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Case Report

A newborn case with carnitine palmitoyltransferase II deficiency initially judged as unaffected by acylcarnitine analysis soon after birth

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ABSTRACT

Carnitine palmitoyltransferase II (CPT-2) deficiency, an autosomal recessive disorder of fatty acid oxidation, can be detected by newborn screening using tandem mass spectrometry (TMS).

Our case was a boy born at 38 weeks and 6 days of gestation via normal vaginal delivery; his elder sister was affected with CPT-2 deficiency. Acylcarnitine (AC) was analyzed in both dried blood spots (DBS) and serum 2 h after birth to determine whether the boy was also affected. His C16 and C18:1 AC levels in DBS were in the normal range, while his serum long-chain AC levels were marginally increased but lower than those of his sister. After the samples were taken, he was treated with glucose infusion to prevent any catabolism for 2 days. On day 4, the long-chain AC levels in both DBS and serum obtained were higher than those on day 0 and were equivalent to those of his sister. Genetic testing confirmed the presence of the same mutation found in his sister, a homozygous F383Y mutation in the *CPT2* gene, thus leading to the diagnosis of CPT-2 deficiency.

The sample for TMS should be taken between days 1 and 7. If the sample is not obtained at an appropriate time, correct diagnosis may not be made, as in our case. Although early diagnosis is required, samples taken within 24 h after birth should not be used for TMS.

1. Introduction

The carnitine palmitoyltransferase II (CPT-2) enzyme is located in the inner mitochondrial membrane and is associated with β-oxidation of long-chain fatty acids [1,2]. CPT-2 converts long-chain acylcarnitine (LCAC), which is transferred from long-chain acyl-CoA by CPT-1, back to long-chain acyl-CoA [3]. CPT-2 deficiency is an autosomal recessive disease and is clinically classified into three types, namely, the lethal neonatal, infantile, and adult forms [4,5]. Patients with the neonatal form of CPT-2 deficiency develop liver failure with hypoketotic hypoglycemia, cardiomyopathy, facial abnormalities or other structural malformations, and seizures soon after birth, often leading to fatality in early infancy. The infantile form is characterized by intermittent attacks of hypoketotic hypoglycemia, liver failure, or occasional sudden unexpected death triggered by infection, diarrhea, or long fasting. In the adult form, patients mainly exhibit recurrent episodes of myalgia, myoglobinuria, or rhabdomyolysis. Moreover, because of the increased prevalence of newborn screening (NBS) using tandem mass spectrometry (TMS), an asymptomatic patient with CPT-2 deficiency was also reported [6]. However, affected babies are occasionally missed because of normal acylcarnitine (AC) profiles in dried blood spots (DBS) obtained from patients in stable condition [7,8].

Here, we report a Japanese newborn case with CPT-2 deficiency that was initially judged as “unaffected” based on the AC profiles in both DBS and serum that were collected immediately after birth on day 0. Informed consent was obtained from the patient's parents in this case.

2. Case report

The patient was a boy born at 38 weeks and 6 days of gestation via normal vaginal delivery. His birth weight was 3402 g, and his Apgar scores were 8 and 9 at 1 and 5 min, respectively. His older sister was affected with CPT-2 deficiency that had been detected by NBS. Genetic testing on his sister revealed a homozygous F383Y mutation in the *CPT2* gene, and the parents were both heterozygous for the F383Y substitution but were not consanguineous. This mutation has been

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previously reported in Japanese cases with a milder form [5,9,10]. The patient's sister had been frequently hospitalized due to elevated creatine kinase (CK) levels caused by fever or vomiting since her early infancy to prevent severe attacks. Her CK level was sometimes increased to several thousands, but myopathic symptoms such as muscular pain or weakness had never been observed. The parents did not elect prenatal diagnosis but requested the diagnosis for CPT-2 deficiency of the present case at the earliest opportunity after birth.

At 2 h after birth, the blood sample was collected. Then, the patient was given a glucose infusion, although a routine laboratory examination was normal except for an elevated CK level (aspartate aminotransferase 38 IU/L (reference value used for adult patients in Shimane University Hospital 10–38), lactic dehydrogenase 380 IU/L (reference 100–215), CK 782 IU/L (reference 36–216), and blood glucose 64 mg/dL (reference 60–109)). This treatment had been scheduled prior to his birth, irrespective of his condition, to prevent any catabolism. Although transient tachypnea was observed after birth, the symptoms rapidly disappeared. The infusion was discontinued on day 2 because of sufficient breastfeeding.

The AC levels in the DBS and serum samples that were obtained on day 0 were analyzed to determine whether he was affected with CPT-2 deficiency. The AC profiles of the DBS from day 0 showed that the levels of C0 (15.24 μM; cutoff, > 10 μM), C16 (2.80 μM; cutoff, < 3.0 μM), and C18:1 (1.4 μM; cutoff, < 2.8 μM) were in a normal range, whereas the (C16 + C18:1)/C2 ratio was elevated to 1.39 (cutoff, < 0.5) (Table 1). Although the levels of C16 and C18:1 and the (C16 + C18:1)/C2 ratio in the serum were elevated to 1.45 μM, 1.01 μM, and 0.65, respectively (cutoffs, < 0.3 μM, < 0.3 μM, and < 0.36, respectively), we suspected that he was only a carrier of CPT-2 deficiency because these elevated serum AC levels were lower than those of his sister (Table 1).

On day 4, the AC profiles in DBS and serum were re-evaluated. The C16 and C18:1 levels and the (C16 + C18)/C2 ratio were elevated in both DBS and serum relative to their corresponding values on day 0, while the level of C0 was decreased despite sufficient breastfeeding (Table 1). The day 4 profiles were comparable to those observed in his sister, strongly suggesting that the boy was affected with CPT-2 deficiency. Genetic testing revealed the homozygous F383Y mutation in the *CPT2* gene, thus confirming his diagnosis of CPT2 deficiency. He has not yet experienced metabolic attacks as of age 4 months.

3. Discussion

Our case suggests that the AC profiles of DBS collected soon after birth can lead to misdiagnosis of CPT-2 deficiency. A general recommendation is to collect the sample for newborn screening using TMS between days 1 and 7. Because catabolism is considered to be maximum on day 2 to 3 [11], these days are the most sensitive time to detect an increase in ACs for minimizing the probability of a false negative. Therefore, the first acylcarnitine profile should have been taken > 24 h after birth even though the guardian requested diagnosis as early as possible. Nevertheless, sampling for TMS should not be delayed because previous report showed the blood collected later than day 4 of life could fail to reflect very long-chain acyl-CoA dehydrogenase deficiency [12].

Although the first sample taken on day 0 was normal, the second sample taken on day 4 showed obvious abnormality in our case. These results suggest that correct diagnosis can be reached if sampling is done at an appropriate time. Meanwhile, the possibility of false negative due to anabolism resulting from glucose infusion or sufficient feeding should also be considered [13,14]. In our case, the LCAC level was abnormally increased on day 4 in spite of good feeding and glucose infusion until day 2. If glucose infusion had been continued until blood sampling, the results may have shown as negative even though the time of blood collection was appropriate. Additionally, Hori et al. [15] reported that LCAC levels increased over time in a case of CPT-2 deficiency that was supplemented with L-carnitine from day 0. It is important to keep in mind that several factors, such as sampling time and some treatments, can interfere with the results of AC profile. This holds true in analyzing serum that is generally more reliable than DBS for the diagnosis of long-chain fatty acid oxidation disorders [16].

Genetic testing or measurement of enzyme activity is usually used to make a definite diagnosis of CPT-2 deficiency [4]. In this regard, genetic testing should have been examined first in our case. However, we chose TMS to obtain a preliminary diagnosis because genetic testing usually takes a few days, whereas AC analysis takes a few hours in our laboratory. In hindsight, this choice led to misdiagnosis. Although TMS is very useful in screening for several inherited metabolic diseases, our case suggests that it should not be used for the diagnosis. Moreover, because glucose infusion that can normalize AC profile is often administered after birth to those suspected inherited metabolic disease, gene analysis may be the best strategy for accurate and early diagnosis if the mutation is known, as in our case.

4. Conclusion

Our case suggests the following two points. 1) If the blood sample for NBS using TMS is collected too early, the correct diagnosis may be missed, but 2) if the sample is obtained at an appropriate time, a reliable result can be reached. AC profile is influenced not only by sampling time but also by anabolism such as glucose infusion.

Table 1
The results of acylcarnitine in dried blood spots and serum on day 0 and 4, and those in the sister on day 22.

	Dried blood spots (μM)				Serum (μM)			
	Day 0	Day 4	(Reference)[a]	Sister	Day 0	Day 4	(Reference)[b]	Sister
C0	15.24	9.05	(10–60)	6.68	25.59	16.68	(25–100)	12.39
C2	3.02	3.09	(5–45)	7.61	3.78	3.88	(4–60)	9.61
C4	0.08	0.05	(< 1.4)	0.042	0.12	0.09	(< 1.0)	0.12
C8	0.03	0.04	(< 0.3)	0.069	0.14	0.11	(< 0.3)	0.15
C10	0.11	0.18	(< 0.25)	0.18	0.28	0.41	(< 0.3)	0.64
C12	0.35	0.78	(< 0.4)	0.6	0.40	1.37	(< 0.2)	0.91
C14	0.43	0.69	(< 0.4)	0.37	0.45	1.07	(< 0.2)	0.53
C14:1	0.17	0.18	(< 0.3)	0.15	0.29	0.29	(< 0.2)	0.24
C16	2.80	4.78	(< 3.0)	2.29	1.45	4.35	(< 0.3)	2.44
C18	1.39	1.74	(< 2.0)	1.25	0.43	1.02	(< 0.3)	0.97
C18:1	1.40	2.75	(< 2.8)	2.06	1.01	3.37	(< 0.4)	3.57
(C16 + C18:1)/C2	1.39	2.43	(< 0.5)	0.57	0.65	1.99	(< 0.36)	0.63

The samples of the elder sister were obtained on day 22 of her age.
Underlined values are abnormal values.
[a] Control values for neonatal mass screening at Shimane University were used as a reference.
[b] Control values for infantile cases with high risk of fatty acid oxidation disorders at Shimane University were used as a reference.

Therefore, even though early diagnosis is required, TMS should not be used as a diagnostic tool.

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