診療の秘訣

小児の診療における尿ケトン

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尿ケトンの測定は小児科ではよく行われる検査である．発熱時や嘔吐下痢症などで尿ケトンを含めた尿試験紙検査をすることは，有意義であり，その点について述べたい．

ケトン体はグルコースをセーブするための代替エネルギー源であり，血糖の低下を防ぐために肝臓にて産生される．脂肪組織から動員された遊離脂肪酸をもとに肝臓のミトコンドリアでβ酸化を経てケトン体（アセト酢酸，3-ヒドロキシ酪酸）は産生され，血液中に放出される．これが血中ケトン体である．血中ケトン体は肝外組織に取り込まれ，再びアセチル-CoAとなり，TCAサイクルに入ってエネルギーとなる．インスリンはこのケトン体産生を強く抑え，カテコールアミン，グルカゴンは促進する．このためインスリンが優位な食後や高インスリン血症ではケトン体産生は抑制され，カテコールアミン，グルカゴンが優位な空腹，感染，ストレスなどの状況ではケトン体産生は亢進する．このケトン体産生亢進は特に学童期以降に比べ乳幼児期で顕著である．血中のケトンが高くなると尿に排泄されるが，グルコースのような明らかな閾値は存在しない．尿試験紙法はアセト酢酸を検出しており，3-ヒドロキシ酪酸には反応しない．

尿ケトンの臨床的意味は以下のようである．通常尿ケトン陽性のほうが異常として注目されるが，注意したいのは尿ケトン陰性の場合のほうである．

尿ケトン体強陽性であれば，飢餓やストレスに反応してケトン体産生の亢進状態で，少なくとも脂肪酸β酸化—ケトン体産生系は機能しており，長鎖脂肪酸代謝異常症やケトン体産生系異常症の可能性は少ないこと．そしてインスリン分泌亢進状態ではない（インスリン不足状態を含む）といえる．もちろん尿糖と尿ケトンがともに陽性であれば，糖尿病性ケトアシドーシスを疑わなくてはいけない．

尿ケトン体陰性であれば，食事（糖分）がとれて，インスリンが機能していると一般には考えられるが，本来ケトン尿が有るべき飢餓，低血糖時であれば，インスリン過分泌，長鎖脂肪酸代謝異常症やケトン体産生系異常症を疑うことになる．

もし胃腸炎症状で，ぐったりして食事をとれていない児で，尿ケトンが強陽性（＋＋～＋＋＋）であれば，むしろそれは当然であり，低血糖を防ぐためにケトン産生が亢進しているので，いちじるしい多呼吸や意識障害など重篤感がなければ，生理的反応といえる．低血糖のチェックとブドウ糖を含む輸液を考慮することが望ましい．いちじるしい多呼吸や意識障害など重篤感があれば，ケトアシドーシスが疑われ，さらなる精査加療を必要とする．一方この状態で尿ケトンが陰性であれば，むしろ異常であり，ケトン体産生に異常がある可能性が高い．すなわち脂肪酸β酸化やケトン体産生の異常を疑い，精査加療を必要とする．病歴や症状から，ケトン体強陽性になっておかしくない状況で陰性や弱陽性であるときに不自然だと感じられることが重要である．

また幼児期～10歳ごろまでは，いわゆる周期性嘔吐症という病態やケトン血性低血糖という病態があり，発作時尿ケトンは強陽性である．反復することが特徴であるが，一度は基礎疾患の有無について先天代謝異常症や内分泌の専門の小児科医にコンサルトしておくほうがよい．

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Clinical and molecular investigation of 19 Japanese cases of glutaric acidemia type 1

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ABSTRACT

Glutaric acidemia type 1 (GA1) is a metabolic disease caused by a deficiency of glutaryl-CoA dehydrogenase (GCDH). Untreated patients mostly develop severe striatal degeneration. More than 200 mutations have been reported in the *GCDH* gene, and common R402W and IVS10-2A>C were found in Caucasian and Chinese/Taiwanese, respectively. However, in Japan, genetic mutations have only been reported in a few cases. Herein, we report the clinical and molecular basis of GA1 in 19 Japanese patients, including six previously reported patients. All cases showed high urinary glutaric acid excretion. Eleven patients were severely impaired (three patients died), three had mild impairment, and five showed normal development. Four of 5 patients that developed normally were detected in the presymptomatic stage by neonatal or sibling screening. Nineteen mutations in 26 alleles were identified, and eight of them (89 or 90delC, Y155C, IVS4+2T C, G244S, Q352X, G354A, K361E, and 1144-1145delGC) were novel. S305L (12.1%, 4/34 alleles) was found in several cases, suggesting that this mutation is a common mutation. In contrast, R402W was not identified and IVS10-2A>C was only found in one allele, suggesting that Japanese patients with GA1 show allelic heterogeneity and have a different genetic background to patients from other countries. One of a pair of sisters with the same mutations (M339V/S305L) lacking residual activity was severely retarded, whereas the older girl remains asymptomatic at 22 years of age, indicating that genotype does not necessarily predict GA1 phenotype. We consistently found that there was no association between genotype and phenotype. However, children with mild impairment were diagnosed and treated earlier than severely impaired cases {4.7±2.5 months (range: 2–8 months) vs. 11.6±12.7 months (range: 4–51 months)}. Our results suggest that early detection and treatment but not genotype are associated with better patient outcome, reinforcing the importance of neonatal screening.

1. Introduction

Glutaric aciduria type 1 (GA1, OMIN 231670) is an autosomal recessive metabolic disorder caused by deficiency of glutaryl-CoA dehydrogenase (GCDH, EC 1.3.99.7) [1,2]. GCDH is located in the mitochondrial matrix and acts in the intermediate steps of lysine, hydroxylysine, and tryptophan metabolisms [3]. The clinical manifestations of GA1 include extrapyramidal symptoms, developmental regression, and macrocephaly, appearing most often after acute encephalopathic crises, which are accompanied by bilateral marked enlargement of the sylvian fissure and degeneration of the striatum [1], and in addition, extrastriatal abnormalities [4] and abnormal hemodynamic changes [5]. Its biochemical characteristics include the accumulation of glutaric acid (GA), and 3-hydroxyglutaric acid, which can be detected by gas chromatography (GC/MS), and glutarylcarnitine, which can be identified by electrospray ionization/tandem mass spectrometry (MS/MS) [1,2]. It has been reported that GA1 can be classified into two types based on the level of excreted GA: the high excretion form (GA>100 mmol/mol creatine) and the low excretion form (GA<100 mmol/mol creatine) [6].

Since GA1 was first described in 1975 [3], more than 200 different mutations have been reported [7–9], and its frequency was estimated to be approximately 1 in 100,000 newborns [2]. Although almost all mutations are private, several common mutations have been identified, including A421V in the Amish Community [10], IVS 1+5G T in Canadian Oji-Cree Indians [11], and E365K in Irish travelers [8]. R402W is the most frequent mutation in the European population [6,8], and IVS10-2A C is relatively common in China [12] and Taiwan [13]. In Japan, the frequency of GA1 has been estimated to be approximately 1 in 210, 000 newborns, based on a newborn screening pilot study [14,15]. However, mutations have only been characterized in a few cases [16] since the first description of a Japanese case in 1987 [17]. Herein, we investigated the clinical and molecular aspects of 19 Japanese patients with GA1.

2. Subjects and methods

2.1. Subjects

We studied 19 Japanese patients who were diagnosed with GA1 based on their urinary organic acid profiles and/or blood acylcarnitine

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analysis. The diagnoses were confirmed by analyzing the *GCDH* gene and/or GCDH activity.

The mutations of 6 cases (cases 2–5, 12, and 19) were reported previously (cases 4, 12, and 19: [16], cases 2, 3, and 5: Japanese domestic journal). In this study, we analyzed the mutations in 13 cases (cases 1, 6–11, and 13–18). Among the 13 patients, 4 cases (case 6, 7, 10, and 11) were previously described in case reports [18,19]. No family demonstrated consanguineous marriage.

2.2. DNA sequencing

Genomic DNA was isolated from skin fibroblasts using a Qiamp DNA Microkit (QIAGEN GmbH, Hilden, Germany) and from peripheral blood lymphocytes using the DNA Quick II kit (Dainippon Pharmaceuticals, Osaka, Japan). Each exon of *GCDH* including the intron/exon boundaries was PCR-amplified for 30 cycles using the conditions shown in Supplemental Table 1. The PCR products were purified using a QIAquick PCR Purification Kit (QIAGEN GmbH, Hilden, Germany) and sequenced using the ABI PRISM 310 Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA) or the CEQ 8000 Genetic Analysis System (Beckman Coulter Inc., Fullerton, CA, USA). The structure of the human *GCDH* gene was obtained from the GenBank database (ENSG00000105607). Informed consent to perform DNA analysis was obtained from the parents of the patients. Our study protocol was approved by the Ethics Committee of the Shimane University Faculty of Medicine.

3. Results

3.1. Clinical characteristics

The clinical features of 19 Japanese GA1 patients (10 boys and 9 girls) are summarized in Table 1. Cases 4 and 19 and cases 15 and 18 were siblings. Fifteen of the 19 cases were symptomatic patients. Three (cases 1–3) of 19 cases were detected in a newborn screening pilot study, and one (case 4) was an asymptomatic sibling case that was detected at 2 years of age. To evaluate their outcomes, we classified them into three groups based on disability score [20] that included motor disability, cognitive function, and speech: a) the severe handicap group (disability score 7–9), b) the mild impairment group (disability score 4–6), and c) the normal developmental group (disability score 3) (Supplemental Table 2).

Eleven of the 19 cases were classified into severe handicap group (three of them died), 3 cases belonged to mild impairment group, and 5 cases showed normal development (Fig. 1). The mean age at onset of the symptomatic cases was 5.7 m (range: 4–8 m) in the severe handicap group, 2.3 m (range: 2–3 m) in the mild impairment group, and 6 m in case 4 of the normal development group who suffered from macrocephaly. The mean age at diagnosis was 11.6 m (range: 4–51 m) in the severe handicap group, 4.7 m (range: 2–8 m) in the mild impairment group, and 27 m (range: 24–30 m) in the normal development group, except for the 3 cases diagnosed by newborn screening. Macrocephaly was observed in 31.6% of patients (6/19). All 19 cases showed high urinary glutaric acid excretion. Cranial CT and/or MRI demonstrated frontotemporal atrophy and striatum signal abnormalities in all cases involving mild impairment or severe handicap. In contrast, three of five cases in the normal development group demonstrated mild changes by neuroimaging.

3.2. Clinical manifestations of patients

No cases had a past history except for cases 1, 6, 7, and 9. None of the cases showed abnormal development before the onset of GA1. Immediately after the diagnosis of GA1, all cases were treated with dietary restriction, L-carnitine administration, and prompt intravenous fluid infusions for catabolic states such as recurrent vomiting and diarrhea. In addition, a GABA analogue and vitamin B2 were given to the 14 and 8 cases, respectively.

3.2.1. Normal development group

Cases 1–3 were detected prior to displaying any specific symptoms by a newborn screening program using MS/MS. Case 1 weighed 2952 g when she was born at a gestational age of 39 weeks and 2 days. Abruptio placentae occurred during her birth and she suffered from asphyxia (Apgar score: 3/4). She recovered following hypothermia treatment for hypoxic–ischemic encephalopathy. Cases 2 [21] and 3 [21] had no remarkable delivery events. In these 3 cases, no signs of neurologic complications were evident at 4 months, 5 years, and 7 years old, respectively.

Case 4 was the nonsymptomatic older sister of case 19, who was severely handicapped [16]. She was diagnosed with GA1 by a sibling GC/MS screening in the presymptomatic stage at 2 years old.

Case 5 was hospitalized because of macrocephaly (47.6 cm, +2.5 S.D.) at 6 months. There was no sign of neurologic complications or developmental delay, but cranial CT suggested a subarachnoid cyst and a subdural hematoma. Thereafter, the subarachnoid cyst and subdural hematoma became smaller. At 2.5 years, he was referred to the pediatric department due to progressive macrocephaly (56.5 cm, +3.0 S.D.). Brain CT demonstrated widening of the Sylvian fissures, which in fact had been found by CT at 7 months.

3.2.2. Mild impairment group

Case 6 was treated for initial vomiting and idiopathic hyperbilirubinemia during the neonatal period [18]. Screening by brain echography identified dilated ventricles.

Case 7 was delivered at 27 weeks of dizygotic twin gestation [18]. His birth weight was 998 g. Macrocephaly and convulsions were noticed at 2 and 3 months, respectively. Following treatment, his development caught up.

In case 8, progressive macrocephaly was noticed at 3 months old. Her head circumference was +5.0 S.D. at 7 months old. Her regression and hypotonia, which were accompanied by seizures at 8 months old, improved gradually after treatment.

3.2.3. Severe handicap group

Cases 10, 11, and 13 died. Case 10 displayed a lack of head control at 4 months old [17,18] and irritability and sleeplessness at 5 months old. She died suddenly at 5 years old after developing a common cold. Cases 11 [19] and 13 presented encephalitis-like disease at 5 and 7 months, respectively. Case 11 died suddenly at the age of 3 years. Case 13 died of airway obstruction due to choking after developing an infection at 3 years old.

Similarly, no treatment was effective for the neurological symptoms of the severely handicapped patients that survived, all of whom are bedridden, require tube feeding, and smile spontaneously. Case 9 was born at 35 weeks with an Apgar score of 6/9 by cesarean delivery for premature membrane rupture and breech presentation. His birth weight was 2235 g. He was diagnosed with GA1 at 4 months after an episode of convulsions. He required mechanical ventilation and a tracheostomy for respiratory distress at 10 months old. Case 12 suffered from encephalitis-like symptoms including convulsions, unconsciousness, and rigidity following fever and an upper respiratory tract infection at 5 months old [16]. Case 14 was affected with Kawasaki disease at 5 months old. Intravenous immunoglobulin resulted in rapid defervescence, but his regression, involuntary movement, and irritability accompanied by fever were irreversible. Case 16 was affected by viral encephalitis with hyperpyrexia, consciousness disturbance, and hypertonia at 7 months of age. Case 17 was found to have subependymal pseudocysts and temporal lobe hypoplasia at 1 month. Transient regression was observed at 7 months after gastroenteritis. Thereafter, progressive neurological regression, hypotonia, and rigidity were observed following convulsions associated with pneumonia at 8 months. Case 19 was the younger sister of

Table 1
Clinical manifestations and genetic characteristics of Japanese patients with glutaric acidemia type 1.

Case I.D	Sex	Age at onset	Age at diagnosis	Precipitating factor	Clinical symptoms	Macrocephaly	Treatment	Outcome	Urine GA	C5DC (<0.3)	Neuroimaging	Exon or intron affected	Base change	Effect	GCDH activity
Normal development group															
(Newborn screening cases)															
1	F	–	1m	None	Normal development	–	L-carnitine	Normal (4m)	High	1.08	Typical	**Exon9 / Exon10**	**1064 G>A / 1147 C>T**	**R355H/R383C**	N.D
2	F	–	1 m	None	Normal development	–	L-carnitine	Normal (5y4m)	High	2.22	Mild	Exon6 / Exon8	556 A>T / 914 C>T	S186C / S305L	Deficiency
3	F	–	1 m	None	Normal development	–	L-carnitine	Normal (7y6m)	High	1.95	Mild	Exon3 / Exon10	215 G>T / 1237 T>G	R72L /Y413D	Deficiency
(Sibling screening cases)															
a 4	F	–	2 y 0 m	None	Normal development	–	L-carnitine, GABA analogue	Normal (22y)	High	N.D	Mild	Exon8 / Exon9	914C >T / 1015A> G	S305L / M339V	Deficiency
(other cases except for screening)															
5	M	6	2 y 6 m	None	Normal development	+	L-carnitine, vitamin B2	Normal (6y11m)	High	4.4	Typical	Exon5 / ?	416C >T / ?	S139L / ?	Deficiency
Mild impairment group															
6	M	2 m	2 m	None	Enlargement of ventricles	+	L-carnitine, vitamin B2, GABA analogue	Mild (23y)	High	N.D	Typical	**Exon8 / Exon10**	**914C >T / 1147 C>T**	**S305L / R383C**	Deficiency
7	M	2 m	4 m	None	Seizure	+	L-carnitine, GABA analogue	Mild (25y)	High	N.D	Typical	**Exon5 / Exon5**	**413G>A / 416C>T**	**R138K / S139L**	Deficiency
8	F	3 m	8 m	None	Seizure, regression	+	L-carnitine, GABA analogue, antiepileptic	Mild (3y2m)	High	3.36	Typical	**Exon8/ Exon9**	**914C >T / 1081A> G**	**S305L / K361E**	N.D
Severe handicap group															
9	M	4 m	4 m	None	Seizure, regression	+	L-carnitine, vitamin B2, antiepileptic	Severe (1y4m)	High	N.D	Typical	**Intron4 / Exon6**	**IVS4+2T>C / 532G>A**	**Truncated (Splicing) / G178R**	N.D
10	F	4 m	7 m	None	Regression, irritability, sleeplessness, dystonia	–	L-carnitine, vitamin B2, GABA analogue	Severe (5y:died)	High	N.D	Typical	**Exon9 / Exon9**	**1054C > T / 1054C> T**	**Truncated(Q352stop) / Truncated(Q352stop)**	Deficiency
11	M	5 m	6 m	None	Seizure, regression, hypotonia, dystonia	–	L-carnitine, GABA analogue	Severe (3y:died)	High	N.D	Typical	**Exon3 / Exon7**	**226C>T / 730G>A**	**Truncated (Q76stop) / G244S**	Deficiency
12	M	5 m	6 m	Infection, fever	Seizure, dystonic	–	L-carnitine, vitamin B2, GABA analogue	Severe (14y6m)	High	N.D	Typical	Exon9 / Exon9	1064G >A / 1064G >A	R355H / R355H	N.D
13	F	5 m	7 m	Infection	Seizure, regression, hypertonia	–	L-carnitine, GABA analogue	Severe (3y9m: died)	High	N.D	Typical	**Exon9 / Intron10**	**1061G > C / IVS10-2 A>C**	**G354A / Truncated (splicing)**	Deficiency
14	M	5 m	7 m	Kawasaki disease	Regression, dystonia	–	L-carnitine, GABA analogue, antiepileptic	Severe (7y)	High	1.76	Typical	**Exon5 / Exon7**	**416C >T / 769C>T**	**S139L / R257W**	Deficiency
b 15	M	5 m	4 y 3 m	Fever of unknown origin	Regression, dystonia	–	L-carnitine, vitamin B2, GABA analogue	Severe (5y2m)	High	0.38	Typical	**Exon1 / Exon5**	**89 or 90delC / 461A>G**	**Truncated (frame shift) / Y155C**	N.D
16	M	7 m	7 m	Infection, fever	Unconscious, dystonia	–	L-carnitine, GABA analogue	Severe (1y1m)	High	0.57	Typical	**Exon10 / Exon11**	**1144-1145delGC / 1298C>T**	**Truncated (frame shift) / A433V**	N.D
17	M	7 m	12 m	Gastroenteritis	Seizure, regression, dystonia, hypotonia	+	L-carnitine, GABA analogue, antiepileptic	Severe (2y5m)	High	N.D	Typical	**Exon5 / Exon10**	**383G>A/ 1147C>T**	**R128Q / R383C**	Deficiency
b 18	F	8 m	9 m	Polio vaccine, infection, fever	Regression, dystonia	–	L-carnitine, vitamin B2, GABA analogue	Severe (1y8m)	High	0.41	Typical	**Exon1 / Exon5**	**89 or 90delC / 461A>G**	**Truncated (frame shift) / Y155C**	Deficiency
a 19	F	8 m	12 m	Gastroenteritis	Coma, dystonia	–	L-carnitine, vitamin B2, GABA analogue	Severe (20y)	High	N.D	Typical	Exon8 / Exon9	914C >T / 1015A>G	S305L / M339V	Deficiency

Abbreviations: a,b, siblings; M, male; F, female; Age, y (years); m (months); Treatment, except for dietary restriction ; GA, glutaric acid; C5DC, glutarylcarnitine in dried spots (nmol/ml) when the patient was diagnosed; GCDH, glutaryl-CoA dehydrogenase.
Novel mutations are underlined. The mutations highlighted in bold were identified in this study; Deficiency: GCDH activity ≤5%.
N.D: not determined.

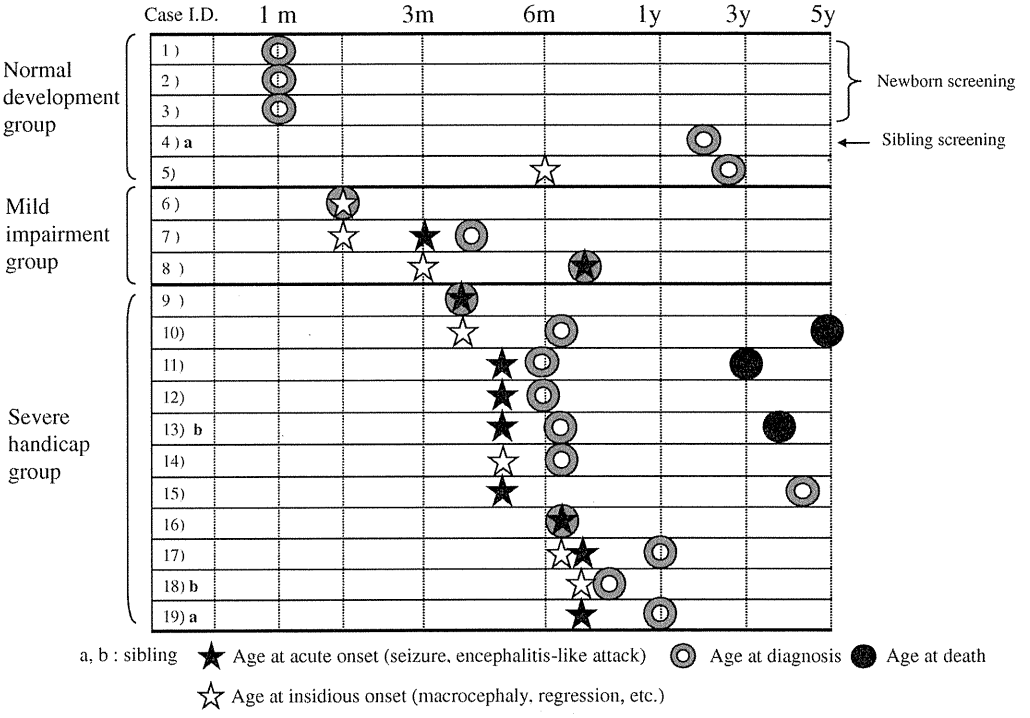

Fig. 1. Age at onset and diagnosis in three groups with different outcomes. The mild impairment group was diagnosed earlier than the severe handicap group (4.7 m (2–8 m) vs. 11.6 m (4–51 m)). Three cases (cases 10, 11, and 13) died.

case 4. At 8 months old, she suffered an encephalopathic crisis after gastroenteritis, which lasted for several days [16]. Cases 15 and 18 were siblings. Case 15, the older brother, was hospitalized for fever of unknown origin at 5 months of age and treated with antibiotics for 10 days. In addition to hypotonia, which appeared at the time of discharge, his regression, rigidity, and involuntary movement worsened every month that he suffered from fever. Although idiopathic encephalopathy was initially suspected, a diagnosis of GA1 was made in a sibling screening program by GC/MS, and treatment was initiated at 4 years and 3 months. Case 18 suffered from fever after polio vaccination at 8 months. Thereafter, she became unable to support her head and roll over. Her neurological skills deteriorated every month that she suffered from fever. The diagnosis of GA1 was made by GC/MS at 9 months of age.

3.3. Gene mutations in GCDH

Nineteen mutations were identified in 13 cases, and 8 of them were novel. These included four missense mutations (Y155C, G244S, G354A, and K361E), a nonsense change (Q352X), a splice site alteration (IVS4+2T>C), and frame shift mutations (89 or 90delC, and 1144-1145delGC). These novel mutations were not detected in 100 chromosomes from unaffected Japanese individuals.

All mutations are summarized in Table 1 and Supplemental Fig. 1, together with information on 6 cases whose genetic alterations were reported previously ([16] and Japanese domestic journal). Only two unrelated patients out of 19 cases had homozygous mutations (Q352X, R355H). In 34 independent alleles, the frequency of S305L was 12.1% (4/34 alleles), S139L, R355H, and R383C had frequencies of 8.8% (3/34 alleles), respectively and Q352X were found in 2 alleles (5.8%) each. Another 19 mutations were only found in a single allele.

4. Discussion

Since it has been remaining unknown whether there are common mutations and a phenotype/genotype correlation in Japanese GA1 cases, we investigated the relationship between clinical and mutational spectrums of 19 Japanese patients with GA1. Japanese are relatively homogenous ethnic population on islands isolated from other countries. We found a few common mutations distinct from other nations. We also found that mutations in Japanese cases are different from what have been reported in the Caucasian cases, indicating specific genetic information unique for Japanese cases are crucial for their diagnosis in the future. The current study also indicates that earlier detection of the disease followed by appropriate medicare is crucial for the better outcome than the genotype, reinforcing the importance of neonatal screening for GA1. This is a first report that studied the largest cohort of Japanese patients with GA1.

In this study, we identified 19 mutations in 24 independent alleles including eight novel mutations. The amino acids affected by these new mutations are highly conserved among different species (Pan troglodytes, mice, Xenopus, and Bordetella parapepertusis) including humans, suggesting that the region plays an important functional role in GCDH activity. It is highly likely that Q352X, 89 or 90 delC, 1144-1145delGC, and IVS4+2T C abolish GCDH activity, because these mutations result in truncation of the peptide. G354S and Y155H, which affect the same positions as G354A and Y155C, respectively, were reported to have no enzymatic activity [6,7]. The homology of the peptide's structure indicates that G244, G354, and K361 are conserved in the acyl-CoA dehydrogenase group [22]. These findings suggested that all 8 novel mutations in this study have little GCDH activity. In the 19 Japanese cases of GA1 including 6 previously reported patients, Q352X and R355H were homozygous mutations and found in 2 alleles. The frequency of S305L was 12.1% (4/34 alleles), suggesting that this mutation is common in Japanese, in contrast to the very few reports of this mutation from other countries. S139L, R355H, and R383C were also found on 8.8% (3/34 alleles), respectively, implicating that these mutations may be also common, respectively. Additionally, mutations in exon 9 were found more frequently in Japanese GA1 compared with the report by the HGMD (http://www.hgmd.cf.ac.uk/ac/index.php). It is highly likely that understanding common mutations

will facilitate rapid and accurate diagnosis of Japanese cases with GA1. Furthermore, this information may be useful for other Asian countries as well, since some of them are shared with patients from other Asian countries. Newborn screening using MS/MS is becoming popular, and the number of patients will become larger in Asian countries [23] as well as the other countries [24–26]. R402W, the most common mutation in Caucasians, in whom it shows an allele frequency of 12–25% [6,8], was not found in our Japanese cases. IVS10-2A C, a common mutation in China (30%, 3/10 alleles) [10] and Taiwan (66.7%, 4/6 alleles) [13], was also only found in a single allele in our study. Collectively, these findings suggest that Japanese GA1 patients show allelic heterogeneity and have different genetic backgrounds to GA1 patients from other countries. However, S139L, R355H and G178R, in addition to IVS10-2A C, may be common mutations among oriental populations, since S139L have been discovered in 2 of 4 alleles in Korean cases [27], and R355H and G178R were detected in one allele in Chinese case, respectively [10].

All 19 cases demonstrated a high-excretor phenotype in urinary organic acid analysis by GC/MS, suggesting that their mutations resulted in lower enzyme activity (≤5%) [6]. In fact, an enzyme assay confirmed 0–5% residual GCDH activity in 11 cases (Cases 2–7, 10–11, 13–14, and 19) [19,21,28,29]. Furthermore, an *in vitro* probe assay using cultured fibroblasts and MS/MS demonstrated a deficiency of GCDH in 10 cases (cases 4, 6, 7, 10, 11, 13–14, and 17–19) [30]. Although all 19 cases were assumed to have barely detectable enzyme activity, their clinical outcomes were diverse, ranging from normal development, through mild impairment, to severe handicap. This study suggests that the phenotypes of Japanese GA1 patients are not associated with a specific genotype. A previous study also showed that there is no clear correlation between genotype, biochemical phenotype, and the clinical severity of GA1 [6,24]. Frequency (31.6%: 6/19 cases) of macrocephaly of this study is lower than other reports (65–75%) [2,31]. This may represent unique phenotype in Japanese patients with GA1, which have genetic backgrounds distinct from other nations. However, additional case studies are warranted to validate whether this is indeed the cases.

All symptomatic cases except for case 5 had mild impairment or severe handicap indicating that the neurological sequelae of symptomatic cases are poor in Japanese GA1 patients, as reported in previous cases [24,31–33]. With respect to the grounds for the neurological manifestation, we were not able to completely rule out hypoxic–ischemic encephalopathy, hyperbilirubinemia, prematurity, very low birth weight, or encephalitis. However, since there was no sign of neurologic complications or developmental delay before the onset in any cases, we suspect that the neurological symptoms are not a consequence of these conditions. Importantly, the mild impairment group was diagnosed earlier than the severe handicap group {4.7 ± 2.5 m (2–8 m) vs. 11.6 m ± 12.7 m (4–51 m)}, suggesting that a better outcome was induced by early diagnosis. The reason for the better outcome seen in the patients who were diagnosed younger age was considered that early diagnosis led to an earlier initiation of the treatment and/or intervention in a timely manner for any medical conditions, which in turn prevented patients from neurological impairment. The frequency of macrocephaly was higher in the mild impairment group (3/3 cases) than in the severe impairment group (2/11 cases), making it likely that macrocephaly led to an early diagnosis of GA1. Furthermore, there was a notable difference in the phenotypes of siblings with the same mutations: case 4 showed normal development, whereas case 19 showed severe retardation (Supplemental Fig. 2), indicating that genotype does not predict clinical outcome. Taken together, these findings strongly suggest that early diagnosis and treatment but not genotype are associated with a better patient outcome.

Because the diagnosis was made by newborn screening only in 15.8% (3/19 cases), there is no direct evidence that newborn screening has neuroprotective effect for the patients with GA1 in this study. However, our study indicates that genotype does not necessarily predict clinical outcome and that early diagnosis and treatment are critical for a better outcome. While indirect findings, these observations strongly suggest that earliest diagnosis by the newborn screening will also be beneficial for a better outcome. In this regard, it is very important to expand newborn screening by MS/MS to improve the outcome of Japanese GA1 patients.

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