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Successful management of pregnancy with very-long-chain acyl-coenzyme A dehydrogenase deficiency

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Abstract

Very-long-chain acyl-coenzyme A dehydrogenase deficiency (VLCADD) is a rare and life-threatening disease characterized by an enzymatic defect in the fatty acid β-oxidation pathway. A nulliparous woman with VLCADD showed improvements in serum levels of the long-chain acylcarnitine moiety (C14:1) during pregnancy and successfully delivered a healthy infant vaginally. Pregnancy and vaginal delivery can be successfully completed in patients with VLCADD with careful management.

Key words: placenta, pregnancy, very-long-chain acyl-coenzyme A dehydrogenase deficiency.

Introduction

β-oxidation of fatty acids (FA) plays a crucial role in energy production and involves a variety of FA oxidation enzymes. Very-long-chain acyl-coenzyme A dehydrogenase deficiency (VLCADD), a mitochondrial β-oxidation deficiency, presents with neonatal cardiomyopathy and hypoglycemia in its severe early-onset form, or with myalgia and exercise-induced rhabdomyolysis in later-onset episodic myopathic form.[1–3] This enzymatic deficiency shows an autosomal recessive inheritance pattern with an estimated incidence ranging from 1 in 13 500 to 1 in 125 000 live births.[4,5] We report the case of a woman with myopathic-form VLCADD who successfully completed a pregnancy without major complications, and discuss the clinical course and obstetric management of this disease with reference to previous reports.

Case Report

The patient was referred to our hospital at 12 years old because of generalized myalgia and recurrent elevation of plasma levels of creatine kinase (CK). Her medical and family histories were non-contributory and her intelligence developed normally. The diagnosis of VLCADD was made based on findings of elevated C14:1. Palmitoyl-coenzyme A oxidation was used to test VLCAD activity in lymphocytes (assay performed at Hiroshima University, Tajima, Japan). Palmitoyl-coenzyme A dehydrogenase level in lymphocytes was low (3.6 pmol/min/10^6 lymphocytes; reference value, 59.5 ± 12.8 pmol/min/10^6 lymphocytes) and sequence analysis of the *ACADVL* gene revealed a novel c.696C>G (Pro266Ala) mutation and a known pathogenic c.1153C>T (Arg385Trp) mutation.[5] Her free carnitine level was low (19 μM) at diagnosis and carnitine

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supplementation was initiated. Free carnitine level subsequently remained stable within the range of 27–42 μM during pregnancy. She was treated with carnitine and a low-fat diet supplemented with medium chain triglyceride formula. She had only been able to attend high school 2–3 days a week in the year before she became pregnant due to severe myalgia.

With a complaint of amenorrhea at 17 years old, she visited an obstetrician, and 25 weeks' gestation was confirmed according to fetal size measured on ultrasonography. The fetus showed no abnormalities. The patient suffered severe myalgia and rhabdomyolysis with elevated plasma CK levels (3250 U/L) in the first trimester, which resolved with bed rest and continuous i.v. infusion using 5% glucose-containing solutions. She was admitted to hospital in gestational week 28, after a short uterine cervix was recognized. We decided against using tocolytic agents, and bed rest was maintained until gestational week 35, allowing slow walks around the ward and showers. The day after discharge during gestational week 35, she again complained of myalgia, and elevated CK levels (3388 U/L) were seen, so she remained in hospital until delivery. During gestational week 38, labor was induced with i.v. administration of oxytocin under epidural anesthesia using levobupivacaine hydrochloride. A healthy female infant weighing 3070 g was delivered. During labor and delivery, the patient was given access to drinks and food (~1700 kcal/day) with medium chain triglyceride formula (360 kcal/day) as desired. In addition, we provided i.v. glucose-containing solutions as a supplemental energy source (400 kcal/day), continuing for 1 week after delivery. She stopped breastfeeding, and her course and that of her baby remained uneventful until the follow-up visit 1 month later. Episodic myalgia and rhabdomyolysis, however, started after the visit. Serum C14:1 level decreased during pregnancy, then again increased from 2 weeks after delivery (Fig. 1). During late pregnancy, basal serum CK levels were normalized (30 U/L) for the first time since she was first diagnosed with VLCADD, but returned to the increased level after the delivery in the same manner as for C14:1 (data not shown). Although C16 and C18:1 levels were elevated before pregnancy and had normalized by the time of delivery, other acylcarnitine moieties remained within normal ranges throughout the clinical course.

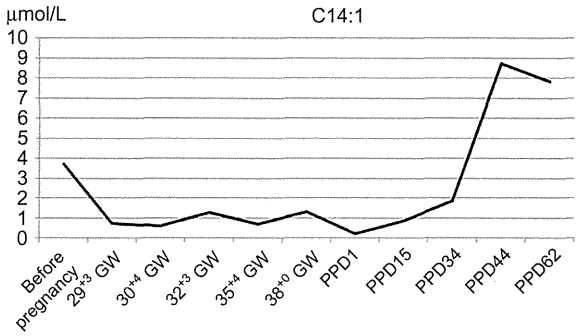

Figure 1 Serum concentration (μmol/L) of (—) C14:1 in the patient. Note the marked improvement in C14:1 level during late pregnancy, returning to abnormal from 34 days after delivery. GW, gestational weeks; PPD, post-partum day.

Discussion

Two major concerns arise in women with VLCADD. The first is whether pregnancy can be risked with this life-threatening disease, and the second is how and when to make the decision to deliver the baby. Laforêt *et al.* reported long-term follow-up of 13 patients with VLCADD.[6] A total of five women delivered nine newborns, with cesarean section performed for two. Three women suffered from delayed myalgia after the first delivery, with elevated levels of CK or myoglobinuria, but renal function remained normal. Although Laforêt *et al.* did not mention indications or strategies underlying the choice of vaginal delivery or cesarean section, mode of delivery did not seem to severely impact maternal or neonatal outcomes in those cases.

Mendez-Figueroa *et al.* described a case of VLCADD in which the patient showed clinical and biochemical improvement during pregnancy.[7] They speculated that VLCAD activity within the placenta metabolized the maternal FA and resulted in a transient decrease in C14:1, with a return to abnormal levels after delivery. In fact, VLCAD has been found to be highly expressed in the human placenta from early gestation.[8] Our case likewise showed improved serum concentrations of C14:1 after the identification of pregnancy. However, as seen in the first trimester of this case, emesis or loss of appetite could lead to a catabolic condition triggering rhabdomyolysis. Caution is also warranted after delivery and during puerperium. Previously, three of five patients with VCLADD were reported to experience myoglobinuria after their first delivery.[7] Cardiac failure during the postpartum period has also been reported.[9] In our case, myalgia started around the same time that she stopped breastfeeding and/or started menses. Hormonal changes during puerperium may thus represent a risk factor for exacerbation of the disease condition.

In conclusion, pregnancy and vaginal delivery seem to be acceptable with careful management in women with VLCADD. Epidural anesthesia can reduce fatigue during labor. Cesarean section should be considered only based on obstetric indications, and perioperative fasting could present a risk for the disease. This case report might provide encouraging information for patients wishing to bear children, and further cases should be accumulated to establish protocols of pregnancy management for this disease.

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Disclosure

The authors declare no conflicts of interest.

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Letter to the editor

Amelioration of acylcarnitine profile using bezafibrate and riboflavin in a case of adult-onset glutaric acidemia type 2 with novel mutations of the electron transfer flavoprotein dehydrogenase (*ETFDH*) gene

Keywords:
Glutaric acidemia type 2
Acylcarnitine profile
Adult onset
Bezafibrate

1. Introduction

Multiple acyl-coenzyme A dehydrogenase deficiency (MADD), also known as glutaric acidemia type 2 (GA2), was first described in 1976 [1]. GA2 is a rare autosomal recessive disorder whose biochemical abnormalities result from a deficiency of one of the two electron transfer flavoproteins (ETF and ETFDH) that transfer electrons from acyl-CoA dehydrogenases to the respiratory chain [2]. The disorder affects multiple metabolic pathways involving branched amino acids, fatty acids, and tryptophan, and results in a variety of distinctive organic acids being discharged. The heterogeneous clinical features of patients with GA2 fall into three subclasses: two neonatal-onset forms (types I/II) and a late-onset form (type III) [3]. The late-onset form is typically characterized by intermittent vomiting, hypoglycemia, hepatomegaly, metabolic acidosis, and/or hyperammonemia, symptoms that are often triggered by general infections or catabolic conditions [4].

Here, we describe the case of a man with lipid-storage myopathy, low muscle carnitine, and an adult-onset form of GA2 with two novel mutations in the *ETFDH* gene. In this case, a combination of a hypolipidemic drug (bezafibrate), riboflavin, and L-carnitine was effective in treating the disease.

2. Case report

A 31-year-old man was referred to our hospital because of muscle weakness and limb fatigability. Nine months earlier, he had gradually developed proximal muscle weakness and fatigability. He exhibited normal psychomotor development. His relatives had no history of neuromuscular disease. Physical examination on admission showed a normally developed, well-nourished man (185 cm, 73 kg) without hepatosplenomegaly. Neurological examination revealed mild muscle weakness in his left iliopsoas muscle (grade 5−). Muscle amyotrophy and myalgia were not noted. The following serum biochemistry markers were elevated: creatine kinase (CK), 689 U/L (normal <230); creatine kinase-MB, 50 U/L (<10); aldolase, 8.9 IU/L (<5.9); myoglobin, 107 ng/mL (<72.0); and triglycerides, 315 mg/dL (<149). The full blood count, blood glucose, renal and thyroid function, immunoglobulins, inflammatory markers, and antinuclear antibodies were normal. Echocardiography, pulmonary function tests, and a brain MRI were normal. Abdominal echography revealed only the fatty liver. A muscle MRI showed a high-density area in the bilateral lower limb muscles in short-T1 inversion recovery (STIR) (Fig. 1A). Atrophy of the biceps was suspected based on a muscle CT scan. Electromyography of the left vastus lateralis muscle and the tibialis anterior muscle displayed myopathic patterns. In the muscle biopsy specimen from the biceps brachii, neither lymphocytic infiltration nor endomysial fibrosis was observed (Fig. 1B), although some fibers contained many vacuoles. These were positively stained with Oil Red O, suggesting a lipid storage myopathy (Fig. 1C).

Total and free carnitine concentrations in muscle specimens were severely decreased at 3.5 (control 15.7 ± 2.8) and 1.7 (12.9 ± 3.7) nmol/mg non-collagen protein (NCP), respectively. Activity of acyl-CoA dehydrogenases was normal. Analysis of urinary organic acids showed increased 2-OH-glutarate, ethylmalonate, and 3-OH-propionate. The acylcarnitine profile of the patient's serum showed a broad-range elevation of acylcarnitines, but no abnormalities were observed in the amino acid profile. This indicated a multiple-dehydrogenation abnormality, which is consistent with GA2. After receiving informed consent, the patient's skin fibroblasts were isolated and cultured, as described previously [5]. Genetic analysis identified novel, compound heterozygous missense mutations in the *ETFDH* gene (890G > T/W297L and 950C > G/P317R). Western blot analysis showed decreased production of ETFDH in the patient's fibroblasts (Fig. 1D). This indicated that the mutations would be pathogenic.

Following treatment with L-carnitine alone, the patient's serum CK reached nearly normal levels. However, his serum acylcarnitine profile remained abnormal (Fig. 1E, left panel). The L-carnitine treatment was then supplemented with riboflavin at 105 mg/day or with bezafibrate (BEZ; 600 mg/day) because the patient showed mild hyperlipidemia, and because this hypolipidemic drug was effective for adolescent GA2 patients [5]. However, the combined treatment of L-carnitine and riboflavin, or L-carnitine and BEZ, failed to improve the acylcarnitine profile (Fig. 1E, left panel) and the patient's symptoms remained stable. For the next 7 months, the patient was treated with L-carnitine alone. During this period, he felt fatigability and his serum CK increased mildly. BEZ was again added to his treatment regimen. His serum acylcarnitine profile improved, but his serum CK remained high and he occasionally complained of fatigue (Fig. 1E, right panel). After 15 months, riboflavin was added to the L-carnitine and BEZ. His serum CK and acylcarnitine profile returned to normal within one month, and his symptoms completely disappeared. This amelioration has continued beyond 6 months.

3. Discussion

We diagnosed a patient with GA2 based on observations of the muscle pathology, acylcarnitine analysis, and *ETFDH* gene mutations. In the

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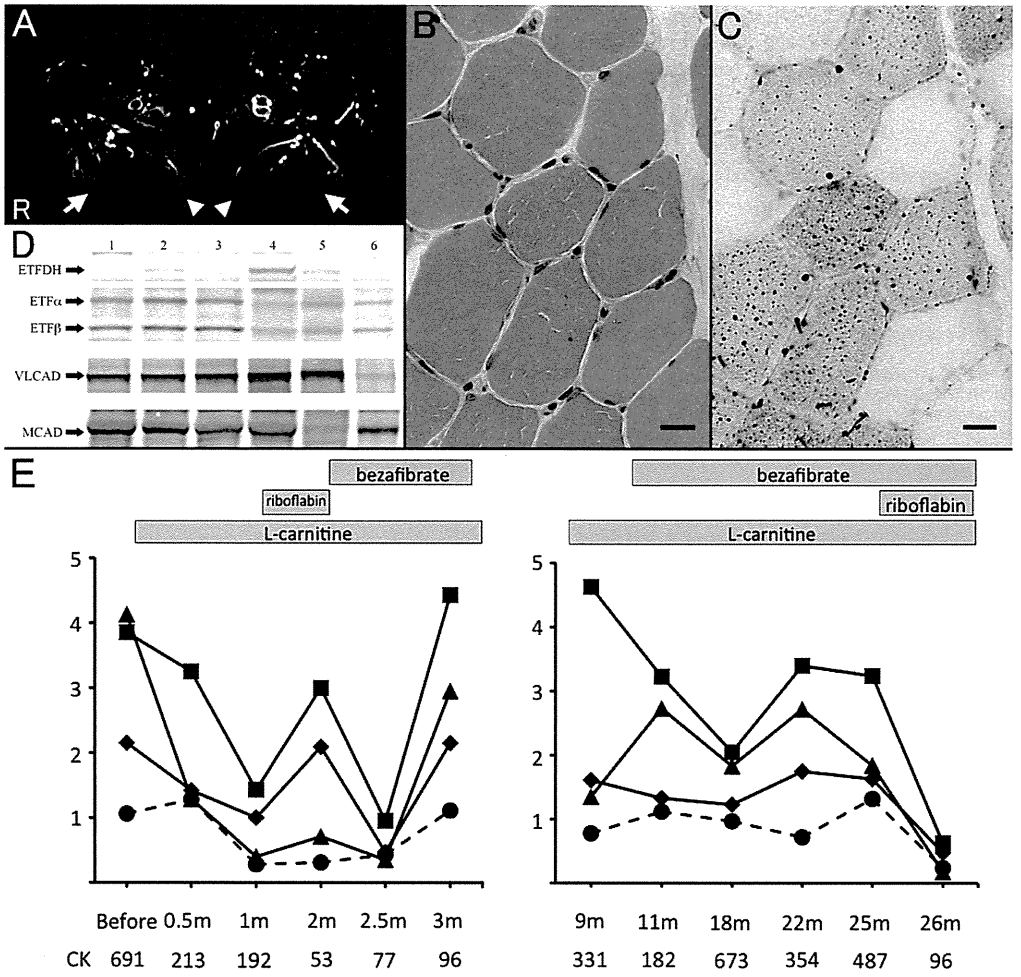

Fig. 1. A. A muscle MRI showed an area of high intensity in the bilateral biceps femoris muscle (arrow) and semimembranous muscle (arrowheads) in short-T1 inversion recovery (STIR). This indicated that increased water content in these muscles due to cellular lysis or fluid accumulation secondary to inflammation [10]. B, C. Biopsy of the patient's right biceps muscle. (B) Hematoxylin and eosin staining showed multiple optically empty vacuoles. (C) Oil Red O staining revealed excessive lipid droplets. The scale bar represents 20 μm. D. Western blot analyses of proteins in the patient's fibroblasts. The patient's fibroblasts were prepared as described previously [5,11]. For analysis of ETFDH, ETFα, and ETFβ, 25 μg of protein was applied to the gel. For analysis of very long-chain acyl-CoA dehydrogenase (VLCAD) and medium-chain acyl-CoA dehydrogenase (MCAD), 10 μg of protein was applied to the gel. Lane 1, patient's fibroblasts; lane 2, control (normal) fibroblasts; lane 3, ETFDH-defective fibroblasts; lane 4, ETFβ-defective fibroblasts; lane 5, MCAD-defective fibroblasts; lane 6, VLCAD-defective fibroblasts. Note that lane 1 from this patient, and lane 3 from the negative control, lack the band corresponding to ETFDH. This indicates that this patient had no ETFDH protein. Compared to control, the patient's fibroblasts showed no change in the expression of ETFα, ETFβ, VLCAD, or MCAD proteins. E. Changes in blood acylcarnitines with various treatments. The acylcarnitine profile of the patient's serum before treatment showed a broad-range elevation of acylcarnitines, including C6, C8, C10, C12, C14, and C16 acylcarnitine at 1.06 nmol/mL (normal <0.46), 2.15 (<1), 3.84 (<0.8), 4.13 (<0.4), 2.81 (<0.3), and 2.22 (<0.5), respectively. In the left panel, BEZ or riboflavin combined with L-carnitine, partially improved serum CK and serum acylcarnitine levels. Combining all three agents completely restored to normal the patient's acylcarnitine profile (right panel). During the seven-month period between the results shown in panels E and F, the patient was treated with L-carnitine alone. Units for acylcarnitine are nmol/mL and for CK are U/L. "m" indicates month. ●, C4; ◆, C8; ■, C10; ▲, C12.

adult myopathic form of GA2, patients sometimes do not show rhabdomyolysis, and there is no typical biochemical examination that can help us to consider the presence of a fatty acid oxidation disorder (FAO), as was observed here. Muscle biopsy and acylcarnitine analysis provide useful information and should be employed without hesitation.

Intake of L-carnitine has been reported to either exacerbate symptoms or to be effective for GA2 patients [6,7]. In the present case, oral carnitine alone leads to only partial improvement based on amelioration of the patient's muscle weakness and decreases in his serum CK and acyl-CoA. Riboflavin supplementation produces improvements in the symptoms and metabolic profiles of GA2 patients with *ETFDH* mutations, and the late-onset form [2]. BEZ is a hypolipidemic drug that is as an agonist of the peroxisome proliferating activator receptor, and was found to be beneficial in Japanese children with *ETFDH* gene mutations exhibiting GA2 [5]. Several mechanisms for the effectiveness of BEZ for FAO have been reported including upregulating mRNA and the activity of several FAO enzymes [8,9]. In the present case, BEZ, L-carnitine, and riboflavin each showed partial effectiveness and produced partial remission in a patient with GA2. In children, BEZ has been administered at doses from 17 to 25 mg/kg/day [5]. In the current patient, 600 mg/day of BEZ was administered, corresponding to only 8.2 mg/kg/day. This low dose was used because of the limitations of BEZ as a hypolipidemic drug and may explain the limited effectiveness of BEZ for our patient. A combination of BEZ, riboflavin, and L-carnitine produced complete remission in this patient, not only of his symptoms and serum CK, but also of his defect in fatty acid metabolism.

This case supports a new option for the treatment of GA2 patients, even in adults. Additional clinical studies and experimental investigation of the mechanisms of action of these drugs are required.

Conflict of interest

The authors have no conflicts of interest to declare.

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Clinical characteristics and mutation analysis of propionic acidemia in Thailand

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Original article

Background: **Propionic acidemia (PA) is caused by a deficiency of propionyl CoA carboxylase. A characteristic urine organic acid profile includes 3-hydroxypropionate, methylcitrate, tiglylglycine, and propionylglycine. The diagnosis of PA is confirmed by detection of mutations in the *PCCA* or *PCCB* genes. We herein report the clinical and molecular findings of four Thai patients with PA.**

Methods: **Clinical findings of four Thai patients with PA were retrospectively reviewed. Urine organic acids were analyzed by gas chromatography-mass spectrometry. PCR-sequencing analyses of encoding exons and intron/exon boundaries of the *PCCA* and *PCCB* genes were performed.**

Results: **All patients had neonatal onset of PA. One patient died of cardiomyopathy, and another one of pneumonia and metabolic decompensation. The remainder experienced significant neurocognitive impairment. Mutation analysis of the *PCCA* gene identified homozygous c.1284+1G>A in patient 1, c.230G>A (p.R77Q) and c.1855C>T (p.R619X) in patient 2, homozygous c.2125T>C (p.S709P) in patient 3, and only one mutant allele, c.231+1G>T in patient 4. No *PCCB* mutation was identified. Four mutations including c.230G>A, c.231+1G>T, c.1855C>T, and c.2125T>C have not been reported previously.**

Conclusions: **The clinical and molecular study of these Thai patients provided additional knowledge of the genotype and phenotype characteristics of PA. The results of the study suggested that *PCCA* mutations in Asian populations were distinct from those of other populations.**

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Key words: **mutations; propionic acidemia; Thailand**

Introduction

Propionic acidemia (PA; MIM 606054) is an autosomal-recessive disorder caused by deficiency of propionyl CoA carboxylase (PCC; EC 6.4.1.3), a mitochondrial biotin-dependent enzyme in the catabolic pathway of amino acids, including valine, threonine, isoleucine, cholesterol side chains, and odd-chain fatty acids.[1] PCC catalyzes the carboxylation of propionyl CoA to yield D-methylmalonyl-CoA. PCC comprises a α6β6 structure.[2] The α and β subunits are encoded by the *PCCA* and *PCCB* genes localized on chromosomes 13q32 and 3q21-q22, respectively.[3,4] To date, 81 mutations and 17 polymorphisms, and 86 mutations and 7 polymorphisms have been identified in the *PCCA* and *PCCB* genes, respectively.[5] Missense mutations are predominant (~40%), followed by small insertions/deletions and splicing mutations and, in the case of the *PCCA* gene, large genomic deletions.[6]

The clinical picture of PA is heterogeneous. Affected patients most commonly present within the neonatal period with nonspecific clinical signs, such as vomiting, refusal to feed, lethargy, hypotonia, seizures, metabolic acidosis and/or hyperammonemia. Patients can deteriorate quickly and lapse into coma, then death if the disorder is not recognized or if patients are already too severely compromised to respond to therapy.[7] Late-onset PA may be characterized by failure to thrive, developmental delay, and various neurological symptoms.[8] Brief clinical summaries and the urine organic acid profiles of only two Thai PA patients have been described,[9] but their causative mutations have not

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been reported. Here we report the clinical course and mutation spectrum of four Thai patients with PA.

Methods

Patients

We retrospectively reviewed the cases of four unrelated Thai patients diagnosed with PA at Siriraj and Phramongkutklao Hospitals between 2000 and 2010. All patients were diagnosed via urine organic acid analysis using urease treatment extraction with gas chromatography-mass spectrometry (GC-MS)[10] after presenting clinical symptoms, and not through neonatal screening. Informed consent was obtained from the families of the patients. The study was approved by the Institutional Ethical Review Board of Faculties of Medicine of Siriraj Hospital.

Mutation analysis of the *PCCA* and *PCCB* genes

Genomic DNA was extracted from peripheral blood. All coding exons as well as flanking introns in the *PCCA* and *PCCB* genes were PCR-amplified and directly sequenced as described previously.[11,12]

Results

Case presentations

Case 1 was a boy of first-degree-cousin parents. He was born full term with birth weight of 3 kg. He had a tachypnea at 10 hours, then developed vomiting and coma. Basic blood chemistries showed metabolic acidosis and hyperammonemia. A metabolic work-up at 2 months of age yielded the following: urine organic acids showed increased excretions of methylcitrate, propionylglycine, and 3-hydroxypropionate; plasma amino acids showed increased glycine (3405 μmol/L, reference range: 94-463 μmol/L); and acylcarnitine profile revealed increased C3-acylcarnitine (22.7 μmol/L, reference: <8.17 μmol/L), and C3/C2 acylcarnitine ratio (8.54, reference: <1.8). He was initially treated with intravenous fluid, low-isoleucine, -methionine, -threonine, and -valine special formulas, oral carnitine, and biotin. He developed seizures at 2 years of age. Thereafter, he had several episodes of infections, diarrhea, metabolic acidosis and epilepsy. Gastrostomy tube placement with fundoplication was performed at the age of 8 years which improved caloric intake, and increased his weight from the 3rd percentile to 25th percentile. However, this did not decrease the frequency of his hospital admissions due to infections and metabolic decompensation. He is now 12 years old with profound mental retardation. His most recent cardiac evaluation did not show long QT or cardiomyopathy.

Case 2 was a girl of non-consanguineous parents. She was diagnosed with neonatal sepsis on 2 days of life. At 25 days, she developed skin abscesses and seizures. Laboratory revealed pancytopenia [hemoglobin (Hb), 11 g/dL; white blood cells (WBC), 2200/mm^3; absolute neutrophil count (ANC), 576/mm^3; platelets 83 000/mm^3]. Blood chemistries did not show acidosis (bicarbonate, 24 mmol/L) but urine analysis revealed ketonuria. Blood ammonia was not obtained at that time. She was admitted in the hospital several times due to recurrent urinary and respiratory tract infections, and neutropenia. Bone marrow aspiration indicated maturation arrest of myeloid series. This led to a diagnosis of cyclic neutropenia. She had mild developmental delay with normal growth parameters. During an admission at 10 months of age, blood chemistry revealed mild metabolic acidosis (bicarbonate, 18 mmol/L) and slight hyperammonemia (ammonia, 127 μmol/L, reference: 11.2-48.2 μmol/L). This prompted a metabolic work-up. Urine organic acids revealed increased excretions of propionylglycine, 3-hydroxypropionate, and methylcitrate, which indicated propionic acidemia. Plasma amino acids showed increased glycine (707 μmol/L, reference: 90-221 μmol/L). The patient was treated with low-isoleucine, -methionine, -threonine, and -valine diet, L-carnitine (100 mg/kg per day), and biotin (10 mg/day). Thereafter, she experienced only a few metabolic crises and mild developmental delay. She died at 6 years of age from sudden cardiac arrest probably secondary to cardiomyopathy. Free carnitine level was not measured before the event. The parents refused postmortem autopsy.

Case 3 was a boy of a consanguineous marriage. He was born at term after an uneventful delivery with a birth weight of 2.76 kg. He developed tachypnea at day 11, and was diagnosed with presumed sepsis. At one month of age, he had generalized tonic clonic seizures, poor weight gain, and metabolic acidosis (bicarbonate, 16 mmol/L). Blood ammonia was mildly elevated (84 μmol/L). Complete blood count (CBC) showed leukopenia (WBC, 3350/mm^3). Urine organic acids at 2 months of age demonstrated increased excretions of 3-hydroxypropionate, methylcitrate, tiglylglycine, and propionylglycine. He was treated with low-isoleucine, -methionine, -threonine, and -valine special formulas, L-carnitine, biotin, and metronidazole. The patient could not achieve good metabolic control due in part to inadequate caloric intake. The patient's weight and height remained below the third percentile since one month of age. The parents refused a gastrostomy tube placement. The patient's cognitive development was severely delayed at 2.5 years of age. The patient's most recent echocardiogram revealed no cardiomyopathy. He died at 3 years of age from pneumonia and metabolic decompensation.

Case 4 was a girl of non-consanguineous parents. She was born at term with a birth weight of 3325 g. She developed lethargy at 7 days of life. Physical examination showed nothing remarkable except for hypotonia. Initial investigations showed metabolic acidosis with a wide anion gap, and severe hyperammonemia (ammonia > 700 μmol/L). Urine organic acids at that time revealed increased excretion of only 3-hydroxypropionate and lactate. She was treated since 2 weeks of age. When she developed metabolic decompensation again at 10 months, urine organic acids showed the typical profile of PA including increased levels of 3-hydroxy propionate, propionylglycine, tiglylglycine, and methylcitrate. Her cognitive development was moderately delayed at age of 2.5 years.

Mutation analysis

Five different *PCCA* mutations and no *PCCB* mutations were identified in this study. Two mutant alleles were identified in three patients (case 1-3), which confirmed a clinical diagnosis of PA. For case 1, homozygous c.1284+1G>A mutation of the *PCCA* gene was identified, and the heterozygous mutation was identified in both parents. Compound heterozygosity of c.230G>A (p.R77Q) and c.1855C>T (p.R619X) mutations of the *PCCA* gene were identified in case 2. The heterozygous c.230G>A and c.1855C>T mutations were identified in the father and mother, respectively. Homozygous c.2125T>C (p.S709P) mutation of the *PCCA* gene was identified in case 3, and the heterozygous mutation was identified in both parents. Only one mutant allele, heterozygous c.231+1G>T, was identified in case 4 but the clinical and urine organic acid findings strongly suggested PA. The heterozygous c.231+1G>T mutation was also identified in the father, and no *PCCA* mutation was identified in the mother. Clinical findings and genotypes are summarized in the Table and Fig.

Table. Summary of the clinical features and genotypes of the patients with propionic acidemia

Case *no.*	Consanguinity	Onset	Diagnosis	Current age	Outcome	Mutations identified					
						Allele 1	Predicted protein change	Ex./ In.	Allele 2	Predicted protein change	Ex./In.
1	+	10 h	2 mon	12 y	Profound MR, epilepsy	c.1284+1G>A	Ex. 13-14 skipping	In. 14	c.1284+1G>A	Ex. 13-14 skipping	In. 14
2	-	2 d	11 mon	Died at 6 y	Mild DD, possible cardiomyopathy	c.230G>A	p.R77Q	Ex. 3	c.1855C>T	p.R619X	Ex. 21
3	+	11 d	1 mon	Died at 3 y	Severe DD, growth failure, epilepsy	c.2125T>C	p.S709P	Ex. 24	c.2125T>C	p.S709P	Ex. 24
4	-	2 d	11 d	2 y 11 mon	Moderate DD, normal growth	c.231+1G>T	Ex. 3-4 skipping	In. 3	ND	-	-

MR: mental retardation; DD: developmental delay; ND: not detected; In.: intron; Ex.: exon.

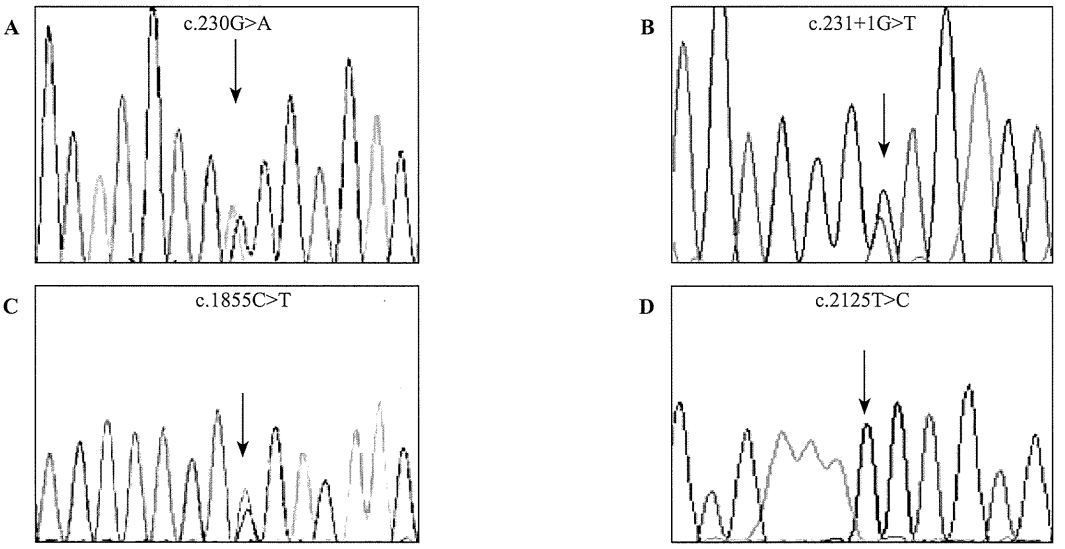

Fig. Novel *PCCA* mutations. Chromatograms demonstrating the nucleotide changes detected. Black arrows indiate the c.230G>A (**A**), c.231+1G>T (**B**), c.1855C>T (**C**), and c.2125T>C (**D**).

Discussion

In Thailand, as in other developing countries, there has been a gradual development of technologies to diagnose inborn errors of metabolism over the last decade.[13] In this study, all PA cases presented in an acute neonatal form. Patient 2 initially presented with severe neutropenia without metabolic acidosis but her blood ammonia level was not measured. This resulted in a delay until an accurate diagnosis, during which the patient had recurrent infections and metabolic acidosis appeared. Neutropenia/anemia/pancytopenia in PA and other organic acidemias might be a consequence of bone marrow suppression. This is probably due to accumulation of CoA esters which inhibits the maturation of bone marrow precursors.[14] Metabolic acidosis was documented in only two thirds of PA patients whereas hyperammonemia was found in >90% of patients.[15] Therefore, blood ammonia could have been elevated in patient 2, and this should be a better screening test for PA rather than serum electrolytes alone. For the final outcomes, two patients died and the survivors had moderate to profound developmental delay/mental retardation. The unfavorable outcomes of our patients are partly related to the delayed diagnosis (three of the four patients were diagnosed at or after 1 month of age). According to a study by Grünert et al,[15] the mortality rate was reduced in patients identified by newborn screening; however, the clinical course or neurocognitive outcome was not improved. Cardiomyopathy, which is a common long-term complication in PA, was suspected in case 2 and was probably a cause of death.[16]

To our knowledge, our study is the first mutation study of PA in Southeast Asia. Among Asian countries, common mutations in the *PCCA* and *PCCB* genes have been identified in Japanese and Korean patients.[17,18] In our study, we identified five different mutations in the *PCCA* gene, whereas no mutation in the *PCCB* gene was identified. The same mutations were identified only as homozygosity in patients with parental consanguinity. In case 4, only one mutant allele of the *PCCA* gene was identified. However, the clinical and organic acid findings were typical for PA. An unidentified mutation in another allele of patient 4 could be due to many possibilities: for example, a mutation is located in unexplored regions, such as regulatory element, promoter, or intronic regions; or a mutation is a large genomic deletion, rendering it undetectable by our methods. However, the unidentified mutation is most likely to be a large deletion, which contributes to the high percentage (-20%) of alleles in *PCCA*-deficient patients.[6] Therefore, without using multiple ligation probe amplification (MLPA) and/or Southern blot analysis to detect large genomic copy variations, a substantial number of *PCCA* mutations would be missed. The c.1284+1G>A mutation has been previously reported[11] that is located at the splice donor site of intron 14, and causes exons 13-14 skipping which affects the biotin carboxylase domain.[11] The c.230G>A, c.231+1G>T, c.1855C>T, and c.2125T>C mutations are novel. Effects of the novel missense mutations on protein are predicted by in silico prediction programs [PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://sift.jcvi.org/)]. For the p.R77Q mutation, it is predicted by PolyPhen-2 to be probably damaging with a score of 1.00, and by SIFT to be damaging with a score of 0. For the p.S709P mutation, it is predicted by PolyPhen-2 to be possibly damaging with a score of 0.555, and by SIFT to be damaging with a score of 0.03. Both amino acid residues are located at the important functional domains, namely biotin carboxylase and biotinylation domains, respectively. Therefore, p.R77Q and p.S709P are likely to be pathogenic mutations. Another *PCCA* mutation at codon 77, namely c.229C>T (p.R77W), has been reported in several populations.[17,19,20] The recurrent mutations at the same codon 77 could be explained by a mutation hot spot (CpG sequence). The c.231+1G>T mutation is located at the splice donor site of intron 3, and predicted to cause splicing aberration. The previously reported mutation at the same site, namely c.231+1G>C, results in exons 3-4 skipping which also affects the biotin carboxylase domain.[21] The p.R619X mutation is located in exon 21, and predicted to cause premature termination codon. Therefore, both are likely to be pathogenic mutations.

In summary, the clinical and molecular study of these four Thai patients provides additional knowledge of the genotype and phenotype of PA. Our data suggest that *PCCA* mutations in Asian populations are distinct from other populations. In addition, four novel mutations, c.230G>A, c.231+1G>T, c.1855C>T, and c.2125T>C in the *PCCA* gene, were identified, expanding the mutation spectrum of this gene.

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analysis, interpretation, and manuscript preparation. Liammongkolkul S analyzed urine organic acids. Sakamoto O performed mutation analysis. Kamolsilp M and Sathienkijkanchai A provided clinical data. Wasant P initiated this study.

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Case report

Early replacement therapy in a first Japanese case with autosomal recessive guanosine triphosphate cyclohydrolase I deficiency with a novel point mutation

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Abstract

Autosomal recessive guanosine triphosphate cyclohydrolase I (GTPCH) deficiency is an inborn error of tetrahydrobiopterin (BH4) synthesis from GTP. GTPCH deficiency causes severe reduction of BH4, resulting in hyperphenylalaninemia (HPA) and decreased dopamine and serotonin synthesis. Without treatment, a patient with GTPCH deficiency develops complex neurological dysfunctions, including dystonia and developmental delays. The first Japanese patient with GTPCH deficiency was discovered by HPA during asymptomatic newborn screening. The phenylalanine level at the age of 5 days was 1273 μmol/L (cutoff value, 180.0 μmol/L). The high serum phenylalanine level was decreased to normal after adequate BH4 oral supplementation. Serum and urinary pteridine examination revealed very low levels of neopterin and biopterin. Sequence analysis of *GCH1* revealed compound heterozygous point mutations, including a novel point mutation (p.R235W). Replacement therapy with BH4 and L-dopa/carbidopa were started at the age of 1 month, and 5-hydroxytryptophan (5-HTP) was started at the age of 5 months. At 10 months of age, the patient showed slight dystonia but no obvious developmental delay. Cerebrospinal fluid should be examined to determine the appropriate dosage of supplement drugs. In conclusion, it is important to control the serum phenylalanine level and perform early replacement of neurotransmitters to prevent neurological dysfunction.

Keywords: Autosomal recessive guanosine triphosphate cyclohydrolase I (GTPCH); Tetrahydrobiopterin (BH4); Hyperphenylalaninemia; Early replacement therapy

1. Introduction

Tetrahydrobiopterin (BH4) is an essential cofactor in the enzymatic hydroxylation of phenylalanine, tyrosine, and tryptophan. The BH4 loading test is performed to distinguish BH4 deficiency from hyperphenylalaninemia (HPA) during newborn screening. BH4 deficiency causes HPA and decreased production of the neurotransmitters dopamine and serotonin. Five types of enzyme deficiencies have been reported in BH4 deficiency: guanosine triphosphate cyclohydrolase I (GTPCH), 6-pyruvoyl-tetrahydropterin synthase (PTPS), sepiapterin reductase (SR), dihydropteridine reductase (DHPR), and pterin-4a-carbinolamine dehydratase (PCD) [1]. GTPCH deficiency is an error of BH4 synthesis. *GCH1*, the gene

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symbol of GTPCH, is located on chromosome 14q22.1-q22.2 and comprises six exons. GTPCH deficiency has autosomal dominant and autosomal recessive forms. The autosomal dominant (AD) form is known as dopa-responsive dystonia (DRD, Segawa disease), whereas the autosomal recessive (AR) form results in swallowing difficulties, truncal hypotonia, seizures, mental retardation, and developmental delays. The residual GTPCH enzyme activity is thought to be the cause of clinical severity between the AD and the AR form. The AR form is so rare that only 17 cases are listed in the BIOMDB Database [1], and no case has been reported in Japan. The combination of BH4 replacement and neurotransmitter precursor supplementation of both L-dopa/carbidopa and 5-hydroxytryptophan (5-HTP) is a common therapeutic approach.

We experienced the first Japanese case of autosomal recessive GTPCH deficiency with HPA during newborn screening. We herein describe the clinical symptoms and treatments with a review of previous reports.

2. Case report

The patient was the first child of healthy, nonconsanguineous parents. He was born in the 40th week of pregnancy by spontaneous delivery (birth weight, 2744 g; birth height, 50 cm). At the age of 11 days, the patient was hospitalized because of HPA detected by newborn screening. The phenylalanine level measured by Guthrie test at the age of 5 days was 1273 μmol/L (cutoff value, 180.0 μmol/L). There were no abnormalities on physical or neurological examinations. Laboratory examinations showed HPA. The serum phenylalanine level was 2206 μmol/L (reference interval, 61.2 ± 14 μmol/L). A BH4 loading test with 10 mg/kg of sapropterin hydrochloride was performed. The serum phenylalanine level was decreased to normal 8 h after adequate BH4 oral supplementation (Fig. 1). Serum and urinary pteridine examination revealed very low levels of neopterin and biopterin. The serum levels of neopterin and biopterin were 5.76 nM (reference interval, 33.8 ± 4.9 nM) and 3.31 nM (reference interval, 15.0 ± 1.6 nM), respectively. The urinary level of neopterin and biopterin were 0.14 mmol/mol creat. (reference interval, 2.09 ± 0.52 mmol/mol creat.) and 0.64 mmol/mol creat. (reference interval, 1.08 ± 0.36 mmol/mol creat.). The cerebrospinal fluid (CSF) concentration of 5-hydroxyindoleacetic acid (5-HIAA) was 114 nmol/L (reference interval, 746 ± 207 nmol/L), and that of homovanillic acid (HVA) was 21 nmol/L (reference interval, 1083 ± 339 nmol/L) (Table 1); both were reduced.

Molecular genetic analysis of *GCH1* revealed compound heterozygous point mutations in exon 5 of *GCH1* (p.R184H) and exon 6 of *GCH1* (p.R235W) (Fig. 2). His father was heterozygous for p.R184H, and his mother was heterozygous for p.R235W. The p.R235W has not been previously reported in patients with GTPCH deficiency.

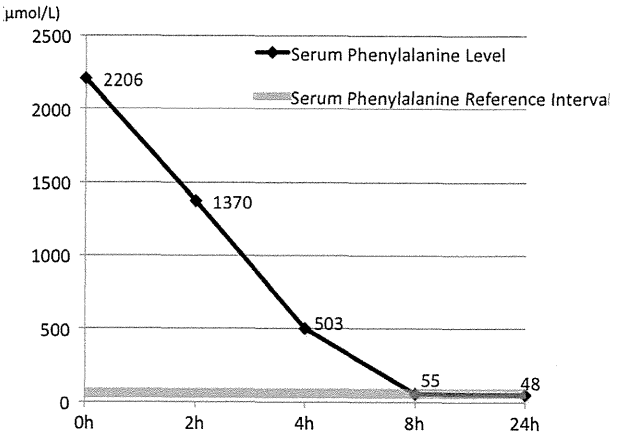

Fig. 1. Response of serum phenylalanine level by oral BH4 loading. Serum phenylalanine level was decreased to reference interval (61.2 ± 14 μmol/L) at the 8 h after BH_4 supplement.

At the age of 1 month, treatments with L-dopa/carbidopa and BH4 were started, with initial doses of 2 and 5 mg/kg/day, respectively. Mild dystonia appeared at the age of 3 months. It was slightly improved by increasing the dose of L-dopa/carbidopa supplementation. Because 5-HTP is not an approved medicine in Japan, we began supplemental therapy with 5-HTP at the age of 5 months (4 mg/kg/day) after approval by our hospital ethics committee. We adjusted the dose of BH4 according to the level of serum phenylalanine, L-dopa/carbidopa according to the clinical symptom of dystonia and serum prolactin and CSF HVA levels, and 5-HTP according to the CSF 5-HIAA level. At 8 months of age, a brain MRI was normal, and the CSF concentrations of 5-HIAA and HVA were improved (5-HIAA, 143 nmol/L; HVA, 403 nmol/L). In the most recent examination at 10 months old, he still had slight dystonia but no obvious developmental delay (sitting without support and playing toy with babbling) under treatment with BH4 (5 mg/kg/day), L-dopa/carbidopa (16 mg/kg/day), and 5-HTP (4 mg/kg/day).

3. Discussion

We herein report the first Japanese case of autosomal recessive GTPCH deficiency detected by newborn screening and presenting as HPA. We identified a novel point mutation of *GCH1*. The patient had no family history, and his parents were asymptomatic carriers. Although five *GCH1* mutations (p.Q110X (exon 1), p.R184H (exon 5), p.M213T (exon 6), p.M211V (exon 6), and p.M211I (exon 6)) have been reported in patients with HPA (BIOMDB Database) [2], there seem to be no obvious phenotype or genotype correlations.

Table 1
Clinical features and therapies for autosomal recessive GTPCH deficiency with HPA.

Report	Our case	Blau et al. [3]	Bandmann et al. [4]	Matalon et al. [5]
Age at diagnosis	1 mo	5 mo	4.5 mo	6 mo
GCH1 mutation	R184H/R235 W	M211I/M211I	M211 V/M211 V	R184H/R184H
Serum phenylalanine level (μmol/L) (reference interval: 61.2 ± 14 μmol/L)	2206	1488	3500	>2400
CSF 5-HIAA Level (nmol/L) (reference interval: 746 ± 207 nmol/L)	114	92	Normal	Unknown
CSF HVA level (nmol/L) (reference interval: 1083 ± 339 nmol/L)	20.9	50	Decreased level	Unknown
Age at therapy	1 mo	9 mo	Unknown	Unknown
Therapy & initial dose	L-dopa/carbidopa: 2 mg/kg/day BH4: 5 mg/kg/day 5-HTP: 4 mg/kg/day	L-dopa/carbidopa: 5.8 mg/kg/day BH4: 3.5 mg/kg/day 5-HTP: 3 mg/kg/day	L-dopa/carbidopa: unknown dose BH4: unknown dose 5-HTP: unknown dose	L-dopa/carbidopa: unknown dose BH4: unknown dose 5-HTP: unknown dose
Symptoms before therapy	1 mo: no symptoms	Generalized hypotonia, dystonia	Feeding problems	0 mo: feeding problems 6 mo: delayed in development 2 y: be unable to walk, seizure, choreoayhetosis
Symptoms after therapy	3 mo: slight dystonia 10 mo: no mental retardation, slight dystonia	15 mo: slight axial hypotonia 33 mo: slight mental retardation	5 y: learning difficulties, moving disorder (stiffening when patient is tired or upset)	Improved the choreoayhetosis 10 y: died

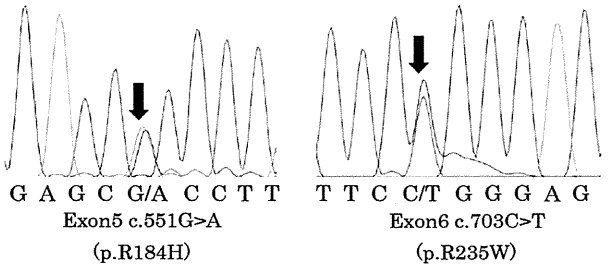

Fig. 2. Gene analysis of *GCH1*. Sequence analysis revealed the compound heterozygous mutation, G-to-A transition (c.551G>A) in exon 5 causing an amino acid substitution of p.R184H and C-to-T transition (c.703C>T) in exon 6 causing an amino acid substitution of p.R235W.

Table 1 shows a comparison of our case with previous reports on autosomal recessive GTPCH deficiency with HPA. The age at diagnosis was 3–6 months in previous reports. Feeding problems, hypotonia, and dystonia were often observed before replacement therapy. After replacement therapy with BH4, L-dopa/carbidopa, and 5-HTP was started, the feeding problems, hypotonia, and dystonia were mildly improved.

We began replacement therapy from the age of 1 month, earlier than previous reports [3–5]. Mild dystonia appeared at the age of 3 months. We then increased the dose of L-dopa/carbidopa. The dose of L-dopa/carbidopa should be adjusted according to the level of HVA in the CSF. However, CSF sampling is invasive and difficult to perform frequently. The serum prolactin level is reportedly a more sensitive and pre-symptomatic marker than is the CSF level of HVA with respect to guiding the drug adjustment of L-dopa/carbidopa in patients with PTPS deficiency [6]. Although the serum prolactin level immediately decreased to the normal range after L-dopa/carbidopa supplementation, the CSF HVA level remained low, and dystonia was not completely improved. Moreover, an increase in the 5-HTP dose may be required because of a low level of CSF 5-HIAA. We consider that scheduled CSF sampling is necessary for adjustment of drugs to prevent development of neurological symptoms.

In the most recent examination at 10 months old, the patient showed normal psychological development. Blau et al. reported that slight mental retardation remained at the age of 33 months [3]. The other cases also had neurological impairments such as speech delays, learning difficulties, and moving disorders past the age of 3 years [4]. These reports indicate that mild mental retardation and/or learning difficulties may remain. Early diagnosis and starting medical treatment during early infancy are key to prevention of mental impairment.

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Scientific Research

Successful Treatment of Cardiac Failure Due to Cardiomyopathy in Propionic Acidemia by Cardiac Resynchronization Therapy and Hemodialysis in a Young Adult

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Abstract

Propionic acidemia is an autosomal recessive disorder that is due to deficiency in the enzyme propionyl-CoA carboxylase. Cardiomyopathy is a well-known phenomenon in propionic acidemia that it may rapidly progress to death. Here we describe a case of propionic acidemia in a 27-year-old man who developed adult-onset secondary dilated cardiomyopathy. In early infancy he was diagnosed with propionic acidemia and was later noted to have mild mental retardation, mild renal failure, and optic nerve atrophy. Although he was in good energy status with a low-protein diet and carnitine supplementation, he was admitted to our university hospital with decompensate heart failure, which resulted in low-output cardiac syndrome with massive mitral regurgitation and left ventricular dyssynchrony. Cardiac resynchronization therapy (CRT) and continuous hemodiafiltration followed by hemodialysis (HD) dramatically improved his clinical status.

Keywords

Propionic Acidemia; Cardiomyopathy; Cardiac Resynchronization Therapy; Hemodialysis

1. Introduction

Propionic acidemia is a relatively rare autosomal recessive disorder characterized by an accumulation of propio-

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nic acid caused by a deficiency in propionyl CoA carboxylase (PCC), an enzyme that catalyzes valine, isoleucine, methionine, threonine, and odd-chain fatty acids in the mitochondrial matrix [1]. PCC deficiency causes an accumulation of propionic acid in the blood and urine, and without appropriate treatment, can lead to metabolic acidosis, lethargy, respiratory distress, and death. Recent advances have enabled patients with acute presentation to live beyond the neonatal period. As patients age, however, the natural progression of propionic acidemia gives rise to intellectual difficulties and increases the risks for a number of complications, including chronic kidney disease and neurological, cardiac, and gastrointestinal disorders [2]-[4]. The initial onset of cardiomyopathy typically occurs later, during school age or in adulthood, but despite its later onset, it is a serious complication as it can rapidly progress to death [5].

Cardiac resynchronization therapy (CRT) is an established adjunct to optimal pharmacological therapy in eligible patients with severe heart failure, but its role in the treatment of heart failure due to secondary cardiomyopathy has not been clarified [6] [7]. Here we describe the case of a young adult with propionic acidemia complicated by severe heart failure and renal dysfunction who was treated successfully by combination therapy CRT and HD. This combined approach may be a viable option for patients with metabolic disorders such as propionic acidemia complicated advanced secondary cardiomyopathy and renal failure.

2. Case Report

A 27-year-old Japanese man with propionic acidemia presented to our university hospital with shortness of breath and nocturnal dyspnea. He was the third child of a nonconsanguineous marriage [8]. His older sister had died of unknown causes during the neonatal period. He was born by normal delivery with a weight of 3200 g. Shortly after birth, he exhibited poor feeding, lethargy, and respiratory distress and underwent peritoneal dialysis together with other standard measures to control metabolic disorder. Diagnosis of propionic acidemia was confirmed by plasma and urinary amino acid analysis. Mutation analysis revealed homozygosity for p.Thr428Ile in the PCCB gene, which codes the beta subunit of PCC. He grew normally on a low-protein diet supplemented with L-carnitine, but had mild mental retardation. At age 15 years, he collapsed and was diagnosed with epilepsy on the basis of abnormal electroencephalography findings. Zonisamide was administered to control his convulsive seizures and he managed to work part time until losing his vision due to optic atrophy at age 22. Cardiomegaly became apparent at age 23, with no family history of cardiomyopathy.

On admission, physical examination revealed height of 1.57 m, 56.5 kg weight, clear consciousness, tachypnea, alternating pulse, and clammy skin at the extremities. A systolic murmur (Levine II/VI) with a gallop rhythm was audible at the apex. Chest radiography showed cardiomegaly and bilateral pulmonary congestion with a cardiothoracic ratio of 60% (**Figure 1(a)**). Electrocardiography demonstrated sinus tachycardia with normal QRS (100 ms in V6) and QT (425 ms in V5) intervals. Echocardiography showed notable global left ventricular dysfunction, characterized by a left ventricular ejection fraction of 15.6% and a markedly dilated left ventricular cavity. Doppler echocardiography revealed severe mitral regurgitation (**Figure 1(c)**). The results of blood gas analysis were as follows: pH, 7.409; PCO_2, 32.5 mmHg; PO_2, 48.1 mmHg; bicarbonate, 18.5 mmol/1; BE, –9.5; glucose, 125 mg/dl; and lactate, 14.5 mg/dl. Blood ammonia was within the normal range, serum creatinine was mildly elevated (1.8 mg/dl), and brain natriuretic peptide was notably elevated (2000 pg/ml). Diuretics, carperitide, catecholamines, and nasal continuous positive airway pressure did not improve his respiratory distress or heart failure. He was intubated and mechanically ventilated due to severe respiratory distress with pulmonary congestion. He developed oliguria and his serum creatinine level was elevated to 3.4 (mg/dl). Continuous hemofiltration was initiated to treat low cardiac output and pulmonary congestion and significantly improved his clinical status and pulmonary congestion. However, he needed regular hemodialysis (HD) to remove the excess preload. He also experienced ventricular fibrillation and required resuscitation on a general ward, but showed no precursory change. Implantation of a CRT defibrillator system (Medtronic Protecta™ XT, Medtronic Inc, Minneapolis, USA) and periodic HD gradually improved his cardiac function. He was discharged with the same neurological status admission with a cardiothoracic ratio of 50% (**Figure 1(b)**) and has not had any cardiac events for 3 years with periodic HD three times a week.

3. Discussion

Cardiomyopathy as a complication of organic acidemia, particularly propionic acidemia, was first described in 1993 by Massoud *et al.* in 6 of 19 pediatric patients with propionic academia [2] and is now a well-established

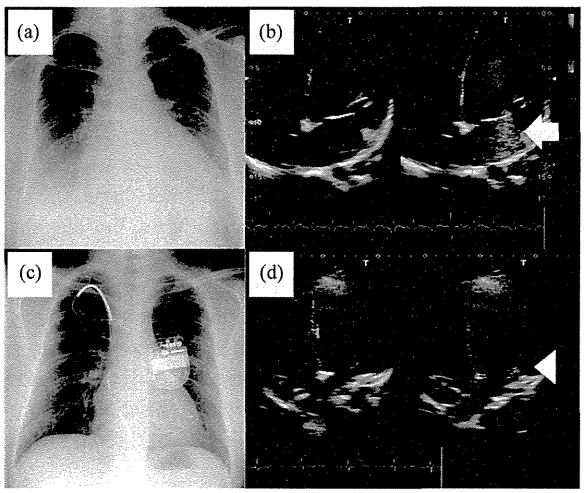

Figure 1. Chest X-rays and echocardiographs on admission (a, b) and at 7 months after introducing the cardiac resynchronization therapy defibrillator (CRTD) system (c, d). (a) Cardiomegaly (cardiothoracic ratio, 60%) with pulmonary congestion. (b) Apical four-chamber images of severe mitral insufficiency showing mitral annular dilatation (left panel) and severe mitral regurgitation on color flow Doppler (right panel, arrow). (c) cardiothoracic ratio of 50% with no pulmonary congestion. (d) Apical four-chamber images of mild mitral regurgitation on color flow Doppler (right panel, arrowhead) showing a marked reduction in mitral regurgitation after introducing the CRTD system and hemodialysis.

phenomenon [Pena, Burton 2012]. Among their 6 patients, 3 died of cardiomyopathy and the other 3 fully recovered. A recent survey of propionic acidemia complications found that cardiomyopathy occurred in 10 of 54 (19%) patients with propionic acidemia, aged 2 - 33 years old [3]. Importantly, propionic acidemia was listed as the cause of death in 70% of the deceased patients, a finding indicative of significant morbidity and mortality in this population.

To date, the cause of cardiomyopathy has not been elucidated, and although carnitine deficiency has been observed in patients with cardiomyopathy, carnitine therapy has not shown any beneficial effects in patients with propionic acidemia-related cardiomyopathy [2]. Mardach *et al.* reported very low levels of cardiac muscle carnitine despite normal plasma carnitine levels, and subsequently proposed that depletion of cardiac muscle carnitine is a precursor of cardiomyopathy [9]. However, as cardiac myocyte findings were limited, further research is needed to clarify any potential relationship. Mitochondrial electron transport chain dysfunction and secondary respiratory chain deficiency followed by an accumulation of toxic metabolites in tissues from patients with propionic acidemia are potential etiologic factors in the development of cardiomyopathy and other complications [10]. While liver transplantation has been shown to ameliorate cardiomyopathy, the absence of a correlation between their metabolic state and this heart complication suggests that different mechanisms may be involved [11]. Biventricular stimulation (*i.e.*, CRT) has been shown to improve cardiac function, reduce the frequency of hospitalization for heart failure, and enhance the quality of life in many patients with severe left ventricular systolic dysfunction and intraventricular conduction disease [6] [7]. However, it remains controversial as to whether CRT benefits patients with heart failure due to secondary cardiomyopathy such as metabolic and/or degenerative disease. In the present case, the introduction of a CRT device significantly improved the ejection fraction, magnitude of mitral regurgitation, and left ventricular dyssynchrony (Figure 2). It is possible that combination therapy, preload reduction, the removal of toxic substances by HD, and the application of CRT to assist cardiac contractions and rectify cardiac dyssynchrony have a synergistic effect.

In summary, combination therapy with CRT and HD dramatically improved the condition of a young man with propionic acidemia complicated by severe left heart failure and renal dysfunction. This combined approach

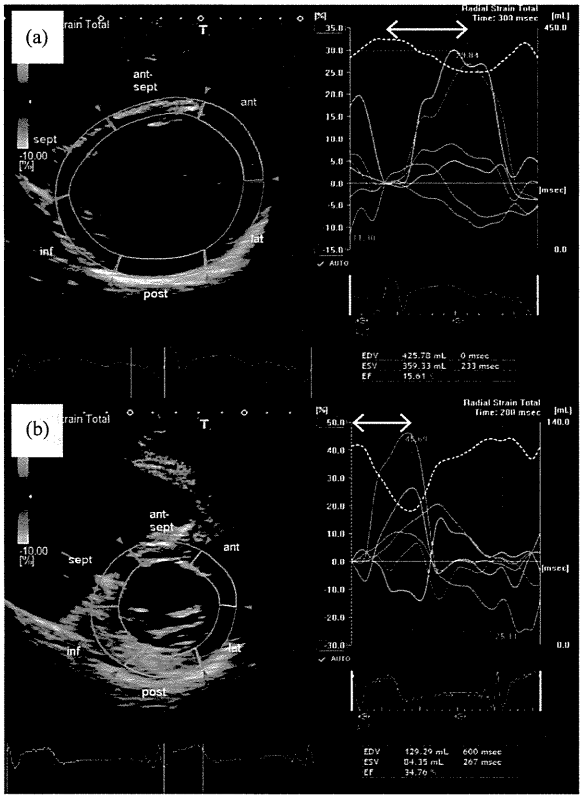

Figure 2. Speckle-tracking images showing dyssynchrony of peak segmental radial strain on admission (a) and at 7 months after introducing the CRTD system (b). Dyssynchrony was assessed using proprietary software (Toshiba Medical Systems Corporation, Tokyo, Japan). a: Dyssynchrony is shown as the time difference (arrow) between time to peak strain in the anterior (yellow outline) and lateral (blue outline) walls and time to peak strain in the inferior wall (purple outline) and septum (pink outline). Data in the table indicate marked cardiomegaly with an end diastolic volume of 426 ml. (b) CTRD placement resulted in improved synchrony of segmental shortening accompanied by a global systolic function left ventricular ejection fraction of 34.8% (compared with 15.6% in panel a).

may be a viable option for individuals with metabolic diseases such as propionic acidemia complicated by advanced secondary cardiomyopathy with renal dysfunction.

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