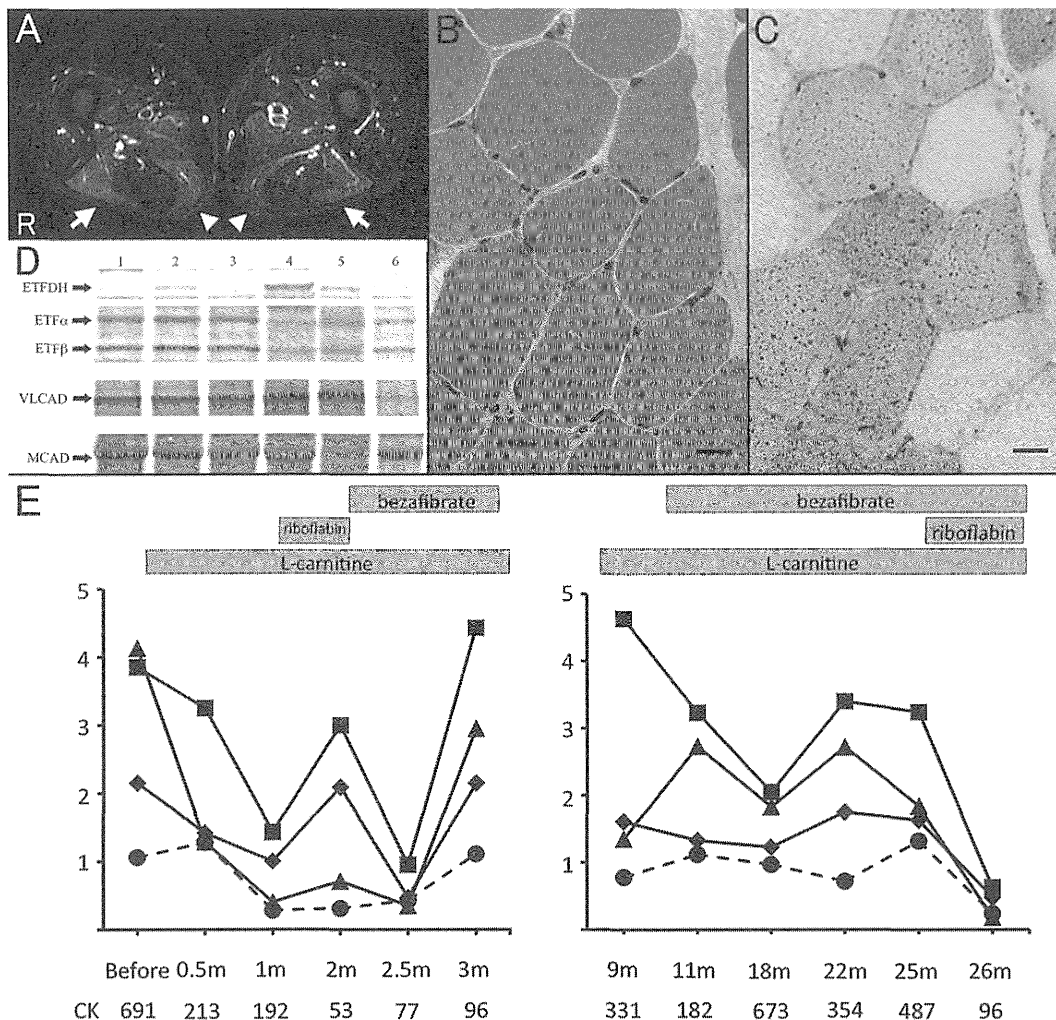

Fig. 1. A. A muscle MRI showed an area of high intensity in the bilateral biceps femoris muscle (arrow) and semimembranous muscle (arrowheads) in short-T1 inversion recovery (STIR). This indicated that increased water content in these muscles due to cellular lysis or fluid accumulation secondary to inflammation [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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Original article

Carnitine–acylcarnitine translocase deficiency: Two neonatal cases with common splicing mutation and *in vitro* bezafibrate response

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Abstract

Background: Mitochondrial fatty acid oxidation (FAO) disorders are among the causes of acute encephalopathy- or myopathy-like illness. Carnitine–acylcarnitine translocase (CACT) deficiency is a rare FAO disorder, which represent an energy production insufficiency during prolonged fasting, febrile illness, or increased muscular activity. CACT deficiency is caused by mutations of the *SLC25A20* gene. Most patients developed severe metabolic decompensation in the neonatal period and died in infancy despite aggressive treatment.

Patients and methods: We herein report the clinical findings of two unrelated cases of CACT deficiency with mutation confirmation, and *in vitro* bezafibrate responses using *in vitro* probe acylcarnitine (IVP) assay. Patients 1 and 2 are products of nonconsanguineous parents. Both patients developed cardiac arrest at day 3 of life but survived the initial events. Their blood chemistry revealed hypoglycemia and metabolic acidosis. The acylcarnitine profiles in both patients demonstrated increased long-chain acylcarnitines, suggesting CACT or carnitine palmitoyltransferase-2 (CPT2) deficiency.

Results: The mutation analysis identified homozygous IVS2-10T>G in the *SLC25A20* gene in both patients, confirming the diagnosis of CACT deficiency. The IVP assay revealed increased C16, C16:1, but decreased C2 with improvement by bezafibrate in the cultured fibroblasts. The short-term clinical trial of bezafibrate in Patient 1 did not show clinical improvement, and died after starting the trial for 6 months.

Conclusion: This splicing mutation has been identified in other Asian populations indicating a possible founder effect. IVP assay of cultured fibroblasts could determine a response to bezafibrate treatment. A long-term clinical trial of more enrolled patients is required for evaluation of this therapy.
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Keywords: CACT deficiency; *SLC25A20* mutation; IVP assay; Bezafibrate

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1. Introduction

Mitochondrial fatty acid oxidation (FAO) disorders are among the causes of neuromuscular symptoms as well as acute encephalopathy or even sudden death. In particular, the carnitine cycle is important in energy-producing pathway for cardiac and skeletal muscle and for preventing from hypoglycemia especially during prolonged fasting or increased muscular exercise. Carnitine–acylcarnitine translocase (CACT, EC 2.3.1.21) is one of the enzymes in the carnitine cycle, which catalyzes the transfer of the long-chain fatty acylcarnitines across the inner mitochondrial membrane in exchange of free carnitine. CACT deficiency (OMIM 212138) was first described in 1992 [1]. It is an autosomal-recessive disease caused by mutations of the *SLC25A20* gene located in chromosome 3p21.31 [2]. The gene consists of 9 exons and encodes protein comprising 301 amino acids [3]. CACT deficiency is a very rare disorder with so far as approximately 30 patients have been described, and accounted for 10% of patients with FAO disorders in French population [4]. However, it might be a common FAO disorder in some East Asian countries such as Hong Kong with the estimated incidence of 1 in 60,000 live births, and accounted for 33% of patients with FAO disorders [5]. Most patients develop neonatal-onset encephalopathy with nonketotic hypoglycemia, hyperammonemia, and hypothermia, or sudden death from cardiac arrhythmias. Cardiomyopathy and hepatic dysfunction may be the associated complications. CACT deficiency could be detected by elevations of C16 and C18 acylcarnitines, and low free carnitine in acylcarnitine profiles. However, the same profile could be found in neonatal carnitine palmitoyltransferase-2 (CPT2) deficiency. Therefore, confirmation of diagnosis requires CACT enzyme assay or molecular analysis of the *SLC25A20* gene [6]. Treatment includes intravenous glucose for acute decompensation, and avoidance of long fasting with frequent meals. Long-chain fatty acids may be restricted in diet, but medium-chain triglyceride (MCT) oil is supplemented instead. Carnitine therapy is still controversial. Despite aggressive treatment, most patients still died in infancy [7]. However, there have been some patients who received early treatment with good outcomes [8,9]. Novel therapy for FAOD using bezafibrate, which is a hypolipedimic drug acting as a peroxisome proliferator-activated receptor (PPAR) agonist has been reported. The clinical trials of bezafibrate showed clinical improvement in adult patients with CPT2 deficiency [10], and a child with glutaric acidemia type 2 (GA2) [11]. *In vitro* probe acylcarnitine (IVP) assay can be used to evaluate FAO disorders [12], and determine the effect of bezafibrate [13]. We herein report the clinical findings of two unrelated cases with neonatal-onset CACT deficiency, and *in vitro* bezafibrate response using the IVP assay.

2. Patients and methods

2.1. Patients

2.1.1. Case 1

This patient was the first child of possibly consanguineous parents from the southern province of Thailand. He was born at 37 weeks of gestation with birth weight of 2460 g (25th percentile), length 48 cm (3rd percentile), and head circumference 30 cm (<3rd percentile). He developed hypothermia at 10 h of age. Sepsis was suspected, but the patient rapidly responded to rewarming treatment. However, after rooming-in with the mother, he developed hypothermia again. At 60 h after birth, he had cardiac arrest. On physical examination, no abnormalities were found. Serum glucose was 1.2 mmol/L and acetoacetate was 0 mmol/L. Venous blood pH was 7.24 and serum bicarbonate was 13 mmol/L with an anion gap of 20. Plasma ammonia was 471 μmol/L (normal, <110 μmol/L). There were mildly elevated liver enzymes aspartate aminotransferase (AST) (97 U/L; normal, 0–32) and alanine aminotransferase (ALT) (78 U/L; normal, 0–33). Serum creatine kinase was 4439 U/L (normal, <190). He had a good response to treatment with intravenous glucose administration. Urine organic acids were unremarkable. A dried blood spot acylcarnitine profile by tandem mass spectrometry (MS/MS) showed free carnitine (C0), 5.26 μM (10–60); C16-acylcarnitine, 14.14 μM (0.6–7); C18-acylcarnitine, 2.71 μM (0.15–2.1); C18:1-acylcarnitine, 4.3 μM (0.3–3.2); and a (C16 + C18)/C0 ratio, 3.21 (0.007–0.5). The profile was consistent with CPT2 or CACT deficiency. The patient has been treated with a modular medical formula, which has been composed of modified fats (long-chain fatty acid restriction along with supplementation of 83% of fat as medium-chain triglyceride oil), protein, maltodextrins, minerals, and fat-, and water-soluble vitamins. L-Carnitine at a daily dosage of 100–150 mg/kg has been supplemented. Thereafter, he has had several episodes of hypoglycemia, hyperammonemia, and metabolic acidosis following infections. At 8 months of age, he developed cholestasis and hepatomegaly. At 9 months of age, an echocardiogram revealed hypertrophic cardiomyopathy. At the age of 15 months, he had mild developmental delay and generalized hypotonia. He could stand with support, put block in cup, and say one word. Then he had a metabolic crisis, and developed generalized weakness. After he recovered from encephalopathy, neurologic examination revealed normal cranial nerves, muscle weakness (grade 3/5), and decreased muscle tone and deep tendon reflexes (1+) in all extremities. A brain computed tomography scan was normal. Serum creatine kinase was elevated (1419 U/L). A nerve conduction study showed no evidence of demyelination. He had been ventilator-dependent since then. At 2½ years of

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age, he had several complications including chronic liver disease, upper gastrointestinal bleeding, and osteoporosis. He died at the age of 2 years and 8 months from upper gastrointestinal bleeding and metabolic decompensation.

2.1.2. Case 2

The patient was the first child of nonconsanguineous parents. She was born at 35 weeks of gestation with a birth weight of 2.3 kg (50th percentile), length 44 cm (25th percentile), and head circumference 30 cm (10th percentile). At 2 days after birth, she developed lethargy, poor feeding, and cardiac arrest. Blood glucose was 0.56 mmol/L. She responded to cardiac resuscitation and intravenous glucose infusion. Serum acetoacetate was 0 mmol/L. Venous blood pH was 7.39 and serum bicarbonate was 13 mmol/L with an anion gap of 20. Plasma ammonia was 157 μmol/L (normal, <110 μmol/L). There were elevated liver enzymes AST (638 U/L; normal, 0–32) and ALT (83 U/L; normal, 0–33). Plasma lactate dehydrogenase (LDH) was 522 U/L (normal, 240–480). An echocardiogram revealed no cardiomyopathy. A dried blood spot acylcarnitine profile by MS/MS analysis showed C0, 13.8 μM (10–60); C16-acylcanitine, 15 μM (0.6–7); C18-acylcarnitine, 4.3 μM (0.15–2.1); C18:1-acylcarnitine, 5.9 μM (0.3–3.2); and a (C16 + C18)/C0 ratio, 1.4 (0.007–0.5). The profile was consistent with either CPT2 or CACT deficiency. The patient had been treated with a high-MCT formula (Portagen®, Mead Johnson Nutritionals), and 100 mg/kg/day of L-carnitine. At 1 month of age, she developed anemia from Hb AE Bart's disease – a thalassemia intermedia resulting from the interaction between α-thalassemia and heterozygous Hb E, which required monthly blood transfusion. At the age of 4 months, she had poor feeding and cardiac arrest. Blood glucose was 0.5 mmol/L. The patient died without any response to resuscitation. An autopsy revealed left ventricular hypertrophy, micro/macrovesicular steatosis of the liver with focal areas of bridging fibrosis, and abnormal lipid accumulation in skeletal muscles and the proximal renal tubules.

2.2. Materials and methods

This study was approved by the Siriraj Institutional Review Board. The written informed consents for the mutation analysis, IVP assay, and bezafibrate trial were obtained from the parents. Genomic DNA was extracted from leukocytes. Mutation analyses of the *CPT2* and *SLC25A20* genes were performed in case 1, and only *SLC25A20* gene in case 2. All coding exons and their flanking intron sequences (up to 20 bases for both sides) of the *CPT2* and *SLC25A20* genes were PCR-amplified and directly sequenced according to the previously described method [14]. The IVP assay was performed using the skin fibroblasts in the absence and presence of bezafibrate according to the previously described method [11].

3. Results

3.1. Mutation analysis and IVP assay

Mutation analysis of the *SLC25A20* gene identified homozygous c.199-10T>G (IVS2-10T>G) mutation in both patients, and heterozygous mutation in their parents (Fig. 1). Mutation analysis of the *CPT2* gene revealed no pathogenic mutation in Case 1. The IVP assay profiles revealed increased C16, C16:1 acylcarnitines, and decreased C2 (acetylcarnitine) indicating a typical pattern of CPT2 or CACT deficiency, with substantial reduction of long-chain acylcarnitines by the presence of bezafibrate in the cultured fibroblasts from both patients (Fig. 2). However, C2 acylcarnitine did not increase as expected.

3.2. Clinical trial of bezafibrate

We started a clinical trial of bezafibrate in case 1 at age of 2 years and 2 months, after the IVP assay which

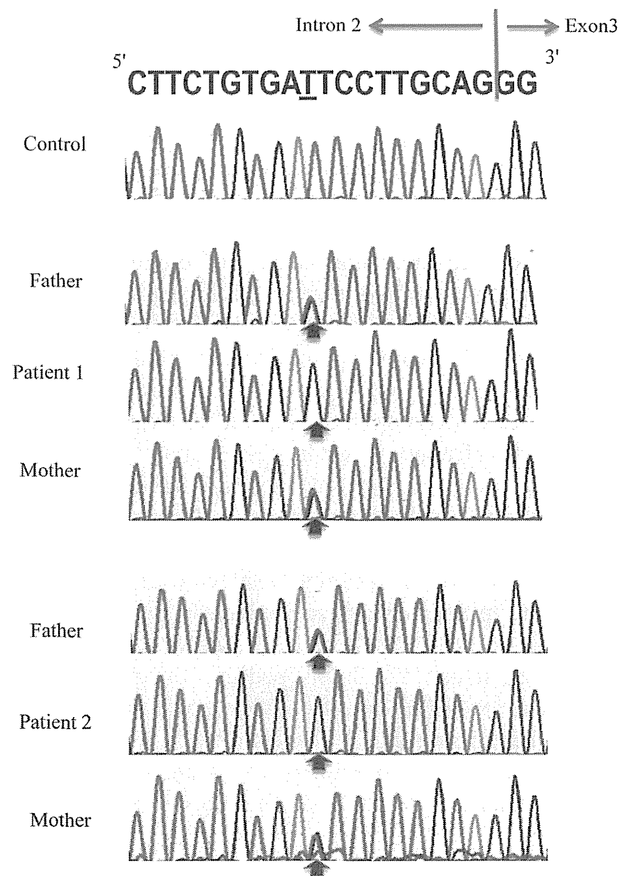

Fig. 1. The reference DNA sequence of an intron 2/exon 3 boundary of the *SLC25A20* gene, and the IVS2-10T>G mutation identified in both patients and their parents denoted by black arrows and the underlined letter.

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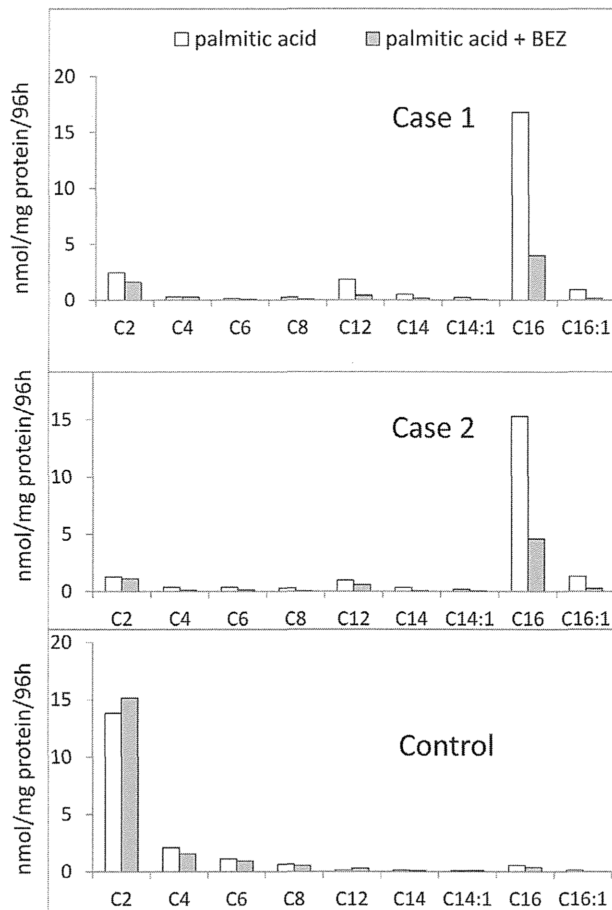

Fig. 2. Acylcarnitine profiles of IVP assay in the presence and absence of bezafibrate (BEZ) of cases 1, 2, and normal control respectively. Unit of vertical lines, nmol/mg protein of acylcarnitines (ACs); the horizontal lines represent acylcarnitines from C2, C4, C6, C8, C12, C14, C14:1, C16, and C16:1. The experiments for each were performed in triplicate, and the mean values of ACs are illustrated with bars.

showed some improvement in acylcarnitine profiles with bezafibrate. We used a dosage of 17–25 mg/kg/day as previously described [11]. Monitoring of liver functions, lactate dehydrogenase (LDH), creatine kinase (CK), and lipid profiles showed no adverse effects of bezafibrate. A short-term evaluation, after 6 months of the trial, did not show clinical improvement except for slightly increased back muscle strength noted by the mother. An echocardiography showed stable but no improvement in a left ventricular mass index. Acylcarnitine profiles in dried blood spots and other biochemical parameters did not show improvement (data not shown). Case 2 died before a clinical trial was considered.

4. Discussion

We report 2 unrelated cases of CACT deficiency with molecular confirmation first identified in Thailand. The c.199-10T>G (IVS2-10T>G) nucleotide change was the most prevalent mutation and identified in 14 out of 76 mutant alleles [15]. This mutation was homozygously identified in three Vietnamese and three Chinese patients. In the present study, in spite that two families had no consanguineous history, both patients were also a homozygotes of the c.199-10T>G mutation. In Japan, three CACT deficient patients have been described. Among them the same mutation was identified heterozygously in only one patient [14]. We propose that this mutation is a founder mutation in Asian populations. Clinical history of the three Chinese patients with homozygous c.199-10T>G mutation were reported [16]. All of them developed cardiac arrest within two days of age, as well as our two patients. Hence the phenotype of homozygotes of c.199-10T>G mutation is severe. This mutation was suggested to reside at a consensus lariat branch point sequence resulting in skipping of exons 3 and 4 or exon 3 alone, which leads to truncation of the protein [17].

Although our cases 1 and 2 were homozygotes of the same mutation, Case 1 survived until 2 years and 8 months and Case 2 died at 4 months of age. Several factors might attribute to their different clinical outcomes: (1) Thalassemia disease in case 2 which required repeated blood transfusions might affect cardiac functions by chronic hypoxia, iron overload, or decreased carnitine [18]; (2) differences in possible modifier genes such as *SLC25A29* gene (CACT-like, CACL) which has palmitoyl-carnitine transporting activity [19]; and (3) different formulas using in our cases, one is a synthetic modular formula and the other is a commercial formula. However, the rationale of both special formulas for diet therapy is a reduction in long-chain fatty acids together with supplementation of medium-chain triglyceride oil to be a caloric source shunting an obstruction of long-chain fatty acid β-oxidation.

Although increased FAO flux induced by bezafibrate was clearly shown in fibroblasts only from patients with mild phenotypes of FAO disorders, increased mRNA expression after bezafibrate exposure also occurred in cell lines from patients with severe phenotypes [20]. This could explain *in vitro* response to bezafibrate observed in fibroblasts of patient 1 and 2. Despite the severe genotype leading to barely detectable enzyme activity [21], we believe that there should be some FAO flux which could be enhanced by bezafibrate in these patients. Our hypothesis is if there is entirely absent FAO flux in these patients, they should have anomalies like those found in a lethal neonatal form of CPT2 deficiency or GA2 [22], even though there has been no report of such findings in CACT deficiency. To our knowledge, patient 1 is the first case of neonatal-onset CACT deficiency who underwent a clinical trial of bezafibrate after showing an *in vitro* response by IVP assay. However, no beneficial short-term effect was shown. This might indicate the irreversible damage of the affected organs esp. the cardiac and skeletal muscles, and liver. Moreover, the difference between the *in vitro* and *in vivo* responses is

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probably due to the difference of bezafibrate concentration used in the IVP assay (400 μmol/L) and typical concentrations obtained in patients on bezafibrate therapy (50–200 μmol/L) [23]. Another possible reason is inadequate acetyl-CoA production despite bezafibrate treatment. This hypothesis is supported by persistently low C2 acylcarnitines in IVP assays of our cases and a previous case with CACT deficiency [11]. Moreover, C16 acylcarnitine did not decrease to the control level after bezafibrate treatment. Overall, although some improvement of acylcarnitine profile was shown in the patient 1 and 2's fibroblasts in IVP assay with bezafibrate, the effect of bezafibrate was less than those in fibroblasts from patients with mild forms of FAO disorders [11,24]. Hence clinical improvement in this patient was thought to be limited. Since CACT-deficient patients who developed metabolic decompensation in early neonatal period had poor prognosis with routine management [7], we decided to use bezafibrate treatment in patient 1. He survived until two years of age with bezafibrate treatment. However, it is uncertain whether this longer survival owed to the effect of bezafibrate treatment or not, since no apparent improvement of clinical laboratory data was obtained.

In conclusion, CACT deficiency may be a common FAO disorder in East Asian populations probably from a founder effect. IVP assay of fibroblasts could determine a response to bezafibrate treatment. A long-term clinical trial and more enrolled patients are required for evaluation of this therapy.

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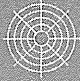

〔タンデムマス・スクリーニングの二次検査〕

血清および尿のアシルカルニチン分析

重松陽介* 畑 郁江**

はじめに

タンデムマス・スクリーニング陽性例に対して，二次検査として血清でのアシルカルニチン分析が実施される対象疾患を表に示した。ほとんどの脂肪酸酸化異常症では必須の検査であり，有機酸代謝異常症では尿有機酸分析が主であるが，重症度判定などのためアシルカルニチン分析も同時に行う。

表 タンデムマス・スクリーニング陽性時に血清アシルカルニチン分析を行う疾患

脂肪酸酸化異常症	<主な診断指標>
MCAD 欠損症	C8 の増加
VLCAD 欠損症	C14:1 の増加
TFP 欠損症	C16-OH，C18:1-OH の増加
CPT2 欠損症	C16，C18:1 の増加
TRANS 欠損症	C16，C18:1 の増加
全身性カルニチン欠乏症	C0 の減少
(グルタル酸血症 2 型)	C10 を中心に全般的な増加
有機酸代謝異常症	**<主な診断指標>**
プロピオン酸血症	C3 の増加
メチルマロン酸血症	C3 の増加
グルタル酸血症 1 型	C5-DC の増加
メチルクロトニルグリシン尿症	C5-OH の増加
マルチプルカルボキシラーゼ欠損症	C5-OH の増加
HMG 血症	C5-OH の増加

I. 血清と濾紙血の違い

血清と濾紙血ではアシルカルニチン濃度が異なる。**図 1** に示したとおり，濾紙血の分析は多くの赤血球を含む全血分析である。赤血球は赤芽球から成熟していく過程で核やミトコンドリアを失うが，ミトコンドリア内などに存在していた長鎖アシルカルニチンを中心とするアシルカルニチンや遊離カルニチンはそのまま赤血球内に留まる。赤血球寿命は通常 3 か月程度（新生児期はもっと短い）であり，新旧の赤血球はそれぞれ赤芽球だったころの病態代謝で生じたアシルカルニチンをそのまま保持しているので，濾紙血アシルカルニチン分析では，過去の赤芽球の代謝の経過が多少なりとも反映されることになる。

一方，血清アシルカルニチン分析では直近の病態代謝が確認できる。また，長鎖アシルカルニチンは細胞内に局在し血清中にはほとんど存在しないので，長鎖アシルカルニチンが病的に細胞内から漏れ出す疾患（長鎖脂肪酸酸化異常症）の診断[1]には血清アシルカルニチン分析が有用である（**図 2**）。

ただし，CPT1 欠損症の診断には，細胞内長鎖アシルカルニチンの減少を確認する必要があるので，血清ではなく濾紙血のアシルカルニチンを分析する[1]。

尿中には長鎖アシルカルニチンは排泄されない。尿アシルカルニチン分析により，短・中鎖アシルカルニチンの異常増加により有機酸代謝異常症を診断することがある。また，カルニチントラ

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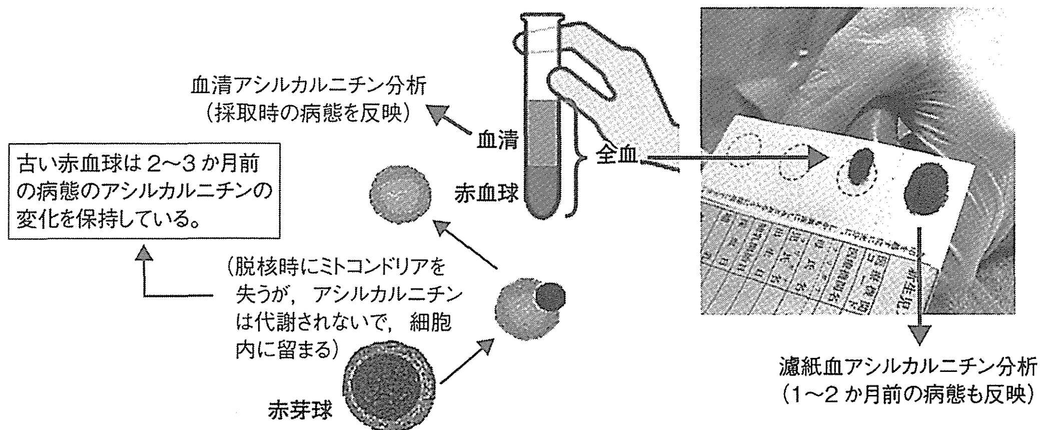

図 1　濾紙血と血清のアシルカルニチン値の違い

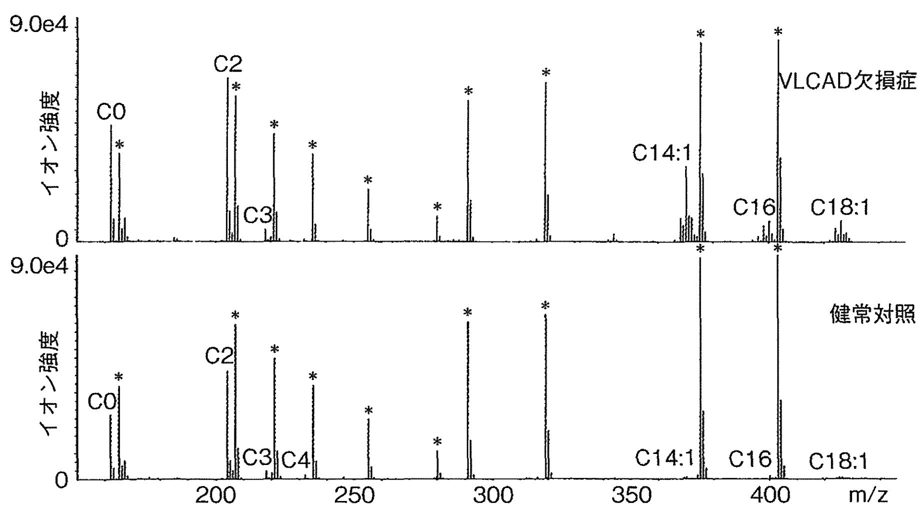

図 2　血清中アシルカルニチン・プロファイル
＊：安定同位体標識内部標準　m/z：質量数

ンスポータ異常症を診断するためには尿中遊離カルニチンを測定する。

II. 血清アシルカルニチン分析の実際

1．実施機関

血清アシルカルニチン分析は，「NPO法人タンデムマス・スクリーニング普及協会」を通じて福井大学小児科[3)]や島根大学小児科[4)]に外注検査として依頼する。また，一部のタンデムマス・スクリーニング検査機関でも実施しているので，確認されたい。測定値については，検査機関ごとに測定機器間差があるので，それぞれの異常判定値基準[5)]により判定することになる。

2．検体量と保存法

血清は0.1 mLあれば検査可能であるが，血清分離後速やかに冷凍保存する必要がある。室温で放置すると短鎖アシルカルニチン（とくにアセチルカルニチン）が壊れやすく，脂肪酸酸化異常症の診断が難しくなることがある。カルニチン腎排泄率測定には尿が必要であるが，クレアチニン濃度も測定する必要があるので，尿は最低1〜2 mL必要である。

3．血清アシルカルニチン分析陽性例の確定診断

陽性例については，酵素活性測定あるいは遺伝子解析で診断を確定する[6]。

III．脂肪酸酸化異常症の化学診断

1．中鎖アシル CoA 脱水素酵素（MCAD）欠損症

C8 アシルカルニチンが最も増加し，C6 と C10 も軽度増加する。アセチルカルニチン（C2）は減少傾向であるが，軽症例では急性発症時に増加することもある。鑑別診断として，低出生体重児の MCT 摂取に注意。本症保因者でも，飢餓時には C8 が軽症患者のレベルに増加することがある。逆に，本症軽症例では非飢餓時には C8 の異常増加がみられなくなることも知られている。

2．極長鎖アシル CoA 脱水素酵素（VLCAD）欠損症

通常，C14:1 が最も増加し C16 や C18:1 も軽度増加する。アセチルカルニチン（C2）は減少傾向であるが，軽症例では急性発症時に増加することもある。重症例の急性発症時には C16 や C18:1 のほうが C14:1 よりも増加するが，C14:1 も著増している。本症保因者でも，飢餓時には C14:1 が軽症患者のレベルに増加することがある。逆に，本症軽症例では非飢餓時には C14:1 の異常増加がみられなくなることも知られている。

3．三頭酵素（TFP）欠損症

C16-OH と C18:1-OH が増加している。軽症例では，非飢餓時には C16-OH や C18:1-OH の異常増加がみられなくなることが多いので，疑わしいときには尿有機酸分析や酵素活性測定，遺伝子解析で診断を確認する必要がある。

4．カルニチンパルミトイルトランスフェラーゼ（CPT）2 欠損症

C16 と C18:1 が増加している。軽症例では，非飢餓時には C16 や C18:1 の異常増加がみられなくなることもあり，疑わしいときには酵素活性測定や遺伝子解析で診断を確認する必要がある。

5．カルニチンアシルカルニチントランスロカーゼ（TRANS）欠損症

CPT2 欠損症と同様の所見を示す。確定診断には遺伝子解析が必要である。

6．全身性カルニチン欠乏症

遊離カルニチン（C0）は通常 10 μM 以下である。カルニチン腎排泄率は，

(尿遊離カルニチン)/(尿クレアチン)/[(血清遊離カルニチン)/(血清クレアチン)]×100（%）

で計算する。健常人では 2%以下である。この検査はカルニチンを服用していないときに実施する必要がある。遺伝子解析で診断を確定する。

7．グルタル酸血症 2 型

C2 が減少気味で，C10 アシルカルニチンを中心に短鎖から長鎖のアシルカルニチンが増加するが，すべてが増加しているわけではない[1]。健常児でも飢餓状態では C10 を中心に中・長鎖アシルカルニチンが軽度〜中程度増加するが，この場合は C2 が著増している。尿有機酸分析や遺伝子解析で診断を確定する。

IV．有機酸代謝異常症の化学診断補助

グルタル酸血症 1 型では，軽症型では尿中グルタル酸排泄が軽微で診断が困難な場合もある。このような場合，カルニチン欠乏状態であることがあり，カルニチンを服用させて血清アシルカルニチン分析を行うと C5-DC の上昇が明確になり診断に役立つ。その他の有機酸代謝異常症でも二次性カルニチン欠乏を呈していることがあり，重症度の判定や治療法の選択において血清カルニチン分析結果が役立つ。

また，**マルチプルカルボキシラーゼ欠損症**や**ビオチン欠乏**に対するビオチン治療の効果判定には，濾紙血ではなく血清のアシルカルニチン分析での C5-OH 濃度を用いる（図 1）。

V．乳幼児期突然死例の化学診断

乳幼児期の原因不明の急死について，剖検時に採取された血清や胆汁のアシルカルニチン分析が脂肪酸酸化異常症や有機酸代謝異常症の診断に利用される。とくに，長鎖アシルカルニチンは尿中ではなく胆汁中に排泄されるので，胆汁のアシルカルニチン分析は診断上有用である[7)]。

Key Points

❶ 血清アシルカルニチン分析は，脂肪酸酸化異常症や有機酸代謝異常症の化学診断を目的として行われる。

❷ 軽症型の脂肪酸酸化異常症患者では，安静・非飢餓時にはアシルカルニチン異常がみられないことがある。

❸ 非患者でも飢餓時の血清アシルカルニチン・プロファイルはグルタル酸血症２型でのプロファイルに類似し，鑑別診断には尿有機酸分析などが必要となる。

❹ CPT-1 欠損症の化学診断には，濾紙血アシルカルニチン分析が必要である。

❺ カルニチントランスポータ異常症の診断には，尿中遊離カルニチンも分析する必要がある。

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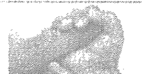

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Brief Report

Biotin and carnitine deficiency due to hypoallergenic formula nutrition in infants with milk allergy

Hisako Hayashi,[1] Shuko Tokuriki,[1] Takashi Okuno,[1] Yosuke Shigematsu,[1] Akiba Yasushi,[2] Go Matsuyama,[3] Ken Sawada[2] and Yusei Ohshima[1]

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Abstract Amino acid formulas and hydrolyzed formulas given to infants in Japan with milk allergies theoretically contain little, if any, biotin and carnitine. We assessed biotin and carnitine insufficiency in six infants with milk allergy who were fed amino acid formulas and/or hydrolyzed formulas, by measuring urine 3-hydroxyisovaleric acid (3-HIA) and serum free carnitine (C0), respectively. All patients presented with elevated urine 3-HIA and lowered serum C0 compared with post-menstrual age-matched infants who were fed breast milk or standard infant formulas. Supplementation with biotin and L-carnitine immediately improved the insufficiency. Care should be taken to avoid biotin and carnitine deficiency in allergic infants fed amino acid or hydrolyzed formulas.

Key words amino acid formula, biotin deficiency, carnitine deficiency, hydrolyzed formula, milk allergy.

Carnitine is an important cofactor for β-oxidation, and in the form of acylcarnitine facilitates the transport of long-chain fatty acids into the mitochondrial matrix.[1] Carnitine could be considered a conditionally essential nutrient in neonates due to their reduced ability to synthesize carnitine.[2] Biotin is a vitamin that serves as a covalently bound coenzyme for carboxylases, which catalyze essential steps in gluconeogenesis, fatty acid synthesis, and metabolism of odd-chain fatty acids and some amino acids. Concerns have arisen that some types of milk and enteral nutrition formulas used by patients with cow's milk allergies lack sufficient biotin and carnitine, and that use of these products can lead to nutrient deficiency.[1]

We recently found that serum carnitine profile and urine 3-hydroxyisovaleric acid (3-HIA) are useful markers for detection of biotin deficiency in preterm infants.[3] Moreover, we found that serum free carnitine (C0) in preterm infants was significantly lower than in term infants. Chronic biotin insufficiency frequently occurs in preterm infants, even those fed with maternal milk or standard infant formulas. In this context, preterm infants with milk allergy are at heightened risk for development of biotin and carnitine deficiency after starting hypoallergenic formula nutrition. Herein we report on six infants with milk allergy who developed both biotin and carnitine deficiency during treatment with amino acid formulas and/or hydrolyzed formulas.

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Methods

Six infants born at the University of Fukui Hospital and Toho University Sakura Hospital between July 2010 and March 2012, and diagnosed as having cow's milk allergy were enrolled (Table 1). The diagnosis of cow's milk allergy was confirmed when the following criteria were satisfied: (i) no other causes of gastrointestinal symptoms; and (ii) disappearance of gastrointestinal symptoms after changing from a standard cow's milk formula to either amino acid or hydrolyzed milk formula, and recurrence of symptoms after the re-introduction of standard formula. Lymphocyte stimulation tests using cow's milk protein were performed in patients 1, 2, and 3, were positive.

3-Hydroxyisovaleric acid was measured on gas chromatography–mass spectrometry with urease-treated urine, and serum C0 on tandem mass spectrometry using the non-derivation method, according to previous reports.[4,5] Given that serum C0 level in preterm infants is dependent on post-menstrual age, the serum C0 level of post-menstrual age-matched control infants who received enteral feeding with maternal milk and/or standard formula made in Japan was used as a reference value.[3]

Biotin deficiency reduces biotin-dependent enzyme methylcrotonyl-CoA carboxylase activity, resulting in increased urinary excretion of 3-HIA.[6] Elevated urinary 3-HIA outside the normal range (3.4–12.5 μg/mg creatinine) is considered a marker of biotin deficiency.

The study was approved by the Institutional Ethics Committee at the University of Fukui. The parents of the infants gave written consent.

Table 1 Demographic data for infants with milk allergy

Patient	1	2	3	4	5	6
Gender	Male	Male	Male	Female	Female	Female
Birth weight (g)	2146	2327	966	1133	1046	880
Gestational age	35 weeks + 5 days (250 days)	32 weeks + 6 days (230 days)	26 weeks + 5 days (187 days)	30 weeks + 0 days (210 days)	24 weeks + 5 days (173 days)	26 weeks + 3 days (185 days)
Time of onset of milk allergy	Day 6	Day 1	Day 18	Day 8	Day 7	Day 14
Symptoms of milk allergy	Poor sucking, failure to thrive	Bile vomiting, hematochezia	Bile vomiting, hematochezia	Hematochezia, meteorism	Bile vomiting	Bile vomiting, hematochezia
During treatment with hypoallergenic formula						
Formula (time of commencement of the formula)	AA → HM (day 11 → day 23, [expiry on 68])	AA → HM (day 16 → day 40)	HM → AA (day 20 → day 76)	AA (day 15)	AA (day 11)	HA → AA (day 14 → day 27)
Clinical manifestation	–	Well-circumscribed erosive erythema, hair loss	–	–	Erosive erythema	Alopecia, hypopigmented hair
Lowest Hb (g/dL)	10.4	8.8	6.2	7.9	7.4	6.1
Lowest MCV	80.3	79.0	86	87	84	84
Highest CK (U/L)	147	199	224	Not determined	145	356
First detection of high 3-HIA	Day 153	Day 186	Day 123	Day 37	Day 49	Day 107
First detection of low C0	Day 69	Day 67	Day 123	Day 37	Day 49	Day 107
Time of commencement of supplementation	Not done	Day 193	Day 14	Day 59	Day 82	Day 121

3-HIA, 3-hydroxyisovaleric acid; AA, amino acid formula; C0, free carnitine; CK, creatine kinase; HM, hydrolyzed formula; MCV, mean corpuscular volume.

Results

Median gestational age was 199 days (range, 173–250 days) and median birth weight was 1089 g (range, 880–2327 g; Table 1). All six patients had elevated urine 3-HIA on day 115 (median), after commencement of amino acid or hydrolyzed milk formula feeding (Fig. 1).

Serum C0 level generally increases with post-menstrual age in preterm infants fed breast milk or standard infant formulas.[3] Three patients, however, did not have any significant increase in serum C0 level during hypoallergenic formula feeding (Fig. 2). After a median 68 days (range, 37–123 days) of treatment with hypoallergenic formula, all patients had serum C0 <20 nmol/mL or the reference level for post-menstrual age-matched control infants.

Symptoms of carnitine deficiency such as rhabdomyolysis, hypoglycemia, and convulsion, were not observed in any of the patients. Although patients 3 and 6 had mild elevation of serum creatine kinase, each patient's muscle tonus was normal. Patient 2 had well-circumscribed erosive erythema in the anogenital and circumorbital regions and hair loss. Patient 5 presented with erosive erythema on the face, and patient 6 developed alopecia with hypopigmented hair. Five patients, all except patient 1, were given oral supplementation with both biotin (0.5–5 mg/day) and L-carnitine (15–99 mg/kg per day). Patient 1 outgrew the milk allergy, so standard infant formula was started at 392 days of post-menstrual age without any supplementation. The skin lesions in patients 2, 5 and 6 immediately disappeared after commencement of supplementation and did not recur.

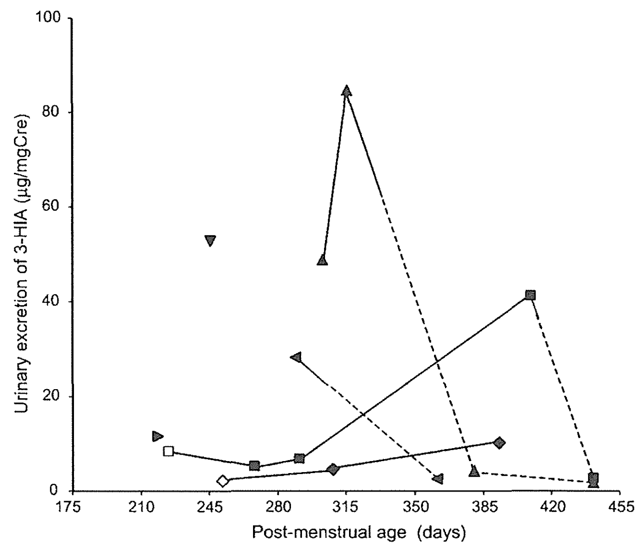

Fig. 1 Urinary excretion of 3-hydroxyisovaleric acid (3-HIA) increased in infants fed amino acid formula and/or hydrolyzed formula. Urinary excretion of 3-HIA (open symbols) before and (closed symbols) after commencement of hypoallergenic formula nutrition is given as urinary concentration of 3-HIA adjusted for urinary concentration of creatinine. Broken lines, period of biotin supplementation. Shading, normal range of 3.4–12.5 µg/mg Cre reported by Mock *et al.*[6] ◆, patient 1; ■, patient 2; ▲, patient 3; ▼, patient 4; ▶, patient 5; ◀, patient 6.

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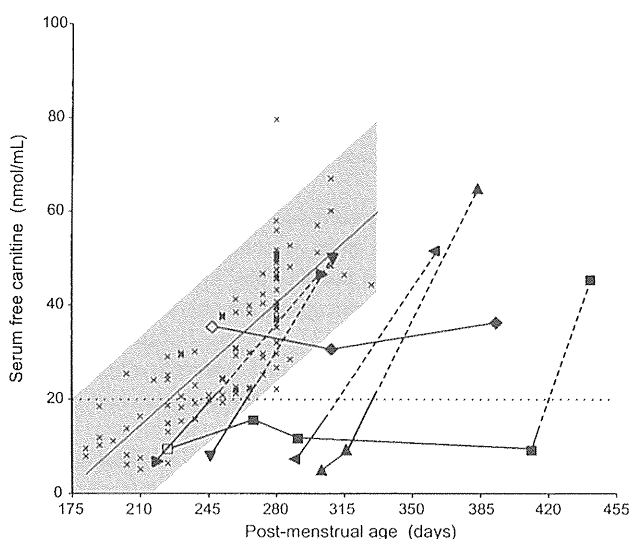

Fig. 2 Serum free carnitine (C0) decreased in infants fed with amino acid and/or hydrolyzed formula. (open symbols), Serum C0 concentration before and (closed symbols) after commencement of hypoallergenic formula nutrition. Broken lines, period of carnitine supplementation; (x), reference values for post-menstrual age-matched control infants who were fed maternal milk or standard infant formula made in Japan;[3] solid line, mean of reference values; shaded polygon, 95% limit of agreement. ◆, patient 1; ■, patient 2; ▲, patient 3; ▼, patient 4; ▶, patient 5; ◀, patient 6.

Discussion

There are several reports exclusively in Japan of biotin deficiency occurring in infants fed amino acid or hydrolyzed formula due to milk allergy.[1,7] The observation that biotin deficiency due to hypoallergenic infant formula nutrition is rare in other countries may be due to the fact that supplementation of hypoallergenic formulas with biotin as a food additive is not permitted in Japan. We recently showed that there is a risk of biotin deficiency even in preterm infants fed maternal milk or standard infant formulas.[3] In the present study, although none of the infants had severe symptoms of biotin deficiency such as seizures, hypotonia, lactic acidosis, or organic aciduria, half of them presented with skin lesions. The anogenital erosive erythema and hypopigmented hair might be caused by zinc and copper deficiency, respectively. They disappeared, however, after commencement of biotin but not after zinc or copper supplementation, suggesting that biotin deficiency is common in preterm infants fed hypoallergenic formulas.

In contrast to the skin lesions characteristic of biotin deficiency, carnitine deficiency is difficult to recognize in the infant period, because its early symptoms are failure to thrive and impaired function of organs such as cardiac muscle and skeletal muscle that are highly dependent on fatty acid oxidation for fuel.[8] Once vital energy is exhausted upon fasting or starvation, defective β-oxidation of fatty acids due to carnitine deficiency may produce pathological situations such as sudden infant death syndrome, Reye-like episodes, hypoketotic hypoglycemic coma, muscle weakness, and profound cardiac dysfunction. Given that the patients developed carnitine insufficiency coincident with or prior to biotin deficiency, physicians should be aware of the possible comorbidity of carnitine deficiency in milk-allergic infants treated with hypoallergenic formulas when typical skin lesions associated with biotin deficiency develop.

Parenteral nutrition without carnitine supplementation in both term and premature infants resulted in decreased carnitine plasma concentration, and carnitine supplementation in parenteral nutrition enhanced fatty acid oxidation and clearance, improved lipid tolerance, and increased nitrogen balance in the neonates.[2] These findings suggest that carnitine supplementation might allow for more rapid growth and better fat utilization in infants fed amino acid or hydrolyzed formulas.

Weaknesses of the current study include the small sample size and methodologic limitations, specifically with respect to assessment of metabolic and anthropometric measurements, and the dosing regimens for carnitine supplementation. For ethical reasons, the patients were treated with different doses of carnitine with the goal of maintaining serum C0 concentration >20 nmol/mL, which is the reference value for diagnosis of carnitine deficiency.[1,9] Future studies evaluating the effects of carnitine and biotin supplementation on infant morbidity parameters should include an assessment of different dosing regimens.

Conclusion

When treating milk-allergic infants with amino acid or hydrolyzed formulas, supplementation of biotin and carnitine is recommended. It would be beneficial if hydrolyzed and amino acid formulas produced in Japan could be fortified with biotin and carnitine.

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CPT Ⅱ欠損症の新生児スクリーニング．見逃し例経験後の指標変更の影響

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【要　旨】

カルニチンパルミトイルトランスフェラーゼII (CPT II) 欠損症は，現時点ではタンデムマス・スクリーニング一次対象疾患となっていない．広島県では試験研究以来スクリーニングを試みてきたが，新生児期に陽性と判断できず急性発症した乳児例が出現したため，以後スクリーニング指標の変更を行った．

スクリーニングを開始した1999年から指標変更を行うまでの約11年間に陽性者は皆無であったが，2011年1月の指標変更から2014年7月までの約3年半には81,466新生児中13例が陽性となった．精査段階では6例に血清長鎖アシルカルニチン高値を，5例に脂肪酸代謝能の軽度低下を認め，うち2例に*CPT II*遺伝子の塩基置換を認めた．今回の指標変更により，非罹患と断定することの難しい症例が多数出現する結果となった．引き続きさらなるスクリーニングおよび診断精度向上のための方策が求められる．

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【背　景】

空腹時，脂肪酸β酸化はエネルギー産生の大きな割合を占める．長鎖脂肪酸が細胞質からβ酸化の場であるミトコンドリア内へ取り込まれるためには，アシルCoAから一旦アシルカルニチン (AC) に変換され，ミトコンドリア内膜に存在するカルニチンパルミトイルトランスフェラーゼII (CPT II) によって再びアシルCoAに変換される必要がある[1]．

CPT II欠損症は思春期以降に骨格筋症状で発症する遅発型が多いが，より重症型である新生児期発症型や乳幼児期発症型も知られている．乳幼児期発症型は生後6-24ヶ月頃に感染／発熱／飢餓時間の遷延を契機に発症し，低ケトン性低血糖症とそれに伴う意識障害／痙攣／Reye様症候群などを呈し，死亡したり，重度の神経学的後遺症を遺したりする．心肥大や不整脈を伴うこともある[1]．

従って，本疾患も乳幼児期発症型を中心に新生児スクリーニングで発見する意義は大きく，広島県では1999年の試験研究開始以来，本疾患の発見に努めてきた．しかしながら，陽性例を全く経験しないまま，2010年に急性発症例が県内で診断され[2]，新生児スクリーニングで正常と判定されていたことが判明した．

【症例呈示】

見逃し例の詳細を示す．生後7か月時に発熱し，その後3時間で嘔吐，7時間でけいれんが出現した．救急搬送され，血液検査により代謝性アシドーシス，低ケトン性低血糖症，アンモニアおよびクレアチンキナーゼの高値を認めた．

受付日：平成26年9月1日　受理日：平成26年11月13日
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ブドウ糖輸液によりアシドーシスおよび低血糖は速やかに改善したが，HHV-6による脳症の関与もあり，けいれんの抑制に難渋し，救命できたが重度の神経学的後遺症を残した．急性期の血清を用いたAC分析では長鎖ACが高値であり，患者培養リンパ球を用いた脂肪酸酸化能測定では低下を認めた．また*CPT II*遺伝子に複合ヘテロ接合性変異を同定した[2]．本症例は新生児期にタンデムマス・スクリーニングを受検しているが，異常は指摘されていなかった．このような症例の再度の出現を防ぐべく，スクリーニング指標の改訂を行った．

【方　法】

2010年12月までの初回濾紙血スクリーニング基準は「C16-AC 6.3nmol/mL以上かつC18:1-AC 3.0nmol/mL」としていた．見逃し症例出現後，2011年1月からはスクリーニング基準を「C16-AC　3.0nmol/mL以上かつ(C16+C18:1)/C2 0.62以上」に変更した（表1）．濾紙血AC分析は試験研究以来福井大学で行っていたが，タンデムマス・スクリーニングが自治体事業化された2013年2月以降は先ず広島市医師会臨床検査センターで濾紙血AC分析を行い，同施設でのスクリーニング基準値「C16-AC 2.5nmol/mL以上かつ(C16+C18:1)/C2 0.40以上」で陽性となった濾紙血を福井大学で分析している．

見逃し例出現後のスクリーニング基準変更以降，2014年7月末現在までの約3年半に81,466件のスクリーニングを施行した．13例を精査対象と判断した（表2）．症例1は指標変更後最初のケースで，新たな基準のうちC16-ACしか基準を満たしていないが，従来の基準を満たしていたため精査対象とした．症例7および11は広島市医師会臨床検査センターの基準で陽性と判断され，福井大学での同一濾紙血検体を用いた検査では基準値を下回っていた．症例13はやはり広島市医師会臨床検査センターの基準で陽性と判断され，福井大学での血清AC分析は実施しているが，濾紙血検査を実施していない．症例8はC0-AC低値で精査となった症例で，精査時の血清AC分析でC16-AC　>0.10nmol/mL, C18:1-AC　>0.10nmol/mLを繰り返したため，CPT II欠損症の精査を実施している．

今回の検討で得られた濾紙血AC分析結果について，これまでに他府県で確定診断された7症例（急性発症例の急性期検体3例，スクリーニングによる診断4例）との比較検討も行った．

精査では血清AC分析を行い，「C16-ACおよびC18:1がいずれも0.10nmol/mL以上」を異常と判断し，参考所見として(C16+C18:1)/C2も評価している（0.09以上を高値）．保護者の同意が得られた場合は，培養リンパ球による脂肪酸酸化能測定およびダイレクトシークエンス法によるCPT II遺伝子解析を実施した．

表1．スクリーニング基準の変更

スクリーニング指標	Cutoff 値	
	～2010	**2011.1～**
C16-AC (nmol/mL)	>6.3	**>3.0**
C18 : 1-AC (nmol/mL)	>3.0	
(C16+C18 : 1) / C2		**>0.62**

【結　果】

濾紙血スクリーニング基準変更以降に陽性となった症例のC16-ACおよび(C16+C18:1)/C2値は，見逃し症例に比し両指標とも高値のものが3例，片方のみ高値のものが6例，いずれの指標とも低値のものが3例であった（図1）．これまでに他府県でCPT II欠損症と確定診断された7症例の濾紙血データを（図1）に加えたものを（図2）として再掲する．変更した濾紙血スクリーニング基準が，これら7症例を漏れなく検出できることがわかる．同時に我々の経験した見逃し例が，極めて発見の難しいケースであったこともわかる．

濾紙血によるスクリーニングで精査対象となった症例に対する血清AC分析では6例が基準を上回っていた（表2）．うち症例5は重症新生児仮死で出生し，重篤な神経学的後遺症を残している．ほかの5例は現在まで何ら症状を認めていない．これら6例の血清AC指標は，いずれも見逃し例急性期検体と比較すると両指標とも1/10以下の，軽度の上昇にとどまっている（表2）．

表2. 見逃し症例とその後の県内精査対象者（スクリーニング総数81,466件）

Case	Year of birth	Day of DBS collection	AC-profie (DBS) C16 (nmol/mL) cutoff 3.0	AC-profie (DBS) (C16+C18:1) / C2 cutoff 0.62	AC-profile (serum) C16 (nmol/mL) cutoff 0.10	AC-profile (serum) C18:1 cutoff 0.10	AC-profile (serum) (C16+C18:1) / C2 (cutoff 0.09)	capacity of beta-oxidation	Gene analysis of *CPT II*
見逃し例	2010	Day 5	3.45	0.75	3.01	3.92	7.93	↓↓	R161W, F383Y
1	2011	Day 5	9.67	0.46＊	**0.11**	**0.1**	0.032	→	WT
2	2011	Day 4	5.18	0.84	0.09	0.07	0.027	→	WT
3	2011	Day 5	3.12	0.94	0.08	0.07	0.021	N.A.	N.A.
4	2012	Day 4	5.33	0.64	0.09	0.11	0.025	→	N.A.
5	2012	Day 4	3.51	0.77	**0.29**	**0.15**	0.062	↓	WT
6	2013	Day 4	3.41	0.64	**0.17**	**0.24**	0.057	↓	WT
7	2013	Day 6	2.98＊	0.59＊	N.A.	N.A.	N.A.	→	
8	2013	Day 4	3.58	0.51＊	**0.12**	**0.1**	0.035	↓	T509A hetero
9	2013	Day 4	3.11	0.83	0.09	0.12	0.026	N.A.	N.A.
10	2013	Day 6	4.21	0.86	**0.1**	**0.1**	0.025	N.A.	N.A.
11	2013	Day 5	2.74＊	0.66	0.09	0.06	0.021	↓	N.A.
12	2013	Day 5	4.44	0.65	0.09	0.09	0.02	→	V368I (SNP) homo, F352C (SNP) hetero
13	2014	Day 5	N.A.	N.A.	**0.13**	**0.18**	0.035	↓	N.A.

W.T.; wild type, N.A.; not analyzed

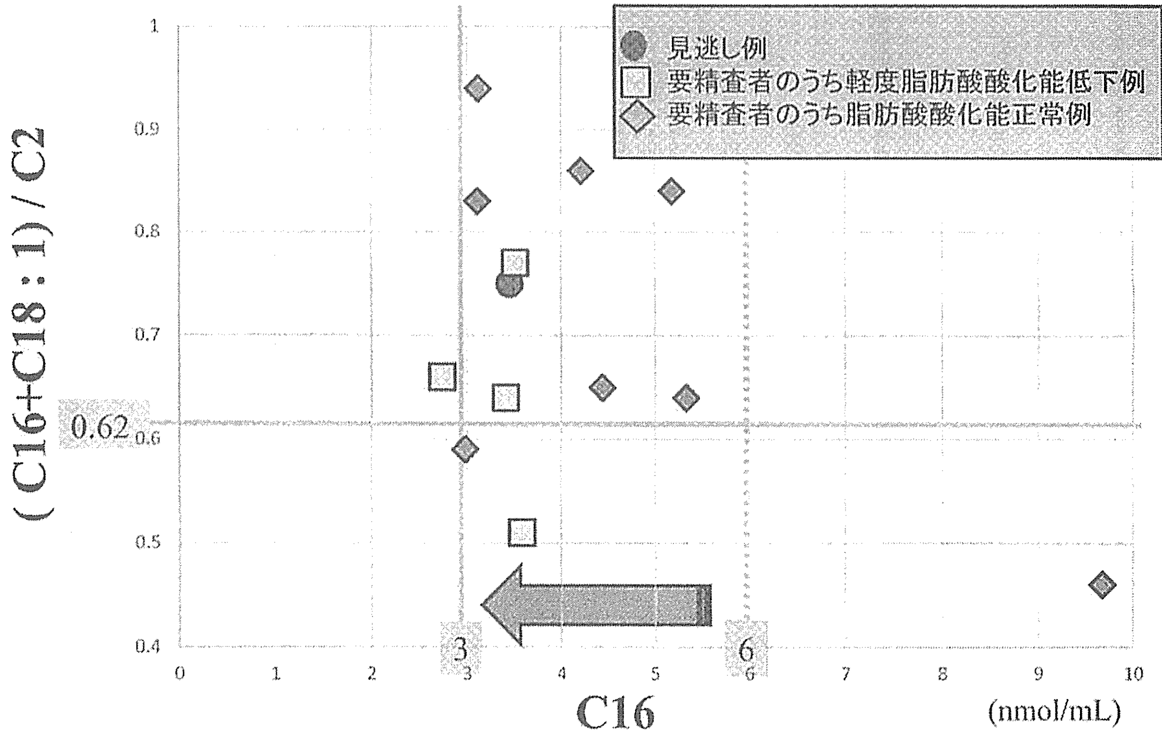

図1．初回濾紙血AC指標の分布

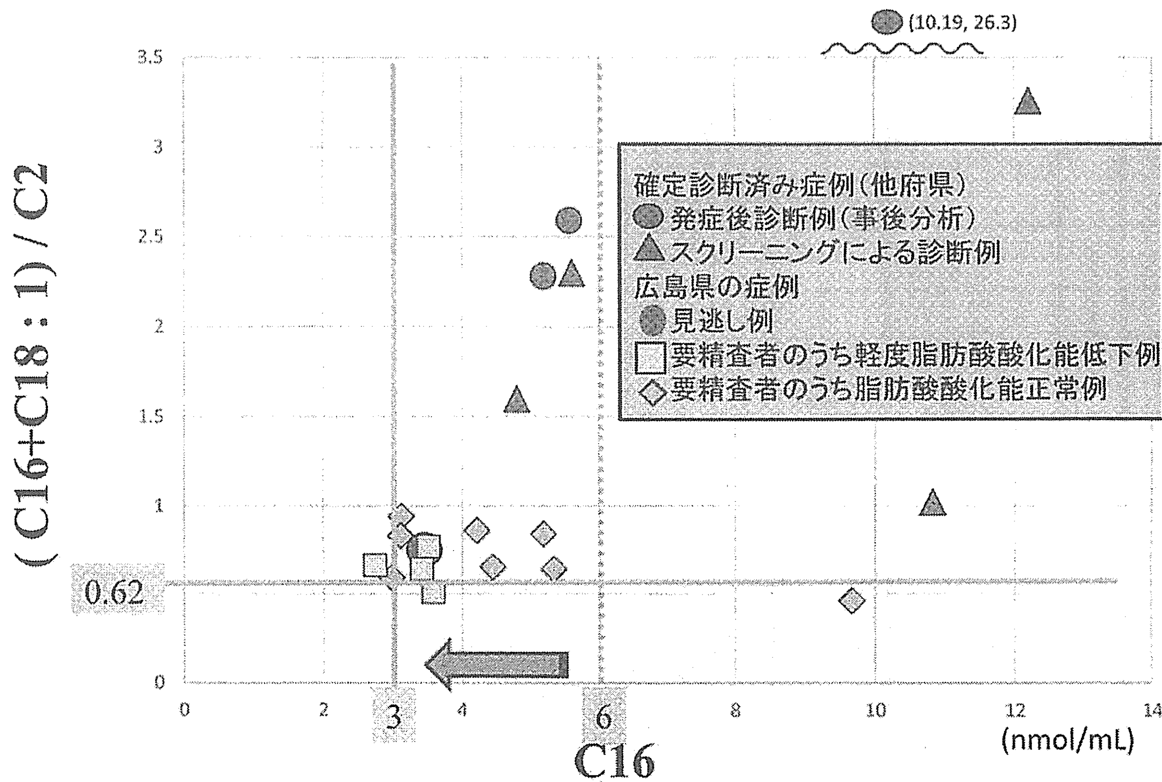

図2．初回濾紙血AC指標の分布（他府県で確定診断された症例のデータを加えたもの）

見逃し例は急性発症後の安定期にC16-AC 0.76 nmol/mL, C18:1-AC 0.76nmol/mLという結果が得られており，これと比較しても6例ともかなり低い値となった．今回詳細を提示していないが，本邦における10例程度の発症後診断例は，両指標とも無症状期0.4以上，急性期2-10程度である．

精査対象となった症例に対する脂肪酸酸化能測定は10例に実施でき，5例に軽度低下を認めた．見逃し症例では明らかな低下を認めている．濾紙血におけるスクリーニング指標値と，精査における脂肪酸酸化能測定結果には，特に相関は認められない（図1)．

精査対象となった症例のうち7例に*CPT II*遺伝子検索を行った．C0-AC低値から疑った症例8に新規変異c.1525A>G(p.T509A)をヘテロ接合性に同定した．症例12には1塩基置換を2種類認めたが，いずれもSNPとして記載のあるものであった．見逃し症例には日本人患者における好発変異F383Y[3,4]を含む複合ヘテロ接合性変異を同定している．脂肪酸酸化能の軽度低下を認めた5例については，同様のAC分析結果を呈しうるカルニチン／アシルカルニチントランスロカーゼ（CACT）欠損症を鑑別するため，遺伝子解析の追加実施を検討している．

【考　察】

基準変更によっても濾紙血データから罹患／非罹患や罹患であった場合の重症度を推測することは困難であった．のみならず，基準変更により出現した要精査者からは，血清AC分析でも判断に苦慮する症例が多発している．de Sain-van der Velden MGらはCPT II欠損症患者4名の検体を用いた検討で，血漿での検査は濾紙血よりC16-ACおよびC18-AC高値がはっきりし，診断に，より望ましいとしている[5]が，我々の検討ではそのような印象は得られなかった．やはりCPT II欠損症のスクリーニングは現状ではまだまだ困難である．タンデムマスの先進地域である欧州においてもCPT IIを対象疾患としているのは37の新生児スクリーニングプログラムのうち7つに過ぎない[6]．

久保田らはCPT II欠損症児の濾紙血および血清AC profileを生後継時的に検討した結果，濾紙血長鎖アシルカルニチンは日齢3にピークを示し，その後漸減することを示し，出生後より早期に濾紙血を採取することがスクリーニング精度向上に有用と提案している[7]．しかし他疾患のスクリーニングとの兼ね合いもあり，国内での実現は難しいと思われる．

効率的なCPT II欠損症スクリーニングのためには新たに明快な確定検査法を確立することが不可欠である．今般，陽性者リンパ球を用いた，より簡便なCPT II酵素活性測定法の条件設定が完了したので，今後実際の症例への応用を進める予定である．

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