ATP産生が低下している状況下で，哺乳による負荷が加わったため肝不全が急激に進行し，劇症肝不全にまで至ったものと考えられた．

ミトコンドリア呼吸鎖異常症はBN-PAGEによるイムノブロット解析により診断が迅速に行えるようになった．新生児における原因不明の肝不全では，ミトコンドリア呼吸鎖異常症も考え，呼吸鎖酵素活性を測定することが診断に有用であると考えられた．

日本小児科学会の定める利益相反に関する開示事項はありません．

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Case report

Two Japanese patients with Leigh syndrome caused by novel *SURF1* mutations

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Abstract

We report two patients with Leigh syndrome that showed a combination of facial dysmorphism and MRI imaging indicating an *SURF1* deficiency, which was confirmed by sequence analysis. Case 1 is a 3-year-old girl with failure to thrive and developmental delay. She presented with tachypnea at rest and displayed facial dysmorphism including frontal bossing, lateral displacement of inner canthi, esotropia, maxillary hypoplasia, slightly upturned nostril, and hypertrichosis dominant on the forehead and extremities. Case 2 is an 8-year-old boy with respiratory failure. He had been diagnosed as selective complex IV deficiency. Case 2 displayed facial dysmorphism and hypertrichosis. Since both patients displayed characteristic facial dysmorphism and MRI findings, we sequenced the *SURF1* gene and identified two heterozygous mutations; c.49+1 G>T and c.752_753del in Case 1, and homozygous c.743 C>A in Case 2. For patients with Leigh syndrome showing these facial dysmorphism and hypertrichosis, sequence analysis of the *SURF1* gene may be useful.
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Keywords: Leigh syndrome; *SURF1* deficiency; Facial dysmorphism; Hypertrichosis

1. Introduction

Leigh syndrome (OMIM 256000) is a progressive neurodegenerative disorder with the usual onset in infancy or early childhood. It is a genetically heterogeneous disease and the most common cause is a molecular defect in mitochondrial energy production system, including the respiratory chain complexes and pyruvate dehydrogenase complex. An isolated generalized defect of complex IV, (Cytochrome C oxidase) is the most common biochemical abnormalities found in Leigh syndrome [1]. Leigh syndrome with *SURF1* mutations, which encode the putative assembly protein of complex IV, have been reported [2] with specific clinical features of facial dysmorphism [3], hypertrichosis [4], and MRI findings [5]. Here, we report two patients with these clinical features and novel *SURF1* mutations.

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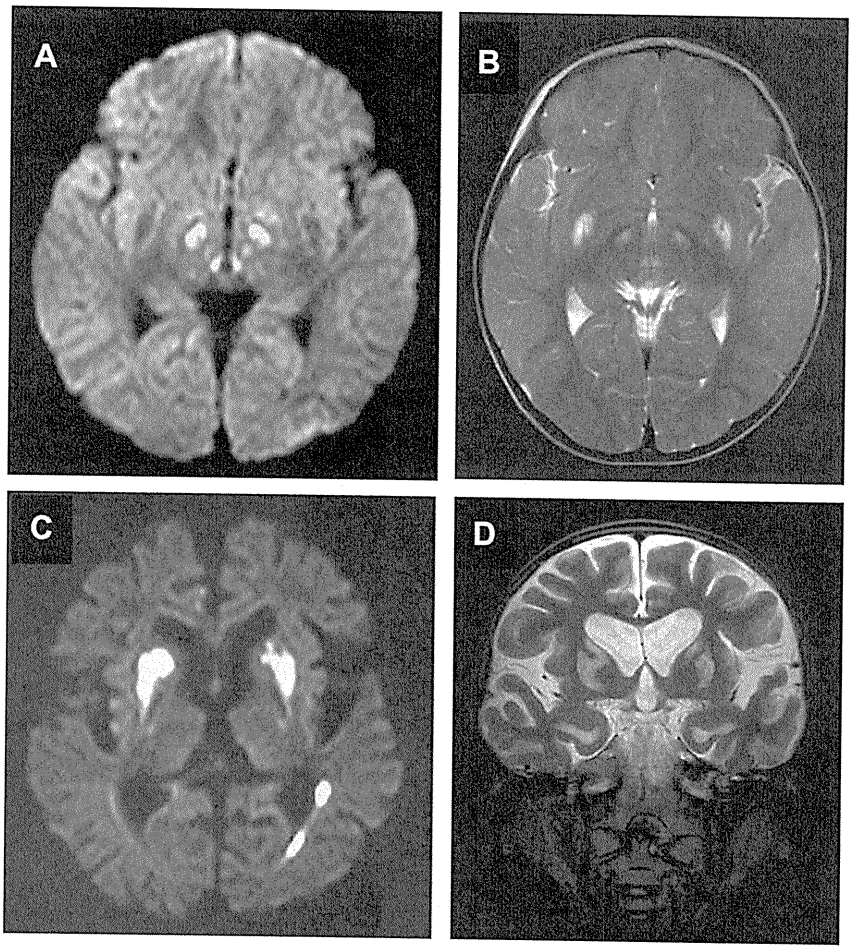

Fig. 1. Diffusion-weighted (A and C) and T2-weighted (B and D) magnetic resonance imaging of the brain in Case 1 at 2 years and 5 months of age (A and B), and in Case 2 at 7 years and 6 months of age (C and D). In Case 1, the bilateral substantia nigra (A), subthalamic nucleus (A and B), red nucleus (A), medial parts of the midbrain (A and B) and putamen (B) show the signal hyperintensity. In Case 2, bilateral striatum reveal hypeintensity (C and D). The left optic radiation is also involved in Case 2 (C) and the global cerebral hemisphere is atrophic (C and D).

2. Case reports

2.1. Case 1

Case 1 is 3-year-old female that was referred to our hospital for an evaluation of failure to thrive and developmental delay at 2 years. She was born to healthy nonconsanguineous Japanese parents. The neonatal period was unremarkable. She held her head upright at 3 months of age, and sat at the 6 months. At the 9 months, she was able to walk independently while holding on to furniture. Her development did not progress thereafter, and she has not walked alone and only speaks using jargon. She was conscious, alert and presented with tachypnea at rest. She displayed facial dysmorphism including frontal bossing, lateral displacement of inner canthi, esotropia, maxillary hypoplasia, slightly upturned nostril, and hypertrichosis dominant on the forehead and extremities. Mild opthalmoplegia and ptosis were noted. She manifested generalized mild hypotonia, truncal ataxia and normal deep tendon reflexes with negative Babinski's signs. Serum lactate was elevated at 35.7 mg/dl. MRI showed signal hyperintensity of the bilateral putamen, subthalamic nucleus, red nucleus and brain stem on T2-weighted images (T2WI) and diffusion-weighted images (DWI) (Fig. 1). The enzyme analysis of the respiratory chain complexes were not performed in this patient.

2.2. Case 2

Case 2 is 8-year-old male on ventilation that was transferred to our hospital for tracheostomy. He was born at term to healthy, nonconsanguineous parents. He had been able to get cruising by 12 months. At 19 months, he presented with neurodevelopmental regression and ataxia. Laboratory investigation revealed elevated cerebrospinal fluid lactate and pyruvate. Brain MRI showed signal hyperintensity of the bilateral basal ganglia, midbrain and medulla oblongata on T2WI. Fibroblast analysis confirmed a decreased amount and activity of complex IV in the respiratory chain complexes

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(Fig. 2). He displayed facial dysmorphism including synophrys and micrognathia, hypertrichosis, thoracic deformity and generalized hypotonia and elevated deep tendon reflexes with positive Babinski's signs. MRI showed that the bilateral cerebral hemisphere were globally atrophic and signal hyperintensity of the bilateral optic radiation, putamen, basal ganglia including subthalamic nucleus, and brain stem on T2WI. The left optic radiation, bilateral putamen and globus pallidus also showed high signal intensity on DWI (Fig. 1).

3. Genomic DNA sequencing, RT-PCR and sequencing

Genomic DNA was prepared from white blood cells using the Wizard Genomic DNA purification kit (Promega, Madison, WI, USA). PCR of all exons and exon–intron boundaries of the *SURF1* gene was performed with specific primers using Ex Taq PCR version 1.0 kit (Takara, Shiga, Japan) according to the manufactures instruction (Suppl. Table 1). Total RNA was extracted from leukocytes using Trizol reagent and amplified with the SMART™ mRNA amplification method (Clontech, Mountain View, CA). The amplified mRNA was subjected to reverse transcription with Prime Script reverse transcriptase (Takara, Shiga, Japan) using Oligo (dT) primers. RT-PCR was performed using primers at exons 1 and 9 of the *SURF1* gene, according to the manufacture's instruction (Suppl. Table 1). Patients and families participating in the gene analysis gave written informed consent to the gene analysis, which was approved by the ethical committee of Kanagawa Children's Medical Center.

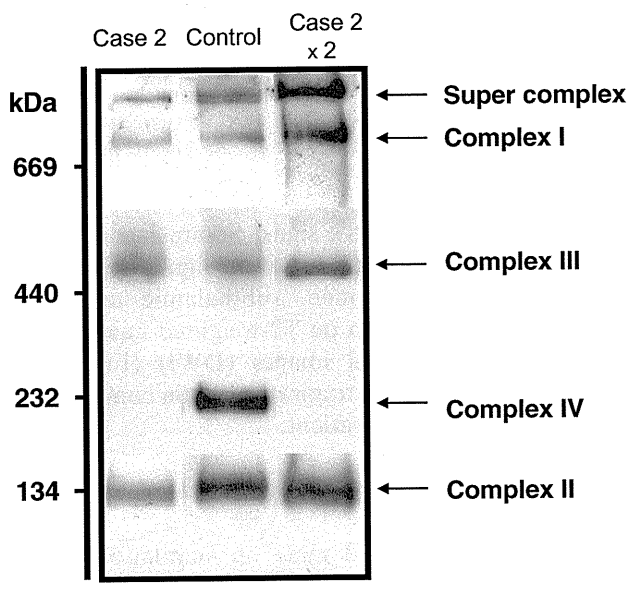

Fig. 2. Analysis of respiratory chain complex amount by blue native polyacrylamide gel electrophoresis in Case 2. Mitochondria isolated from Case 2 and normal control fibroblasts were solubilized in dodecyl maltoside and subjected to BN-PAGE and Western blotting [9]. In x 2 lane, the amount of protein loaded was twice. The amount of fully assembled complex IV was shown to be dramatically decreased in Case 2. The amount of complexes I, II, and III were all comparable to those in the normal control. In vitro enzyme assay [10] also revealed deficiencies of complex IV: the activities of complex I, II, III and IV relative to that of citrate synthase were 137%, 238%, 124% and 12%, respectively.

4. Results

4.1. Case 1

We identified two novel heterozygous mutations: a maternal c.49+1 G>T splice site mutation in intron 1 and a paternal c.752_753del in exon 8. This deletion resulted in a frame shift at amino acid 251(Gln251) causing a stop codon in exon 8 (Fig. 3). The c.49+1 G>T splice site mutation changes the highly conserved G nucleotide at position +1 of the donor splice site (5′ss) in intron 1. We attempted to characterize the splicing outcome of this sequence variation by RT-PCR analysis from patient's blood. Sequence analysis of the RT-PCR reaction detected only the allele with the c.752_753delAG mutation, which implies the presence of a nonsense mediated decay or instability of mRNA from the allele with the c.49+1 G>T splice site mutation.

4.2. Case 2

Sequence analysis of the *SURF1* gene revealed a novel homozygous c.743 C>A, p.Ala248Asp in exon 7. Both parents of this patient were heterozygous for this mutation (Fig. 3). This mutation changes highly conserved Alanine to Aspartate. This mutation was not found in 100 control alleles.

5. Discussion

Molecular elucidation of Leigh syndrome is challenging since many enzymes are involved, such as mitochondrial respiratory chain complexes I, II, III, IV, and V, and components of the pyruvate dehydrogenase complex. Mutation analysis in DNA is more complicated, even after focusing on respiratory complex IV. Mitochondrial-encoded *MTCO3* and nuclear-encoded *COX10*, *COX15*, *SCO2*, and *SURF1*, have been reported as the cause of Leigh syndrome [6,7]. Our two cases presented with mental retardation, failure to thrive, respiratory dysfunction, facial dysmorphism and hypertrichosis. Facial dysmorphism including micrognathia and hypertrichosis especially in the extremities have been reported to be distinctive and characteristic feature of *SURF1* gene mutation [3,4]. Our two cases underscore the importance of *SURF1* analysis in Leigh syndrome with facial dysmorphism and hypertrichosis. However, not all patients with this gene mutation carry these symptoms. Although facial dysmorphism has been also

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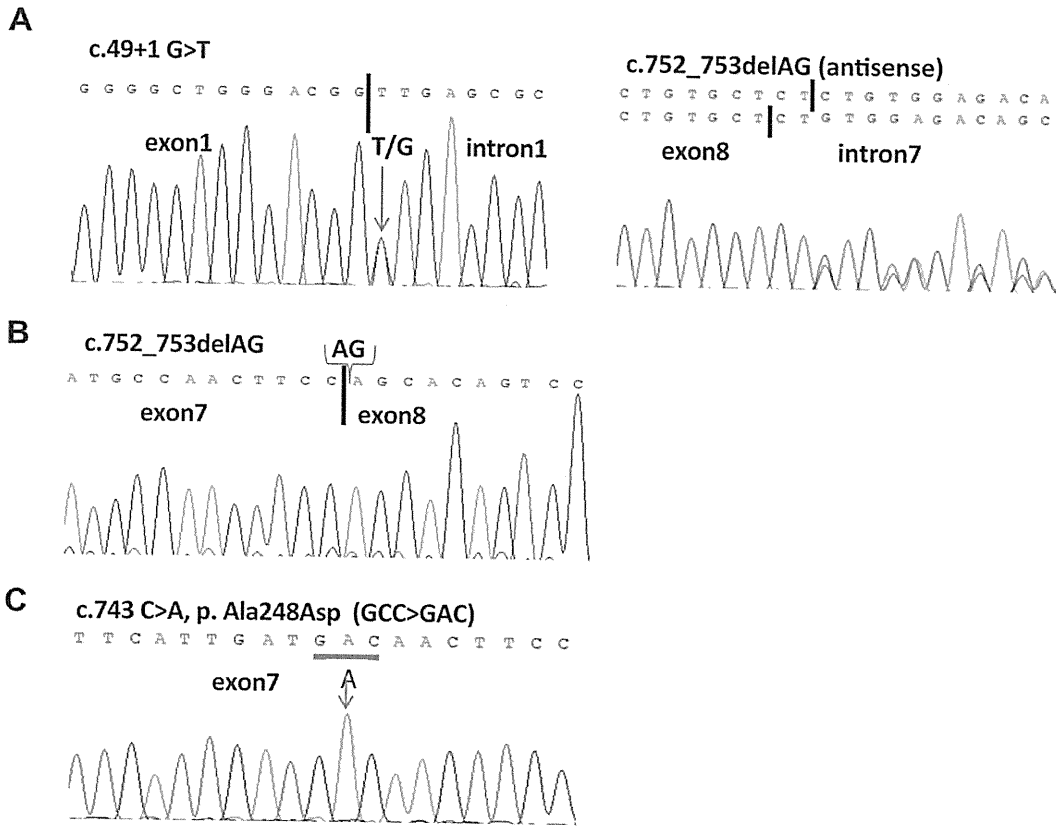

Fig. 3. Analysis of the *SURF1* gene. A chromatogram of the two novel heterozygous mutations; c.49+1 G>T and c.752_753del in Case 1 (A) and homozygous c.743 C>A in Case 2 (C). Panel B shows the chromatogram of cDNA from Case 1. The mutations are shown on the sense strand except for the right panel of A (antisense).

reported in Leigh syndrome with pyruvate dehydrogenase complex, hypertrichosis has not been described [8].

To date, more than 100 patients of Leigh disease with *SURF1* mutations have been reported [6,7]. To our knowledge, this is the first report of a mutation in intron 1, suggesting the need to scan whole exons and exon/intron boundaries.

Common MRI findings of Leigh syndrome are symmetric lesions in the brainstem, basal ganglia, thalamus and spinal cord, Leigh syndrome with *SURF1* mutation have been reported to involved the subthalamic nuclei, medulla, inferior cerebellar peduncles, and substantia nigra [5]. In addition, Case 2 showed signal hyperintensities in bilateral optic radiation on T2WI and DWI, which has not been reported previously in Leigh syndrome with *SURF1* mutations. Since Case 2 had never shown severe hypoxemia, this finding may be significant in patients with *SURF1* mutation or may appear in a progressed stage of disease.

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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.braindev.2012.02.007.

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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 β-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 $pmol \cdot min^{-1} \cdot 10^{6}$ $lymphocytes^{-1}$ (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 ß-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

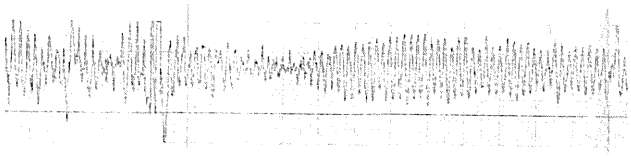

Figure 2. Sequence analysis

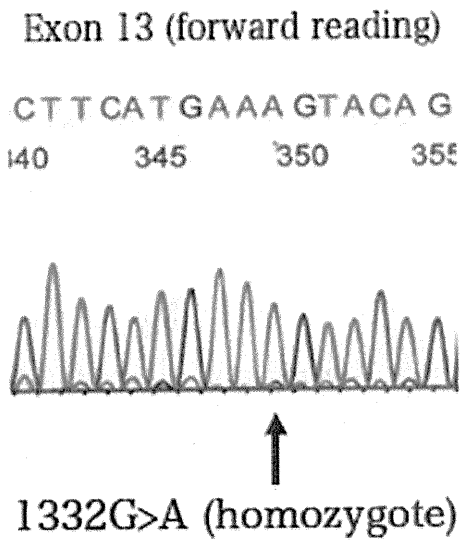

Table 1. Tandem mass analysis

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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