Metabolic Survey of Hidden Inherited Metabolic Diseases in Children With Apparent Life-Threatening Event (ALTE) or Sudden Unexpected Death in Infancy (SUDI) by Analyses of Organic Acids and Acylcarnitines Using Mass Spectrometries

Tomoo TAKAHASHI , Yuki HASEGAWA , Kenji YAMADA , Ryosuke BO , Hironori KOBAYASHI , Takeshi TAKETANI , Seiji FUKUDA and Seiji YAMAGUCHI

Department of Pediatrics, Shimane University Faculty of Medicine, Izumo, 693-8501, Japan

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To determine the relation between sudden unexpected death in infancy (SUDI) or apparent life-threatening events (ALTE) and inborn errors of metabolism (IMD), we investigated clinical and biochemical features in patients presenting with SUDI or ALTE, who were diagnosed with having hidden IMD. Subjects were infants between aged from 2 days and 3 years, detected during the period between January 2004 and December 2014. The diagnosis of IMD was based on the findings of gas chromatography/mass spectrometry and/or tandem mass spectrometry. IMDs were detected in 3 of 239 (1.2%) patients with SUDI, and in 22 of 219 (10.0%) patients with ALTE. These patients had a history of some prodromal illness and/or abnormalities of routine laboratory tests. When a case with SUDI or ALTE is encountered at an emergency unit, it is essential to take detailed medical histories and to measure biochemistry tests including mass spectrometric analysis as well as genetic testing.

Key words: inherited metabolic diseases, sudden unexpected death in infancy, apparent life-threatening events, gas chromatography/mass, tandem mass spectrometry

Address for correspondence and reprint request: Tomoo Takahashi
Department of Pediatrics, Shimane University Faculty of Medicine, 89-1 Enya-cho, Izumo, Shimane 693-8501, Japan
Tel: +81-853-20-2219
Fax: +81-853-20-2215
E-mail : tomoo00094@yahoo.co.jp

1. INTRODUCTION

Inherited metabolic disease (IMD) is caused by inherited defect of metabolic enzymes, and many of such diseases result in impairments in multiple organs including central nervous system (CNS), liver, or skeletal muscles. Children with such diseases may die in early infancy or childhood, or have severely handicaps. Recently, new groups of IMD which may cause sudden infant death syndrome (SIDS)-like illness or apparent life-threatening event (ALTE), have been attracted attention. Especially, organic acidemia (OA-emia) or fatty acid oxidation defect (FAOD) are famous as causative diseases of SIDS or ALTE, with recent development in diagnostic tools, including analyses of urinary organic acids (OA) or blood acylcarnitines (AC) using gas chromatography mass spectrometry (GC/MS), or tandem mass spectrometry (MS/MS), respectively.

SIDS is defined as the sudden, unexpected death of infant whose causes cannot be explained based on previous medical history or symptoms of the infant. According to recent study, SIDS is the third leading cause of overall infant mortality in Japan, following congenital anomalies, and perinatal disorders. Sudden death may occur not only in infants with no prodromal symptoms but also in previously healthy infants following infections or diarrhea [1-5]. The latter cases are not considered to represent SIDS in a strictly defined sense, but the other term, sudden unexpected death in infancy (SUDI), is applied. ALTE is defined as life-threatening episode in infant that does not result in death, following sudden arrest of breath, skin color change, muscle tone change, coughing, and so on. ALTE includes a con-

dition that was previously called “near-miss SIDS”.

Recently, expanded newborn mass screening for OA-emia and FAODs as well as amino acidemias (AA-emia), using MS/MS, popular worldwide. Furthermore, GC/MS and MS/MS have been applied for survey of the metabolic causes in SUDI or ALTE cases. GC/MS is particularly useful in diagnosis of OA-emias, and MS/MS is used for screening for OA-emias, FAODs as well as AA-emias.

Previously, we reported 10 children with SUDI or ALTE, in which IMDs were identified retrospectively [6]. In this study we surveyed hidden IMDs by analyses of urinary OA and/or blood AC using GC/MS and/or MS/MS, respectively, in children presenting SUDI or ALTE. Further, disease types, and the clinical features in the acute condition were investigated.

2. METHODS

2.1 Subjects

Infants who presented SUDI or ALTE, aged at neonates to 3 years or less and were diagnosed with IMSs by analyses of OA and/or AC, were investigated. Samples of urine and/or blood were introduced to the Department of Pediatrics, Shimane University from all over Japan, during the period between January 2004 and December 2014. The criteria for our survey were as follows: (a) ages between 2 days and 3 years, (b) clinical diagnosis of SUDI (or SIDS) or ALTE, and (c) established diagnosis of OA-emia or FAOD. Furthermore, we also surveyed cases that were strongly suspected of IMDs but the final diagnosis was not confirmed because of less information [7, 8].

2.2 Urinary organic acid analysis using GC/MS

Urine samples for the analysis of OA using GC/MS were pretreated as described previously. Briefly, to an aliquot of urine equivalent to 0.2 mg of creatinine, 20 μg each of heptadecanic acid and tetracosane (C24), and 40 μg of 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 analysis was performed using GCMS QP2010 PLUS (Shimadzu Corporation, Kyoto, Japan). The column (30 m × 0.25 mm i.d.) was DB-5 (J&W Scientific Inc., CA, USA). The oven temperature was initially held at 100°C for 4 min, and was then raised to 290°C at an increasing rate of 4°C/min [11, 12].

2.3 Blood acylcarnitine analysis using MS/MS

ACs were analyzed by MS/MS after butyl derivatization. Serum sample aliquots of 10 μL were analyzed according to the method described previously [13]. MS/MS analysis was carried out using an API 3000 (Lub Solution, Applied Biosystems, Foster City, CA, USA) or Shimadzu LC-MSMS 8040 (Kyoto, Japan). Data were analyzed employing (Labsolution LCMS/ Applied Biosystems/MDS SCIEX, Toronto, Canada).

3. RESULTS

3.1 Age distribution of cases with SUDI or ALTE

We studied a total of 458 infants including 239 with SUDI and 219 with ALTE. Figure 1 shows the age distribution at onset. The numbers of cases and age group, A to H, were as follows: group A over 2 days, under 7 days after birth, 59 (22 and 37 with SUDI and ALTE, respectively); group B over 7 days, under 30 days of age, 49 (22 and 27 with SUDI and ALTE, respectively); group C over 30 days, under 3 months, 82 (40 and 42 with SUDI and ALTE, respectively); group D over 3 months, under 6 months, 87 (41 and 46 with SUDI and ALTE, respectively); group E over 6 months, under 12 months, 86 (62 and 24 with SUDI and ALTE, respectively); group F over 1 year, under 1 year 6 months of age, 48 (30 and 18 with SUDI and ALTE, respectively); group G over 1 year 6 months, under 2 years of age, 25 (12 and 13 with SUDI and ALTE, respectively); group H over 2 years, under 3 years of age, 22 (10 and 12 with SUDI and ALTE, respectively).

3.2 Incidence of inherited metabolic diseases (IMDs) confirmed

Analyses of urinary OAs by GC/MS, and ACs by MS/MS, and/or genetic testing yielded a diagnosis of IMDs in 25 (5.4%) of the total 458 infants. Of

the 25 cases, there were 3 cases with SUDI, and 22 with ALTE.

3.3 Age at onset of cases with IMD

Among these 25 infants, 12 were in the group A; 3 in the group B; 1 in the group C, 5 in the group E; 1 in the group F; and 3 in the group G.

3.4 Disease types

Among the above 25 infants, 3 were SUDI cases while the remaining 22 were ALTE. 2 cases had carnitine palmitoyltransferase type II (CPT2) deficiency and 1 case had medium-chain acyl-CoA dehydrogenase (MCAD) deficiency. On the other hand, disease types in 22 ALTE cases were as follows: 9 cases with urea cycle disorders (UCD), followed by 8 cases with methylmalonic acidemia (MMA), 2 cases with 3-hydroxy-3-methylglutaryl-CoA synthase (HMGS) deficiency, and 1 case each with MCAD deficiency, trifunctional protein (TFP) deficiency and 3-methylglutaconic acidemia (MGC), respectively, as shown in Table 1. The diagnosis of UCD was based on elevation of uracil and orotic acid in urinary organic acid finding as well as hyperammonemia in clinical findings. Among UCD cases, specific diagnosis such as 3 cases of citrullinemia type I was made according to the findings of blood amino acid analysis using MS/MS.

3.5 Clinical history and findings in the acute condition

Prodromal symptoms seen in 14 of 15 neonates (Cases 1 to 15 in Table 1) were as follows: poor feeding in 8 neonates; weight loss, and hypotonia in 3 each; vomiting, and lethargy in 2 each. Loss of Moro reflex was noted in 2 neonates. One infant (Case 12) had a family history of acute encephalopathy of the sibling.

In 9 of 10 children between ages of 1 month and 3 years (cases 16 to 25 in Table 1), common cold-like symptoms such as fever, cough, rhinorrhea, and vomiting were noted as prodromal symptoms. As acute symptoms of the 9 infants, intractable vomiting was seen in 4 cases; medical history of episodic hypoglycemia in 3 (cases 21, 22 and 23); and episode of cyanosis during the neonatal period in one (case 21).

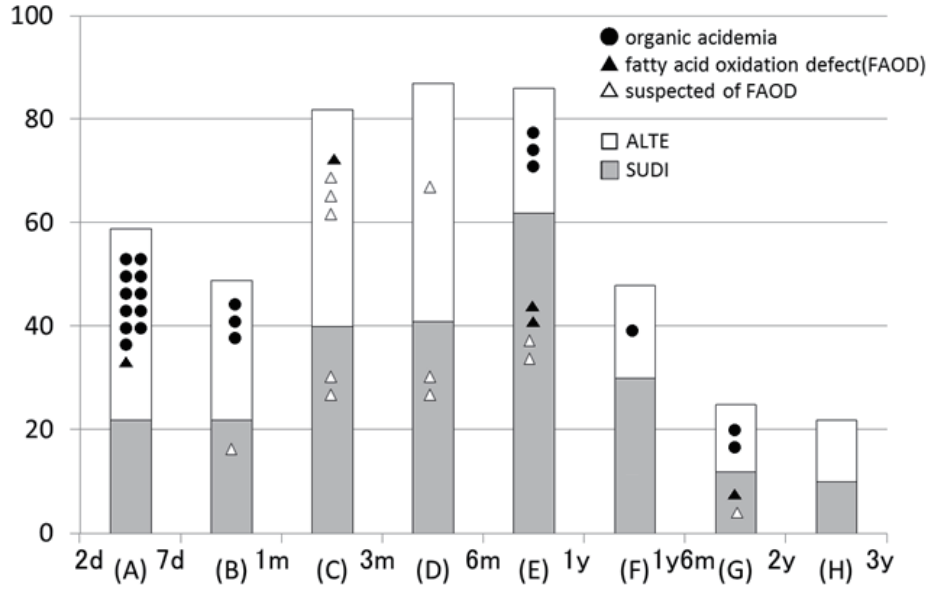

Figure1. Age distribution of inherited metabolic diseases identified in cases with SUDI and ALTE
ALTE are 219 and SUDI are 239 cases. Inborn errors of organic acidemia are shown circle(●) and fatty acid oxidation defect are show triangle(▲), and fatty acid oxidation defect (△). White column (□) indicates cases of ALTE, and gray column (▩) does cases of SUDI.
Abbreviations: ALTE, acute life threatening event; SUDI sudden unexpected death in infancy

Table 1. Symptoms and clinical course of patients presenting ALTE or SUDI diagnosed with inherited metabolic disease by analysis using GC/MS and/or MS/MS

Case	Age at onset	Sex	Diagnosis	GC/MS	MS/MS	Prodromal illness	Acute symptom
ALTE							
1	2d	F	UCD (Cit)	?	◎	vomiting, poor feeding	seizure
2	2d	F	UCD	◎	◎	poor feeding	resp. failure
3	2d	?	UCD	◎	◎	weight loss	resp. failure seizure
4	2d	M	UCD	◎	◎	vomiting	cyanosis
5	2d	M	MCAD def*2	◎	◎	poor feeding	resp. failure seizure
6	3d	F	UCD	◎	◎	hypotonia	resp. failure
7	3d	M	MMA	◎	◎	nl	resp. failure
8	4d	M	UCD	◎	◎	poor feeding	resp. failure
9	4d	M	MMA	◎	◎	weight loss	unconscious
10	5d	F	MMA	◎	◎	hypotonia, poor feeding	unconscious
11	6d	F	MMA	◎	◎	hypotonia, poor feeding	resp. failure
12	6d	F	UCD (Cit)	◎	◎	weight loss	resp. failure
13*1	7d	F	MMA	◎	◎	poor feeding	
14*1	8d	F	UCD (Cit)	◎	◎	lethargy	resp. failure
15*1	8d	F	UCD	◎	◎	poor feeding, lethargy	unconscious
16*1	1m	M	TFP def*2	◎	◎	nl	resp. failure
17	6m	M	HMGS def*2	◎	?	fever, diarrhea	unconscious
18	7m	M	MGC	◎	?	vomiting	unconscious
19*1	8m	M	MMA	◎	◎	vomiting	resp. failure, unconscious
20	1y0m	M	HMGS def*2	◎	?	fever	resp. failure, unconscious
21*1	1y8m	F	MMA	◎	◎	cough, vomiting	unconscious, cyanosis
22*1	1y10m	M	MMA	◎	◎	fever, vomiting	resp. failure
SUDI							
23*1	6m	F	CPT2 def*2	◎	◎	cough	unconscious
24	9m	M	CPT2 def*2	◎	◎	fever	resp. failure
25*1	1y8m	M	MCAD def*2	◎	◎	fever, cough	unconscious

*1, previously reported cases by Takahashi el al; *2, confirmed by gene analysis
Abbreviations: UCD, Urea cycle disorder (elevation of uracil and orotic acid in urinary organic acid analysis by GC/MS); Cit, citrullinemia identified by MS/MS analysis ; MCAD, Medium-chain acyl-CoA dehydrogenase: MMA, Methylmalonic acidemia; TFP, mitochondrial trifunctional protein; GA2, Glutaric acidemia type II; HMGS, 3-hydroxy-3-methylglutaryl-CoA synthetase; MGC, 3-methlglutaconic acidemia ; CPT2, Carnitine palmitoyltransferase II; ?, data not available; resp. failure, respiratory failure; nl, normal; GC/MS, gas chromatography mass spectrometry; MS/MS, tandem mass spectrometry. ◎, analyzed by GC/MS or MS/MS

Table 2. Laboratory data in the acute stage

Case	Age	Diagnosis	pH	Ketone	AST (IU/L)	ALT (IU/L)	CK (IU/L)	NH_3 (μg/dL)	BS (mg/dL)
ALTE									
1	2d	UCD (Cit)	7.3	?	44	13	?	>1000	nl
2	2d	UCD	nl	nl	26	18	614	592	nl
3	2d	UCD	ab	?	283	64	2332	597	nl
4	2d	UCD	nl	?	ab	ab	ab	>1000	ab
5	2d	MCAD def*2	7.2	nl	56	9	2413	25	3
6	3d	UCD	7.0	nl	200	?	1113	723	?
7	3d	MMA	nl	?	292	246	nl	1118	59
8	4d	UCD	ab	nl	ab	ab	?	442	nl
9	4d	MMA	7.2	nl	ab	ab	nl	2050	ab
10	5d	MMA	7.3	nl	65	53	nl	674	nl
11	6d	MMA	7.2	nl	70	10	726	326	nl
12	6d	UCD (Cit)	ab	nl	145	52	662	2006	40
13*1	7d	MMA	7.3	nl	65	53	245	674	143
14*1	8d	UCD (Cit)	ab	?	55	33	308	2006	79
15*1	8d	UCD	nl	+	36	16	284	1035	78
16*1	1m	TFP def*2	nl	?	153	71	8077	nl	71
17	6m	HMGS def*2	?	+	219	120	?	195	?
18	7m	MGC	7.4	?	nl	nl	1305	116	?
19*1	8m	MMA	7.1	+	nl	nl	1084	?	63
20	1y0m	HMGS def*2	7.0	?	511	126	nl	179	nl
21*1	1y8m	MMA	7.2	+	40	43	560	162	30
22*1	1y10m	MMA	6.9	+	52	17	100	188	88
SUDI									
23*1	6m	CPT2 def*2	7.37	nl	353	178	203	147	203
24	9m	CPT2 def*2	7.2	?	3144	1712	1100	?	17
25*1	1y8m	MCAD def*2	7.2	?	80	40	107	1640	107

*1, *2, previously reported as in Table 1.
Abbreviations: nl, normal; ab, abnormality; ?, data not available; the other are the same as those of Table1.

3.6 Routine laboratory findings

Abnormalities in routine laboratory tests during the acute phase were as follows: metabolic acidosis (pH 6.9-7.3) was seen in 17 of 24 infants, positive ketone bodies (in urine or blood) in 5 of 15 children tested, liver dysfunction (AST, 52 to 3144 IU/L; ALT, 43 to 1712 IU/L) in 20 of 25 cases tested, high blood creatine kinase (CK) levels (203 to 8,077 IU/L) in 16 of 22 cases tested, hyperammonemia (147 to 2,006 μg/dL) in 21 of 23 cases tested, and hypoglycemia (blood glucose 3-30 mg/dL) in 5 of 22 cases tested (Table 2).

3.7 Diseases types in cases suspected of IMDs but not confirmed

In addition to the above described 25 infants, at least 12 infants were strongly suspected of IMDs, but lacked a definitive diagnosis because of less information/data, as shown in Table 3. Of the 12 cases, 4 were ALTE cases, while 8 SIDS cases.

Suspected diseases were as follows: carnitine uptake defect (CUD, systemic carnitine deficiency) in 4 cases; GA2 in 2; carnitine-acylcarnitine translocase (CACT) deficiency or CPT2 deficiency in 2; GA2 or VLCAD deficiency, TFP deficiency, MCAD deficiency, short-chain acyl-CoA dehydrogenase (SCAD) deficiency in 1 each, respectively.

4. DISCUSSION

The incidence of SIDS in Japan is estimated to be one in about 6,000 to 7,000 births recently. It is reported that ages at onset of SIDS is most common from 2 months to 6 months after birth [14]. In the present study, 3 (1.2 %) of 239 infants with SUDI and 22 (10.0%) of 219 infants with ALTE were diagnosed as having IMDs. The total incidence of IMDs was 25 (5.4%) of 458 cases with SUDI and ALTE. Cases retrospectively identified IMDs became symptomatic most commonly in the neonatal period, followed by under 1 month of age. The reason why neonatal cases were most common is considered that SUDI cases were not strictly defined SIDS.

Chace *et al*. reported that IMDs were identified in 66 (0.9%) of 7,058 infants with SUDI, while Bole *et al*. found FAODs in 27 (6.4%) of the SIDS in their study [16-19]. In our study, 22 of 25 cases were ALTE, many cases were life-saving. This suggests that it is possible to save such children by early diagnosis even after symptomatic. On the other hand, 4 cases, ALTE (3 cases) or SUDI (1 cases), developed in 4 patients after 1 year of age. It suggests that we should pay attention to IMDs in children with ALTE or SUDI, even in children aged over 1 year.

15 (60%) of 25 cases with IMDs were neonates

Table 3. Profiles of suspected cases of inherited metabolic disorders but not confirmed

Case	Age at onset	Sex	Suspected disease	GC/MS (abnormal OA)	MS/MS (abnormal AC)	Prodromal illness	Acute symptom
ALTE							
1	1m	M	CUD	?	C0 (↓)	poor feeding	resp. failure
2	1m	M	TFP def	?	C16:1-OH C18:1-OH C18-OH	?	resp. failure
3	2m	M	GA2/ or VLCAD def	PA, LA,NKDA	C10-16	fever	seizure resp. failure
4	4m	M	MCAD def	PA, KA, DA	C8-C10	fever vomiting	CPA
SIDS							
5	20d	M	CUD	?	C0 (↓)	poor feeding	CPA
6	1m	F	SCAD def	?	C4	?	CPA
7	2m	F	GA2	?	Short to long ACs	fever	CPA
8	4m	M	CUD	PA, LA, DA	C0 (↓)	fever	CPA
9	4m	M	CACT def/ or CPT2 def	?	C18:1 C16;1	?	CPA
10	9m	F	GA2	?	Medium to long ACs	diarrhea vomiting	CPA
11	9m	M	CACT def/ or CPT2 def	PA, NKDA	C18:1 C16:1	fever	CPA
12	1y9m	F	CUD	PA, LA, DA	C0 (↓)	fever	CPA

Abbreviations: CUD, carnitine uptake defect (systemic carnitine deficiency); VLCAD, very long-chain acyl-CoA dehydrogenase; SCAD, short-chain acyl-CoA dehydrogenase; CACT, Carnitine-acylcarnitine translocase; (↓), decreased; poor feed, poor feeding.; AC, acylcarnitine; ?, data not available; resp., respiratory; CPA, cardiopulmonary arrest; PA, pyruvic acid; LA, lactic acid; NKDA, non-ketotic dicarboxylic aciduria; DA, dicarboxylic aciduria

(under 1 month). IMDs with onset in the neonatal period are often severe and fatal [15]. There is a possibility that cases introduced might have been biased in clinical setting, and samples after death might have rarely been introduced. In 9 (60%) of the 15 neonatal cases, UCD was detected, followed by 5 cases of MMA (33%). Both diseases often show hyperammonemia in the acute stage, and in this study, all but one case had hyperammonemia. The characteristic symptoms of neonatal cases were not common cold-like symptoms such as fever up or cough, but hypotonia, poor feeding or lethargy. It indicates that hyperammonemia and common cold-like symptom are observed in neonatal cases.

3-Hydroxy-3-hydroxyglyaryl-CoA synthetase (HMGS) deficiency was a newly identified disease from previous study [6]. In our study, HMGS deficiency was detected in two cases by gene analysis retrospectively. In these cases, they had history of episodic profound hypoglycemia with ketosis and elevation of C2 in AC analysis. However, we should pay attention to repeated episodes of profound hypoglycemia with ketosis, and AC findings. Hyperammonemia and liver dysfunction were very often seen in the acute stage of IMD cases, and these findings may be a clue to approach the diagnosis of IMDs.

Definitive diagnosis could not be made in at least 12 infants due to several factors such as lack of specimens or not enough clinical information, although the results of analyses of urinary OAs or blood ACs were suggestive of IMDs. This may suggest that infants with OA-emia or FAODs may account for at least 1.5% of those presenting with SUDI or ALTE. Unfortunately, however, data of blood ammonia or urinary ketone body levels were not available in these cases. Even in many cases, blood samples were collected, but urine was not collected. Therefore, data of urinary OAs or urinary ketone body levels were not available. When we come across the cases with ALTE or SUDI, collection of urine even by suprapubic bladder puncture should be tried.

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