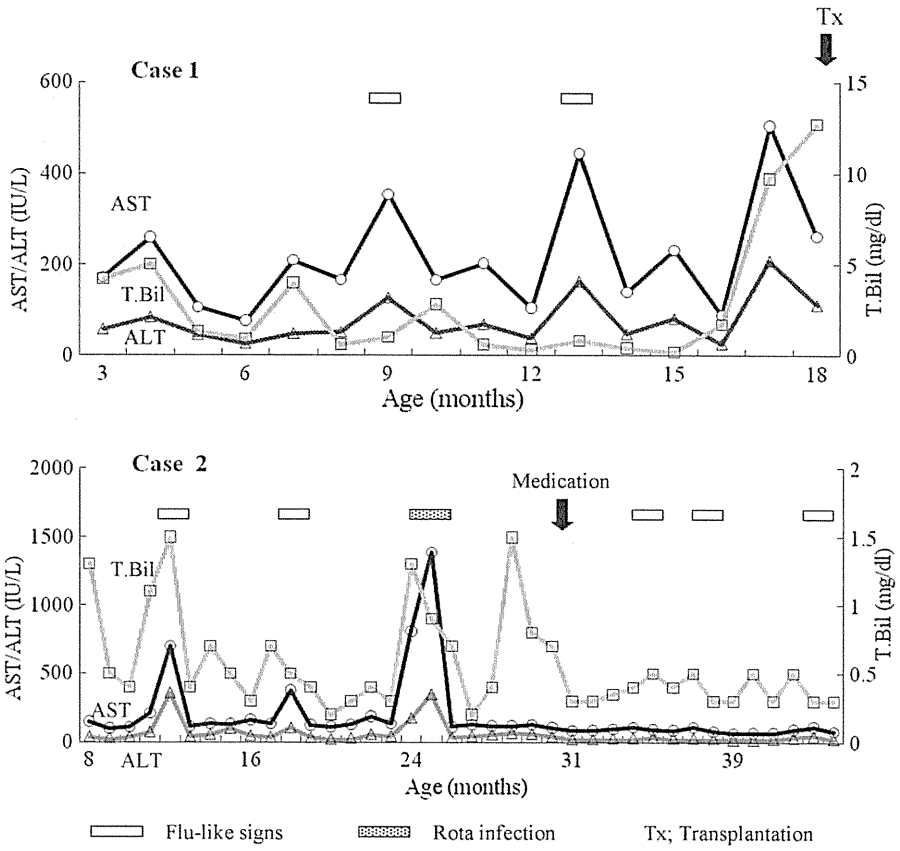

Fig. 1. Clinical courses of cases 1 (upper panel) and 2 (lower panel).

Determination of enzyme activities

Activities of RC Co I, II, III, and IV were assayed for the crude post-600 g supernatant of the liver samples as described previously [6,7]. The activity of each complex was presented as a percentage of the mean value obtained from 35 healthy controls. For each patient, the percentages of Co I, II, III, and IV activities relative to that of citrate synthase (CS) as a mitochondrial enzyme marker or Co II activity were calculated [6].

BN-PAGE Western blotting

Expression levels of the mitochondrial RC Co I, II, III, and IV proteins in the liver were examined by Western blotting using blue native polyacrylamide gel electrophoresis (BN-PAGE) according to the methods described previously [8,9]. Ten micrograms of the protein in the mitochondria-enriched fraction was separated by BN-PAGE. Immunostaining was performed using a monoclonal antibody specific for the 39 kD subunit of Co I, the 70 kD subunit of Co II, the core 1 subunit of Co III, and the subunit 1 of Co IV (Molecular Probes, Eugene, OR).

Quantitative PCR

mtDNA was quantitatively estimated by the real-time amplification of fragments of ND1 in the mtDNA genome, as previously described [10,11]. To determine the overall abundance of mtDNA, we compared the real-time amplification of ND1 with a single-

Table 1
Enzyme assay of respiratory chain and quantitative mtDNA evaluation by qPCR.

%	Co I	Co II	Co III	Co IV	CS	mtDNA/nDNA (%)
Elder Brother (17 months)						7.8
% of normal	0	80	13	41	300	
CS ratio	0	27	4	14	–	
Co II ratio	0	–	16	50	–	
Younger Brother (30 months)						6.6
% of normal	22	80	34	83	397	
CS ratio	6	36	9	21	–	
Co II ratio	15	–	24	57	–	
Younger Brother (37 months)						
% of normal	23	170	28	75	254	
CS ratio	9	67	11	29	–	
Co II ratio	13	–	16	43	–	–

Co I; complex I, Co II; complex II, Co III; complex III, Co IV; complex IV, CS; citrate synthase.
Enzyme activities are expressed as % of mean normal control activity relative to protein, relative to CS, and relative to Co II.

copy nuclear reference gene (exon 24 of the CFTR gene, chosen because it lacks single-nucleotide polymorphisms). For both experiments, DNA from six adult liver samples (from needle biopsies, obtained with informed consent) was used as controls. The results presented were the means of four independent runs, with samples assayed in triplicate in each run.

Mutation detection

Genomic DNA was extracted from liver or peripheral blood leukocyte according to the standard procedures. Detailed sequencing methods appear in the supplemental materials.

Results

Enzyme activities

Both affected siblings showed low activity levels of RC Co I, III, and IV. In particular, their Co I activities were strikingly low. In contrast, their Co II activities were maintained at normal, and those of citrate synthase were greatly elevated (Table 1). Co III and Co IV activity levels were higher in the younger brother than those in the elder brother.

BN-PAGE Western blot analysis

Fig. S2 shows the RC Co amounts by BN-PAGE in each brother. In both brothers, the band corresponding to either assembled Co I or assembled Co IV was invisible, and the band corresponding to the assembled Co III was strikingly weak. On the other hand, the intensity of the Co II band remained normal in both brothers.

Quantitative PCR

Quantitative PCR revealed that liver mtDNA was markedly decreased in both brothers (Table 1). The ratio of ND1 to CFTR in the liver of each brother was lower than those of the six controls (mean ± SD: 7.8 ± 4.6% for the elder brother, 6.6 ± 1.5% for the younger brother).

Mutations in MPV17

Both brothers were confirmed to be compound heterozygotes for c.451insC/c.509C > T (Fig. S3). c.451insC in exon 6 causes a frame-shift predicting an elongated gene product p.Leu151fsX189 (p.Leu151PhefsX39, according to the standard mutation nomenclature guidelines at http://www.genomic.unimelb.edu.au/mdi/mutnomen/). The c. 509C > T in exon 7 causes an amino acid substitution (Ser170Phe). These variations had not registered as genetic polymorphisms in the ensembl_mart_47 database (martdb.ensembl.org) and had not been reported as disease-causing mutations. Moreover, the alignment shows that both amino acid residues (Leu151 and Ser170) mutated in the affected siblings are absolutely conserved in all species (Fig. S4). Therefore, we consider these variations to be novel mutations. A single allele of c.451insC was present in all three siblings and their mother, whereas c. 509C > T was detected in both affected siblings and their father (Fig. S3). The fact that two such mutations were inherited from each parent independently indicated that these mutations were compound heterozygous in both affected siblings. The parents and the unaffected sibling had only one mutation, and had no obvious phenotype (Fig. S3). These observations support an autosomal recessive manner of inheritance for the hepatic dysfunction phenotype segregating within this family.

Discussion

Both brothers had novel compound heterozygous mutations, c.451insC/c.509C > T, but their clinical courses differed greatly. The phenotype of the elder brother was classified as possibly the infantile form, characterized by early onset liver disease that rapidly progresses to liver failure within the first few years of life [4,5].

In contrast, the younger brother exhibited a rather mild course. His liver damage was relatively mild, and he did not show any apparent neurological abnormality.

Such a great difference in the clinical courses between these brothers might be explained, in part, by the differences in their RC activity levels. The degree of reduction in RC activity was generally milder in the younger brother than in the elder. However, several studies have shown that RC activities were not correlated with the clinical course [4,5].

In our MPV17 mutant patients, the fluctuations in liver function were associated with infections that may cause oxidative stress. It was likely that cytomegalovirus infection promoted the onset and progression of liver disease in the elder brother, and that the liver dysfunctions in these siblings were greatly exacerbated by viral infections, in particular rotavirus infection.

Taken together, our experiences with these cases allowed us to assume that the clinical course and prognosis of MDS caused by MPV17 mutations were determined not only by the mutation but also by other factors. We postulated that many complicating factors may arise, including infection.

An effective treatment for mitochondrial RC disorders involving MDS has yet to be established. Liver transplantation is not so promising [6,12–14]. Besides the surgical complication, neurological regression after transplantation has been reported. Collectively, the survival rate is less than 50% [14].

For the younger brother, we tried to administer medications targeting the RC system, including succinate and coenzyme Q. Simultaneously, a lipid-rich carbohydrate-restricted diet using ketone milk was initiated. This combined treatment improved his liver disease biochemically and histologically. However, his liver RC activities did not improve.

Initially, he received a carbohydrate-rich, lipid-restricted diet and fat-soluble vitamins, together with UDCA, as had his elder brother. Recently, Parini et al. reported that glucose administration to avoid hypoglycemia is efficient in slowing the progression of liver disease [5]. However, this dietary treatment did not achieve favorable effects for our patients. Therefore, we resorted to another treatment for the younger brother.

The efficacy of medications with succinate and coenzyme Q, together with a lipid-rich diet, was possibly explained by their biochemical features. The mitochondrial RC system comprises Co I, II, III, and IV. Co I activity was markedly reduced in the younger brother, while his Co III activity remained mildly or moderately decreased. On the other hand, his Co II activity was entirely normal. Co I, an electron and proton acceptor from NADH and H^+, respectively, is the most important reduction-type hydrogen carrier, generating ATP by glucose oxidation [13]. From this context, glucose should hardly have been used as an energy source in the liver of the younger brother. On the other hand, succinate might donate electrons and protons to Co II connected to ubiquinone via $FADH_2$ [15]. In addition, a lipid-rich diet was expected to donate electrons and protons to ETF (electron-transfer flavoprotein): QO (ubiquinone oxidoreductase) connected to ubiquinone by promotion of $FADH_2$ production.

In summary, these cases suggested that the clinical course of MPV17 mutation is not determined solely by the mutation but rather is greatly influenced by viral infection, and that medications targeting Co II, together with a lipid-rich diet, may be beneficial in the clinical management of patients with MDS.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ymgme.2009.04.014.

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Very-long-chain acyl-coenzyme A dehydrogenase (VLCAD) deficiency in a patient who recovered from ventricular fibrillation, but died suddenly of an RSV infection

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Summary

The neonatal onset of inborn metabolism deficiencies sometimes follows a severe clinical course. Sudden death is observed in most of these cases. Here, we report a newborn boy with very-long-chain acyl-coenzyme A dehydrogenase (VLCAD) deficiency, a fatty acid beta-oxidation disorder with high morbidity and mortality. Tachypnea and grunting were observed in the patient on the day of birth. After a few minutes, he had a sudden onset of ventricular fibrillation. We successfully treated him with cardiopulmonary resuscitation and administration of medications. He showed normal development under follow-up with long-term dietary therapy, supplementation of carnitine and medium-chain triglyceride (MCT) oil. However, the patient suddenly died after infection of the respiratory syncytial virus. This case suggests that the follow-up of a patient with severe VLCAD deficiency can seem positive but can be difficult, especially in stress management such as in common infections.

Abbreviations

VLCAD	Very-long-chain acyl-coenzyme A Dehydrogenase

Introduction

Patients with fatty acid oxidation disorders may present with early onset of a severe form usually associated with cardiomyopathy and leading to sudden death in some cases (Mathur et al 1999). In infants, the disease course can be rapid and is difficult to diagnose in the emergency department. Very-long-chain acyl-coenzyme A (CoA) dehydrogenase (VLCAD) deficiency (OMIM #201475) is an autosomal recessive disorder affecting fatty acid oxidation. The phenotype of VLCAD deficiency is classified into 3 clinical forms on the basis of the onset of symptoms: a severe form with neonatal onset, a milder form with childhood onset, and a late-onset form. The severe neonatal form is the most common, and patients present with cardiomyopathy, hepatopathy, and skeletal myopathy. This form has a higher mortality rate than the other 2 forms. Arrhythmia is normally merely symptomatic, but can be fatal in the neonatal form. The childhood-onset form presents with hypoketotic hypoglycemia, and the late-onset form shows recurrent rhabdomyolysis and myoglobinuria (Gregersen et al 2001). VLCAD deficiency may cause sudden infant death because of cardiac or hepatic involvement (Roe et al 2000). Here, we report the case of a newborn infant with VLCAD deficiency who developed ventricular fibrillation (VF), which was successfully treated by intensive care. However, the patient suddenly died after a respiratory syncytial virus infection.

Case Report

Our patient was a boy weighing 3566 g at birth who was born at 39 weeks and 4 days of gestation following an unremarkable pregnancy. There was no significant family history or consanguinity. On the first day of life, tachypnea and grunting were noted. These findings suggested pneumonia, but the patient did not improve with intravenous administration of antibiotics. The patient was not responding well and was therefore transferred to the pediatric emergency center for further examinations. He showed slightly delayed capillary refill, oxygen saturation of 99%, heart rate of 118 beats/min, and respiratory rate of 80 breaths/min. Laboratory analysis revealed blood glucose and potassium levels of 42 mg/dL (2.33 mmol/L) and 7.05 mmol/L, respectively, and blood gas measurement showed metabolic acidosis with pH 7.294, pCO_2 29.4 mmHg, pO_2 35.6 mmHg, HCO_3 13.8 mmol/L, BE -11.1 mmol/L, and anion gap 25.2 mEq/L. ECG monitoring revealed a sudden onset of VF (Figure 1). Cardiac pulmonary resuscitation was attempted, with administration of calcium gluconate and epinephrine, and after 30 minutes, the patient showed recovered to sinus rhythm. Administration of sodium bicarbonate followed by glucose-insulin therapy was initiated. The patient was then transferred to our neonatal intensive care unit. After arrival, hypoglycemia, hyperkalemia, and metabolic acidosis recovered quickly. Cardiac function required more

time for complete recovery, but the patient did not experience arrhythmia. A blood spot taken at administration was sent to the laboratory for tandem mass spectrometry (MS/MS) and revealed abnormal acylcarnitine values: C14:1, 4.08 μmol/L; C16, 13.38 μmol/L; and (C16+C18)/C2, 1.67 (Table 1). These findings suggested that the newborn patient may probably have VLCAD deficiency. Fatty acid ß-oxidation analysis in cultured lymphocytes was therefore performed (Table 2). Increase in $d_{27}C14$ and $d_{31}C16$ values and decrease in $d_{23}C12/d_{27}C14$ and $dC2/d_{31}C16$ values confirmed the suspected VLCAD deficiency. An enzyme activity assay was performed, and the activity was found to be low at 0.42 pmol·min^{-1}·10^{6} lymphocytes^{-1} (Table 3). Gene analysis revealed a homozygote c.1332G>A mutation in the exon-intron junction of the acyl-CoA dehydrogenase, very-long-chain (ACADVL) gene (Figure 2), indicating a splicing abnormality. After the correct diagnosis, the patient showed normal development with long-term dietary therapy and supplementation of carnitine and medium-chain triglyceride (MCT) oil. Vomiting and diarrhea was sometimes associated with metabolic acidosis, but he recovered quickly after rapid transfusion of glucose and electrolytes. However, at the age of 2 years, he was affected by respiratory syncytial virus. He only showed coughing and wheezing and was therefore not admitted to the hospital. On the next morning, he became unconscious and died suddenly in our hospital. We speculate

that his death was due to arrhythmia.

Discussion

VLCAD deficiency is an autosomal recessive disorder affecting the first step in the mitochondrial fatty acid β-oxidation system. The phenotype of VLCAD deficiency is heterogeneous. Patients are classified into 3 forms on the basis of the onset of symptoms and clinical findings: a severe form with onset in neonates, a milder childhood-onset form, and a mild late-onset form. The prevalence of VLCAD has been estimated to be 1 in 150,000. It is believed that a severe neonatal-form of this disease produces hypoglycemia, hyperammonemia, cardiomyopathy, muscle damage, and sudden death (Mathur et al 1999). VF and respiratory arrest have been reported in patients who develop VLCAD within a year of birth (Bonnet et al 1999). In the present case, the patient developed VF and was rescued by cardiopulmonary resuscitation, because the pediatrician was at his bedside during the development of VF. When the patient was transferred to our hospital, metabolic acidosis was improved by glucose transfusion. First, we suspected mitochondrial diseases and secondary cardiac disorders. MS/MS was very useful for the final diagnosis of VLCAD.

In the past 5 years in Kumamoto, the analysis of MS/MS was initiated as a

pilot study, and MS/MS was introduced for mass screening of newborns with approximately 100% agreement. Because the genetic abnormality in this case was observed 2 days after birth, the patient was not covered by our standard screening. The abnormality was detected only when post-symptom high-risk screening was performed. Elevations in C14:1, C16, C16+18/C2 values were found by MS/MS, and VLCAD deficiency was suspected. At this point, the patient was given MCT milk and carnitine. Next, we performed a fatty acid ß-oxidation assay and found that the metabolism of C14 to C12 was abnormal. We also performed a VLCAD enzyme assay and ACADVL gene analysis (Tajima et al 2008). Palmitoyl-CoA dehydrogenase activity of this patient was found to be severely decreased. Molecular analysis of the ACADVL gene encoding VLCAD showed that the patient had a single base mutation, c,1332G>A, at the exon-intron junction. To the best of our knowledge, this case presents a novel mutation. We did not perform splicing examinations and mRNA analysis. However, we assumed that it was a mutation causing exon-skipping or connection to a new junction (Coughlin et al 2010).

An inborn error in metabolism is one of the differential diagnoses of unknown cardiomyopathy or arrhythmia. In this case, MS/MS was insufficient for preclinical diagnosis because of the delayed time of sampling to detect early-onset VLCAD;

however, it was very useful for accurate diagnosis (Spiekerkoetter et al 2003).

It is possible to prevent secondary complications of VLCAD with intake of MCT milk and carnitine supplementation and with diet therapy. We can expect normal development with careful follow-up for most patients (Touma et al 2001). It is important to start a glucose infusion, especially in cases with gastroenteritis and starvation. The present patient experienced a metabolic crisis accompanied by gastroenteritis at 1 year of age, but recovered quickly following treatment with IV glucose. The prognosis shows that control of VLCAD deficiency is very challenging even after successfully resolving several crises.

Conclusion

We report the case of a newborn with VLCAD deficiency who developed VF as an initial symptom. We successfully treated VF with rapid resuscitation and then diagnosed the patient with VLCAD deficiency, which is a lethal disease. On correct diagnosis, it was possible to prevent secondary complications with daily management and medication. Normal development can be expected in most patients; however, the disease is hard to manage. It is difficult to identify preclinical early-onset VLCAD deficiency by MS/MS, but MS/MS was useful for diagnosis of the present case.

Figure 1. ECG showing VF

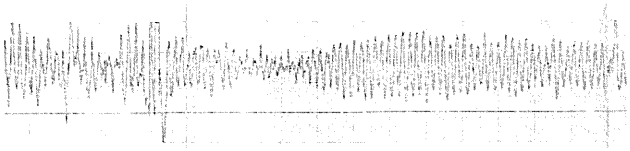

Figure 2. Sequence analysis

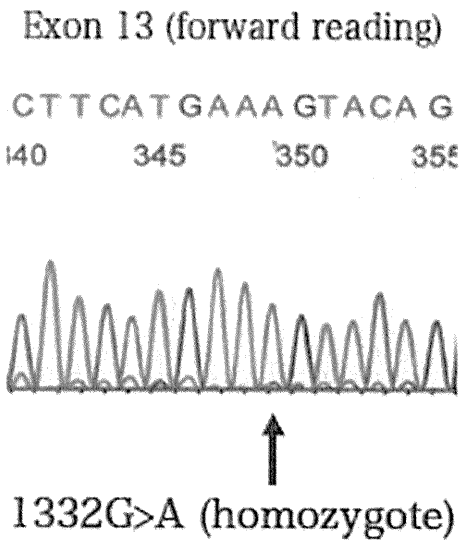

Table 1. Tandem mass analysis

Index	Data (nmol/mL)	Cut off
C10	0.78	>0.35
C14:1	4.08	>0.4
C16	13.38	>6
OH-C16	0.12	>0.05
C18:1	3.3	>3
C16 + C18/C2	1.67	>0.62

Table 2. Fatty acid ß-oxidation in cultured lymphocytes

Index	Mean ± SD	Patient
dC2	271.2 ± 124.8	115.7
$d_{23}C12$	13.95 ± 8.53	15.24
$d_{27}C14$	20.06 ± 10.91	574.71
$d_{31}C16$	70.76 ± 41.97	485.04
$d_{23}C12/d_{27}C14$	0.632 ± 0.2	0.027
$dC2/d_{31}C16$	4.91 ± 2.56	0.24

Table 3. Enzyme activity

Subject	Palmitoyl-CoA dehydrogenase activity ($pmol \cdot min^{-1} \cdot 10^6$ $lymphocytes^{-1}$)
Patient	0.42
Control	25.1
Normal (n = 31)	54.5 ± 17.5

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Carnitine Palmitoyltransferase 2 Deficiency: The Time-Course of Blood and Urinary Acylcarnitine Levels during Initial L-Carnitine Supplementation

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Carnitine palmitoyltransferase 2 (CPT2) deficiency is one of the most common mitochondrial beta-oxidation defects. A female patient with an infantile form of CPT2 deficiency first presented as having a Reye-like syndrome with hypoglycemic convulsions. Oral L-carnitine supplementation was administered since serum free carnitine level was very low (less than 10 μmol/L), indicating secondary carnitine deficiency. Her serum and urinary acylcarnitine profiles were analyzed successively to evaluate time-course effects of L-carnitine supplementation. After the first two days of L-carnitine supplementation, the serum level of free carnitine was elevated; however, the serum levels of acylcarnitines and the urinary excretion of both free carnitine and acylcarnitines remained low. A peak of the serum free carnitine level was detected on day 5, followed by a peak of acetylcarnitine on day 7, and peaks of long-chain acylcarnitines, such as C16, C18, C18:1 and C18:2 carnitines, on day 9. Thereafter free carnitine became predominant again. These peaks of the serum levels corresponded to urinary excretion peaks of free carnitine, acetylcarnitine, and medium-chain dicarboxylic carnitines, respectively. It took several days for oral L-carnitine administration to increase the serum carnitine levels, probably because the intracellular stores were depleted. Thereafter, the administration increased the excretion of abnormal acylcarnitines, some of which had accumulated within the tissues. The excretion of medium-chain dicarboxylic carnitines dramatically decreased on day 13, suggesting improvement of tissue acylcarnitine accumulation. These time-course changes in blood and urinary acylcarnitine levels after L-carnitine supplementation support the effectiveness of L-carnitine supplementation to CPT2-deficient patients.

Keywords: carnitine palmitoyltransferase 2; CPT2; L-carnitine; acylcarnitine profile; carnitine administration
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Carnitine palmitoyltransferase 2 (CPT2) deficiency (EC 2.3.1.21, OMIM 600650) is one of the most common disorders of mitochondrial fatty acid oxidation. CPT2 deficiency has several clinical presentations (Bonnefont et al. 1999). The adult form is characterized by episodes of rhabdomyolysis triggered by prolonged exercise. The infantile form presents as severe attacks of hypoketotic hypoglycemia, occasionally associated with sudden infant death or a Reye-like syndrome (Demaugre et al. 1991; Hug et al. 1991). The most severe kind, the neonatal form, is almost always lethal during the first month of life.

Secondary carnitine deficiency, characterized by low levels of total and free carnitines associated with an increase in the long-chain acylcarnitine fraction, is observed in the infantile form of CPT2-deficient patients (Bonnefont et al. 2004; Longo et al. 2006). Hence, L-carnitine supply might be useful in severe CPT2 deficiencies (Bonnefont et al. 2004), although supplementation with L-carnitine in patients with beta-oxidation defects of long-chain acyl-CoA has long been a matter of controversy (Costa et al. 1998;

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Liebig et al. 2006; Primassin et al. 2008).

In this report, we describe a CPT2-deficient patient who presented as having a Reye-like syndrome with secondary carnitine deficiency. We focused on time-dependent changes in the serum and urinary acylcarnitine profiles after intial L-carnitine supplementation.

Clinical Report

The patient, a female, was born to nonconsanguineous Japanese parents. She had been well until 15 months of age when she suddenly had tonic-clonic convulsions at 3:00 a.m. for about 30 minutes and became unconscious. Ten days before the convulsions, she had a cold and was given Cefteram pivoxil (CFTM-PI) for four days. When she arrived at another hospital, she had hypoglycemia (blood glucose 1.1 mmol/L), hepatic dysfunction (AST 85 IU/L, ALT 55 IU/L, LDH 402 IU/L), and mild hyperammoniemia (NH3 84 μmol/L). Urinary ketones were not detected. Brain MRI and cerebrospinal fluid were normal. She was suspected of being affected by a Reye-like syndrome and transferred to Gifu University Hospital.

On admission, her height was 72 cm (−1.5s.d.) and her weight was 10 kg (+0.73s.d.). She had a fever (38.3°C) and exhibited lethargy. Physical examination revealed mild hepatomegaly. A laboratory test showed AST 382 IU/L, ALT 441 IU/L, LDH 557 IU/L, PT 31%, NH3 84 μmol/L, and blood glucose 4.7 mmol/L.

We tentatively diagnosed her as having a Reye-like syndrome and treated her with intravenous glucose. Her consciousness level became clear on the 4th hospital day and she started oral intake of food. An abdominal CT scan still showed hepatomegaly and a fatty liver (20HU) on the 6th hospital day. The finding of cardiac ultrasonography was normal. Urinary organic acid analysis during the hypoglycemic condition showed hypoketotic dicarboxylic aciduria. The initial measurements of serum free carnitine and acyl-

Table 1. Time-course of serum and urinary acylcarnitine levels measured by tandem MS.

	Day	- 1	3	5	7	9	13
Serum (μmol/L)	range						
C0	10 - 55	2.98	12.70	40.75	24.31	18.49	58.22
C2	4 - 60	2.25	3.85	14.87	20.15	8.37	14.8
C8	- 1.0	0.035	0.024	0.088	0.058	0.073	0.10
C8DC	- 0.25	0.035	0.046	0.12	0.89	0.97	0.063
C10	- 0.8	0.055	0.062	0.25	0.12	0.17	0.21
C10DC	- 0.1	0.063	0.12	0.24	0.33	0.53	0.19
C12:1	- 0.2	0.038	0.038	0.18	0.15	0.15	0.091
C12DC	- 0.05	0.053	0.064	0.19	0.14	0.27	0.054
C14:1	- 0.1	0.075	0.16	0.47	0.58	0.68	0.18
C16	- 0.5	1.01	1.29	2.99	4.45	8.07	2.56
C18	- 0.3	0.49	0.65	1.46	1.67	3.07	0.99
C18:1	- 0.46	1.50	1.84	4.21	6.09	10.03	3.62
C18:2	- 0.3	0.46	0.67	1.47	1.43	2.05	0.98
(C16+C18:1)/C2	- 0.36	1.12	0.81	0.48	0.52	2.16	0.42
C total		12.35	26.74	86.07	84.99	67.46	85.52
Urine (μmol/mmol Cr)	range*						
C0	5.67 - 56.09	0.61	1.31	82.33	37.85	45.95	329.15
C2	6.87 - 60.48	0.56	0.02	25.44	128.00	41.83	53.58
C4	0.07 - 0.74	0.31	0.47	0.92	0.47	1.38	2.32
C6	0.04 - 0.48	0.18	0.09	0.21	0.22	0.61	0.23
C6DC		1.25	1.34	1.63	15.69	83.33	2.93
C8	0.05 - 0.39	0.00	0.02	0.33	0.98	1.33	0.62
C8DC		0.25	0.52	0.83	23.90	122.99	1.11
C10	0.03 - 0.36	0.05	0.06	0.11	2.66	1.76	0.12
C10DC		0.11	0.02	0.10	0.75	4.03	0.08
C12DC		0.00	0.02	0.01	0.23	1.52	0.01
C16	0.05 - 1.55	0.04	0.02	0.02	0.18	0.63	0.08
C total		4.75	6.86	122.16	226.51	344.91	408.34

* Reference values for urine acylcarnitines were obtained from data reported by Mueller et al. (2003) (10th - 90th percentile)