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Case Report

Combination of postmortem mass spectrometry imaging and genetic analysis reveals very long-chain acyl-CoA dehydrogenase deficiency in a case of infant death with liver steatosis☆,☆☆,☆☆☆

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ABSTRACT

Case history: A 3-month-old infant was found dead in his bed. A postmortem computed tomography (CT) scan suggested fatty attenuation in the liver parenchyma, but no other potentially fatal changes were found. To clarify the cause of death, a medicolegal autopsy was carried out.
Autopsy findings: Internal examination confirmed the presence of liver steatosis as well as hepatomegaly. There were no other significant findings including encephalitis or brain edema.
Mass spectrometry analysis: To clarify the mechanism underlying lipid accumulation in the liver, matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI–IMS) analysis was conducted. This indicated a significant accumulation of C14:1 acylcarnitine in the liver of the deceased, suggesting very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency.
Genetic analysis: To find the cause of the VLCAD deficiency, genetic analysis of the responsible gene, *acyl-CoA dehydrogenase, very long chain* (*ACADVL*), was performed. This revealed two novel mutations that may have accounted for the disease.
Conclusion: A combination of these data revealed that the liver steatosis in this case might have been caused by VLCAD deficiency based on genetic mutations of *ACADVL*. Thus, the deceased might have been vulnerable to energy crisis and sudden infant death. The present findings show that MALDI–IMS analysis as well as genetic analysis can be useful for elucidating the cause of death.

1. Introduction

A significant proportion of sudden and unexpected infant deaths have been reported to be associated with fatty acid oxidation (FAO) deficiency [1–5]. Among the enzymes and cofactors involved in FAO, very long-chain acyl-CoA dehydrogenase (VLCAD) is a dimeric molecule comprising two 70-kDa subunits, loosely bound to the mitochondrial inner membrane, and catalyzes the major part of mitochondrial palmitoylcoenzyme A dehydrogenation [6]. Mutation of the enzyme causes VLCAD deficiency, which is identified as an increased level of C14:1 acylcarnitine in the blood, and can lead to several symptoms such as hypoglycemia, acidosis, cardiomyopathy, and hepatic dysfunction as well as liver steatosis [7–9]. On the other hand, liver steatosis can also be caused by other diseases such as alcoholic liver disease, type 1 diabetes mellitus, infection, or drugs and toxins (including Reye's syndrome) [8]. Thus, in order to understand the pathogenesis of liver steatosis, it is important to detect the type of lipid that accumulates in the steatotic liver cells.

☆ Genetic analysis was conducted after obtaining informed consent, and all procedures of this study were approved by the Ethics Committees at the Gunma University Graduate School of Medicine.
☆☆ Postmortem computed tomography (CT) was performed at the Autopsy Imaging Facility, Gunma University, Graduate School of Medicine.
☆☆☆ The nucleotide sequences reported in this article have been submitted to the GenBank with accession Numbers KJ909280 and KJ909281.

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A recently developed modality, matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI–IMS), is able to facilitate the acquisition of comprehensive mass spectra directly from tissue specimens and can provide reconstructed density maps of detected ions [10]. This new modality may be applied to liver sections to analyze lipid accumulation in a steatotic liver and may assist forensic pathologists to identify the cause of liver steatosis as well as the cause of death.

Here, we report a case of sudden infant death accompanied by liver steatosis, where a combination of MALDI–IMS and genetic analysis was useful for demonstrating that VLCAD deficiency might have been the cause.

2. Case history

The case subject was a 3-month-old Japanese infant who had been born with a low birth weight of 2212 g. He had no particular history of illness, and his older 2-year-old brother also had no significant medical issues. On the day before his death, the deceased had been ill-tempered, continued crying, and sometimes vomited, although it was unclear whether or not a high fever had been present. On the following morning, he had been found in a state of respiratory arrest in his bed 3 h after being placed there by his mother. During and after attempted but unsuccessful resuscitation, no laboratory tests had been performed. A postmortem computed tomography (CT) scan indicated fatty attenuation in the liver parenchyma (Fig. 1), but no other potentially fatal changes, including brain edema, were found. To clarify the cause of death, a medicolegal autopsy was carried out 1 day after death.

3. Autopsy findings

The body measured 56 cm and weighed 4.8 kg. External examination revealed no significant changes other than traces caused by medical treatment and attempted resuscitation. Internal examination confirmed hepatomegaly, steatotic liver (Fig. 2A), and congestion of the organs. The liver weighed 360 g. Histologically, the liver showed diffuse severe steatosis with a microvesicular pattern predominantly in the centrilobular regions, while a macrovesicular pattern was present in the periportal areas (Fig. 2B). There was no evidence of hepatitis, hepatocellular necrosis, fibrosis, or ductular reaction. Notably, we did not find any evidence of encephalitis or brain edema. Most of the organs showed moderate congestion, but no other significant findings were observed. Thus, the present case appeared to be one of sudden infant death associated with severe steatosis.

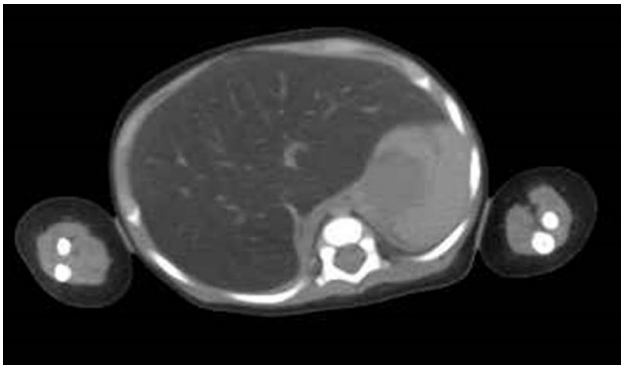

Fig. 1. Postmortem CT imaging of the abdomen. Postmortem CT of the entire body was performed before autopsy. All scans were performed using a four-slice CT scanner (Asteion/TSX-021B/4A, Toshiba, Tokyo, Japan) with a slice thickness of 1 mm and settings of 120 kV and 225 mAs for the head and 120 kV and 100 mAs for the body. These CT images were interpreted by radiologists. Postmortem CT demonstrated lower absorbance in the whole liver, suggesting fatty attenuation.

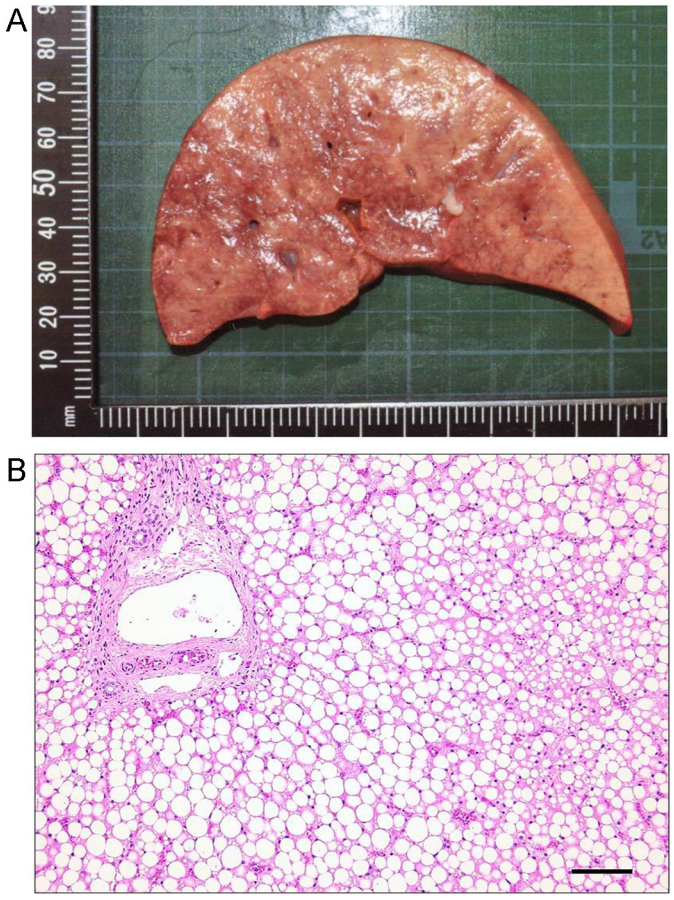

Fig. 2. Macroscopic and microscopic observations of liver tissue. (A) Macroscopic examination of the cut surface of the liver of the deceased. The surface appears somewhat yellowish, but does not show granular changes or fibrosis. (B) Microscopic examination of the liver with hematoxylin–eosin staining. The liver shows diffuse severe steatosis. A microvesicular pattern of steatosis is more dominant in the centrilobular regions, while a macrovesicular pattern is evident in the periportal areas. Neither infiltration of inflammatory cells nor fibrosis is observed. Scale bar, 100 μm.

4. Mass spectrometry analysis

To clarify the type of lipid that had accumulated in the steatotic liver, we conducted MALDI–IMS analysis. For this purpose, the liver of the deceased was frozen on a cryostat at −20 °C with a little optimal cutting temperature (OCT) compound. Control liver tissue from a child who had been killed in a traffic accident was also prepared similarly. Sequential frozen sections of 10-μm thickness were mounted on conductive glass slides, and their surfaces were sprayed with α-cyano-4-hydroxy-cinnamic acid (HCCA) matrix (7 g/L in 50% acetonitrile (ACN) with 0.2% trifluoroacetic acid (TFA)) using Image Prep™ (Bruker Daltonics, Billerica, MA, USA) (Fig. 3A). Subsequently, MALDI–IMS was performed using ultrafleXtreme™ (Bruker Daltonics, Billerica, MA, USA). Signals between m/z 0 and 2000 were collected, while raster scans of the tissue surfaces were performed automatically. Each data point was the total response of 1000 laser irradiations per location. Imaging reconstruction was performed using flexImaging 4.0™ (Bruker Daltonics, Billerica, MA, USA). The analysis indicated much more accumulation of C14:1 acylcarnitine (m/z 408.25) in the liver tissue of the deceased in comparison with the control sample (Fig. 3B). These data suggested that the deceased had suffered from VLCAD deficiency.

Consistently, when we analyzed the acylcarnitine profiles from a blood specimen of the deceased spotted on a newborn screening card using conventional tandem mass spectrometry, it also