

- In this case series, the modified Atkins diet (MAD) achieved sufficient ketosis to have a therapeutic effect.
- In combination with a single antiepileptic drug, the MAD fully controlled epileptic seizures.
- Treatment with the MAD improved cognitive function significantly in at least two of this study's younger participants.
- The paper shows that the MAD was readily accepted by the participants and their families and tolerable for long-term application.

Table I: Clinical summary of the six individuals with GLUT1-DS

Participant	1	2	3	4	5	6
Age (y:mo)	8:7	10:10	14:11	15:1	15:2	19:3
Sex	M	M	M	M	M	M
Age at diagnosis (y:mo)	7:5	7:0	6:9	12:2	14:7	11:7
Age at onset (y:mo)	1:0	0:4	0:4	0:8	0:6	0:2
Clinical presentation						
Abnormal episodic eye movement	–	Rotatory nystagmus[a]	–	Horizontal or rotatory nystagmus[a]	Opsoclonus[a]	–
Epilepsy	+	+	+	+	+	+
Age at onset (y:mo)	2:10	0:7	0:4	2	1:9	0:2
Initial seizure type	PS	AS	MS[a]	MS	PS	MS[a]
Present seizure types	–	AA	AS, Ab	AA	Ab	PS
Other paroxysmal events	Somnolence, dyskinesia, ataxia, spastic paraplegia	Somnolence	Right hemiplegia	Somnolence, vomiting, headache	Left hemiplegia, vomiting, dyskinesia	Vomiting
Hypotonia	–	+	+	+	+	+
Spasticity	–	Diplegia	Diplegia	Paraplegia	Diplegia	Tetraplegia
Dystonia on action	–	+	+	+	+	+
Cerebellar ataxia	+	+	+	+	+	+
Dysarthria	+	+	+	+	+	+
Intellectual disability	Mild	Severe	Severe	Mild to moderate	Severe	Profound
Precipitating factors	Fat, Ex, HD, CZP, Tri	Fas, HD, weekend, PB	Ba, Fas, Fat, Fe, HD	Fas, Fe	Fas, Fe, Theo, Tri	Fas, PB
Alleviating factors	Ea, sleep	Ea, sleep	Ea, sleep	Ea, sleep	Ea, aging, sleep	Ea, aging, sleep
Ratio CSF:blood glucose	0.37	0.36	0.3 (4y), 0.4	0.45	0.29 (5y), 0.41	0.3 (0y9m), 0.3
Mutation analysis	p.Ser324Leu	p.Arg330X	p.Arg249fs	Not detected	p.Ile193Ile fsX36	p.Tyr 28X

[a]Onset symptom. M, male; +, present; –, absent; Ab, absence seizure; AA, atypical absence; AS, atonic seizure; MS, myoclonic seizure; PS, partial seizure; Ba, bathing; Ea, eating; Ex, exertion; Fa, fatigue; Fas, fasting; Fe, fever; HD, hot day; CZP, clonazepam; PB, phenobarbital; Theo, theophylline; Tri, trichlorethyl sodium phosphate; CSF, cerebrospinal fluid; fs, frame shift; X, stop codon.

Table II: Effectiveness of the modified Atkins diet (MAD) for the six individuals with GLUT1-DS

Participant	1	2	3	4	5	6
Age at starting MAD (y:mo)	8:1	7:4	13:9	13:0	15:1	16:9
Duration of MAD treatment (mo)	6	42	14	25	1	30
Clinical presentation						
Vigilance level	↑	↑	↑	↑	↑	↑
Comprehension	↑↑	↑↑	↑	↑	→	↑
Concentration	↑↑	↑↑	↑↑	↑	→	→
Motivation	↑↑	↑↑	↑↑	↑↑	↑	↑↑
Epileptic seizures	–					
Seizure frequency before MAD		AA: 2–3/wk	AS: 1/mo; Ab: 40–60/wk	AA: 20–30/wk	Ab: 40–50/d	PS: 4–5/y
Seizure frequency after MAD		AA: disappeared	AS: disappeared; Ab: 0–6/wk	AA: 0–3/wk	Ab: 4–5/d	PS: 0–1/y
Ataxia	↓↓	↓↓	↓↓↓	↓↓↓	↓	↓
Spasticity	–	↓	↓↓	↓↓↓	↓	↓
Gait disturbance	↓↓	↓↓	↓↓	↓↓	↓	↓
Kinetic dystonia	–	↓	↓	↓	→	↓
Slurred speech	↓	↓	↓↓	↓↓↓	↓↓	↓
Aggravation observed before meals	–	↓	↓	↓	↓	→
Investigation						
Qualitative test for urine ketone bodies (morning/after breakfast)	+2/+2~3	+2~3/+3~4	+2~3/+3	+2~3/+3	+3/+3	+2/+2~3
IQ before MAD	65 (TBS)	33 (TIS)	30 (TBS)	48 (WISC-III)	NE	12 (Owaki scale)
IQ after MAD	67 (TBS)	41 (TBS)	35 (TBS)	50 (WISC-III)	NE	NE
Interictal EEG analyses						
Frequency of background activity	↑	↑	↑	↑	↑	↑
Frequency of epileptic discharges	↓	–	↓	↓	↓	–
Aggravation of EEG abnormalities before meals	Disappeared	Disappeared	Disappeared	Disappeared	Disappeared	NE
Adverse effects						
Initial phase	Constipation, UA ↑, hypocarnitinaemia	Vomiting, TG ↑, TC ↑, UA ↑, hypercalcinuria	Hungry, fatigue, TG ↑	Nausea, fatigue, headache, TG ↑	Nausea, constipation, transient opsoclonus	–
Maintenance phase	–	Hypocarnitinaemia TC ↑	–	UA ↑	–	–
Treatment						
Antiepileptic drugs discontinued	CZP	VPA	CLB	ZNS		
Antiepileptic drugs using at present	VPA	CZP	VPA	CZP	VPA	CLB

–, absent; ↑, increased; ↑↑, further increased; ↓, decreased; ↓↓–↓↓↓, further decreased; → , unchangeable; Ab, absence seizure; AA, atypical absence; AS, atonic seizure; PS, partial seizure; TBS, Tanaka–Binet Scale; TIS, Tsumori–Inage Scale; WISC-III, Wechsler Intelligence Scale for Children – 3rd edn; NE, not examined; TG ↑, hypertriglyceridaemia; TC ↑, hypercholesterolaemia; UA ↑, hyperuricaemia; CLB, clobazam; CZP, clonazepam; VPA, valproic acid; ZNS, zonisamide.

Following an overnight fast, waking EEGs were recorded in the morning before and 2 hours after breakfast. The EEGs were recorded with silver–silver chloride electrodes positioned according to the 10 to 20 International System with a reference montage (time constant 0.1s; high-frequency cut-off at 70Hz). The EEG was digitized with a sampling rate of 500Hz and stored on a hard disk. Then, artefact-free and stationary EEG tracings lasting for longer than 40 seconds were selected visually from the monitor and used for power spectrum analysis with the Nihon Kohden program (Nihon Kohden Corporation, Tokyo, Japan). The differences in each frequency band activity (absolute and relative values of delta, theta, alpha, and beta frequency bands) between the EEG samples before and after breakfast were also calculated.

RESULTS

Effectiveness of the MAD

Neuropsychological activity

The parents or caregivers recognized that the participants' neuropsychological activity generally improved along with an increasing vigilance level (Table II). They all showed increased concentration in terms of attention, thinking, and listening, and motivation eliciting a positive attitude and perseverance. This improvement was documented before the reduction of

antiepileptic drugs. Follow-up IQ examinations revealed a favourable effect of the MAD, especially in participants 2 and 3 (from 33–41 and from 30–35 respectively).

Epilepsy

Epileptic seizures reduced in frequency by more than 90% in all five individuals with active epilepsy at starting the MAD (Table II). Antiepileptic drug therapy could be converted to monotherapy, but not discontinued completely, in all individuals because seizures recurred without medication. Interictal EEG examinations showed an improvement in background activity and the disappearance of epileptic discharges. Worsening of EEG background activity before meals was not observed after the introduction of the MAD (Fig. 1).

Neurological status

Non-paroxysmal permanent ataxia, spasticity, dysarthria, and dystonia were moderately improved in participants 1 to 4 and slightly improved in participants 5 and 6, whereas paroxysmal ataxia and spasticity induced by long-duration walking in participant 1, the individual with the mildest form of GLUT1-DS, reduced markedly (Table II). However, all individuals generally became dexterous at manual operations. Participants 1 to 5 were able to walk faster over a longer distance with a proper posture, and participants 2 and 3 could climb up and down stairs without support. Their slurred speech became articulate and audible, and vocabulary and multiword sentences increased, leading to conversations. In GLUT1-DS, paroxysmal events have generally tended to improve until adolescence regardless of whether or not the individual follows a ketogenic diet.[1,2] Even so, paroxysmal episodes of somnolence (participants 1, 2, and 4), vomiting (participants 4 and 5), headache (participant 4), and non-kinesigenic dyskinesia (participant 1) disappeared immediately after the introduction of the MAD. The aggravation of neurological symptoms before meals (or at times after bathing) disappeared after the introduction of the MAD. In participant 3, a prolonged latency in the wave 5 component of auditory brainstem-evoked responses improved 6 months after the introduction of the MAD.

Other information

In participant 6, clinical symptoms had already improved to some extent following a medium-chain triglycerides–ketogenic diet (2:1) and with age. However, the change to the MAD resulted in further improvement in terms of a reduced frequency of seizures and increased body weight and physical vigour. In participant 5, the MAD was involuntarily suspended when this individual was sent to boarding school, during which time his speech once again became inarticulate and absence seizures reappeared frequently.

Ketosis

All individuals exhibited urinary ketosis as determined by the ketostick test, with values ranging from +2 to +3 in the morning before breakfast, although that of participant 1 was constantly +2 (Table II). All participants remained in good neurological and physical condition, with total plasma ketone bodies higher than 2.5mM or more.

Adverse effects of the MAD

There were no serious side-effects of the MAD (Table II). In the early phase after the introduction of the MAD, nausea, vomiting, fatigue, headache, constipation, opsoclonus, hyper-

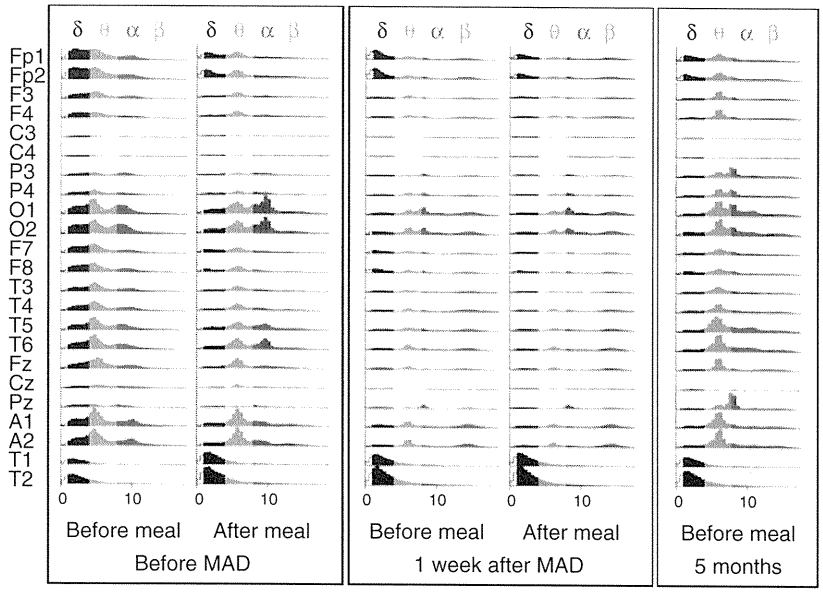

Figure 1: Power spectral analysis of background electroencephalogram in participant 4. The power spectrum analysis was performed before and after meals and also before, 1 week after, and 5 months after the introduction of the modified Atkins diet (MAD). A marked reduction in delta activity was clearly shown in the power spectrum analysis conducted before meals at 5 months after the introduction of the MAD compared with that before the diet.

lipidaemia, and hyperuricaemia occurred temporarily in some individuals. In the maintenance phase, serum carnitine levels were within the lower normal limits, but the acylcarnitine–free carnitine ratio in participant 2 was elevated to 1.18 (normal <0.8). Participant 2 received supplementation with levocarnitine. Participant 4 developed hyperuricaemia and was treated by limiting high-purine foods and uricosuric drugs.

DISCUSSION

The conventional ketogenic diet currently remains as the only fundamental and first-line treatment for individuals with GLUT1-DS.[7] Alternative ketogenic diets have also been utilized for the treatment of GLUT1-DS: the medium-chain triglycerides–ketogenic diet (2:1) in 2005[6] and the MAD in 2008.[12] In this study, we studied the efficacy and adverse effects of the MAD in six individuals with GLUT1-DS at our institution.

The most significant clinical benefits obtained by the introduction of the MAD were an improvement in the participants' cognitive activity and epilepsy as well as the ease of maintaining the diet. Along with an increased vigilance level, comprehension, concentration, and motivation were significantly improved in all individuals. Participant 2 was able to speak many meaningful words immediately and two-word sentences 6 months after the introduction of the MAD. The effects of a ketogenic diet on cognition in individuals with GLUT1-DS have been reported to be less significant.[1,2,7,8] However, the MAD appeared to have a beneficial effect on cognitive function, although this could not be formally assessed because of the severity of their intellectual disability or young age of the participants. The greatest improvement in cognition was observed in participants 1 and 2, the youngest participants. The intelligence level of individuals varied greatly and precluded applying the same IQ tests in all individuals. A significant increase in IQ values was not achieved within the period of this study in all individuals. However, it is suggested that the participants developed sufficiently at their own pace, without deterioration in their ability.

In accordance with previous reports on ketogenic diets,[1,3,4,7,8] epileptic seizures were reduced in frequency but not completely controlled without the help of an antiepileptic drugs. Constant and sufficient ketosis, in combination with an effective antiepileptic drug, is essential for full control of epileptic seizures. Interictal EEG activity after the MAD treatment showed an improvement in background activity and epileptic discharges even before meals. The improvement in cognition found in this study was attributed primarily to the MAD because it was recognized after the introduction of the MAD and before the reduction of antiepileptic drugs. However, weaning of antiepileptic drugs also resulted in improved cognition.

The improvement in other neurological manifestations such as ataxia, spasticity, dysarthria, and dystonia appeared less striking than the improvement in cognition and seizure control in this study. Nevertheless, the individuals' families, teachers, and medical staff recognized improvements in participants' manual ability, walking, and speaking. In general, paroxysmal events tend to improve towards adolescence.[1,2] However, paroxysmal somnolence, vomiting, headache, and paroxysmal non-kinesigenic dyskinesia disappeared after the introduction of the MAD. Interestingly, a prolonged latency in the wave 5 component of auditory brainstem-evoked responses improved 6 months after the introduction of the MAD in participant 3. The improvement in general neurological status contributed to a better of quality of life, leading to increased motivation and a positive attitude as well as the acquisition of further abilities.

All individuals displayed +2 to +3 urinary ketosis on ketostick testing in the morning before breakfast, which was usually less than that achieved with the conventional ketogenic diet. One individual (participant 1) showed a missense mutation and a mild phenotype, the correlation between which Leen et al.[4] identified recently. Although this individual was rarely able to produce urinary ketosis of more than +3, this moderate ketosis was sufficiently effective to alleviate his neurological manifestation and maintain a good physical condition.

The change from the medium-chain triglycerides–ketogenic diet to the MAD brought significant benefits to two individuals' families: the individuals no longer complained of being hungry owing to the increased calorie intake and meal volume, and parents could prepare the diet more easily than the more restricted ketogenic diet. All individuals and their families were pleased that they could eat together because the MAD appears similar to a standard diet. The participants became less interested in the ordinary diet that other family members or friends were consuming together, probably because they understood that the MAD improved their disabilities and it was also more palatable than the previous ketogenic diet. It is recommended that, in those with GLUT1-DS, a ketogenic diet should be introduced as early as possible to meet the energy demands of the developing brain, and then maintained into adolescence.[1,2,4,6] In addition, the MAD should be tried as the first choice because it has many advantages over the conventional ketogenic diet, especially because it is easier to maintain for many years, even after adolescence.

CONCLUSION

Compared with the conventional ketogenic diet, the MAD is less restrictive in terms of the total protein and calories consumed, is more palatable, and is easily prepared by caregivers. The effectiveness of the MAD was similar to that of the conventional ketogenic diet. No serious adverse effects were observed. Thus, the MAD is very promising for individuals with GLUT1-DS and their families and seems to be tolerable for long-term application.

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Original article

T295M-associated Glut1 deficiency syndrome with normal erythrocyte 3-OMG uptake

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Abstract

Purpose: Glucose transporter type 1 (Glut1) is expressed in vascular endothelial cells comprising blood–brain barrier. Glut1 deficiency syndrome is characterized by low cerebrospinal fluid (CSF) concentration of glucose with normoglycemia, infantile seizure, acquired microcephaly, developmental delay and ataxia. As Glut1 is also expressed in erythrocytes, the diagnosis is confirmed by a decreased uptake of 3-O-methylglucose (3-OMG) into erythrocytes. However, patients with T295M mutation in the Glut1 gene show normal 3-OMG uptake. An *in vitro* study has proved that the T295M affects efflux rather than influx of glucose, explaining the discrepancy. However, the normal 3-OMG uptake in erythrocytes still indicates a possibility that the phenotype associated with this particular mutation may be milder. We compared the phenotype of three T295M-associated patients with that of other Glut1-deficient patients. *Patients and methods:* Two patients are from our clinic and one is a patient reported elsewhere. The phenotype and biochemical data of patients with mutations other than T295M were obtained from a review and our previous report. *Results:* Despite the normal 3-OMG uptake into erythrocytes, all patients with T295M showed decreased glucose levels in CSF (33, 31 and 38 mg/dl, respectively). The levels were comparable to those in patients with mutations other than T295M (31 ± 4.3 mg/dl ($n = 45$)). All patients had convulsion, ataxia, speech delay, microcephaly and spasticity. *Conclusion:* Despite the normal 3-OMG uptake in erythrocytes, phenotype of T295M-associated Glut1 deficiency was not significantly different from that of patients with a deficient 3-OMG uptake, indicating that T295M affects the glucose transport at the blood–brain barrier as much as other mutations.

Keywords: Glut1DS; Glucose transporter type 1 deficiency; T295M mutation; SLC2A1; 3-OMG

1. Introduction

Glucose transporter type 1 (Glut1) is expressed in vascular endothelial cells comprising blood–brain barrier. The transporter facilitates glucose transport across the barrier into brain. Glut1 deficiency syndrome (Glut1DS; MIM 606777), first reported by De Vivo et al., is characterized by low cerebrospinal fluid (CSF) concentration of glucose (hypoglycorrhachia) with normoglycemia, infantile seizure, acquired microcephaly, developmental delay and ataxia [1,2]. As the Glut1 gene (SLC2A1; MIM 138140) is also expressed in erythrocytes, the diagnosis is confirmed by a decreased uptake of 3-O-methylglucose (3-OMG) into erythrocytes. Pathogenic mutations cause haploinsufficiency, thus decreas-

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ing the 3-OMG uptake by approximately 50% [2]. However, patients with T295M mutation in SLC2A1 show normal 3-OMG uptake in erythrocytes [3,4]. Although pathogenicity of this particular mutation has been confirmed by the experiment using *Xenopus laevis* oocyte expression system [5], normal 3-OMG uptake still indicates a possibility that the phenotype with T295M may be milder. In this study, we compared the phenotype of three T295M-associated patients with that of other Glut1-deficient patients.

2. Patients and methods

The biochemical and molecular studies were performed after a written informed consent was obtained from the parents.

2.1. Patient 1

A 10-year-old girl was born to healthy non-consanguineous parents after an uneventful pregnancy and delivery with a normal birth-weight. Her head circumference at birth was 32 cm (about 25 percentile). The circumference during infancy was between 3 and 10 percentile while the height was above average. She walked at 16 months of age, spoke words from the age 2 years and spoke two-word sentences at 6 years of age. At 2 years of age, she developed intermittent ataxic gait. The symptom appeared mostly before meals and improved after a meal. At the same age, she also presented with attacks of general weakness and eye-blinking followed by vomiting which persisted several hours. The attack became frequent since 5 years of age. She had also developed convulsion during fast twice since 3 years of age. Electroencephalography (EEG) revealed frontal dominant diffuse spikes and waves. Magnetic resonance imaging (MRI) taken at 3 years of age showed multiple high-signal areas in the cerebral white matter on T2-weighted images. At 9 years of age, Glut1DS was suspected and a spinal tap was performed. The CSF-glucose level was 33 mg/dl and blood-glucose level was 80 mg/dl, thus a CSF/blood glucose ratio was 0.41. Developmental quotient examined at age 10 years was 42. The patient was referred to our hospital at 10 years of age. Physical examinations revealed increased patellar tendon reflexes and ankle clonus. Her speech was slow and slurred. Truncal ataxia was not noted (the examination was done shortly after a meal). She did not have tremor or dystonia. She was obese (height, 141 cm; weight, 44.8 kg) because she tended to eat sweets at the time of the intermittent symptoms.

2.2. Patient 2

This 13-year-old girl has been described in detail elsewhere [4]. Briefly, she had acquired microcephaly (−2 SD after 2 years of age) and developed monthly generalized convulsion since 5 months of age. Since age 2 years, convulsions were followed by nausea and vomiting that persisted for about 6 h. She also had attacks of generalized weakness or transient hemiplegia without a loss of consciousness. EEG showed spike discharges over the bilateral central areas. She walked independently at age 15 months, but had intermittent ataxic gait which improved after a meal. She also had spasticity in her legs. She had speech delay, cerebellar dysarthria, and mild mental retardation. MRI at 5 years of age was normal. The CSF-glucose level was 31 mg/dl and the blood-glucose level was 77 mg/dl resulting in a CSF/blood glucose ratio of 0.4. The CSF-lactate level was 1.0 mM.

2.3. Patient 3

A brief profile of this 12-year-old boy was reported elsewhere in a table [3]. He was born after an uneventful pregnancy and delivery at term with a normal birth-weight. He sat at 6 months and walked unaided at 21 months of age. His coordination was poor and his speech development was delayed. He had mild microcephaly. At age 1 year, he developed myoclonic jerks. By 4 years of age, the jerks became increasingly evident upon awakening during which time he appeared dazed. The symptom improved after taking sugar-containing foods. EEG revealed bursts of 3 Hz spike-and-wave discharges. At age 3 and half years, he also developed an intermittent gait disturbance. The disturbance included writhing movements of the limbs, body and neck, and gyrations of the pelvis lasting minutes or an hour, which improved with sweet foods. Deep tendon reflexes were increased. MRI at 5 years of age was normal. At age 8 years, a lumber puncture showed low CSF-glucose (38 mg/dl) and low-normal CSF-lactate (0.97 mM) levels. The blood-glucose level was normal (94 mg/dl), thus a CSF/blood glucose ratio was 0.4.

2.4. Non-T295M patients

The clinical and biochemical data of the Glut1DS patients with mutations other than T295M were obtained from a review article and our previous report [2,4,6]. Among the patients in the review, we selected cases with definite mutations only.

2.5. Erythrocytes zero-trans 3-OMG uptake

Blood samples from our patients and parents as controls were taken at the same time, shipped under the same conditions and tested within 24 h of collection. The uptake studies were performed according to the previously described method [1,7].

2.6. Mutational analysis of SLC2A1 gene

White blood cells were used to extract genomic DNA. The mutational analysis of SLC2A1 gene was performed as described previously [8]. Linkage analysis was not performed for any of the T295M patients and their family member.

3. Results

From the review article and our previous report, the CSF-glucose levels in patients with mutations other than T295M ($n = 45$) was 31 ± 4.3 mg/dl (mean ± SD; range, 16–40 mg/dl). The ratio of CSF/blood glucose in those patients (only the ratio was available in one patient, therefore, $n = 46$) was 0.36 ± 0.061 (range, 0.20–0.48). The 3-OMG uptake (% of controls) was 52.5 ± 14.8% ($n = 36$; range, 30–109%). Among these 36 patients, the uptake was 109% in a patient with S285insQQLS, 80% with R218H and 75% with G130S. If these particular patients were deleted, the uptake was 49.2 ± 9.6% ($n = 33$; range, 30–68%), indicating haploinsufficiency. The average age of seizure onset was 7.4 months ($n = 37$; range, 1–77 months).

As shown in Table 1, all patients with T295M showed normal to near normal 3-OMG uptake into erythrocytes (100%, 94% and 75%, respectively). However, all showed decreased glucose levels in CSF (33, 31 and 38 mg/dl, respectively). The levels were within the same range shown in 45 patients with other mutations. The CSF/blood glucose ratios in T295M patients (0.41, 0.40 and 0.40, respectively) were higher than the mean of the ones in non-T295M patients but fell within the same range of non-T295M (within 0.82 SD). All had seizures, ataxia, speech delay, microcephaly and spasticity, similar to other typical Glut1-deficient patients. One patient (Patient 3) had intermittent dystonic movement which the other two did not present. The ages of seizure onset were varied (5 months, 13 months and 2 years), but were still comparable to those in non-T295M patients. Thus, other than the 3-OMG uptake, the clinical and biochemical findings of T295M-associated Glut1DS were the same as those found in non-T295M patients.

4. Discussion

Glut1 is a facilitative glucose transporter which has two distinct molecular forms, one with an apparent molecular weight of 55 kDa and another with 45 kDa [9]. The 55 kDa form is expressed in brain microvascular endothelial cells as well as in erythrocytes. The Glut1 at the endothelial cells functions as a blood–brain barrier. With the help of the Glut1, glucose crosses the endothelial luminal membrane into cytoplasm, and then crosses the abluminal membrane into the brain extracellular pool. With a deficiency of Glut1, blood-glucose cannot cross this barrier into brain, causing a dysfunction in the central nervous system, namely Glut1DS. As the 55 kDa Glut1 is expressed in erythrocytes as well, glucose uptake into erythrocytes is also disturbed in Glut1DS, making it possible to diagnose Glut1DS by means of a 3-OMG uptake study utilizing erythrocytes. Glut1DS is an autosomal dominant disorder, and majority of patients as shown in this study show a decrease in 3-OMG uptake approximately by 50%, indicating a haploinsufficiency [2,10,11]. The 3-OMG uptake study in T295M patients, however, shows normal or near normal uptake, which made a confirmation of the diagnosis difficult. When the T295M was first reported (Patient 3 with the 3-OMG uptake of 75%), it was considered that this particular mutation mildly affects the Glut1 function. Another possibility of somatic mosaicism was also discussed because such a case had been confirmed in a patient with R333W [3]. On the other hand, given the fact that the 3-OMG uptake rates among non-T295M patients are widely spread (among 36 molecularly confirmed patients, three had the uptake rate of 75%, 80% and 109%, respectively, which made the value of 2.5 SD as high as 89.5%), one could argue that the uptake rate of 75% in a T295M patient is simply within an error of the uptake study. However, having two more patients with T295M who showed normal 3-OMG uptake, it is unlikely that the normal or near normal uptake rates in T295M patients were due to the inter-experimental variability of the uptake study nor was it likely that the mosaicism was the cause. One can also argue that a missense mutation with a normal 3-OMG uptake means that the mutation is not pathogenic. However, further studies utilizing *X.*

Table 1
Biochemical features of patients with T295M and ones with other mutations.

Patients	CSF-glucose (mg/dl)	CSF-/blood-glucose ratio	CSF-lactate (mM)	3-OMG uptake (% of normal)
Patient 1	33	0.41	N.D.	100
Patient 2	31	0.40	1.0	94
Patient 3	38	0.40	0.97	75
Non-T295M patients (mean ± SD (range))	31 ± 4.3 (16–40) ($n = 45$)	0.36 ± 0.061 (0.20–0.48) ($n = 46$)	1.1 ± 0.34 (0.6–1.5) ($n = 33$)	52.5 ± 14.8 (30–109) ($n = 36$)
Disease control ($n = 318$)	62 ± 0.96	0.65 ± 0.01	1.3 ± 0.017	100

Values of non-T295M patients and disease controls were obtained from Refs. [2,4,6]. N.D., not done.

laevis oocytes that incorporated the T295M mutant protein in the plasma membrane revealed that the T295M affects glucose efflux disproportionately [5]. Under zero-trans influx condition in this experiment, the T295M V_{max} was 79% of the wild type value, similar to the 3-OMG uptake in the T295M patients. On the other hand, under zero-trans efflux conditions, the T295M V_{max} was as low as 16% of the wild type value. A blockage of the extracellular gate for glucose efflux by the side chain of M295 was predicted to explain this disproportionate dysfunction.

Although the disturbance of glucose efflux was confirmed by the *in vitro* experiment, we still do not know how much the efflux is affected in the brain microvascular endothelial cells by the T295M mutation. Our study found that the CSF-glucose levels in T295M patients (33, 31 and 38 mg/dl, respectively) were lower than normal, but the same or slightly higher than the mean CSF-glucose values of non-T295M patients (31 ± 4.3 mg/dl). Similarly, the CSF/blood glucose ratios (approximately 0.4) were also slightly higher than the mean value of non-T295M patients (0.36). Still, the CSF-glucose levels and the CSF/blood ratio in T295M patients fell within the same range as those found in non-T295M patients. On the other hand, if the glucose efflux in the endothelia cells was as low as 16% like in the *Xenopus* oocytes, these values are expected to be lower than the means of non-T295M Glut1DS. We cannot explain this discrepancy. The clinical phenotype of T295M patients was not significantly different from non-T295M patients with classic phenotype; all had seizures, ataxia, speech delay, microcephaly and spasticity. One patient (Patient 3) had intermittent dystonic or dyskinetic movement as well, which has been reported in non-T295M patients [12]. Including the age of seizure onset, the phenotype was thus similar to classical Glut1DS. We could not, however, compare the severity of each symptom in this study because severities of each symptom in different patients were not reported using a standardized evaluation method. Wang et al. compared the phenotype of 16 patients including a patient with T295M using a severity rating system [3]. According to this report, the severities of seizures, hypotonia/spasticity, ataxia, language deficit and microcephaly in T295M (Patient 3 in our report) were relatively milder because the 3-OMG uptake was higher. However, a N34S-patient with the uptake of 55% had even milder phenotype [3], and a R218H-patient with 80% uptake had classic phenotype [13], indicating that the uptake rate may not always related to severity. More precise and standardized clinical evaluation is necessary to compare the phenotype of T295M and other mutations to conclude if the T295M causes milder severity or not.

Among the molecularly confirmed non-T295M patients, three were reported to have shown a high 3-OMG uptake; patients with G130S, R218H and S285insQQLS showed the 3-OMG uptake of 75%, 80% and 109%, respectively [3,4,13]. Considering that the other non-T295M mutations showed haploinsufficiency with the uptake of 49.2 ± 9.6%, the uptake rates in these three mutations may be unusual like in T295M. The CSF-glucose levels in these mutations (30, 25 and 38 mg/dl, respectively), however, were comparative to the ones in other mutations. Therefore, there is a possibility that these three mutations may also affect the Glut1 function disproportionally. Further study is necessary to investigate this possibility.

In conclusion, the 3-OMG uptake into erythrocytes in T295M patients was normal to near normal (75–100%). However, the CSF-glucose levels and CSF/blood glucose ratio were not significantly different from those in non-T295M patients, although the values were slightly higher than the mean values of non-T295M Glut1DS. Symptoms of T295M patients were not different from classical Glut1DS. Considering that the CSF-glucose levels in T295M were slightly higher than the mean of non-T295M, there was a possibility that the glucose efflux at the brain microvascular endothelial cells in T295M patients were not as severely affected as in the T295M incorporated *X. laevis* oocytes in which the efflux was as low as 16% of wild type value. Further study is necessary to prove this possibility.

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