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Sudden infant death from neonate carnitine palmitoyl transferase II deficiency

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Highlights

- We present the case of CPT2 deficiency, extremely rare in forensic practice.
- HE and Sudan III staining showed that significant steatosis of important organs.
- TMS analysis and genetic testing were performed to estimate the CPT2 deficiency
- A parental genetic testing can be helpful in the diagnosis of this disease.

Abstract

A full-term female baby born to parents who gave birth three years prior to a girl who survived only 31 hours postpartum died 36 hours after birth. An autopsy showed that the heart was markedly hypertrophic (32 g). Microscopically, the myocardium, liver and kidney cells exhibited extensive vacuolar degeneration. Sudan III staining was positive in cardiac muscle, liver and kidney tissue. Tandem mass spectrometry analysis revealed that the deceased patient had a carnitine palmitoyl transferase II (CPT2) deficiency or a carnitine-acylcarnitine translocase deficiency. Genetic testing of the parents revealed heterozygous CPT2 mutations, indicating that their offspring would have a 25% chance of having a CPT2 deficiency. Therefore, we speculated that CPT2 deficiency might be the cause of death based on the results of staining, tandem mass spectrometry analysis and parental genetic testing.

Keywords: Carnitine palmitoyl transferase Ⅱ deficiency; Sudden death in infancy; Cell steatosis; Genetic test

Introduction

Carnitine palmitoyl transferase II (CPT2) deficiency is a fatty acid oxidation deficiency (FAD) caused by lack of CPT2, which is used in fatty acid beta oxidation. Fatty acids provide energy for cells and participate in a series of biochemical reactions through beta oxidation. The fatty acid beta oxidation process includes a series of enzymes, and any defects in these enzymes can lead to fatty acid oxidation metabolic disorders. Long chain fatty acids are a major constituent of the fatty acids. They are normally transported into the mitochondrial matrix for beta oxidation by a system that relies on carnitine [1]. The carnitine-dependent transportation system

consists of three components: CPT1, CPT2 and carnitine acylcarnitine translocase. Without CPT2, fatty acylcarnitine cannot be converted to the corresponding fatty acyl coenzyme A, leading to accumulation of long-chain acylcarnitine in the mitochondria. Microscopically, this phenomenon can be observed as tissue steatosis [1-3].

CPT2 deficiency is a disease with autosomal recessive inheritance [4-6]. Depending on the age of onset and clinical symptoms, CPT2 deficiency can be divided into adult, child, infant and neonatal forms [6]. The most serious form is the neonatal type, for which clinical manifestations include coma, convulsions, hypoketotic hypoglycaemia, cardiomegaly and multiple organ abnormalities. CPT2-deficient infants often die days after birth, and most also exhibit anomalous cardiovascular development [2].

The pathologic changes associated with CPT2 deficiency have been widely reported. However, the combined discussion of autopsy findings and genetic test results of a CPT2-deficient patient is rare. Here, we present a case of sudden infant death due to suspected CPT2 deficiency, with a diagnosis aided by parental genetic testing.

Case report

A female baby born at 39^{+3} weeks weighed 3.1 kg and had an Apgar score of 10 at birth. When the nurse fed the infant a moderate amount of milk for the first time postpartum, she started crying angrily. During the first 12 to 15 hours after birth, the infant continued crying, seemed drowsy, and consumed small amounts of milk. She continued crying and stopped feeding until 36 hours after birth. Suddenly, her face turned white and then black. Despite emergency rescue efforts, she died at 36 hours postpartum. The doctor suspected that she had asphyxiated due to milk ingestion.

An inquiry into the family history revealed that her mother had undergone two abortions. In addition, the older sibling died of similar circumstances at 31 hours postpartum three years previously. The doctors considered the death to be due to acute

suffocation caused by milk, and no autopsy was performed. The family was concerned due to the occurrence of a similar previous tragedy. To clarify the cause of death, an autopsy was performed at the request of the family.

Autopsy findings

An autopsy was performed five days after death. The findings showed a well-developed infant, 53 cm in height and 3.1 kg in weight. On internal examination, the heart was markedly hypertrophic (32 g), and the right atrial epicardium exhibited scattered punctate bleeding. No ventricular septal defect was observed. The foramen ovale was not closed and measured 0.8×0.3 cm. The left ventricular wall thickness was 0.55 cm, and the right ventricular wall measured 0.35 cm. Each valve was intact, and the coronary sinus was normal. The left and right coronary artery and its branches appeared to have no abnormalities. The lungs weighed 160 g, and both exhibited congestion and oedema macroscopically. Small amounts of oedema fluid and milk components were visible in the bronchial lumen.

No significant pathological changes were observed in other organs except for congestion and oedema.

Histopathology

1. Hematoxylin-Eosin staining (H.E. staining)

The myocardial interstitium showed mild broadening, and interstitial congestion was visible in the small blood vessels. The myocardium also exhibited extensive vacuolar degeneration [Fig. 1a]. The bilateral coronary arteries and their branches displayed no abnormalities microscopically. The conduction system also showed no abnormalities.

Most of the alveolar cavity was clear with local atelectasis. Some alveolar cavities showed mononuclear cell infiltration. Additionally, several foreign bodies could be observed, and a small number of free bronchial epithelial cells were evident.

The liver cells exhibited diffuse vacuolar degeneration [Fig. 1b].

The renal cortex and medullary structures were clear. Extensive vacuolar degeneration was evident within the renal tubular epithelial cells [Fig. 1c].

2. Sudan III staining (fat staining)

Sudan III staining was performed on heart, liver and kidney tissues. These tissues all exhibited positive results, indicating diffuse steatosis [Fig. 1d-f].

Tandem mass spectrometry analysis (TMS analysis)

The results of TMS analysis of the patient's cardiac blood are shown in the attached table. The levels of Tyr, C12, C14, C16, C18, C20, C5/C3, C16:1-OH, C18:1, C18:1-OH and (C16+C18)/C0 in the plasma were elevated. These changes suggested either CPT2 deficiency or carnitine acylcarnitine transferase deficiency (CACTD). A relevant enzyme activity test or genetic tests were recommended to confirm the diagnosis [Table 1].

Genetic testing

We performed genetic tests of the cardiac blood and skin tissue. Unfortunately, no results were obtained due to putrefaction. We then suggested that the parents undergo genetic testing. The results showed that both parents were CPT2 gene mutation carriers. Sanger sequencing revealed two mutations in the paternal CPT2 gene: in Exon 4, c.1102G>A (p.V368I) and in Exon 5, c.1939A>G (p.M647V). Additionally, two mutations were identified in the maternal CPT2 gene: in Exon 4, c.1055T>G (p.F352C) and c.1102G>A (p.V368I) [Table 2].

Discussion

This case illustrates the diagnostic challenges of neonatal CPT2 deficiency. Due to the absence of characteristic clinical symptoms or specific findings on biochemical

examinations, prenatal diagnosis is difficult for paediatricians. Thus, an autopsy is very important for diagnosis.

However, forensic experts have a limited understanding of CPT2 deficiency. In general, during a systemic autopsy, only abnormal development of the cardiovascular system is observed, otherwise no obvious pathologic changes are evident. If cardiovascular system dysplasia is noted, a genetic disease such as CPT2 deficiency is likely to be misdiagnosed as congenital heart dysplasia. In contrast, histopathology is more relevant for the diagnosis of this disease. Microscopically, significant steatosis of important organs such as the heart, liver and kidneys can be observed [7]. It is suggested that in these cases, forensic experts should be highly suspicious of abnormal fat metabolism diseases. Using the autopsy results combined with genetic metabolic enzyme testing, forensic experts can establish a preliminary diagnosis of CPT2 deficiency [8].

In this case, through anatomical and histopathological examination, we observed the significant cardiac findings of a patent foramen ovale and patent ductus arteriosus as well as multiple organ steatosis. Patent foramen ovale and patent ductus arteriosus are often present during the neonatal period. Therefore, they are not typically considered dysplastic features responsible for death. However, sufficient attention should be focused on the significant steatosis of multiple organs, and genetic metabolic enzyme testing should actively be performed. A parental genetic metabolic examination can be helpful in the diagnosis of this disease. In this case, the parents had heterozygous CPT2 gene mutations, giving the child a one-quarter likelihood of having a CPT2 deficiency. Based on this result and the pathologic changes, a diagnosis of CPT2 deficiency could be established. Genetic metabolic testing of the parents not only helped to diagnose the cause of death but also had important guiding significance for

the next pregnancy.

The adult myopathic form of CPT2 deficiency is relatively benign and difficult to diagnose [9, 10]. Among the different clinical subtypes of CPT2 deficiency, the adult-onset myopathic form shows mild clinical manifestations characterized by recurrent rhabdomyolysis or myoglobinuria [11, 12]. A previous study reported characteristic profiles of different types of CPT2 deficiency [13]. In this case, sequencing of the CPT2 gene revealed a novel missense mutation, c.1939A>G (p.M647V), which is reported here for the first time.

In summary, diagnosis of CPT2 deficiency as a cause of death requires a systemic anatomical and histopathological examination as well as genetic metabolic enzyme and chromosome examinations. In some circumstances, it also may be necessary to perform genetic testing of the parents of the deceased.

Conflict of interest

The authors declare that they have no conflicts of interest.

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Figure Legends

Figure 1: H.E. staining of the heart (a), liver (b) and kidney (c) (magnification, 40×), extensive vacuolar degeneration was evident within the tissues. Sudan III dyeing (fat dyeing) of the heart (d), liver (e) and kidney (f) (magnification, 20×), these tissues all exhibited positive results, indicating diffuse steatosis.

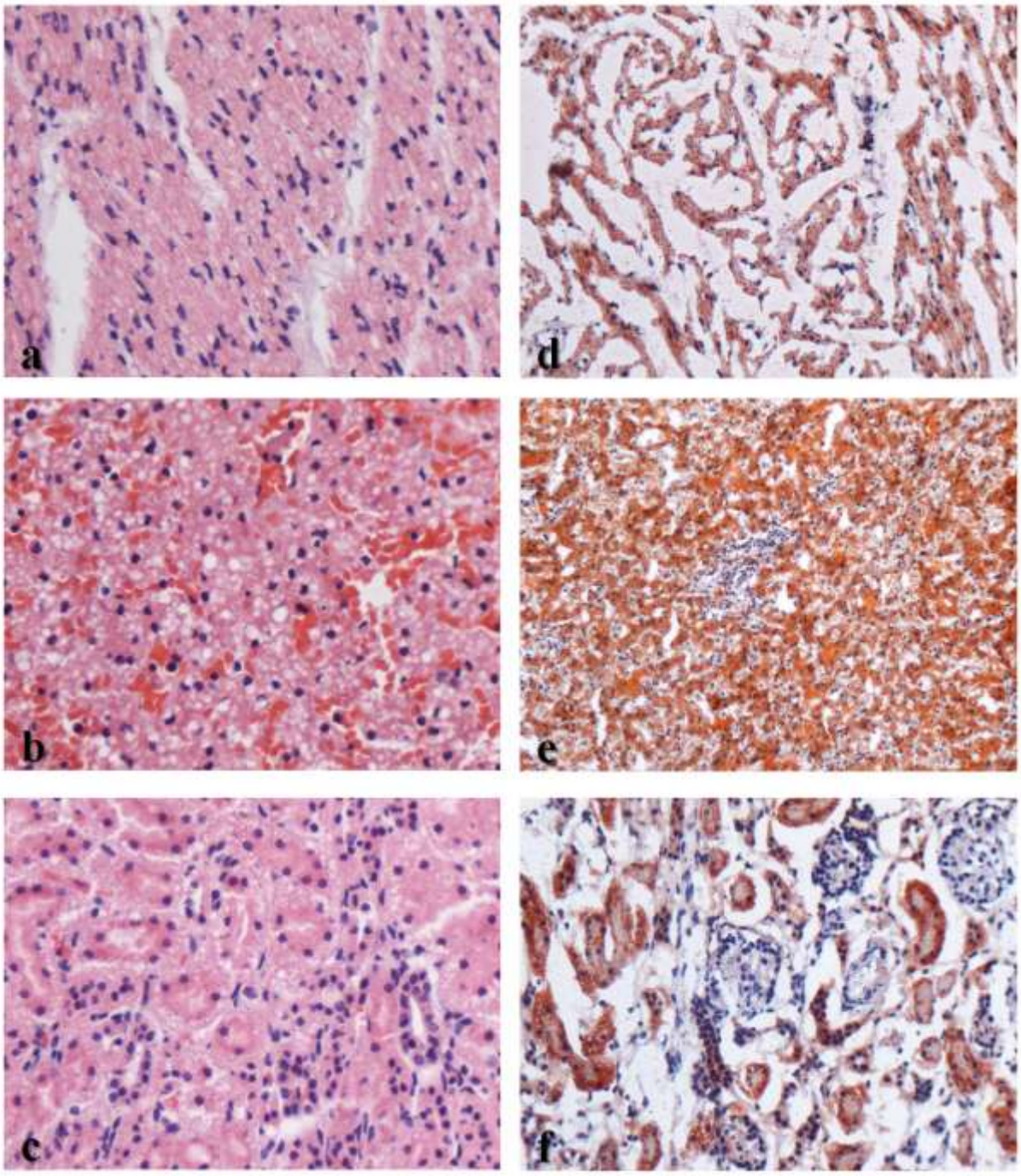

Table Legends

Table 1. Results of tandem mass spectrometry analysis

Tyr, C12, C14, C16, C18, C20, C5/C3, C16:1-OH, C18:1, C18:1-OH, (C16+C18)/C0 plasma levels elevated, respectively.

Detection Indicator	**Reference range**	**Results**	
Tyr	20-200	230.09	↑
C12	0.02-0.4	0.48	↑
C14	0.05-0.6	1.07	↑
C16	0.5-5.0	13.16	↑
C16:1-OH	0.02-0.2	0.28	↑
C18	0.25-1.85	2.73	↑
C20	0.01-0.2	0.23	↑
C5/C3	0.01-0.4	0.67	↑
C18:1	0.2-2.5	3.6	↑
C18:1-OH	0.02-0.13	0.14	↑
(C16+C18)/C0	0.03-0.24	0.88	↑

Table 2. Genetic test results of the parents

Both of the parents are CPT2 gene mutation carriers.

	Gene	**Location**	**Mutation**
The mother	GenBank : NC_000001.10 Gene ID: 1376	Exon 4	c.1055T>G (p.F352C)
		Exon 4	c.1102G>A (p.V368I)
The father	GenBank : NC_000001.10 Gene ID: 1376	Exon 4	c.1102G>A (p.V368I)
		Exon 5	c.1939A>G (p.M647V)