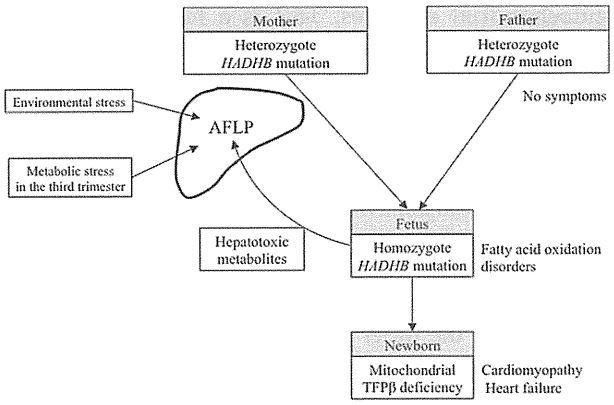

Figure 3 Possible mechanisms of the pathogenesis of acute fatty liver of pregnancy (AFLP) in our case. Toxic fatty acid metabolites from the fetus lacking trifunctional protein (TFP) activity via the placenta return to the mother's circulation, resulting in the symptoms of AFLP. Environmental stress including a high-fat diet or metabolic stress in the third trimester of pregnancy may lead to the further accumulation of toxic metabolites in the genetically susceptible mother.

cardiomyopathy caused by severe cardiac mitochondrial proliferation in TFP deficiency may lead to lethality.[12]

In summary, AFLP is a serious maternal disorder occurring in the third trimester of pregnancy with significant perinatal mortality. Recent evidence demonstrates that fetal fatty acid oxidative disorders are one of the mechanisms underlying the pathophysiology of AFLP. Early detection and treatment are essential for better prognosis for both mother and newborn. Genetic counseling should be provided to the parents in subsequent pregnancies including pre-implantation genetic diagnosis.

Disclosure

The authors have no conflict of interest.

References

1. Ibdah JA. Acute fatty liver of pregnancy: an update on pathogenesis and clinical implications. *World J Gastroenterol* 2006; **12**: 7397–7404.
2. Schoeman MN, Batey RG, Wilcken B. Recurrent acute fatty liver of pregnancy associated with a fatty-acid oxidation defect in the offspring. *Gastroenterology* 1991; **100**: 544–548.
3. Treem WR, Rinaldo P, Hale DE *et al.* Acute fatty liver of pregnancy and long-chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency. *Hepatology* 1994; **19**: 339–345.
4. Chakrapani A, Olpin S, Cleary M, Walter JH, Wraith JE, Besley GT. Trifunctional protein deficiency: three families with significant maternal hepatic dysfunction in pregnancy not associated with E474Q mutation. *J Inherit Metab Dis* 2000; **23**: 826–834.
5. Isaacs JD Jr, Sims HF, Powell CK. Maternal acute fatty liver of pregnancy associated with fetal trifunctional protein deficiency: molecular characterization of a novel maternal mutant allele. *Pediatr Res* 1996; **40**: 393–398.
6. Ch'ng CL, Morgan M, Hainsworth I, Kingham JG. Prospective study of liver dysfunction in pregnancy in Southwest Wales. *Gut* 2002; **51**: 876–880.
7. Ko H, Yoshida EM. Acute fatty liver of pregnancy. *Can J Gastroenterol* 2006; **20**: 25–30.
8. Browning MF, Levy HL, Wilkins-Haug LE, Larson C, Shih VE. Fetal fatty acid oxidation defects and maternal liver disease in pregnancy. *Obstet Gynecol* 2006; **107**: 115–120.
9. Jamerson PA. The association between acute fatty liver of pregnancy and fatty acid oxidation disorders. *J Obstet Gynecol Neonatal Nurs* 2005; **34**: 87–92.
10. Ushikubo S, Aoyama T, Kamijo T *et al.* Molecular characterization of mitochondrial trifunctional protein deficiency: formation of the enzyme complex is important for stabilization of both alpha- and beta-subunits. *Am J Hum Genet* 1996; **58**: 979–988.
11. Purevsuren J, Fukao T, Hasegawa Y *et al.* Clinical and molecular aspects of Japanese patients with mitochondrial trifunctional protein deficiency. *Mol Genet Metab* 2009; **98**: 372–377.
12. Spiekerkoetter U, Mueller M, Cloppenburg E. Intrauterine cardiomyopathy and cardiac mitochondrial proliferation in mitochondrial trifunctional protein (TFP) deficiency. *Mol Genet Metab* 2008; **94**: 428–430.

PEDIATRICS INTERNATIONAL Official Journal of the Japan Pediatric Society

Pediatrics International (2015) **57**, 348–353 doi: 10.1111/ped.12660

Review Article

Metabolic disease in 10 patients with sudden unexpected death in infancy or acute life-threatening events

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Abstract In order to determine the associations between sudden unexpected death in infancy (SUDI) or acute life-threatening events (ALTE) and inborn errors of metabolism, particularly organic acidemia and fatty acid oxidation disorders, we evaluated clinical features in patients with SUDI or ALTE. The subjects were infants between the ages of 7 days and 3 years who developed SUDI or ALTE between January 2004 and December 2013. They were then diagnosed as having inborn errors of metabolism on gas chromatography–mass spectrometry (GC/MS) and/or tandem mass spectrometry (MS/MS). The age distribution, onset forms, and clinical findings were evaluated during the acute phase. Inborn errors of metabolism were detected in three of 196 patients with SUDI, and in seven of 167 patients with ALTE. Of these 10 patients, nine had a history of poor feeding and somnolence during the neonatal period, and symptoms of infection such as cough, fever or vomiting during infancy. Routine laboratory tests during an acute phase indicated hyperammonemia, liver dysfunction, increased blood creatine kinase, acidosis, positive ketone bodies in urine or blood, or hypoglycemia. When SUDI or ALTE are encountered in the emergency unit, it is essential that a detailed medical history is taken, particularly with regard to the neonatal period, and that specific abnormalities are investigated on routine laboratory tests. Moreover, samples such as urine, serum, and filter paper blood specimens should be collected for GC/MS and/or MS/MS of organic acids and acylcarnitines, to identify inborn metabolic disorders.

Key words apparent life-threatening event, gas chromatography, inborn error of metabolism, sudden unexpected death in infancy, tandem mass spectrometry.

Sudden infant death syndrome (SIDS) is defined as the sudden, unexpected death of an infant that cannot be explained based on previous medical history or symptoms. The cause of the death cannot be specified on autopsy. A recent study found SIDS to be the third leading cause of overall infant mortality in Japan, following congenital anomalies, and perinatal disorders. A sudden death may occur not only in infants with no prodromal symptoms but also in previously healthy infants who develop infections or diarrhea.[1–6] The latter cases are not considered to represent SIDS in a strictly defined sense, but the broad term "sudden unexpected death in infancy" (SUDI) is applied. An apparent life-threatening event (ALTE) is defined as a life-threatening episode in an infant that does not lead to death and is characterized by sudden apnea resulting in skin color change, muscle tone change, coughing, and so on. ALTE includes a condition that was previously called near-miss SIDS.

Newborn mass screening on tandem mass spectrometry (MS/MS) has been widely used and newborns can now be screened for organic acid and fatty acid disorders in addition to amino acidemias. Some infants with organic acid and fatty acid disorders are known to develop symptoms similar to those of SUDI or ALTE.

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Received 11 November 2014; revised 18 February 2015; accepted 2 March 2015.

Given that newborn mass screening on MS/MS would allow early detection and intervention prior to the development of symptoms of organic acid and fatty acid disorders, this form of mass screening is expected to prevent sudden deaths due to such disorders.

We have been analyzing metabolic disorders using gas chromatography–mass spectrometry (GC/MS) and/or MS/MS in children who present with symptoms similar to those of acute encephalopathy, SUDI, or ALTE. Thus, we conducted the present study with the aims of improving the early detection and the treatment of inborn metabolic disorders underlying SUDI or ALTE. We thus evaluated detection frequency, age distribution, and clinical features in infants <3 years of age who presented with symptoms similar to those of SUDI or ALTE, and were diagnosed as having organic acid and fatty acid disorders on GC/MS or MS/MS.

Method

Subjects

Among infants who were referred to the Department of Pediatrics, Shimane University for GC/MS of urinary organic acids or MS/MS of acylcarnitines during the 10 year period between January 2004 and December 2013, those who met the following criteria were included: (i) age between 7 days and 3 years; (i) clinical diagnosis of SUDI or ALTE; and (iii) established diagnosis of organic acid or fatty acid disorders.

Neonates before day 7 of birth were excluded because congenital abnormalities and perinatal disorders might have contributed to their condition.[7,8]

Sample preparation and GC/MS

Organic acid analysis

Urine samples for GC/MS of urinary organic acids were pretreated as described previously. Briefly, to an aliquot of urine 0.2 mg equivalent of creatinine, 20 μg heptadecanic acid, 20 μg tetracosane (C24), and 40 μg tropic acid were added as internal standards. Distilled water was added to yield 2.0 mL of the mixture, and solvent extraction, oximation, and trimethylsilyl derivatization were performed.[9,10]

GC/MS was performed using GCMS QP2010 Plus (Shimadzu, Kyoto, Japan). The column (30 m × 1.0 mm i.d.) was DB-5 (J&W Scientific, Folsom, CA, USA). The oven temperature was initially 100°C and was then raised to 290°C at a rate of 4°C/min.

Glycerol metabolite analysis

In 30 patients with residual urine samples, glycerol-3-phosphate was measured according to a method described previously.[11,12] Briefly, to achieve urea decomposition, 20 μg tropic acid and 20 units of urease were added to an aliquot of urine equivalent to 0.1 mg creatinine. In total, 500 μL ethanol was then added for deproteinization, and the solution was dried under a nitrogen stream at 50°C. The dried residue of organic acids was trimethylsilylated. GC/MS was performed under the same conditions as aforedescribed.

Quantitative acylcarnitine analysis

Acylcarnitines were analyzed on MS/MS after butyl derivatization had been performed in serum sample aliquots of 10 μL according to a method described previously.[13] MS/MS was carried out using an API 3000 (Applied Biosystems, Foster City, CA, USA). Data were analyzed with ChemoView™ (Applied Biosystems/MDS SCIEX, Toronto, Canada).

Results

Age distribution

We studied a total of 363 infants, including 196 with SUDI (GC/MS and MS/MS in 57, GC/MS only in 10, MS/MS only in 129), and 167 with ALTE (GC/MS and MS/MS in 95, GC/MS only in 13, MS/MS only in 59).

Figure 1 shows the age distribution. The numbers of infants in each age group were as follows: 7–28 days (neonates), n = 45 (SUDI, n = 20; ALTE, n = 25); 1–6 months, n = 177 (SUDI, n = 91; ALTE, n = 86); 6–12 months, n = 55 (SUDI, n = 39; ALTE, n = 16); 1–2 years, n = 66 (SUDI, n = 37; ALTE, n = 29); 2–3 years, n = 20 (SUDI, n = 9; ALTE, n = 11).

Confirmed metabolic disorders

The GC/MS of urinary organic acids, MS/MS of acylcarnitines, and genetic testing yielded a diagnosis of inborn errors of metabolism in 10 (2.7%) of the total 363 infants. Among these 10 patients, three were newborns, three were 1 month–1 year of age, and four were ≥1 year of age.

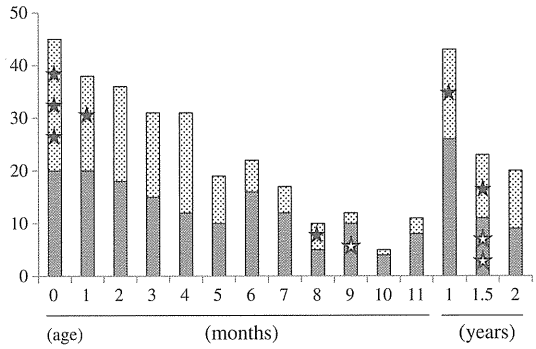

Fig. 1 Age distribution in infants with (▨) acute life-threatening events (n = 167) or (▩) sudden unexpected death (n = 196). ★ or ☆, inborn error of metabolism.

Among these 10 patients, two had medium-chain acyl-CoA dehydrogenase (MCAD) deficiency, one had carnitine palmitoyltransferase type 2 (CPT2) deficiency presenting with SUDI, four had methylmalonic acidemia (MMA), two had urea cycle disorders, and one had mitochondrial trifunction protein (TFP) deficiency with ALTE (Table 1).

Clinical findings

Poor feeding and somnolence were common in three neonates (patients 1–3) as prodromal symptoms. Of the seven patients between the ages of 1 month and 3 years (patients 4–10), six had cold symptoms such as fever, cough, rhinorrhea, and vomiting as prodromal symptoms. Of these seven patients, three had intractable vomiting; one had a medical history of transient hypoglycemia; and one had an episode of cyanosis during the neonatal period.

Laboratory results during an acute phase included acidosis (pH, 6.9–7.3) in seven of 10 infants; positive ketone bodies (in urine or blood) in four of six infants who received ketone testing; liver dysfunction (hyper blood aspartate aminotransferase (AST; 52-3144IU/L); hyper blood alanine aminotransferase (ALT; 43-1712IU/L) in eight of 10 infants; high blood creatine kinase (CK; 203–8077 IU/L) in eight of 10 infants; hyperammonemia (147–2006 μg/dL) in seven of eight whose data were available; and hypoglycemia (blood glucose, ≤17–30 mg/dL) in three of 10 infants (Table 2).

Two of the three newborns (patients 1,3) had loss of the Moro reflex. Only one infant (patient 8) had a family history of abnormality (acute encephalopathy).

In addition to the aforedescribed 10 infants, there were at least 10 other infants who lacked definitive diagnosis but were strongly suspected to have inborn errors of metabolic disease (Table 3). The results were suggestive of glutaric acidemia type 2, very long-chain acyl-CoA dehydrogenase deficiency, and primary carnitine deficiency, but a definitive diagnosis could not be obtained.

Table 1 Organic or fatty acid disorder patient profiles

Patient ID no.	Age at onset	Sex	Diagnosis	ALTE/SUDI	GC/MS (abnormal OA)	MS/MS (elevated AC)	Abnormality in neonatal period	Prodrome
1	7 days	F	MMA	ALTE	MM, MC, 3HP	C3, C3/C2	Poor sucking	Poor sucking
2	8 days	F	UCD	ALTE	Orotic, uracil	Cit	Lethargy	Lethargy
3	8 days	F	UCD	ALTE	Normal	Cit	Poor sucking	Poor sucking
4	1 month	M	TFP def.	ALTE	NKDA, LA, PA	C14-OH, C16-OH, C18-OH, C18:1-OH	Cyanosis	Non-specific
5	8 months	M	MMA	ALTE	MM, MC, 3HP	WNL	WNL	Vomiting
6	9 months	M	CPT2 def.	SUDI	NKDA	C14,C16,C18	WNL	Fever
7	1 year 1 month	F	CPT2 def.	SUDI	WNL	C16	WNL	Cough
8	1 year 8 months	F	MMA	ALTE	MM, MC, 3HP, PG	C3, C3/C2	WNL	Cough, vomiting
9	1 year 8 months	M	MCAD def.	SUDI	NKDA, HG, SG	C6, C8, C10	Hypoglycemia	Fever, cough
10	1 year 10 months	M	MMA	ALTE	MM, MC, 3HP, PG	C3, C3/C2	ND	Fever, vomiting

3HP, 3-OH-propionate; AC, acylcarnitine; ALTE, acute life threatening event; CPT2, carnitine palmitoyltransferase type 2; def., deficiency; GA2, glutaric acidemia type 2; GC/MS, gas chromatography–mass spectrometry; HG, hexanoylglycine; KDA, ketotic dicarboxylic aciduria; LC, long-chain; MC, methylcitrate; MCA, medium chain acylcarnitine; MCAD, medium chain acyl-CoA dehydrogenase; MM, methylmalonate; MMA, methylmalonic acidemia; MS/MS, tandem mass spectrometry; NA, not analyzed; ND, no data; NKDA, non-ketotic dicarboxylic aciduria; OA, organic acid; PG, propionylglycine; SG, suberylglycine; SUDI, sudden unexpected death in infancy; TFP, trifunctional protein; UCD, urea cycle disorder; WNL, within normal limits.

Table 2 Acute stage laboratory data at admission

Patient ID no.	Age at onset	Diagnosis	pH	Ketosis	AST (IU/L)	ALT (IU/L)	CK (IU/L)	NH_3 (μg/dL)	Glucose (mg/dL)	Gene analysis
1	7 days	MMA	7.3	–	65	53	245	674	143	NA
2	8 days	UCD	Acidosis	NA	55	33	308	2006	79	NA
3	8 days	UCD	WNL	+	36	16	284	1035	78	NA
4	1 month	TFP def.	WNL	NA	153	71	8077	WNL	71	c.1364T>G (homo)
5	8 months	MMA	7.1	+	WNL	WNL	1084	ND	63	NA
6	9 months	CPT2 def.	7.2	NA	3144	1712	1100	ND	17	c.520G>A (homo)
7	1 year 1 month	CPT2 def.	7.37	–	353	178	203	147	98	c.745delG/ c.1148T>A
8	1 year 8 months	MMA	7.2	+	40	43	560	162	30	NA
9	1 year 8 months	MCAD def.	7.2	NA	80	40	107	1640	Not detected	c.449_452 delCTGA(homo)
10	1 year 10 months	MMA	6.9	+	52	17	100	188	88	NA

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; CPT2, carnitine palmitoyltransferase type 2; def., deficiency; MCAD, medium chain acyl-CoA dehydrogenase; MMA, methylmalonic acidemia; NA, not available; ND, no data; TFP, trifunctional protein; UCD, urea cycle disorder; WNL, within normal limits.

Table 3 Suspected inherited metabolic disease in SUDI/ALTE patients

Patient ID no.	Age	Sex	ALTE or SUDI	Suspected disease	GC/MS (OA abnormality)	MS/MS (AC abnormality)
11	14 days	F	ALTE	GA2	LA, PA, KDA	C4, C6, C8, C10
12	20 days	M	SUDI	VLCAD def. or GA2	NA	LC AC
13	28 days	F	ALTE	PCD	PA,KDA	Low C0 (6.8)
14	1 month	M	ALTE	PCD	NA	Low C0 (13.97)
15	1 month	M	ALTE	GA2	LA, KDA	MC–LC AC
16	4 months	F	SUDI	PCD	NA	Low C0 (17.27)
17	4 months	M	SUDI	GA2 or MCAD def.	NA	MC AC
18	5 months	M	ALTE	VLCAD def. or GA2	NA	LC AC
19	1 year 2 months	M	SUDI	GA2	LA, PA, KDA	SC–LC AC
20	1 year 4 months	M	ALTE	GA2	NA	SC–LC AC

AC, acylcarnitine; ALTE, acute life threatening event; def., deficiency; GA2, glutaric acidemia type 2; GC/MS, gas chromatography–mass spectrometry; KDA, ketotic dicarboxylic aciduria; LA, lactic acid; LC, long-chain; MC, medium-chain; MS/MS, tandem mass spectrometry; NA, not analyzed; OA, organic acid; PA, pyruvic acid; PCD, primary carnitine deficiency; SC, short-chain; SUDI, sudden unexpected death in infancy; VLCAD, very long chain acyl-CoA dehydrogenase.

Step 1: Case reports

Case 1

Patient 1 was a 7-day-old girl who had been admitted to hospital on the seventh day after birth because of poor feeding noticed on the fifth day of life. She was born at 40 weeks 4 days of gestation without asphyxia and with a birthweight of 2770 g. She showed closing of her eyes, poor response to painful stimuli, asterixis, loss of the Moro reflex, and marked dehydration. GC/MS and MS/MS yielded a diagnosis of MMA. Her condition improved with arginine treatment and exchange transfusion.

Case 2

Patient 2 was an 8-day-old girl, born at 39 weeks 2 days of gestation with a birthweight of 3170 g. Apgar scores were 9 at 1 min and 10 at 5 min. Reduced activity and somnolence developed from the second day of life. On the seventh day of life, marked somnolence recurred, and grunting plus a low body temperature (34.0°C) were also noticed. GC/MS of organic acids showed elevated orotic acid and uracil. MS/MS indicated citrulline elevation. Eventually, a diagnosis of citrullinemia type 1 was made. She was treated with continuous hemodiafiltration, and survived.

Case 3

Patient 3 was an 8-day-old girl. She was born at 38 weeks 6 days of gestation without asphyxia and the birthweight was 2350 g. Poor feeding and weak crying were apparent on the fourth day of life. Shallow and superficial respiration developed on the eighth day of life and the Moro reflex could not be elicited. GC/MS showed no special abnormalities whereas MS/MS indicated elevated citrulline. Citrullinemia type 1 was thus diagnosed. Her condition improved with dialysis.

Case 4

Patient 4 was a 1-month-old boy. Cyanosis developed on the fifth day of life but then improved. Respiratory failure suddenly developed on the 32nd day of life. GC/MS showed hypoketotic dicarboxylic aciduria. MS/MS indicated increased C14-OH, C16-OH, C18-OH and C18:1-OH. Genetic tests showed homozygosity for the c.1364T>G mutation on *HADHB* [Hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase (trifunctional protein), beta subunit] gene (Table 2). Thus, diagnosis of mitochondrial TFP deficiency was made. His condition improved with appropriate treatment.

Case 5

Patient 5 was an 8-month-old boy. His growth and development had been normal until the sudden occurrence of vomiting, tachypnea, somnolence and impaired consciousness. Magnetic resonance imaging (MRI) of the brain showed a high-intensity area in the globus pallidus. GC/MS yielded a diagnosis of MMA. His condition improved with appropriate treatment.

Case 6

Patient 6 was a 9-month-old boy. No abnormalities in his growth and development had been noted. He was taken to a nearby physician because of fever, and influenza A was diagnosed. Shallow respiration rapidly worsened thereafter, and cardiopulmonary arrest occurred. MS/MS showed an increase in C14, C16 and C18. Genetic testing indicated homozygosity for the 520G>A (E174K) mutation on *CPT2*. Thus, CPT2 deficiency was diagnosed. The infant had no response to the treatments given, and died.

Case 7

Patient 7, a girl, was 13 months old. She had received ambulatory medical care for the common cold because of cough and rhinorrhea. Her mother found that she was lethargic at 06:00 hours on the eighth day of this illness. The infant was transported to a hospital by ambulance. Convulsions developed, and the anterior fontanelle bulged. MRI of the brain showed brain edema. MS/MS indicated elevation of C14, C16 and C18. Genetic testing of *CPT2* indicated compound heterozygous mutations of c.745G/c.1148T>A. Thus, diagnosis of CPT2 deficiency was made. She died 10 h after admission, showing no response to resuscitation.

Case 8

Patient 8, a girl, was 1 year 8 months old. She first presented with cough and vomiting. Somnolence appeared and gradually

worsened. Decreased consciousness and cyanosis were apparent on the fourth day of this illness, and she was admitted to hospital. GC/MS and MS/MS yielded a diagnosis of MMA. After admission, consciousness improved with appropriate treatment.

Case 9

Patient 9 was a 1-year and 8-month-old boy. He had cold-like symptom of cough and rhinorrhea, with pyrexia developing on day 3–5 of this illness. During a daytime nap on the sixth day of illness, disturbance of consciousness suddenly developed. He was transported to the emergency department, where he died 2 h later. His sibling had a psychosomatic disorder that had developed after acute encephalopathy of unknown cause. Organic acid included in the urine analysis showed hypoketotic dicarboxylic acids, uric with evaluation of hexanoylglycine, and suberylglycine. Blood filter paper acylcarnitines analysis showed elevation of C8 and C10 acylcarnitines. Genetic testing indicated homozygosity for c.449_452delCTGA of acyl-Coenzyme A dehydrogenase (*ACADM*). Thus, postmortem diagnosis of MCAD deficiency was made.

Case 10

Patient 10 was a 1-year and 10-month-old boy. Pyrexia had developed, but resolved 3 days later. Vomiting and diarrhea occurred on the fifth day after pyrexia onset. On the seventh day of illness, tachypnea and retractive breathing developed, and he was taken to a clinic. GC/MS and MS/MS yielded a diagnosis of MMA. His condition improved with appropriate treatment.

Discussion

The Japanese Ministry of Health, Labour and Welfare started a campaign to prevent SIDS in 1999 through the implementation of three key intervention strategies: (i) avoidance of prone sleeping; (ii) cessation of family smoking; and (iii) breast-feeding to the greatest extent possible. Consequently, the reported number of SIDS cases was reduced from 412 to 148 per year, in 1999–2011, respectively.[14] SIDS in infants aged less than 1 year has received attention but there are also some cases attributable to heart disease and infection among infants aged more than 1 year who had no prodromal symptoms.[15]

In the present study, clinical features were investigated in 10 infants with inborn errors of metabolism, in which symptoms similar to those of SUDI/ALTE allowed confirmation of the diagnoses of metabolic disorders. Three newborns aged between 7 and 28 days were identified as having inborn errors of metabolism. Inborn errors of metabolism that manifest during the neonatal period generally have a high mortality rate.[16] These three infants included one with MMA and two with urea cycle disorders, but they all survived with emergency treatment despite disease onset being a few days after discharge from obstetric institutions. This suggests that an early diagnosis can be life saving, even after the development of symptoms.

Two of seven infants aged more than 1 month had prodromal episodes including transient hypoglycemia during the neonatal period. Meticulous history taking to document even minor episodes during the neonatal period, including transient cyanosis and hypoglycemia, may be diagnostically useful in the search for the cause of SUDI or ALTE.

The most common disease was MMA, which was detected in four infants. This disease commonly manifests during early infancy. Some infants have onset at ≥1 year of age.[17] In addition to the four infants with MMA, there were two with urea cycle disorder, and four with fatty acid oxidation disorder.

Features during the clinical course included poor feeding and somnolence during the neonatal period, as well as acute onset patterns with symptoms such as intractable vomiting, convulsions, and impaired consciousness following fever, cough, and diarrhea, in most of the infants. Given that hypercatabolism of proteins and fatty acids frequently accelerates during these illnesses, prevention of the hypercatabolism with hypertonic glucose solution at a very early stage is essential for prevention of SUDI and ALTE.

Only one patient (patient 8) with MCAD deficiency was found to have a family history of an abnormality. A meticulously taken family history may provide important information when dealing with a potential case of SUDI or ALTE.

Routine laboratory findings, characteristically observed during an acute phase of SUDI or ALTE in cases of underlying inborn errors of metabolism, included ketoacidosis and hyperammonemia in organic acidemia patients. In the patients with fatty acid oxidation disorder, increased blood CK, AST, or ALT reflect abnormalities in the skeletal muscles, myocardium, and liver, because these are β-oxidation-dependent organs.

Chace *et al.* reported that inborn errors of metabolism were identified in 66 (0.9%) of 7058 infants with SUDI, while Boles *et al.* identified such inborn errors in 27 (6.4%) of the 418 infants in their study.[16,18–21] In the present study, three (1.5%) of 196 infants with SUDI were diagnosed as having inborn errors of metabolism.

A definitive diagnosis could not be made in at least another nine infants due to reasons such as shortage of specimens, although urinary organic acid and blood acylcarnitine analyses were suggestive of inborn errors of metabolism. This indicates that infants with organic acid and fatty acid disorders may account for at least 1.5% of those presenting with SUDI. Neither blood ammonia nor urinary ketone bodies were measured in the clinical setting in many cases. Moreover, in a number of infants, urinary organic acids could not be analyzed due to difficulty in collecting urine, thus only MS/MS of blood acylcarnitines could be performed.

When SUDI or ALTE is encountered, it is necessary to measure blood glucose, blood gases, ammonia, and CK as well as liver function. At the same time, urine and blood specimens should be routinely collected for GC/MS and MS/MS. For such analyses, urine, serum and filter paper blood (blood spots on Guthrie cards) samples are useful. In the event of a patient's death, urine should be obtained by even suprapubic aspiration of the bladder and bile should be reserved because it contains high levels of acylcarnitines.

Metabolic disorders were diagnosed more frequently in ALTE than SUDI. If diagnosis in ALTE is delayed, the patient is more likely to die, therefore it is important to distinguish metabolic disease in patients with ALTE.

In addition, if peripheral lymphocytes or skin biopsy specimens (for the purpose of culturing skin fibroblasts) are cleanly obtained and sent to a specialized center, then measurement of enzyme

activity[22] or *in vitro* probe assay using cultured cells and MS/MS,[23,24] as well as gene analysis, can be performed.

Detection of inborn errors of metabolism using next-generation sequencing technology has become widespread. Unlike routine genetic analysis, this technology requires the DNA of parents and siblings to be analyzed simultaneously because the aim is a comprehensive genetic analysis for causative genes. This genetic technology is especially worth using in the case of siblings of patients with ALTE or SUDI. MS/MS newborn mass screening was useful in identifying diseases in the present subjects. Benefits of MS/MS screening are expected, in terms of the prevention of SUDI or ALTE, with more extensive newborn mass screening in the future.

Acknowledgments

This study was supported in part by grants from the Ministry of Health, Labor and Welfare, and from the Ministry of Education, Science, and Culture, Further, we thank Professor S. Fukuda, Department of Pediatrics, Shimane University for comments on this manuscript, and Ms M. Furui, N. Tomita, Y. Ito, and E. Mizuno, Department of Pediatrics, Shimane University, for technical assistance.

References

1 Willinger M, James LS, Catz C. Defining the sudden infant death syndrome (SIDS): Deliberations of an expert panel convened by the National Institute of Child Health and Human Development. *Pediatr. Pathol.* 1991; **11**: 677–84.
2 Weber MA, Ashworth MT, Risdon RA *et al.* The role of postmortem investigations in determining the cause of sudden unexpected death in infancy. *Arch. Dis. Child.* 2008; **93**: 1048–53.
3 Trachtenberg FL, Haas EA, Kinney HC, Stanley C, Krous HF. Risk factor changes for sudden infant death syndrome after initiation of Back-to-Sleep campaign. *Pediatrics* 2012; **129**: 630–38.
4 Côté A. Investigating sudden unexpected death in infancy and early childhood. *Paediatr. Respir. Rev.* 2010; **11**: 219–25.
5 Berkowitz CD. Sudden infant death syndrome, sudden unexpected infant death, and apparent life-threatening events. *Adv. Pediatr.* 2012; **59**: 183–208.
6 McGarvey CM, O'Regan M, Cryan J *et al.* Sudden unexplained death in childhood (1-4 years) in Ireland: An epidemiological profile and comparison with SIDS. *Arch. Dis. Child.* 2012; **97**: 692–7.
7 Rodríguez-Alarcón J, Melchor JC, Linares A *et al.* Early neonatal sudden death or near death syndrome. An epidemiological study of 29 cases. *Acta Paediatr.* 1994; **83**: 704–8.
8 Polberger S, Svenningsen NW. Early neonatal sudden infant death and near death of fullterm infants in maternity wards. *Acta Paediatr. Scand.* 1985; **74**: 861–6.
9 Kimura M, Yamamoto T, Yamaguchi S. Automated metabolic profiling and interpretation of GC/MS data for organic acidemia screening: A personal computer-based system. *Tohoku J. Exp. Med.* 1999; **188**: 317–34.
10 Fu X, Iga M, Kimura M *et al.* Simplified screening for organic acidemia using GC/MS and dried urine filter paper: A study on neonatal mass screening. *Early Hum. Dev.* 2000; **58**: 41–55.
11 Matsumoto I, Kuhara T. A new chemical diagnostic method for inborn errors of metabolism by mass spectrometry-rapid, practical and simultaneous urinary metabolites analysis. *Mass Spectrom. Rev.* 1996; **15**: 43–57.
12 Iga M, Kimura M, Ohura T *et al.* Rapid, simplified and sensitive method for screening fructose-1,6-diphosphatase deficiency by analyzing urinary metabolites in urease/direct preparations and gas chromatography-mass spectrometry in the selected-ion monitoring mode. *J. Chromatogr. B Biomed. Sci. Appl.* 2000; **746**: 75–82.
13 Li H, Fukuda S, Hasegawa Y *et al.* Effect of heat stress and bezafibrate on mitochondrial beta-oxidation: Comparison between cultured cells from normal and mitochondrial fatty acid oxidation disorder children using in vitro probe acylcarnitine profiling assay. *Brain Dev.* 2010; **32**: 362–70.
14 Health, Labour and Welfare Ministry. Sudden infant death syndrome. Available from URL: http://www.mhlw.go.jp/stf/houdou/2r9852000002mqh0.html
15 Hori D, Hasegawa Y, Kimura M *et al.* Clinical onset and prognosis of Asian children with organic acidemias, as detected by analysis of urinary organic acids using GC/MS, instead of mass screening. *Brain Dev.* 2005; **27**: 39–45.
16 Lundemose JB, Kølvraa S, Gregersen N *et al.* Fatty acid oxidation disorders as primary cause of sudden and unexpected death in infants and young children: An investigation performed on cultured fibroblasts from 79 children who died aged between 0-4 years. *Mol. Pathol.* 1997; **50**: 212–17.
17 Hörster F, Baumgartner MR, Viardot C *et al.* Long-term outcome in methylmalonic acidurias is influenced by the underlying defect (mut0, mut-, cblA, cblB). *Pediatr. Res.* 2007; **62**: 225–30.
18 Chace DH, DiPerna JC, Mitchell BL *et al.* Electrospray tandem mass spectrometry for analysis of acylcarnitines in dried postmortem blood specimens collected at autopsy from infants with unexplained cause of death. *Clin. Chem.* 2001; **47**: 1166–82.
19 Boles RG, Buck EA, Blitzer MG *et al.* Retrospective biochemical screening of fatty acid oxidation disorders in postmortem livers of 418 cases of sudden death in the first year of life. *J. Pediatr.* 1998; **132**: 924–33.
20 Bennett MJ, Powell S. Metabolic disease and sudden, unexpected death in infancy. *Hum. Pathol.* 1994; **25**: 742–6.
21 Wilcox RL, Nelson CC, Stenzel P *et al.* Postmortem screening for fatty acid oxidation disorders by analysis of Guthrie cards with tandem mass spectrometry in sudden unexpected death in infancy. *J. Pediatr.* 2002; **141**: 833–6.
22 Tajima G, Sakura N, Yofune H *et al.* Enzymatic diagnosis of medium-chain acyl-CoA dehydrogenase deficiency by detecting 2-octenoyl-CoA production using high-performance liquid chromatography: A practical confirmatory test for tandem mass spectrometry newborn screening in Japan. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 2005; **823**: 122–30.
23 Okun JG, Kölker S, Schulze A *et al.* A method for quantitative acylcarnitine profiling in human skin fibroblasts using unlabelled palmitic acid: Diagnosis of fatty acid oxidation disorders and differentiation between biochemical phenotypes of MCAD deficiency. *Biochim. Biophys. Acta* 2002; **1584**: 91–8.
24 Giak Sim K, Carpenter K, Hammond J *et al.* Quantitative fibroblast acylcarnitine profiles in mitochondrial fatty acid beta-oxidation defects: Phenotype/metabolite correlations. *Mol. Genet. Metab.* 2002; **76**: 327–34.

Molecular Genetics and Metabolism 116 (2015) 192–194

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

Original Article

Elevation of pivaloylcarnitine by sivelestat sodium in two children

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ARTICLE INFO

Article history:
Received 9 August 2015
Received in revised form 24 September 2015
Accepted 24 September 2015
Available online 26 September 2015

Keywords:
Sivelestat sodium
Antibiotics containing pivalic acid
Pivaloylcarnitine
Isovaleric acidemia
Tandem mass spectrometry
Newborn screening

ABSTRACT

Background: Sivelestat sodium (sivelestat), a neutrophil elastase inhibitor, is used to treat acute respiratory distress syndrome (ARDS). We report two cases that developed elevated C5-acylcarnitine (C5-AC) levels following treatment with sivelestat.
Case 1 was a 14-day-old female infant born at 25 weeks and 1 day of gestation who was treated with sivelestat for the prophylaxis of Wilson–Mikity syndrome soon after birth. Isovaleric acidemia (IVA) was suspected based on a newborn screening using tandem mass spectrometry (MS/MS). Her C5-AC level was elevated to 4.49 μM (cut-off, <1.0) after treatment with sivelestat. Case 2 was a 4-year-old female with pneumocystis pneumonia that developed during chemotherapy for disseminated medulloblastoma. Sivelestat was given for the complication of ARDS. Her C5-AC level increased (1.09 μM) after eight days of treatment with sivelestat.
Results: In both cases, IVA was ruled out because isovalerylglycine was not observed in the urinary organic acid analysis. Case 1 was associated with carnitine deficiency (C0 9.16 μM; reference value, 10–60). Liquid chromatography-MS/MS confirmed elevated pivaloylcarnitine (PVC) in both cases.
Discussion: Similar to antibiotics containing pivalic acid (PVA), sivelestat contains PVA, which has the potential to cause secondary carnitine deficiency. In addition, elevated PVC can lead to false positive findings of IVA in newborns screened using MS/MS.

1. Introduction

Some oral antibiotics contain esterified pivalic acid (PVA) to increase their intestinal absorption rate [1]. Previous reports have demonstrated that long-term treatment with PVA-containing antibiotics can induce severe hypoglycemia or encephalopathy [2,3]. This is because the serum carnitine level is reduced by esterification with PVA, which is excreted as pivaloylcarnitine (PVC) in the urine [4,5].

Sivelestat sodium (sivelestat) is a specific inhibitor of neutrophil elastase, and it is an effective treatment for acute respiratory distress syndrome (ARDS) [6]. Sivelestat is generally used in adult patients, but it has recently been widely used in pediatric cases as well [7]. However, it is unknown whether sivelestat influences the metabolism of carnitine. We herein report two cases with elevated C5-acylcarnitine (C5-AC) levels after treatment with sivelestat.

2. Case report

2.1. Case 1

A 14-day-old female was born with an extremely low birth weight (782 g) via a Cesarean section at 25 weeks and 1 day of gestation due to an intrauterine infection. She was treated with sivelestat for the prophylaxis of Wilson–Mikity syndrome soon after birth for 14 days because the level of immunoglobulin M in the serum was elevated at birth (36 mg/dL, reference value < 20 [8]). Newborn mass screening using tandem mass spectrometry (MS/MS) on day 7 was conducted at another agency and revealed elevated C5-AC levels (14.4 μM, cut-off: <1.0), which strongly suggested that she was affected by isovaleric acidemia (IVA). Fourteen days after birth, she was further evaluated for the presence of IVA in our laboratory. She died of necrotizing enterocolitis on day 99, which had developed on day 36 of her life.

2.2. Case 2

A 4-year-old female was diagnosed with medulloblastoma at 2 years and 6 months of age. She suffered from tumor dissemination to the meninges when she was 3 years and 6 months old. She developed ARDS

Abbreviations: AC, acylcarnitine; IVA, isovaleric acidemia; PVA, pivalic acid; PVC, pivaloylcarnitine.

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http://dx.doi.org/10.1016/j.ymgme.2015.09.009
1096-7192/

because of pneumocystis pneumonia when she was 4 years old, and she was administered sivelestat and placed on mechanical ventilation. Her AC levels were analyzed because of the potential risk of a secondary carnitine deficiency as experienced with Case 1. Free carnitine level was normal (C0 43.07 μM, cut-off: <20), whereas C5-AC levels were found to be elevated after 8 days of treatment with sivelestat (C5-AC 1.09 μM). Mechanical ventilation and administration with sivelestat were provided for 8 and 10 days, respectively. She passed away 1 month after sivelestat treatment due to the exacerbation of medulloblastoma.

3. Methods

AC on dried blood spots was pre-treated using an underivatized method as described previously [9,10]. D9-isovalerylcarnitine (d9-IVC) was purchased from Cambridge Isotopes Laboratories to use as an internal standard. PVC and 2-metyhlbutyrylcarnitine (2MBC) standard solutions were provided by Dr. Hideki Nakajima (National Center for Child Health and Development, Tokyo, Japan).

Samples (10 μL) were analyzed using MS/MS (TQ Detector; Waters, Milford, MA, USA) via high performance liquid chromatography (HPLC) (Alliance 2795; Waters) using two ion exchange multi-mode octadecylsilyl columns (SS-C18; Imtakt, Kyoto, Japan). The isomers (IVC, PVC, and 2MBC) were chromatographically separated, and acquisition in the mass spectrometer was achieved by multiple reaction monitoring, recording one unique transition (246 > 85) for all isomers and another one (255 > 85) for the internal standard.

4. Results

In Case 1, an increase of the C5-AC levels (4.49 μM) and a mild decrease in the free carnitine levels (C0 9.06 μM, cut-off: <10) were observed following 14 days of treatment with sivelestat. These findings strongly suggested that the patient had IVA with secondary carnitine deficiency. However, IVA was considered to be unlikely because isovalerylglycine was not detected by a urinary organic acid analysis (Table 1). Moreover, no abnormal body smell, hypoglycemia, or elevation of the serum creatine kinase levels were noted in the patient, all of which are typical symptoms of IVA or carnitine deficiency.

No typical clinical features for congenital metabolic disease were observed in Case 2. The blood levels of C0 and C5-AC were normal (22.9 μM and 0.52 μM, respectively) before the administration of sivelestat. However, mild elevation of the C5-AC levels (1.09 μM) was observed after 8 days of treatment with sivelestat, whereas the C0 level remained in the normal range (43.07 μM). Isovalerylglycine was not detected by a urinary organic acid analysis (Table 1).

In both cases, the increased C5-AC levels were not believed to have derived from IVC because neither of the cases had any symptoms that suggested metabolic disease and isovalerylglycine was not detected in the urine. It is known that elevated C5-AC levels are sometimes caused by antibiotics containing PVA, but neither of the present cases nor the mother of Case 1 had been administered oral antibiotics. Nevertheless, we suspected that the elevation of C5-AC was caused by a drug, and we found that sivelestat contained PVA. Subsequently, HPLC-MS/MS was used to separate the C5-isomers: IVC, PVC, and 2MBC. A large PVC peak and a small IVC peak were detected in both cases. Case 2 also presented with a small 2MBC peak (Fig. 1). C5-AC detected in both cases predominantly consisted of PVC because the IVC and 2MBC levels in both cases remained within a range in normal controls.

5. Discussion

This report described two cases with elevated PVC following the administration of sivelestat, which had never been reported before. Sivelestat is separated into a pharmacologically active form and PVA following administration in vivo. Although the pharmacologically active form is conjugated with glucoronate, the PVA would be bound to free carnitine to generate PVC, which is subsequently excreted in the urine. Therefore, sivelestat can cause secondary carnitine deficiency, as has previously been reported for antibiotics containing PVA [2,3,5]. Indeed, mild carnitine deficiency was observed in Case 1 but not in Case 2. The carnitine level was potentially low in Case 1 because she was a premature neonate. In contrast, the free carnitine was likely maintained in Case 2 via the tube feeding of formula containing L-carnitine (approximately 1 mg/kg/day). Moreover, the risk of developing a secondary carnitine deficiency during treatment with 4.8 mg/kg/day sivelestat for 14 days (according to product instructions) is smaller compared to that using antibiotics containing PVA because the amount of PVA during sivelestat treatment is smaller as well.

Case 1 was first suspected to have IVA because sivelestat had been given before the neonatal screening test, indicating that the administration of sivelestat can cause a false positive finding of IVA in newborn mass screening. This is because IVC cannot be distinguished from PVC by a conventional MS/MS analysis that utilizes flow injection [11]. Indeed, previous reports have indicated that false positive results for IVA were observed in infants who had been treated (or whose mothers had been treated) with antibiotics containing PVA [12,13].

Table 1
The levels of acylcarnitine and urine organic acid.

	Case 1 (14-day-old girl)	Case 2 (4-year-old girl)	Reference[a]
Acylcarnitine on dried blood spots (nmol/mL)			
C0	9.16	43.07	10–60
C2	19.97	48.39	5–45
C3	0.66	11.8	<5.25
C4	0.37	1.13	<1.4
C5	4.49	1.09	<1.0
C5:1	0.022	0.026	<0.08
C5-OH	0.17	0.28	<1.0
C6	0.13	0.24	<0.25
C8	0.08	0	<0.35
C10	0.029	0.082	<0.4
C12	0.022	0.11	<0.3
C14	0.092	0.14	<0.7
C14:1	0.049	0.059	<0.4
C16	0.89	2.05	0.6–7.0
C18	0.5	0.54	0.15–2.1
Urinary organic acid			
Slight elevation of lactate and 5-oxoproline		Mild elevation of glycerol, thiodiglycolate and isobutyrylglycine	

[a] The reference values used at Shimane University. Abnormal findings are underlined.

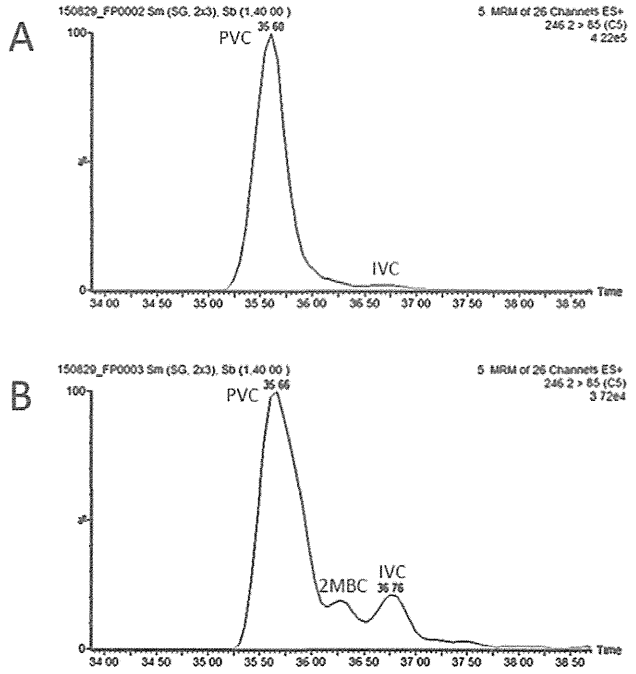

Fig. 1. A chromatogram of the C5-acylcarnitine isomers. (A): A sample from Case 1 that was collected after sivelestat treatment for 14 days. (B): A sample from Case 2 that was collected after sivelestat treatment for 8 days. PVC: pivaloylcarnitine; IVC: isovalerylcarnitine; 2MBC: 2-methylbutyrylcarnitine.

It is not widely known that PVA, which facilitates intestinal absorption, is also present in several drugs other than oral antibiotics. Although sivelestat was designed to be administered via intravenous injection, the reason for conjugating PVA to sivelestat is unknown and remains proprietary information. Of note, a recent report demonstrated that ingestion of "neopentanoate", which is PVA within an ointment or cosmetic agent, could also lead to elevated C5-AC levels [14]. Physicians must be aware that some medications, such as sivelestat, contain PVA, which increases the risk of secondary carnitine deficiency.

Conflicts of interest

The authors have no conflicts of interest to declare.

Role of the funding source

This report was partially supported by grants from the Ministry of Education, Culture, Sports, Science and Technology (S.Y. and K.Y.) and the Ministry of Health, Labor and Welfare (S.Y.) of Japan.

Acknowledgments

The separation of PVC was performed at the Tokyo Health Service Association based on the method developed by Dr. Hideki Nakajima (National Center for Child Health and Development).

References

[1] T. Ito, N. Sugiyama, M. Kobayashi, K. Kidouchi, T. Itoh, O. Uemura, K. Sugiyama, H. Togari, Alteration of ammonia and carnitine levels in short-term treatment with pivalic acid-containing prodrug, Tohoku J. Exp. Med. 175 (1995) 43–53.
[2] E. Holme, J. Greter, C.E. Jacobson, S. Lindstedt, I. Nordin, B. Kristiansson, U. Jodal, Carnitine deficiency induced by pivampicillin and pivmecillinam therapy, Lancet 2 (1989) 469–473.
[3] Y. Makino, T. Sugiura, T. Ito, N. Sugiyama, N. Koyama, Carnitine-associated encephalopathy caused by long-term treatment with an antibiotic containing pivalic acid, Pediatrics 120 (2007) e739–e741.
[4] E.P. Brass, M.D. Mayer, D.J. Mulford, T.K. Stickler, C.L. Hoppel, Impact on carnitine homeostasis of short-term treatment with the pivalate prodrug cefditoren pivoxil, Clin. Pharmacol. Ther. 73 (2003) 338–347.
[5] E. Holme, U. Jodal, S. Linstedt, I. Nordin, Effects of pivalic acid-containing prodrugs on carnitine homeostasis and on response to fasting in children, Scand. J. Clin. Lab. Invest. 52 (1992) 361–372.
[6] T. Sakuma, K. Takahashi, N. Ohya, K. Usuda, M. Handa, T. Abe, ONO-5046 is a potent inhibitor of neutrophil elastase in human pleural effusion after lobectomy, Eur. J. Pharmacol. 353 (1998) 273–279.
[7] T. Kikuchi, T. Kugimiya, S. Katashima, Usefulness of sivelestat sodium administration in patients with aspiration pneumonia, J. Anesth. 20 (2006) 355.
[8] Stiehm ER, Immunological Disorders in Infants and Children, ed. by Stiehm ER, Fulginiti VA, WB Saunders, Philadelphia, 1980.
[9] G. Paglia, O. D'Apolito, G. Corso, Precursor ion scan profiles of acylcarnitines by atmospheric pressure thermal desorption chemical ionization tandem mass spectrometry, Rapid Commun. Mass Spectrom. 22 (2008) 3809–3815.
[10] S. Forni, X. Fu, S.E. Palmer, L. Sweetman, Rapid determination of C4-acylcarnitine and C5-acylcarnitine isomers in plasma and dried blood spots by UPLC-MS/MS as a second tier test following flow-injection MS/MS acylcarnitine profile analysis, Mol. Genet. Metab. 101 (2010) 25–32.
[11] N. Janzen, U. Steuerwald, S. Sander, M. Terhardt, M. Peter, J. Sander, UPLC-MS/MS analysis of C5-acylcarnitines in dried blood spots, Clin. Chim. Acta 421 (2013) 41–45.
[12] J.E. Abdenur, N.A. Chamoles, A.E. Guinle, A.B. Schenone, A.N. Fuertes, Diagnosis of isovaleric acidaemia by tandem mass spectrometry: false positive result due to pivaloylcarnitine in a newborn screening programme, J. Inherit. Metab. Dis. 21 (1998) 624–630.
[13] Y. Shigematsu, I. Hata, Y. Kikawa, M. Mayumi, Y. Tanaka, M. Sudo, N. Kado, Modifications in electrospray tandem mass spectrometry for a neonatal-screening pilot study in Japan, J. Chromatogr. B Biomed. Sci. Appl. 731 (1999) 97–103.
[14] F. Boemer, R. Schoos, V. de Halleux, M. Kalenga, F.G. Debray, Surprising causes of C5-carnitine false positive results in newborn screening, Mol. Genet. Metab. 111 (2014) 52–54.

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Brain & Development 38 (2016) 293–301

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

www.elsevier.com/locate/braindev

Original article

Clinical, biochemical and molecular investigation of adult-onset glutaric acidemia type II: Characteristics in comparison with pediatric cases

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Received 22 July 2015; received in revised form 6 August 2015; accepted 13 August 2015

Abstract

Introduction: An increasing number of adult patients have been diagnosed with fatty acid β-oxidation disorders with the rising use of diagnostic technologies. In this study, clinical, biochemical, and molecular characteristics of 2 Japanese patients with adult-onset glutaric acidemia type II (GA2) were investigated and compared with those of pediatric cases.

Methods: The patients were a 58-year-old male and a 31-year-old male. In both cases, episodes of myopathic symptoms, including myalgia, muscle weakness, and liver dysfunction of unknown cause, had been noted for the past several years. Muscle biopsy, urinary organic acid analysis (OA), acylcarnitine (AC) analysis in dried blood spots (DBS) and serum, immunoblotting, genetic analysis, and an *in vitro* probe acylcarnitine (IVP) assay were used for diagnosis and investigation.

Results: In both cases, there was no obvious abnormality of AC in DBS or urinary OA, although there was a increase in medium- and long-chain ACs in serum; also, fat deposits were observed in the muscle biopsy. Immunoblotting and gene analysis revealed that both patients had GA2 due to a defect in electron transfer flavoprotein dehydrogenase (ETFDH). The IVP assay indicated no special abnormalities in either case.

Conclusion: Late-onset GA2 is separated into the intermediate and myopathic forms. In the myopathic form, episodic muscular symptoms or liver dysfunction are primarily exhibited after later childhood. Muscle biopsy and serum (or plasma) AC analysis allow accurate diagnosis in contrast with other biochemical tests, such as analysis of AC in DBS, urinary OA, or the IVP assay, which show fewer abnormalities in the myopathic form compared to intermediate form.

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http://dx.doi.org/10.1016/j.braindev.2015.08.011

Keywords: Multiple acyl-CoA dehydrogenase deficiency (glutaric acidemia type II); Adult onset; Myopathy; Serum acylcarnitine; Immunoblotting; *In vitro* probe acylcarnitine assay

1. Introduction

Many organic acidemias or fatty acid oxidation disorders (FAODs) are often believed to be symptomatic in childhood, especially in early infancy [1]. However, an increasing number of adult patients with inherited metabolic diseases (IMDs) has recently been identified with new developments in diagnostic technologies, including mass spectrometry, and the spread of knowledge regarding IMDs, even in the field of adult neurology.

Glutaric acidemia type II (GA2) is an autosomal recessive disease caused by a defect in electron transfer flavoprotein (ETF) or ETF dehydrogenase (ETFDH), resulting in deficiencies in multiple acyl-CoA dehydrogenases, such as short-, medium-, and long-chain acyl CoA dehydrogenases, as well as isovaleryl-CoA dehydrogenase, glutaryl-CoA dehydrogenase, and sarcosine dehydrogenase [2,3]. GA2 has been clinically classified into 2 types: (1) the neonatal-onset type, which develops during the neonatal period or early infancy and is often severe, and (2) the late-onset type, which develops after the infantile period [4].

Patients with the neonatal-onset type of GA2 develop severe respiratory failure, cardiomyopathy, hypotonia, metabolic acidosis, and profound hypoglycemia soon after birth, and they often have a fatal outcome in early infancy. Some patients with this type have congenital anomalies, including Potter's face or polycystic kidney disease [5,6]. In the late-onset type, intermittent episodic attacks of lethargy, hypoglycemia, and hyperammonemia, or, occasionally, acute encephalopathy or sudden death triggered by infection, diarrhea, or long fasting are seen starting in early childhood [7–9].

Recently, several adult-onset GA2 cases have been reported [10–13]. However, it is not always easy to establish the correct diagnosis. In this study, the clinical, biochemical, and pathological characteristics of 2 cases of adult-onset GA2 were investigated and compared with those of pediatric cases.

2. Materials and methods

2.1. Patients

Case 1 was a 58-year-old male with chief complaints of episodic myalgia and muscle weakness. The clinical course of case 1 has been reported previously [14]. His younger brother died unexpectedly from an unknown cause in his 30s. The patient sometimes had general fatigue, myalgia, or muscle weakness as early as in his 40s. Those symptoms progressively worsened in his 50s, and he began to use a wheelchair because of persistent muscle weakness and myalgia. Furthermore, he had 3 episodes of unconsciousness after the age of 50. Although he was hospitalized at the third episode, there were no obvious abnormalities in routine biochemical tests, including blood sugar and liver function. He visited several neurology clinics and hospitals to undergo a more detailed examination. However, no abnormality was found, except for the occasional elevation of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and creatine kinase (CK). The diagnosis was "myopathy of unknown cause". Then, as he repeatedly developed liver dysfunction and rhabdomyolysis, he was hospitalized at age 58 for detailed examination, including muscle biopsy.

On admission, his level of consciousness was normal, and his vital signs and intelligence were normal. No hepatosplenomegaly was noted. Muscle tenderness and atrophy with mild sensory dysfunction were observed in his limbs, especially in the lower limbs, as neurological findings. The deep tendon reflex was normal, and he was able to walk with support. In manual muscle testing, his muscle strength was level 2 for the deltoid and iliopsoas muscles and 3+ to 4 for other upper and lower limb muscles.

Routine blood examination indicated the elevation of liver and muscle enzymes, such as AST (197 IU/L, normal range 10–38), ALT (215 IU/L, normal 5–40), LDH (2903 IU/L, normal 100–215), and CK (2364 IU/L, normal 36–216), as shown in Table 1.

Case 2 was a 31-year-old male with episodic muscle weakness and myalgia similar to case 1. No abnormalities in his past and family history were noted. He was formerly a baseball player on a non-professional team, but he developed muscle weakness after retiring from the baseball team at 29 years of age. Then, his exertional muscle weakness worsened gradually, and he began to experience difficulty in his daily activities. Although he visited several neurology clinics or hospitals, only liver dysfunction of unknown cause was occasionally noted. He was hospitalized to undergo further examination at 31 years of age.

His level of consciousness and his intellectual level were normal. Abnormalities in vital signs and hepatosplenomegaly were not observed. His patellar and Achilles tendon reflexes were slightly reduced, but no pathological reflex or muscle atrophy was observed.

Table 1
Outlines of the patients and results of routine laboratory tests.

	Case 1	Case 2	(Reference value[a])
Onset age	40s	31	
Sex	M	M	
Clinical features			
	Myalgia Muscle weakness Rhabdomyolysis	Myalgia Muscle weakness	
Routine blood examination			
CBC			
WBC (/μL)	4800	5000	(3300–8600)
RBC ($\times 10^4$/μL)	370	539	(385–438)
Hb (g/dL)	12.3	16.5	(11.0–14.8)
Plt ($\times 10^4$/μL)	18.7	20.7	(15.8–35.3)
Biochemical data			
T-Bil (mg/dL)	0.3	0.8	(0.2–1.2)
TP (g/dL)	5.6	7.3	(6.5–8.2)
Alb (g/dL)	3.4	5.1	(3.8–5.1)
AST (IU/L)	197	71	(10–38)
ALT (IU/L)	215	84	(5–40)
LDH (IU/L)	2903	684	(100–215)
ALP (IU/L)	178	152	(110–340)
CK (IU/L)	2364	689	(36–216)
BUN (mg/dL)	7	10.9	(8.0–21.0)
Cre (mg/dL)	0.35	0.5	(0.44–0.83)
Na (mEq/L)	138	139	(137–146)
K (mEq/L)	3.4	4.1	(3.5–4.9)
Cl (mEq/L)	101	103	(98–109)
Ca (mg/dL)	8.8	10.6	(8.6–10.3)
BS (mg/dL)	90	104	(60–109)

[a] The reference values used at Shimane University. Abnormal findings are underlined.

The results of manual muscle testing were also within the normal range.

Blood examination indicated a slight elevation of liver and muscle enzymes (AST 71 IU/L, ALT 84 IU/L, LDH 684 IU/L, and CK 689 IU/L), although no abnormalities were observed in other tests.

This study was conducted with the approval of the Institutional Review Board of Shimane University and consent from the patients.

2.2. Urinary organic acid analysis

The urinary organic acids (OAs) were analyzed using gas chromatography mass spectrometry (GC/MS; QP-2010 plus; Shimadzu, Kyoto, Japan) at Shimane University, Japan, after solvent extraction and oxime-trimethylsilyl derivatization of urine samples as previously described [1,15].

2.3. Blood acylcarnitine analysis

Acylcarnitine (AC) in dried blood spots (DBS) or serum was analyzed using tandem mass spectrometry (MS/MS) (API-3000; Applied Biosystems, Foster City, CA, USA) after butyl-derivatization of samples, as previously described [16,17].

2.4. Histological studies

Muscle biopsies were performed using the rectus femoris muscle and biceps brachii in cases 1 and 2, respectively. The biopsied materials were frozen and cryostat-sectioned for Oil-Red O staining [18].

2.5. Cell culture

Skin fibroblasts were cultured in Eagle's minimal essential medium (MEM) (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) supplemented with 2 mmol/L glutamine, 10% fetal bovine serum, and 1% penicillin/streptomycin at 37 °C in a humidified 5% CO_2/95% air incubator until confluence [19,20].

2.6. Immunoblotting

Twenty five micrograms of protein derived from the cellular extract of a pellet of cultured fibroblasts was subjected to 12.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS/PAGE). Immunoblotting was performed according to a routine protocol using rabbit polyclonal antibodies against ETF, which were a gift from Dr. T. Hashimoto (Professor Emeritus of Shinshu University, Matsumoto, Japan),

and ETFDH, which was purchased from Japan Bio Services Co., Ltd. (Saitama, Japan), as the primary antibodies. Blots were visualized using the Immuno-Pure NBT/BCIP Substrate Kit TM (Promega, Madison WI, USA) [19,21].

2.7. Gene analysis of ETFDH

Genomic DNA was isolated from fibroblasts using a QIAamp DNA Microkit (QIAGEN GmbH, Hilden, Germany). Each exon of *ETFA*, *ETFB*, and *ETFDH*, including intron/exon boundaries, was PCR-amplified for 30 cycles. Primers for *ETFDH* were prepared as previously reported [2,14]. The PCR products were purified with a QIAquick PCR Purification Kit (QIAGEN GmbH, Hilden, Germany) and sequenced using an ABI PRISM 310 Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA) or CEQ 8000 Genetic Analysis System (Beckman Coulter Inc., Fullerton, CA, USA).

2.8. In vitro probe acylcarnitine (IVP) assay

An IVP assay to evaluate the β-oxidation capacity was performed as previously described [20]. Briefly, confluent cells were harvested by trypsinization and seeded onto 6-well microplates with fresh medium (described above) until they again reached confluence. Thereafter, cells were washed twice with D-PBS and cultured at 37 °C in 1 mL of experimental MEM containing 0.4% essential fatty acid-free BSA, 0.4 mmol/L L-carnitine, and 1% penicillin/streptomycin with 0.2 mmol/L unlabeled palmitic acid. The concentration of ACs in 10 μL of the culture medium after incubation for 96 h was determined by MS/MS.

3. Results

3.1. Urinary organic acid analysis

No obvious abnormalities were found for urinary OAs under stable conditions for both cases 1 and 2 (Table 2).

3.2. Blood acylcarnitine analysis

In the AC profiles in DBS, there were no obvious abnormalities in case 1, while there was slight elevation from C4 to C18 in case 2 (Table 3).

In contrast, in the serum AC analysis, slight elevation of C8 and C10 was observed, even under the stable conditions of case 1, and remarkable elevation from C8 to C18 was observed in case 2 (Table 3).

3.3. Histological studies

Muscle tissues stained with Oil-Red O revealed abundant fat deposition in both cases 1 and 2, suggesting metabolic myopathy (Fig. 1A and B).

3.4. Immunoblotting

In both cases 1 and 2, ETFDH protein was not detected, while both ETFα and ETFβ proteins were

Table 2
Results of special examinations.

	Case 1	Case 2
Muscle biopsy	Lipid deposit	Lipid deposit
Urinary organic acid analysis	Normal	Non-specific finding
Blood acylcarnitine analysis (dried blood spots)	Normal	Mild elevation of C4-C18
Gene analysis of *ETFDH*	c.1367C>T (p.P456L) (homozygote)	c.890G>T (p.W297L)/c.950C>G (p.P317R)

Table 3
Comparison of free carnitine and acylcarnitine in DBS and serum.

	Dried blood spot			Serum		
	Case 1	Case 2	(Reference)	Case 1	Case 2	(Reference)
C0	37.94	45.37	(20–60)	32.79	52.35	(10–55)
C2	28.07	46.19	(5–45)	11.56	33.02	(4–60)
C4	0.37	1.77	(<1.4)	0.27	0.78	(<1.65)
C8	0.06	0.98	(<0.25)	1.92	1.61	(<0.46)
C10	0.18	2.03	(<0.35)	1.88	4.63	(<0.8)
C12	0.09	0.8	(<0.4)	0.24	1.35	(<0.4)
C14	0.38	1.01	(<0.7)	0.08	3.29	(<0.3)
C16	2.90	3.12	(<7.0)	0.22	1.19	(<0.5)
C18	1.14	2.32	(<2.1)	0.06	0.55	(<0.3)

The reference values reported here are those used at Shimane University. Values judged as abnormal are underlined.

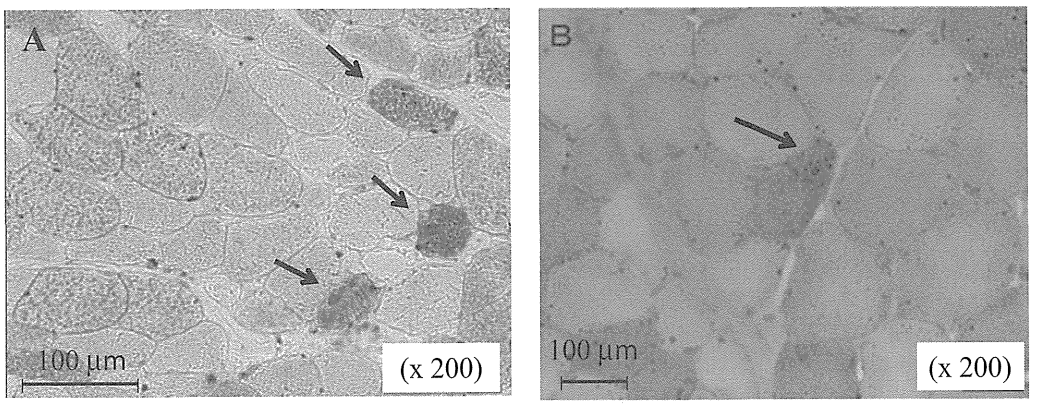

Fig. 1. Pathological findings from the muscle biopsy (Oil-red O stain). (A) Case 1 and (B) case 2. Arrows indicate lipid deposits.

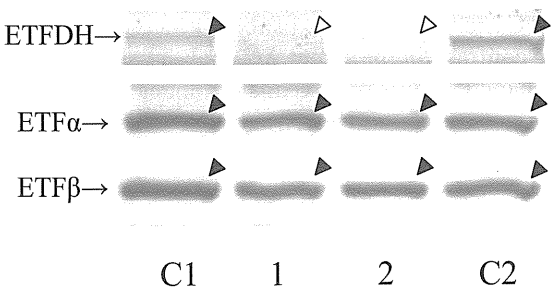

Fig. 2. Immunoblots of ETFDH and ETF proteins using fibroblasts. Lanes C1 and C2, normal controls; lanes 1 and 2, cases 1 and 2, respectively. Black and white triangles indicate a presence and absence of the protein, respectively.

observed to be normal. These findings strongly suggested that both patients had GA2 due to a defect in ETFDH (Fig. 2).

3.5. Gene analysis of ETFDH

Mutation analysis revealed that case 1 was a homozygote of c.1367C>T (p.P456L), and case 2 was a compound heterozygote of c.890G>T (p.W297L) and c.950C>G (p.P317R). Eventually, both cases were diagnosed with GA2 due to a defect in ETFDH (Table 2).

3.6. In vitro probe acylcarnitine assay

Only a slight elevation in C10 was observed in case 2, and the elevation of short- to long-chain ACs, which is a characteristic profile for the IVP assay in pediatric cases of GA2, was not observed in either case (Fig. 3A and B).

4. Discussion

In this study, we report the clinical, biochemical, and molecular aspects of the adult-onset myopathic form of GA2 in 2 cases. Our cases exhibited the following characteristics compared with pediatric cases: (1) repeated episodes of general fatigue, myalgia, or muscular hypotonia after adulthood (approximately 30 or 40 years of age); (2) in routine laboratory findings, slight or moderate elevation of AST, ALT, LDH, and CK; (3) no specific abnormalities for urinary OA analysis under stable conditions; (4) no or barely observable abnormalities in the AC analysis in DBS; (5) significant abnormalities for ACs in the serum; (6) lipid deposition in the muscular biopsy as an initial hint suggesting a GA2 diagnosis; and (7) no abnormalities in the IVP assay for adult-onset cases.

In both cases, few or no abnormalities were detected in several examinations, including urinary OA analysis and AC analysis in DBS. Indeed, cases of adult-onset GA2 with little biochemical abnormality have been previously reported [22,23], suggesting that a biochemical diagnosis of adult-onset GA2 is challenging. Therefore, a number of adult-onset GA2 patients with myopathy of unknown cause might be hidden. Likewise, there is a possibility of overlooking adult-onset GA2 in neonatal mass screening using DBS.

Serum AC analysis appeared to be more informative than DBS for diagnosing adult-onset GA2. There are previous reports that serum or plasma AC analysis could be more useful than DBS for diagnosing long-chain FAODs, such as very long-chain acyl-CoA dehydrogenase deficiency or carnitine palmitoyltransferase-II deficiency [17,24].

The histological findings of lipid deposition provided an initial clue for the diagnosis of GA2 in both of our cases. If fatty degeneration is revealed by muscle biopsy in patients with myopathy of unknown cause, the possibility of FAODs should be considered, even in adult cases.

We previously reported that pediatric cases of GA2 could be classified into the severe or milder form using the results of the IVP assay [25]. However, the profiles for the IVP assay in our cases were different from those of the severe or milder forms. In other words, the biochemical characteristics of adult-onset GA2 are different

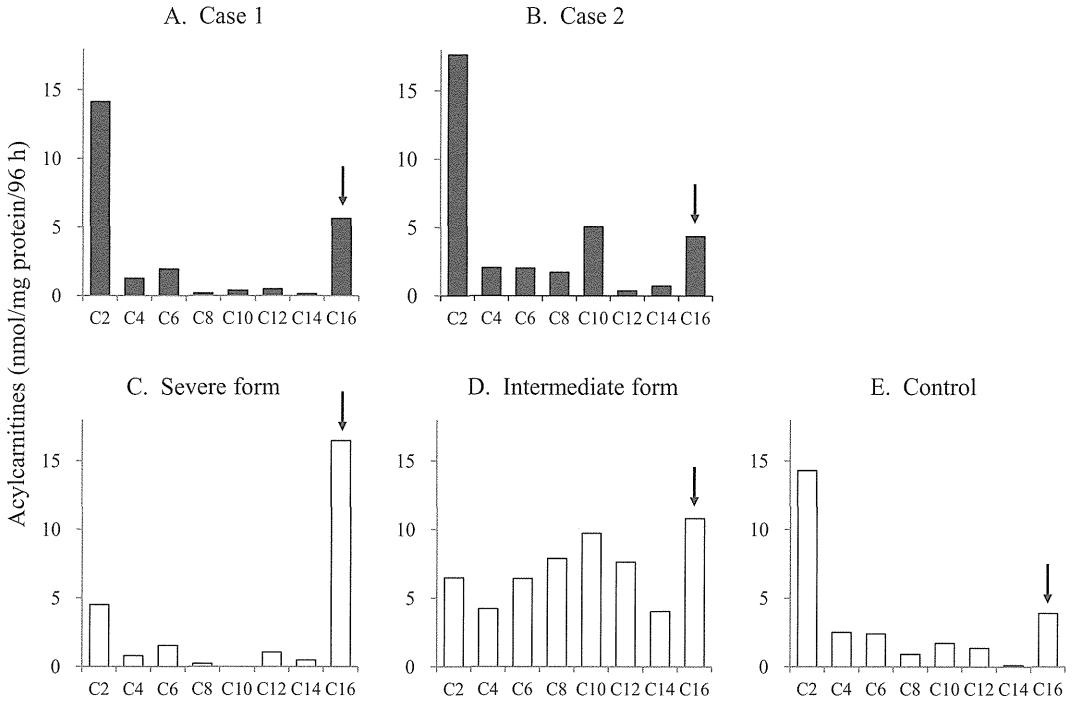

Fig. 3. Profiles of the *in vitro* probe assay. Arrows indicate loaded fatty acid (palmitic acid). The *Y*-axis represents values of acylcarnitines expressed as nmol/mg protein/96 h. (A) Case 1; (B) case 2; (C) patient with a severe form of GA2 due to defect of *ETFA* with homozygote of IVS6-1G>C (frame shift); (D) patient with an intermediate form of GA2 due to defect of *ETFDH* with compound heterozygote of c.G1078C (p.A360P) and c. T1519G (p.Y505D); and (E) healthy controls. Black and white columns indicate our cases and previously tested cases of the severe form, the intermediate form, and the control, respectively.

from those of pediatric cases. Additionally, we determined whether abnormal findings in the IVP assay could be improved by bezafibrate [26], but it may be difficult to evaluate the efficacy of bezafibrate for adult-onset GA2 because the profile of the IVP assay in adult-onset GA2 does not encompass specific abnormalities. However, treating the patients with adult-onset GA2 using bezafibrate may be helpful, even though efficacy of bezafibrate cannot be estimated *in vitro*, because bezafibrate was effective for a pediatric case which is more serious than the adult-onset type [26].

The clinical findings in case 1 included at least three episodes of unconsciousness, which were estimated to be caused by a hypoglycemic attack. Moreover, the younger brother of case 1 had previously died suddenly from an unknown cause in his 30s, suggesting that he might also have had GA2 and then developed profound hypoglycemia or arrhythmia, leading to sudden death. There are previous case reports of adult-onset GA2 cases with serious complications, including a 25-year-old female who was treated with a ventilator due to respiratory muscle failure [27] and a 19-year-old female patient who had repeated hypoglycemic attacks [28]. These cases indicate that critical symptoms can occur in the adult-onset type.

Clinical and biochemical features of adult-onset GA2 have recently been reported, as shown in Table 4. All were myopathic cases associated with ETFDH deficiency. However, there is also a report of a late-onset type other than ETFDH deficiency, although this is very rare [29]. It is considered that GA2 due to defect of ETFDH tend to be milder form in particular in Asian peoples, although some patients with defect of ETFDH occasionally exhibited severe clinical features [14]. The clinical severity varied; severe general symptoms manifested in patients with adult-onset GA2 despite few biochemical abnormalities, as in case 1 reported here and a case reported by Rosenbohm et al. [27], suggesting an unlikely association between the degree of clinical severity and biochemical abnormality.

GA2 has been roughly classified into the neonate-onset and late-onset types [4]. However, the clinical course of the "late-onset type" differs substantially among individuals; some cases have encephalopathy or sudden death during the infantile period, while others may only have muscular symptoms in adulthood, as was the case with the patients reported here. Therefore, we propose to distinguish the late-onset type of GA2 between the intermediate and myopathic forms, as shown in Table 5, according to the results of the IVP assay as well as age at onset, fatality, and clinical characteristics. The intermediate form (juvenile-onset form) exhibits intermittent attacks, including hypotonia, hypoglycemia, hyperammonemia, and acute

Table 4
Recently reported clinical and biochemical features for adult-onset GA2.

No	Sex	Age at onset (year)	Myalgia	Muscle weakness	Other symptoms	Laboratory data			Increased urinary organic acid	Elevated acylcarnitines		Gene	Gene mutation		Refs.
						Elevated trans-aminase	LDH (IU/L)	CK(IU/L)		DBS	Serum (plasma)		Allele 1	Allele 2	
Our cases															
1	M	40s	+	+	Coma	+	2903	3000	Normal	Normal	C8–C10	ETFDH	p.P456L	p.P456L	Our case
2	M	31	+	+	No	+	2860	1897	Normal	C4–C12	C8–C18	ETFDH	p.W297L	p.P317R	Our case
Previously reported cases															
3	M	42	+	+	No	N/A	942	1855	GA, 2HG, EMA	C4, C5, C8, C10, C14	N/A	ETFDH	p.I243T	p.T294I	Köppel et al. [10]
4	F	24	+	+	No	N/A	N/A	677	N/A	C8–C12	N/A	ETFDH	p.L409F	p.V291G	Wen et al. [23]
5	F	23	+	+	Vomiting	N/A	N/A	513	N/A	C8–C12	N/A	ETFDH	p.L409F	p.V291G	Wen et al. [23]
6	F	48	+	+	Vomiting	N/A	N/A	128	N/A	C0 (↓), C8–C10	N/A	ETFDH	p.Y257C	Not detected	Wen et al. [23]
7	F	22	–	+	No	N/A	N/A	478	GA, 2HG, EMA, DCA, KB	C4-OH, C10–C14	N/A	ETFDH	p.Y257C	p.V291G	Wen et al. [23]
8	F	33	+	+	No	N/A	N/A	352	GA, 2HG, EMA, DCA, KB	C0 (↓), C12–C14	N/A	ETFDH	p.Y257C	p.325del48	Wen et al. [23]
9	F	63	–	+	No	N/A	N/A	2120	GA,2HG, EMA, DCA	C0, C5–C14	N/A	ETFDH	IVS3+1G>A heterozygote	None	Wen et al. [23]
10	F	23	+	+	Vomiting	N/A	N/A	1998	GA, 2HG, EMA, DCA, KB	C8–C14	N/A	ETFDH	p.M404T	Not detected	Wen et al. [23]
11	F	22	+	–	No	N/A	N/A	339	normal	C0	N/A	ETFDH	p.L409F	Not detected	Wen et al. [23]
12	M	46	+	+	Difficulty in breathing	+	543	5995	GA, 2HG, DCA	N/A	N/A	ETFDH	p.M404T	p.D596N	Izumi et al. [11]
13	F	55	+	+	No	N/A	N/A	8000	normal	N/A	C4–C18	ETFDH	p.H293D	Not detected	Kaminsky et al. [22]
14	M	36	–	–	Exercise intolerance	N/A	1161	3055	2HG, 2-OH adipate	N/A	N/A	ETFDH	p.D511 N	p.W603X	Sugai et al. [12]
15	M	53	+	+	Osphyalgia, nausea	+	600	571	GA, 2HG, EMA	C8–C12	N/A	ETFDH	p.P508T	p. N528KfsX3	Zhao et al. [13]
16	F	24	+	+	Vomiting, respiratory insufficiency	+	N/A	20,000	2HG, EMA, DCA, HG, SG	N/A	C2 (↓), C14:1	ETFDH	p.S515I	p.S515I	Rosenbohm et al. [27]

LDH: lactate dehydrogenase, CK: creatine kinase, DBS: dried blood spot, N/A: not available, GA: glutarate, HG: 2-hydroxyglutarate, EMA: ethylmalonate, DCA: dicarboxylate, KB: ketone body, HG: hexanoylglycine, SG: suberylglycine, and (↓): decreased.

Table 5
Classification of glutaric acidemia type II based on the severity and IVP assay results.

Clinical form	Age at onset	Clinical course	Mortality	Biochemical abnormality	*In vitro* probe assay with C16 loaded
1. Severe from (neonatal-onset)	Soon after birth	Rapid onset and early death after birth hyperammonemia, hypoglycemia, or cardiomyopathy	++	++	Marked elevation of C16
2. Intermediate form (juvenile-onset)	Infantile or childhood	Episodes of lethargy, liver dysfunction, or hypoglycemia occasionally encephalopathy or even sudden death	+	+	Elevation of C4–C16
3. Myopathic form (adult-onset)	School-age or adulthood	Episodes of myalgia, muscle weakness, fatigue, or liver dysfunction	–	±	Almost normal

encephalopathy-like attack, with typical biochemical abnormalities and relatively high mortality following metabolic stress from an infection or diarrhea in infancy or young childhood. The IVP assay for the intermediate form reveals the elevation of broad ranges in acylcarnitine (C4–C16) when palmitate is loaded (Fig. 3D) [25]. The myopathic form (adult-onset form), in which the patients primarily present with intermittent muscular symptoms after adolescence or adulthood with normal intelligence, offers a favorable life prognosis in many cases. However, it should be noted that muscle symptoms are sometimes exhibited during the infantile period even in the myopathic form [30].

The above classification based on the IVP assay can also be used for preclinical risk control of GA2 detected in neonatal mass screening. Moreover, it is considered that making diagnosis using IVP assay is useful because clinical form cannot be predicted only by the genotype. It is expected that, with the spread of knowledge regarding the clinical characteristics of adult-onset GA2, such a form of GA2 will be found among patients with "myopathy of unknown origin" in the future.

Conflict of interest

The authors indicate no potential conflict of interest.

Acknowledgements

This study was partially supported by grants from the Ministry of Education, Culture, Sports, Science, and Technology (S.Y. and K.Y.) and the Ministry of Health, Labor and Welfare (S.Y.) of Japan. The authors thank Ms. Furui M., Hattori M., Ito Y., and Tomita N. for technical assistance. We also thank Dr. Takashi Hashimoto, Professor Emeritus of Shinsyu University, for the kind gift of purified enzymes and antibodies against ETF and for comments on this study.

References

[1] Hori D, Hasegawa Y, Kimura M, Yang Y, Verma IC, Yamaguchi S. Clinical onset and prognosis of Asian children with organic acidemias, as detected by analysis of urinary organic acids using GC/MS, instead of mass screening. Brain Dev 2005;27:39–45.

[2] Goodman SI, Binard RJ, Woontner MR, Frerman FE. Glutaric acidemia type II: gene structure and mutations of the electron transfer flavoprotein:ubiquinone oxidoreductase (ETF:QO) gene. Mol Genet Metab 2002;77:86–90.

[3] Goodman SI, Frerman FE. Glutaric acidaemia type II (multiple acyl-CoA dehydrogenation deficiency). J Inherit Metab Dis 1984;7(Suppl. 1):33–7.

[4] Frerman FE, Goodman SI. Deficiency of electron transfer flavoprotein or electron transfer flavoprotein:ubiquinone oxidoreductase in glutaric acidemia type II fibroblasts. Proc Natl Acad Sci USA 1985;82:4517–20.

[5] Lehnert W, Wendel U, Lindenmaier S, Böhm N. Multiple acyl-CoA dehydrogenation deficiency (glutaric aciduria type II), congenital polycystic kidneys, and symmetric warty dysplasia of the cerebral cortex in two brothers. I. Clinical, metabolical, and biochemical findings. Eur J Pediatr 1982;139:56–9.
[6] Sweetman L, Nyhan WL, Tauner DA, Merritt TA, Singh M. Glutaric aciduria type II. J Pediatr 1980;96:1020–6.
[7] Mantagos S, Genel M, Tanaka K. Ethylmalonic-adipic aciduria. In vivo and in vitro studies indicating deficiency of activities of multiple acyl-CoA dehydrogenases. J Clin Invest 1979;64:1580–9.
[8] Niederwieser A, Steinmann B, Exner U, Neuheiser F, Redweik U, Wang M, et al. Multiple acyl-Co A dehydrogenation deficiency (MADD) in a boy with nonketotic hypoglycemia, hepatomegaly, muscle hypotonia and cardiomyopathy. Detection of N-isovalerylglutamic acid and its monoamide. Helv Paediatr Acta 1983;38:9–26.
[9] Rinaldo P, Matern D, Bennett MJ. Fatty acid oxidation disorders. Annu Rev Physiol 2002;64:477–502.
[10] Koppel S, Gottschalk J, Hoffmann GF, Waterham HR, Blobel H, Kolker S. Late-onset multiple acyl-CoA dehydrogenase deficiency: a frequently missed diagnosis? Neurology 2006;67:1519.
[11] Izumi R, Suzuki N, Nagata M, Hasegawa T, Abe Y, Saito Y, et al. A case of late onset riboflavin-responsive multiple acyl-CoA dehydrogenase deficiency manifesting as recurrent rhabdomyolysis and acute renal failure. Intern Med 2011;50:2663–8.
[12] Sugai F, Baba K, Toyooka K, Liang WC, Nishino I, Yamadera M, et al. Adult-onset multiple acyl CoA dehydrogenation deficiency associated with an abnormal isoenzyme pattern of serum lactate dehydrogenase. Neuromuscul Disord 2012;22:159–61.
[13] Zhao ZN, Bao MX, Ma GT, Liu XM, Xu WJ, Sun ZW, et al. A case of late-onset riboflavin responsive multiple acyl-CoA dehydrogenase deficiency with novel mutations in ETFDH gene. CNS Neurosci Ther 2012;18:952–4.
[14] Yotsumoto Y, Hasegawa Y, Fukuda S, Kobayashi H, Endo M, Fukao T, et al. Clinical and molecular investigations of Japanese cases of glutaric acidemia type 2. Mol Genet Metab 2008;94:61–7.
[15] Kimura M, Yamamoto T, Yamaguchi S. Automated metabolic profiling and interpretation of GC/MS data for organic acidemia screening: a personal computer-based system. Tohoku J Exp Med 1999;188:317–34.
[16] Shigematsu Y, Hirano S, Hata I, Tanaka Y, Sudo M, Sakura N, et al. Newborn mass screening and selective screening using electrospray tandem mass spectrometry in Japan. J Chromatogr B 2002;776:39–48.
[17] Al-Thihli K, Sinclair G, Sirrs S, Mezei M, Nelson J, Vallance H. Performance of serum and dried blood spot acylcarnitine profiles for detection of fatty acid β-oxidation disorders in adult patients with rhabdomyolysis. J Inherit Metab Dis 2014;37:207–13.
[18] Shapira Y, Deckelbaum R, Statter M, Tennenbaum A, Aker M, Yarom R. Reye's syndrome; diagnosis by muscle biopsy? Arch Dis Child 1981;56:287–91.
[19] Purevsuren J, Fukao T, Hasegawa Y, Kobayashi H, Li H, Mushimoto Y, et al. Clinical and molecular aspects of Japanese patients with mitochondrial trifunctional protein deficiency. Mol Genet Metab 2009;98:372–7.
[20] Li H, Fukuda S, Hasegawa Y, Purevsuren J, Kobayashi H, Mushimoto Y, et al. Heat stress deteriorates mitochondrial beta-oxidation of long-chain fatty acids in cultured fibroblasts with fatty acid beta-oxidation disorders. J Chromatogr B 2010;878:1669–72.
[21] Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 1979;76:4350–4.
[22] Kaminsky P, Acquaviva-Bourdain C, Jonas J, Pruna L, Chaloub GE, Rigal O, et al. Subacute myopathy in a mature patient due to multiple acyl-coenzyme A dehydrogenase deficiency. Muscle Nerve 2011;43:444–6.
[23] Wen B, Dai T, Li W, Zhao Y, Liu S, Zhang C, et al. Riboflavin-responsive lipid-storage myopathy caused by ETFDH gene mutations. J Neurol Neurosurg Psychiatry 2010;81:231–6.
[24] de Sain-van der Velden MG, Diekman EF, Jans JJ, van-der-Ham M, Visser G, et al. Differences between acylcarnitine profiles in plasma and bloodspots. Mol Genet Metab 2013;110:116–21.
[25] Endo M, Hasegawa Y, Fukuda S, Kobayashi H, Yotsumoto Y, Mushimoto Y, et al. In vitro probe acylcarnitine profiling assay using cultured fibroblasts and electrospray ionization tandem mass spectrometry predicts severity of patients with glutaric aciduria type 2. J Chromatogr B 2010;878:1673–6.
[26] Yamaguchi S, Li H, Purevsuren J, Yamada K, Furui M, Takahashi T, et al. Bezafibrate can be a new treatment option for mitochondrial fatty acid oxidation disorders: evaluation by in vitro probe acylcarnitine assay. Mol Genet Metab 2012;107:87–91.
[27] Rosenbohm A, Sussmuth SD, Kassubek J, Muller HP, Pontes C, Abicht A, et al. Novel ETFDH mutation and imaging findings in an adult with glutaric aciduria type II. Muscle Nerve 2014;49:446–50.
[28] Dusheiko G, Kew MC, Joffe BI, Lewin JR, Mantagos S, Tanaka K. Recurrent hypoglycemia associated with glutaric aciduria type II in an adult. N Engl J Med 1979;301:1405–9.
[29] Grunert SC. Clinical and genetical heterogeneity of late-onset multiple acyl-coenzyme A dehydrogenase deficiency. Orphanet J Rare Dis 2014;9:117.
[30] Xi J, Wen B, Lin J, Zhu W, Luo S, Zhao C, et al. Clinical features and ETFDH mutation spectrum in a cohort of 90 Chinese patients with late-onset multiple acyl-CoA dehydrogenase deficiency. J Inherit Metab Dis 2014;37:399–404.

Molecular Genetics and Metabolism Reports 6 (2016) 1–4

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

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Case Report

A fetus with mitochondrial trifunctional protein deficiency: Elevation of 3-OH-acylcarnitines in amniotic fluid functionally assured the genetic diagnosis

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ARTICLE INFO

Article history:
Received 22 October 2015
Received in revised form 25 November 2015
Accepted 25 November 2015
Available online xxxx

Keywords:
Prenatal diagnosis
Mitochondrial trifunctional protein (TFP)
Acylcarnitine analysis
Fatty acid oxidation
HADHA gene

GenBank and other databases:
HADHA OMIM: 600890, GDB: 434026, GenBank: NM_000182
HADHB OMIM: 143450, GDB: 344953, GenBank: NM_000183

ABSTRACT

Mitochondrial trifunctional protein (TFP) is a multienzyme complex that catalyzes the last three steps of the β-oxidation cycle of long-chain fatty acids. In the prenatal diagnosis of TFP deficiency, acylcarnitine (AC) analysis has been considered difficult because of limited excretion of long-chain ACs into the fetal urine and hence into the amniotic fluid. Here, we report our experience with prenatally diagnosing TFP deficiency using AC analysis of amniotic fluid. The index case was a boy born at 38 weeks gestation and weighing 2588 g. He suddenly became unconscious and hypoglycemic and died on day 6 of life. Postmortem blood AC analysis and gene sequencing revealed TFP deficiency. Therefore, the parents underwent prenatal diagnoses for their subsequent 2 pregnancies. Mutation analysis suggested that one (Case 1) was affected and the other (Case 2) was not. AC analysis also demonstrated identical results, with significantly elevated 3-hydroxy-AC levels in the amniotic fluid of the affected pregnancy compared with those of heterozygotes and normal controls (n = 2 for heterozygotes and n = 8 for normal controls). Our findings suggest that AC analysis can functionally confirm results even in families with unidentified mutations, without raising issues related to maternal cell contamination. During prenatal diagnosis, misdiagnosis has to be avoided, and combining AC analysis with gene sequencing may result in more accurate prenatal diagnosis of TFP deficiency.

1. Introduction

Mitochondrial trifunctional protein (TFP) is a multienzyme complex consisting of trans-2,3-long-chain enoyl-CoA hydratase (LCEH, EC 4.2.1.74), long-chain 3-OH-acyl-CoA dehydrogenase (LCHAD, EC 1.1.1.211) located in the TFP α-subunit (HADHA, OMIM: 600890), and long-chain 3-ketoacyl-CoA thiolase (LCKT, EC 2.3.1.16) located in the TFP β-subunit (HADHB, OMIM: 143450). These enzymes catalyze the last three steps of the β-oxidation cycle of long-chain fatty acids [1,2]. TFP deficiency is clinically classified into three types: 1) lethal type (neonatal-onset form), which includes the development of profound hypoglycemia, lactic acidosis and cardiomyopathy during the neonatal period; 2) intermediate type (infant-onset form), which is accompanied by hypoketotic hypoglycemia that is generally observed following infection or long periods of fasting during the infantile period; and 3) myopathic type (adult-onset form), which includes muscular symptoms, such as intermittent myalgia or rhabdomyolysis, that are associated with prolonged exercise after adolescence. The neonatal form is normally lethal during the neonatal period, irrespective of any intensive treatments [3]. Therefore, families who have had such an affected child often undergo genetic counseling for prenatal diagnosis during subsequent pregnancies.

TFP deficiency is usually diagnosed based on increased levels of long-chain 3-OH-acylcarnitines (3-OH-ACs), such as C16-OH or C18:1-OH, which can be measured by blood acylcarnitine (AC) analysis using tandem mass spectrometry (MS/MS). However, instead of AC analysis, gene analysis is usually performed for the prenatal diagnosis of TFP deficiency [4]. Herein, we report our experience with prenatally diagnosing TFP deficiency using AC analysis and gene analysis. Our data indicate that AC analysis of amniotic fluid is useful for the prenatal diagnosis of TFP deficiency.

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http://dx.doi.org/10.1016/j.ymgmr.2015.11.005