Molecular Genetics and Metabolism Reports 24 (2020) 100611

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: www.elsevier.com/locate/ymgmr

Case Report

Need for strict clinical management of patients with carnitine palmitoyltransferase II deficiency: Experience with two cases detected by expanded newborn screening

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ARTICLE INFO

Keywords:
Carnitine palmitoyltransferase II deficiency
Metabolic decompensation
Fatty acid oxidation disorders
Sudden unexpected death in infancy (SUDI)

ABSTRACT

In Japan, carnitine palmitoyltransferase II (CPTII) deficiency has been included as one of the primary target diseases in the expanded newborn mass screening program since 2018. However, many cases of the severe infantile hepatocardiomuscular form of CPTII deficiency showed severe neurodevelopmental delay or sudden death, which indicated that management of CPTII deficiency in the acute phase remains to be studied in detail. Herein, we discuss two cases diagnosed by newborn mass screening. Patient 1 was under strict clinical management from the neonatal period, with > 20 admissions in 14 months, while Patient 2 was managed using a relatively relaxed approach, with only 2 admissions in the same period. Patient 1 showed normal development; however, Patient 2 expired at the age of 1 year 2 months. To develop strategies for preventing sudden deaths in patients with CPTII deficiency, this retrospective study focused on detailed clinical management practices and biochemical findings during the acute phase. We also investigated the correlation between conventional biomarkers (such as creatine kinase) and long-chain acylcarnitines. We propose that strict monitoring and immediate medical attention, even in case of slight fever or minor abdominal symptoms, can help prevent sudden death in patients with CPTII deficiency. Considering the higher morbidity rate of such patients, strict and acute management of CPTII deficiency cannot be overemphasized.

1. Introduction

Carnitine palmitoyltransferase II (CPTII, EC 2.3.1.21) plays a pivotal role in converting long-chain acylcarnitine (AC) to long-chain acyl-CoA on the mitochondrial inner membrane, where it is used as a substrate for β-oxidation [2]. CPTII deficiency (OMIM 600650) is a fatty acid oxidation disorder, which is clinically classified into three forms: (1) lethal neonatal form, (2) severe infantile hepatocardiomuscular form, (3) mild myopathic form [3]. Cases of CPTII deficiency identified in Japan are etiologically different from those identified in Caucasian patients, with the severe infantile hepatocardiomuscular form being more prevalent in the former group because of a common mutation in the *CPT2* gene [1,4,5]. Reports of multiple cases of infant deaths due to metabolic imbalance associated with CPTII deficiency led Japan to include CPTII deficiency as one of the primary target diseases in the expanded newborn screening (NBS) program in 2018 [1].

Some of the prescribed measures to prevent metabolic decompensation associated with the severe infantile hepatocardiomuscular form of CPTII deficiency include avoidance of long fasting hours and frequent glucose supply [6]. However, investigations using autopsy and postmortem metabolic examination diagnosed more cases of sudden death in infancy associated with CPTII deficiency than with other fatty acid oxidation disorders [7–10]. Patients detected by NBS, even those at the preclinical stage, reportedly died unexpectedly because of infectious diseases [1]. Thus, unresolved issues remain in the management of patients with CPTII deficiency, especially in the acute phase, and more concrete clinical management practices are required.

Because of catabolic situations requiring increased ATP production,

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https://doi.org/10.1016/j.ymgmr.2020.100611
Received 30 March 2020; Received in revised form 21 May 2020; Accepted 22 May 2020

such as high fever and long hours of fasting, the levels of long-chain ACs increase, which may lead to metabolic decompensation. Accumulation of long-chain ACs reportedly induces arrhythmias and hepatic dysfunction [11,12]. Although long-chain ACs could be a reliable biomarker for metabolic status, many hospitals lack the infrastructure to perform immediate AC analysis. In contrast, serum creatine kinase (CK) levels that can be easily measured have been reported to significantly correlate with long-chain ACs in other fatty acid oxidation disorders, and are recommended as laboratory biomarkers in the acute phase of these disorders [13,14].

Herein, we present the clinical course of two patients with CPTII deficiency detected as part of the NBS program in two different hospitals. Patient 1 was under strict clinical management from the neonatal period with frequent admissions, while Patient 2 was managed with a relatively relaxed approach. To develop strategies for preventing sudden death of patients with CPTII deficiency, this study retrospectively focused on detailed clinical management practices and biochemical findings during the acute phase. We also investigated the correlation between biochemical parameters and serum ACs to identify biomarkers with prognostic value for CPTII deficiency.

2. Materials and methods

We enrolled two patients with CPTII deficiency from two different medical facilities. Diagnosis of both patients was confirmed by *CPTII* analysis. The clinical, biological, and genetic background of both patients is presented in Table 1.

The serum acylcarnitine level of Patient 1 was analyzed using tandem mass spectrometry (MS/MS) (API-3000; Applied Biosystems, Foster City, California) at Shimane University, Japan, as previously described [15], while serum AC analysis of Patient 2 was performed using API 4000 LC/MS/MS system (Applied Biosystems, Tokyo, Japan) at Fukui University, Japan, as previously described [16].

Genomic DNA was extracted from peripheral white blood cells. All exons and flanking intron regions comprising the *CPT2* gene were PCR-amplified, and the products were sequenced directly using the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) and ABI PRISM 310 genetic analyzer (Applied Biosystems), as previously described [17].

Moreover, the correlation between long-chain ACs and several laboratory parameters such as serum levels of acetylcarnine (C2), CK, aspartate aminotransferase (AST), 3-hydroxy butyrate (3-OHB), and free fatty acids (FFA) was analyzed for Patient 1 at the time of admission. Informed consent was obtained from the patients' parents, and the study design was approved by the Ethical Committee of each hospital.

Statistical analysis was performed using Spearman's correlation analysis using GraphPad PRISM 8 (GraphPad Software). For all statistical analyses, $P < .05$ was considered as significant.

Table 1
Clinical, biological, and genetic background of Patients 1 and 2.

Parameter	Patient 1	Patient 2
Sex	Female	Male
Birth weight (g)	2810	3242
Gestational age	37 wk. 5 d	39 wk
Delivery	Vacuum extraction	Normal
Perinatal course	No problem	No problem
Enzyme assay	13%	Not tested
CPT2 analysis	p.Phe383Tyr/ p.Phe 383Tyr	p.Phe 383Tyr / p.Arg151Trp
NBS data		
C16 (μM)	4.98 ([a]cutoff, < 3.0)	9.93 ([a]cutoff, < 6.5)
C18:1 (μM)	3.22 (cutoff, < 2.8)	5.25 (cutoff, < 2.8)
C16 + C18:1/C2	3.27 (cutoff, < 0.5)	3.44 (cutoff, < 0.6)

Abbreviations: wk., weeks; d, day.
[a] Different cutoff values of NBS were used because Patients 1 and 2 were born at distant areas, using different analytical facilities.

Table 2
Comparison of clinical course and biological data for Patients 1 and 2 (until 1 y 3 mo).

Parameters	Patient 1	Patient 2
Number of hospitalizations	24 (until 1 y 3 mo)	2
Average hospital stay (range)	3.4 d (2 to 7)	4.0 (2 to 6)
Treatments	From 1 mo L-carnitine (15 mg/kg/day) MCT milk From 1 y 10 mo Uncooked cornstarch	From 2 mo L-carnitine (10 mg/kg/day) From 1 y MCT milk
Hospitalization criteria	Elevation of serum CK levels Diarrhea and/or vomiting (including mild symptoms) High fever (even in low-grade)	Diarrhea and/or vomiting High fever
Laboratory findings (at admission day)	Average (range)	Median (range)
BS (mM)	5.66 (5.17 to 6.56)	5.8 (3.0, 8.6)
AST (U/L)	54 (42 to 88)	40 (37, 42)
CK (U/L)	357 (213 to 1879)	112 (102,121)
LDH (U/L)	358 (312 to 465)	328 (309, 355)
Lactate (mg/dL)	15 (9 to 26)	–
3-OHB (μM)	390 (29 to 706)	131 (20, 241)
FFA (μEq/L)	1070 (520 to 2480)	2383 (1363, 3372)
FFA/TKB ratio	3.03 (1.36 to 7.86)	21.0 (9.39, 32.6)
Outcome	Normal development (until 5 y of age)	Death (at the age of 1 y 3 mo)

Abbreviations: mo, month; y, year; d, day; BS, blood sugar; AST, aspartate aminotransferase; CK, creatine kinase; LDH, lactate dehydrogenase; 3-OHB, 3-hydroxy butyrate; FFA, free fatty acids; TKB, total ketone body; MCT, medium-chain triglyceride; n/a, not available. The average values of BS, AST, CK, LDH, and lactate in Patient 1 were calculated by 24-reading tests, while those of 3-OHB, FFA, and FFA/TKB ratio were calculated by 8- and 11-, 9- reading tests, respectively. BS, AST, CK, LDH, 3-OHB, and FFA levels in Patient 2 were tested two times.

3. Results

Clinical course, biological findings, and clinical management plans for both Patients 1 and 2 are shown in Table 2.

3.1. Case presentation

Patient 1 was born to non-consanguineous parents at 37 weeks and 5 days of gestation via vacuum extraction and weighed 2.81 kg. As the family history, her younger brother was also diagnosed with CPTII deficiency and previously reported [15]. Elevated levels of palmitoylcarnitine (C16) (4.98 μM; cutoff, < 3.0 μM), oleylcarnitine (C18:1) (3.22 μM; cutoff, < 2.8 μM), and (C16 + C18:1)/C2 ratio (3,27; cutoff, < 0.5) were observed during NBS. Serum AC analysis showed elevated levels of C16 (2.44 μM; cutoff, < 0.3 μM), C18:1 (3.57 μM; cutoff, < 0.4 μM), and (C16 + C18:1)/C2 (0.63; cutoff, < 0.36). Genetic evaluation revealed a homozygous mutation in the *CPT2* gene (c.1148T > A, p.Phe383Tyr), and CPTII enzyme activity was at only 13% of the control, confirming CPTII deficiency. Each of her parents was heterozygous for p.Phe383Tyr. The attending physicians emphasized the importance of frequent feeding to her parents. She was treated with L-carnitine and medium-chain triglyceride (MCT) milk since a month after birth and was administered uncooked corn starch from 22 months. In addition, early interventions such as glucose infusion and hospitalization were indicated even during low-grade fever or mild gastrointestinal symptoms such as a single episode of vomiting and/or soft stools, irrespective of specific biochemical findings. Under the strict clinical management plan as outlined above, she was hospitalized 24 times during 15 months. The average duration of hospitalization was

3.4 days. Now at 5 years of age, she has achieved normal physical growth and mental development.

Patient 2 was 15 months old at the time of death. No family history of other neonatal deaths and/or congenital metabolic diseases was noted. He was born to non-consanguineous parents at 39 weeks of gestation via vaginal delivery and weighed 3.24 kg with stable perinatal clinical course. Increase in C16 (9.93 μM; cutoff, < 6.5 μM), C18:1 (5.25 μM; cutoff, < 2.8 μM), and (C16 + C18:1)/C2 ratio (3.44; cutoff, < 0.62) was detected in dried blood filter paper (DBS) collected at day 4 after birth. At 12 days after birth, serum AC analysis also showed an increase in C16 (3.18 μM; cutoff, < 0.13 μM), C18:1 (2.31 μM; cutoff, < 0.16 μM), and (C16 + C18:1)/C2 ratio (1.307; cutoff, < 0.09). In addition, genetic analysis identified compound heterozygosity for c.451C > T(p.Arg151Trp) and c.1148T > A (p.Phe383Tyr) in *CPT2*. Genetic analysis of his parents showed p.Arg151Trp and p.Phe383Tyr in his father and his mother, respectively. Eventually, he was diagnosed with CPTII deficiency. He was administered low-dose L-carnitine (about 10 mg/kg/day) from 2 months of age. His parents were informed of the need for early intervention with glucose infusion at an episode of pyrexia or vomiting. At 1 year of age, he was hospitalized with severe vomiting and diarrhea with hypoketotic hypoglycemia (BS, 3 mM; 3-OHB, 359 μM) due to norovirus enteritis. Glucose infusion and oral L-carnitine administration improved his condition in a few days. Subsequently, MCT milk was introduced in his diet. At 1 year 3 months, he was hospitalized with hyperpyrexia and upper respiratory symptoms, and underwent intravenous hydration, including glucose infusion. No remarkable abnormality was noted in the levels of AST, CK, and LDH at the time of admission (Table 2), and he was discharged with normal general appearance and adequate oral intake on Day 2 of admission. However, a day later, he suffered acute cardiopulmonary arrest, and died despite intensive therapy. When he was admitted for the cardiopulmonary arrest, AC analysis was not performed due to insufficient sample volume. His parents declined pathological autopsy.

3.2. Correlation between long-chain ACs and laboratory data of Patient 1

Fig. 1 indicates the correlation between long-chain ACs and other biochemical findings in Patient 1 on the day of admission. We observed significant correlation between C18:1 and C16 ($r = 0.758, P < .0001$), although no correlations were noted between C18:1 and the levels of C2, CK, AST, 3-OHB, and FFA ($r = 0.03, -0.12, 0.035, -0.209$, and 0.419 respectively). When she was hospitalized at the age of 11 months, because of upper respiratory tract-related symptoms, significant elevations were observed in C16 and C18:1 (14.9 and 23.4 μM, respectively), but not in CK.

4. Discussion

Persisting issues in the management of patients with CPTII deficiency, especially during the acute phase, emphasizes the need to optimize clinical management practices. This study demonstrated the clinical course, status of biological parameters such as serum ACs, and clinical management of two patients with CPTII deficiency detected as part of the NBS program.

Further, we detected two types of variants (p.Arg151Trp and p.Phe383Tyr). Patients homozygous for the variant p.Arg151Trp reportedly develop the lethal neonatal form of CPT2 deficiency [18], while those homozygous for the mutation p.Phe383Tyr develop the severe infantile hepatocardiomuscular form [15,17]. However, since each of the patients had at least one allele carrying p.Phe383Tyr, it can be considered that both Patients 1 and 2 had the severe infantile hepatocardiomuscular form [17] wherein it has been reported that prompt treatment could probably prevent the sudden death and the developmental problems secondary to recurrent metabolic decompensation [19]. Nevertheless, different prognoses were obtained for the two patients not from genetic background, but from clinical management after confirming the diagnosis and avoiding the catabolic situations. In fact, Patient 1 was under strict clinical management that included glucose infusion and hospitalization. On the other hand, Patient 2 was hospitalized only twice, the first time being attributed to

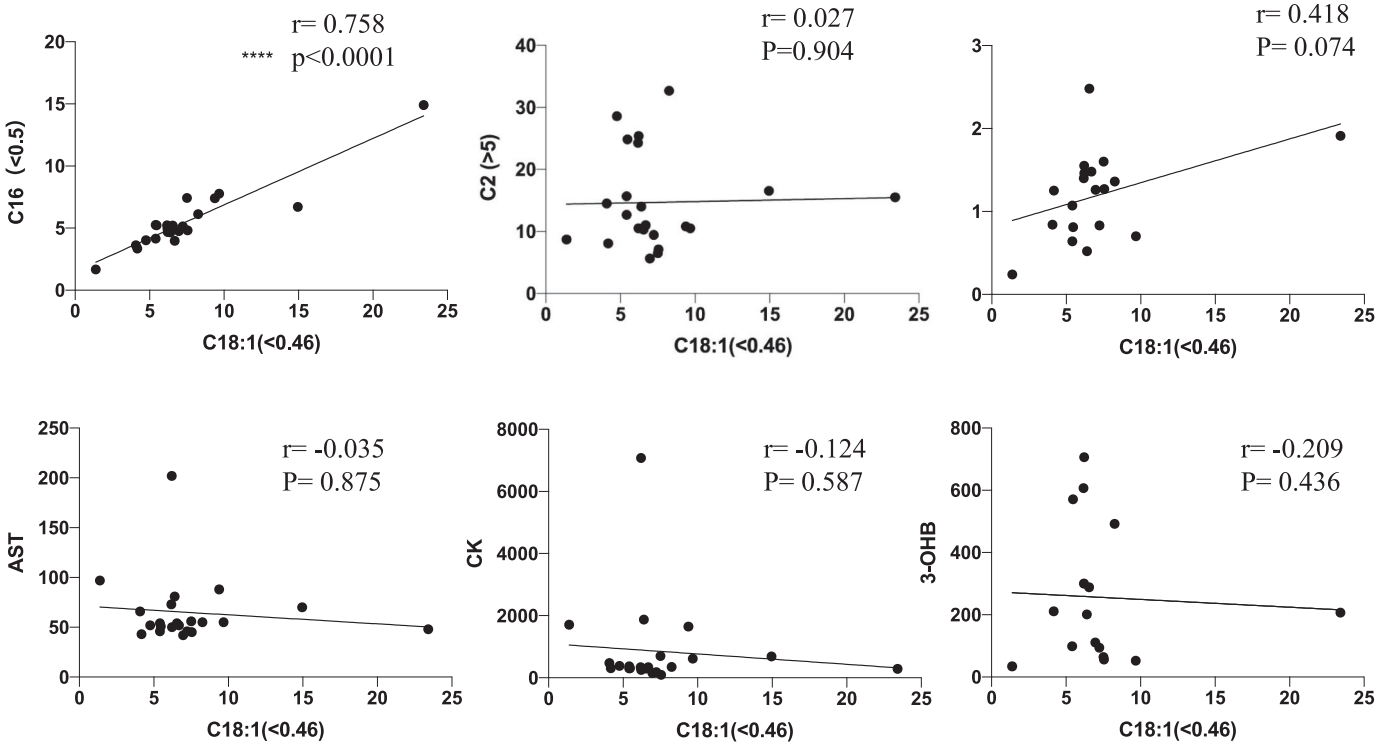

Fig. 1. Correlation between plasma ACs and laboratory findings in Patient 1 Linear regression analysis was performed using the indicated parameters.

hypoketotic hypoglycemia due to delayed therapeutic intervention. Moreover, during the next round of hospitalization, he was discharged on Day 2 despite the risk of metabolic decompensation. These differences indicate the importance of strict clinical management during the acute phase and suggest that attending physicians should repeatedly emphasize the importance of glucose supply and close monitoring to the parents of such patients.

In addition, Patient 2 had an almost normal general appearance, and enough oral intake was assured at the time of discharge. However, he suddenly died the next day, suggesting that general appearance might not be a reliable indicator for the degree of metabolic decompensation. In fact, Table 2 showed almost normal findings in the acute phase. In addition, data for Patient 1 collected on the day of admission did not indicate any correlation between the levels of long-chain ACs and CK. Similarly, other biomarkers (C2, AST, 3HBA, and FFA) did not show any correlation with ACs. CK is often considered as the most suitable biomarker [14]; however, it might be difficult to predict disease progression on the basis of these conventional biochemical findings. Our results show that, currently, no biomarker can completely reflect the degree of catabolic conditions except AC profiles.

This report has two limitations. One is the small number of targeted patients with CPTII deficiency, and therefore, additional studies with a larger population are needed. In addition, when Patient 2 was admitted for cardiopulmonary arrest, we could not take enough samples to analyze biological data precisely.

Management of CPTII deficiency entails several difficulties because the clinical findings, including immediate laboratory results, do not indicate the metabolic condition of patients. However, based on our findings, we propose that strict monitoring and immediate medical attention, even in case of slight fever or minor abdominal symptoms, can help prevent sudden death in patients with CPTII deficiency.

5. Conclusion

CPTII deficiency can cause sudden death or severe metabolic decompensation in some cases of minor infection or abdominal symptoms. Clinical decisions based on apparent general conditions and laboratory data can underestimate the metabolic status of the patient. Considering the higher morbidity rate of such patients, strict and acute management of CPTII deficiency cannot be overemphasized.

Acknowledgments

We thank Ms. M. Furui, K. Konada, H. Kajitani, and T. Esumi (Department of Pediatrics, Shimane University) for their technical assistance. We also thank Dr. Go Tajima (Division of Neonatal Screening, Research Institute, National Center for Child Health and Development) for assisting with CPTII enzyme assay. This research was partially supported by Grant Number 15K09593 (Chief Investigator: Seiji Yamaguchi) and 19K17298 (Chief Investigator: Ryosuke Bo) from the Ministry of Health, Labor and Welfare of Japan.

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